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Peanut-based diets and growth performance of pond-cultured Nile tilapia fish (*Oreochromis niloticus* L.) at Busoga University farm, Eastern Uganda

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Abstract
Despite the local availability of protein rich peanut-based ingredients, fish meal has persisted as the conventional source of dietary protein for aqua feeds in Uganda. A 20-week field experiment was conducted in to determine the Relative Growth Rates of Nile tilapia fish fed on fish meal and peanut-based diets in earthen ponds at Busoga University farmland in Iganga district of Eastern Uganda. Six-week old juvenile fish of mean weight of 22.0 grams were stocked and fed at 5% of their body weight (BW) daily. Three test diets were utilized namely the fishmeal (FM)-based diet and the peanut based diets which included; peanut meal (PNM)-based diet & mixed plant meal (MPM)-based diet. The existing locally available commercial feed (CF) for Nile tilapia acted as the control diet. Although the mean Relative Growth Rate (RGR) of fish fed on the FM-based diet was the best, it was not significantly different ($p>0.05$) from that fed on the MPM-based diet. A significant difference ($p<0.05$) in mean RGR's existed between the FM and PNM-based diets and between MPM and PNM-based diets. Basing on the RGR's of the experimental fish, the MPM-based diet should completely substitute fish meal in the diet of Nile tilapia.

Keywords: Peanut-based diets, Growth performance, Nile tilapia, aqua feeds, Relative Growth Rates.

1. Introduction
Growth performance is the most important parameter in fish nutrient requirement studies ([23]. Fast growth of fish is an indicator of a high quality of feed. Relative Growth Rate (RGR) is one of the methods utilized for measuring the growth performance of cultured fish. It refers to the growth rate relative to the size of an organism. It can also be a comparison of various increases in size of similar organisms, tissues or structures. In aquaculture, RGR is obtained when the weight gain of fish is divided by the initial body weight and converted into a percentage [11]. According to [13], Relative Growth Rate is calculated as follows;

$$\text{RGR (\%)} = \frac{Wf - Wi}{Wi} \times 100$$

Where;
Wf = final weight of fish at the end of the experiment (g)
Wi = initial weight of fish at the beginning of the experiment (g)

In fish diets, dietary protein is the main ingredient utilized for promotion of animal growth [10]. The growth performance of fish is greatly influenced by the nutritional quality of dietary protein. Fishmeal is the commonest ingredient used to provide dietary protein in aqua feeds [37, 20, 19].
Fish meal has persisted as the conventional source of dietary protein for pond cultured *Oreochromis niloticus* L. It is still utilized as a sole source of dietary protein in aqua feeds for most cultured fish species [23]. It is a preferred ingredient in aqua feeds because it contains the required ingredients in perfect balance [30]. This explains why in many feeding trials, formulations containing fish meal have always promoted fast growth of fish. In complete diets, fish meal features prominently because of the good growth response it induces in cultivated fish species [1]. There has been a growing interest among investigators to replace fish meal in aqua feeds with alternatives of plant origin (Robinson & Stickney, 1984) [29].
Plant-based alternatives are regarded as cost-effective sources of dietary protein compared to

those of animal origin [16]. However, most plant-based ingredients have not been effectively developed into aqua feed because of several limitations. These include mainly the presence of protein inhibitors [8] and deficiency of key amino acids such lysine, and tryphan (Aqua-training [6]. Such deficiencies always result into poor utilization of dietary protein and retardation of fish growth [36]. In spite of the limited success, formulation of feeds containing high levels of plant proteins has become an important objective in fish nutrition research [28].

Oil seeds have been the commonest plant-based products that have been studied as fish meal alternatives in aqua feeds. They include mainly soybean, cotton seed, sunflower seed, mastered seed, lin seed, and coconut. Attempts to replace fish meal with the alternative protein sources resulted in variable success. Most of the results have indicated reduced fish growth [33]. Among the single sources of dietary protein, it is only soybean meal which has produced excellent results [17]. In most African countries, oil seeds such as peanuts exhibit a relatively higher advantage of replacing fish meal in aqua feeds because they are produced in large quantities [26]. Generally, the feeding value of peanut meal as an aqua feed ingredient has been poorly investigated [12, 1] although it has sometimes been a constituent of fish diets. Peanuts have already been utilized to replace 25% of fish meal in the diet of *Oreochromis mossambicus* without any significant effect on growth performance [14].

