Effect of substitution of fishmeal with Napier grass protein on performance and carcass characteristics of broiler chicken

Muhammad Kiggundu (kiggundumuhammad@gmail.com)
National Livestock Resources Research Institute https://orcid.org/0000-0003-4187-9894
Zainah Nampijja
Makerere University College of Agricultural and Environmental Sciences
Abasi Kigozi
National Agricultural Research Organization, National Livestock Resources Research Institute
Swidiq Mugerwa
National Agricultural Research Organization, National Livestock Resources Research Institute https://orcid.org/0000-0001-9882-809X
Moly Allen
National Agricultural Research Organization; National Livestock Resources Research Institute
Clementine Namazzi
National Agricultural Research Organization, National Livestock Resources Research Institute
Siraj Ismail Kayondo
National Agricultural Research Organization, National Livestock Resources Research Institute
Faitwa Walugembe
National Agricultural Research Organization, National Livestock Resources Research Institute
Alexander Bombom
National Agricultural Research Organization, National Livestock resources Research Institute

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Abstract

This study evaluated the performance and carcass characteristics of broiler chickens fed diets with varying substitution levels of fishmeal (FM) 0, 250, 500, 750, and 1000 g/kg DM with Napier grass protein (NGP). Treatment diets included T0 (control diet), T250, T500, T750, and T1000 to represent the different substitution levels. After the starter phase, birds were finished on a conventional fishmeal-based diet for 14 days. Feed intake, weight gain, and hot carcass decreased ($P < 0.01$) at a decreasing rate with increasing substitution in starter diets. Feed intake, weight gain, and hot carcass were highest ($P < 0.01$) in T0 birds, followed by T250 birds. Feed conversion ratio (FCR), protein, and energy utilization efficiencies were highest ($P < 0.01$) in T0 birds followed by T250 birds and lowest for T1000 birds. Ileal CP digestibility (862.3 g/kg DM) was highest for T0 birds but comparable ($P > 0.05$) to T500 birds (716.4 g/kg DM). Similarly, ME digestibility was highest in T0 birds (819.5 g/kg DM) although comparable ($P > 0.05$) to T250 birds and T500 birds. In contrast, T250 and T500 birds showed higher ($P < 0.05$) crude fiber digestibility (645 g/kg DM) than T0 birds (402 g/kg DM). Hot carcass weight decreased ($P < 0.01$) with FM substitution with the heaviest carcasses recorded for T0 birds. As percentage of the hot carcass, weight of breast and thigh reduced ($P < 0.05$) with increasing substitution of FM. Percentage weights of empty gizzard, caeca, and pancreas for T1000 birds were higher ($P < 0.05$) and double compared with T0 birds. In the finisher phase, there was no difference ($P > 0.05$) in weight gains of T0 and T250 birds, although T250 birds had numerically higher weight gain compared with T0 birds. However, percentage change in weight gain in the finisher phase was influenced ($P < 0.05$) by MFP substitution level in the starter phase. In the finisher phase, T1000 birds had the highest percentage change in weight gain (71.6%) compared to 40.7% for T0 birds. Nonetheless, regression analysis of FCR data in the starter phase showed that optimal FCR is attained at 150 g/kg NGP substitution in broiler diets. These results suggest that NGP is of low biological value in broiler diets, probably due to its high fiber content and deficiency in methionine and lysine, which are limiting essential amino acids in broiler diets.

1. Introduction

Protein remains a major nutrient influencing production, productivity, and profitability of poultry production enterprises (Beski et al., 2015). The use of soybean and silverfish present with challenges, including competition with humans for food, contamination and or adulteration, unsustainable depletion of fish stocks, and high prices (Nampijja, 2018). Alternative protein sources are being explored, including the use of insect meals like black soldier larvae (Schiavone et al., 2017), housefly larvae (Pretorius, 2011), and earthworms (Prayogi, 2011). However, the use of insect meals in poultry diets is limited by low throughput of insect rearing technologies and facilities (Huis, 2014), ethical consumer considerations (Veldkamp and Bosch, 2015), high chitin and fat contents of insect meals (Nampijja, 2018). The high fat content lowers feed intake while the chitin digestibility lowers feed digestibility (Tabata et al., 2018).

Elsewhere, research efforts have assessed the potential for the production of green proteins from forage biomass as a source of protein for poultry. Plant-derived protein produced from Gliricidia (*Gliricidia sepium*) has been explored as an alternative to fishmeal. Replacement levels of up to 250 g/kg of G. *sepium* derived protein were found optimal with no effects on growth and carcass characteristics in poultry (Agbede and Aletor, 2003). Napier grass (*Pennisetum purpureum*) is a native forage resource in Uganda high in biomass, adapted to diverse environments, and with a crude protein content of 67 to 92 g/kg DM depending on the growth stage. Biorefining can sustainably produce high-quality protein from Napier grass with crude protein values between 150 to 280 g/kg DM. However, no initiatives have been carried out to biorefine and produce plant-based protein from Napier grass as a sustainable, low-cost protein and potential substitute for silverfish meal and soya bean in poultry diets. This study sought to explore the use of plant-derived protein from Napier grass as a potential substitute for fishmeal in poultry diets and determine its effect on performance and carcass characteristics of broiler chickens.

2. Materials And Methods

2.1. Production of protein from Napier grass

Freshly cut Napier grass was harvested and collected from farmer fields, crushed and pressed using second-generation biorefinery equipment (Grassa, Netherlands) at Kabarole Research and Resource Center (KRC) in Fort Portal, Western Uganda. The resulting juice was separated from the press cake, processed, and proteins concentrated by heating and subsequently dried using a solar drier. The plant-based protein obtained by biorefining Napier grass is herein referred to as Napier grass protein (NGP). After that, the NGP was used to formulate diets along with other ingredients. The proximate composition of NGP and FM, is presented in Table 1.

