Received: 7 August 2019 | Revised: 14 November 2019 | Accepted: 7 December 2019

DOI: 10.1111/are.14464

ORIGINAL ARTICLE

Aquaculture Research WILEY

The effect of plant protein-based diets on apparent nutrient digestibility, growth response, egesta quantity, postprandial ammonia excretion rate and serum quality of Nile tilapia

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Funding information
Danida Fellowship Centre, Grant/Award Number: 13-PO1-GHA

Abstract

The study assessed the effect of oilseed meal mixtures on the biological value, faecal matter output, ammonia excretion rates and serum biochemistry of Nile tilapia over 63 days. The experimental diets (PPB 1, PPB 2 and PPB 3) were formulated using four selected oilseed meals that were mixed in different protein proportions to contribute 80% of total dietary crude protein. In each diet, either one or two of the oilseed meals were the dominant protein contributor. A commercial tilapia feed was used as the control diet (CTRL). Sex-reversed Nile tilapia fingerlings (35 g) were stocked at 20 fish per tank in a recirculation system and fed at 3% body weight of their respective diets. At the end of the study, ADCs of dry matter, crude lipid and ash were significantly ($p < .05$) lower in all the plant-based diets compared with the control diet. Fish fed the control diet had significantly higher weight gain and SGR compared with the plant-based diets. Egesta output was 127% higher in the plant-based diets compared with the control diet. All dietary treatments exhibited a similar trend in changes in ammonia nitrogen. Nevertheless, serum metabolites levels indicated no significant differences among treatments. Although the PPBs did not affect ADC of protein and serum profile, fish growth was reduced while faecal output increased.

KEYWORDS

biological value, Oilseed meal, *Oreochromis niloticus*, serum biochemistry, TAN excretion

1 | INTRODUCTION

Aquaculture currently contributes 47% of global food fish production, and the trend is expected to increase owing to the stagnation of yields from inland and marine fisheries. Moreover, the importance of aquaculture to global food security, human nutrition and health, and employment cannot be overemphasized (FAO, 2018a). Nonetheless, the sustainability of the aquaculture industry is threatened by the relatively expensive fish feeds due to the limited supply and high cost of fishmeal (FM)—the primary protein source in fish feeds (Tacon & Metian, 2008). This requires immediate attention if the UN sustainable development goals 1, 2 and 14 are to be achieved, that is reducing hunger, food insecurity, malnutrition and poverty while protecting life below water. This challenge has pioneered research into low-cost alternative ingredients that could either partially or completely replace FM in fish feeds. This study utilized soya bean meal (SBM), copra meal (CM), groundnut meal (GNM) and cottonseed meal (CSM) because they are among the potential plant protein ingredients that have been identified due to their availability in large quantities (Gabriel, Akinrotimi, Bekibele, Onunkwo, & Anyanwu, 2007; Obirikorang, Amisah, Fialor, & Skov, 2015a; Wang, Wang, Zhang, & Song, 2017), have relatively good nutritional quality, palatable and unlimited supply and are less expensive (Agbo, 2008) compared with fishmeal. Studies have indicated the

replacement of fishmeal with plant proteins and recommended the utilization of plant protein mixtures over a single plant protein ingredient due to the ability of the different ingredients to compensate for essential amino acid deficiencies and avoidance of a high inclusion level of any single anti-nutritional factor (Agbo, Madalla, & Jauncey, 2015; Borgeson, Racz, Wilkie, White, & Drew, 2006; De Silva & Anderson, 1995; Larsen, Dalsgaard, & Pedersen, 2012). In this regard, some studies have affirmed that the feeding of mixtures of different plant proteins in diets to fish had comparable growth performance to a fishmeal-based diet and further stated that using plant protein mixtures could significantly reduce or completely replace fishmeal in fish feeds (Aoki, Watanabe, Furuichi, & Tsuda, 1997; Burr, Wolters, Barrows, & Hardy, 2012; Olukunle, 1982). This has been established especially for tertiary consumers like the Nile tilapia that feed efficiently at low trophic levels (Tacon, 1994) and has a fast growth rate, relatively cheap and an important source of sustainable protein for human consumption (El-Sayed, 2006). As a result, Nile tilapia is ranked second to carps as the most cultured species worldwide. Globally, in 2016, total production of the tilapias was reported to be approximately 5.9 million metric tonnes with a farm gate value of US$ 11.3 billion (FAO, 2018b). Furthermore, Tacon (2018) estimated growth rates of 6% and 5% corresponding to total productions of 7.4 and 9.5 million metric tonnes by 2020 and 2025 respectively. Subsequently, 11.3 and 15.2 million metric tonnes of feed inputs would be required to achieve the target production levels (Tacon, 2018).

Nonetheless, López (1997) and Ip and Chew (2010) indicated that feed consumed by fish is partly assimilated and between 40% and 60% of nitrogen intake is excreted as ammonia within 24 hr postprandial. With the anticipated rise in feed input in fish farming, a corresponding increase in pressure on both the culture environment and receiving waters from nutrient inputs is expected. Increased waste production in the form of ammonia and phosphorus can lead to poor water quality resulting in depressed fish growth and mass mortality in the event of ammonia toxicity (Ip & Chew, 2010). Additionally, excessive input of faecal matter may raise the levels of suspended solids which may lead to eutrophication through nutrient leaching and anoxic conditions from high oxygen demand affecting the general health of the culture environment and the receiving aquatic environment (López, 1997). With the quest to develop sustainable, low-cost and quality fish feeds to meet the growing demand of global aquaculture, it is important that this is done in a way that does not compromise fish and environmental health.

