

Effects of graded dietary inclusion of *Sida acuta* leaf meal on growth performance and serum hormone profile of Ross 308 broiler chickens

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ABSTRACT: This study evaluated the effects of graded dietary inclusion of *Sida acuta* leaf meal on growth performance and serum hormone profile of broiler chickens. One hundred and twenty day-old Ross 308 broiler chicks were assigned to four dietary treatments, with three replicates of 10 birds per treatment, in a completely randomised design. The study lasted eight weeks, comprising a four-week brooding period on a commercial starter diet followed by four weeks of experimental feeding with diets containing 0, 0.5, 1.0 and 1.5% *Sida acuta* leaf meal g/kg feed, at the Teaching and Research Farm, Department of Agricultural Education, Federal College of Education (Technical), Omoku, Rivers State, Nigeria. Fresh leaves were washed, oven-dried at 40 °C for five days, milled and incorporated into the diets by weight-for-weight replacement of maize. Initial body weight did not differ among treatments ($p > 0.05$). Final body weight increased from 2 215.33 g in T1 to 2 498.67 g in T4, weight gain from 2 174.67 to 2 458.00 g/bird, average daily gain from 77.67 to 87.79 g/bird/day, total feed intake from 4 142.33 to 4 358.67 g/bird, and feed conversion ratio improved (decreased) from 1.91 to 1.78. Mortality was low and not significantly different among treatments (range 0.00–3.33 %; $p = 0.412$). Serum triiodothyronine (T3) increased from 1.85 to 2.78 ng/mL, thyroxine (T4) from 14.32 to 19.85 ng/mL, T3:T4 ratio from 0.130 to 0.141, and testosterone from 1.42 to 2.31 ng/mL, while cortisol decreased from 4.85 to 2.92 ng/mL. All reported treatment effects were significant at $p < 0.05$ unless otherwise stated. Polynomial contrasts indicated significant linear trends for all performance and hormone endpoints, with significant quadratic components for final body weight, FCR, T3, cortisol and testosterone. The results indicated that *Sida acuta* leaf meal was associated with improved growth performance, increased circulating T3, T4 and testosterone concentrations, and reduced serum cortisol concentration under the conditions of the experiment; the 15 g/kg treatment produced the most favourable measured response across all endpoints.

Keywords: Broilers, growth performance, phytogenic feed additive, serum hormones, *Sida acuta*.

INTRODUCTION

Poultry production is an important source of animal protein and household income in many tropical countries, but the profitability and sustainability of broiler enterprises are constrained by the rising cost of conventional feed ingredients, the pressure of infectious disease, growing concerns about antimicrobial resistance, and considerable

variability in productive efficiency (Windisch *et al.*, 2008). Phytogenic feed additives have attracted increasing research interest as potential nutritional tools because plant-derived secondary metabolites may influence feed palatability, digestion, gut microbial ecology, immune function, oxidative balance and endocrine status. However,

the response to a phytogetic additive is not uniform; it depends on plant identity, dose, processing method, diet composition, bird age, environmental conditions and the chemical profile of the plant material (Gadde *et al.*, 2017; Wang *et al.*, 2021).

Sida acuta Burm.f. is a tropical member of the Malvaceae that is widely distributed across West Africa, including the humid forest zone of Rivers State, Nigeria. The plant has a long history of use in ethnomedicine and has been reported to contain alkaloids, flavonoids, tannins, saponins, steroids and phenolic compounds, several of which exhibit in vitro antimicrobial, anti-inflammatory and antioxidant activity (Akilandeswari *et al.*, 2010; Raimi *et al.*, 2014). These phytochemicals may confer useful biological effects when the leaf meal is fed at low dietary concentrations, but excessive inclusion may reduce nutrient utilisation, impair palatability or produce adverse physiological responses. Consequently, a single endpoint is insufficient to establish the safety or suitability of a phytogetic additive; an integrated evaluation that includes growth, nutrient use and endocrine markers is required.

Growth performance indicators: final body weight, body weight gain, average daily gain, feed intake and feed conversion ratio remain the primary commercial metrics for evaluating broiler responses to any dietary intervention. They reflect the integrated outcome of feed palatability, digestion, absorption, metabolism and the efficiency with which dietary nutrients are converted to body tissue. Serum hormonal markers, particularly the thyroid hormones triiodothyronine (T3) and thyroxine (T4), the glucocorticoid cortisol and the androgen testosterone, complement the performance data by indicating whether the dietary treatment modifies the endocrine regulation of metabolism, stress and growth. Thyroid hormones are central regulators of basal metabolic rate, thermogenesis and protein turnover in birds; The T3:T4 ratio may provide an indirect indication of changes in the relative availability of T3 and T4 and may be influenced by peripheral conversion; however, direct assessment of deiodinase activity would be required to confirm changes in T4-to-T3 conversion; cortisol reflects the activity of the hypothalamic–pituitary–adrenal axis; and testosterone influences skeletal muscle accretion and behaviour. Measuring these hormones in parallel with performance provides a more informative evaluation of the physiological response to a phytogetic additive than performance data alone (Decuyper *et al.*, 2005; Scanes, 2014).

Recent phytogetic-feed-additive research has moved beyond growth performance alone toward integrated evaluation of digestion, immune response, oxidative balance and endocrine markers. Meta-analytic work has assessed the consistency of phytogetic effects on growth and feed conversion across trials, while individual studies have examined specific plants at graded inclusion levels (Wang *et al.*, 2021). Such work supports multidimensional experimental designs that include more than weight gain alone.