Table 1
Proximate composition of Napier grass protein and fish meal (FM) used in experimental diets

Nutrient, g/kg DM	NGP	FM
Dry Matter	890.9	861.8
Crude Protein	201.2	521.4
Crude fat	26.6	70.2
Crude Fiber	241.0	7.4
Total Ash	139.6	349.0
NFE	282.5	113.8
ME (Kcal/kg)	1920.0	2819.9

Table 1

2.2. Formulation of experimental diets

Five iso-nitrogenous and iso-carolic diets were formulated (Table 2) to meet nutritional requirements of broiler chickens as recommended by (NRC, 1994) using Creative Formulation Concepts software educational version 8.01.01. Napier grass protein used in this study was a fine meal and would easily be blown away in the form of dust during feed mixing. To overcome this challenge, we pelleted (1.5 mm pellet aperture) the diet as opposed to feeding as a marsh in order to minimize wastage and also to encourage a high fed intake of the experimental diets (Svihus, 2014).

Table 2
Ingredient composition (kg) of experimental diets

Ingredient (kg)	Diets (g/kg of FM substitution)				
	T0	T250	T500	T750	T1000
Maize	11.5	10.0	8.0	10.0	8.0
Maize bran	55.0	43.1	30.0	20.0	12.0
Soya	5.0	8.0	13.0	15.0	16.0
cotton seed cake	9.0	9.0	9.0	5.0	4.0
NGE	0.0	14.9	29.7	44.6	59.4
Fish	18.0	13.5	9.0	4.5	0.0
Shells	0.5	0.5	0.5	0.5	0.5
Salt	0.5	0.5	0.5	0.5	0.5
Vitamin premix	0.5	0.5	0.5	0.5	0.5

Table 2

Treatment diets T250, T500, T750, and T1000 were constituted by substituting 250, 500, 750, and 1000 g/kg of FM with NGP, respectively. The control diet (T0) contained 1000 g/kg FM and 0 g/kg NGP. The determined nutrient composition of the formulated experimental diets (Table 3) was done according to the Association of Official Analytical Chemists standards (AOAC, 1990).

Table 3
Determined nutrient composition of diets used in the experiment

Nutrient, g/kg DM	Diets (g/kg of FM substitution)					Finisher Diet
	T0	T250	T500	T750	T1000	
Dry Matter	967.5	970.0	972.1	972.5	979.3	914.8
Crude Protein	215.0	214.2	200.0	200.0	191.6	197.5
Crude fat	86.5	89.1	78.2	71.3	13.7	100.0
Crude Fiber	290	741	10.9	1263	1401	70.6
Total Ash	132.6	117.2	104.1	103.1	102.3	124.3
NFE	530.8	550.2	590.8	596.5	702.3	469.2
ME (Kcal/kg)	3257.3	3175.8	3051.6	2961.4	2887.4	3271.5
E:P (Kcal/ g CP)	15.2	14.8	15.3	14.8	15.1	16.6

Table 3

2.3. Birds and experimental design

Three hundred Cobb 500 Day old chicks with an average initial weight of 42.43 ± 0.24 g were obtained from Ugachick Poultry Breeders, Uganda. The chicks were randomly assigned to one of the five treatment diets T0, T250, T500, T750, and T1000 in a Completely Randomized Design (CRD). Subsequently, in the text herein, birds fed on treatment diets T0, T250, T500, T750, and T1000 are referred to as T0 birds, T250 birds, T500 birds, T750 birds, and T1000 birds, respectively. Each treatment had three replicates with 20 birds per replicate in a male: female ratio of 1:1. The stocking rate was 10 birds per square meter in the brooder and 5 birds per square meter in the finishing unit. Brooding of the day-old chicks was carried out for two weeks under a deep litter system with coffee husks (7.5 cm deep) as litter material over a concrete floor. Birds were provided with soluble vitamins in drinking water during the first week of brooding to correct for any deficiencies from the breeder firm. A 24hour lighting program was followed using fluorescent tubes. Vaccination of all birds was carried out following the Ugachick Poultry Breeders' recommended vaccination schedule, that is, Newcastle Disease (NCD) and Infectious Bronchitis (IB) – days 1 and 21; Infectious Bursal Diseases (IBD) – days 7 and 14. The starter diet was fed to the birds for 28 days after which, all birds in the respective treatment groups were fed on a fishmeal based finisher diet for an additional 14 days.

Data collection and Sampling

Feed intake was recorded on a cage basis daily as the difference between feed offered and the feed refusal while bodyweight of the birds was recorded on a weekly interval by weighing each bird per cage using a digital balance. From this data, average daily feed intake, body weight gain, feed conversion ratio (FCR) calculated as feed per unit gain in body weight, protein efficiency ratio (PER) and energy efficiency ratio (EER) were calculated. Three birds per replicate were slaughtered following Halal procedure to allow for sampling of the ileal digesta (Ahmed et al., 2014). The digesta was collected from the portion of ileum between Meckel's diverticulum and 2.0 cm from the ileo-caecal junction. The ileal digesta samples were immediately placed on ice in cooler boxes and later transported to the laboratory for analysis.

2.4. Nutrient digestibility

Titanium Dioxide (TiO_2) was added to test diets as an indigestible marker at a rate of 5 g/kg of diet on the 21st day of the trial and was used in the determination of ileal nutrient digestibility (Short et al., 1995; Myers et al., 2004). Proximate analysis following standard methods of the AOAC (1990) was applied to the diets and digesta to determine the CP, EE, and ME digestibility.

