Develop Feeding Strategies for *Moringa Oleifera* and *Leucaena Leucocephala* as Protein Sources in Tilapia Diets

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ABSTRACT

Digestibility and feeding trials were performed to evaluate *Moringa oleifera* and *Leucaena leucocephala* leaf meals as protein sources (compared to soybean meal) in Nile tilapia diets. Both leaf meals were obtained from Tanzania. The leaf meals were soaked in water to reduce anti-nutritional factors, dried, and ground to a small particle size before incorporation into diets. Five diets were made for both trials: the control diet contained 50% soybean meal (SOY), and diets 15 MOR and 30 MOR were made by substituting 15 and 30% of the soybean protein with *Moringa* protein. Diets 15 LEU and 30 LEU were made by substituting 15 and 30% of the soybean protein with *Leucaena* protein. For the digestibility trial, diets containing 32% crude protein were used. Diets with 36% protein were used for the feeding trial due to the small initial fish size. The digestibility trial was carried out using mixed sex Nile tilapia of 200-400 g in 150-L indoor tanks in a recirculating system with dechlorinated municipal water maintained at 28^0C. Other water quality parameters were kept at optimum levels for Nile tilapia. Fish were conditioned to the experimental diets and the fecal removal process (fresh, floating feces were collected with a net) for 1 week. Fecal collection was conducted 8-10 hours after the last meal in the evening, as well as the next morning before feeding. The fecal samples were dried at 50^0C for 12 hours and then frozen until analysis. The apparent protein digestibility (APD, mean±SE, %) of the SOY diet (86.35±0.87) was higher than all others. The APD of the 15 MOR (84.69±0.59) and 30 MOR (83.34±1.10) diets were similar to each other and higher than the APD of the 15 LEU (78.49±0.91) and 30 LEU (74.70±0.52) diets. The APD of 30 LEU was also lower than that of the 15 LEU diet. Overall, leaf meals reduced protein digestibility compared to soybean meal, but diets containing *Moringa* were digested better than those containing *Leucaena*. There was no difference in the apparent lipid digestibility (%) of the SOY (95.83±0.34) and 15 MOR (95.19±0.62) diets. Lipid digestibility of the SOY diet was higher than that of 30 MOR (92.99±1.17), 15 LEU (94.46±0.06), and 30 LEU (92.98±0.24) diets. Lipid digestibility of the 30 MOR and 30 LEU diets was lower than that of the other diets. Both protein and lipid digestibility were inversely related to concentration of dietary fiber. For the feeding trial, 100 mixed-sex Nile tilapia, averaging 5.16 g individually were stocked in 1500-L outdoor circular plastic tanks supplied with reservoir water. The

tanks were maintained as static systems except for periodic flushing when water quality parameters fell below the optimum for Nile tilapia. Subsamples of 30-50 fish were weighed every two weeks and water quality parameters were monitored weekly. The trial was terminated after 60 days. There were no differences in growth, feed conversion, survival, lysozyme, or proteolytic enzyme activity among treatments. Proximate and fatty acid composition of whole fish is still in progress.

INTRODUCTION

Over the last 30 years, aquaculture has grown worldwide faster than any other animal production sector (FAO 2007a). The average annual growth rate of aquacultural production was 10% compared with 3% in the cattle industry and 1.6% in captured aquatic species from natural environments. The rapid rise in aquaculture has generated an increase in the production of aquatic animal feeds. The estimated increase in production of aquaculture feeds from 2000 to 2010 was from about 13 million metric tonnes (mmt) to 30 mmt (Francis et al. 2001). Securing raw materials for these feeds will be a continuous challenge for the aquaculture industry.

In Africa, economic studies have demonstrated that fish farming is a viable enterprise combining high gains with minimal costs (Molnar et al. 1991). Wijkstrom and MacPherson (1990) indicated that large-scale and intensive aquaculture enterprises are beyond the means of most farmers in Africa, but small-scale aquaculture with commercial orientation can be a profitable economic activity.

