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Effects of false yam tuber meals and charcoal on broiler chicken production and blood parameters

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Abstract

The authors investigated the effects of replacing a portion of a commercial broiler feed with false yam tuber meals on broiler growth performance, feed conversion rate (FCR) and blood parameters. Furthermore, wood charcoal was added at various levels to the meals to explore their potential to attenuate toxic effects. One hundred and sixty-eight 28-day-old healthy female broiler chickens (average initial bodyweight 1081.1 ± 66.20 g) were randomly assigned to 28 experimental groups (7 dietary treatments, 4 replicates) of six birds each, using a randomized complete block design. Dietary treatments included the control diet (commercial broiler feed) (C), raw false yam tuber meal (RFY) replacing 50 g/kg of the commercial broiler feed, false yam tuber meal soaked in water (SFY) replacing 150 g/kg of the commercial broiler feed, RFY with 30 g/kg and 60 g/kg wood charcoal, and SFY with 30 g/kg and 60 g/kg wood charcoal. Growth performance, feed intake and FCR were assessed over four weeks. At the end of the experiment, blood samples were collected from 21 birds (three from each dietary treatment) to analyse haematological and serum biochemical parameters. Analysis of variance, Kruskal-Wallis tests, and simple regressions were used to evaluate the effects of the meals and charcoal. The results indicated that broilers fed 150 g/kg SFY had a significantly lower growth rate and poorest FCR. Consequently, highest bodyweights were observed for C and RFY diets. Additionally, blood serum proteins were below the references ranges for birds fed SFY, particularly with additional charcoal. In contrast, RFY could be included at 50 g/kg in broiler chicken diets without any adverse effects on their performance and blood (serum) parameters. Anti-nutritional substances contained in SFY at this substitution level are harmful to the birds, irrespective of whether charcoal is added or not. In contrast, RFY could replace commercial feed at the studied level (50 g/kg).

Keywords: Feed efficiency, growth performance, haematology, serum biochemistry, terpenes
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Introduction

False yam (*Icacina oliviformis* (Poiret) J. Raynal, synonym *I. senegalensis* A. Juss*,* family Icacinaceae) is a drought-resistant shrub that is native to West and Central Africa (NRC, 2008). It belongs to the family of Icacinaceae, the phylogenetic relationships of which are still under discussion (Byng *et al.,* 2014). It provides three edible products that are appreciated for human consumption, namely fruits, seeds, and large starch-rich tubers (NRC, 2008). Sun-dried false yam tuber meals contain 486.3 g/kg starch, high levels of neutral detergent fibre (286.1 g/kg dry matter (DM)), but low levels of crude protein (CP) (54.1 g/kg DM) (Dei *et al.*,

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2011). Umoh & Iwe (2014) reported that false yam is a good source of the micronutrients that are necessary for human nutrition such as potassium, sodium, calcium and zinc. Recently, extensive research has focused on the phytochemical profile of *Icacina trichantha* Oliv. It revealed pimarane-type diterpenoid compounds belonging to the small subclasses of 17-norpimarane, (9*β*H)-pimarane, (9*β*H)-17-norpimarane, 16,17-di-nor- and 17,19-di-nor-pimarane, a rare compound class in nature (Onakpa *et al*., 2014*;* Zhao *et al*., 2015; Guo *et al*., 2016). Pimarane-type momilactones are also found in rice plants (*Oryza sativa*) and mosses (*Hypnum plumaeforme* and *Pseudoleskeella papillosa*).These secondary metabolites are known for their cytotoxic and antitumor activities (Onakpa *et al*., 2014; Kim *et al*., 2007) and are reported to function as phytoalexins and allelochemicals (Nozaki *et al.*, 2007; Liu *et al.*, 2012; Kato-Noguchi & Peters, 2013). Hence several *Icacina* species are used in popular herbal medicine to treat food poisoning, constipation and malaria and as a starch source during famine for the people of tropical West Africa (Asuzu *et al.*, 1999; Sarr *et al.*, 2011).

Seeds, leaves and tubers of false yam have been described as low-cost alternative feeds for poultry and rabbit production in Ghana (Ansah *et al.*, 2012; Dei *et al.*, 2015a; Alhassan, 2015). However, despite the false yam's abundance in the northern part of the country, its use as alternative poultry feed has been limited. This is probably due to anti-nutritional factors in raw false yam meals (Dei *et al.*, 2011), which affect final bodyweights and carcass characteristics of broiler chickens negatively (Teye *et al.*, 2011).

Traditional food processing methods such as soaking have been suggested to wash toxic substances out of false yam tubers. For instance, Dei *et al.* (2015a) and Dei *et al.* (2015b) proved that broiler chickens fed tubers that were soaked in water or a saltpetre solution had similar performances to broiler chickens fed a normal diet. Adding dietary wood charcoal to false yam meals might further reduce the negative effects of toxic substances (Gerlach & Schmidt, 2012). Although charcoal as a general feed additive has received a great deal of interest in recent years, scientific studies that address this topic in broiler chickens are scarce (Kutlu *et al.*, 2001, Kana *et al.*, 2011). The objectives of the present experiment were therefore i) to compare growth performance, feed intake and feed conversion rate, ii) to compare haematological and serological profiles of female broiler chickens fed raw and soaked false yam tuber meals, and iii) to evaluate whether wood charcoal could attenuate the toxic effects of the two false yam tuber meals.

