Heliyon 9 (2023) e17341

Contents lists available at ScienceDirect

Heliyon

journal homepage: www.cell.com/heliyon

Dietary *Dialium guineense* stem-bark supplementation improves growth performance and haemato-biochemical characteristics of broiler chickens

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ARTICLE INFO

Keywords:
Broiler chickens
Velvet tamarind
Chemical composition
Growth parameters
Blood variables

ABSTRACT

Poultry farmers, particularly in developing countries, are concerned about the rising cost of conventional feed additives such as antibiotics. This has sparked a lot of interest in the search for phytogenic feed additives (PFAs) that can be used as alternative to antibiotic growth promoter in chicken diets. Thus, the purpose of this study was to evaluate the chemical composition of *Dialium guineense* **stem-bark** (DGSB) one of such PFAs, and its effect of its supplementation on the performance of Ross 308 broiler chickens. Fresh *D. guineense* stem barks were manually harvested, dried on shade and thereafter milled into DGSB powder. The DGSB was chemically analysed and standard broiler chicken diets were supplemented with DGSB at 0 (T1), 0.5 (T2), 1.0 (T3) and 1.5 (T4) g kg^{-1} feed. 200 day-old chicks were raised on starter mash from days 1–21 and finisher mash from days 22–47. Results suggested that DGSB is relatively high in fibre, ash and important phytochemicals. Average daily feed intake (ADFI) responded to incremental levels of DGSB linearly. Maximum ADFI was achieved at 1.5 g DGSB/kg feed. Birds in group T2 recorded the lowest feed conversion ratio (FCR) of 1.90, while those in group T4 had the highest FCR of 2.68. Broiler chickens on 0.5 g/kg feed attained higher ($P < 0.05$) final live weight (FLW) and average daily gain (ADG). Results indicated that dietary DGSB supplementation influenced aspects of the blood traits of Ross 308 broiler chickens. Results also show that DGSB had a quadratic effect on FLW, ADG, aspects of red blood cell indices, platelets, white blood cells, lymphocytes, glucose, cholesterol, alanine transaminase (ALT) and aspartate transaminase (AST) in broiler chickens. In conclusion, DGSB is high in fibre, ash and some beneficial phytochemicals and may be incorporated to broiler chicken nutrition at a supplementation level not beyond 0.5 g/kg feed for best growth performance and blood characteristics.

Abbreviations: PFA, Phytogenic feed additives; DGSB, Dialium guineense stem bark; ADFI, Average daily feed intake; ADG, Average daily gain; FCR, Feed conversion ratio; ADL, Acid detergent lignin; ADF, Acid detergent fibre; NDF, Neutral detergent fobre; FLW, Final live weight; ILW, Initial live weight; CV, Coeeficient of variation; AST, Aspartate transaminase; ALT, Alanine transaminase; AGP, Antibiotic growth promoters; DM, Dry matter; CF, Crude fibre; CP, Crude protein; EE, Ether extract; Hb, Haemoglobin; PCV, Packed cell volume; RBC, Red blood cell; WBC, White blood cell; MCV, Mean cell volume; MCH, Mean cell haemoglobin; MCHC, Mean cell haemoglobin concentration.

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https://doi.org/10.1016/j.heliyon.2023.e17341
Received 4 January 2023; Received in revised form 14 June 2023; Accepted 14 June 2023
Available online 16 June 2023

1. Introduction

Poultry farmers, particularly in developing countries, are concerned about the rising cost of conventional feed additives such as antibiotics. Many countries have imposed health restrictions on the use of poultry produced with antibiotics. Antibiotic growth promoters are incorporated in the ration at sub-therapeutic concentrations to improve productivity by lowering the activity of harmful gut microbes [1]. However, the continued usage of in-feed antibiotics is linked to an increase in bacterial resistance to several antibiotics [2]. The widespread public health concern over antibiotics resistance and perceived negative impacts of increased residue in animal products resulted in its removal in livestock feed as growth promoters in livestock feed in many European countries by the European Surveillance of Veterinary Antimicrobial Consumption (ESVAC) [3].

The call for stopping the inclusion of antibiotic growth promoters (AGPs) in livestock ration has sparked a lot of interest in the search for phytogenic feed additives (PFAs) that can be used as alternative to AGPs in chicken diets [4,5]. PFAs are herbs or plant-derived products added to animal feeds to achieve better growth, immune system and reduced stress responses through improvement in digestibility, nutrient uptake, product quality and antimicrobial activity [6–8]. The very common among these PFAs is *D. guineense* Wild. It is also called black velvet tamarind in English. It is a tropical legume that belongs to the family Fabaceae and sub-family Caesalpinioideae. It also grows well in the rainforest zone of West Africa. The leaves and fruits of *D. guineense* are utilized in traditional medicines to treat a variety of diseases [9]. The leaves are used to treat several ailments in folklore medicines [10].

D. guineense has attracted much attention in humans and animals due to its many nutritional and medicinal benefits. The plant is rich in malic acid, ascorbic acid, tartaric acid, sugar, and citric acids [10,11] as well as important phytochemicals [9,12]. The whole seed of *D. guineense* is abundant in energy and moderate in crude fibre and crude protein, but limited in ash and proteins, while *D. guineense* leaf is modest in energy. Similarly, the pulp is low in protein and fibre but moderate in energy and ash. There is evidence that *D. guineense* leaves are high in nutrients and beneficial bioactive compounds [13] which may affect the efficiency of feed digestion, nutrient utilization, and blood indices of chickens.

Blood provides a valuable medium for clinical investigation and nutritional status of animal hence its use in the medical and nutritional assessments [14,15]. Ogbuewu et al. [16] found that blood indices usually influenced by nutrition include packed cell volume, haemoglobin, glucose, cholesterol, urea and among others. However, available data on the use of *D. guineense* stem-bark as feed additive in chicken diets is lacking in the literature. This experiment therefore aimed at evaluating the chemical composition of *D. guineense* stem-bark, and additive value on growth performance and blood parameters of Ross 308 broiler chickens.