2.5. Carcass characteristics and relative weight of internal organs

On the last day of starter phase d 28, 6 birds (2 birds per replicate) from each treatment were sampled for analysis of carcass characteristics. The birds were fasted for 12 hours to allow for emptying of the gut and weighed before slaughter. The fasted birds were weighed and killed following Halal procedures (Ahmed et al., 2014), bled for three minutes, scalded at 63˚C for approximately two minutes, de-feathered, and eviscerated. The weights of hot carcasses after evisceration, internal organs (visceral, liver, gizzard, heart, proventriculus, spleen, lungs, caeca, crop, pancreas, and bladder), head, feet and carcass parts (breast, wings, back, neck, thighs, and drumsticks) were measured using a precision balance (Eco's equipment, Hs-300S model, South Korea) and recorded. Relative weights of organs were expressed as percentage of live body weight while weights of the carcass parts were expressed as percentages of the hot carcass. Carcass samples of about 10 g each were collected from breasts and thighs of each of the slaughtered bird and pooled according to the treatments. Collected samples were kept at -20˚C until further proximate analysis. Skinning of the samples was done before analysis for dry matter, crude protein, crude fat, and ash was carried out.

2.6. Chemical analysis

Feed and meat samples were oven-dried at 100°C for 24 hours and ground to pass through a 1.0 mm sieve (Cyclotec 1093 sample mill Foss Tecator). Similarly, ileal samples were oven-dried at 65°C for 3 days (AOAC, 1990), ground to pass through a 1.0 mm sieve, and stored in airtight containers for determination of ileal nutrient digestibility. Dry matter

(DM), crude protein (CP), ether extract (EE), crude fiber (CF), and ash of the samples were analyzed according to standard methods, 967.03, 984.13, 920.39, 978.10 and 942.05, of (AOAC, 1990), respectively. The Metabolizable energy (ME) of the samples was calculated using the NRC (1994).

2.7. Statistical analysis

Data collected on feed intake, body weight, and weight gain were analyzed using PROC GLM for repeated measures of SAS, (2003) while data on FCR, PER, EER, and ileal digestibility was subjected to analysis of variance (ANOVA) using PROC GLM of SAS, (2003). For significant effects, LSMEANS of parameters were separated using the PDIFF option of SAS, (2003). The following model was used;

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

Where Y_{ij} was the overall observation on the j^{th} pen of the i^{th} substitution level of Napier grass extract, μ was the overall mean, T_i the effect of the i^{th} substitution level and e_{ij} the error term. The linear and quadratic effects of inclusion of NGP (i = 0, 250, 500, 75, and 1000 g/kg) were determined by applying polynomial orthogonal contrasts using the CONTRAST options within SAS. To determine the optimal inclusion levels of NGP in broiler diets, second-order polynomial regressions were fitted to data on feed conversion ratio (FCR) in order to represent treatment response, and the regression equation was used to determine the optimal response point.

3. Results

Growth performance and feed utilization efficiency of birds on starter diets

Growth performance parameters of broiler chickens in the starter phase (Table 4) followed a curvilinear trend (P < 0.01). Final weight, daily weight gain, hot carcass, and feed intake decreased at a decreasing rate with increasing level of inclusion NGP in broiler diets. Control birds had the highest final live weight, daily weight gain, hot carcass, and feed intake, while T1000 birds had the lowest final live weight, daily gain, hot carcass, and feed intake.

Table 4

Growth, feed utilization and nutrient digestibility of birds in the starter phase (0–28 d)

Parameter	Diets (g/kg of FM substitution)					SEM	*P-value*		
	T0	T250	T500	T750	T1000		Overall	Lin	Quad
Initial weight (g)	42.32	42.43	42.55	42.43	42.42	0.191	Ns	nd	nd
Final weight (g)	1034.62[a]	548.40[b]	438.23[c]	282.49[d]	128.14[e]	15.288	< .0001	< 0.0001	< 0.0001
Weight gain (g)	992.30[a]	505.97[b]	395.68[c]	240.06[d]	85.72[e]	15.156	< .0001	< .0001	< .0001
Hot carcass (g)	830.50[a]	476.67[b]	300.83[c]	166.17[d]	48.50[e]	21.797	< 0.0001	< 0.0001	< 0.0001
Feed intake (g)	1811.31[a]	1286.56[b]	1111.52[c]	878.44[d]	581.80[d]	38.020	< .0001	< 0.0001	< 0.0001
Feed utilization efficiency									
FCR	1.83[d]	2.54[c]	2.82[c]	3.67[b]	6.84[a]	0.150	< 0.0001	ns	< 0.0001
PER	2.55[a]	1.84[b]	1.78[b]	1.36[c]	0.77[d]	0.050	< .0001	< .0001	< .0001
EER	16.33[a]	11.45[b]	10.38[c]	8.02[d]	4.75[e]	1.032	< .0001	< 0.0001	< 0.0001
Nutrient digestibility									
DM	721.4[a]	543.5[b]	437.6[b]	235.2[c]	65.5[d]	76.95	< 0.0001	0.0003	0.00285
CP	862.3[a]	792.7[a]	716.4[a]	528.1[b]	468.6[b]	51.40	0.0007	ns	< 0.0001
EE	873.2[a]	868.3[a]	890.9[a]	753.0[ab]	625.0[b]	34.93	0.0042	0.0241	0.0008
CF	401.5[b]	637.6[a]	652.1[a]	572.1[ab]	595.8[ab]	32.84	0.0357	ns	ns
ME	819.5[a]	686.9[b]	622.7[b]	449.1[c]	237.8[d]	67.65	< 0.0001	0.0037	< 0.0001

[a.b.c.d] *Means with different superscripts in the row differ significantly (p ≤ 0.05). FCR: Feed Conversion Ration; PER: Protein Efficiency Ration; ME: Metabolizable Energy; EER: Energy Efficiency; ns: not significant*

Table 4

The FCR of birds on the different treatments followed a quadratic trend ($P < 0.01$) while PER and EER followed a curvilinear trend with increasing level of NGP in the starter diets. The lowest FCR was recorded in T0 birds while the highest was recorded in T1000 birds. However, the fitted minimum FCR of 1.96 corresponded to 150 g/kg of fishmeal substitution with NGP (Fig. 1). On the contrary, no significant differences ($P > 0.05$) were observed in FCR, PER, and EER of T250 and T500 birds.