Some of the problems that make tilapia farming in Tanzania unprofitable include low productivity resulting from poor management, and low seed and feed quality (FAO 2006). Low productivity has a nutritional component due to the imbalance of energy and protein in fish diets commonly used by small-scale farmers. In Tanzania, farmers use naturally available feeds or feedstuffs in fish culture. Ponds are commonly fertilized with domesticated animal droppings or tender leaves as compost manure to stimulate plankton growth. The most frequently used feeds are rice and maize bran, kitchen leftovers, and garden remains. These are of low quality, and fish reared on these feeds are unable to meet their maintenance and production requirements, especially for protein. This prolongs the time to reach market weight and consequently leads to production of poor-quality fish and hence, low profitability of fish farming.

In formulation of fish feeds, the nutrient requirements of fish, as well as the nutrient composition of feedstuffs, price, palatability quality, and availability of various feedstuffs should be considered (De Silva and Anderson 1998). Protein is a critical component in complete fish diets and is always given a high priority in formulation of complete feeds. Protein inadequacy may lead to poor growth and generally inferior performance. Protein is also usually the most expensive component of fish feeds, accounting for more than 50% of total feed costs in intensive aquaculture (Thompson et al. 2005). In semi-intensive systems with abundant natural foods, the protein content of supplementary feeds may be reduced and the carbohydrate content increased (Hepher 1988).

Consequently, efforts have been shifted to evaluating potential alternative protein sources for use in fish diets. Alternative protein sources include soybean meals, protein concentrates (Refstie et al. 1998), meat meals (Williams et al. 2003) and blood meals (Bureau et al. 1999). Soybean meal has been the focus of attention for fish meal substitution because of its advantage in terms of protein quality, competitive price and adequate supply. However, soybean meals and agro-industrial by-products have not been readily

adopted by small-scale fish farmers in sub-Saharan Africa due to cost and limited supplies. There is a need to identify less expensive alternative sources of protein from locally available feed resources and to select protein sources that do not conflict with human food security interests (El-Sayed 1999; El-Saidy and Gaber 2002). For instance, fish meal, soybean meal, meat meal and blood meal are likely to be reserved for human rather than animal diets in Tanzania.

Leguminous tree leaves and their pods seem to be appropriate alternative protein sources to fish meal and soybean meal (Fernandes et al. 1999; El-Saidy and Gaber 2003; Kaushik et al. 2004). In particular *Leucaena leucocephala* (Pantastico and Baldia 1980; Ferraris et al. 1986; Santiago and Lovell 1988) and *Moringa oleifera* leaf meals (Richter et al. 2003; Afuang et al. 2003) may lower feed costs and increase the profitability of fish farming enterprises because leaf meals are cheaper than fish meal or soybean meal.

Moringa oleifera (hereafter referred to as *Moringa*) has positive agronomic attributes such as the ability to resist adverse soil and climatic conditions and yet sustain a reasonably high yield (Palada and Chang 2003). More than 11,300 hectares of *Moringa* trees have been planted in various parts of Tanzania, primarily for its oil seeds (Creighton, 2001). The potential of *Moringa oleifera* as an ingredient in fish feed formulations lies in its local availability, affordability and relatively good nutritional profile. Another advantage is its multiple uses, which potentially could serve as a source of additional income to the farmers. For instance, oil from *Moringa* seeds is used in perfumes and lubrication of fine machinery, while powder from the seed kernel has coagulant properties which can be used to clarify turbid water (Palada and Chang 2003).

The amino acid pattern of *Leucaena* (hereafter referred to as *Leucaena*) is comparable to that of soybean and fish meal (Ter' Meulen et al. 1979) or other animal feed sources available in developing countries (D'Mello and Thomas 1977; Ter' Meulen et al. 1979; Kale et al. 1987). The protein concentration in *Leucaena* is about 23.5-31.5% (Kimbi 1997; El-Hassan et al. 2000; Kimoro 2003). *Leucaena* is now being grown in most parts of Tanzania due to its outstanding nutritional value to ruminants ((1994). Overall, the information on the feeding value of *Leucaena* and *Moringa* is not conclusive; particularly, the digestibility and the effects of *Leucaena* and *Moringa* on overall performance and enzyme activity in Nile tilapia. Hence there is a need to find out the extent to which these leguminous tree leaves can replace fish meal or soybean meal as protein sources in small-scale tilapia production.