2. Materials and methods

2.1. Experimental site and ethics approval

This feeding experiment was performed at Federal University of Technology Owerri (latitude $4^{o}4'$ and $6^{o}3'$N and longitude: $6^{o}15'$ and $8^{o}15'$E) following the protocols outlined by the Institution's Animal Ethics Committee. The mean day temperature, relative humidity, and annual rain fall at the study site are 27 °C, 75% and 2500 mm, respectively. This study was conducted during the dry season period (December–February).

2.2. Source and preparation of Dialium guineense stem bark (DGSB) powder

The DGSB used in this study was harvested from the Botanical garden of FUTO. The plant was identified and given the voucher number CST-2023-154, and then kept at the Herbarium of Crop Science and Technology Department, FUTO for future reference. The fresh DGSB powder were washed and sliced into tiny pieces using a kitchen knife to increase the surface area and facilitate drying. The sliced DGSB were spread on black polyethylene and allowed to dry under shade for 5–10 days to a constant weight. The shade-dried samples were then milled into fine particle mass using a hammer mill fitted with a 0.01 mm sieve. The powdery mass was stored in an air-tight plastic container at room temperature until used.

2.3. Chemical analysis of DGSB

The AOAC [17] method was used for proximate analyse of DGSB samples in triplicates to determine the dry matter (DM) value (method no 930.15), crude fibre (CF) (method no 978.10), ash (method no 924.05), ether extract (EE) (method 954.02) and nitrogen content (method no 984.13). Crude protein (CP) was computed by multiplying N content by 6.25. All the proximate values were recorded in percentages (%). Fibre composition was determined in triplicates using ANKOM^{200} Fibre Analyzer (Model: ANKOM200 Fibre Analyser 220v 50Hz, New York) as described by Van Soest et al. [18] and reported in percentages (%). Terpenoids, cyanogenic glycosides and alkaloid content were determined using the Salkowski test, Keller-Killiani test and Wagner's test, respectively. Tannins, total phenols, saponins and trypsin Inhibitors were determined using standard methods [19–22]. Total flavonoid content was analysed via the aluminum colorimetric method [23,24] with some modifications using quercetin as the standard. The modification was based on the earlier report that quercetin solutions produce calibration curves with higher coefficients of determination [23]. The antioxidant capacity of DGSB was assessed using 2,2-diphenyl-1-picryl-hydrazyl-hydrate (DPPH) assay according to the method described by Abdul-Wahab et al. [25].

2.4. Housing and management of chickens

Two hundred unsexed day-old Ross 308 broiler chicks weighing averagely 40.0 g ± 1 g were used for the feeding study. The birds were bought from one of the poultry dealers in Owerri Imo State, Nigeria. The broiler chicks were housed in an open sided poultry house situated along an east-west direction for proper ventilation. The pens were cleaned and disinfected with Izal® 14 days before the study began. Feeding and watering troughs were installed 5 days before the chicks arrived. The pen floor measuring 2 m × 2 m was covered with litter materials to a height of 3 cm. Furthermore, the birds were managed under in a deep litter system. Drinkers and feeders were cleaned daily in the morning before being used. The broiler chickens were reared following the procedures outlined in the Ross broilers management guidelines [26]. Brooding and vaccination programme was implemented as reported by Ahiwe et al. [27]. The study lasted for 47 days and experimental birds have unrestricted access to feed and water.

2.5. Experimental design and diet

Experimental broiler chicks (200 Ross 308) were weighed using a weighing balance and divided into four experimental groups with 50 birds in each. Each group was further subdivided into 5 replicates with 10 birds per a replicate. Four diets (starter and finisher) were prepared as shown in Table 1 viz T1 (Control), T2 (0.5 g DGSB powder), T3 (1.0 g DGSB powder) and T4 (1.5 g DGSB powder). Each experimental diet was allotted to 5 replicate groups of 10 birds. DGSB powder was mixed with the standard diets using locally fabricated stainless steel poultry feeding mixer machine (Maximum capacity: 100 kg/batch). The decision to use these low levels of DGSB in the present study was premised on the fact that this was first study to the best our knowledge to ascertain the responses of broiler chickens to DGSB supplementation. Experimental rations were presented in mash form based on a maize-soybean meal and were formulated to meet the requirements of Ross broilers [28,29]. The Avigen [29] and the NRC [28] composition tables were utilized to compute the metabolisable energy (ME), CP, ash, CF, and EE of the starter and finisher diets. Furthermore, the proximate values were determined via AOAC [17] method. The metabolisable energy was computed using a standard formular [30].

Table 1
Composition of the experimental starter and finisher diets with calculated and analysed nutrient composition of the diets as fed.[a]

Ingredients	Starter (d 1–21)	Finisher (d 22–47)
Maize	52.00	60
Soybean meal	30.00	26
Wheat offal	3.50	2
Palm kernel cake	4.00	3
Spent grain	3.50	1
Fish meal	3.00	3
Bone meal	2.50	3
Lime stone/oyster shell	0.50	1
Common salt	0.25	0.25
Vitamin/mineral premix[b]	0.25	0.25
Lysine	0.25	0.25
Methionine	0.25	0.25
Total	100	100
Calculated nutrient composition of the experimental diets (%)		
Crude protein	23.90	20.39
Metabolisable energy (Kcal/kg)	3003	3100
Crude fibre	4.08	4.08
Crude fat	4.44	4.44
Ash	1.66	1.66
Calcium	1.63	1.63
Phosphorus	1.04	1.04
Determined nutrient composition of the experimental diets (%)[c]		
Crude protein	23.98	20.43
ME (Kcal/kg)	3003	3100
Crude fibre	4.18	4.21
Crude fat	4.45	4.43
Ash	1.67	1.66
Calcium	1.64	1.65
Phosphorus	1.15	1.13