Figure 1

Nutrient digestibility in starter diets

Dry matter digestibility of experimental diets varied (P < 0.05) with substitution of FM with NGP. The highest DM digestibility (721.4 g/kg DM) was recorded in the T0 birds while the lowest (65.5 g/kg DM) was recorded in T1000 birds. Similarly, the lowest CP digestibility was also observed in T1000 birds. Digestibility of metabolizable energy of diets followed a curvilinear trend and was measured to be highest (819.5 g/kg) and lowest (237.8 g/kg) for the T0 birds and T1000 birds, respectively. However, no significant differences (P > 0.05) were observed in DM, CP, and ME digestibility in T250 and T500 birds.

Growth performance and feed utilization during the finishing phase

Irrespective of treatment, there was a drastic increase in the weight gain of all birds when a conventional diet was introduced during the finishing phase (Table 5). No significant difference was observed in the weight gain of T0, T250, and T500 birds. T1000 birds had the highest percentage change in weight gain in the finisher phase of 71.6% compared to 40.7% for T0 birds. No significant difference was observed in the feed intake of T0 and T250 birds.

Table 5
Growth and feed utilization of birds fed starter diets and finished on a conventional diet in the finisher phase (29 to 42 d)

Parameter	Diets (g/kg of FM substitution)					SEM	*P-value*		
	0	250	500	750	1000		Overall	Lin	Quad
Growth Performance									
Initial weight (g)	1034.62^{a}	548.40^{b}	438.23^{c}	282.49^{d}	128.14^{e}	15.288	< .0001	< 0.0001	< 0.0001
Final weight (g)	1746.11^{a}	1284.75^{b}	1103.52^{c}	772.24^{d}	452.54^{e}	18.446	< .0001	< .0001	< .0001
Weight gain (g)	705.19^{a}	736.53^{a}	671.99^{a}	489.93^{b}	323.66^{c}	14.930	< .0001	ns	ns
Feed intake (g)	1882.80^{a}	1679.89^{ab}	1518.52^{b}	1137.06^{c}	730.97^{d}	53.131	< .0001	0.0025	ns
Feed utilization efficiency									
FCR	2.67^{a}	2.28^{b}	2.26^{b}	2.32^{b}	2.26^{b}	0.062	0.0111	0.0109	0.0066
PER	1.91^{b}	2.22^{a}	2.24^{a}	2.18^{a}	2.24^{a}	0.052	0.0122	0.0136	0.0065
EER	11.50^{b}	13.41^{a}	13.49^{a}	13.17^{a}	13.53^{a}	0.315	0.0122	0.0136	0.0065

$^{a.b.c.d}$ *Means with different superscripts in the row differ significantly (p ≤ 0.05). FCR: Feed Conversion Ration; PER: Protein Efficiency Ration; EER: Energy Efficiency Ratio; ns: not significant.*

Table 5

There was an improvement in feed utilization of all birds when the conventional diet was introduced, with T1000 birds having a better FCR than T0 birds (Table 5). Irrespective of substitution level, T250, T500, T750, and T1000 birds in the finisher phase had comparable FCR values (P > 0.05), averaging 2.28 but lower (P < 0.05) than for T0 birds which had an average FCR value of 2.67. On the contrary, T250, T500, T750, and T1000 birds had similar (P > 0.05) but higher PER and EER values than T0 birds. The PER and EER values of T0 birds averaged at 1.91 and 11.50, while for where FM was substituted with NGP, values averaged at 2.22 and 13.4, respectively.

Yield of carcass quality and yield characteristics

Dressing percentage and yield of broiler carcass parts expressed as a percentage of the hot carcass was significantly ($P < 0.05$) affected by level of FM substitution in poultry diets (Table 6). The highest carcasses yield was observed in T0 birds while the lightest carcasses were observed in T1000 birds. The weight of thigh linearly decreased with increasing inclusion of NGP. Control birds yielded heavier thighs, although no significant differences were observed in T500, T750, and T1000 birds. Similarly, breast muscle followed a similar trend with T0 and T250 birds having heavier breasts compared to T500 and T750 birds. Conversely, T1000 birds had significantly ($P < 0.05$) heavier backs and necks compared to T0 and T250 birds.