The greater abundance of vegetable products has attracted research interest as ingredients for fish feed production (El-Sayed 1999). The use of raw vegetables is limited, however, by their antinutritional factors (Table 2) which are grouped into three categories: (1) those affecting protein utilization and digestion; (2) those affecting mineral utilization; and (3) anti-vitamins and toxic substances (Francis et al. 2001). Higher replacement levels (>50%) of fish meal or soybean meal by plant ingredients can also reduce palatability (Hassan et al. 1997), nutrient utilisation (Eusebio et al. 2004), growth (Eusebio and Coloso 2000), or cause poor reproductive performance (Santiago and Lovell 1988).

In this study we hypothesize that the replacement of soybean meal with *Leucaena* leaf meal and *Moringa* leaf meal as protein sources in fish diets may lower feed costs and hence increase the profitability of fish

farming enterprises. The major objective of this study is to evaluate the feeding value of *Leucaena* and *Moringa* leaf meals as protein sources in Nile tilapia diets as assessed by the following sub-objectives:

1) To compare feeding levels at 5, 7.5, and 10% of body weight on growth performance and feed conversion efficiency of tilapia fed diets containing sunflower seed cake, Moringa oleifera and Leucaena leucocephala as protein sources (Sokoine University of Agriculture, Tanzania).
2) To compare alternate day feeding with daily feeding on growth performance and feed conversion efficiency of tilapia fed diets containing sunflower seed cake, Moringa oleifera and Leucaena leucocephala as protein sources (Sokoine University of Agriculture, Tanzania).
3) To evaluate the digestibility of *Moringa oleifera* and *Leucaena leucocephala* leaf meals using mixed sex Nile tilapia *Oreochromis niloticus* (University of Arkansas at Pine Bluff, USA).
4) To evaluate the effect of feeding *Moringa oleifera* and *Leucaena leucocephala* on growth performance, feed conversion ratio, survival, proximate and fatty acid composition of whole body, nonspecific immune responses, and proteolytic enzyme activity of mixed sex Nile tilapia (University of Arkansas at Pine Bluff, USA).

METHODS

Objectives 1 and 2

These objectives were being achieved from studies in Tanzania at Sokoine University of Agriculture (SUA). A preliminary study first developed ten different diets from three protein sources, i.e., soybean, sunflower seed cake and *Moringa* leaf (Table 1). The diets were then tested at different inclusion levels of the three protein sources. Preliminary results suggest Diet 3 (Table 1) performed better compared to the other diets, and consequently is being used in experiments to address objectives 2 & 3 in pond feeding trials using SUA's standard protocols. These experiments did not begin on schedule due to funding delays, and could not be conducted concurrently due to limitations on numbers of ponds available for the trials. The expected completion date for these objectives is April, 2012.

Objectives 3 & 4

The study consisted of two different trials: 1) A digestibility trial; and 2) A feeding trial. The digestibility trial was carried out in a recirculating system in indoor tanks in the UAPB fish nutrition wet lab, and the growth trial was conducted in a static system in outdoor tanks at the UAPB aquaculture research station. After the trials were completed, all live fish were returned to the UAPB aquaculture research station.

Source of fish

Oreochromis niloticus (2500) from stocks maintained at the UAPB aquaculture station were used for the digestibility study, and smaller fish for the growth trial were obtained from a commercial farm in Alabama.