Until now, it is less clear how mixtures of plant proteins in a single diet affect both the culture and receiving environments, and fish health in terms of ammonia excretion rates, faecal matter output and serum biochemistry. This study therefore determined the effect of almost total replacement of fishmeal with dietary protein blend diets of soya bean meal, cottonseed meal, groundnut meal and copra meal on apparent nutrient digestibility, growth performance, faecal matter output, ammonia excretion rates and the serum metabolite profile of the Nile tilapia (*Oreochromis niloticus*).

2 | MATERIALS AND METHODS

2.1 | Ethical statement

All fish handling and husbandry procedures were in compliance with the 'Guide for care and use of laboratory animals' by the USA National Research Council (NRC, 2011).

2.2 | Experimental ingredients, diets formulation and preparation

Mechanically extracted groundnut meal (GNM), solvent-extracted cottonseed meal (CSM), screw-pressed soya bean meal (SBM) and copra meal (CM) were the main dietary protein sources; wheat bran (WB) and tuna fishmeal (FM) were procured from commercial sources in Kumasi, Ghana. Table 1 summarizes the chemical composition of the basic ingredients used in diet formulation and preparation. Nitrogen-free extract and gross energy contents of the ingredients and diets were calculated according NRC (1993).

TABLE 1 Proximate composition (% as fed) of basal ingredients used in the formulation and preparation of experimental diets

	Ingredients					
Proximate composition	**FM**	**CSM**	**GNM**	**SBM**	**CM**	**WB**
Dry matter	90.17 ± 0.24	90.35 ± 0.07	89.75 ± 0.35	90.50 ± 0.00	88.00 ± 0.00	90.50 ± 0.71
Crude protein	57.60 ± 0.40	37.90 ± 0.57	42.46 ± 0.31	44.92 ± 0.00	20.42 ± 0.00	16.19 ± 0.00
Crude lipid	10.25 ± 0.35	9.53 ± 0.04	18.00 ± 1.41	9.25 ± 0.35	13.00 ± 0.71	4.50 ± 0.71
Ash	18.10 ± 0.14	7.28 ± 0.06	2.17 ± 0.23	7.00 ± 0.47	8.33 ± 0.47	4.33 ± 0.00
Crude fibre	1.05 ± 0.64	11.35 ± 1.06	3.26 ± 0.62	4.80 ± 0.55	8.16 ± 174	5.82 ± 1.15
NFE[a]	4.41 ± 0.40	35.65 ± 0.73	23.66 ± 0.09	29.19 ± 1.45	46.11 ± 1.17	65.48 ± 1.41

Note: Values are means (±*SD*) of duplicate samples.

Abbreviations: CM, copra meal; CSM, cottonseed meal; FM, fishmeal; GNM, groundnut meal; NFE, nitrogen-free extract; SBM, soya bean meal; WB, wheat bran.

[a]NFE calculated as: 100 − (CP + CL + Ash + CF + Moisture).

In the formulation of the diets, GNM, CSM, CM and SBM were blended at different proportions to contribute a minimum total of 80% combined protein relative to the dietary protein content. In the first plant protein blend (PPB 1) diet, SBM was the highest protein contributor whereas in PPB 2 both CM and SBM contributed equal proportions of protein. In PPB 3, all dietary protein was supplied by SBM (Table 2). All the PPBs together in each diet represented about 24% of the target dietary protein content. The control diet (CTRL) was a commonly used commercial diet which according to the manufacturer's label contains 'products and by-products of poultry, oilseeds and cereals, vitamins and minerals'.

All experimental diets were formulated to contain similar quantity of protein and lipid (Table 3) and to meet the nutritional requirements (Kamal, Rosentrater, & Brown, 2010; NRC, 1993) of Nile tilapia juveniles using formulation software (WINFEED v. 2.8, WinFeed Ltd.). The ingredients were weighed and mixed in a kitchen food mixer (LINEA PLU, GTM-6118, Coop A/S, Denmark) for 20 min. The diets were extruded between 90 and 127°C (extruder barrel temperature) and a screw speed of 336 x*g* using a twin-screw extruder (CLEXTRAL, BC 21, Firminy, France) fitted with a 3.0 mm die. The extruded diets were oven-dried at 60°C for 24 hr, bagged in airtight Ziploc® bags and stored in a freezer until feeding commenced.