Specifically no single oil seed meal is sufficiently nutritionally complete to enable it to fully supply the dietary protein requirements of tilapias [23]. Peanuts have proved to be an acceptable protein source only at low inclusion levels in tilapia diets [1]. Combining peanuts with other sources of dietary protein can improve on the biological performance of a diet. For example, the growth performance of *Oreochromis mossambicus* was improved when single ingredient sources namely peanut meal, sunflower meal and sesame meal were combined. Mixed plant protein sources of dietary protein will act as better alternatives to fish meal in aqua feeds in the near future.

Therefore, the main objective of this study is to utilize growth performance as a tool of measurement of nutritional quality of peanut-based diets of pond-cultured Nile tilapia, *Oreochromis niloticus* L.

Materials and Methods

Study area

The 20 week experiment was set up at Busoga University farm land located 3 km from Iganga town in Busoga sub-region of Eastern Uganda. The experimental station consisted of 40 earthen ponds of size of 3m x 4m x 1m established on two study sites. Each of the study sites consisted of 20 ponds. Study site 1 was referred to as the Dry Season Stock (DSS) on December 17th 2012. This was the first phase of the experiment. Study site 2 was stocked at the beginning of the main rains or wet season (WSS) on February 13th 2013.

Fig 1: Phase 1 of the experiment (Dry Season Stock)

Fig 2: Phase 2 of the experiment (Wet Season Stock).

Research Design

Completely Randomized Design

The Completely Randomized Design (CRD) was used for setting up of the field experiment at the study because;

a) There were more than two treatments per experiment which required a complex design particularly CRD as guided by [31].
b) Following Bailey [7], the CRD was the most appropriate for the environment with a lot of non-experimental variables such as the earthen ponds.

Earthen pond units were utilized for the field experiment following [3]. Each experiment had four treatment groups based on test diets for Nile tilapia. There were 3 replicated experimental ponds at the study sites. Out of the 20 pond units at each study site, only 12 were utilized as experimental ponds. The remaining eight the ponds were utilized as follows; Four acted as fish reserves and the other four were water reserves.

Non-experimental variables were controlled as follows:

- Only 'all-male' fish were stocked in preference to the mixed-sex stock. This controlled irrelevant breeding and possible population variations in the stocked pond units.
- The Nile tilapia stock was collected from a single source as a pure breed.
- Fish fingerlings of the same age and size were stocked.
- All pond units at a particular study site were stocked at the same time.
- All the pond units at the study sites were uniform in size.
- Water parameters were measured and monitored periodically in order to ensure that they fall in ranges acceptable for optimum growth of Nile tilapia
- Fish was fed at 5% of its total wet body weight as the daily feeding ration (DFR), following [9]. Feeds were introduced at 9.00 am in the morning and at 5.00 pm in

the evening during cool water temperatures to encourage optimum feed consumption

- For each treatment group, fish reserve ponds (FRP) were utilized to sustain the initial fish stock density and any decline in pond water levels. The FRP method utilized one FRP per treatment group for instant substitution of fish in pond units that had experienced fish mortality. Monitoring of the initial fish stock density was periodically carried out after every seven days.
- Weekly seining was done using seine nets to extract all fish in every pond unit in ascertaining the existence of the correct or initial stocking density and to eliminate foreign organisms.
- The seine nets were specific for a particular treatment group in order to eliminate possibilities pond water contaminations between the groups.

Preparation of test feeds

Proximate analysis of feed ingredients

This was carried out for the dietary ingredients at the Faculty of Agriculture at Makerere University. The relevant nutrients in test ingredients especially crude protein, fat, fiber dry matter and ash were analysed as shown in the table below;

Table 1: Results for proximate analysis of test Feed ingredients

Type of nutrient	% of nutrient in test feed ingredient			
	PNM	MPM	FM	MB
Crude protein	55.16	44.5	38.68	6.8
Fat	35.07	29.81	4.58	
Fiber	9.44	9.18	0.46	
Dry matter	93.69	90.51	94.51	
Ash	2.82	3.26	20.15	

Formulation of test diets

Test diets that constituted the treatment groups included the fish meal-based diet (FM) and two peanut-based diets; peanut meal-based diet (PNM) and mixed plant meal-based diet (MPM). The control diet or the standard commercial diet was also utilized following [5].