Source and preparation of Leucaena and Moringa meals

The test ingredients (*Moringa* and *Leucaena*) were obtained from Tanzania. Prior to shipment to the US, Sebastian Chenyambuga (Soloine University, Tanzania) soaked the leaves in water to reduce antinutrients and sun-dried them. Upon arrival at UAPB, the leaf meals were soaked again (for 3 days at room temperature) to further reduce mimosine (Hassan et al. 1994; Wee and Wang 1987), a toxin in *Leucaena*, and saponins in Moringa (Tacon 1985). During soaking at UAPB, the leaf meal-water mixtures were stirred for 1 h daily. After three days of soaking, the mixtures were filtered through a 0.5-mm sieve. The

residue was fan-dried for 24 h and then lyophilized for 60 h using an MD3053 model freeze drier (Mill Rock Technologies., San Diego, California). The dried leaves of *Moringa* and *Leucaena* were then finely ground before incorporation into diets.

Proximate composition of Moringa and Leucaena meals

Protein, crude fiber, dry matter and ash content of the two leaf meals, formulated diets, feces, and whole fish from the feeding trial were analyzed according to Standard Methods (AOAC 1995). The Folch method (Folch et al. 1957) was used to analyze the total lipids. The Kjeldahl method (AOAC 1995) was used for crude protein, and the Ankom 200 fiber analyzer (Ankom Technology Corp., Fairport, New York) was used for crude fiber. Lipid extracts from the leaf meals, diets, and whole body will be used for fatty acid analysis (leaf meals and diets only). Fatty acid methyl esters (FAME) were analyzed (Morrison and Smith 1964) using a flame ionization gas chromatograph (Varian, Model CP-3800 fitted with a CP-8200 autosampler, Walnut Creek, CA) with helium as the carrier gas. The FAMEs were separated on a fused silica capillary column (15m x 0.25 mm ID; Varian CP select for Fame #CP8510). 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. Each sample had a total analysis time of 60 min. The FAMEs were identified and quantified by comparing the retention time and peak area to those of serially diluted mixtures of reference standards (GLC-96, GLC-473b, Nu-Check Prep, Elysian, MN). The results of the individual fatty acids were expressed as g/100g of total identified FAMEs.

Diet composition

Five isonitrogenous (32% crude protein) and isocaloric diets (18 KJ/g) were formulated for the digestibility trial, whereas diets for the feeding trial contained 36% protein because smaller fish were used. The protein-to-energy ratio of the diets will be 100 mg of protein per Kcal of energy (Suresh 2003). The reference diet contained soybean meal as the primary protein source (Table 3). Four other diets contained the alternative protein sources *Leucaena* or *Moringa***.** These ingredients replaced soybean meal on an equal protein basis, at 15% or 30%. This resulted in a total of five diets: diet 1 was the reference diet, and 15 % and 30% of the protein in soybean meal in the reference diet was replaced with *Moringa* to make diets 2 and 3. Diets 4 and 5 were produced by replacing 15% and 30% of the protein in the soybean meal diet with the protein in *Leucaena*. Similar diet formulas (with the exception of protein amount) were used for both the digestibility and feeding trials. For the digestibility trial chromic oxide was added as an inert marker, and for the growth trial, chromic oxide was replaced by wheat middlings.

Preparation of diet

All ingredients were finely ground (1-2 mm) in a Wiley mill at UAPB prior to inclusion in diets. The diets were prepared in the fish nutrition lab at UAPB by slowly adding the micro-ingredients (vitamin and mineral premixes) to the macro-ingredients to ensure a homogenous mixture. Between 400 and 450 mL of distilled water was added per kilogram of diet to achieve a consistent mixture that produced stable pellets. A meat grinder fitted with 6.25- or 3.125-cm dies was used to produce different sized pellets, which were fan dried for 8 h and stored at -18^{o}C until use. The bigger pellets were used for the digestibility trial.