2.3 | Experimental system and source of fish

To ensure minimum pain, distress and suffering, fish handling and husbandry were conducted according to the procedure outlined in the American Fisheries Society's guidelines for the use of fishes in research (AFS, 2004). The experiment was conducted at the Faculty of Renewable Natural Resources fish farm located on the campus of Kwame Nkrumah University of Science and Technology for a period of 63 days in a modified recirculation aquaculture system made up of twelve 150 L cylindro-conical plastic tanks. The sex-reversed Nile tilapia fingerlings used for the study were obtained at 1-g size from a commercial supplier and nursed in an earthen pond. The fry were fed with a commercial Tilapia starter feed containing 48% crude protein for 2 months before the start of the experiment. At the onset of the study, 240 tilapia fingerlings of mean weight (35.0 ± 0.23 g) were stocked at 20 individuals per tank and acclimatized to the tank environment for 1 week. Throughout the study, fish were exposed to a 12-hr light and 12-hr darkness regime using preset 7 W light bulbs overhanging each tank.

TABLE 2 Percentage contribution of plant protein sources in experimental diets

Experimental diet	FM	GNM	CSM	CM	SBM	Total
PPB 1	0	5	15	25	35	80
PPB 2	0	10	10	30	30	80
PPB 3	0	0	0	0	80	80

Abbreviations: CM, copra meal; CSM, cottonseed meal; FM, fishmeal; GNM, groundnut meal; SBM, soya bean meal.

TABLE 3 Feed formulation (g/kg), proximate composition (%) and gross energy (MJ/kg) contents of the experimental diets

	Experimental diet			
Ingredients	CTRL	PPB 1	PPB 2	PPB 3
Fishmeal		63.10	69.50	0
Wheat bran		108.80	81.00	367.40
Cottonseed meal		106.60	74.50	0
Groundnut meal		33.10	63.80	0
Soya bean meal		235.10	203.5	532.6
Copra meal		370.00	428.10	0
Cassava flour (binder)		54.90	54.60	61.5
Soya bean oil		24.90	21.50	35.0
Vitamin and Mineral premix		3.50	3.50	3.50
Proximate composition				
Dry matter	89.11	89.19	89.12	88.66
Crude protein	29.89	31.20	31.58	30.88
Crude lipid	6.35	10.07	10.86	11.39
Ash	7.99	12.04	11.90	13.15
Crude fibre	2.00	6.17	11.53	4.69
NFE[a]	42.88	31.70	30.14	33.11
Gross energy[b]	17.33	17.50	17.80	17.54

Note: CTRL = Control, contains products and by-products of poultry, oilseeds and cereals, vitamins and minerals, according to the manufacturer's label.

[a]Nitrogen-free extract (NFE), calculated as: 100 − (crude protein% + crude lipid% + Ash% + Moisture%).

[b]Gross energy, calculated as: crude protein% × 23.6 + crude lipid% × 39.5 + Nitrogen-free extract (NFE) × 17.2 (NRC, 1993).

2.4 | Experimental design, feeding and faecal matter estimation

The completely randomized design was used in the study where the four dietary treatments were randomly assigned to the tanks in triplicate. Fish were fed twice daily at 9:00 a.m. and 4:00 p.m. at 3% of body weight. Feeding was carefully done to avoid feed wastage, and any uneaten pellets were removed 5 min after feeding. Faeces collection for faecal matter estimation followed the procedure described in Obirikorang, Amisah, Fialor, and Skov (2015b) commenced at the onset of the growth experiment and lasted for 6 days. During this period, faeces produced were allowed to settle in a sedimentation column beneath each tank submerged in ice flakes kept in a styrofoam box to ensure minimal microbial degradation of the collected

faecal matter. All faeces produced were collected into 1.5-L sampling bottles every morning at 8:00 a.m. daily before the next feeding commenced. All collected samples were kept frozen until the end of the collection period where frozen samples were thawed to room temperature, poured into 2-L beakers and allowed to stand for an hour before excess water was decanted. Samples were oven-dried at 105°C for 24 hr. Faecal matter load was expressed as percentage faecal matter relative to the amount of feed consumed within that period. This is given as;

$$\text{Faecal matter production}(\%) = \frac{\text{Dry weight of faecal matter (g)}}{\text{Weight of feed ingested (g)}} \times 100$$

2.5 | Faecal collection for digestibility assessment

Immediately after the faecal collection for digestibility study by the direct method (Jobling, 1994) was carried out similar to how the collection was done for the egesta estimation; however, in this case, the faeces collection from each tank s done for 9 days but pooled in 3-day intervals as described previously in Larsen et al. (2012) and kept frozen until needed for analysis. Faeces were collected prior to feeding each morning (8:00 a.m.) into 1.5-L sampling bottles. Approximately 15 min after feeding, all faeces collection columns were inspected to ensure that no uneaten feed pellets remained in the sedimentation column. Total uneaten pellets were quantified by multiplying the average weight of a pellet by the number of uneaten pellets.