[a] Formulated according to Ross 308 nutrition specifications (2019) (Aviagen 2019).
[b] To provide the following per kg feed: vitamin A - 12000 IU; vitamin B1 – 1.43 mg; vitamin D3 – 3500 IU; vitamin B3 – 40.17 mg; vitamin E – 44.7 IU; vitamin B2 – 3.44 mg; pantothenic acid – 6.46 mg; vitamin B6 – 2.29 mg; biotin – 0.05 mg; folic acid – 0.56 mg; vitamin B12 – 0.05 mg; vitamin K3 – 2.29 mg; iron – 120 mg; zinc – 120 mg; copper – 15 mg; manganese – 150 mg; cobalt – 0.4 mg; selenium – 0.3 mg; iodine – 1.5 mg.
[c] Chemical analysis was done using the method of AOAC (2007).

2.6. Data collection

2.6.1. Growth performance

The initial live weight of chickens in each group was recorded at the onset of the feeding trial and then on a weekly basis. These live weights were used to compute the average daily gain (ADG). Average daily feed intake (ADFI) was determined by deducting the amount of diet provided to the chickens from the left over the following morning. Feed conversion ratio (FCR) was then determined by dividing ADG with ADFI.

2.6.2. Blood analysis

On day 47 of the experiment, 5 broiler chickens were selected at random from each treatment, and 5 ml of blood was drawn from their brachial vein of each chicken using a hypodermic syringe and needles. About 2 ml of blood was aspirated into an ethylenediaminetetraacetic acid (EDTA) treated collection tube for haematological assay, while the remaining 3 ml was aspirated into bijou bottles not treated with EDTA for clinical chemistry assay. In the current study, the EDTA concentration was 1.5 mg per ml of blood [31]. Blood samples for analyses were prepared following the procedures described by the AGAPPE test kit (LiquiCHEK ™). Blood constituents were assayed using the Beckman Coulter Ac-T 10 Laboratory Haematology Blood Analyzer and Semi-auto chemistry Analyser (BA-88A model, Mindray, Nansha, Shenzhen, China), respectively. Haematological parameters analysed were haemoglobin (Hb) concentration, packed cell volume (PCV), red blood cells (RBCs), RBC indices [MCH, MCV and mean cell haemoglobin concentration (MCHC)], platelets, white blood cell (WBC), differential WBC (lymphocytes, heterophils, monocytes and eosinophils). RBC indices were calculated using the formulae of Jain [32]: MCV = 10 × PCV/RBC, MCH = Hb/RBC and MCHC = Hb × 100/PCV. Serum biochemical variables determined were total protein, albumin and globulin, glucose, cholesterol, urea, creatinine, ALT and AST. Serum biochemical parameters were determined using AGAPPE kit: urea (Urease/GLDH method), creatinine (Jaffe's method), glucose (GOD-PAP method), AST (International Federation of Clinical Chemistry recommended procedure), ALT (International Federation of Clinical Chemistry recommended method), cholesterol (CHOD-PAP method), total protein (Biuret method), and albumin (Bromocresol green method) and globulin were determined as the difference between the total protein and albumin values. The detection limits for the kits were: urea (300 mg/dl), creatinine (24 mg/dl), glucose (600 mg/dl), AST (1000 U/L), ALT (1000 U/L), cholesterol (600 mg/dl), total protein (15 gm/dL), and albumin (6 g/dl). Serum biochemical samples were analysed through Semi-auto chemistry Analyser (BA-88A model, Mindray, Nansha, Shenzhen, China) according to the methods of Ruiz-Jimenez et al. [33] and Fischbach and Dunning [34].

2.7. Data analysis

The data obtained on proximate and phytochemical contents were statistically analysed and displayed as means ± standard deviation and assessed by analysis of variance via General Linear Model procedure of SAS software [35]. In addition, data generated on growth performance (i.e., live body weight, ADFI, ADG and FCR) and blood variables (Hb concentration, PCV, RBC, RBC, MCH, MCV, MCHC, platelets, WBC, and differential WBC) were analysed using the general linear models (GLM) procedure of SAS [35]. Significant means were separated using Duncan' test and linear model employed was: $Y_{ijk} = \mu + T_i + E_{ijk}$, where Y_{ijk} is the response variable (growth performance and blood variables), μ is the general mean, T_i is the fixed effect of the DGSB supplemented diets (i = 4; T1, T2, T3

Table 2
Proximate composition (%) and phytochemical composition (mg/100 g) of DGSB.

Parameters	Mean ± standard deviation
Dry matter	86.54 ± 0.13
Moisture	13.46 ± 0.26
Crude protein	6.42 ± 0.15
Ether extract	0.47 ± 0.04
Crude fibre	30.65 ± 0.41
Total ash	9.35 ± 0.02
Nitrogen free extract	39.67 ± 0.24
Neutral detergent fibre	70.64 ± 0.63
Acid detergent fibre	46.98 ± 0.06
Acid detergent lignin	22.12 ± 0.34
Cellulose %	24.86 ± 0.28
Hemicellulose %	23.66 ± 0.57
Neutral detergent fibre	70.64 ± 0.63
Tannins	955.57 ± 1.13
Total phenol	2021.79 ± 2.92
Flavonoid	337.64 ± 8.89
Trypsin Inhibitor	44.81 ± 0.08
Terpenoid	3.98 ± 0.22
Cyanogenic glycosides	8.73 ± 0.13
Alkaloids (%)	5.83 ± 0.21
Saponins (%)	4.04 ± 0.10
Total Antioxidant (% DPPH Scavenged) μg/ml	45.95 ± 0.11

and T4) and E_{ijk} is the random error linked with observation. The supplementation-related responses to increasing supplementation levels of DGSB was modeled using the quadratic formula: $Y = a + c_1x + c_2x^2$ via, where y = response parameters (growth performance and blood variables); a = intercept; x = supplementation levels of DGSB; c_1 and c_2 = coefficients of the quadratic equation; and $-c_1/2c_2$ = DGSB level for optimum productivity. This equation (Y = a + bx) was used to determine the relationship between ADFI and DGSB, where Y = ADFI, a = intercept, b = coefficient of the linear equation and x = DGSB supplementation level. These models were used because they gave the highest coefficient of determination.