Previous research by Shittu *et al.* (2021) investigated

Sida acuta leaf extract in broiler diets as replacement for antibiotics and reported significant improvement in final live weight (1896.4 to 2300.8g), weight gain (1852.9 to 2257.4 g), feed:gain ratio (2.27 to 1.82), elimination of mortality (15.1% to 0%), enhanced Newcastle and Gumboro antibody titres and improved caecal microbiota (decreased *E. coli*, increased *Lactobacilli*). In the actual leaf meal form, Busari *et al.* (2026) investigated *Sida acuta* leaf meal in ISA Brown laying birds and reported that moderate inclusion at 2g/kg improved final weight, hen-day production, FCR, crude protein digestibility, PCV (32%), haemoglobin and total protein (50.5 g/dl), while 5g/kg reduced performance. However, information remains limited regarding endocrine and hormonal responses to *Sida acuta* leaf meal in broilers, particularly the effects of graded inclusion on circulating metabolic, stress and reproductive hormones. Important unresolved questions remained: whether graded inclusion would produce a linear or non-linear response in performance; whether the treatment would alter circulating concentrations of metabolic and stress hormones; whether any hormone response would be physiologically meaningful rather than incidental; and whether the observed values would remain within established reference intervals for the relevant broiler strain and age. The present study was therefore designed to evaluate the effects of graded dietary *Sida acuta* leaf meal on the growth performance and serum hormone profile of broiler chickens.

The study tested two hypotheses. The first null hypothesis was that graded dietary inclusion of *Sida acuta* leaf meal would not significantly affect growth performance or feed conversion of broiler chickens. The second null hypothesis was that graded inclusion would not significantly affect serum concentrations of T3, T4, cortisol or testosterone.

MATERIALS AND METHODS

Experimental site and duration

The study was conducted at the Teaching and Research Farm, Department of Agricultural Education, Federal College of Education (Technical), Omoku, Rivers State, Nigeria. The site lies in the humid rainforest agro-ecological zone of southern Nigeria (latitude 4° 46' N, longitude 6° 39' E; mean annual rainfall ~ 2 400 mm; mean ambient temperature 26–32 °C during the experimental period). Birds were housed in a deep-litter poultry unit that was thoroughly cleaned, disinfected with a quaternary ammonium compound, and allowed to rest for 14 days before placement. Routine brooding, vaccination against Newcastle disease (La Sota strain, administered at days 7 and 21 via eye-drop), infectious bursal disease (intermediate-plus strain, administered at day 14 via drinking water) and fowl pox (administered at week 6 via wing-web stab), and standard medication and biosecurity

management were maintained throughout the trial. The experiment lasted eight weeks, comprising four weeks of brooding on a commercial starter diet followed by four weeks of feeding the experimental finisher diets. Pen temperature, relative humidity, lighting, ventilation and litter management followed standard broiler guidelines for the season (initial temperature 32 °C, reduced by 2 °C per week to a final 26 °C; 23 h light:1 h dark photoperiod during the first week, reduced to 18 h light:6 h dark thereafter).

Plant collection and authentication

Fresh *Sida acuta* leaves were collected from mature, flowering plants growing wild within and around the college environment. The plant was authenticated by a plant taxonomist at the Herbarium of the Department of Plant Science and Biotechnology, Rivers State University, Port Harcourt, and a voucher specimen (No. RSUH/2024/042) was deposited in the herbarium for future reference. The leaves were washed thoroughly with clean water to remove dust and debris, oven-dried at 40 °C for five days in a forced-air convection oven (Model DHG-9101) until brittle, milled into a fine powder using a hammer mill (Model 912, Thomas-Wiley, Philadelphia, PA, USA) fitted with a 1 mm screen, and stored in labelled airtight plastic containers at room temperature until incorporation into the diets.

Proximate and phytochemical analyses

The proximate composition of *Sida acuta* leaf meal was determined using the current applicable AOAC procedures (AOAC International, 2019): moisture by oven-drying at 105 °C to constant weight (Method 934.01); crude protein by the Kjeldahl procedure ($N \times 6.25$; Method 988.05); crude fibre by acid–alkali digestion (Method 991.43); ether extract by Soxhlet extraction with petroleum ether (Method 920.39); ash by incineration in a muffle furnace at 550 °C (Method 942.05); and nitrogen-free extract by difference. All proximate values are reported on a dry-matter basis.

Phytochemical screening and quantification of the leaf meal were performed using the following procedures: total phenolics by the Folin–Ciocalteu colorimetric method with gallic acid as standard and results expressed as mg gallic acid equivalents (GAE)/g dry matter (Singleton *et al.*, 1999); total flavonoids by the aluminium chloride colorimetric method with quercetin as standard and results expressed as mg quercetin equivalents (QE)/g dry matter (Zhishen *et al.*, 1999); condensed tannins by the vanillin–HCl method with catechin as standard and results expressed as mg catechin equivalents (CE)/g dry matter (Price *et al.*, 1978); saponins by the vanillin–sulphuric acid method with diosgenin as standard and results expressed as mg/g dry matter (Ezeabara *et al.*, 2014); alkaloids by the gravimetric method after precipitation with Mayer's reagent and results expressed as mg/g dry matter

Table 1. Proximate and phytochemical composition of **Sida acuta** leaf meal (dry-matter basis)

Component	Value	Unit
Moisture	9.40	%
Crude protein	18.62	%
Crude fibre	14.35	%
Ether extract	3.78	%
Ash	8.42	%
Nitrogen-free extract	54.83	%
Total phenolics	42.18	mg GAE/g
Total flavonoids	22.46	mg QE/g
Condensed tannins	12.74	mg CE/g
Saponins	18.32	mg/g
Alkaloids	14.85	mg/g
Oxalate	9.62	mg/g
DPPH IC50	78.55	µg/mL
FRAP	286.41	µmol Fe ²⁺ /g

Values are means of three analytical determinations. GAE = gallic acid equivalents; QE = quercetin equivalents; CE = catechin equivalents; DPPH = 2,2-diphenyl-1-picrylhydrazyl; FRAP = ferric-reducing antioxidant power.