Table 6
Caracas parts as a percentage of hot carcass weight of experimental birds in the 28 d starter phase

Carcass part (%)	**Diets (g/kg of FM substitution)**					**SEM**	***P-Value***		
	0	**250**	**500**	**750**	**1000**		**Overall**	**Lin**	**Quad**
Dressing percentage	63.38	57.82	57.13	49.43	39.17	2.011	< 0.0001	0.1277	0.0789
Drumstick	12.29	12.18	13.03	12.46	14.77	0.311	< 0.0001	ns	0.0203
Thigh	16.17^{a}	15.32^{ab}	14.43^{b}	14.09^{b}	14.76^{b}	0.346	0.0027	0.0004	ns
Wings	10.82^{b}	12.59^{a}	13.47^{a}	13.57^{a}	13.42^{a}	0.377	< 0.0001	< 0.0001	0.0165
Breast	30.40^{a}	29.58^{a}	28.08^{ab}	24.79^{b}	19.13^{c}	1.267	< 0.0001	ns	ns
Back	20.90^{ab}	20.88^{ab}	19.85^{b}	22.03^{ab}	23.82^{a}	0.894	0.0140	ns	ns
Neck	9.41^{b}	9.46^{b}	11.14^{ab}	13.06^{a}	14.11^{a}	0.904	0.0033	ns	ns

[a.b.c.d] *Means with different superscripts in the row differ significantly ($p \leq 0.05$)*

Table 6

Nutrient composition of drumstick and breast muscle following the starter phase

The chemical composition of the drumstick and breast muscles of the experimental birds as influenced by the level of substitution of fish meal with NGP is shown in Table 7. The crude protein content of drumsticks was highest ($P < 0.05$) in T1000 birds but lowest in T0 birds. Conversely, the fat content in the drumstick was highest in T0 birds but lowest in T1000 birds.

Table 7

Effect of starter diets on Nutrient composition (g/kg DM) of drumstick and breast Muscle of birds at the end of the 28 d starter phase

Nutrient, g/kg DM	Diets (g/kg of FM substitution)						
	0	**250**	**500**	**750**	**1000**	**SEM**	***P-value***
Drum sticks							
CP	750.6^{d}	780.4^{c}	792.230^{c}	824.2^{b}	846.5^{a}	8.92	< .0001
EE	177.6^{a}	149.7^{ab}	93.8^{ab}	71.6^{ab}	34.4^{b}	16.15	0.0276
Ash	26.4	28.2	30.8	23.2	28.2	1.24	0.3478
Breast							
CP	866.2	890.8	910.2	883.5	825.5	8.94	0.1578
EE	87.1	68.4	58.5	47.4	45.6	5.66	0.0819
Ash	31.7^{b}	32.7^{b}	34.0^{b}	35.3^{b}	52.7^{a}	1.73	0.0099

$^{a.b.c}$ *Means with different superscripts in a row column differ significantly ($p \leq 0.05$)*

Table 7

Weight of internal organs

The weight of external and internal organs as a percentage of the final live weight of birds is presented in Table 8. The weight of heads increased ($P < 0.001$) at an increasing rate with an increase in substitution of FM with NGP in broiler starter diets. However, no differences ($P > 0.05$) were observed in the relative weight of heads of T0 and T250 birds. Weight of the visceral increased ($P < 0.001$) at an increasing rate with an increase in substitution of FM with NGP in broiler starter diets. Among the visceral organs, the relative weight of the empty gizzard increased at an increasing rate with the substitution of FM with NGP. Control and T1000 birds had the smallest and highest percentage weight of the empty gizzards, respectively. Similarly, experimental diets had a significant influence ($P < 0.05$) on the weight of the proventriculus, ceca, and pancreas. Overall, these organs were heavier for T1000 birds compared with T0 birds, although no significant differences ($P > 0.05$) were observed between T0 and T250 birds.

Table 8
Weights of internal organs expressed as a percentage of final live weight of birds slaughtered at the end of the 28 d starter phase

Organs weight (%)	Diets (g/kg of FM substitution)					SEM	*P-value*		
	0	250	500	750	1000		Overall	Lin	Quad
External Organs									
Head	2.63^{c}	3.03^{c}	4.01^{b}	4.52^{b}	7.71^{a}	0.345	< .0001	0.9165	0.0003
Feet	3.83	3.75	4.19	4.09	3.6823	0.270	0.6076	ns	ns
Internal Organs									
Visceral	15.84^{d}	17.89^{d}	22.32^{c}	26.18^{b}	32.66^{a}	1.200	< .0001	0.0037	0.0861
Empty Gizzard	2.39^{d}	3.02^{d}	3.75^{c}	4.65^{b}	6.21^{a}	0.267	< .0001	0.0030	0.0154
Liver	2.1548	2.1962	2.3369	2.2778	2.1785	0.046	0.7359	ns	ns
Proventriculus	0.59^{c}	0.79^{bc}	1.02^{ab}	1.27^{a}	1.18^{a}	0.107	0.0005	0.001	ns
Heart	0.58	0.59	0.65	0.59	0.63	0.022	0.1719	ns	ns
Caeca	0.75^{b}	1.11^{ab}	1.21^{ab}	0.88^{b}	1.67^{a}	0.181	0.0097	ns	0.0056
Lungs	0.45	0.40	0.34	0.50	0.44	0.046	0.1648	ns	ns
Empty Crop	0.72	0.65	0.80	1.14	1.14	0.149	0.0810	ns	ns
Pancreas	0.28^{b}	0.40^{b}	0.46^{ab}	0.46^{ab}	0.610^{a}	0.049	0.0039	ns	0.0411
Spleen	0.09	0.09	0.10	0.13	0.11	0.019	0.5212	ns	ns
Bladder	0.11	0.11	0.20	0.17	0.30	0.042	0.0638	ns	ns

$^{a.b.c.d}$ *Means with different superscripts in the row differ significantly ($p \leq 0.05$), ns: not significant*

Table 8

Discussion

Non-ruminants are less efficient at utilizing protein from forage-based sources due to lack of appropriate microbes and enzymes in their digestive tract that can ferment and digest fiber (Knudsen, 1997). In this study, the biorefinery of Napier grass was used to produce the plant-based protein. The protein from the extracted juice was recovered by drying the residue, which was lower in fiber content compared with the original Napier grass. As such, the NGP had significantly lower fiber and higher crude protein content compared with whole Napier grass forage. Nonetheless, when compared with fish meal, the NGP contained 97% more fiber.