Materials and methods

The study was conducted in February/March 2016 at the University for Development Studies (UDS), Tamale, northern Ghana. The outdoor temperature during the experiment averaged 32.1 ± 1.4 °C, with a minimum of 28.8 °C and maximum of 34.8 °C. The average relative humidity was 31.0 ± 18.1%, ranging between 7.3% and 69.1%. The poultry house was open sided to allow for natural ventilation. Light was provided 24 h d^{-1}, as is common practice in northern Ghana to stimulate feed intake during cooler night temperatures (Dei *et al*., 2011). The intensity of light was 10 lx. Ethical clearance was obtained on 14 January 2016 from UDS (code number ANS/FOA/02/14012016). The experiment was conducted in compliance with regulations for animal experiments of UDS, and was closely supervised by a veterinarian.

At 28 days of age, 168 healthy female broiler chickens (Cobb 500 strain) were selected on a weight equalization basis and randomly assigned to 28 experimental groups with six birds each, using a randomized complete block design. Two blocks consisted of 14 floor pens with deep litter (1.65 × 0.84 m^2) and the other two blocks of 14 pens with wire mesh floor (1.8 × 0.9 m^2). Each dietary treatment was replicated four times. The seven dietary treatments included i) C: commercial broiler finisher diet (control); ii) RFY-0: raw false yam tuber meal, replacing 50 g/kg of the commercial broiler feed; iii) SFY-0: false yam tuber meal soaked in water, replacing 150 g/kg of the commercial broiler feed; iv) RFY-30: RFY with 30 g/kg wood charcoal (WC); v) SFY-30: SFY with 30 g/kg WC; vi) RFY-60: RFY with 60 g/kg WC; and vii) SFY-60: SFY with 60 g/kg WC. The birds were adapted to the diets for one week. Feed and water were provided *ad libitum*.

All dietary treatments were based on a commercial finisher feed with added vitamin premix and phytase. It was purchased from Agricare Ltd., Kumasi, Ghana, and contained 11.7 MJ metabolizable energy/kg according to the manufacturers' declaration. False yam tubers were harvested at UDS Nyankpala campus. They were washed with water, peeled and sliced into chips. A portion of the chips was sun-dried and milled into gritty flour. The other portion was soaked in water (1 part fresh false yam tuber chips to 2 parts water) for 12 days. Water was changed every three days. Finally, the soaked false yam tuber chips were washed, sun-dried and milled into gritty flour. The WC was purchased from Tamale wood and charcoal market, crushed and milled into gritty powder to pass a 1-mm sieve and included in the false yam diets.

Representative samples of each dietary treatment and the pure WC and false yam tuber meals were collected, weighed using an electronic precision balance (3,500 g weighing capacity, 0.01 g resolution; Kern PCB, Kern und Sohn GmbH, Balingen, Germany), and dried in a hot-air drying oven (80 °C) to constant weight, weighed again, and ground to pass a 1-mm sieve (Cyclotec, FOSS, Hamburg, Germany) before analysis. Following the standard procedures of the Association of German Agricultural Analytic and Research Institutes (VDLUFA, 2006), the DM and organic matter (OM) concentrations of the feedstuffs were determined in two replicates per sample by consecutively drying the materials at 105 °C overnight, weighing the residual, and incinerating it at 550 °C for 3 hours. The OM concentration was calculated based on the resulting ash weight. A semi-automated Ankom 220 Fibre Analyser (ANKOM Technology, Macedon, NY, USA) served to determine, in two independent steps, the concentrations of ash-free neutral detergent fibre (NDF) and acid detergent fibre (ADFom). Although heat-stable amylase was used for NDF determination (aNDFom), decalin and sodium sulphite were not added to the detergent solutions (Van Soest *et al*., 1991).

Dumas combustion was used to analyse the samples for their carbon (C) and nitrogen (N) concentration (C/N–TCD analyser, Elementar Analysensysteme GmbH, Hanau, Germany). The N was multiplied by factor 6.25 to obtain the concentration of CP. Phosphorus (P) content in samples was determined by the Vanadate Molybdate method (Hitachi U-2000 photometer, Hitachi Co. Ltd., Tokyo, Japan) (Table 1).

Table 1 Analysed chemical composition of the commercial finisher feed and the two false yam tuber meals with varying levels of wood charcoal used in the broiler chicken experiment (g/kg DM)

Item	**Composition (g/kg DM)**					
	OM	**aNDFom**	**ADFom**	**CP**	**C**	**P**
WC	875	nd	nd	43	726	1.6
RFY	973	191	102	36	432	0.4
SFY	974	210	134	35	431	0.5
C	898	163	65	201	416	8.1
RFY-0	902	167	80	197	414	8.0
SFY-0	917	168	76	171	423	7.4
RFY-30	908	167	88	190	430	7.6
SFY-30	918	177	90	172	439	7.0
RFY-60	912	195	100	176	437	7.4
SFY-60	917	211	111	168	441	6.7

WC: wood charcoal; RFY: pure raw false yam tuber meal; SFY: pure soaked false yam tuber meal; C: commercial broiler finisher feed (control); RFY-0: RFY without WC; SFY-0: SFY without WC; RFY-30: RFY with 30 g/kg WC; SFY-30: SFY with 30 g/kg WC; RFY-60: RFY with 60 g/kg WC; SFY-60: SFY with 60 g/kg WC. DM: dry matter; OM: organic matter; aNDF-NDFom: neutral detergent fibre assayed with heat-stable amylase and exclusive of residual ash; ADF-ADFom: acid detergent fibre exclusive of residual ash; CP: crude protein, calculated as nitrogen × 6.25; C: total carbon, P: phosphorus; nd: not determined

To determine essential amino acids (except tryptophan), ion chromatographic methods were used that conformed with the German Food and Feed Code (§64 LFGB L 49.07-2). Tryptophan was quantified in accordance with procedures specified by the Association of German Agricultural Analytic and Research Institutes using HPLC methods (VDLUFA, 2006). Amino acid concentrations were determined for dietary treatments without WC (C, RFY-0, and SFY-0) and extrapolated for treatments with WC (RFY-30, RFY-60, SFY-30, and SFY-60) (Table 2).

