



Dietary Fishmeal Substitution by Peanut-Based Meals in Nile Tilapia (*Oreochromis Niloticus L.*): Effect of Pond Water Quality on Biomass Production

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Abstract. A 16 week experiment was conducted in earthen ponds in Iganga District of Eastern Uganda to investigate the effect of water quality on biomass production in Nile tilapia (*Oreochromis niloticus L.*) fed on peanut-based meals as alternative dietary fishmeal. Iso-nitrogenous diets containing 30% and 25% Crude Protein were applied for the first 12 weeks and last four weeks while the control was a local diet for Nile tilapia of 25% Crude Protein throughout the experiment. Treatments included the fishmeal-based diet and two peanut-based diets; peanut meal-based diet and mixed meal-based diet. Each of the 16 pond units measuring 3.0 x 4.0 x 1.0 were stocked with 48 fish fingerlings of 21.7 grams mean weight. Significant differences ($p \leq 0.05$) in mean values occurred among targeted parameters; pH, Dissolved Oxygen, nitrite nitrogen and unionized ammonia with exception to temperature. Apart from the unionized ammonia, the significant variations ($p \leq 0.05$) in water quality parameters did not significantly affect ($p \geq 0.05$) biomass production because they were maintained in suitable ranges for Nile tilapia.

Keywords: Nile Tilapia, water quality, peanut-based meals.

Introduction

Hassan (2001), Wu-chang *et al* (2004), Coyle *et al* (2004) and Olfasen (2006) concluded that fishmeal is essential in fish feed formulations. The popularity of the dietary ingredient is linked to stimulation of high fish productivity in farmed fish. According to Miles and Chapman (2006) and Khan (2011), fishmeal inclusion in fish diets induces optimal growth and yield in cultured Nile tilapia. The unrivalled biological performance in fish species fed on dietary fishmeal links to the following factors: Possession of all the Essential Amino Acids (Miles and Chapman, 2006), high levels of Crude Protein, energy, minerals, vitamins and essential fatty acids (Robinson *et al*, 2001), good palatability (Bureau, 2007), digestibility (Bainempaka, 2006) and perfect nutrient composition (Rust *et al.*, 2012). Generally, the relevant and appropriate nutrients levels characteristic to fishmeal is its relevant advantage as a protein source in aquaculture diets.

Plant resources have been consistently tested to substitute dietary fishmeal in farmed fish. Among the cultured species, Nile tilapia (*Oreochromis niloticus* L.) has largely been targeted for the plant-derived feeds (Nordahl and Pickering, 2004; El-Sayed, 2006; Agbo *et al.*, 2011; FAO, 2013; Al-thobaiti *et al.*, 2017). The herbivorous character in Nile tilapia (El-Sayed, 2006) renders the species suitable for the vegetable resources. According to Obirikoranga *et al.* (2016), the comparative advantage of low cost among plant protein alternatives is largely offset by poor fish growth. Even soybean meal that was comparable to dietary fishmeal (Nordahl and Pickering, 2004; Uga-chick, 2014) became relatively expensive due to low production in Uganda (Agricultural Planning Department, 2010).

Further research on suitable protein sources is justified since none of the tested plant-based alternatives to dietary fishmeal has been sustained in cultured fish. According to Moehl and Hawart (2005), intensification of human activities has led to depletion of wild plant resources. Subsequently, irrespective of higher demand in human food (Agricultural Planning Department, 2010), aqua feed is destined for crop-derived ingredients. Chakraborty *et al.* (2019) reported that peanut meal exhibits a high possibility for future incorporation in fish feed. There is need to exploit the high potential of soybean meal any successful replacement of dietary fishmeal in aqua feed. Specifically, the combination of peanut meal and soybean meal can improve on feed performance (Gosh and Mandal, 2015).