The level of feed consumption is a critical determinant of the rate of growth of broiler birds. Although energy intake is known to be the primary driver of feed intake in commercial broiler lines (Ferket and Gernet, 2006), it is, however, unlikely that the observed slight differences in determined energy and/or energy to protein ratios were responsible for the observed variations in feed intake of birds. The observed differences in feed intake strongly support this even among treatments with similar energy and energy protein ratios (T250, T750). Therefore, the observed variations in feed intake suggest that other factors could be responsible for the observed differences in feed intake of birds. The reduction in feed intake with increased substitution FM was attributed to the increase in dietary crude fiber content of the diets (Melesse et al., 2013; Walugembe et al., 2015; Oloruntola, 2018). Connectedly, Chiesa, and Gnansounou. (2011) reported that the green chloroplastic fraction of plant protein has an extremely limited digestibility of less than 500 g/kg DM due to association with indigestible components. High levels of dietary fiber above 100 g/kg in broiler diets have been reported to result in gut fill, which in turn lowers feed intake (Hetland et al., 2002). The reduction in weight gain with substitution was attributed to the reduced feed intake and lower nutrient digestibility of the diet with increasing substitution with NGP. Also, the low quality of NGP compared to FM (Beski et al., 2015), could have contributed to the observed reduction in feed intake and the resultant weight gain. Methionine, a largely deficient amino acid in plant protein, influences feed intake and gain in chickens (Hashemi et al., 2014), and its deficiency results in reduced feed intake and weight gain (Hashemi et al., 2014). This is further supported by the fact that although birds fed up to 500 g/kg NGP had comparable CP digestibility, the weight gains of the birds significantly differed. Moreover, when birds were switched to a fish meal-based diet, their feed intake and weight gain significantly increased, suggesting compensatory growth. This compensatory growth was also observed by Jariyahatthakij et al. (2018).

Birds on the control diet and those fed up to 500 g/kg NGP in the starter phase, had comparable weight gain in the finisher phase due to better feed utilization efficiency when birds were switched to a conventional fishmeal based diet. This compensatory growth rate in birds could be due to the superior quality of fish protein and, thus, a better protein utilization efficiency. Fishmeal protein is rich in key limiting essential amino acids like methionine and lysine (Beski et al., 2015).

Carcass weight of birds reduced with increasing substitution with NGP in broiler diets due to low feed intake and poor feed digestibility. For all the different carcass parts, the reduction in weight of the carcass parts is attributable to reduced feed intake and lower conversion and deposition of nutrients in the diet into muscle mass. In broiler diets, energy and protein ratio is a critical consideration to optimize the conversion of nutrients in muscle. However, due to the low ME and CP digestibility, it is plausible that although the required energy: protein ratio based on calculated figures was met, the observed poor feed conversion especially where the substitution exceeded 500 g/kg was possibly due to imbalance in the amino acid profile particularly methionine and lysine which are needed for meat yield (Mateos et al., 2014; Jariyahatthakij et al., 2018). Indeed Tesseraud et al. (1999) showed that deficiency of lysine results in a 50–55% reduction in weight of the breast and 34–45% in leg muscles in chicken. Furthermore, Tesseraud et al. (1998) demonstrated that deficiency of essential amino acids like lysine is responsible for poor muscle growth and protein turn over in breast and leg muscles. As a percent of the hot carcass, the weight of the back and neck increased with substitution. This indicates that while nutrient availability may be limiting in the diet of birds, there is a tendency to prioritize the development of the skeleton and less on the deposition of muscle. The crude fat content of both drumstick and breast muscle decreased with increasing NGP in broiler diets, possibly because NGP has a very low-fat content compared with fishmeal and thus the inverse relationship between carcass fat content and NGP substitution. This indicates that where nutrients are limiting, the tendency is to divert nutrients to grow important muscles like those for locomotion while deposit less in breast muscle.

The functionality of the gastrointestinal tract of birds is influenced by diet (Svihus, 2014). The weight of internal organs was relatively higher for birds in which NGP was added. In poultry, the proventriculus and gizzard are considered the stomach compartment of the GIT (Svihus, 2014). Birds on the control and 250 g/kg NGP diets had significantly lighter gizzards, proventriculus, caeca, and pancreas compared with higher NGP substitution levels possibly because with NGP above 500 g/kg had significantly heavier gizzard, proventriculus, caeca and pancreas possibly because these organs are involved in fiber digestion in poultry. This finding is consistent with earlier reports that birds fed high fiber diets had significantly longer and expanded the gastrointestinal tract compared with birds on conventional low fiber diets (Jørgensen et al., 1996; Mpofu et al., 2016). Meanwhile, research has shown that mean retention time, and rate of passage of feed in the GIT of birds is influenced by the nature and composition of the diet (Jørgensen et al., 1996; Svihus, 2014). Highly digestible low fiber diets have a shorter retention time in the gizzard and the caeca compared with high fiber diets. Indeed, high fiber diets have been reported to result in autrophy of the gizzard in poultry (Oloruntola, 2018). Furthermore, studies have shown that the gizzard may double in size when birds are fed high fiber compared to low fiber diets (Amerah et al., 2008). The increase in the size of the gizzard is a strategy to increase the retention time, increase the size reduction of particles, and consequently improve nutrient utilization of the feed (Svihus, 2014). Likewise, the heavier caeca are an adaptation to optimize fiber fermentation and nutrient absorption from the feed before it is passed out as excreta (Svihus, 2014).