(Harborne, 1998); and oxalate by the permanganate titration method and results expressed as mg/g dry matter (Iwuoha and Kalu (1995). The in vitro antioxidant capacity of the leaf meal was determined using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) free-radical-scavenging assay (Blois, 1958) and the ferric-reducing antioxidant power (FRAP) assay (Benzie and Strain, 1996), with ascorbic acid as the reference standard. The DPPH result is expressed as the concentration of extract required to scavenge 50 % of the DPPH radical (IC50, µg/mL) and the FRAP result as µmol Fe²⁺/g dry matter. All phytochemical and antioxidant assays were performed in triplicate, and the procedures, standards, units and basis of expression (dry-matter basis) are recorded in Table 1.

Experimental animals and management

A total of 120 unsexed day-old Ross 308 broiler chicks were obtained from a reputable commercial hatchery in Port Harcourt, Rivers State. The chicks comprised a mixed-sex population of both males and females, which were distributed among the treatment groups as evenly as possible. On arrival, the chicks were weighed individually, and any chick with a body weight more than two standard deviations from the cohort mean was excluded. Following initial weighing, chicks were randomly allocated to 12 replicate pens, with 10 birds per pen. Treatments and pen positions were assigned using a randomisation procedure designed to minimise allocation and location bias. The treatments were:

Table 2. Gross ingredient and analysed chemical composition of the basal diet.

Ingredient	Inclusion (%)
Maize	50.00
Soybean meal (44% crude protein)	20.00
Palm-kernel cake	10.00
Wheat offal	10.00
Fish meal (72% crude protein)	3.00
Bone meal	2.50
Limestone	1.50
Salt	0.25
Vitamin–mineral premix	0.25
L-lysine	0.25
DL-methionine	0.25
Total	100.00
Chemical composition (analysed)	Value
Crude protein (%)	18.20
Metabolizable energy (kcal/kg)	2950
Crude fibre (%)	4.50
Ether extract (%)	3.80
Calcium (%)	0.85
Available phosphorus (%)	0.45

Maize was substituted by 0, 5, 10 and 15 g/kg of *Sida acuta* leaf meal in T1, T2, T3 and T4, respectively. The vitamin–mineral premix supplied (per kg of diet): retinol 3.6 mg, cholecalciferol 75 µg, α-tocopherol 30 mg, menadione 2 mg, thiamine 2 mg, riboflavin 6 mg, pyridoxine 4 mg, cobalamin 20 µg, niacin 35 mg, pantothenic acid 12 mg, folic acid 1.5 mg, biotin 100 µg, iron 80 mg, copper 10 mg, zinc 80 mg, manganese 80 mg, iodine 0.8 mg, selenium 0.3 mg.

- T1: basal diet, 0 % *Sida acuta* g/kg feed (control);
- T2: basal diet +0. 5% *Sida acuta* g/kg feed;
- T3: basal diet + 1.0% *Sida acuta* g/kg feed;
- T4: basal diet + 1.5% *Sida acuta* g/kg feed.

Feed and clean drinking water were provided ad libitum throughout the trial. Routine vaccination, medication and brooding management were maintained for all birds. Mortality was recorded daily, and dead birds were weighed and the weight recorded. The replicate pen was used as the experimental unit. (Percie du Sert *et al.*, 2020)

Experimental diets

The basal diet was formulated to meet the nutrient requirements of growing broiler chickens for the finisher phase (NRC, 1994). The experimental diets contained 0, 5, 10 and 15 g *Sida acuta* leaf meal g/kg diet, corresponding to 0, 0.5, 1.0 and 1.5% inclusion, respectively, so that the basal ingredient composition of the diet remained otherwise unchanged. The complete ingredient and analysed nutrient composition of the basal diet are presented in Table 2. The four treatment diets

were analysed for crude protein, crude fibre, ether extract, ash, calcium and total phosphorus, and the results are reported on an as-fed basis (Table 2).

Performance measurements

Body weight and feed intake were monitored throughout the study; however, treatment effects were evaluated primarily during the four-week period in which the experimental diets were administered using a digital balance (0.1 g precision; Model EK-600H, AND, Tokyo, Japan). The following equations express each performance measurements.

Body Weight Gain (BWG)

BWG (g/bird) = Final body weight (g/bird) – Initial body weight (g/bird)

Average Daily Gain (ADG)

ADG (g/bird/day) = BWG (g/bird) ÷ Number of feeding days

For the finisher phase described, the number of feeding days is 28.

Total Feed Intake (TFI) per Pen

TFI (g/pen) = Total feed offered (g/pen) – Total feed refused (g/pen)

Average Daily Feed Intake (ADFI)

ADFI (g/bird/day) = TFI (g/pen) ÷ [Feeding days × Average number of birds in the pen]

Feed Conversion Ratio (FCR)

FCR (g feed/g weight gain) = TFI (g/pen) ÷ Body-weight gain of birds in the pen (g/pen)

Mortality (%)

Mortality (%) = [Number of birds that died during the trial ÷ Number of birds placed at the start] × 100

Mortality-Corrected Feed Conversion Ratio (FCR_{adj})

FCR_{adj} = Total feed consumed per pen (g) ÷ [Total weight gain of surviving birds (g) + Total weight gain of deceased birds (g)]

Total Weight Gain of Deceased Birds

Weight gain of deceased birds (g) = Body weight at death (g) – Initial body weight at Day 0 (g)

If more than one bird died, calculate the weight gain for each deceased bird and sum those gains for the pen.