Feed input in fish production systems can affect water quality yet The Fish Site (2015) indicated that water quality is one of the factors affecting performance in farmed fish. Among the feed ingredients, protein sources account for the state of water quality (Torres-Beristain *et al.*, 2004). Consequently, poor water quality in fish production is largely due to dietary protein (Sergeant, 2014). The stressful culturing environment consequent to the compulsory inclusion of protein in aqua feed negatively affects fish biomass production. Although conventional protein sources such as fishmeal induce high growth and yield in farmed fish (Miles and Chapman, 2006), the excellent performance can be counteracted by poor water quality. For example, phosphorous pollution and corresponding reduction in Dissolved Oxygen levels in fish ponds due to dietary fishmeal (Abou *et al.*, 2012) may account for the unfavourable environment for proper growth and survival in farmed fish. Specifically, poor water quality lowers fish yield due to retardations in growth and survival (Onada *et al.*, 2015). Earthen ponds that are largely preferred by fish farmers (FAO, 2013) are characterized by poor growth and uncontrolled mortalities (Onada, 2015) due to degraded water quality.

Investigations on substitution of dietary fishmeal have largely been based on production performance (Allan *et al.*, 2000; Gonzalex-Felix *et al.*, 2010; Hardy, 2010; Al-thobati, *et al.*, 2018) without considering water quality. According to Goda *et al.* (2007) conflicting results in trials aimed at replacing dietary fishmeal in farmed fish may attribute to variation in culture conditions. Therefore, the current study aimed at investigating the effects of water quality on biomass production in pond cultured Nile tilapia fed on peanut-based diets as alternatives to dietary fishmeal.

Materials and Methods

Study Area

The field experiment was established at Busoga University farm in Iganga district in Busoga sub-region located 0° 30'-1° 00' North, 33° 00'-34° 00' East (The comprehensive Atlas, 2015) approximately 120 kilometres east of Kampala. The study lasted for 16 weeks from March to

July 2016. Sixteen rectangular shaped earthen ponds of uniform in size; 3.0 x 4.0 x 1.0 were used for culturing the experimental fish (Plate 1). The shallower and deeper ends measured 1.0 and 1.2 meters respectively.



Plate 1. Earthen ponds at the experimental site

Proximate Analysis, Standardization Feed Formulation and Rationing

Three dried feed ingredient samples each contained 100 gram package were taken for proximate analysis at the Faculty of Agriculture of Makerere University prior to feed formulation. The procedure was guided by Abdulrazak *et al.* (2014). The process analysed the presence and levels of Crude Protein, Crude Fat, and Crude Ash for the following feed ingredients; maize bran, fish meal, peanut meal and mixed plant meal.

In the fishmeal (FM)-based diet and peanut meal (PNM) based diets, sole protein sources of FM and PNM were used respectively at 100% inclusion levels. The mixed plant (MPM)-based diet contained combined protein sources: (PNM and soybean meal (SBM) in a ratio of 50: 50. Following Ajonina & Nyambi (2013), Pearson Square Method standardized the Crude Protein (CP) contents of test diets. Consequently, iso-nitrogenous test diets resulted when maize bran (MB) (basal ingredient) mixed with the protein ingredients. During the first rationing phase (12 weeks after stocking), test diets were standardized to 30% CP content when 23.2 kg of FM, PNM and MPM were mixed with 8.68, 26.16 and 14.5 kg of maize bran (MB) respectively.

Due to declining demand for protein as fish grows (Fitzsimmon, 2009) the CP content reduced to 25% until end of the feeding trial. This was achieved when 18.2 kg of the protein ingredients (FM, PNM MPM) mixed with 13.68, 30.16 and 19.5 kg of MB respectively. Prior to mixing, a top loading electronic balance (version 3.1, 2009) weighed the ingredients. The Nile tilapia commercial feed (CF) from Uga-chick Limited that acted as control diet maintained a CP content of 25% throughout the experiment. Micronutrients (vitamin and mineral premixes) and cassava flour binder constituted 2.5% and 7.5% of the test diets respectively. Provision of

all the essential ingredients aimed at satisfying nutritional requirements for cultured Nile tilapia. Feed pellets were sun dried at room temperature for eight hours before storage as recommended by Suleiman & Ahmed (2011). Shelf life of the pellets was maintained at 28 days.

Daily Feeding Ratio of 5% of mean body weight of the experimental fish was used following Nandal & Pickering (2004). The experimental fish fed twice daily at 9.00 am and 5.00 pm. Basing on Opiyo *et al* (2014), ration adjustments occurred on the 28th, 56th, 84th and 112th days of the feeding trial.