To determine terpenic constituents, 10 g each of pure tuber meals RFY and SFY were exhaustively extracted with ethanol three times for 30 minutes in a shaking water bath at 30 °C. The ethanol extract was evaporated (<40 °C) and partitioned between *n*-hexane–H_2O, $CHCl_3$–H_2O, EtOAc–H_2O and *n*-butanol–H_2O. The subfractions were evaporated (<40 °C) and their amounts for RFY meal were 204.0 mg (*n*-hexane subfraction), 27.8 mg ($CHCl_3$ subfraction), 6.8 mg (EtOAc subfraction), 3.6 mg (*n*-butanol subfraction), and 6.0 mg (H_2O-subfraction. For the SFY meal the amounts were 202.2 mg (*n*-hexane-subfraction), 21.2 mg ($CHCl_3$ subfraction), 6.5 mg (EtOAc subfraction), 1.4 mg (*n*-butanol subfraction), and 4.5 mg (H_2O subfraction). Ultra-performance liquid chromatography-mass spectrometry (UPLC-MS) analyses of *Icacina* extracts were performed on a Q Exactive Plus hybrid quadrupole-orbitrap mass spectrometer (Thermo Fisher Scientific, Bremen, Germany) equipped with an Ultimate 3000 series RSLC (Dionex, Sunnyvale, CA, USA) chromatograph. An Acclaim C18 column (150 × 2.1 mm, 2.2 µm particles with 120 Å pore diameter, Dionex, Sunnyvale, CA, USA) with a flow rate of 300 µL min^{-1} in a binary solvent system of water (Solvent A) and acetonitrile (Solvent B), both containing 0.1% (v/v) formic acid, was used to separate the extracts chromatographically. Fifteen µL of each extract, diluted 1 : 100, were loaded onto the column and eluted with this gradient: linear increase from 0% B to 100% B within 15 minutes – 100% B constant for 5 minutes – equilibration time at 0% B for 5 minutes. The mass spectrometer was operated in positive and negative ionization modes using heated-electrospray ionization (H-ESI) in the mass range of *m/z* 100 to 1,000 using 70,000 m/Δm resolving power in the Orbitrap mass analyser. H-ESI source parameters were set to 4 kV for spray voltage, 35 V for transfer capillary voltage at a capillary temperature of 300 °C. Data were evaluated and interpreted using Xcalibur v.3.0.63 software (Thermo Fisher Scientific, Waltham, MA, USA) (Hölscher *et al*., 2017).

Table 2 Amino acid profile of the commercial finisher feed and the two false yam tuber meals with varying levels of wood charcoal used in the broiler experiment

Amino acid[*] (g/kg DM)	**Dietary treatment**						
	C	**RFY-0**	**SFY-0**	**RFY-30**	**SFY-30**	**RFY-60**	**SFY-60**
Arginine	12.2	12.2	10.8	11.8	10.5	11.5	10.2
Histidine	6.1	6.1	5.8	5.9	5.6	5.7	5.5
Isoleucine	8.6	8.2	7.5	8.0	7.3	7.7	7.1
Leucine	19.7	18.3	16.9	17.8	16.4	17.2	15.9
Lysine	12.1	12.2	11.3	11.8	11.0	11.5	10.6
Methionine	3.9	3.7	3.4	3.6	3.3	3.5	3.2
Phenylalanine	10.5	10.1	9.1	9.8	8.8	9.5	8.6
Threonine	7.6	7.2	6.7	7.0	6.5	6.8	6.3
Valine	10.0	9.3	8.7	9.0	8.4	8.7	8.2
Tryptophan	1.9	1.9	1.7	1.8	1.6	1.8	1.6

[*] Calculated for RFY-30, RFY-60, SFY-30 and SFY-60. C: commercial broiler finisher feed (control); RFY-0: raw false yam tuber meal (50 g/kg; RFY) without wood charcoal (WC); SFY-0: false yam tuber meal soaked in water (150 g/kg; SFY) without WC; RFY-30: RFY and 30 g/kg WC; SFY-30: SFY and 30 g/kg WC; RFY-60: RFY and 60 g/kg WC; SFY-60: SFY and 60 g/kg WC. DM: dry matter

For the evaluation of the UPLC-MS-data, a database was created of 25 reported pimarane-type diterpenoids identified from *Icacina* species producing such compounds. These secondary metabolites were listed according to their exact mass, with isomers grouped together. The evaluation of subfractions of raw and soaked false yam tuber material revealed the exact mass of all listed isomer groups of pimarane-type candidate structures (Table 3). Because of the lack of reference compounds, differentiation between candidates with an identical molecular formula was not possible. In terms of reported isomers, two (Nos. 4 and 11 in Table 3) have been identified in *I. oliviformis* (Vanhaelen *et al*., 1987). The exact mass of *m/z* 391.17622 can be assigned to $[M+H]^+$ of icacinol and the *m/z* 359.11371 is attributable to icacenone, two pimarane diterpenoids isolated from *I. oliviformis*. This method provides rapid information about the phytochemical constituents of biological samples.