Serum hormone analyses

At the end of the 8-week trial, two birds per replicate (six birds per treatment, 24 birds in total) were randomly

selected, fasted for 12 h. and the experimental procedures were reviewed and approved by the Research Ethics Committee of the Faculty of Agriculture, Rivers State University. All procedures involving animals complied with applicable institutional and national guidelines established by the Nigerian Institute of Animal Science (NIAS) and the Rivers State Ministry of Agriculture regarding animal care, handling, and humane slaughtering practices. Blood samples (approximately 5 mL per bird) were collected by jugular venepuncture into plain, non-heparinised tubes, allowed to clot at room temperature for 30 min and centrifuged at $3\,000 \times g$ for 10 min. The serum was separated into labelled cryovials, stored at $-20\text{ }^{\circ}\text{C}$ and analysed within 14 days of collection.

Serum concentrations of triiodothyronine, thyroxine, cortisol, and testosterone were determined using validated competitive commercial enzyme-linked immunosorbent assay (ELISA) kits obtained from Cloud-Clone Corp. (Wuhan, China; Catalogue Nos. CEA452Ge for T3, CEA453Ge for T4, CEA462Ge for cortisol, and CEA458Ge for testosterone). The assays were specifically designed and validated by the manufacturer for multi-species application, including chicken (*Gallus gallus*) serum, with matrix spike-recovery rates ranging between 91% and 105% and cross-reactivity with non-target steroids and thyroid analogues verified at less than 1.0%.

Analytical sensitivities (minimum detectable limits) were 0.45 ng/mL for T3, 1.23 ng/mL for T4, 1.15 ng/mL for cortisol, and 0.28 ng/mL for testosterone. Intra-assay coefficients of variation (CV) were 4.5%, 4.8%, 5.1%, and 5.4% for T3, T4, cortisol, and testosterone, respectively. Inter-assay CVs were 8.2% for T3, 8.6% for T4, 9.3% for cortisol, and 9.7% for testosterone, confirming high analytical precision (CV < 10.0).

All assays were performed in duplicate following the manufacturer's protocol, with reagent blanks, kit standards, and internal quality control samples included on each plate. Optical density was measured at 450nm, and sample concentrations were calculated against standard curves generated using four-parameter logistic (4PL) regression curve fitting. Expressed units (ng/mL for all hormones) strictly correspond to the manufacturer's purified reference calibrators. The T3:T4 ratio was calculated as a unitless value from the measured T3 and T4 concentrations of each sample. General procedures for avian endocrine profiling and validation followed the principles described by Decuypere *et al.* (2005), Wild (2013) and Scanes (2014).

Statistical analysis

All data were analysed using IBM SPSS Statistics version 26.0 (IBM Corp., Armonk, NY, USA). For serum hormone variables, the two birds sampled from each replicate pen were treated as subsamples, and the replicate pen was retained as the experimental unit. Pen-level means were

subjected to one-way ANOVA. The statistical model was:

$$Y_{ij} = \mu + T_i + e_{ij}$$

Where Y_{ij} is the observed response, μ is the overall mean, T_i is the fixed effect of dietary treatment ($i = 1, 2, 3, 4$), and e_{ij} is the residual error. For hormone variables, dietary treatment was fitted as a fixed effect and replicate pen as a random effect, with individual birds nested within replicate pens. Residual normality was assessed using the Shapiro-Wilk test, and homogeneity of variance was assessed using Levene's test. Treatment means were compared by one-way analysis of variance (ANOVA). Where the treatment effect was significant, means were separated using Tukey's honestly significant difference (HSD) test. Linear and quadratic polynomial contrasts were used to assess the nature of the response to graded *Sida acuta* inclusion. Significance was declared at $p < 0.05$. Results are presented as mean $\pm$ standard error of the mean (SEM). The statistical procedures followed the general principles described by Montgomery (2017).

RESULTS

Proximate and phytochemical composition of *Sida acuta* leaf meal

The proximate and phytochemical composition of *Sida acuta* leaf meal is presented in Table 1. The leaf meal contained 9.40 % moisture, 18.62 % crude protein, 14.35 % crude fibre, 3.78 % ether extract, 8.42 % ash and 54.83 % nitrogen-free extract on a dry-matter basis. The phytochemical profile included 42.18 mg GAE/g total phenolics, 22.46 mg QE/g total flavonoids, 12.74 mg CE/g condensed tannins, 18.32 mg/g saponins, 14.85 mg/g alkaloids and 9.62 mg/g oxalate. The in vitro antioxidant capacity of the leaf meal, expressed as DPPH IC₅₀, was 78.55 $\mu\text{g/mL}$, and the FRAP value was 286.41 $\mu\text{mol Fe}^{2+}/\text{g}$ dry matter.

Growth performance

The growth performance responses are presented in Table 3. Initial body weight did not differ significantly among treatments ($p = 0.978$), indicating comparable starting body weights across dietary treatments. Final body weight increased from 2 215.33 g in T1 to 2 498.67 g in T4, a relative increase of 12.79 %. Body weight gain increased from 2 174.67 g in T1 to 2 458.00 g in T4, and average daily gain increased from 77.67 g/bird/day in T1 to 87.79 g/bird/day in T4. Total feed intake increased from 4 142.33 g in T1 to 4 358.67 g in T4, and average daily feed intake increased from 147.94 to 155.67 g/bird/day. Feed conversion ratio improved (decreased) from 1.91 in T1 to 1.78 in T4, a relative improvement of 6.81 %.

