ENHANCING THE NUTRITIONAL VALUE OF TILAPIA FOR HUMAN HEALTH

Sustainable Feed Technology and Nutrient Input Systems/Experiment/13SFT02PU

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ABSTRACT

Fish have traditionally been regarded as an important and wholesome source of nutrition for humans. Ghana's fish intake is known to exceed that of the world average. Cultured fish are known to have higher levels of n-6 than n-3 fatty acids and this is directly linked with the quality of the ingredients used to prepare feeds for growing such fish. Therefore, by altering the diets of cultured fish to have higher n-3 fatty acids it is likely to enhance the nutritional value of the fish grown and increase the benefits derived by consumers. This study focused on identifying local sources of feed ingredients high in n-3 fatty acids, determining the impact of diets high in n-3 fatty acids on the flesh quality of fish, and benefits to consumers. To determine sources of feed ingredients rich in n-3 fatty acids, a survey was conducted with targeting oilseed sellers and processors. Additionally, secondary data on oilseeds were obtained from three leading research institutions and from general literature search because there was no database that had such information. Fatty acid composition of a select group of eight seeds was determined. An 8-week growth study was conducted in $1m^3$ hapas using n-3-rich oils from oilseeds. In all six of the diets that were tested: five were formulated using three different oils and the sixth was a commercial control. The diets were randomly assigned to hapas stocked with Nile tilapia *Oreochromis niloticus* fingerlings of size 28±4.0 g. Fatty acid composition of test diets, control fish and experimental fish carcass were quantified. Although, the select ingredients had high levels of lipids, none of them had appreciable levels of n-3 fatty acids (> 1%).

INTRODUCTION

Both n-3 and n-6 fatty acids are essential nutrients for humans, but there is a striking imbalance in the intake of n-3 and n-6 fatty acids globally. Intake of n-6 fats far exceeds that of n-3 fats due to the widespread consumption of plant oils and grains that contain more n-6 than n-3 fatty acids (Trushenski and Lochmann 2009). Thus, traditional diets can lead to marginal to severe deficiencies of n-3 fatty acids and a variety of associated health problems, such as cardiovascular disease, arthritis, atherosclerosis, diabetes, and cancer (Horrocks and Yeo, 1999; Arterburn et al. 2006; Simopoulos, 2008). In infants, deficiency of n-3 fatty acids can be especially destructive, as fatty acids such as DHA are crucial for normal brain development, behaviour, and cognitive function (Innis, 2007).

In Ghana, n-3 deficiency is more common in infants and pregnant or lactating women than in adult males (Siekmann and Huffman, 2011). Currently, the recommended n-3 range is from 500-1500 mg/day depending on factors such as age, gender, reproductive status, and prior history of coronary problems.

Fish are the primary practical source of n-3 fatty acids in most countries (Tocher, 2003), and fish supplies approximately 60% of the protein for the population of Ghana. It is well known that fish acquire the fatty acid signatures of their diets (they "are what they eat") (Turchini et al. 2011). Farmed tilapia have been criticized for being too high in n-6 and too low in n-3 fatty acids (Weaver et al. 2008). Fish feed typically contain large amounts of plant ingredients high in n-6 fatty acids. Although, these ingredients are considered environmentally sustainable to use in fish diets, they lack

the healthy n-3 long-chain polyunsaturated fatty acids (LC-PUFAs) found in marine fish products. With the global and local decline in fish catches it would be preferable to identify viable plant sources of n-3 fatty acids for long-term growth and sustainability of the aquaculture industry in Ghana. Fortunately, tilapia can elongate and desaturate 18:3n-3 found in plant oils to form n-3 LC-PUFAs such as 20:5n-3 and 22:6n-3. Therefore, inclusion of preformed LC-PUFAs is not necessary for the general performance or health of the fish (NRC, 2011).

The suitability of feed ingredients for commercial production of both fish and feed are based not only on nutrient content, but on economics and availability, as well as palatability to the fish (Hardy and Barrows, 2002). In Ghana, a large number of oilseed and cereal by-products are available for screening as potential sources of n-3 fatty acids for fish feeds (Nelson and Wallace, 1998; Hecht, 2007). A few plants found in Ghana such as *Leucaena leucocephala* and *Moringa oleifera* contain more than 30% 18:3n-3 in their lipids. There is some information on the feeding value of *Leucaena* and *Moringa* in Nile tilapia (Adeparusi and Agbede, 2005; Madalla, 2008), but more information is needed to optimize the inclusion levels of these leaf meals in diets to obtain both profitable production and improved product quality (i.e., enhanced content of n-3 fatty acids). A recent study at the University of Arkansas at Pine Bluff (Kasiga, 2012) showed that leaf meals made from these plants could be substituted for up to 30% of the protein in soybean meal in diets of Nile tilapia without reducing fish performance. The leaf-meal diets also significantly increased the concentration of total n-3 fatty acids and n-3 LC-PUFAS in the fish. In addition to the leaf meals, a survey should be conducted to identify any other potential feed ingredients available in Ghana that would enhance the n-3 fatty acid content of the fish and meet the other criteria discussed previously.