3. Results

3.1. Chemical composition of DGSB

The concentrations of DM, CP, CF, EE and total ash were 86.54, 6.42, 30.65, 0.47 and 9.35%, respectively as described in Table 2. The predominant fibre content detected in DGSB in the current study follows the order of NDF (70.64%) > acid detergent fibre, ADF (46.98%) > cellulose (24.86%) > hemicellulose (23.66) > acid detergent lignin, ADL (22.12%). The phytochemical results indicate that DGSB is high in tannins (955.57 ± 1.13 mg/100 g), total phenol (2021.79 ± 2.92 mg/100 g) and flavonoids (337.64 ± 8.89 mg/100 g), and moderate in trypsin inhibitors (44.81 ± 0.08 mg/100 g) and total antioxidant activity (45.95 ± 0.11 μg/ml), and low in terpenoid, cyanogenic glycosides, alkaloids and saponins.

3.2. Growth performance

The initial live weight (ILW), FLW, ADFI, ADG and FCR of birds offered DGSB is illustrated in Table 3. The mean ILW, FLW, ADG, ADFI and FCR were 42.77 g/b/d, 2897.48 g/b/d, 67.75 g/b/d, 154.75 g/b/d and 2.31. In comparison with control (T1), birds in group T2 had better ($P < 0.05$) FLW and FCR. Similarly, birds in group T2 experienced better ADG than those on the other three groups. However, broiler chickens fed 0, 1.0 and 1.5 g DGSB/kg diet had similar ADG. ADFI responded to incremental levels of DGSB linearly. The highest ADFI was achieved at the 1.5 g DGSB/kg. The coefficient of variation (CV) values across growth performance data were low and ranged from 4.00 to 12.71%.

3.3. Blood indices

Table 4 shows the haematological characteristics of broiler chickens on DGSB supplementation. Inclusion of DGSB in broiler chicken feed had no influence on Hb, RBC, PCV, MCHC, heterophils (H), monocytes and eosinophils values. However, broiler chickens fed a diet without DGSB supplementation had the highest MCV value which differed significantly ($P < 0.05$) from those offered a diet supplemented with DGSB at 1.5 g/kg diet, but similar ($P > 0.05$) to birds in groups T2 and T3. Conversely, birds in group T3 recoded the lowest MCH value which differed significantly ($P < 0.05$) from those in the other three groups, but similar ($P > 0.05$) to those that received a diet without DGSB supplementation. Birds in group T2 and T4 had lower ($P < 0.05$) platelets than birds in group T3. However, birds in groups T1, T3 and T4 had similar ($P > 0.05$) platelet values. Birds in group T2 had the highest WBC counts; whereas, birds fed the other 3 diets had similar ($p > 0.05$) WBC values. Birds in groups T1 and T2 recorded the highest lymphocytes (L), which differed statistically ($p < 0.05$) from those in group T4. Maximum H/L ratio was obtained from in birds in group T3; whereas the minimum was recorded from birds in group T2. The CV values were low to high and ranged from 2.20 to 73.20%.

The influence of dietary DGSB supplementation on serum biochemical variables of broiler chickens is displayed in Table 5. Mean total protein, albumin (A), globulin (G), A/G ratio, glucose, creatinine, urea, cholesterol, ALT and AST were 49.83 g/dl, 19.25 g/dl, 30.58 g/dl, 0.63, 172.50, mg/dl, 0.37 mg/dl, 6.50 mg/dl, 87.42 mg/dl, 464.09 U/L and 20.33 U/L, respectively. Dietary DGSB did not affect ($P > 0.05$) urea, total proteins, albumin, globulin, and creatinine in broiler chickens. However, birds in groups had statistically elevated ($P < 0.05$) glucose levels in comparison with broiler chickens in groups T and T5. However, broilers in groups T2 and 4 had elevated ($P < 0.05$) cholesterol than their counterparts given diet having 0 and 0.5 GGSB/kg. In addition, ALT values were highest ($p < 0.05$) among broiler chickens fed diets supplemented with DGSB 0 when compared to broiler chickens fed a control diet (i.e., without DGSB supplementation). Birds in group T1 had lower AST ($P < 0.05$) than those in groups T2-T4 which did not differ significantly from

Table 3
Performance characteristics of broiler chickens offered DGSB supplemented diets.

Parameters	DGSB supplementation levels				Mean	SD	CV (%)	SEM	*p*-value
	T1	T2	T3	T4					
ILW (g/b/d)	43.50	42.63	42.63	42.30	42.77	2.02	4.00	1.01	0.1612
FLW (g/b/d)	2934.60^{b}	3002.11^{a}	2869.06^{c}	2784.15^{c}	2897.48	21.58	8.14	12.62	0.0456
ADG (g/b/d)	67.29^{b}	78.23^{a}	65.07^{b}	60.40^{b}	67.75	6.54	9.66	3.78	0.0226
ADFI (g/b/d)	147.47^{b}	148.87^{b}	160.73^{a}	161.93^{a}	154.75	6.61	4.27	3.82	0.0345
FCR (g/g)	2.19^{b}	1.90^{c}	2.47^{a}	2.68^{a}	2.31	0.29	12.71	0.17	0.0134

^{a,b,c} Means in the same row different superscript are significantly different ($p < 0.05$), ILW – initial live weight; FLW – final live weight; ADFI –average daily feed intake; ADG – average daily gain; FCR-feed conversion ratio; SD: standard deviation, CV coefficient of variation, SEM: standard error of the mean.