Broiler chickens were weighed individually each week using an electronic precision balance (3,500 g weighing capacity, 0.01 g resolution; Kern PCB, Kern und Sohn GmbH, Balingen, Germany). The average daily weight gain (ADG) (g/d) was calculated by deducting the individual bird weight at the end of the previous week from the weight at the end of the experimental week, divided by seven days. The average weight was calculated per treatment, corrected for mortalities. Feed intake was assessed weekly for each replicate, and calculated as the difference between the amount of feed offered during the week and feed refused at the end of the week. This was divided by the number of days (7). The average daily feed intake (DFI) was obtained (g/bird and day). The FCR was calculated per replicate as unit of feed consumed daily per unit of ADG (g/g).

At the end of the experimental period (at 63 days of age), one bird from three replicates of each treatment was randomly selected for blood sampling. The selected birds were restrained and 2 mL of blood were drawn from their wing veins with a syringe and needle. Blood samples for haematological evaluation were collected into EDTA tubes, while blood samples for blood chemistry evaluation were collected without anticoagulant. Samples were kept in cooled condition prior to analysis. These haematological parameters were assessed: packed cell volume (PCV, %) following Mukherjee (2005), red blood cell count (RBC ×

10^6/µL) following Dacie & Lewis (2000), white blood cell count (WBC × 10^3/µL) following Holfbrand & Petit (2000), haemoglobin (Hb, g/dL) following Cheesbrough (2001), white blood cell differentials (heterophils, lymphocytes, eosinophils, monocytes, basophils; all %), mean corpuscular haemoglobin concentration (MCHC, g/dL), mean corpuscular haemoglobin (MCH, pg), mean corpuscular volume (MCV, µm³) and platelets (PLAT, × 10^3/µL) using a haemo-analyser (Sysmex Hematology Analyser, XS-500i, Sysmex Europe GmbH, Norderstedt, Germany). The serum biochemical assay was carried out by spectrophotometry. The parameters included total serum proteins (g/L), albumin (g/L), globulins (g/L), alkaline phosphate (units (U)/L), aspartate transferase (U/L) and alanine transferase (U/L).

Table 3 Pimarane-type diterpenoid compound candidates detected in false yam tubers (UPLC-MS)

No.	Name	Molecular formula	Measured $[M-H]^-$/$[M+H]^+$ monoisotopic mass (u); MS (ppm) in brackets
01	Icacine	$C_{22}H_{30}O_6N$	404.20810 (0.592)
02	17-Hydroxyicacinol*	$C_{20}H_{26}O_8$	395.17029 (0.622)
03	14α-Methoxyhumirianthol	$C_{21}H_{28}O_7$	391.17622 (-0.016)
04	Icacinol*	$C_{20}H_{26}O_7$	379.17535 (0.581)
05	7α-Hydroxyicacenone	$C_{19}H_{20}O_8$	375.10840 (-0.375)
06	Icaceine	$C_{22}H_{32}O_4N$	374.23340 (2.178)
07	Icacinlactone C* Icacinlactone D* 7β-Hydroxyicacinlactone B*	$C_{20}H_{20}O_7$	373.12853 (0.940)
08	Icacinlactone L Icacintrichantholide	$C_{20}H_{18}O_7$	369.09727 (-1.859)
09	Humirianthol	$C_{20}H_{26}O_6$	361.16536 (-0.005)
10	2β-Hydroxyhumirianthenolide C	$C_{19}H_{22}O_7$	361.12958 (0.841)
11	Icacenone Icacinlactone F Icacinlactone K	$C_{19}H_{20}O_7$	359.11371 (0.233)
12	Icacinlactone B	$C_{20}H_{20}O_6$	355.11856 (-0.427)
13	Humirianthenolide C Icacinlactone G Icacinlactone I Icacinlactone J	$C_{19}H_{22}O_6$	345.13434 (-0.063)
14	Icacinlactone E Icacintrichanone	$C_{19}H_{20}O_6$	343.11856 (-0.442)
15	12-Hydroxyicacinlactone A	$C_{19}H_{18}O_6$	341.10260 (-1.353)
16	Icacinlactone A	$C_{19}H_{18}O_5$	325.10746 (-2.113)

UPLC-MS: ultra-performance liquid chromatography-mass spectrometry
*Detected as $[M+H]^+$

Data were assessed for normality by the Shapiro-Wilk test and for constant variance by the Bartlett test. Normally distributed data with homogenous variance were subjected to one-way analysis of variance (ANOVA) and *post-hoc* Tukey's honest significant difference (HSD) test with 95% family-wise confidence level. All other data were subjected to the Kruskal-Wallis rank sum test, followed by the Wilcoxon rank sum test for pairwise comparison of treatments. No block effect was observed and therefore was not further considered in the models. First step analyses included the control and the false yam tuber meals without additional WC (treatments C, RFY-0, SFY-0), next step analyses compared the false yam tuber meals without (RFY-0, SFY-0) and with additional WC (RFY-30, SFY-30, RFY-60, SFY-60). Finally, MCV values were fitted into a simple linear regression model to predict relationship with increasing inclusion level of charcoal to the false yam meals (RFY, SFY). All statistical analyses were performed in R version 3.3.0 (The R Foundation for Statistical Computing). The same software was used to created graphs.

Results

Replacing part of the commercial broiler feed with SFY tuber meal reduced bird weight gain (*P* <0.001) (Table 4), while RFY tuber meal at the studied inclusion level had no effect on bird weight gain.