Aside from whole plant ingredients, it is possible to add isolated lipid sources to the diets also, such as flaxseed oil. This oil contains more than 50% 18:3n-3, and does not inhibit tilapia growth (Karapanagiotidis et al. 2007). Linseed oil (53% 18:3n-3) is another available source of n-3 fatty acids in Ghana. It is also possible to maximize the retention of the desirable n-3 LC-PUFAs in fish by supplying most of their dietary fat as saturated fat such as coconut oil or palm kernel oil (Trushenski et al. 2009). These oils are widely available in tropical regions and could be used in combination with an n-3 lipid like linseed oil to optimize the n-3 content of the fish, while keeping diet cost as low as possible.

In this study, 20% fish meal is used in diets. The strategy has not been tested in tilapia using diets without fish meal and oil, so further verification of the strategy using diets without marine products is needed. The degree of enrichment and the time needed to achieve a target level of enrichment will depend on the amount of n-3 fatty acids in the diet, the feeding rate, and length of time the fish are fed the diets. The diets will also need to support optimal growth, have high feed conversion rates and survival of the tilapia to minimize cost-of-gain and support production profitability.

The ability to produce tilapia enriched with n-3 acids as a functional food could be a key factor in mitigating widespread health problems associated with essential fatty acid deficiencies in Ghana and other developing countries. The challenge is to identify n-3 sources that will support fish performance and enhance the quality of farmed tilapia for human health while maintaining production profitability. In this project, we sought to identify and nutritionally characterize locally available, cost-effective ingredients that will add n-3 fatty acids to the diets of tilapia, and create an n-3 enhanced product that has greater potential to enhance the health of consumers.

OBJECTIVES

1. Assess the availability and distribution of potential fish feed ingredients containing n-3 fatty acids for use in Nile tilapia diets in Ghana.

2. Determine the proximate composition and fatty acid composition of potential feed ingredients, and recommend specific inclusion rates for diets to be tested in feeding trials with Nile tilapia in Ghana.
3. Analyze the proximate and fatty acid composition of experimental diets and fish from feeding trials in Ghana and determine cost-of-gain of the different diets.
4. Determine the amount of tilapia that would need to be consumed by humans to obtain the target amount of n-3 fatty acids (500-1500 mg/day) for health benefits.

Null hypothesis: There will be no difference in n-3 fatty acid content among fish fed diets with different ingredients.

METHODS AND MATERIALS

Study 1 – Identification and Characterization of Local Sources of Potential Ingredients with High Omega-3 Content

The initial plan was to do a desk study to identify these ingredients, but with the few published studies in Ghana and no database available on possible sources, the search was expanded to include a search of documents in Ministry of Food and Agriculture in Ghana, research institutes and university libraries.

Data collection

In addition to the secondary data acquired from the government and research institutions, primary data on oilseeds were obtained from oilseed sellers and processors (nine) by means of a survey. The survey instrument was a semi-structured questionnaire. The investigation focused on the major crop production and sale areas in Ghana; Brong-Ahafo, Ashanti, Greater Accra, and Upper East regions (5 of 10 administrative regions) of the country.

Proximate, fatty and amino acid determination

Eight of the potential ingredients were selected for further investigations because they were the least researched ones (Table 1). Proximate composition of the ingredients was determined following methods described in AOAC (2005). Lipid extracts from the ingredients were used for the fatty acid analysis. Ten mL of the lipid extracts were evaporated under nitrogen and then trans-esterified with 14% boron trifluoride. The resulting fatty acid methyl esters (FAMEs) was analyzed (Morrison and Smith 1964) using a flame ionization gas chromatograph with helium as the carrier gas. The FAMEs were separated on a fused silica capillary column (15 m x 0.25 mm internal diameter). The injection volume was 1 µl, with an injector and detector temperature of 250°C and 315°C, respectively. The column temperature was held initially at 100°C for 10 min, increased to 160°C at a rate of 15°C/min and held for 4 min, then increased to 250°C at a rate of 2.5°C/min. The FAMEs were identified and quantified by comparing the retention time and peak area to those of serially diluted mixtures of reference standards. Amino acid analysis was conducted at the University of Missouri.

Study 2 - Growth trial; growth performance of fish fed n-3 enhanced diets and nutritional composition of the fish fed these diets

As there were no appreciable levels of omega-3 fatty acids in the ingredients selected in Study 1, locally available oils (linseed, palm, and soy) were used to replace fish oil in the diets for Study 2. Five diets with different inclusion levels of the selected oils were formulated and compared with a commercial control.