Digestibility trial

Mixed sex fish (N=10), weighing 200-400 g in individual weight were stocked in each 150-L tank. The gender of the fish was manually determined and a fixed gender ratio of 9 males to 1 female was used in

all tanks. Three replicate tanks were used per diet. Fish were maintained in a recirculating system supplied with dechlorinated municipal tap water. The temperature was maintained near 28˚C, which is considered the optimal temperature for growth for *Oreochromis niloticus* (Popma and Masser 1999). Temperature will be recorded daily. The flow rate in each tank was set at 1.1 L/min, which resulted in the total replacement of tank water volume every 2 hours to ensure proper clearance of ammonia. Each tank had individual water and air valves and was aerated by individual air stones. The water quality parameters monitored were pH (UB-10 pH/ mV meter, Denver Instruments, Colorado), total hardness (EDTA/ManVer method), dissolved oxygen (YSI 55, YSI Incorporated, Yellow springs, Ohio), and total ammonia nitrogen (TAN) (salicylate/cyanurate method, pH adjusted to 7, DR/890 colorimeter/high range Test'N® Tube, Hach Company, Loveland, Colorado). Equations from Emerson et al. (1975) were used to calculate the percentage of unionized ammonia from total ammonia. Calcium chloride was added to the water to maintain hardness $\geq$ 50 mg/L as calcium carbonate. The fish were fed their respective diets to apparent satiation once daily for 5-7 days prior to collection of feces to allow them to adjust to the diets.

Sampling feces

We attempted stripping feces from the fish, but they were apparently stressed by the procedure and refused feed for days after each stripping period. Therefore, evacuated feces were collected by siphoning each day for a week instead. Fecal collection was conducted 8-10 hours after the last meal in the evening, as well as the next morning before feeding. All feces collected from each tank were combined in the same aluminum pan and frozen between collections. Once sufficient feces were collected (>10grams per tank), the pans containing feces were dried in the oven at 50^0C for 12 hours, and then stored at -20^0C until analyzed.

Calculation of apparent digestibility

We assumed that there was no variation in the content of chromic oxide in the feces. Chromic oxide concentrations in the diets and the feces were determined by AOAC (1995) methods.

The concentration factor (CF) was determined by the method of Sugiura (2000):
CF= chromic oxide concentration in feces / chromic oxide concentration in diet.
The CF indicates a portion of the feces that corresponds to a unit amount of the diet, therefore the nutrient content in the feces was divided by the CF.
Digestibility (%) = 100X {nutrient concentration in the diet - Concentration of nutrients in feces/CF}/nutrient concentration in the diet.

Growth trial

Stocking density and feeding for the growth trial

The growth trial was conducted using mixed-sex fish, but the fish were too small to identify gender prior to stocking so fixed ratios of males and females could not be achieved. Individual initial mean weight of fish was 5.2$\pm$0.02. One hundred fish were stocked into 20 1500-L outdoor tanks (4 tanks per diet) in a static system supplied with reservoir water. Fish were fed twice daily to satiation for 60 days. Water quality parameters were monitored as described for the digestibility trial. In addition, chlorophyll a (chloroform–methanol extraction, Lloyd and Tucker 1988) was measured in the feeding trial. When water quality in any tank fell below acceptable limits for Nile tilapia, (> 0.05 ppm un-ionized ammonia, > 8 or < 6.5 pH), 50% of the water in all the pools was flushed with fresh water to restore the water quality to acceptable conditions. Each tank was filled separately with a tap and had an individual stand pipe for

drainage and air stone for aeration. Subsamples of fish were weighed every two weeks throughout the trial to assess growth and adjust feed rations. Feed intake and mortalities were recorded daily. The average protein deposition was calculated by determining the difference in the protein content of fish before and after the growth trial. The average protein intake was calculated as the dry protein in feed (g) in the feed consumed. At harvest, the overall growth, feed conversion, survival, non-specific immune function, digestive enzyme activity, and body composition were evaluated.

Determination of growth rate and individual weight gain
Growth rate = 100X (final weight-initial weight) / days in cycle.
The mean individual weight gain = mean final individual weight-mean initial individual weight.

Determination of feed conversion ratio (FCR)
FCR= Feed consumed (g of dry matter) / live weight gain (g).

Determination of apparent net protein utilization (ANPU)
ANPU= Average protein deposition/ average protein intake.