Table 4
Haematological values of broiler chickens on dietary DGBS supplementation.

Parameters	DGSB supplementation levels				Mean	SD	CV (%)	SEM	*p*-value
	T1	T2	T3	T4					
Hb (g/dl)	17.23	16.73	16.33	17.23	16.88	0.70	4.29	0.41	0.3963
PCV (%)	29.27	29.03	27.40	28.97	28.67	1.00	4.49	0.58	0.2242
RBC ($\times 10^{12}$/L)	2.80	2.81	2.67	2.77	2.76	0.12	4.97	0.07	0.3362
MCV (fl)	105.40^{a}	104.77^{ab}	104.67^{ab}	103.37^{b}	104.55	2.70	2.51	0.01	0.0370
MCH (pg)	61.60^{ab}	62.30^{a}	62.37^{a}	59.70^{b}	61.49	0.86	2.20	0.11	0.0193
MCHC (g/dl)	58.47	57.80	59.60	59.50	58.84	1.37	2.40	0.79	0.3780
Platelets ($\times 10^{9}$/L)	54.33^{ab}	52.67^{b}	56.00^{a}	53.67^{b}	54.17	5.34	9.07	0.55	0.0063
WBC ($\times 10^{9}$/L)	91.67^{b}	94.67^{a}	92.33^{b}	91.33^{b}	92.50	2.96	2.95	1.01	0.0327
Lymphocytes (%)	76.00^{a}	78.33^{a}	75.00^{ab}	74.00^{b}	75.83	3.45	4.80	0.09	0.0171
Heterophils (%)	21.00	19.33	22.33	20.67	20.83	4.02	19.48	2.32	0.7278
H/L ratio	0.28	0.26	0.30	0.28	0.28	0.13	2.10	0.03	0.5432
Monocytes (%)	1.67	1.33	1.67	1.67	1.59	0.58	–	0.33	0.8592
Eosinophils (%)	1.33	1.00	1.00	1.00	1.08	0.79	73.20	0.46	0.9578

[a,b,c] Means in the same row not sharing a common superscript are significantly different ($p < 0.05$), Hb – haemoglobin; PCV – packed cell volume; RBC – red blood cell; H – heterophils; L– lymphocyte; WBC – white blood cell; MCH – mean cell haemoglobin; MCV – mean cell volume; AST-aspartate transaminase; ALT – alanine transaminase; SD: standard deviation, CV coefficient of variation, SEM: standard error of the mean.

Table 5
Effect of DGSB supplementation levels on serum biochemical indices of broiler chickens.

Parameters	DGSB supplementation levels				Mean	SD	CV (%)	SEM	*p*-value
	T1	T2	T3	T4					
Total proteins (g/dl)	50.67	50.00	48.33	50.33	49.83	4.18	8.11	2.42	0.9253
Albumin (g/dl)	20.00	20.33	18.33	18.33	19.25	0.92	6.69	2.53	0.0731
Globulin (g/dl)	30.67	29.67	30.00	32.00	30.58	0.42	5.13	1.91	0.0710
A/G ratio	0.65	0.69	0.61	0.57	0.63	0.32	7.63	0.10	0.0672
Glucose (mg/dl)	165.33^{b}	180.00^{a}	186.00^{a}	158.67^{b}	172.50	12.95	10.21	7.48	0.0188
Creatinine (mg/dl)	0.30	0.50	0.40	0.27	0.37	0.15	47.00	0.09	0.3778
Urea (mg/dl)	6.00	6.33	7.00	6.67	6.50	0.54	10.37	0.31	0.3300
Cholesterol (mg/dl)	83.67^{b}	84.00^{b}	92.33^{a}	89.67^{a}	87.42	4.57	6.97	2.64	0.0246
ALT (U/L)	393.67^{c}	467.33^{b}	537.67^{a}	457.67^{b}	464.09	74.29	18.81	42.89	0.0368
AST (U/L)	15.67^{b}	23.33^{a}	22.33^{a}	20.00^{a}	20.33	2.36	18.68	1.36	0.0282

[a,bc] Means in the same row not sharing a common superscript are significantly different ($p < 0.05$), A – albumin; G – globulin; AST-aspartate transaminase; ALT – alanine transaminase; SD: standard deviation, CV coefficient of variation, SEM: standard error of the mean.

each other.

3.4. Optimization functions

The impact of dietary DGSB supplementation levels on FLW and ADG as shown in Table 6 revealed that FLW and ADG were optimised at DGSB levels of 1.73 g/kg feed and 2.07 g/kg feed, respectively. Table 7 shows strong and positive relationship between DGSB and ADFI in broiler chickens. Table 8 shows the influence of DGSB on MCH, MCV, platelets, WBC and lymphocytes of broiler chickens. MCV and MCH were optimised at DGSB levels of 2.58 ($r^2 = 0.44$; $P = 0.0370$) and 1.95 ($r^2 = 0.43$; $P = 0.0193$) g/kg feed, respectively. Quadratic results showed that DGSB was optimised at 2.90 ($r^2 = 0.03$; $P = 0.0063$), 1.90 ($r^2 = 0.74$; $P = 0.0327$) and 1.94 ($r^2 = 0.69$; $P = 0.0171$) g/kg feed for platelets, WBC and lymphocytes, respectively in broiler chickens. DGSB had a quadratic influence ($P < 0.05$) on glucose, cholesterol, AST and ALT in broiler chickens. Glucose, cholesterol, AST and ALT were optimised at 2.43 ($r^2 = 0.94$; $P = 0.0188$), 4.26 ($r^2 = 0.67$; $P = 0.0246$), 2.84 ($r^2 = 0.90$; $P = 0.0368$) and 2.74 ($r^2 = 0.92$; $P = 0.0282$) g DGSB/kg feed, respectively in broiler chickens. The results indicate that all the significant blood values had a moderate (0.03) to high (0.94) coefficient of determination (r^2).