2.6 | Total ammonia nitrogen analysis

Total ammonia nitrogen (TAN) determination followed the procedure of Obirikorang, Amisah, Fialor, and Skov (2015c) immediately after faecal collection for digestibility. Before TAN sampling, fish were starved for 24 hr to allow complete emptying of the gastro-intestinal tract and all the tanks were thoroughly cleaned to eliminate faecal and feed traces. Water flow to the tanks was stopped by means of an angle seat valve to prevent water exchange. Each dietary treatment was administered to their respective tanks once at 2% of the total biomass. Water samples for TAN estimation were taken at twelve different time periods at 0, 1, 2, 3, 5, 7, 9, 12, 15, 18, 21 and 24 hr postprandial. Total ammonia nitrogen concentrations were determined spectrophotometrically (HACH Lange DR 3900, Germany) according to the specifications of ISO.11905-1997(E) at 680 nm in duplicate before applying the static system formula to calculate for TAN excretion rate for each treatment group:

$$\text{TAN}\left(\text{mg kg}^{-1}\,\text{hr}^{-1}\right) = \frac{(N_2 - N_1) \times V}{T \times M}$$

where N_2 and N_1 are final and initial TAN concentrations (mg/L) in the water; V is the volume of water (L) in the tank during the flux period; T is the time (hr) between the flux periods; and M is the bulk mass of fish (kg).

2.7 | Determination of fish growth and feed utilization

Feeding of fish for the growth experiment continued after the digestibility study until the end of the experiment. During this period, the bulk weight of fish from each tank was recorded with a digital scale (OHAUS Navigator XL, Ohaus Corporation) every 14 days to monitor fish growth and adjust feed quantities accordingly. All experimental tanks were cleaned at the end of every week and about 50% of the water in each tank replaced with fresh water. Mortalities during the experiment were recorded, and all dead fish were disposed by burying. Water quality variables were monitored weekly with a multi-parameter probe (HACH, Hach Lange GmbH, Düsseldorf, HQ40d, Germany), and the following mean measures were recorded: temperature: 27 ± 0.78°C, dissolved oxygen: 5.84 ± 0.79 mg/L, pH: 5.73 ± 0.43 and conductivity: 299.80 ± 65.94 μS/m. Growth performance and feed utilization were determined by the following formulae:

$$\text{Weight gain}(\%) = \frac{(\text{Final weight (g)} - \text{Initial weight (g)})}{\text{Initial body weight (g)}} \times 100$$

$$\text{Specific growth rate}\left(\%\,\text{day}^{-1}\right) = \frac{(\text{In Final weight (g)} - \text{In Initial weight (g)})}{\text{Experimental days}} \times 100$$

$$\text{Feed intake}\left(\text{g fish}^{-1}\right) = \frac{\text{Feed Consumed (g)}}{\text{Weight gain (g)}}$$

$$\text{Feed conversion ratio}\left(\text{FCR}\right) = \frac{\text{Feed fed (g)}}{\text{Weight gain (g)}}$$

$$\text{Protein efficiency ratio}\left(\text{PER}\right) = \frac{\text{Weight gain (g)}}{\text{Protein intake (g)}}$$

$$\text{Survival rate}\ (\%) = \frac{\text{Number of fish at the end of the experiment}}{\text{Number of fish at the start of the experiment}} \times 100$$

2.8 | Blood sampling for serum metabolite analysis

On the day of termination of the experiment, five fish from each tank were carefully sampled and anesthetized in aerated water containing 10 mg/L of propofol (PROFOL®, Claris Injectables Ltd). Two millilitres of blood samples were drawn twice from the caudal vein of each fish by puncture with a heparinized syringe into separate Vacutainer tubes (BECTON DICKINSON, Franklin Lakes, NJ, USA) without ethylenediaminetetraacetic acid (EDTA) as anticoagulant, for serum biochemical analysis. Haemolyzed or insufficient sample volumes were discarded. After sampling, fish were placed in tanks of aerated fresh water for recovery. The blood samples collected into the tubes without EDTA were allowed to clot for 10 min and centrifuged at 3,739 x*g* for 10 min to separate serum from cells. Photometric assays were immediately done on the serum samples using a spectrophotometer (JENWAY 6305, Cole Palmer, Staffordshire, UK) to determine glucose, albumin

and total cholesterol levels (Hrubec, Cardinale, & Smith, 2000). Sample concentrations for the serum biochemical parameters were determined using commercial diagnostic reagent kits (ELITech Diagnostics, ELITech Group, Puteaux, France), following the manufacturer's instructions. Globulins were calculated as the difference between total protein and albumin values (Hrubec et al., 2000).

2.9 | Proximate composition of experimental ingredients, diets fish and faeces samples

The dry matter, crude protein and ash contents of the experimental ingredients, diets and faecal matter were analysed using standard procedures of the Association of Official Analytical Chemists (AOAC, 2005): dry matter after oven-drying at 105°C for 24 hr; ash content was determined by incineration of the samples in a muffle furnace (NABERTHERM GmbH 30–3,000°C, Lilienthal, Germany) at 550°C for 6 hr; crude protein ($N \times 6.25$) by the micro Kjeldahl method digestion (FOSS Digestor 2520 Auto, Hillerød, Denmark), distillation (FOSS Kjeltec 8200, Hillerød, Denmark) and titration (Metrohm Autotitrator Ti-Touch 916, Herisau, Switzerland). Crude lipid in ingredients and diets were determined using the Soxhlet extraction method by petroleum ether whereas the procedure described by Bligh and Dyer (1959) was used for lipid extraction in wet faecal samples with chloroform and methanol. Nitrogen-free extract was calculated as the dry matter minus the sum of crude protein, crude lipid and ash (NRC, 1993).