Lysozyme activity
Blood samples (1mL) were obtained from 3 randomly selected fish per tank at the end of the growth trial. Blood was drawn from the caudal vasculature using heparinized syringes. The blood plasma was assayed for lysozyme activity (Hutchinson and Manning 1996). Bled carcasses were frozen for later proximate analyses.

Digestive proteolytic enzyme activity
Three additional fish per tank were used for the proteolytic enzyme activity assays on whole intestines at the end of the growth trial. The fish were anaesthetized by adding tricaine methanesulfonate at a rate of 30 mg/L and then killed by cervical separation, about 24h after the last meal. The guts were removed by dissection, placed on ice, and uncoiled. The stomachs were excised. The whole intestine was then frozen in liquid nitrogen. The entire procedure for each fish was completed in about 3 min and the samples were stored at −80°C until analyzed. Frozen intestine were homogenized by sonification for 1 min, and centrifuged at 9,400×*g* for 2 min at 4°C. Following centrifugation, the homogenate was collected and stored in small aliquots (100–200 µl) at −80°C until just before use in colorimetric assays of proteolytic enzyme activities. All pH values listed for buffers were measured at room temperature (22°C), and all reactions were run at saturating substrate concentrations as determined for the proteolytic enzyme at 4°C. Blanks consisting of substrate only and homogenate only (in buffer) were conducted simultaneously to account for endogenous substrate and/or product in the tissue homogenates and substrate solutions (German et al. 2009).

Total proteolytic activity
Total protease activity was quantified by detection of primary amines resulting from proteolysis. Succinylated casein was used as a substrate for protein hydrolysis by all proteases within the intestine and compared to trypsin standards. Hydrolysis of the casein substrate results in the release of peptide fragments with free amino-terminal groups. These peptides react with trinitrobenzene sulfonic acid, which forms a colorimetric reaction and formation of yellow trinitrobenzene-peptide products. The color

intensity was measured at 540 nm and was directly proportional to the enzyme activity of proteases in the sample (Bubnis and Ofner 1992; Hatakeyama et al. 1992).

Proximate and fatty acid analyses of the whole body

The body composition of 5 individual fish was analyzed initially before starting the growth trial in outdoor tanks, and after the trial 3 fish per tank (12 per diet) were reserved from the health assays for proximate and fatty acid analysis. The reserved fish were finely ground to get one homogenous sample per tank and subjected to the same procedures described in the digestibility trial, except that fiber analysis was not performed on fish.

Statistical analysis of the digestibility trial data

The mean data per replicate for digestibility, body composition, weight gain (growth rate), survival, PDC, FCR, ANPU, lysozyme activity, and proteolytic enzyme activity will be analyzed by one way Analysis of Variance (ANOVA) with StatView (SAS Institute Inc., Cary, North Carolina) to test for differences among experimental groups. When the differences among treatment means are significant ($P \leq 0.05$ for the indoor digestibility trial; $P<0.01$ for the outdoor feeding trial), Fisher's least significant difference test was used to identify specific treatment differences. Water quality data from the feeding trial was analyzed using repeated measures ANOVA.

RESULTS

Objective 3

Digestibility trial

The apparent protein digestibility (APD, mean±SE, %) of the SOY diet (86.35±0.87) was higher than all others. The APD of the 15 MOR (84.69±0.59) and 30 MOR (83.34±1.10) diets were similar to each other and higher than the APD of the 15 LEU (78.49±0.91) and 30 LEU (74.70±0.52) diets. The APD of 30 LEU was also lower than that of the 15 LEU diet. Overall, leaf meals reduced protein digestibility compared to soybean meal, but diets containing *Moringa* were digested better than those containing *Leucaena*. There was no difference in the apparent lipid digestibility (%) of the SOY (95.83±0.34) and 15 MOR (95.19±0.62) diets. Lipid digestibility of the SOY diet was higher than that of 30 MOR (92.99±1.17), 15 LEU (94.46±0.06), and 30 LEU (92.98±0.24) diets. Lipid digestibility of the 30 MOR and 30 LEU diets was lower than that of the other diets. Both protein and lipid digestibility were inversely related to concentration of dietary fiber.