Table 3. Growth performance of broiler chickens fed graded levels of *Sida acuta* leaf meal

Parameter	T1 (0 g/kg)	T2 (5 g/kg)	T3 (10 g/kg)	T4 (15 g/kg)	SEM	p-value
Initial body weight (g/bird)	40.67±1.20	40.33±1.10	40.67±1.15	40.67±1.05	0.55	0.978
Final body weight (g/bird)	2215.33±22.45 ^a	2286.67±21.85 ^a	2412.33±19.78 ^b	2498.67±18.92 ^c	10.25	< 0.001
Body weight gain (g/bird)	2174.67±22.65 ^a	2246.33±21.92 ^a	2371.67±19.65 ^b	2458.00±18.74 ^c	10.34	< 0.001
Average daily gain (g/bird/day)	77.67±0.81 ^a	80.23±0.78 ^a	84.70±0.70 ^b	87.79±0.67 ^c	0.37	< 0.001
Total feed intake (g/bird)	4142.33±28.45 ^a	4198.67±25.78 ^a	4285.33±23.45 ^b	4358.67±21.65 ^c	12.50	< 0.001
Average daily feed intake (g/bird/day)	147.94±1.02 ^a	149.95±0.92 ^{ab}	153.05±0.84 ^{bc}	155.67±0.77 ^c	0.45	< 0.001
Feed conversion ratio (feed/gain)	1.91±0.02 ^a	1.87±0.02 ^{ab}	1.81±0.01 ^{bc}	1.78±0.01 ^c	0.01	< 0.001
Mortality (%)	3.33±1.92	3.33±1.92	0.00±0.00	0.00±0.00	0.86	0.412

Values are means ± SEM of three replicate pens per treatment (n = 3pens/treatment; 10 birds/pen, total N = 120 birds). The replicate pen was the experimental unit for all performance variables (feed intake, body weight gain, and feed conversion ratio).

Table 4. Serum hormone profile of broiler chickens fed graded levels of *Sida acuta* leaf meal

Parameter	T1 (0 g/kg)	T2 (5 g/kg)	T3 (10 g/kg)	T4 (15 g/kg)	SEM	p-value
Triiodothyronine, T3 (ng/mL)	1.85 ± 0.07 ^a	2.15 ± 0.08 ^{ab}	2.48 ± 0.09 ^{bc}	2.78 ± 0.10 ^c	0.12	< 0.001
Thyroxine, T4 (ng/mL)	14.32 ± 0.45 ^a	15.78 ± 0.48 ^a	17.65 ± 0.52 ^{ab}	19.85 ± 0.58 ^b	0.62	< 0.00
T3:T4 ratio	0.130 ± 0.004 ^a	0.137 ± 0.004 ^a	0.140 ± 0.004 ^a	0.141 ± 0.004 ^a	0.005	0.312
Cortisol (ng/mL)	4.85 ± 0.18 ^a	4.12 ± 0.16 ^{ab}	3.48 ± 0.14 ^{bc}	2.92 ± 0.12 ^c	0.21	< 0.001
Testosterone (ng/mL)	1.42 ± 0.07 ^a	1.71 ± 0.08 ^{ab}	2.02 ± 0.09 ^{bc}	2.31 ± 0.10 ^c	0.11	< 0.001

Values represent pen-level means of 2 birds sampled per replicate pen n = 3 replicate pens per treatment; 6 birds total per treatment). Birds within replicate pens were treated as subsamples, with the replicate pen considered the experimental unit. Within a row, means with different superscript letters a,b,c differ significantly according to Tukey's HSD test p < 0.05. Pooled standard error of the mean SEM is based on n = 3 experimental units per treatment. Polynomial contrasts: significant linear trend linear < 0.01 for T3, T4, cortisol, and testosterone.

Mortality was low in all treatments and did not differ significantly among treatments (range 0.00–3.33 %; p = 0.412).

Treatment effects on final body weight, body weight gain, average daily gain, total feed intake, average daily feed intake and feed conversion ratio were all significant (p<0.05). The Tukey HSD test indicated that T4 differed significantly from T1 and T2 for all performance parameters, while T3 differed significantly from T1 for final body weight, body weight gain, average daily gain, feed conversion ratio and total feed intake. T2 did not differ significantly from T1 for any performance parameter. Polynomial contrasts indicated a significant linear trend for all performance parameters (p_{linear} < 0.01) and a significant quadratic component for final body weight, body weight gain, average daily gain and feed conversion ratio (p_{quadratic} < 0.05). The significant linear and quadratic components indicate a curvilinear response across the tested inclusion levels

increased from 0.130 in T1 to 0.141 in T4 (relative increase 8.46 %). Testosterone increased from 1.42 ng/mL in T1 to 2.31 ng/mL in T4 (relative increase 62.68 %). In contrast, cortisol decreased from 4.85 ng/mL in T1 to 2.92 ng/mL in T4 (relative decrease 39.79 %).

Treatment effects on T3, T4, T3:T4 ratio, cortisol and testosterone were all significant (p < 0.05). The Tukey HSD test indicated that T4 differed significantly from T1, T2 and T3 for all five hormone endpoints, while T3 differed significantly from T1 for T3, T4, cortisol and testosterone. T2 did not differ significantly from T1 for T4 or the T3:T4 ratio but differed significantly from T1 for T3, cortisol and testosterone. Polynomial contrasts indicated a significant linear trend for all five hormone endpoints (p_{linear} < 0.001) and a significant quadratic component for T3, cortisol and testosterone (p_{quadratic} < 0.05), consistent with a dose-dependent response that began to plateau at the highest inclusion level for the thyroid hormones and testosterone but continued to decline for cortisol.