Experimental Design

The growth study was conducted at the Faculty of Renewable Natural Resources farm, Kwame Nkrumah University of Science and Technology (KNUST), Ghana. In all, six diets were tested including a commercial control, each with four replicates. The experimental units were 1 m x 1 m x 1

m hapas, which were mounted in two ponds (blocks) of size 30 m x 10 m each; two ponds were used to prevent overcrowding. The diets were randomly assigned to the hapas in the ponds and the variables that were monitored for growth were food conversion ratio (FCR), specific growth rate (SGR), and weight gain percentage. To determine the effects of the diet on the fatty acid composition of the Nile tilapia, the fatty acid profile of the diets and fish fed the different test diets were also determined.

Test Diets

Five test diets were formulated (Table 5) with the plant oils; linseed oil, soybean oil, and palm oil were used as lipid sources in the feed and a commercial floating feed was used as the control diet. The Winfeed 2.8 feed formulation software was used in formulating the diets. For each feed, the appropriate ingredients were weighed using an electronic scale (Ohaus Navigator XL model NVL2101/1) and these were well mixed in a Kenwood KVC5000 standmixer (Plate 3.2 B) for about 10 minutes. Then 100 mL of water was added to the mixture for easy pelletability and re-mixed in the mixer. The resulting mixture was then passed through a Bosch meat mincer (Propower MFW67440) fitted with 2.0 mm die size; then oven dried at 60°C for 12 hours. The strands of pellets produced were further broken into smaller pieces that could be eaten by the juvenile fish.

Pond preparation

Ponds used for the experiments were completely drained and dried after which the ponds were limed at a rate of 1 kg agriculture lime per 10 m^2. Ponds were then filled with water from a borehole and water levels were maintained above 1 m throughout the experiment. Ponds were fertilized with Mono Ammonium Phosphate (MAP) at 2 g per m^2 and Urea at 3 g per m^2.

Stocking and Feeding

Each 1 m^3 hapa was stocked with 25 all male Nile tilapia fingerlings of average weight of 28±4.0 g procured from the Pilot Aquaculture Centre, Tano-Odumasi and fed rations corresponding to 3% body weight per day of their respective experimental diet randomly assigned them. Feeding was done twice daily at 09:00 am and 4:00 pm.

Fish and Water Sampling

Fish were sampled every two weeks. At each sampling the bulk weight of all the fish in each hapa were taken. The following water quality variables were monitored throughout the study to ensure that water quality remained well within limits recommended for Nile tilapia culture. Water temperature (ºC), pH, and dissolved oxygen (mg/L) were measured twice a week using Hanna (HI 9828) multi-parameter probe. Total ammonia nitrogen and nitrate levels in the pond were determined using the Indophenol and photometric method respectively with a Wagtech photometer whereas nitrate levels were determined using the diazonium method.

Nutritional analyses

The nutritional composition of the ingredients, test diets, control fish, and experimental fish at the end of the study were determined following methods described above.

Cost to gain

Only the lipid sources varied between diets (the control diet was not included in this calculation due to the unknown composition). Since the total cost of the experimental diets were unknown, relative cost-of-gain was determined based only on the cost of lipids in relation to the FCR obtained on each diet.

Statistical Analyses
Data on growth were analyzed using a two-way ANOVA test at an alpha of 0.05, to test for differences due to the treatment (diets) and block effect and a Bonferroni posttest for post hoc comparison. All data were expressed as means ± SD, all graphs and analyses were executed using the Graphpad Prism 5.01 for windows statistical software.

RESULTS

Study 1 – Identification and Characterization of Local Sources of Potential Ingredients with High Omega-3 Content
In all, 23 ingredients locally produced in Ghana were identified based on the survey that was conducted and secondary data sources. Most of these were abundant for short periods (1-2 months) in the year with the exception of Sheanut and palm nut seed/oil, which were abundant for four months of the year.

Of the eight ingredients selected for further investigations, two of them; *Cucumeropsis mannii* (Egusi) and *Citrillus lanatus var neri* (Neri) were common to all the five regions sampled in the study, but the other six potential ingredients were found only in the Brong Ahafo and Upper East Regions. Unfortunately, none of the ingredients contained significant amounts of n-3 fatty acids (<0.5%; Table 2). Hence, a supplemental lipid, linseed oil, was added to the diets to enrich the fish and their human consumers with n-3 fatty acids. Protein content of the ingredients was high, ranging from 21-39% (Table 3). This compares favourably with other plant products such as distiller's grains, but is lower than others such as soybean meal.

