

Formulation and Manufacture of Practical Feeds for Western Kenya

Sustainable Feed Technology and Nutrient Input Systems/Experiment/13SFT07AU

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ABSTRACT

Information about proximate composition of local feed ingredient for farm made fish feed is usually limited and not reliable. Farmers depend on the existing information about the feed composition given by different fish feeds manufacturers. Unbalanced dietary amino acid contents could result in an increased deamination and can increase ammonia levels released in water. This study intended to formulate on farm diets using locally available ingredients and balancing the Essential Amino Acids (EAA) to enhance both the physical quality and nutritive value for culture of *Oreochromis niloticus*. The study tested the four diets, comprising methionin+lysine and lysine supplemented diets at 24.5 g kg⁻¹ to non-EAA supplemented and commercial tilapia diets, for 105 days in hapas installed in earthen ponds at the University of Eldoret Fish Farm from June to September 2015. The practical diets consisted of 48% wheat bran, 30% freshwater shrimp, 18% cotton seed meal, 2% fish oil, and 1% vitamin/mineral premix. The four diets were tested for growth performance of *O. niloticus* for 105 days in 1 m³ hapas in three earthen ponds, each measuring 10 m by 15 m (150 m²) in a complete randomized block design. Even though there were significant variations in temperature and pH, the values were still within suitable range for tilapia at 18°C to 20°C and 7.2 to 7.0. Dissolved oxygen (DO) did not vary significantly over the period (4.8 to 6.2 mg L⁻¹). All experimental test diets had 30% crude protein (CP) before EAA supplementation. The diets were estimated to provide about 17.17 MJ kg⁻¹ with about 22.9% digestible CP level, 8.03% ash, and 90.7% dry matter (DM). Diet 2 with lysine supplement exhibited better growth than all the other diets with a Phi prime (Ø') of 3.441, Body Weight Gain (BWG) of 289.8, Average Daily Weight Gain (DWG) of 1.92, Specific Growth Rate (SGR) of 5.4, Food Conversion Ratio (FCR) of 1.24, and Protein Efficiency Ratio (PER) of 2.68. The results show there is a high potential for on-farm fish feed formulation and processing, and the present knowledge will benefit more than 1000 fish farmers in formulation nutritionally balanced diets to improve growth and production of tilapia in Western Kenya. The present protocol will also be adopted in the new hatchery under construction at the University of Eldoret to provide quality fingerlings to more than 600 fish farmers in Uasin Gishu County.

INTRODUCTION

Tilapia farming poses a substantial challenge to fish farming in Sub-Saharan Africa, despite the huge technological leaps achieved worldwide on improved strains. According to Craig and Helfrich (2002), high cost of fish feeds, comprising of over 50% of the production costs limits tilapia production and sustenance. Sustainability and success of aquaculture depends on type of feed used and management. Success of intensive fish culture depends to a large extent on adequate information on nutrient requirements, especially dietary protein, which is the most expensive component in artificial diets (Tacon et al. 2009, 2011). Tilapia feed cost depends dietary protein level, the source, and type of ingredients derived from plant or animal sources.

Fish meal is very expensive thus increasing the cost of fish production. It competes with man for food and for use in aquaculture feeds. The demand and growth of tilapia farming has resulted in the expansion of

nutrient requirement data and improvements in feed formulations (NRC 1993). Selection of a protein ingredient is not limited to only assessing the crude protein levels of feed ingredients, but also involves in-depth knowledge in their amino acid profile and bioavailability. Commercially available ingredients have significant amounts of anti-nutritional factors and digestibility of the proteins is highly variable. It has been generally noted that nutrient are lost during feed manufacturing and improper storage of aqua feeds in low-technology production systems. Fish growth and survival can be compromised by poorly digested feed that can deteriorate water quality. Pellet type and manufacturing process also influence water quality and digestion efficiency of fish species under culture.

Standard nutrient requirement cannot be applied to practical feed formulation since it is not adequate for high density commercial rearing situations. Most of the research is actually done in aquaria and not ponds, therefore, the recommendations are for intensive systems. Many commercial feeds for tilapia contain lower protein levels (17% to 25%), which are considerably below recommended levels to reduce the cost of production (NRC1993). Unbalanced dietary amino acid contents could result in an increased de-amination and can increase ammonia levels released into the water (Hasan et al. 2007). Information about proximate composition of locally feed ingredient for farm made fish feed is usually limited and not reliable. Thus, farmers depends only on the existing information about the feed composition given by different fish feeds manufacturers. There have, however, been several attempts to profile the nutritional value of different feed ingredients from different agro-ecological and geographical locations in the world (Hasan et al. 2007, Tacon et al. 2009, 2011).

Fish diets can be improved considerably by inclusion of essential amino acids (EAAs) and EAA supplements whenever they become limiting for fish growth. It is also important to compose and process a balanced and biologically available levels of EAAs that meet the targeted species nutrient requirements (Nunes et al. 2014).