Serum hormone profile

The serum hormone responses are presented in Table 4. Triiodothyronine (T3) increased from 1.85 ng/mL in T1 to 2.78 ng/mL in T4 (relative increase 50.27 %), and thyroxine (T4) increased from 14.32 ng/mL in T1 to 19.85 ng/mL in T4 (relative increase 38.62 %). The T3:T4 ratio

Comparison of observed values with published broiler reference intervals

The comparison of the observed performance and hormone values with published broiler reference intervals is presented in Table 5. Final body weight in T1 and T2 fell within the published reference interval for Ross 308

Table 5. Comparison of observed performance and hormone values with published broiler reference intervals

Parameter	Observed range across treatments	Published reference interval	Comparative interpretation	Reference
Final body weight (g)	2 215.33–2 498.67	2100–2 750 (Ross 308, d 56)	All treatment means fall within expected growth potential for 56-day Ross 308 broilers	Aviagen (2022) Ross 308 performance objectives
Feed conversion ratio (feed/gain)	1.78–1.91	1.65–2.15 (Ross 308, d 56)	All values fall within standard commercial range; higher inclusion levels showed improved efficiency	Aviagen (2022) Ross 308 performance objectives
Triiodothyronine, T3 (ng/mL)	1.85–2.78	1.20–3.50 (broiler, d 56)	All values within physiological baseline; highest supplementation level (T4) approached upper limit	Decuypere et al. (2005); Scanes (2014)
Thyroxine, T4 (ng/mL)	14.32–19.85	10.00–25.00 (broiler, d 56)	All values within physiological baseline progressive increase observed with leaf meal inclusion	Decuypere et al. (2005); Scanes (2014)
Cortisol (ng/mL)	2.92–4.85	1.50–6.00 (unstressed broiler)	All values within the nonstressed basal range; supplementation reduce systematic cortisol levels	Scanes (2014)
Testosterone (ng/mL)	1.42–2.31	0.50–3.00 (broiler cockerel, d 56)	All values falls within the standard endocrine range for late stage broiler	Scanes (2014)

Reference intervals are used as comparative yardsticks rather than diagnostic cut-offs. Differences from a published interval are distinguished from statistically significant differences among treatments. Reference values are affected by bird strain, age, sex, production stage, environment and analytical method.

broilers at 35 days of age, while T3 and T4 approached or marginally exceeded the upper end of the cited interval. Feed conversion ratio values were at the lower (more favourable) end of the published reference interval across all treatments, with T4 at the lower end of the cited range. T3 and T4 concentrations were within the published reference intervals for the relevant broiler strain and age, with the highest values (T4) approaching the upper end of the cited range but not exceeding it. Cortisol values were within the published reference interval for unstressed broilers and did not indicate a stress response in any treatment. Testosterone values were within the published reference interval for broiler cockerels at the relevant age. These comparisons are presented as context only and do not replace the within-experiment statistical analysis.

DISCUSSION

Phytochemical composition of *Sida acuta* leaf meal

The proximate composition of *Sida acuta* leaf meal recorded in the present study (crude protein 18.62 %, crude fibre 14.35 %, ether extract 3.78 %, ash 8.42 %) is consistent with previously published values for *Sida acuta* leaves harvested in West Africa (Akilandeswari *et al.*, 2010; Raimi *et al.*, 2014) and indicates that the leaf meal is a moderately good source of protein, fibre and minerals. The crude protein content of 18.62 % is higher than that reported for many tropical forage leaf meals of comparable fibre content, and suggests that *Sida acuta* leaf meal could

contribute meaningful amounts of nitrogen to the broiler diet at moderate inclusion levels. The crude fibre content of 14.35 % is, however, consistent with the general pattern in tropical browse plants and is the proximate component most likely to limit the upper inclusion level of the leaf meal in monogastric diets.

The phytochemical profile total phenolics 42.18 mg GAE/g, total flavonoids 22.46 mg QE/g, condensed tannins 12.74 mg CE/g, saponins 18.32 mg/g, alkaloids 14.85 mg/g and oxalate 9.62 mg/g is also consistent with published values for *Sida acuta* and is biologically relevant. Phenolics and flavonoids are well-known plant antioxidants that may protect intestinal epithelium from oxidative damage, support tight-junction integrity and improve nutrient absorption (Surai, 2014). Saponins have been reported to modulate rumen and gut microbial populations and to influence nutrient digestion, although the direction and magnitude of effect vary with dose and saponin structure (Francis *et al.*, 2002). Tannins at low concentrations can form reversible complexes with dietary protein and reduce rumen proteolysis, but in monogastrics they are more commonly associated with reduced nutrient utilisation and growth depression at high concentrations (Mueller-Harvey, 2006). Alkaloids can act on the central and peripheral nervous system and on metabolic enzymes, while oxalates can bind dietary calcium. The oxalate value of 9.62 mg/g is well below the threshold typically associated with clinical or subclinical toxicity in poultry and is not expected to compromise mineral utilisation at the inclusion levels tested.

The *in vitro* antioxidant capacity of the leaf meal,

expressed as DPPH IC₅₀ of 78.55 µg/mL and FRAP of 286.41 µmol Fe²⁺/g, is consistent with the recorded phenolic and flavonoid content and indicates that the leaf meal has meaningful free-radical-scavenging and ferric-reducing activity. This is biologically relevant because the in vitro antioxidant activity of phytogetic additives is often correlated with in vivo effects on tissue antioxidant status, gut health and stress resilience. Collectively, the proximate and phytochemical profile of *Sida acuta* leaf meal is consistent with the observed biological responses in the present study, and provides a coherent mechanistic basis for interpreting the performance and hormone results.

Growth performance

The growth performance results of the present study showed a clear, dose-related improvement with increasing dietary inclusion of *Sida acuta* leaf meal. Final body weight, body weight gain, average daily gain, total feed intake and average daily feed intake all increased significantly from T1 to T4, while feed conversion ratio improved (decreased) significantly. Mortality was low in all treatments and was not significantly different among treatments, indicating that the inclusion levels tested were well tolerated and did not produce clinical toxicity.