The similarity in the relative weight of internal organs between the control group and 250 g/kg NGP indicates that at 250 g/kg NGP level of substitution, the fiber content of the diet was not a limiting factor compared with higher substitution levels. Likewise, the significant increase in the relative weight of the pancreas with NGP substitution at levels above 250 g/kg may probably indicate an increase in the level of secretion of pancreatic amylase and protease enzymes (Gracia et al., 2003) which are required for the digestion of carbohydrates and proteins in the diet, respectively.

Conclusion

Substitution of fishmeal with NGP lowered feed intake, feed use efficiency, and carcass yield of broiler birds. Based on the a priori set substitution levels, the best level in broiler starter feed would be 250 g/kg. However, orthogonal polynomial regression analysis revealed that the optimal substitution levels of fishmeal with NGP in starter diets of broilers without compromising the feed use efficiency is approximately 150 g/kg. However, when birds started on NGP were provided a conventional finisher diet, showed potential for compensatory. Nonetheless, there is a need to establish the amino acid composition of the NGP to facilitate adequate supplementation from synthetic amino acid sources. Therefore, these results suggest that NGP should be sparingly used as a substitute for fishmeal and that its use can be applied as a strategy for restriction in broiler diets during the starter phase. Our findings in the study warrant the need to investigate the effect of supplementation of NGP diet with synthetic methionine, lysine, and histidine as well as the use of exogenous enzymes on nutrient digestibility and performance and carcass quality.

Declarations

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Data availability: Data associated with this article are not publicly available due to pending intellectual property application, but can be made available upon resealable request.

Ethics approval: This study was performed in line with the provisions of section 12 of the Uganda animals (preventions and cruelty) act (ULII, 2000).

References

1. Agbede, J.O. and Aletor, V.A., 2003. Evaluation of fish meal replaced with leaf protein concentrate from Gliricidia in diets for broiler - Chicks: Effect on performance, muscle growth, haematology and serum metabolites International Journal of Poultry Science, 2, 242–250
2. Ahmed, A., Zulkifli, I., Farjam, A.S., Abdullah, N., Liang, J.B. and Awad, E.A., 2014. Effect of solid state fermentation on nutrient content and ileal amino acids digestibility of canola meal in broiler chickens. Italian Journal of Animal Science, 13, 410–414
3. Amerah, A.M., Ravindran, V., Lentle, R.G. and Thomas, G.D., 2008. Influence of feed particle size on the performance, energy utilization, digestive tract development, and digesta parameters of broiler starters fed wheat- and cornbased diets Poultry Science, 87, 2320–2328
4. AOAC, 1990. Official Methods of Analysis, 15th ed In:, Official Methods of Analysis, (Association of Official Analytical Chemists: Washington, DC, USA)
5. Beski, S.S.M., Swick, R.A. and Iji, P.A., 2015. Specialized protein products in broiler chicken nutrition: A review 1, 47–53
6. Chiesa, S. and Gnansounou, E., 2011. Protein extraction from biomass in a bioethanol refinery – Possible dietary applications: Use as animal feed and potential extension to human consumption Bioresource Technology, 102, 427–436
7. Ferket, R.P. and Gernet, G.A., 2006. Factors That Affect Feed Intake of Meat Birds: A Review International Journal of Poultry Science, 5, 905–911
8. Gracia, M.I., Aranibar, M.J., Lazaro, R., Medel, P. and Mateos, G.G., 2003. α -Amylase Supplementation of Broiler Diets Based on Corn 1 Poultry Science, 82, 436–442
9. Hashemi, S.M., Loh, T.C., Foo, H.L., Zulkifli, I. and Bejo, M.H., 2014. Effects of putrescine supplementation on growth performance, blood lipids and immune response in broiler chickens fed methionine deficient diet Animal Feed Science and Technology, 194, 151–156
10. Hetland, H., Svihus, B. and Olaisen, V., 2002. Effect of feeding whole cereals on performance, startch digestibility and duodenal particle size distribution in broiler chickens British Poultry Science, 43, 416–423
11. Huis, A. Van, 2014. Potential of Insects as Food and Feed in Assuring Food Security Annual Review of Entomology, 58, 653–568
12. Jariyahatthakij, P., Chomtee, B., Poeikhampha, T., Loongyai, W. and Bunchasak, C., 2018. Methionine supplementation of low-protein diet and subsequent feeding of low-energy diet on the performance and blood chemical profile of broiler chickens Animal Production Science, 58, 878–885
13. Jørgensen, H., Zhao, X.-Q., Knudsen, K.E.B. and Eggum, O.B., 1996. The influence of dietary fibre source and level on the development of the gastrointestinal tract, digestibility and energy metabolism in broiler chickens British