The addition of WC to false yam tuber meals did not show any effect (*P* >0.05), positive or negative, on broiler chickens' ADG (Table 5) and hence final bodyweight.

Table 4 Performances and mortality rate of experimental broiler chickens fed a control diet and various false yam tuber meals without wood charcoal

	Control (C)			**RFY-0**			**SFY-0**			***P*-value**
	Median	**Min.**	**Max.**	**Median**	**Min.**	**Max.**	**Median**	**Min.**	**Max.**	
Weight gain (g/d)	38.9^{a}	27.3	51.6	38.3^{a}	29.5	54.8	31.1^{b}	24.6	37.9	0.001
Feed intake (g/d)	132.0	117.2	138.0	125.0	115.7	185.0	138.1	128.1	171.4	0.472
Feed : gain ratio	3.3	3.1	3.5	3.1	2.4	5.3	4.5	4.0	5.7	0.093
Mortality rate	0.09	0.00	0.33	0.17	0.00	0.50	0.00	0.00	0.17	0.352

Control: commercial broiler finisher diet; RFY-0: raw false yam tuber meal (50 g/kg; RFY) without wood charcoal (WC); SFY-0: false yam tuber meal soaked in water (150 g/kg; SFY) without WC. Kruskal-Wallis test and *post-hoc* Wilcoxon rank sum test. Min.: minimum; Max.: maximum

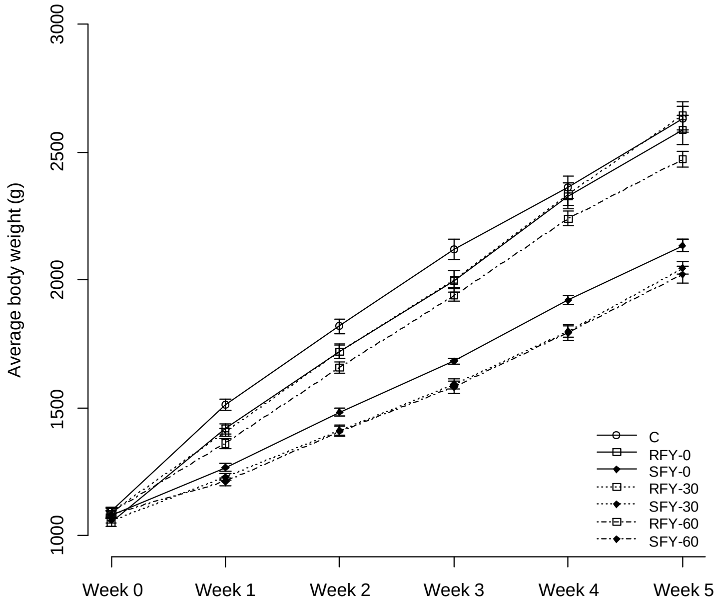
.

Table 5 Effect of varying inclusion levels of wood charcoal to false yam tuber meals on performances and mortality rate of experimental broiler chickens

	Weight gain (g/d)			**Feed intake (g/d)**			**Feed : gain ratio**			**Mortality rate**		
	Median	**Min.**	**Max.**	**Median**	**Min.**	**Max.**	**Median**	**Min.**	**Max.**	**Median**	**Min.**	**Max.**
RFY-0	38.3	29.5	54.8	125.0	115.7	184.7	3.1	2.4	5.3	0.17	0.00	0.50
RFY-30	45.6	30.1	54.3	142.1	137.8	143.6	3.3	2.9	3.7	0.17	0.00	0.33
RFY-60	38.0	29.9	52.1	143.6	135.1	151.9	3.6	3.3	4.0	0.00	0.00	0.00
P-value	0.252			0.368			0.309			0.085		
SFY-0	38.3	29.5	54.8	138.1	128.1	171.4	4.5	4.0	5.7	0.00	0.00	0.17
SFY-30	45.6	30.1	54.3	143.6	139.8	173.7	5.1	4.5	6.2	0.00	0.00	0.17
SFY-60	38.0	30.0	52.1	132.3	112.8	140.0	4.4	4.2	4.9	0.00	0.00	0.00
P-value	0.387			0.118			0.309			0.577		

RFY-0: raw false yam tuber meal (50 g/kg; RFY) without wood charcoal (WC); RFY-30 and RFY-60: raw false yam tuber meal with 30 g/kg and 60 g/kg wood charcoal; SFY-0: false yam tuber meal soaked in water (150 g/kg; SFY) without WC; SFY-30 and SFY-60: soaked false yam tuber meal with 30 g/kg and 60 g/kg wood charcoal. Kruskal-Wallis test and *post-hoc* Wilcoxon rank sum test. Min.: minimum; Max.: maximum