Appropriate dietary methionine and lysine levels improve the use of other Essential Amino Acids because they have the ability to reduce the oxidation rate of other amino acids (NRC 2011). Profiles on amino acid contents of fish feed ingredients provide valuable information necessary for formulating diets that support maximum growth of the fish under various cultural techniques. Commercial manufactures usually produce feeds in bulk, leaving small-scale fish farmers with the option of buying large quantities of expensive feed, which often goes to waste (Pandey 2013). Small quantities of fish feed required for experimental purposes can be easily made in the laboratory or on-farm, with particular ingredients of known nutritional quality, especially the EAAs.

Feed formulators are now adopting modern and environmentally sound formulation techniques based on nutrient value, on supplementation with crystalline EAAs and on animal nutrient requirements. Commercial feed formulation are intended to meet nutritional requirement with quality product at cheaper prices depending on type of fish species grown. In commercial aquaculture production feed costs can be reduced by developing proper feed management and husbandry strategies to improve fish growth. Best management practices (BMPs) in fish husbandry involve proper stocking densities, nutrient ratios, aeration, and water exchange to reduce metabolites that can deteriorate water quality. Plant proteins that are cheap and locally available are used to supplement animal protein at lower cost. Feeds consisting of soybean, wheat and corn meal, canola meal, and extruded pea seed meal, supplemented with methionine have been used for formulation of diets for carps, tilapia, and catfish without influencing growth performance (Tacon and Metian 2008).

Homemade feed is useful when specific diets are needed to improve fish growth performance. The nutritional requirements for protein, lipids and energy for optimum growth of specific fish species are necessary for formulating a balanced diet. Lysine and methionine are essential amino acids that cannot be synthesized by the body, but are obtained from the diet. Several studies have been conducted on the

Amino Acid Nutrition requirements, composition, supplementation and balanced ratios of protein in aqua feeds. (Ketola 1982, NRC 1993, Ahmadr 2008, NRC 2011, Munguti et al. 2012).

OBJECTIVES

- Develop low-cost, improved quality feeds using rice bran and freshwater shrimps (*Caridina niloticus*) as fish meal replacement;
- Assess the costs and benefits of three different feeding regimes in cages;
- Transfer technologies on management of monosex tilapia in cages through training farmers and extension officers; and
- Compare work conducted in this investigation on the use of low-cost supplemental feeds with the accomplishments of 20 years of CRSP-related work in the area.

MATERIALS AND METHODS

Study area. The study was conducted at University of Eldoret fish farm for three and a half months from June 2005 to September 2015 in seven earthen ponds of 150 m² and in fifteen hapas of capacity 1m³ suspended in the pond. The hapas for the experiment were made of foul resistant synthetic netting of mesh size 1.5 mm and were closed from all sides except the top. The study was conducted for 15 weeks (105) days from June to September 2015.

Source of experimental fish. Monosex male *O. niloticus* juveniles were obtained from Sagana National Aquaculture Research and Development Centre (NARDC) and transported to the study area under well oxygenated condition in plastic bags. The juveniles were acclimatized in holding tanks for two weeks prior the experimental stocking in hapas and ponds.

Study design. The first experiment was conducted in twelve hapas suspended in one pond while the second experiment was. *O. niloticus* juveniles were stocked at density of sixty fingerlings per hapa. Complete randomized block design was used for the experiment. Four diets were tested for the experiment in triplicate for each treatment. The experimental ponds 150 m² were limed at the rate of 2,500 kg ha⁻¹ with CaCO₃ and fertilized at a rate of 20 kg N and 8 kg P ha⁻¹ with urea and diammonium phosphate (DAP) to facilitate growth of phytoplankton and zooplankton.

Feed ingredients and acquisition. The feed ingredients consisted of freshwater shrimp (*C. niloticus*), cottonseed cake, wheat bran, fish oil, and vitamin/mineral premix. Diet 1 was supplemented with both lysine and methionine at 2.7 g kg⁻¹ each and 5.1 g kg⁻¹ respectively (Santiago and Lovell 1988, Furuya et al. 2006). Diet 2 supplemented with lysine only at 2.7 g kg⁻¹. Diet 3 did not receive any EAA supplement. Diet 4 was a commercial fish feed with 32% crude protein content. The three experimental diets were formulated at 30% crude protein at the University of Eldoret fish farm of which Diet 3 was used as a control (Table 1). All the diets were tested for three and a half months on monosex *Oreochromis niloticus* stocking densities of 2 fish m⁻² in duplicate for growth performance.

Feed preparation and proximate analysis. Feed ingredients were subjected to proximate analysis before diet formulation to determine the crude protein, moisture content, lipid, crude fibre, ash, and digestible carbohydrate. Experimental diets were formulated using Winfeed Ver. 2.8 software. Dry ingredients were passed through a sieve (0.6 mm diameter hole) before mixing into the diets. The ingredients were weighed and ground to small particle size (approximately 250 µm) and thoroughly mixed with water to obtain a 30% moisture level. Oil, vitamins, and minerals mixture were added to the diets. The diet was dried for 8 h in the open air and broken to appropriate size of crumbles.