Study 2 - Growth trial; growth performance of fish fed n-3 enhanced diets and nutritional composition of the fish fed these diets
Survival was high for the study ranging from 98-100%; there were no significant differences in survival rates for the different diets. Generally, there was a steady growth at almost equal rate at the beginning of the trial for all diets except for the diet with 100% Linseed oil for the first 12 days. After day 12, the control performed better than the experimental diets and among the experimental diets, the one with 100% linseed oil had the poorest growth rate. A similar trend was observed with the FCR and SGR growth parameters.

At the end of the 7-week growth trial, the control diet had the highest weight gain of 92.4±2.0%. The least performing diets in this study were the 100%Lin and the PalmSoy diets with a percentage weight gain of 66.2±2% and 69.2±1% respectively. The rest of the diets had between 70-80% weight gain (Table 6). There were significant differences in weight gain ($p= 0.0012$) between the diets with the differences occurring between the control diet and experimental diets. However, no significant differences were found between the two experimental blocks in the study ($p= 0.859$). The highest SGR was observed in the control diet with 1.32% and the least was 1.02%. in the 100%Lin diet. There were significant differences in SGR ($p= 0.002$) between the control and all the diets except the LinSoy diet. The diet with the best FCR was the control diet with 1.2±0.1 and the least was the LinPalm and 100%Lin diets with 1.62±0.2 and 1.60±0.2, respectively (Table 6). Again, there were significant differences in FCR between the control and all the diets. However, there were no significant differences among the two experimental blocks for both SGR and FCR.

A total of 23 fatty acids were identified in the fish tissues at the end of the experiment. The fatty acids of interest in this study were PUFAs belonging to the omega-3 and omega-6 series. The n-3 content in the fish at the end of the study ranged from as low as approximately 5% in the control diet to a high level of 22.4% in 100% Lin (Figure 2). The diets containing Linseed oil (100% Lin), had high levels of alpha-linolenic acid (n-3) in the fish tissues as compared to the other experimental diets. For the omega-6 fatty acids (n-6), the content in the fish tissues ranged from a low of 17.8% in control diet to

the highest level of 31.5% in 100% Soy. Whereas n-3 fatty acids increased considerably in all diets, with reference to the initial sample, n-6 fatty acid showed a decrease. Long chain derivatives of n-3 fatty acids, that is, EPA and DHA fatty acid of fish fed diets with linseed oil, that is 100% Lin, LinSoy and PalmSoy showed slight increase (2.5-3.9%) when compared to levels in the fish prior to the experiment (2.1%). This was however, not statistically significant ($p < 0.05$). There was a significant difference in n-3 in fish flesh between 100% Lin and all the other diets, as well as the initial fish stock. Significant differences in n-3 also occurred between control diet & 100% Soy and between LinSoy & LinPalm.

Cost of gain:

For the experimental diets the relative cost of gain was in the following order:

100% Lin > LinPalm > 100% Soy > LinSoy > PalmSoy

The diet with 50% palm and 50% soybean oil had the lowest relative cost of gain, whereas that with 100% linseed oil had the highest relative cost of gain. The high cost of linseed oil in combination with the high FCR of fish fed that diet explained the latter result.

However, fish fed the diet with 100% linseed oil had the highest total level of n-3 fatty acids. Fish fed diets with LinSoy or LinPalm had intermediate levels of n-3 fatty acids, whereas those fed 100% Soy or PalmSoy oils had the lowest levels of total n-3 fatty acids (Figure 2). Although, the fish in this study were sub-market size, consumption of about 50 grams of fish fed the 100% Lin diet or the LinSoy or LinPalm diets would provide 500 mg or more of total n-3 fatty acids (the lower end of the recommended level). Consumers would need to eat about 5 times more (250 g) of fish fed the 100% Soy or PalmSoy diets to get the same amount of total n-3 fatty acids.

Although, specific levels of the long-chain n-3 fatty acids (mainly EPA and DHA) were not reported for fish fed each diet, a range of 25 – 39 mg could be calculated. The recommended n-3 fatty acid intake (500–1500 mg/day) is actually for the long-chain n-3 fatty acids. The concentration of long-chain n-3 fatty acids was more similar in fish fed the different diets than the total n-3 fatty acids (dominated by alpha-linolenic acid). Therefore, it is estimated that consumption of 250 g of fish fed any of the diets would meet or exceed the minimum recommended level of 500 mg of long-chain n-3 fatty acids. It must be noted that all diets contained 20% fish meal, which would have provided a basal level of about 2% fish oil in all diets. If the level of fish meal inclusion was reduced (to lower diet cost and improve environmental sustainability of tilapia culture), the results of this study might be very different. The effects of different lipids (differing in fatty acid composition) will vary with the overall diet composition, and would be expected to differ when using diets with little or no fish meal compared to diets with 20% fish meal.