Table 2: Percentage contribution of ingredients as sources of dietary protein in test diets

Test diets	Dietary protein ingredients		
	FM	PNM	SBM
FM-based diet	100	00	00
PNM-based diet	00	100	00
MPM-based diet	00	50	50

Apart from the dietary protein ingredients, all other feed components were kept constant and later for the four test diets. These included the carbohydrate source or maize bran (MB), micronutrients and feed binder (cassava flour) This was aimed at providing complete diets with minimum contents of all the essential nutrients to satisfy the needs of the experimental fish as recommended by [24]. The test diets were grounded into mashes, moistened with hot water before channeling them through a hand operated pelleting machine. These were sun dried and stored at room temperature to serve for only 28 days. The various ingredients that constituted the test diets were mixed in ratios on a dry weight basis following [27]. Test diets possessed the following ratios of major ingredients during the first 84 days (12 weeks) after stocking; dietary protein source (30%), carbohydrate source (60%), feed binder (7.5%) and micronutrients (2.5%). Adjustment in the dietary protein content of test diets was effected since fish fingerlings require higher amounts of dietary protein than sub-adult fish as guided by [18]. Therefore, for the next 56 days (8 weeks before end of the experiment), the ratios of major test feed ingredients were adjusted as follows; dietary protein source (25%), energy source (65%), feed binder (7.5%) and micronutrients (2.5%)

Sampling method

Simple random sampling

Simple Random Sampling (SRS) was applied for the field experiments following Bailey [7] at the study sites 1 and 2. Out of a population of 6000 fish fry at Sun fish farm hatchery of Njeru near Jinja town in Uganda, 2000 fish fry were transferred to the nursing ponds of respective study sites. At the study sites, 16 plastic bowels (PB's) were each packaged with 48 fish scooped from the 2000 fish stock in the nursing ponds. 16 paper cards (PC's) were marked with one of the numbers ranging from 1-16. The PC's were reshuffled several times before assigning them to a row of 16 PB's. The first PB of the row was served with the top most PC and so on. PB's with PC's marked with numbers 1-4 were selected to provide fish stock for the first column of four pond units. PC's with numbers 5-8 stocked the second column of pond units. Those marked with the 9-12, stocked the third column while 13-16 stocked the forth column

Data Collection

Fish mortality and mortality rate

Fish mortality during the experimental period was determined by use of Fish Reserve ponds (FRP,s). The total number of fish harvested minus the number of fish stocked in the four reserve ponds, equaled to the total number of fish lost to the experimental ponds or mortality

Table 3: Calculation of fish mortality using the fish reserve pond method

Treatment	No. of fish stocked in FRP,s	No. of fish harvested at the end of experiment	No. of fish lost to experimental ponds/mortality
FM-based diet	48	23	25
PNM-based diet	48	26	22
MPM-based diet	48	28	20
CF	48	25	23
TOTAL	192	102	90

The total number of fish lost during the two phases of the experiment was 90. Since each phase was stocked with 576 fish, the total number of fish for the two phases of the experiment (DSS & WSS) was 1152. Therefore, the mortality rate was 90/1152 x 100 = 7.8%. Thus, the survival rate was calculated following [32] as follows 100%-7.8% = 92.2%

Measurements of water quality parameters

Several water parameters that influence fish growth and survival were measured and monitored as follows; water temperature and transparency were measured using a mercury thermometer and a sechii disc respectively while dissolved oxygen, ammonia-nitrogen, nitrite nitrogen, carbon dioxide, calcium carbonate Carbon dioxide and pH were all measured as guided by the Fresh water aquaculture test kit. Whenever necessary, adjustments were carried out to reverse water quality conditions regarded as less optimum for the growth of Nile tilapia.

Table 4: Mean values of pond water quality parameters

Treatment/ Test diet	Water quality parameters					
	Sechii depth (cm)	Temp. (O C)	DO (ppm)	pH	NH3 (ppm)	NH2 (ppm)
FM-base diet	16.0	28.0	4.0	7.0	1.8	0.05
PNM-based diet	19.0	27.9	6.0	8.0	1.5	0. 25
MPM-based diet	21.0	27.3	6.0	8.0	1.25	0. 25
CF-based diet	18.0	27.2.	5.0	7.0	1.5	0.05
$\bar{X}$	18.5	27.6	5.25	7.5	1.5	0.04

Measurements of fish growth rate

Measurements of body weight (BW) were carried out using weighing balances sensitive up to 0.1 of one gram. Data was collected as follows; fish specimens were placed in a trough half filled with water. The specimen and the water trough were later placed on a weighing balance. The weight of fish was calculated after the weight of trough had been subtracted from the combined weight of the trough and the fish specimen.