2.10 | Data analysis

The D'Agostino–Pearson omnibus normality test was performed on data for each variable before the averages of the treatments were subjected to a one-way ANOVA at $p < .05$ before the differences between the treatments means were compared using Tukey multiple comparison test. Results of the effect of plant protein blends on nutrient digestibility, growth performance and feed utilization, and serum biochemistry of tilapia is given in tables as means with their standard deviation (*SD*) in whereas the growth curve as well as results for egesta quantity and total ammonia nitrogen excretion are presented in figures. All statistical analysis and graphs were executed using GraphPad Prism version 6.01 for Windows.

3 | RESULTS

3.1 | Apparent digestibility coefficient

The apparent digestibility coefficient (ADC) of dry matter, crude protein, lipid, ash and nitrogen-free extract are given in Table 4. At the end of the study, the ADCs of dry matter, crude lipid and ash were significantly ($p < .05$) lower in all the PPB diets compared with the control diet. Crude protein digestibility did not differ significantly between diets although; the control diet was approximately 3% more digestible than the PPB diets.

3.2 | Growth performance and feed utilization

The growth performances of *O. niloticus* fed the experimental diets are summarized in Table 5. Fish fed the control diet had higher ($p = .0005$) final body weight compared with the PPB diets. This resulted in the control fish group significantly ($p = .0024$) tripling their weight whereas fish that received the PPBs doubled their weights. In all, the specific growth rate, feed intake, feed conversion ratio and protein efficiency ratio were significantly ($p < .05$) lower in the plant protein blend diets compared with the control diet. Nonetheless, PPB 1, among the plant protein diets, recorded the best performance in all parameters assessed.

3.3 | Faecal matter production

Faecal matter production in fish fed the experimental diets is presented in Figure 1. At the end of the 6 days of collection period, faecal output relative to the ingested feed differed significantly ($p = .0002$) among the dietary treatments. The commercial control diet had the lowest faecal output (116.0 ± 4.31 g $DMkg^{-1}$ ingested feed) while the

TABLE 4 Mean ± *SD* of apparent digestibility coefficients (%) of nutrients of plant protein blend diets fed to Nile tilapia

	Experimental diet				
Parameter	CTRL	PPB 1	PPB 2	PPB 3	*p* value
Dry matter	89.23 ± 2.38[a]	83.27 ± 1.53[b]	82.63 ± 1.10[b]	83.40 ± 1.97[b]	.0066
Crude protein	86.90 ± 1.04	84.10 ± 0.79	84.43 ± 1.01	84.90 ± 1.90	.0968
Crude lipid	65.99 ± 10.26	63.92 ± 9.47	50.41 ± 6.65	47.02 ± 4.74	.0477
Ash	76.87 ± 1.98[a]	59.93 ± 4.16[b]	61.23 ± 2.06[b]	48.03 ± 2.26[c]	<.0001
NFE	82.36 ± 3.65	71.32 ± 11.52	64.15 ± 8.79	70.27 ± 6.08	.1203

[a,b,c]Mean values that do not share a common superscript letter within a row differ significantly ($p < .05$). Absence of superscript letters indicates no significant difference between treatments.

Abbreviations: CTRL, control; NFE, nitrogen-free extract; PPB, plant protein blend.

TABLE 5 Growth performance and feed utilization (mean ± *SD*) of Nile tilapia fed plant protein blend diets for 63 days

	Experimental diet				
Parameter	**CTRL**	**PPB 1**	**PPB 2**	**PPB 3**	***p* value**
IBW (g)	34.65 ± 3.61	34.47 ± 1.26	35.00 ± 1.97	34.85 ± 0.23	.9905
FBW (g)	92.73 ± 9.04^{a}	77.63 ± 1.51^{ab}	74.55 ± 3.87^{b}	74.29 ± 7.15^{b}	.0181
Weight gain (%)	167.93 ± 12.73^{a}	125.37 ± 6.53^{b}	113.16 ± 8.80^{b}	113.13 ± 19.75^{b}	.0024
SGR (% day^{-1})	1.56 ± 0.07^{a}	1.29 ± 0.04^{b}	1.20 ± 0.07^{b}	1.19 ± 0.15^{b}	.0040
FI (g/fish)	98.31 ± 7.47^{a}	86.01 ± 3.12^{b}	84.10 ± 2.84^{b}	82.71 ± 1.37^{b}	.0082
FCR	1.59 ± 0.11^{a}	2.33 ± 0.22^{b}	2.75 ± 0.78^{b}	2.51 ± 0.13^{b}	.0017
PER	2.05 ± 0.15^{a}	1.62 ± 0.17^{ab}	1.32 ± 0.33^{b}	1.39 ± 0.07^{b}	.0082
Survival rate (%)	96.67 ± 2.89	93.33 ± 5.77	90.00 ± 8.66	93.33 ± 5.77	.6371

^{a,b}Mean values with different superscript within a row differ significantly ($p < .05$). Absence of superscript letters indicates no significant difference between treatments.

Abbreviations: FBW, final body weight; IBW, initial body weight; PPB, plant protein blend.

highest faecal output (263.40 ± 10.91 g DMkg^{-1}) was recorded in PPB 2 which contained high but equal levels (30% protein contribution each) of copra meal and soya bean meal. It was observed that among the plant protein diets, PPB 3 which had SBM only as the sole protein source produced the least faecal matter. Overall, faecal matter output ranked as follows: CTRL < PPB 3 < PPB 1 < PPB 2.

