

Substituting Fishmeal with Napier Grass Protein: Effects on Intake, Growth Performance and Carcass Characteristics of Broiler Chickens

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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 g/kg, 250 g/kg, 500 g/kg, 750 g/kg, 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 a percentage of the hot carcass, the weight of the 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, the percentage change in weight gain in the finisher phase was influenced ($P < 0.05$) by the 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.

Keywords: Carcass quality, forage biorefinery, green protein, ileal digestibility.

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1. INTRODUCTION

Protein remains a major nutrient influencing the production, productivity, and profitability of poultry production enterprises [1]. The use of soybean and silverfish presents challenges, including competition with humans for food, contamination and or adulteration, unsustainable depletion of fish stocks, and high prices [2]. Alternative protein sources are being explored, including the use of insect meals like black soldier larvae [3], housefly larvae [4], and earthworms [5]. However, the use of insect meals in poultry diets is limited by low throughput of insect rearing technologies and facilities [6], ethical consumer considerations [7], high chitin and fat contents of insect meals [2], [6]–[8]. The high fat content lowers feed intake, while the chitin digestibility lowers feed digestibility [9].

Elsewhere, research efforts have assessed the potential to produce 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 [10]. 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 g/kg DM 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 g/kg DM and 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 the 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 and processed, and proteins were 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 I.

2.2. Formulation of Experimental Diets

Five iso-nitrogenous and iso-caloric diets were formulated (Table II) to meet the nutritional requirements of

TABLE I: 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

broiler chickens as recommended by NRC [11] 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 mash to minimize wastage and to encourage a high feed intake of the experimental diets [12].

Treatment diets T250, T500, T750, and T1000 were constituted by substituting 250 g/kg, 500 g/kg, 750 g/kg, 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 III) was done according to the Association of Official Analytical Chemists standards [13].

2.3. Birds and Experimental Design

Three hundred Cobb 500 days old chicks with an average initial weight of 42.43 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-to-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 24-hour 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

TABLE II: 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 III: 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

by weighing each bird per cage using a digital balance. From this data, average daily feed intake, body weight gain, and 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 [14]. 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 (TiO₂) 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. Proximate analysis following standard methods of the AOAC [13] 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 day 28, six birds (two 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 [14], 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 a 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 the breasts and thighs of each of the slaughtered birds 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 three days [13], 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 [13], respectively. The Metabolizable energy (ME) of the samples was calculated using the [11].

2.7. Statistical Analysis

Data collected on feed intake, body weight, and weight gain were analyzed using PROC GLM for repeated measures of SAS [15], while data on FCR, PER, EER, and ileal digestibility was subjected to analysis of variance (ANOVA) using PROC GLM of SAS [15]. For significant

TABLE IV: GROWTH, FEED UTILIZATION, AND NUTRIENT DIGESTIBILITY OF BIRDS IN THE STARTER PHASE (0 DAY–28 DAY)

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	<0.0001	<0.0001	<0.0001
Weight gain (g)	992.30 ^a	505.97 ^b	395.68 ^c	240.06 ^d	85.72 ^e	15.156	<0.0001	<0.0001	<0.0001
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	<0.0001	<0.0001	<0.0001
	<i>Feed utilization efficiency</i>								
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	<0.0001	<0.0001	<0.0001
EER	16.33 ^a	11.45 ^b	10.38 ^c	8.02 ^d	4.75 ^e	1.032	<0.0001	<0.0001	<0.0001
	<i>Nutrient digestibility</i>								
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

Note: ^{a,b,c,d} means with different superscripts in the row differ significantly ($P \leq 0.05$). FCR means feed conversion ratio, PER means protein efficiency ration, ME means metabolizable energy, EER means energy efficiency, and ns means not significant, and nd means not determined.

effects, LSMEANS of parameters were separated using the PDIFF option of SAS [15]. 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 was the effect of the i^{th} substitution level, and e_{ij} was the error term. The linear and quadratic effects of the inclusion of NGP ($i = 0$ g/kg, 250 g/kg, 500 g/kg, 75 g/kg, 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) to represent treatment response, and the regression equation was used to determine the optimal response point.

3. RESULTS

3.1. Growth Performance and Feed Utilization Efficiency of Birds on Starter Diets

Growth performance parameters of broiler chickens in the starter phase (Table IV) 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.

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 levels 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.