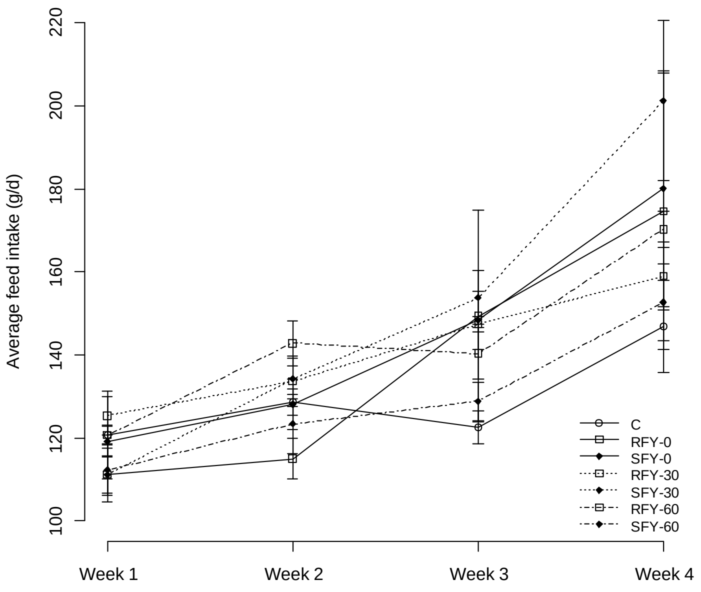
After the adaptation week, bird bodyweights already varied, depending on the diet (Figure 1). If the diets without WC are compared, the highest bodyweights at the start of the first experimental week (week 1) were found for birds fed C. Birds fed RFY-0 diets had lower bodyweights (*P* <0.01) compared with C. The lowest bodyweights were observed for birds fed SFY-0 (*P* <0.001) for which the mean bodyweight remained lowest over the four experimental weeks, resulting in 19% lower final bodyweight compared with birds fed C (*P* <0.05). In contrast, the final bodyweights of birds fed RFY-0 were similar to those of the control diet (*P* >0.05). As could be expected from these results, no effects of WC on bird bodyweights were obtained (*P* >0.05).



C: commercial broiler finisher diet (control); RFY-0: raw false yam tuber meal (50 g/kg; RFY) without wood charcoal (WC); SFY-0: false yam tuber meal soaked in water (150 g/kg; SFY) without WC; RFY-30: RFY with 30 g/kg WC; SFY-30: SFY with 30 g/kg WC; RFY-60: RFY with 60 g/kg WC; SFY-60: SFY with 60 g/kg WC

Figure 1 Average bodyweight of broiler chickens at the start of each week

The average DFI, FCR and mortalities did not differ among dietary treatments without WC (*P* >0.05) (Table 4). As for ADG and bodyweight, no effect of WC at the studied inclusion levels on these variables was observed (*P* >0.05) (Table 5). However, DFI increased more sharply for false yam tuber meals compared with C in experimental weeks 2 to 4, particular for SFY-0 and SFY-30 (Figure 2). As a consequence, FCR was poorest in broiler chickens fed SFY, particularly SFY-30.



C: commercial broiler finisher diet (control); RFY-0: raw false yam tuber meal (50 g/kg; RFY) without wood charcoal (WC); SFY-0: false yam tuber meal soaked in water (150 g/kg; SFY) without WC; RFY-30: RFY with 30 g/kg WC; SFY-30: SFY with 30 g/kg WC; RFY-60: RFY with 60 g/kg WC; SFY-60: SFY with 60 g/kg WC

Figure 2 Average daily feed intake of broiler chickens for each experimental week

With regard to haematological parameters, the authors observed no effects of false yam tuber meals, except a decrease in MCV and an increase in RBC of birds fed SFY-0 compared with birds fed C and RFY-0 diet (*P* <0.05). Furthermore, basophils were detected in the blood of birds fed SFY-0 (Table 6). The inclusion of WC in false yam tuber meals could counterbalance the negative effect in MCV. MCV values for birds fed RFY-30, RFY-60, SFY-30 and SFY-60 were not different from C. A linear increase in MCV was realized with increasing levels of WC. In contrast, no effect of varying levels of WC was observed for RBC values of birds fed the SFY meal (Figure 3).

Table 6 Haematology of experimental broiler chickens fed a control diet and various false yam tuber meals without wood charcoal (arithmetic means)

Variable	Unit	C	RFY-0	SFY-0	SEM	*P*-value
PCV[a]	%	32.77	32.93	32.87	0.229	0.334
RBC[a]	× 10^6/µL	2.37^c	2.66^c	2.78^d	0.071	0.023
Hb[a]	g/dL	7.73	7.93	8.23	0.156	0.482
MCHC[a]	g/dL	23.67	24.10	25.03	0.453	0.484
MCH[a]	pg	32.67	29.93	29.70	0.605	0.068
MCV[b]	µm³	138.63^c	124.10^c	118.37^d	3.469	0.032
Platelets[a]	× 10^3/µL	11.33	10.33	11.33	1.691	0.971
WBC[a]	× 10^3/µL	8.11	13.29	11.02	1.567	0.460
Heterophils[b]	%	51.00	50.67	50.00	0.747	0.888
Lymphocytes[a]	%	48.67	48.67	48.00	0.747	0.935
Eosinophils[b]	%	0.00	0.00	0.33	0.111	0.368
Monocyte[b]	%	0.33	0.67	1.00	0.289	0.656
Basophils[b]	%	0.00	0.00	0.67	0.147	0.102

C: commercial broiler finisher diet (control); RFY-0: raw false yam tuber meal (50 g/kg; RFY) without wood charcoal (WC); SFY-0: false yam tuber meal soaked in water (150 g/kg; SFY) without WC. PCV: packed cell volume; RBC: red blood cell count; Hb: haemoglobin; MCHC: mean corpuscular haemoglobin concentration; MCH: mean corpuscular haemoglobin; MCV: mean corpuscular volume; WBC: white blood cell count. SEM: standard error of mean