3.4 | Ammonia excretion rate

At the end of the sampling period, although total TAN excretion rate was lower ($p < .05$) for the control diet compared with the plant protein diets, yet, all dietary treatments exhibited a similar trend in TAN excretion rates (Figure 2). Peak TAN excretion was observed for all dietary treatments between 5-hr and 7-hr postprandial and ranged from 10.67 ± 1.34 mg NH_3-Nkg^{-1} hr^{-1} for CTRL diet to 13.21 ± 0.67 mg NH_3-Nkg^{-1} hr^{-1} for PPB 2.

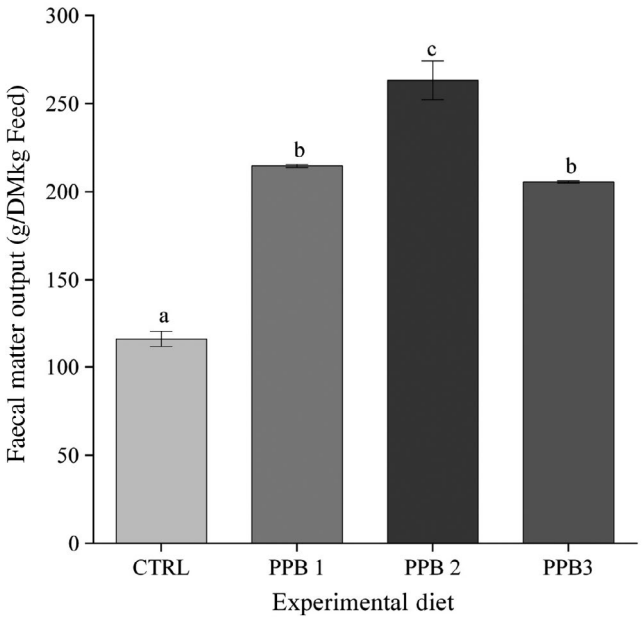

FIGURE 1 Faecal matter production (g DMkg^{-1}) of the Nile tilapia fed on plant protein blend (PPB) diets. ^{a,b}Bars with different superscript letter differ significantly ($p < .05$). Absence of superscript letters indicates no significant difference between treatments

3.5 | Serum biochemical parameters

Plasma metabolite profile in *Oreochromis niloticus* is summarized in Table 6. There were no differences ($p < .05$) in the serum biochemistry of fish fed the experimental diets. The highest total cholesterol (T. Cho) level (114.72 ± 1.22 mg/dl) was observed in the fish fed the control diet while fish fed PPB 3 recorded the lowest T. Cho level of 84.42 ± 0.71 mg/dl. Normal blood sugar levels (80–130 mg/dl) were recorded for fish in all dietary treatments with the most concentration observed in PPB 2 and the least in PPB 1. High-density lipoprotein cholesterol (HDL-Cho) and LDL-Cho were found to be averagely 22.13% and 55.34%, respectively,

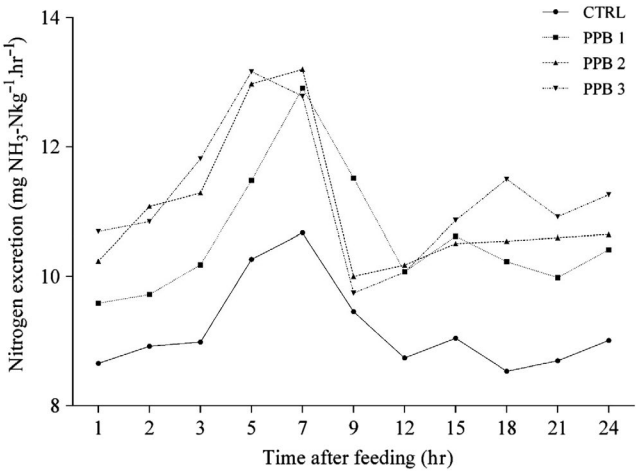

FIGURE 2 TAN excretion rates in Nile tilapia fed different plant protein blend (PPB) diets over 24-hr postprandial

TABLE 6 Mean ± *SD* of Serum biochemical parameters of Nile tilapia fed plant protein blend diets for 63 days