DISCUSSION

The upper East and Brong Ahafo regions are important sources of oilseeds in Ghana. The eight oilseeds selected were high in lipid and protein content, but were not considered as important sources of n-3 fatty acids. They could be partially used to replace more expensive protein sources in the diet of tilapia. The good growth performance in fish fed diets with equal proportions of linseed and palm oils could possibly be due to relatively equal proportions of both n-3 and n-6 PUFA supplied by the linseed and soy oil respectively, and explain why the fish fed diet with 100% soybean oil, which was low n-6, had poor growth performance. According to Takeuchi *et al.* (1983) and Aksoy *et al.* (2009), tilapia has a higher requirement of n-6 to n-3 PUFA. The poor growth rate in the linseed only diet could be observed right after the 1st sampling and continued though the end of the study, whereas, the differences in the rest of the diets were only prominent within the last 12 days of the experiment. This study showed similar results compared to the findings of Chen *et al.* (2013); the muscle fatty acid

composition of Nile tilapia juveniles fed diets with n-3 fatty acids was reflective of the content in the diet. They determined that the optimum requirement of Nile tilapia juveniles was 0.45-0.64% of dry weight.

CONCLUSIONS

Twenty oilseeds were identified in five of the ten regions in Ghana. The Brong Ahafo and Upper East region had the most number of oilseeds. With the Egusi and Neri seeds that were available for four months in a year, the others were available for 1-2 months within the year. The oil seed/ingredients had low n-3 levels (< 0.5%), but high levels n-6 fatty acids; the most abundant fatty acids in all the seed were the linoleic and oleic acids. Protein content was between 21-39%.

The n-3 concentration in the fish at the end of the study were highly reflective of the composition their diet; ranging from 3.5% (control) to 22% (diet with 100% Linseed oil) compared to 17.8% (control) to 31.5% (100% soybean oils diet). The diet with 100% linseed oil had significantly higher levels of n-3 than the rest, however, the high n-3 content seem to suppress the growth of the juvenile tilapia and had the highest relative cost to gain. There were similar trends in the weight gain, feed conversion ration and specific growth rate (SGR) with the control diet generally performing better than the test diets. An exception was the 50:50 linseed and soybean oil diet which had comparable SGR with that of the control. Based on our findings, a person would have to consume 250 g of fish fed any of the test diets to get the minimum recommended level of long chain n-3 fatty acids.

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TABLES AND FIGURES

Table 1. Ingredients from Ghana analyzed for nutrient content to assess potential for tilapia diets

Calabash seeds	(*Lagenaria siceraria*)
Eggplant seeds	(*Cucumis spp.*)
Sesame seeds	(*Sesamum indicum*)
Kidney beans	(*Phaseolus lunatus*)
Neri seeds	(*Citrillus lanatus*)
Egushi seeds	(*Cucumeropsis mannii*)
Cucumber seeds	(*Cucumis sativus*)
African Locust seeds	(*Parkia biglobosa*)

Table 2. Fatty acid composition of ingredients from Ghana with potential use in tilapia diets.

	Fatty acids (% by weight)											
	C14:0	C16:0	C18:0	C20:0	C16:1	C18:1	C18:1	C18:1	C20:1	C18:2	C18:3	Total
	(%)	(%)	(%)	(%)	p-oleic (%)	elaidic acid	oleic (%)	vaccenic	(%)	linoleic(%)	alpha(%)	(sum%)
Sesame seed A		10.1		0.6	0.1	6.1	40.6	0.2	0.2	41.8	0.3	100
Sesame seed B		9.9		0.7	0.1	6.3	41.5	0.1	0.2	40.8	0.4	100
Citrullus lanatus **A**		9.8	12.5	0.4			14	0.3		63		100
Citrullus lanatus **C**		10	12.4	0.4			13.3	0.2		63.9		100.2
Cucumeropsis mannii **A**		15.1	7.7	0.4			16	0.3		60.4	0.1	100
Cucumeropsis mannii **C**		16.1	14.4	0.4			15.9	0.3		52.9		100
Cucumis metulferus **A**		15.9	9.8	0.5			31.1	0.4		42.3		100
Cucumis metulferus **C**		14.8	9.3	0.4			25	0.4		50		99.9
Parkia biglobosa **B**		12.9	17.4	4.1			18.2			47.4		100
Parkia biglobosa **C**		12.4	17.8	5.3			17.1			47.3		99.9
Lagenaria siceraria **A**	0.1	13.8	11	0.9			22.1	0.2		51.8	0.1	100
Lagenaria siceraria **C**		13.3	9.7	0.5			20.1	0.2	0.1	55.9	0.1	99.9
Lagenaria siceraria **D**		14	9.8	0.7			21.4	0.3		53.8	0.1	100.1
Cucurbita spp **A**	0.1	15.7	10.2	0.8			35.4	0.3		37.4	0.2	100.1
Cucurbita spp **C**	0.1	13.9	8.4	0.6	0.2		45.3		0.1	31.3	0.1	100