[a] ANOVA and post-hoc Tukey HSD test

[b] Kruskal-Wallis test

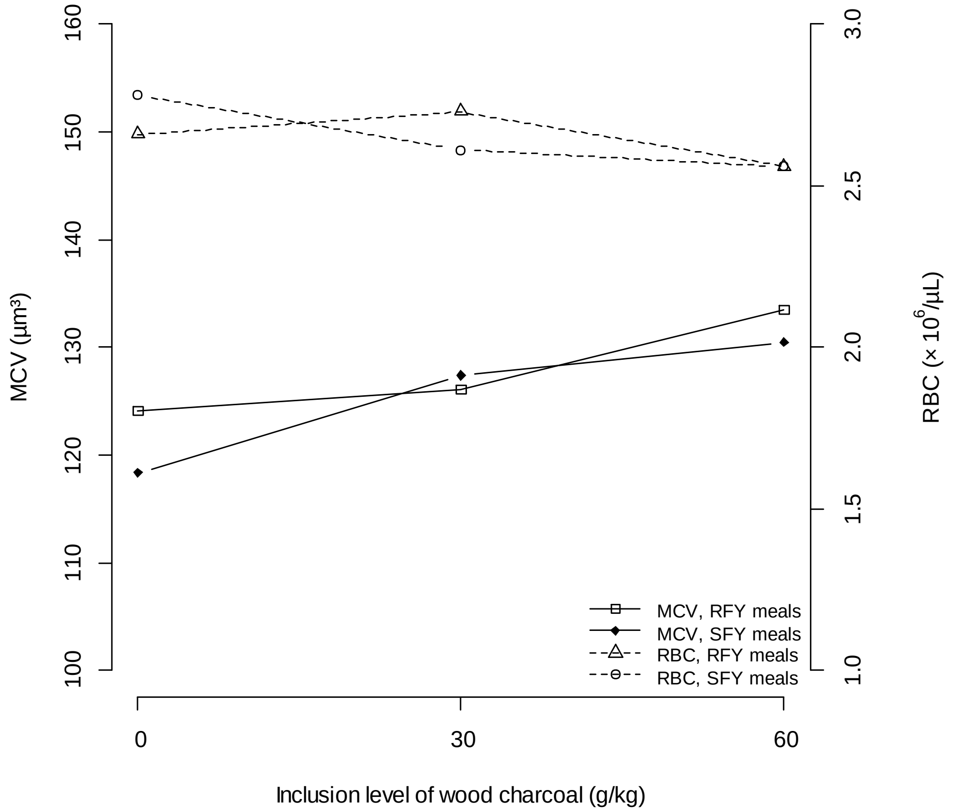
Similar to haematological variables, the type of false yam tuber meal did not affect blood serum proteins, globulin, alkaline phosphate, aspartate transferase and alanine transferase levels of broiler chickens (*P* >0.05) (Table 7). Furthermore, no effects of increasing level of WC to false yam tuber meals were observed for serological parameters (*P* >0.05).

Discussion

The RFY at 50 g/kg inclusion level might be an option to replace part of commercial broiler feed in northern Ghana and other countries where *Icacina oliviformis* is native. No effect of RFY was observed on bird production performances. However, a higher inclusion of RFY would lead to a reduction in broiler production performances (Teye *et al.*, 2011). In contrast, MCV in the blood of experimental birds was reduced if no WC was added, but values were still within the reference range of 100–139 µm³ (Greenacre & Morishita, 2014). The MCV defines the size of RBC and is used to morphologically classify anaemia. Reduced MCV might be an indicator of microcytic anaemia (Sarma, 1990). Nevertheless, experimental birds fed the RFY diet did not show any signs of anaemia. Finally, toxic substances in RFY might lead to slightly higher mortality rates.

In contrast to the results obtained for RFY, the inclusion of SFY in commercial broiler feed at the investigated level of 150 g/kg is not recommended because of the negative effects on bird production performance. Limiting the inclusion level to 120 g/kg, as suggested by Dei *et al.* (2015b), might be an option to avoid negative effects on performance characteristics. In the current study, the decrease in growth performance of birds fed SFY could not be compensated by a comparably higher DFI, which was particularly observed at the end of the finisher phase. This could be explained by the relatively low CP concentrations of

SFY tuber meals, which were below the recommended values of 200 g/kg (4–6 weeks of age) and 180 g/kg (above 6 weeks of age) for broiler chickens (Merck Veterinary Manual, 2016; National Research Council, 1994). Additionally, Bregendahl *et al.* (2002), among others, revealed a lower growth performance, higher feed intake and thus poorer FCR of broiler chickens fed low-protein diets. Hence, more feed and feed additives might be needed to compensate for the low energy and protein content of false yam meals (Dei *et al*., 2015b) and to allow birds to counterbalance the toxic effect of terpenic constituents in false yam tubers (Nersesian *et al*., 2012). In addition to the time needed to soak false yam tubers, this would increase costs and thereby potentially limit the benefits of false yam in poultry production.



RFY: raw false yam tuber meal at 50 g/kg substitution level; SFY: soaked false yam tuber meal at 150 g/kg substitution level in relation to increasing level of included wood charcoal (0 g/kg, 30 g/kg, 60 g/kg)

Figure 3 Mean corpuscular volume and red blood cell count of broiler chickens fed false yam tuber meals

Table 7 Serology of experimental broiler chickens fed a control diet and various false yam tuber meals without wood charcoal (arithmetic means)

	Unit	C	RFY-0	SFY-0	SEM	*P*-value
Total protein	g/L	35.40	32.80	31.57	1.286	0.526
Albumin	g/L	16.17	13.77	13.40	0.563	0.067
Globulin	g/L	19.20	19.07	18.20	1.008	0.929
ALK	U/L	155.10	154.27	168.27	7.730	0.764
ASP	U/L	126.50	91.53	142.50	18.699	0.589
ALA	U/L	10.97	7.87	8.40	1.287	0.640

C: commercial broiler finisher diet (control), RFY-0: raw false yam tuber meal (50 g/kg; RFY) without wood charcoal (WC); SFY-0: false yam tuber meal soaked in water (150 g/kg; SFY) without WC. ALK: alkaline phosphate; ASP: aspartate transferase; ALA: alanine transferase. U: unit. SEM: standard error of the mean. ANOVA test.