Parameter	CTRL	PPB 1	PPB 2	PPB 3	*p* value
Glucose (mg/dl)	82.71 ± 1.66	72.27 ± 1.42	83.13 ± 0.60	72.54 ± 1.61	.9087
Albumin (g/dl)	1.84 ± 0.41	1.80 ± 0.77	1.68 ± 0.34	1.81 ± 2.67	.5698
Globulin (g/dl)	1.54 ± 2.93	1.68 ± 2.89	1.29 ± 0.30	1.54 ± 4.18	.4716
A:G	1.23 ± 0.24	1.09 ± 0.14	1.31 ± 0.06	1.21 ± 0.16	.4647
TP (g/dl)	3.38 ± 3.19	3.48 ± 3.62	2.97 ± 0.11	3.35 ± 6.78	.4986
T. Cho (mg/dl)	114.72 ± 1.22	99.27 ± 0.76	86.10 ± 0.57	84.42 ± 0.71	.1294
HDL-Cho (mg/dl)	43.11 ± 0.56	34.44 ± 0.73	36.21 ± 0.22	35.16 ± 0.25	.6223
LDL-Cho (mg/dl)	41.67 ± 0.70	26.73 ± 0.42	27.30 ± 0.34	26.46 ± 0.18	.1307
Creatinine (mg/dl)	0.21 ± 9.39	0.15 ± 9.63	0.18 ± 6.54	0.20 ± 6.40	.8276
Triglyceride (mM)	1.32 ± 0.18	0.87 ± 0.31	1.21 ± 0.18	0.94 ± 0.38	.2293
Urea (mM)	2.33 ± 0.68	2.28 ± 0.62	2.35 ± 0.62	2.14 ± 0.82	.9828

Abbreviations: A:G, albumin to globulin ratio; HDL-Cho, high-density lipoprotein cholesterol; LDL-Cho, low-density lipoprotein cholesterol; PPB, plant protein blend; T. Cho, total cholesterol; TP, total protein.

more in the control diet than the PPB diets. Triglyceride concentration in fish fed the dietary treatments ranked in the following order PPB 1 < PPB 3 < PPB 2 < CTRL.

4 | DISCUSSION

The high inclusion of the PPBs in this study generally resulted in significantly lower growth performance and feed utilization efficiencies in the treatment groups compared with the control group. Higher nutrient ADCs were associated with the fish fed the commercial control diet compared with those fed the PPB diets. The observed growth differences among the fish groups could be directly attributed to the differences in nutrient digestibilities. The inclusions of plant ingredients in aquafeeds usually result in high levels of indigestible dietary fibre, non-starch polysaccharides (NSPs) and anti-nutritional factors (Duodu et al., 2018; Francis, Makkar, & Becker, 2001). Most fish lack the enzymes to hydrolyse NSPs and complex dietary fibre resulting in decreased gastro-intestinal transit time for ingested feeds and leading to poor hydrolysis and reduced nutrient absorption in the gut (Bairagi, Ghosh, Sen, & Ray, 2002; Obirikorang et al., 2015d). In a study by Soltan, Hanafy, and Wafa (2008), the replacement of fishmeal up to 45% with plant protein mixtures (cottonseed meal, sunflower meal, canola meal, sesame and linseed meal) in diets for Nile tilapia did not adversely affect nutrient ADCs compared with the fishmeal control diet. However, higher fishmeal replacement between 60% and 100% adversely affected dry matter, lipid and protein digestibility as was observed in this study.

Fish faeces are the major source of solid waste in aquaculture systems (Franco-Nava, Blancheton, Deviller, Charrier, & Le-Gall, 2004). Recent trends in aquafeed formulations are usually towards reducing faecal matter production relative to feed intake. In this study, the inclusion of the PPBs resulted in significantly higher faeces production (gDMkg^{-1} feed) which is related to inefficient digestion of fibre and NSP components of the diets (Kokou & Fountoulaki, 2018; Obirikorang et al., 2015c). Even though the high faecal matter production following the ingestion of the PPB diets could have negative implications for water quality through the release of nitrogen and phosphorus (Bureau & Cho, 1999; Wallace, Sanders, & Ferl, 1991), the faecal matter produced by the fish that received the PPB diets were within the 10%–30% reported in culture systems (Chen, Coffin, & Malone, 1997; Cho & Bureau, 1997).

Ammonia is excreted by fish through the gills, or as urine and also from the disintegration or suspension of nutrients from solid waste. However, this study focused on ammonia excretion through the gills and possibly by urine. The results showed that the TAN excretion rate for all diets were similar (Figure 2) over the 24-hr duration but was generally lower for the control diet compared with the plant protein blend diets. Although TAN concentrations in all diets peaked at 7 hr after meals were administered, the levels for the plant-based diets were higher ranging between 12.79 ± 0.94 and 13.21 ± 0.67 mg NH_3-Nkg^{-1} hr^{-1}. Ammonia excretion indices can indicate the impacts of some environmental and nutritional factors on the metabolic fate of exogenous and endogenous protein and provide insights into the nitrogen balance of fish (Engin & Carter, 2001). Additionally, measuring postprandial metabolic responses such as ammonia excretion rates can provide estimates of protein utilization in fish (Thillart & Kesbeke, 1978). The significant differences in TAN excretion rates between the test and control groups support the assertion that plant protein is inefficiently digested by Nile tilapia as evidenced by the significantly lower nutrient ADCs. Although a large proportion of the ammonia excreted by fish originates directly from deamination of dietary amino acids (Cowey & Walton, 1988), the significantly higher TAN excretion rates in the fish fed the PPBs could be linked to unbalanced dietary essential amino acid profiles. Some studies have reported that in instances where a single or multiple amino acids are limiting in a diet, there could be oxidation or interconversion of amino acids to augment the deficiencies which can result in increased ammonia excretion (Médale et al., 1998; Robaina et al., 1999; Yigit, Koshio, Aral, Karaali, & Karayucel, 2003). It is also likely that the mixture

of the different plant proteins might have resulted in the formation of anti-nutritional factors (ANFs) complexes leading to a lower protein metabolism thereby impairing protein utilization (Table 5) resulting in increased nitrogen excretion (Kokou & Fountoulaki, 2018). According to Kokou and Fountoulaki (2018), gossypol found in cottonseed meal is reported to bind with lysine leading to less availability of this very important essential amino acid and thus, causing reduced protein utilization, lower growth rate and increased excretion of nitrogen waste. Phytic acid forms insoluble phytic acid–protein complexes which lowers the availability of dietary protein (Richardson, Higgs, Beames, & McBride, 1985), thus increasing nitrogen excretion into the environment.