Experimental Design

Forty eight 'all male' Nile tilapia fingerlings of average mean weight of 21.7 were grams were stocked in each of the 16 ponds by Simple Random Sampling. The randomization for assignment of stock to pond units followed the procedure of Musita *et al* (2016). There were four dietary treatment groups including a control (commercial feed for Nile tilapia). Each treatment group at consisted of three ponds. Maintenance the pond stock density by use of a fish reserve pond per treatment group and control of non-experimental variables followed the procedures of Musita *et al* (2021).

A weighing balance of a readability of 0.01 grams measured 30 fish specimens from the three ponds of each treatment group. A five-litre plastic and transparent trough partially filled with water accommodated the fish specimens prior to the growth measurements. Live weight measurements were periodic after every 28 days while mortalities in pond units were recorded on a daily basis throughout the feeding trial while

The pond water parameters; temperature, Dissolved Oxygen (DO), pH, unionized ammonia (NH³) and nitrite (NO₂⁻) were measured during daytime throughout the experimental period. Only one pond was involved at a time across treatment groups. The test for water temperature occurred on a daily basis at mid-day (12.00 pm). Following El-Saidy and Gaber (2003). It involved insertion of a cylindrical mercury thermometer for five minutes at 30 centimetres (cm) below the water surface.

Following Ajibonge *et al* (2015), the Lamotte Fresh Water Kit was applied for testing other water parameters. Tests for Dissolved Oxygen (DO) concentrations across dietary treatments were conducted as follows; Using tightly capped plastic bottle a water sample was withdrawn at 10 centimetres cm depth below the water surface. Eight drops of manganous sulphate solution and another eight drops of alkaline potassium iodide azide reagent were added to the half-filled bottle prior to capping. After a two-minute vigorous shaking, the mixture led formed a precipitate. The bottle was opened and eight drops of sulphuric acid were added to dissolve the precipitate. A yellowish colour characterized the fixed sample prior to titration to determine the DO level.

Basing on visual tests of the colorimetric procedure characteristic to the Lamotte Fresh Water Kit was applied for testing levels of NH₃ and NO₂⁻ across dietary treatment groups. It involved withdraws of water samples from pond units followed by treatments with appropriate reagents to produce colour reactions. The colorations were inserted into Octa-Slide Viewers containing respective Octa-Slide Bars to compare with the sets of standard colours.

Indices for Water Quality and Fish Biomass Production

Apart from water temperature and DO where test results were read directly from the scale markings of the glass tubes, indicators of water quality and fish growth performance were calculated as shown below;

- a) $NH_3 = (NH_3 - N) \times 1.2$.
Where; NH_3 =unionized ammonia in the water sample (mg/L-); and (NH_3-N) = ammonium nitrogen (mg/L-).
- b) $NO_2^- = (NO_3^- - N) \times 3.3$.
Where; NO_2^- = Nitrite (mg/L-); and $(NO_3^- - N)$ =nitrite nitrogen (mg/L-)
- c) $MWg = MWh - MWi = \frac{SWgP1+SWGP2S+WGp3}{SD \times 3}$
Where;
MWh=mean final weight of the experimental fish at harvest (g)
MWi=mean initial weight of the experimental fish at stocking (g)
MWg=mean weight gain of the experimental fish during the experiment (g)
SWgP1=Stock weight of pond unit (g/pond-)
SD=stock density=48 fish/pond
- d) $MR = M/SD \times 100$.
Where; MR=mortality rate of the experimental fish (No. /pond-)
M= mortality of the experimental fish (No. /pond-)
SD=stock density of the experimental fish (No. /pond-).

One-way Analysis of Variance (ANOVA) was applied to test possibility a significant difference among all treatment means at $p \leq 0.05$ for water parameters, weight gain and mortality in Nile tilapia following Opiya *et al* (2014). Guided by Amisa *et al.* (2009), the Turkey's Honestly Significant Difference (HSD) was used for post-hoc among pairs mean values where significant differences ($p \leq 0.05$) existed.

Results

Results on test ingredient composition, levels of various water quality parameters and their effects on growth in Nile tilapia are indicated below;

Proximate analysis for the test ingredients

The PNM exhibited the highest Crude Protein and fat contents among test ingredients. It was followed by the MPM, FM and MB. On the contrary, the FM exhibited the highest Crude Ash content followed by the MPM and PNM (Table 1).