Despite their low CP concentrations, the false yam tuber meals met the requirements for most limiting amino acids, as shown by the amino acid profiles of the diets, with the exception of the threonine concentrations of SFY-30 and SFY-60, which were below the recommendations set by NRC (1994). This lack might explain the negative effect on total serum protein, albumin and globulin concentrations, which were below the reference ranges (Greenacre & Morishita, 2014). A decrease in blood proteins reflects decreased protein synthesis owing to malnutrition or lack of essential amino acids, which are required for protein synthesis (Shikora, 2002). Although the authors observed such a decrease for both types of false yam tuber meals, differences were insignificant to the control diet.

Furthermore, terpenes could be an antagonist of essential amino acid receptors in poultry. However, the mechanisms of interaction and effects of terpenes in *Icacina oliviformis* on bird physiology are largely unknown and additional studies are needed to clearly confirm the negative effects of false yam tuber meals on bird performance, serological parameters and consequently health status. In general, studies revealed a large variety of properties of extracts of *Icacina* species. Ethanol extracts of *Icacina trichantha* were reported to protect the kidneys and livers of rats poisoned with tetrachloromethane (Asuzu & Abubakar, 1995). Hydroalcoholic extracts of *Icacina trichantha* tubers showed an inhibition of croton oil-induced ear edema in mice (Asuzu *et al*., 1999). Some subfractions of methanol extracts of leaves of *Icacina oliviformis* inhibited the growth of chloroquine resistant strains of *Plasmodium falciparum*, which cause malaria in humans (Sarr *et al*., 2011). Ethanol and water extracts of leaves *Icacina oliviformis* showed antimicrobial activities against *Escherichia coli*, *Staphylococcus aureus*, *Streptococcus spp*, *Candida albicans* and *Klebsiella pneumonia* (Shagal & Kubmarawa, 2013). Unfortunately, all these results are preliminary, because secondary metabolites have not yet been identified; nor have their properties been tested and proven in *in vivo* studies. In contrast, Zhao *et al*. (2014) reported cytotoxic activities of isolated, purified and identified pimarane-type diterpenoids from tubers of *Icacina trichantha.* Diterpene humirianthenolide C in particular showed activity against the human melanoma cancer (MDA-MB-435) and human breast cancer cells (MDA-MB-231) (Zhao *et al*., 2014).

Soaking false yam tubers in water to remove terpenes before feeding, as suggested by Dei *et al.* (2015b), had almost no effect on the amount of the lipophilic *n*-hexane subfractions, but starting with the $CHCl_3$-subfraction, all other subfractions showed smaller mass data for the soaked biological material than for raw. This demonstrates that water removes hydrophilic compounds from the false yams tubers more easily than lipophilic ones.

Including WC along with false yam tuber meals partially attenuated the anti-nutritive effects of *Icacina oliviformis*, particularly for raw tubers. However, adding WC with false yam tuber meals further reduces the energy and nutrient density per unit feed, which is reflected in lowest final bodyweights and blood protein of birds fed SFY with 30 g/kg and 60 g/kg added WC. Although the inclusion of WC in false yam diets did not further increase the feed intake of birds of these groups in the current study, higher feed intakes and lower FCRs obtained in other studies do not justify the inclusion of WC in broiler diets (Odunsi *et al*., 2007). A study by Chu *et al.* (2013) revealed a negative effect of bamboo charcoal on total blood protein in fattening pigs, while, contrary to the results of the current study, a positive effect on pig growth performance was observed. Finally, the inclusion of WC in livestock feed is supportable, only if sustainable sources of biomass are used as feedstock for its production and there are no alternative uses of this material. A review by Duku *et al.* (2011) showed that Ghana owns a large variety of potential feedstock for the production of charcoal, including agricultural waste (for example rice husks and maize cobs) and forestry residues, as well as wood processing waste such as sawdust.

Conclusions

Anti-nutritional substances contained in SFY at the studied substitution level are harmful to the birds, irrespective of whether WC is added or not. In contrast, including 50 g/kg RFY had no effect on the parameters, except for mortality, which tended to be slightly higher. Wood charcoal could possibly reduce mortality in birds fed RFY, but only at inclusion levels higher than 30 g/kg. It would be of interest to verify the effects of false yam tuber meals and WC on performances and blood parameters of local avian genetic resources because of their adaptation to adverse climatic conditions and husbandry practices and their role in food security of urban and rural households in northern Ghana.

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Authors' Contributions

CS and RR coordinated the project design and were in charge of writing the manuscript. BA and HKD were in charge of supervising the implementation of the study at UDS Nyankpala. LA, ARS and AM were in charge of data collection; AM was responsible for laboratory tests of the blood samples. RCM and DH were responsible for laboratory analysis of terpenic constituents of false yam tuber meals and interpreting related results. RR coordinated laboratory analysis of other feed constituents including the amino acid profiles. All co-authors participated in results, statistics and interpretation.

Conflict of Interest Declaration

The authors declare that there are no conflicts of interest.

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