Journal of Nutrition, 75, 379–395

14. Khusro, M., Andrew, N.R. and Nicholas, A., 2012. Insects as poultry feed: a scoping study for poultry production systems in Australia World's Poultry Science Journal, 68, 435–446
15. Knudsen, K.E.B., 1997. Carbohydrate and lignin contents of plant materials used in animal feeding. Animal Feed Science and Technology, 67, 319–338
16. Mateos, G.G., Mohiti-asli, M., Borda, E., Mirzaie, S. and Frikha, M., 2014. Effect of inclusion of porcine mucosa hydrolysate in diets varying in lysine content on growth performance and ileal histomorphology of broilers Animal Feed Science and Technology., 187, 53–60
17. Melesse, A., Getye, Y., Berihun, K. and Banerjee, S., 2013. Effect of feeding graded levels of Moringa stenopetala leaf meal on growth performance, carcass traits and some serum biochemical parameters of Koekoek chickens Livestock Science, 157, 498–505
18. Mpofu, D.A., Marume, U., Mlambo, V. and Hugo, A., 2016. The effects of Lippia javanica dietary inclusion on growth performance, carcass characteristics and fatty acid pro fi les of broiler chickens Animal Nutrition, 2, 160–167
19. Nampijja, Z., 2018. Effect of substituting fish meal (Rastrineobola argentea) protein with black soldier fly (Hermatia illucens) larvae meal protein on performance of broiler chickens (Makerere University)
20. NRC, 1994. Nutrient requirements of poultry, 9th Revise (National Academy Press, Washington DC, USA)
21. Oloruntola, O.D., 2018. Gliricidia Leaf Meal in Broiler Chickens Diet: Effects on Performance, Carcass, and Haemato-biochemical Parameters Journal of Applied Life Sciences International, 18, 1–9
22. Prayogi, H.S., 2011. The effect of earthworm meal supplementation in the diet on quail's growth performance in attempt to replace the usage of fish meal International Journal of Poultry Science, 10, 804–806
23. Pretorius, Q., 2011. The evaluation of larvae of Musca domestica (common house fly) as protein source for broiler production (Stellenbosch University)
24. SAS, 2003. Statistical Analysis Institute (SAS/STAT). User's Guide. Version 9.1. SAS institute Inc.
25. Schiavone, A., Cullere, M., De Marco, M., Meneguz, M., Biasato, I., Bergagna, S., Dezzutto, D., Gai, F., Dabbou, S., Gasco, L. and Zotte, A.D., 2017. Partial or total replacement of soybean oil by black soldier fly larvae (Hermetia illucens L.) fat in broiler diets: Effect on growth performances, feed-choice, blood traits, carcass characteristics and meat quality Italian Journal of Animal Science, 16, 93–100
26. Svihus, B., 2014. Function of the digestive system Journal of Applied Poultry Research, 23, 306–314
27. Tabata, E., Kashimura, A., Kikuchi, A., Masuda, H., Miyahara, R., Wakita, S., Ohno, M., Sakaguchi, M. and Sugahara, Y., 2018. Chitin digestibility is dependent on feeding behaviors, which determine acidic chitinase mRNA levels in mammalian and poultry stomachs Scientific Reports, 8, 1–11 (Springer US)
28. Tesseraud, S., Le Bihan-Duval, E., Peresson, R., Michel, J. and Chagneau, A.M., 1999. Response of chick lines selected on carcass quality to dietary lysine supply: Live performance and muscle development Poultry Science, 78, 80–84
29. ULII, 2000. The Animals (preventions and cruety) Act, (Uganda Legal Information Institute)
30. Veldkamp, T. and Bosch, G., 2015. Insects: a protein-rich feed ingredient in pig and poultry diets Animal Frontiers, 5, 45–50
31. Walugembe, M., Rothschild, M.F. and Persia, M.E., 2015. Effects of high fiber ingredients on the performance, metabolizable energy and fiber digestibility of broiler and layer chicks Animal Feed Science and Technology, 188, 46–52

Figures

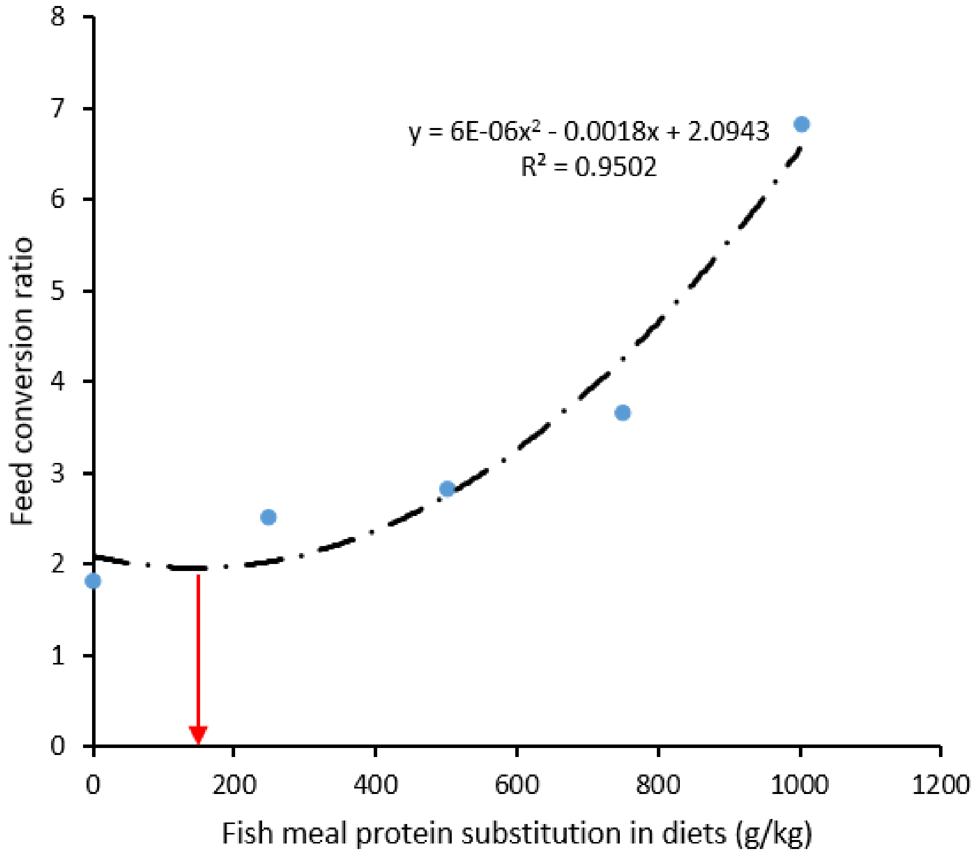

Figure 1

Feed conversion ratio data fitted with polynomial regressions (to the 2nd order) fitted to determine optimal response points