Table 6
DGSB supplementation levels for optimal growth performance indices of broiler chickens.

Variable	Formular	X	Y	r^2	*p*-value
FLW (g/b/d)	$Y = 2853.1 + 132.08\ DGSB - 38.105\ DGSB^2$	1.73	2967.55	0.88	0.0456
ADG (g/b/d)	$Y = 56.693 + 16.129\ DGSB - 3.9025\ DGSB^2$	2.07	73.36	0.69	0.0226

Table 7
Relationship between DGSB supplementation levels and ADFI in broiler chickens.

Variable	Formular	r^2	*p*-value
Average daily feed intake (g/b/d)	Y = 140.94 + 5.524 DGSB	0.87	0.0345

r^2 - coefficient of determination, p value – probability value.

Table 8
DGSB supplementation levels for optimal haemato-biochemical indices of broiler chickens.

Variable	Formular	X	Y	r^2	*p*-value
MCV (fl)	Y = 107.16–2.49158 DGSB + 0.4825 $DGSB^2$	2.58	103.94	0.44	0.0370
MCH (pg)	Y = 62.588–1.805 DGSB + 0.4575 $DGSB^2$	1.97	60.81	0.43	0.0193
Platelets ($\times 10^9$/L)	Y = 52.993 + 0.9725 DGSB - 0.1675 $DGSB^2$	2.90	54.40	0.03	0.0063
WBC ($\times 10^9$/L)	Y = 103.85–2.55 DGSB + 0.67 $DGSB^2$	1.90	101.42	0.74	0.0327
Lymphocytes (%)	Y = 74.003 + 3.2295 DGSB - 0.8325 $DGSB^2$	1.94	77.14	0.69	0.0171
Glucose (mg/dl)	Y = 123.5 + 51.102 DGSB - 10.5 $DGSB^2$	2.43	185.68	0.94	0.0188
Cholesterol (mg/dl)	Y = 77.097 + 6.3705 DGSB - 0.7475 $DGSB^2$	4.26	90.67	0.67	0.0246
AST (U/L)	Y = 206.43 + 218.31 DGSB - 38.415 $DGSB^2$	2.84	516.59	0.90	0.0368
ALT (U/L)	Y = 4.8475 + 13.686 DGSB - 2.4975 $DGSB^2$	2.74	23.60	0.92	0.0282

MCV - mean cell volume; WBC – white blood cell; MCH - mean cell haemoglobin; fl – femtolitre; pg-picogram; AST – aspartate transaminase; ALT – alanine transaminase r^2: coefficient of determination.

4. Discussion

4.1. Nutrient composition

Proximate analysis data are widely used in research cum the industry to quickly assess the nutrient value of feedstuffs. This study revealed that DGSB contained 13.46% moisture, indicating that DGSB could it could be easily ashed. This also suggests that shelf life of DGSB will be extended and microbial deterioration will be limited. The DM content of 86.54% obtained in this study indicates that DGSB was rich in nutrients as DM is used as an indicator of the quantity of nutrients that are available to the animal in a particular feed. The CP value of 6.42% recorded in this experiment is lower than the values of 12.64–18.72% and 17.44% reported in *D. guineense* leaves and whole seeds by Awotedu et al. [36] and Osanaiye et al. [37], respectively, but higher than the value of 3.94% reported in *D. guineense* pulps by Osanaiye et al. [37]. The CP value of 6.42% recorded in this experiment suggests that DGSB contains less nitrogenous compound than the leaves and whole seeds. The low CP content of DGSB lends support to its use as a non-protein source feed additive in monogastric nutrition. The EE value of 1.02–1.31% reported in the leaves of *D. guineense* by Awotedu et al. [36] was lower the value obtained in this investigation. This implies that stem-bark of *D. guineense* plant may be low in essential oils. The ash content of DGSB determined in this study was greater than the levels of 7.80% and 2.55% found by Osakwe et al. [38] and Osanaiye et al. [38] *D. guineense* leaves and whole seeds, respectively. The variation could partly be linked to the plant part used and soil type. Nevertheless, the ash value recorded in this study confirmed that DGSB is moderate in minerals and may be used as a feed additive in livestock and chickens.

The demand for fibre in animal feed is increasing [39] and a proper proportioning of fibre components should be used to assess feed quality. The CF value of 30.65% reported in this study was greater than the concentrations of 13.34–14.29% reported for *D. guineense* leaves by Awotedu et al. [36]. This suggests that DGSB may be incorporated to animal feed to help feed digestion as reported by Jha and Mishra [39] that dietary fibre stimulates gut health and changes gut micro-environment and morphology in animals. The order of fibre elements detected in DGSB in this study is NDF (70.64%) > ADF (46.98%) > cellulose (24.86%) > hemicellulose (23.66%) > ADL (22.12%). NDF is used to estimate the level of lignin, hemicellulose and cellulose in a feed resource. The high NDF and ADF content in DGSB in this experiment, suggest that the incorporation of DGSB in monogastric nutrition may negatively affect voluntary feed intake and allow the simple stomached avian species to fill up their gut more quickly. This necessitates the development of appropriate strategies, such as exogenous enzyme supplementation, to increase the digestibility of DGSB. Hemicellulose defined as cell wall polysaccharides that can bind strongly to cellulose micro-fibrils by hydrogen bonds as obtained in this experiment was higher compared to the amount (16.51%) found in *D. guineense* leaves by other researchers [36–38]. This trend was confirmed by Jung and Allen [40], who discovered that stems have higher cell wall concentration than leaves, which increases as the plant matures. The NDF, ADF, ADL and cellulose values recorded in this study were slightly higher than the values of 61.65, 45.14, 20.48 and 24.66%, respectively reported in *D. guineense* leaves by other investigators [36–38].