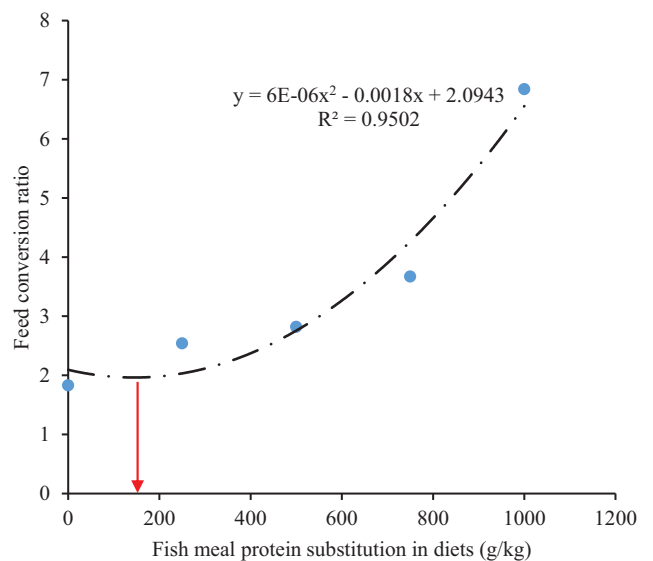


Fig. 1. Feed conversion ratio data fitted with polynomial regressions (to the 2nd order) fitted to determine optimal response points.

3.2. Nutrient Digestibility in Starter Diets

Dry matter digestibility of experimental diets varied ($P < 0.05$) with the 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 the 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.

3.3. 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

TABLE V: GROWTH AND FEED UTILIZATION OF BIRDS FED STARTER DIETS AND FINISHED ON A CONVENTIONAL DIET IN THE FINISHER PHASE (29 DAY TO 42 DAY)

Parameter	Diets (g/kg of FM substitution)					SEM	P-value		
	0	250	500	750	1000		Overall	Lin	Quad
<i>Growth performance</i>									
Initial weight (g)	1034.62 ^a	548.40 ^b	438.23 ^c	282.49 ^d	128.14 ^e	15.288	<0.0001	<0.0001	<0.0001
Final weight (g)	1746.11 ^a	1284.75 ^b	1103.52 ^c	772.24 ^d	452.54 ^e	18.446	<0.0001	<0.0001	<0.0001
Weight gain (g)	705.19 ^a	736.53 ^a	671.99 ^a	489.93 ^b	323.66 ^c	14.930	<0.0001	ns	ns
Feed intake (g)	1882.80 ^a	1679.89 ^{ab}	1518.52 ^b	1137.06 ^c	730.97 ^d	53.131	<0.0001	0.0025	ns
<i>Feed utilization efficiency</i>									
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

Note: ^{a,b,c,d} means with different superscripts in the row differ significantly ($P \leq 0.05$). FCR means feed conversion ratio, PER means protein efficiency ratio EER means energy efficiency ratio, and ns means not significant.

TABLE VI: CARCASS PARTS AS A PERCENTAGE OF HOT CARCASS WEIGHT OF EXPERIMENTAL BIRDS IN THE 28 DAY 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

Note: ^{a,b,c,d} means with different superscripts in the row differ significantly ($P \leq 0.05$).

was introduced during the finishing phase (Table V). 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.

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 V). 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.

3.4. Yield of Carcass Quality and Yield Characteristics

The dressing percentage and yield of broiler carcass parts expressed as a percentage of the hot carcass were significantly ($P < 0.05$) affected by the level of FM substitution in poultry diets (Table VI). The highest carcasses yield was observed in T0 birds, while the lightest carcasses were observed in T1000 birds. The weight of the 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.

3.5. 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 VII. 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.

3.6. 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 VIII. The weight of heads increased ($P < 0.001$) at an increasing rate with an increase in the substitution of FM with NGP in broiler starter diets. However, no differences ($P > 0.05$) were observed in the relative weight of the 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

TABLE VII: EFFECT OF STARTER DIETS ON NUTRIENT COMPOSITION (G/KG DM) OF DRUMSTICK AND BREAST MUSCLE OF BIRDS AT THE END OF THE 28 DAY STARTER PHASE

Nutrient, g/kg DM	Diets (g/kg of FM substitution)					SEM	P-value
	0	250	500	750	1000		
	<i>Drumsticks</i>						
CP	750.6 ^d	780.4 ^c	792.230 ^c	824.2 ^b	846.5 ^a	8.92	<0.0001
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
	<i>Breast</i>						
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

Note: ^{a,b,c} Means with different superscripts in a row column differ significantly ($P \leq 0.05$).