The increase in feed intake from 4 142.33 g in T1 to 4 358.67 g in T4 is consistent with reports that low-to-moderate inclusion of phytogetic additives can improve feed palatability in broilers, possibly through effects on diet aroma, taste or gut sensory signalling (Windisch *et al.*, 2008; Gadde *et al.*, 2017). The increase in body weight gain from 2 174.67 to 2 458.00 g/bird and the improvement in feed conversion ratio from 1.91 to 1.78 indicate that the additional feed intake was converted to body tissue with greater efficiency, and not simply dissipated as metabolic heat or activity. The 6.81 % relative improvement in FCR is biologically meaningful in a commercial setting, where even small improvements in FCR have a large effect on the cost of production.

The improved performance may potentially be related to the phytochemical constituents of *Sida acuta*. However, the present study did not directly assess intestinal integrity, gut microbiota or systemic oxidative status; therefore, these mechanisms remain hypotheses requiring experimental confirmation. The phenolic and flavonoid constituents of the leaf meal may have improved gut health by reducing oxidative damage to the intestinal epithelium, supporting tight-junction integrity and favouring a more balanced gut microbial community. The in vitro antioxidant activity of the leaf meal may have translated into a reduced systemic oxidative load, sparing nutrients that would otherwise be diverted to antioxidant defence and allowing more nutrients to be directed toward growth. The improvement in serum thyroid hormone status (T3 and T4) is biologically consistent with the observed improvement in performance, because thyroid hormones are central regulators of basal metabolic rate and protein turnover in

broilers; an increase in circulating T3 would be expected to support greater protein accretion and feed efficiency. The increase in serum testosterone and the decrease in serum cortisol (see §4.3) would also be expected to favour muscle protein accretion and to reduce the catabolic effect of stress on lean tissue.

The present experiment did not measure feed digestibility, gut microbiota, intestinal morphology, carcass composition or tissue antioxidant status; accordingly, the most defensible interpretation is that the dietary treatment was associated with improved growth performance under the conditions of the experiment, rather than that the leaf meal directly stimulated growth through any specific mechanism. The significant linear trend for all performance parameters is consistent with a true dose response within the tested range, while the significant quadratic component for final body weight, body weight gain, average daily gain and feed conversion ratio is consistent with the response beginning to plateau between 10 and 15 g/kg. The 15 g/kg treatment produced the most favourable measured response among the inclusion levels evaluated; however, an optimum inclusion level cannot be established because higher inclusion levels were not tested.

The performance response observed in the present study is broadly consistent with the direction and magnitude of response reported for other tropical phytogetic additives in broilers (Gadde *et al.*, 2017; Wang *et al.*, 2021), and extends the small body of evidence on *Sida acuta* specifically by demonstrating that the response is dose-dependent and that the highest tested inclusion level (15 g/kg) produced the most favourable measured performance response.

Serum hormone profile

The serum hormone results of the present study showed a consistent and biologically coherent response to graded *Sida acuta* leaf meal inclusion. Triiodothyronine, thyroxine, the T3:T4 ratio and testosterone all increased significantly and dose-dependently from T1 to T4, while cortisol decreased significantly and dose-dependently.

The increase in serum T3 (from 1.85 to 2.78 ng/mL) and T4 (from 14.32 to 19.85 ng/mL) is biologically relevant because thyroid hormones are central regulators of basal metabolic rate, thermogenesis and protein turnover in broilers (Decuyper *et al.*, 2005; Scanes, 2014). An increase in circulating T3 and T4 in well-nourished broilers is generally associated with increased metabolic activity, increased protein turnover and, in the absence of stress, increased lean tissue accretion. The modest increase in the T3:T4 ratio may indicate a change in the relative availability of T3 and T4 and could be consistent with altered peripheral conversion; however, direct measurement of deiodinase activity would be required to establish this mechanism. The increase in thyroid status is consistent with the observed improvement in growth performance, particularly the improvement in feed conversion ratio,

because higher T3 supports more efficient use of dietary energy and protein for growth. The mechanism by which *Sida acuta* leaf meal may have increased thyroid status is not established by the present experiment. Although mineral contributions from the leaf meal could potentially influence endocrine responses, the present study did not determine its iodine, selenium or zinc content. Consequently, this explanation remains speculative. The highest T3 and T4 values (in T4) remained within the published reference intervals for unstressed broilers at the relevant age, indicating that the increase was physiological and did not represent hyperthyroidism or pathological activation of the thyroid axis.

The lower serum cortisol concentration may indicate an altered glucocorticoid response (from 4.85 to 2.92 ng/mL). Cortisol is the principal glucocorticoid in birds and reflects the activity of the hypothalamic–pituitary–adrenal axis; an elevated cortisol concentration is a marker of stress, while a low-normal concentration in well-nourished, healthy birds is generally associated with a more anabolic metabolic state (Scanlan, 2014). The decrease in cortisol is consistent with the increase in testosterone (which is generally anti-glucocorticoid in anabolic action) and with the improvement in growth performance (because cortisol is catabolic and antagonises lean tissue accretion). Plausible explanations for the decrease in cortisol include the antioxidant and anti-inflammatory actions of the phenolic and flavonoid constituents of the leaf meal, the improved nutritional status of the supplemented birds, and the influence of plant constituents on the hypothalamic–pituitary–adrenal axis. As with the thyroid hormones, the cortisol values across all treatments remained within the published reference interval for unstressed broilers. However, because behavioural and physiological stress responses and repeated cortisol measurements were not assessed, the present findings cannot establish that the dietary treatment reduced stress.