The initial body weight (IBW) was determined by weighing all fish stock prior to stocking at every study site. The final body weight (FBW) was determined at the end of the particular experiment. The body weight gain (BWG) of fish was a result of subtracting the IBW from the FBW as shown in table 5.0.

Data analysis

The mean IBW was subtracted from the mean FBW of fish from the various pond units in order to obtain the mean BWG. The RGR's of fish were calculated as guided by [13] using the formula; wf-wi/wi x100

Where; wf = Final Mean BWG

wi = Initial Mean BWG

Significant differences (p<0.05) between mean RGR values were established after a One Way Analysis of Variance (ANOVA). The formulas above were aimed at generating a One-Way ANOVA test statistic (F) following [7] as shown below;

CF= (2)

ΣX/N

SST= 2

ΣX-CF

SSG= 2

(ΣX/n)-CF

SSE = SST-SSG

MSG = SSG/DFG

MSE = SSE/DFE

F = MSG/MSE

The F-statistic revealed a significant difference (p<0.05) between the RGR means. Therefore, Turkey's Post-hoc test called the Honestly Significant Difference (HSD) was utilized to reveal the particular RGR means that were significantly different (p<0.05) from one another.

Results and Discusions

Results

Table 5: Calculation of Relative Growth Rates of *Oreochromis niloticus L*

Test diet	Stock type	Pond No	Mean IBW (gm)	Mean FBW (gm)	Mean BWG (gm)	RGR (%)
FM-based diet	DSS	1	22.0	165.9	143.5	654
		2	22.0	165.1	143.1	650
		3	22.0	169.6	147.6	671
	WSS	1	22.0	174.3	152.3	692
		2	22.0	168.6	146.6	666
		3	22.0	170.5	148.5	675
PNM-based diet	DSS	1	22.0	150.6	128.6	581
		2	22.0	144.1	122.1	554
		3	22.0	155.4	133.4	606
	WSS	1	22.0	140.3	118.3	537
		2	22.0	153.2	131.2	596
		3	22.0	146.7	124.7	563
MPM-based diet	DSS	1	22.0	168.9	146.9	667
		2	22.0	165.6	143.6	653
		3	22.0	170.0	148.0	672
	WSS	1	22.0	165.9	143.9	654
		2	22.0	169.4	147.4	670
		3	22.0	170.2	148.2	673

CF	DSS	1	22.0	153.2	131.2	595
		2	22.0	146.7	124.7	563
		3	22.0	145.5	123.5	559
	WSS	1	22.0	137.5	115.5	525
		2	22.0	139.1	117.1	532
		3	22.0	137.8	115.8	526

Table 6: Calculation of mean Relative Growth Rates of *Oreochromis niloticus* L. fed on different test diets

	FM-based diet	PNM-based diet	MPM-based diet	CF-based diet
	654	581	667	595
	650	554	653	563
	671	606	672	559
	692	537	654	525
	666	596	670	532
	675	563	673	526
ΣX	4008	3437	3989	3300
$\sum X^2$	2,678,502	1,972,267	2,652,427	1,818,800
$\bar{X}$	668	573	665	550

N=24, n=6, k=4, DFG=3, DFE=20

Table 7: One-Way Analysis of Variance for determination of the F-value

Source of variation	Degrees of freedom	Sum of squares	Mean square	F-value
Group	3	67,744	22,581	**51.3**
Error	20	8,804	440.2	
Total	23	76,548		

The ANOVA test statistic or F = 51.3. However, the P-value at @ 0.05; 3, 20 = 3.1 The HO was therefore rejected because of the evidence of a significant difference (p<0.05) between the group means since 51.3 > 3.1

Turkey's HSD was utilized for the post –hoc test using the formula;

HSD=q√ MSE/n as follows; HSD = q√ 440.2 / 6= 73.4 = 8.57

According to the table of distribution of q (studentized range statistic), the value of q at @ 0.05; 3, 20 = 3.58

3.58 x 8.57= 30.7

Table 8: Determination of levels of significance between pairs of group means

Treatments	Treatment means	Mean differences	Comparisons with the HSD value (30.7)	Level of difference
FM & PNM	668 & 573	95	Larger	Significantly different (p<0.05)
FM & MPM	668 & 665	03	Smaller	Not significantly different (p>0.05)
FM & CF	668 & 550	118	Larger	Significantly different (p<0.05)
PNM & MPM	573 & 665	92	Larger	Significantly different (p<0.05)
PNM & CF	573 & 550	23	Smaller	Not significantly different (p>0.05)
MPM & CF	665 & 55O	115	Larger	Significantly different (p<0.05)