4.2. Phytochemical composition

Plants contain a variety of phytochemicals, including phenols, flavonoids, saponins and tannins, among others [41,42]. These phytochemicals are rich in antioxidant, anti-inflammatory and antibacterial activity, but may also contain anti-nutritional factors that reduce the absorption of nutrients such as minerals or amino acids in the gut [41]. The present study revealed that DGSB is high in tannins, total phenol and flavonoids but low in alkaloids, saponins, terpenoids and cyanogenic glycosides. This observation agrees with

Osakwe et al. [38] and Oluwole-Banjo [13], who noticed that DGSB is rich in saponins, phenols, cyanogenic glycosides, and flavonoids. The high phenolic compounds in DGSB indicate that DGSB can be supplemented in animal feed to help protect the cells from oxidative damage [43]. Conversely, DGSB can be used to enhance intestinal health and improve intestinal microbiota as some phenolic compounds have been discovered to stop the proliferation of pathogenic bacteria in the gut by binding minerals required by microbes for survival, or rupturing the cell membranes of the microbes [44,45]. The reported higher content of flavonoids in DGSB in this investigation is in agreement with the findings by Osakwe et al. [38] and Oluwole-Banjo [13]. The saponin content recorded in the current study was lower than the value of 25.82 mg/100 g reported by Osakwe et al. [38] and Oluwole-Banjo [13] for the same plant. DPPH free radical scavenging activity assay examines the ability of the extract to donate hydrogen or to scavenge free radicals [24,42]. This study suggested that DGSB has antioxidant property and this may be ascribed to action of bioactive constituents contained in the test material such as phenolic compounds [41,42].

4.3. Growth performance

This investigation indicate that broiler chickens fed 0.5 g DGSB/kg feed had better FLW, ADG and FCR than those fed the other 3 treatment diets. The antioxidant and antimicrobial properties of DGSB [13,46] may have improved the growth performance of chickens fed 0.5 g/kg feed DGSB. There is evidence in chickens voluntary feed intake is a function of dietary fibre content and characteristics [39]. The significantly higher ADFI in groups T3 and T4 when compared to birds in groups T1 and T2 might be an attempt by the chickens in these groups to utilise the limiting nutrients in the feed to meet their dietary needs. The mechanism underlying the increased ADFI in chickens fed 1.5 g/kg DGSB is not well known. This could be due to nutrient dilution which causes broiler chickens fed 1.5 g/kg feed DGSB to consume more diets to meet their nutrient requirements. The improvement in FCR and ADG in group T2 could be linked to the ability of beneficial bioactive compounds in DGSB to improve the conversion of feed to meat via one or combination of these mechanisms: (1) decreasing the population of gut pathogens by rupturing their cell membrane, (2) stimulating the release of digestive juice and endogenous enzymes, (3) protecting the cells from oxidative stress and direct nutritional influence, and (4) activation of gut immune responses. A low FCR indicates a high-quality feed, whereas a high FCR suggests otherwise. The observed poor FCR for birds in groups T2-T4 could be due to high fibre level in these diets, which is beyond the tolerance level of the chickens [47].

4.4. Blood characteristics

Haematological variables are now used to determine health status of farm animals, and variations in blood constituents may affect feed and feeding [16,48]. Low protein intake lowers Hb and PCV values, but higher protein intake tends to improve them [16]. Results suggested that blood variables measured in this feeding trial is within the value reported for chickens [15,49]. The results also showed that DGSB did not affect Hb, PCV, RBC, MCHC, heterophils, monocytes and eosinophils in broiler chickens. This indicates the non-significant effect of DGSB on these blood variables. This also implies that inclusion of DGSB up to 1.5 g/kg in the diets of chickens had no deleterious impact on these blood components. MCV values in this study were ranged from 103.37 to 105.40 fl which is within the range (90–140 fl) observed for chickens [49]. Furthermore, MCV value in broiler chickens offered 1.5 g/kg feed DGSB was significantly lower than those fed control diet. The significantly lower MCH in birds given 1.5 g/kg DGSB relative to the groups fed 0.5 and 1.0 g/kg DGSB might be ascribed to low uptake of this diet, as revealed by the proximate analysis that DGSB is high in fibre CF and low in crude protein, resulting in poor performance. The significantly lower MCV and MCH levels in broiler chickens fed 1.5 g/kg feed DGSB, suggest hypochromic microcytic anaemia associated with iron deficiency. This may also be due to anti-physiological agents such as trypsin inhibitors found in DGSB, as evidenced by the chemical analysis, which may have exceeded the tolerance level of broiler chickens.

The main function of WBC is to protect the body of the animal from infections. WBC values of broiler chickens in all the treatments were found within the normal range of 50–100 $\times$ 10^9/L for chickens [49]. Although the WBC values in this fall within the concentrations found for chickens [49], the significantly higher WBC in broiler chickens fed 0.5 g/kg feed DGSB indicates enhanced utilization of the experimental diet, as corroborated by other authors [50,51], resulting in better growth performance [16]. This investigation shows that incorporation of DGSB to broiler chicken feed had significant influence on the levels of platelets and lymphocytes. However, these values were within the normal range (40–80 $\times$ 10^9/L and 40–100%, respectively) reported for chickens [49]. In chickens, the H/L ratio is employed to assess nutritional stress as well as immune system status. The lower the H/L ratio, the better the immune system and ability to fight infection [51]. However, in this study, DGSB supplementation did not have significant influence on H/L ratio in Ross 308 broiler chickens.