TABLE VIII: WEIGHTS OF INTERNAL ORGANS EXPRESSED AS A PERCENTAGE OF THE FINAL LIVE WEIGHT OF BIRDS SLAUGHTERED AT THE END OF THE 28 DAY STARTER PHASE

Organs weight (%)	Diets (g/kg of FM substitution)					SEM	P-value		
	0	250	500	750	1000		Overall	Lin	Quad
	<i>External organs</i>								
Head	2.63 ^c	3.03 ^c	4.01 ^b	4.52 ^b	7.71 ^a	0.345	<0.0001	0.9165	0.0003
Feet	3.83	3.75	4.19	4.09	3.6823	0.270	0.6076	ns	ns
	<i>Internal organs</i>								
Visceral	15.84 ^d	17.89 ^d	22.32 ^c	26.18 ^b	32.66 ^a	1.200	<0.0001	0.0037	0.0861
Empty gizzard	2.39 ^d	3.02 ^d	3.75 ^c	4.65 ^b	6.21 ^a	0.267	<0.0001	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

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

significant differences ($P > 0.05$) were observed between T0 and T250 birds.

4. DISCUSSION

Non-ruminants are less efficient at utilizing protein from forage-based sources due to the lack of appropriate microbes and enzymes in their digestive tract that can ferment and digest fiber [16]. 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 [17], 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 [18]–[20]. Connectedly, Chiesa and Gnansounou [21] reported that the green chloroplast fraction of plant protein has an extremely limited digestibility of less than 500 g/kg DM due to its 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 [22]. 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 [1] 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 [23], and its deficiency results in reduced feed intake and weight gain [23]. 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.* [24].

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 [1]. 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 [24], [25]. Indeed, Tesseraud *et al.* [26] showed that deficiency of lysine results in a 50%–55% reduction in breast weight and 34%–45% reduction in leg muscles in chickens. Furthermore, Tesseraud *et al.* [26] demonstrated that a deficiency of essential amino acids like lysine is responsible for poor muscle growth and protein turnover 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 [12]. 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 compartments of the GIT [12]. 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 [27], [28]. Meanwhile, research has shown that mean retention time and rate of passage of feed in the GIT of birds are influenced by the nature and composition

of the diet [12], [27]. 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 atrophy of the gizzard in poultry [20]. Furthermore, studies have shown that the gizzard may double in size when birds are fed high-fiber compared to low-fiber diets [29]. 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 [12]. Likewise, the heavier caeca are an adaptation to optimize fiber fermentation and nutrient absorption from the feed before it is passed out as excreta [12].

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 [30], which are required for the digestion of carbohydrates and proteins in the diet, respectively.

5. 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, they were provided a conventional finisher diet and showed potential for compensation. 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.

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AUTHOR CONTRIBUTIONS

Study conceptualization, Alexander Bombom and Swidiq Mugerwa; methodology, Zainah Nampijja, Muhammad Kiggundu, Abasi Kigozi; formal analysis, Zainah Nampijja, Muhammad Kiggundu; investigation, Zainah Nampijja, Muhammad Kiggundu, and Abasi Kigozi; resources, Alexander Bombom and Swidiq Mugerwa; data curation, Abasi Kigozi, Muhammad Kiggundu, Zainah Nampijja; writing—original draft preparation, Muhammad Kiggundu, Zainah Nampijja, and Abasi Kigozi; writing—review and editing, Muhammad Kiggundu, Zainah Nampijja, Abasi Kigozi, Swidiq Mugerwa, Allen Molly, Clementine Namazzi, Siraj Ismail Kayondo, Walugembe Faitwa, and Alexander Bombom; supervision, Zainah Nampijja, Abasi Kigozi; project administration, Alexander Bombom, and Swidiq Mugerwa; funding acquisition, Alexander Bombom, and Swidiq Mugerwa.

DATA AVAILABILITY

Data associated with this article are not publicly available due to pending intellectual property applications 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 [31].

COMPETING INTEREST

The authors declare that they have no competing interests associated to this work.

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