At the end of the study, plasma glucose postprandial in all treatments ranged between 72.27 ± 1.42–83.13 ± 0.60 mg/dl) higher than the reference interval (30–69 mg/dl) for fish raised in high-density systems but were within the range (39–96 mg/dl) for fish raised in the low-density system reported by Hrubec et al. (2000). The stocking density used in this study (5 g/L) was similar to that of Hrubec et al. (2000). Thus, it could be deduced that the glucose levels recorded in this study indicated a normal functioning of the liver, kidney and heart. Moreover, plasma glucose concentration is used as a bio-marker for stress in fish, and lower to optimum levels are associated with excessive utilization of stored glycogen for metabolic response to stress factors. Similarly, the cholesterol concentrations for all dietary treatments were within the desirable range of 64–299 mg/dl for tilapia. However, albumin and globulin levels were observed to be slightly beyond the optimum range. Nonetheless, this may not be a cause for concern since high levels of albumin in the blood is an indication of a healthy condition of the hepatic and somatic tissues in the fish. Furthermore, the high levels could be linked to the high protein content of the diets. Albumin:globulin (A:G) ratios are used as proxies for determining the well-being of the liver and protein synthetic organelles in fish. Low ratios are an indication of stress on the liver due to excessive utilization of albumins for tissue repairs. The A:G ratios recorded for all the dietary treatments were greater than 1:1, which is considered normal for most fish and an indication of a balance in the total protein synthesis by the liver and other nitrogen synthetic organelles (Shell, 1961). The similar urea content of the blood could be due to the isoproteic nature of the diets. From the study, the plasma creatinine concentrations which serve as blood marker for renal function in fish ranged between 0.15 and 0.21 mg/dl for the different treatments. According to Hrubec et al. (2000), the reference range for plasma creatinine for a healthy tilapia is 0.2–1.1 mg/dl. With the exception of the fish fed the CTRL and PPB 3 diets that recorded slightly lower creatinine levels, PPBs 1 and 2 were well within the normal range. This indicates that the kidneys and renal function of the experimental fish used were not affected by the dietary treatments. The result is in agreement with findings of Mahmoud, Kilany, and Dessouki (2014) who reported no effect of plant proteins on the renal function of the fish in their study to determine the effect of fishmeal replacement with soya bean meal and use of exogenous enzymes in diets of Nile tilapia (*O. niloticus*).

5 | CONCLUSIONS

In conclusion, crude protein digestibility in Nile tilapia was not affected by the dietary treatments; however, growth performance and feed utilization were significantly reduced in fish that received the plant protein blend diets. The incorporation of 30% copra and soya bean meals in PPB 2 resulted in the highest faecal matter output of 26.44% compared with ingested feed. All PPB diets resulted in approximately 77%–127% more faecal output than the control diet. Ammonia-N excretion was highest in PPB 2 and lowest in the control diet although, and all diets exhibited similar TAN excretion pattern over 24 hr. The serum biochemistry profile of all fish was not affected by the dietary treatments although the control diet recorded higher cholesterol levels. Overall, the performance of PPB 1 is recommended for improvement and optimization.

ACKNOWLEDGMENT

This research was funded by the Danish International Development Agency (DANIDA) under the Sustainable Fish Feed Development in Ghana (DFC No. 13-PO1-GHA) project. The authors are grateful to the technicians at the Fish Nutrition laboratory of the Department of Fisheries and Watershed Management, Kwame Nkrumah University of Science and Technology, Ghana for their assistance with samples analyses.

CONFLICT OF INTEREST

The authors declare no conflict of interest in the publication of this scientific paper.

AUTHORS CONTRIBUTIONS

The authors' contributions are as follows: C.P.D. contributed to the planning, design of methodology, interpretation of the data, performed laboratory analysis and critically proofread the manuscript; D.A.B. and K.A.O. contributed to the overall conceptualization of research goals and aims, critically proofread the manuscript and supported the provision of resources; A.K.A. and P.A. performed the experiment, initial analyses and interpretation of the data, and wrote the initial draft of the manuscript; K.A.O. supervised the study and assisted in the designing, planning and interpretation of data.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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How to cite this article: Duodu CP, Adjei-Boateng D, Amponsah AK, Andrews P, Obirikorang KA. The effect of plant protein-based diets on apparent nutrient digestibility, growth response, egesta quantity, postprandial ammonia excretion rate and serum quality of Nile tilapia. *Aquac Res*. 2020;51:1152–1161. https://doi.org/10.1111/are.14464