The ability of phytogenic feed supplements to influence serum cholesterol levels in animals has been highlighted [15,16,45] and this effect has been attributed to a possible effect of plant bioactive compounds to accelerate the removal of total lipids and cholesterol via faeces and/or inhibit cholesterol formation [16]. In contrast, addition of DGSB to broiler chicken feed increased serum cholesterol content in the current study, which is consistent with the findings of Abu et al. [52]. It is likely that DGSB stimulate lipid biosynthesis by enhancing the transport of acetate into the liver cell, resulting in increased substrate (acetate) availability, which is then utilized by the liver cells to form cholesterol. Serum creatinine, total proteins, albumins and urea values in broiler chickens were not influenced by DGSB. The comparable serum total proteins, globulin, albumins, A/G ratio, urea and creatinine values in this study suggest that DGSB supplementation had no significant effect on these serum variables in broiler chickens. Broiler chicken in groups that received 0.5–1.0 g/kg DGSB recorded higher serum glucose levels than their counterpart offered 0 and 1.5 g/kg DGSB. The phytochemical results of this study show that DGSB contains a reasonable amount of beneficial bioactive substances such as flavonoid and phenolic compounds [12]

all of which exhibit antioxidant/anti-inflammatory activity. The mechanism involved in the hyperglycemic activity of DGSB in chickens given 0.5 and 1.0 g/kg feed DGSB is not clear. Hence, further investigation is required in this direction. In contrast, the lower serum glucose level in broilers fed 1.5 g DGSB/kg feed relative to those offered 0.5 and 1.0 g/kg feed DGSB indicates that high inclusion level of DGSB reduced the serum glucose level in broiler chickens. However, this is not a source of concern as serum glucose levels of broiler chickens in all the groups within the normative value (108.3–236.10 mg/dl) found for broiler chickens [53].

The level of the various enzymes involved in the metabolism and function of the heart, liver and kidney are used to assess their effects on these important organs. Serum enzymes are often elevated following cellular damage of smooth muscles, heart, liver and kidneys as a result of enzyme leakage from cells to blood [54]. In toxic cases, the level of serum ALT is typically higher than serum AST [55]. The fact that serum AST is characteristically lower than serum ALT in this feeding trial rule out the chances of toxicity due to DGSB supplementation. The increased serum ALT and AST values in broiler chickens fed DGSB in the current study indicate the inability of DGSB's bioactive components to protect hepatocytes from cellular damage [54] However, more research is needed to determine the actual phytochemicals in DGSB responsible for the increased AST and ALT levels in broiler chickens fed DGSB.

4.5. Optimization function

This investigation suggests that no single DGSB supplementation level optimised growth performance and haemato-biochemical variables in broiler chickens. A strong and positive linear association was found between DGSB levels and ADFI in broiler chickens, suggesting that DGSB had a strong and positive r^2 on ADFI in Ross 308 broiler chickens. The dietary DGSB supplementation level that optimised ADG was higher than the level that optimised FLW. Similarly, the DGSB levels that optimised MCV, platelets, glucose, cholesterol, AST and ALT were higher than the levels for optimum MCH, WBC and lymphocytes. This suggests that the nutrient requirements of broiler chickens are dynamic and dependent on which performance variable is taken into consideration when formulating rations for chickens. Thus, the feeding program for optimal productivity in Ross 308 broiler chickens must take into consideration the primary variable in question. These findings have practical application when incorporating diet DGSB for growth performance and blood variables in broiler chickens to minimise feed additive wastages. The results showed that significant variables had low to high r^2. The moderate to high r^2 value recorded for the FLW, ADG, WBC, lymphocytes, glucose, cholesterol, AST and ALT showed the moderate to high strength of relationship between these variables and DGSB using the quadratic analysis. The significant quadratic effect on FLW, ADG, ADFI, WBC, lymphocytes, glucose, cholesterol, AST and ALT suggest that their concentrations in broiler chickens could be determined at a given supplementation level of DGSB supplemented in the ration. Few studies have utilized quadratic formula to ascertain the optimal amount of feedstuffs or additives that gave the best performance variables in chickens [56, 57]. However, there is scanty published data on the influence of DGSB on productivity of broiler chickens using quadratic formula.

4.6. Limitations and strengths of the study

This feeding trial was conducted on Ross 308 broiler chickens, and the results may not be applicable to other animal species. The effect of sex was not evaluated in this study, which could be a limitation. As a result, more research is required in this direction. Despite these limitations, the results of this feeding trial show that DGSB is high in fibre, ash and beneficial bioactive compounds, and it can be supplemented to the Ross 308 chicken diet at 0.5 g/kg feed to improve growth and blood characteristics.

5. Conclusion

This experiment has demonstrated that DGSB is high in fibre, ash and beneficial phytochemical compounds indicating that DGSB may serve as a feed additive source for chickens. It is recommended that DGSB may be incorporated to the rations of Ross 308 broiler chickens at 0.5 g/kg diet to enhance growth performance and blood characteristics. The quadratic results suggested that no single dose of DGSB optimised all the performance variables in this study, suggesting that the DGSB level for optimum growth performance and blood variables in broiler chickens is a function of production parameter under investigation. This also indicates that nutrient needs of Ross 308 broiler chickens are complex and should be considered when developing a feed additive for broiler chickens. It is therefore concluded that optimizing DGSB levels in the diet of broiler chickens could help enhance their growth performance and haemato-biochemical characteristics.

Declaration

Author contribution statement

Ifeanyichukwu Princewill Ogbuewu: Conceived and designed the experiments; Analysed and interpreted the data; Wrote the paper.

Christain Anayo Mbajiorgu: Performed the experiments; contributed the reagents, materials, analysis tool, analysed and interpreted the data.

Data availability statement

Data will be made available on request.

Additional information

No additional information is available for this paper.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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