Objective 4

Feeding trial

Tank 38 was excluded from analysis of growth performance data because we discovered that the tank was not stocked with 100 fish originally, so fish density was lower and fish in that tank were much larger than other fish fed that diet.

Mean individual weight gain ranged from 30.4-34.7 grams, feed conversion ratio ranged from 1.6-1.9, survival ranged from 91.8-97.3%, and there were no differences among diets. Lysozyme activity ranged from 13.1-14.8 units/25 µl plasma and there were no differences among diets. Total proteolytic enzyme activity ranged from 38.8-47.5 µmol/g tissue and there were no diet effects. However, fish size had a significant effect on enzyme activity, and just by chance larger fish were randomly selected from diet 1 replicates for enzymatic analysis. There was a tendency toward decreasing enzyme activity with

increasing fish size, but further analysis is required to interpret this result. Proximate and fatty analysis of whole fish from the feeding trial is still in progress.

DISCUSSION

Objectives 3 & 4

The most likely explanation for reduced nutrient digestibility of the leaf meals compared with soybean meal is the higher fiber content of the leaf meals. Fiber is indigestible to monogastric animals such as fish and can reduce the overall energy and essential nutrients available from the diet (NRC 2011). Despite differences in nutrient availability among diets, fish growth, feed conversion, survival, lysozyme activity, and proteolytic enzyme activity were similar among treatments in the feeding trial. It is likely that the tilapia compensated for any nutrient deficiencies by consuming algae and other natural foods in the outdoor tanks. It is worthy of note that the tilapia were able to make up for diet differences even at a young age, and young fish are usually more sensitive to diet differences than larger fish. The results look promising for increasing use of *Moringa* and *Leucaena* in tilapia diets to reduce diet cost and improve profitability, but the diets need to be tested in a longer study where fish are grown to market size. In addition, economic analysis is needed to help identify the most cost-effective diets for tilapia production.

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TABLE 1: - The % composition of 10 diets using different ingredients

	Diets								
Ingredients	**D1**	**D2**	**D3**	**D4**	**D5**	**D6**	**D7**	**D8**	**D9**
SFC	84	66	41	21	0	0	0	0	0
SBM	0	0	0	0	0	25	24	20	13
MLM	0	22	41	63	80	0	7	20	39
HM	10	6	12	10	14	69	63	54	42
SFO	3	3	3	3	3	3	3	3	3
MIN.	1	1	1	1	1	1	1	1	1
WM	2	2	2	2	2	2	2	2	2
Total	**100**	**100**	**100**	**100**	**100**	**100**	**100**	**100**	**100**

SFC = Sunflower seed cake, SBM= Soybean meal, MLM= Moringa leaf meal, HM= Hominy meal, SFO= Sunflower oil, MIN= Mineral, WM= Wheat meal

TABLE 2. —The major categories of antinutritional factors (Tacon 1985).

Group	Antinutritional factor
Proteins	Protease inhibitors, hemagglutinins.
Glycosides	Goitrogens, cyanogens, saponins, estrogens.
Phenols	Gossypol, tannins.
Miscellaneous	Anti-minerals (e.g. phytic acid), anti-vitamins, anti-enzymes, food allergens, microbial/plant carcinogens, toxic amino acids

TABLE 3. —Composition of the reference diet for a digestibility trial with Nile tilapia. The control diet for the feeding trial was similar but contained 36% protein and wheat midds in place of chromic oxide.

Ingredients	Percentage inclusion
Soybean meal	50.00
Cottonseed meal	7.50
Corn	16.50
Wheat middlings	22.00
Fish oil/soy oil (1:1)	2.00
Vitamin mixture[a]	1.00
Mineral mixture[a]	1.00
Chromium oxide[b]	0.005

[a] Same as Moon and Gatlin (1991).
[b] Chromic oxide will be purchased from Sigma-Aldrich Corp., St. Louis, Missouri.