Table 3. Protein content (%) of ingredients from Ghana assessed for potential use in tilapia diets

Sample Description	**Protein %**
African Locust Seeds A1-1	28.7
African Locust Seeds A1-2	30.4
African Locust Seeds A1-3	30.8
Egushi Seeds A2-1	36.6
Egushi Seeds A2-2	36.1
Egushi Seeds A2-3	36.6
Eggplant Seeds B1-1	37.0
Eggplant Seeds B1-2	37.3
Eggplant Seeds B1-3	37.6
Calabash Seeds B2-1	28.1
Calabash Seeds B2-2	25.5
Calabash Seeds B2-3	29.6
Kidney Beans B3-1	25.5
Kidney Beans B3-2	24.7
Kidney Beans B3-3	24.4
Cucumber Seeds B4-1	39.1
Cucumber Seeds B4-2	38.1
Cucumber Seeds B4-3	38.4
Neri Seeds B5-1	27.1
Neri Seeds B5-2	25.4
Neri Seeds B5-3	25.8
Sesame Seeds B6-1	21.1
Sesame Seeds B6-2	20.8
Sesame Seeds B6-3	20.9
Range: 21 - 39% protein	

Table 4. Amino acid composition of ingredients from Ghana assessed for potential in tilapia diets

Units	**W/W%**	**W/W%**	**W/W%**	**W/W%**	**W/W%**	**W/W%**	**W/W%**	**W/W%**	**W/W%**	**W/W%**	**W/W%**	**W/W%**	**W/W%**	**W/W%**	**W/W%**	**Reference 1**	**Reference 2**
uapb	Citriullus anatus	Citriullus lanatus	Cucumis metuliferus	Cucumis metuliferus	Cucurbita	Cucurbita	Lagenaria sicerana	Lagenaria sicerana	Lagenaria sicerana	Parika biglobosa	Parika biglobosa	Sesamum indicum	Sesamum indicum	Cucumeropsis mannii	Cucumeropsis mannii	Soybean meal	fish meal (64.5% protein)
Taurine	0.09	0.06	0.12	0.12	0.10	0.08	0.08	0.08	0.09	0.19	0.25	0.04	0.04	0.04	0.03	Not detected	3.55
Hydroxyproline	0.04	0.03	0.06	0.05	0.06	0.04	0.06	0.05	0.05	0.05	0.07	0.03	0.03	0.03	0.03		
Aspartic Acid	1.97	2.11	2.43	2.20	3.20	2.92	2.89	2.61	2.72	2.84	2.68	1.69	1.72	2.42	3.49		
Threonine	0.68	0.73	0.88	0.78	0.86	0.85	1.02	0.91	0.96	0.89	0.86	0.72	0.73	0.87	1.31	2.00	2.64
Serine	0.90	0.97	1.21	1.07	1.49	1.46	1.32	1.15	1.22	1.05	0.98	0.81	0.84	1.08	1.52		
Glutamic Acid	3.55	3.68	4.92	4.52	6.32	5.87	5.33	4.73	5.17	4.63	4.30	3.66	3.81	4.59	6.55		
Proline	0.72	0.79	1.00	0.91	1.11	1.10	1.14	1.00	1.06	1.23	1.14	0.68	0.70	0.94	1.42		
Lanthionine	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00		
Glycine	1.33	1.39	1.65	1.52	2.32	2.05	1.89	1.74	1.78	1.24	1.19	1.10	1.11	1.60	1.96		
Alanine	1.00	1.04	1.35	1.25	1.41	1.37	1.43	1.31	1.37	1.24	1.17	0.96	0.99	1.26	1.71		
Cysteine	0.31	0.33	0.41	0.38	0.45	0.41	0.44	0.40	0.41	0.26	0.29	0.38	0.40	0.37	0.49		
Valine	0.99	1.04	1.38	1.32	1.63	1.60	1.50	1.40	1.46	1.38	1.28	1.06	1.08	1.36	1.79	2.70	3.03
Methionine	0.57	0.62	0.51	0.47	0.73	0.70	0.82	0.74	0.79	0.21	0.20	0.55	0.58	0.72	0.87	0.70	1.77
Isoleucine	0.86	0.89	1.21	1.17	1.29	1.29	1.24	1.17	1.23	1.19	1.10	0.82	0.84	1.16	1.55	2.60	2.57
Leucine	1.46	1.53	2.04	1.90	2.23	2.24	2.11	1.92	2.02	1.98	1.84	1.39	1.43	1.88	2.78	3.80	4.54
Tyrosine	0.52	0.61	0.85	0.68	1.19	1.08	0.87	0.74	0.76	0.63	0.62	0.51	0.54	0.71	1.19	1.25	2.04
Phenylalanine	1.17	1.28	1.34	1.19	1.61	1.63	1.74	1.58	1.65	1.29	1.20	0.94	0.97	1.51	2.01	2.70	2.51
Hydroxylysine	0.05	0.05	0.05	0.03	0.04	0.03	0.03	0.03	0.02	0.03	0.03	0.01	0.01	0.03	0.07		
Ornithine	0.01	0.00	0.01	0.01	0.04	0.02	0.03	0.02	0.01	0.02	0.01	0.01	0.01	0.02	0.03		
Lysine	0.81	0.74	1.15	1.03	1.64	1.46	1.06	1.05	1.07	1.93	1.81	0.63	0.64	0.94	1.21	2.24	4.81
Histidine	0.53	0.56	0.73	0.66	0.81	0.76	0.82	0.75	0.79	0.77	0.73	0.52	0.53	0.72	0.96	1.30	1.78
Arginine	3.00	3.30	4.43	3.91	5.31	5.02	4.93	4.31	4.65	1.78	1.64	2.43	2.54	4.16	5.71	3.60	3.66
Tryptophan	0.38	0.39	0.51	0.48	0.58	0.57	0.54	0.44	0.51	0.11	0.11	0.30	0.31	0.47	0.70	0.70	0.66
Total	20.94	22.14	28.24	25.65	34.42	32.55	31.29	28.13	29.79	24.94	23.50	19.24	19.85	26.88	37.38		
Crude Protein	23.80	24.16	31.06	28.14	39.00	35.28	34.60	30.48	33.22	27.17	27.22	21.16	21.69	30.18	40.01		