The increase in serum testosterone (from 1.42 to 2.31 ng/mL) is consistent with the observed improvement in growth performance, because testosterone supports muscle protein accretion in birds, particularly in cockerels (but also in pullets at lower circulating concentrations, where it acts in synergy with growth hormone and insulin-like growth factor 1 to support lean tissue growth). The mechanism by which *Sida acuta* leaf meal may have increased serum testosterone is not established by the present experiment, but plausible explanations include improved nutritional status (particularly improved availability of dietary energy, protein, zinc and vitamin A precursors), reduced cortisol-mediated suppression of the gonadal axis. The mechanism underlying the increase in testosterone was not established in the present study. Potential explanations may include changes in nutritional status or endocrine regulation, but these possibilities require direct experimental investigation. The testosterone values across all treatments remained within the published reference interval for broiler cockerels at the relevant age

The present hormone analysis has limitations. A single end-point sampling cannot establish cause and effect, and the present data cannot distinguish whether the observed hormone changes were a primary effect of the dietary treatment, a secondary consequence of the improved nutritional and metabolic state of the supplemented birds, or a combination of both. A longitudinal sampling design with stress challenges (e.g., ACTH stimulation, heat stress, handling stress) and direct measurement of deiodinase activity, glucocorticoid-receptor expression and steroidogenic-enzyme activity would be required to confirm the mechanism. The present data should therefore be interpreted as an association between graded *Sida acuta* inclusion and a favourable serum hormone profile under the conditions of the experiment.

Comparison with published reference intervals and overall interpretation

The comparison of the observed values with published broiler reference intervals (Table 5) supported the within-experiment statistical analysis. The final body weight values, although statistically different among treatments, were generally within the published range for Ross 308 broilers at the relevant age, with T3 and T4 at the upper end of that range. The FCR values were at the lower (more favourable) end of the published range across all treatments, with T4 the most favourable. The T3, T4 and testosterone values were within the published reference intervals, while the cortisol values were within the range expected for unstressed broilers in all treatments. The fact that the observed values remained within published physiological intervals across all treatments, including the highest inclusion level. The observed hormone concentrations did not fall outside the published comparative ranges used in this study. These comparisons provide contextual information but do not establish long-term safety or absence of subclinical effects.

The significant linear and quadratic trends in the present dataset indicate that the response to graded *Sida acuta* inclusion was dose-dependent within the tested range, with the response beginning to plateau between 10 and 15 g/kg for several endpoints. The fact that no treatment produced a value outside the published reference interval for any parameter suggests that the upper limit of the tested inclusion range is below the threshold at which adverse physiological effects would be expected, and provides a basis for further evaluation of inclusion levels in the 10–15 g/kg range under commercial conditions.

Collectively, the present results support the following physiological interpretation: graded inclusion of *Sida acuta* leaf meal in a maize–soybean–palm-kernel finisher diet was associated with a dose-related improvement in growth performance, an increase in circulating thyroid hormones and testosterone, and a decrease in circulating cortisol, with all values remaining within the published physiological

reference intervals for the relevant broiler strain and age. The 15 g/kg treatment produced the most favourable measured response across all endpoints.

The interpretation must, however, remain within the limits of the experiment. The data are derived from a single trial, in a single location, with a single broiler strain, a single leaf-processing method and a single set of laboratory conditions. The most defensible interpretation is therefore that graded *Sida acuta* leaf meal at 5, 10 and 15 g/kg was associated with improved growth performance and a favourable serum hormone profile under the conditions of this experiment. The data do not by themselves establish long-term safety, therapeutic efficacy, the active component(s) responsible for the observed effects, or universal suitability for all broiler strains and production systems.

Conclusion

Dietary inclusion of *Sida acuta* leaf meal at 5, 10 and 15 g/kg feed produced significant, dose-related changes in the growth performance and serum hormone profile of Ross 308 broiler chickens under the conditions of this experiment. Dietary inclusion of *Sida acuta* leaf meal at 5–15 g/kg was associated with improved growth performance and altered circulating concentrations of T3, T4, cortisol and testosterone in Ross 308 broilers under the conditions of the experiment. The 15 g/kg treatment produced the most favourable measured responses among the levels tested. The 15 g/kg treatment produced the highest body weight gain and the best (lowest) feed conversion ratio, together with the highest T3, T4 and testosterone values and the lowest cortisol value, and all values remained within the published physiological reference intervals for the relevant broiler strain and age.

The growth performance response was linear for feed intake and curvilinear (linear + significant quadratic) for final body weight, body weight gain, average daily gain and feed conversion ratio, consistent with a dose-dependent response that began to plateau between 10 and 15 g/kg. The hormone response was linear for T3, T4, cortisol and testosterone and curvilinear (linear + significant quadratic) for T3, cortisol and testosterone. No adverse response was detected in the measured growth and serum hormone variables at the tested inclusion levels during the experimental period; they do not by themselves establish long-term safety, therapeutic efficacy, the active component(s) responsible for the observed effects, or universal suitability for all broiler strains and production systems.

Recommendations

1. The 15 g *Sida acuta* leaf meal/kg diet treatment should be subjected to further validation under commercial farm conditions because it produced the most favourable measured performance and hormone responses

among the inclusion levels tested.

2. *Sida acuta* leaf meal should be chemically characterised before each batch is used in feeding trials or on-farm, because phytochemical composition may vary with location, plant age, season, soil and processing method.
3. Future feeding trials should evaluate inclusion levels above and below 15 g/kg using adequate independent replicate pens per treatment, while assessing nutrient digestibility, intestinal morphology, gut microbiota, oxidative-status biomarkers and additional endocrine variables.
4. The selected dietary level should be validated under commercial farm conditions, including different seasons, management systems and broiler strains, before broad recommendation.

CONFLICT OF INTEREST

The author declares that they have no conflict of interest.

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