Table 1. Proximate analyses for major ingredients that were included in test diets

	Composition in the ingredients (%)			
	<i>PNM</i>	<i>MPM</i>	<i>FM</i>	<i>MB</i>
CP	55.16	44.5	38.68	6.8
CF	35.07	29.81	4.58	
CA	2.82	3.26	20.15	

*PNM=peanut meal, MPM= mixed plant meal, FM=fish meal, MB=maize bran, CP=Crude Protein, CF=Crude Fat, CA=Crude Ash.

Dietary treatments, water quality and biomass production in Nile tilapia

Test diets, water parameter levels in ponds of different treatment groups are indicated (Table 2).

Table 2. Mean values of selected water quality parameters of treatment groups at the experimental study sites

DT	Temp. (°C)	DO (mg/L-)	pH	NH ³ (mg/L-)	NO ₂ ⁻ (mg-L)
D1	28.0±0.6 ^a	4.0±0.1 ^b	7.0±0.15 ^b	1.8±0.125 ^b	0.05±0.003 ^b
D2	27.9±0.5 ^a	6.0±0.5 ^a	8.0 ±0.25 ^a	1.5±0.15 ^a	0.025±0.001 ^a
D3	27.3±0.4 ^a	6.0±0.25 ^a	8.0±0.05 ^a	1.5±0.075 ^a	0.025±0.008 ^a
D4	27.2±0.4 ^a	5.0±0.1 ^b	7.0.±0.1 ^b	1.25±0.075 ^b	0.025±0.005 ^a

*DT=dietary treatment, D1=Fishmeal-based diet, D2= Peanut meal-based diet, D3=mixed meal-based diet, D4=commercial feed for Nile tilapia, Temp=Temperature, DO=Dissolved Oxygen, NH³= Unionized ammonia, NO₂⁻=Nitrite nitrogen.

*Pairs of group means having a different superscript denote that the values are significantly different ($p \leq 0.05$) and vice versa.

There was variation among all parameters with in and across treatment groups. There was no significant difference ($p \geq 0.05$) across dietary treatment groups for water temperature. Significant differences ($p \leq 0.05$) occurred among mean values of other parameters; pH, DO, NO₂⁻ and NH³ (Table 3).

Table 3. Pond water quality parameters and growth in Nile tilapia

DT	Pn	Water parameter levels				Biomass production		
		Temp. (°C)	DO (mg/L-)	pH	NH ³ (mg/L-)	NO ₂ ⁻ (mg/L-)	MWg (g)	MR (%)
D1	P1	28.0	3.9	6.85	1.675	0.047	129.5	14.5
	P2	27.4	4.1	7.0	1.800	0.050	128.04	20.8
	P3	28.6	4.0	7.15	1.925	0.053	130.6	16.6
D2	P1	27.9	5.5	8.0	1.350	0.024	111	14.5
	P2	27.4	6.0	7.75	1.500	0.025	109.4	14.5
	P3	28.4	6.5	8.25	1.650	0.026	109.9	16.6
D3	P1	27.3	6.0	8.05	1.425	0.017	127.2	12.5
	P2	26.9	6.25	8.0	1.500	0.025	125.6	10.4
	P3	27.7	5.75	7.95	1.575	0.032	124.9	18.8
D4	P1	27.2	5.1	7.0	1.175	0.020	105.1	14.5
	P2	26.8	4.9	6.9	1.250	0.025	104.9	14.5
	P3	27.6	5.0	7.1	1.325	0.030	104.3	18.8

*DT=dietary treatment, D1=Fishmeal-based diet, D2= Peanut meal-based diet, D3=mixed meal-based diet, D4=commercial feed for Nile tilapia, Temp=Temperature, DO=Dissolved Oxygen, Pn=pond unit serial number, P=pond unit, NH³=Unionized ammonia, NO₂⁻=Nitrite nitrogen, MWg=mean weight gain in fish, MR=mortality rate.

Performance levels induced by the FM-based diet in terms of DO, pH, NH³ and NO₂⁻ were comparable to the control diet. The peanut-based diets (D2 and D3) indicated no significant difference ($p \geq 0.05$) among group means for all the tested parameters.