Discussion

There was variation in the mean RGR's of pond cultured Nile tilapia fed on different sources of dietary protein. By virtue of the differences in nutritive values of the dietary ingredients of test diets, there were variations in RGR's of Nile tilapia. According to the results, the RGR of Nile tilapia that corresponded to the FM-based was the highest. It was significantly higher (p<0.05) than the RGR corresponding to the PNM-based diet but not that of the MPM-based diet. Apart from the appropriate amino acid profile, other factors mainly the comparatively higher protein sparing effect of the non-protein portion and the perfect balance of nutrients in FM, contributed to the higher RGR of Nile tilapia. The findings are concomitant with [10] and [38] who stated that efficiency of utilization of dietary protein is related to the nature of non-protein energy sources especially the high energy lipids that can be utilized for protein sparing in aqua feeds

According to the results, the Mean RGR for Nile tilapia subjected to the MPM-based diet was close to that of the FM-based diet. There was no significant difference (p>0.05) between the RGR's of *Oreochromis niloticus L* fed on MPM and FM-based diets. This indicates that the two diets possess similar two test diets exhibit equal performances in terms of fish growth. However, there was significant difference between the RGR's of *Oreochromis niloticus L* corresponding to the MPM and the PNM-based diets. The highly competitive performance of MPM is attributed to the combined effect of the mixed ingredients of PNM and SBM. For example, PNM improved the palatability, lipid and phosphorus contents of SBM. On the other hand, SBM with a desirable amino acid profile [4] increased the levels of lysine in the lysine deficient amino acid profile characteristic to PNM [14]. Most of the single sources of dietary protein tend to exhibit varying degrees of nutritional deficiency, which affects fish growth. Finding a single source of dietary protein as efficient in promotion of fish growth as FM might be difficult because; no single oilseed meal is nutritionally complete to supply all the dietary protein requirements required for proper growth of tilapias [23], utilization of single sources of dietary protein in tilapia feeds may be limited by dietary minerals such as phosphorus [12]. Combined sources of dietary protein in aqua feeds can provide an alternative to FM as promoters of fast growth in Nile tilapia. The findings and observations are consistent with; [2] who revealed that substituting fish meal in aqua feeds with mixed sources of ingredients provides more balanced nutrients than single ingredients, and [2] who concluded that mixtures of ingredients that mimic the amino acid profile of fishmeal could be successfully utilized for its replacement in aqua feeds. However, the findings contradict Hardy [21] who stated that it is still difficult for plant-based ingredients to maintain rapid growth rates at higher levels of fish meal substitution.

The PNM-based diet exhibited a lowest RGR as compared the FM and MPM-based diets. Similar results were obtained from studies carried out by; [34] where an all-peanut protein diet resulted into poor growth rate for *Tilapia aurea*, [22] who revealed that high inclusion levels of 50-75% of peanut diet reduced the growth rate of tilapias. Fish growth responses always vary depending on the inclusion level of PNM. According to [35], in order to maintain the growth performance of Mozambique tilapia (*Oreochromis mossambicus*) fry, PNM should not substitute more than 20% of FM. The poor growth performance of Nile tilapia fed on PNM was mainly due to the amino acid profile that was deficient in the essential amino acid called lysine. This observation is consistent with those of; [15] where it was concluded that lysine is one of the six essential amino acids that are critical for growth in animals and [14], who stated that the utilization of PNM is limited by the low content of the key an amino acid called lysine. Due to their poor performance in terms of growth of Nile tilapia, PNM-based diets are not suitable substitutes for fishmeal in aqua feeds.

Conclusions and Recommendations

Single sources of plant-based ingredients usually exhibit varying degrees of nutritional deficiencies as sources of dietary protein in aqua feeds. This normally leads to reduced fish growth rates especially in Nile tilapia. A few essential amino acids or mineral nutrients relevant for fish growth usually occur in low amounts in plant-based sources of dietary protein. A nutritionally complete source of dietary protein for aqua feed similar to fish meal, can be obtained by combining different sources of dietary protein that mutually supplement each other particularly soybean meal and peanut meal. When mixed ingredients are utilized, the growth of Nile tilapia is promoted more than when SBM or PNM are utilized as single ingredients. The plant-based mixed source of dietary protein called MPM should be utilized for complete substitution of FM in aqua feeds for Nile tilapia.

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