Table 5.Composition (% inclusion levels) of experimental diets with different plant lipid sources fed to juvenile *Oreochromis niloticus*

Feed Ingredient	100%Lin (Diet 2)	100%Soy (Diet 3)	LinSoy (Diet 4)	LinPalm (Diet 5)	PalmSoy (Diet 6)
Fishmeal	20	20	20	20	20
Soybean meal	33	33	33	33	33
Wheat bran	34.5	34.5	34.5	34.5	34.5
Soy oil	-	9	4.5	-	4.5
Linseed oil	9	-	4.5	4.5	-
Palm oil	-	-		4.5	4.5
Vitamin & mineral premix	1.5	1.5	1.5	1.5	1.5
Binder	2	2	2	2	2

Table 6. An eight-week growth of juvenile *Oreochromis niloticus* fed experimental diets with different plant lipid sources

Variables	Control	100%Lin	100%Soy	LinSoy	LinPalm	PalmSoy
Av. Iintial Wgt (g)	30.2±2	28.45±1	30.1±1	28.0±4	30.4±1	30.7±4
Av.Final Wgt (g)	58.3±2	47.3±1	53.3±1	50.1±4	50.7±1	52.1±3
Av.Wgt Gain (%)	92.4 ± 2^{a}	66 ± 2^{b}	75.7 ± 2^{b}	79.7 ± 1^{b}	70.3 ± 3^{b}	69.2 ± 1^{b}
Specific.Grt Rate(%/day)	1.32 ± 0.3^{a}	1.02 ± 0.2^{b}	1.1 ± 0.2^{b}	1.2 ± 0.2^{ab}	1.1 ± 0.2^{b}	1.1 ± 0.0^{b}
FCR	1.2 ± 0.1^{a}	1.6 ± 0.2^{b}	1.49 ± 0.4^{b}	1.40 ± 03^{b}	1.62 ± 0.2^{b}	1.58 ± 0.6^{b}
Survival (%)	100	98	100	100	98	100