The FM-based diet was characterized by highest levels of water temperature, NH_3 , weight gains and mortality relative to other test diets. Higher insignificantly different ($p \geq 0.05$) weight gains were observed in Nile tilapia fed on for the FM and MPM-based diets. The weight gains corresponding to the two test diets were significantly higher ($p \leq 0.05$) than that of the PNM-based diet. On the contrary, there were insignificant differences ($p \geq 0.05$) among fish mortality rates for all the dietary treatment groups.

Discussion

Similar results indicating insignificant differences ($p \geq 0.05$) in water temperature among dietary treatment groups were reported by FAO (2006). Despite the mild differences, the FM-based diet exhibited the highest water temperature among test diets. In line with the current results, the feeding trial conducted by Mmanda *et al.* (2020) indicated a slightly higher water temperature for the FM-based diet. The results link to differences in structure among the feed ingredients. Unlike the animal-derived fishmeal, proteins isolated from plants are associated with indigestible oligosaccharides and structural fibre components (Miles and Chapman, 2006) that limit the rate of composting in water bodies. Subsequently, the higher decomposition rate of fishmeal relative to its counterparts accounted for the elevation of corresponding water temperature. The findings are consistent with Themelis (2005) who stated that under aerobic conditions, composting of organic wastes is coupled with generation of heat.

The safe water quality limits for Nile tilapia growth and survival; NH_3 (0.01-0.029), NO_2^- (0.46mg/L), pH (6.6-7.2), DO (6.1-7.2 mg/L) and temperature (26-30°C) (Hargreaves and Tucker; 2004; Rhida, 2006) implying that ranges for the tested parameters were largely appropriate for the experimental fish during the current study. Although significant differences ($p \leq 0.05$) in water parameters; pH, DO NH_3 and NO_2^- were observed across dietary treatment groups, they did not significantly affect ($p \geq 0.05$) biomass production because they were retained in the recommended ranges appropriate for the experimental fish with exception to NH_3 . The results are attributed to the intensity of the alterations in water parameters by the test diets that fell below the threshold required for retarding growth or mortality in Nile tilapia. The findings conform to the study of Olapode and Quinn (2019) where only the NH_3 concentration that rose above the recommended range resulted into fish mortality.

The highest NH_3 , weight gain and fish mortality occurred in ponds treated with the FM-based diet. Unlike the plant-based materials, animal-derived ingredients including fishmeal are more prone to decomposition (Themelis, 2005) implying that they can readily release NH_3 . According to Rossana (2019), NH_3 is the first form of nitrogen released when organic matter decays. The NH_3 correlated positively with mortality of the experimental fish because the higher the concentration in water, the more toxic the compound and vice versa. The findings are consistent with Onada *et al.* (2015) who attributed the high fish mortality in earthen ponds to increased level of NH_3 .

According to the results based on water quality parameters, the PNM and MPM-based diets indicated comparable performances. Similar results were obtained by Sayed *et al.* (2018) when insignificant differences ($p \geq 0.05$) were observed in water temperature, pH, DO in cage cultured *labeo rohita* fed on closely related diets. Unlike the FM-based diet, the PNM and MPM-based diets were derived from peanut products. Subsequently, the closer chemical composition of the two test diets largely accounted for the similarity in performances. The current findings are

consistent with the investigation Seargent (2014) where variations in water quality mirrored chemical differences among feed materials introduced.

Although NH³ and NO₂⁻ were positively correlated, they exhibited a negative correlation with DO across the dietary treatment groups. Similar observations have been reported by authors. Sayed *et al* (2018) revealed that increased pond aeration reduced toxic ammonia through accelerated diffusion of the gas while Hargreaves and Tucker (2004) concluded that concentrations of NH³ and NO₂⁻ often correlate directly. Consequently, conditions associated with reduced DO in fish ponds are capable of inducing poor aeration and accumulation of both NH³ and NO₂⁻. The current findings are consistent with Bhatnager and Devi (2013) who stated that low oxygen solubility due to high water temperatures leads to a build-up of ammonium compounds.

Conclusion

Fishmeal and peanut-based meals in the diet of pond cultured Nile tilapia significantly affected ($p \leq 0.05$) water quality parameters; pH, DO NH³ and NO₂⁻ with exception to temperature. Apart from the concentration of NH³ that exceeded recommended levels across dietary treatment groups consequently inducing mortality, other variations in water quality parameters did not significantly affect ($p \geq 0.05$) biomass production in the experimental fish.

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