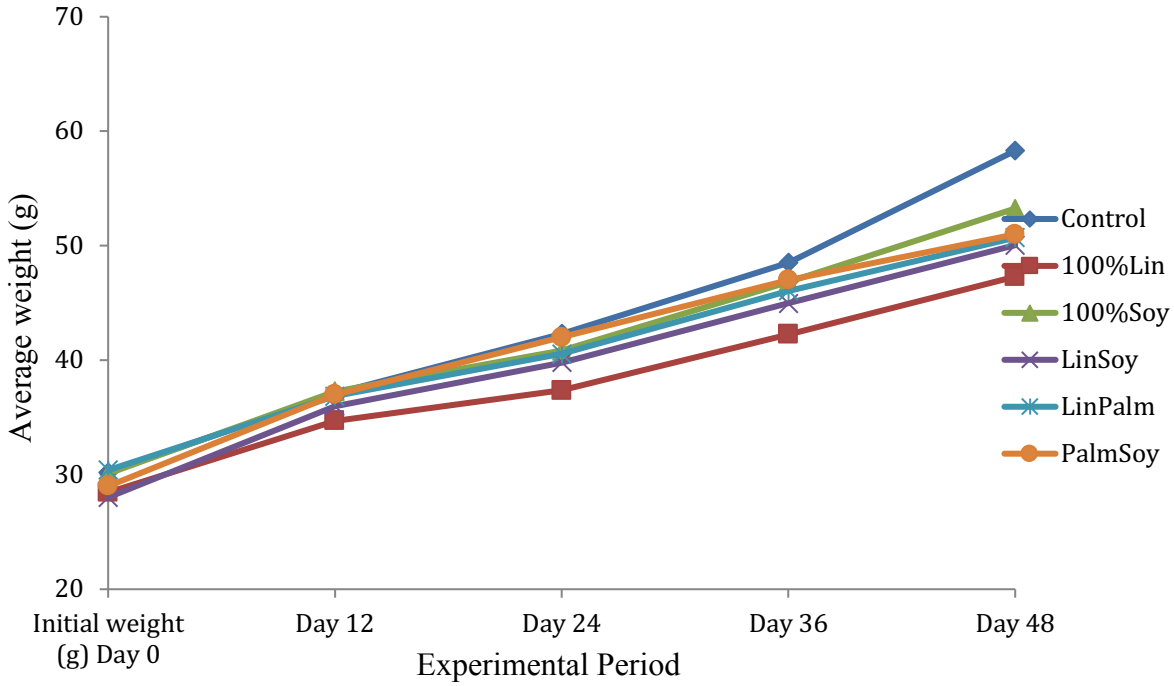

Figure 1. Growth (g) (means ±SD) of juvenile *Oreochromis niloticus* fed experimental diets with different plant lipid sources

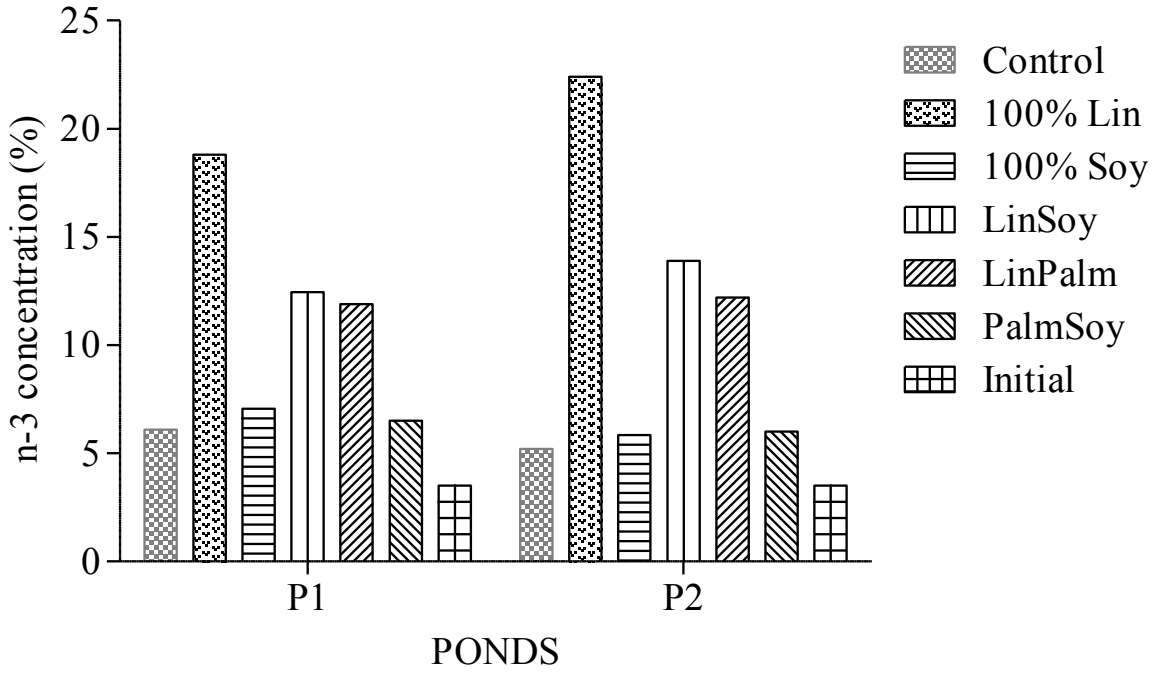

Figure 2. Omega-3 concentration (%) in tissues of juvenile *Oreochromis niloticus* at the end of an 8-week feed trial after being fed with different plant lipid sources