Table 1: Ingredients and chemical composition of experimental diets used for feeding *O. niloticus* fingerlings before methionine and lysine supplements.

	Diet 1	Diet 2	Diet 3
	48.0	48.0	48.0
Caridina niloticus	30.0	30.0	30.0
Cotton seed meal	18.0		18.0
Fish oil	2.0	2.0	2.0
Trace mineral	1.0	1.0	1.0
Lysine%	1.96% + Supplement 2.7 g kg ⁻¹	1.96% + Supplement 2.7 g kg ⁻¹	No supplement
Methionine%	0.95% + Supplement 5.1 g kg ⁻¹	No supplement	No supplement

Feed floatability and quality. The experimental diets were tested for water stability using procedure described by Fagbenro and Jauncey (1995) to determine if the pellet were maintaining its form for at least five minutes before conducting a feeding trial. Water stability was expressed as a percentage of immersed diet weight/initial sample weight (Orire et al. 2010), or as percent loss of dry matter (%LDM) calculated as percent difference in sample weight (minus the initial diet moisture) after re-weighing (Johnston and Johnston 2007), or as percentage of dry matter remaining (%DMR) calculated as $\% \text{ DMR} = \frac{W_t}{W_o} \times (1-M) - W_t / W_o \times (1-M) \times 100$ where W_o = pellet weight as fed, W_t = weight after immersion and drying, and M=moisture content of diet as a proportion (Ruscoe et al. 2005).

Protein digestibility, determination of crude fat, dry matter and ash. The chemical compositions of the formulated diets were determined following AOAC (1990) procedures: dry matter, by drying in an oven at 105°C for 8 hours; crude fat, by Soxhlet extraction with ether; crude ash, by incineration in a muffle furnace at 580°C for 8 hour; crude protein (N× 6.25), by the Kjeldahl method after acid digestion; non digestible proteins, by Kjeldahl method after enzymatic hydrolysis of the digestible protein with pepsin; finally, digestible proteins were obtained as the difference between the crude and nondigestible proteins.

Feeding the experimental fish. Feeds were offered daily in hapas and ponds by broadcasting method at 10:00 h and 16:00 h. Initial feeding was offered at 10% of body weight adjusted to 5% and to 3% body weight, respectively. Feeding rations were adjusted after every two weeks.

Sampling water quality parameters. Water quality parameters to be measured include Dissolved oxygen, temperature, conductivity, total dissolved solid (TDS), pH, and ammonia. Water quality was determined three times a week at the surface, in the middle and pond bottom. Dissolved oxygen was measured using model 57 oxygen meter, pH was measured with a glass electrode-pH meter while total transparency was measured using Secchi disk.

Growth parameters and nutrient utilization. Total length of fish sample was measured in cm while weight measurements was taken monthly using 0.01g sensitive weighing balance. At the end of the experiment, all fish from the ponds were harvested, weighed and counted. Growth parameters and nutrient utilization calculated using the following formulas:

$$\text{i) Body Weight Gain (BWG)} = \frac{[(\text{Final weight (g)} - \text{Initial weight (g)})]}{\text{Initial weight (g)}}$$

$$\text{ii) Daily Weight Gain (DWG)} = \frac{\text{Final weight (g)} - \text{Initial weight (g)}}{\text{Time interval in days (t)}}$$

$$\text{iii) Specific Growth Rate (SGR)} = \frac{[\ln(\text{Final weight (g)}) - \ln(\text{Initial weight (g)})] \times 100}{\text{Time interval in days (t)}}$$

$$\text{iv) Food Conversion Ratio (FCR)} = \frac{\text{Weight of dry feed (g)}}{[\text{Final weight (g)}] - [\text{Initial weight (g)}]}$$

$$\text{v) Protein Efficiency Ratio (PER)} = \frac{\text{Final weight (g)} - \text{Initial weight (g)}}{\text{Protein consumed (g)}}$$

vi) *Length-frequency Analysis*

Munro's method. This method, based on Munro (1982), uses growth increment data to estimate L_{∞} and requires growth increment data file. The approach minimizes the coefficient of variation of:

$$\text{Ratio} = [\ln(L_{\infty} - L_m) - \ln(L_{\infty} - L_r)] / (t_r - t_m)$$

Where L_m is the initial length, L_r is the length at time t_m and t_r the corresponding dates of length measurements. Each growth increment leads to an estimate of K , given L_{∞} , and the variance of this estimate is computed. The value of L_{∞} is selected which minimizes that variance.

Growth performance index (ϕ'). The growth performance index (phi prime) was computed according to the relationship:

$$\phi' = \log_{10}(K) + 2 \cdot \log_{10}(L_{\bar{y}}) \text{ (Pauly 1984)}$$

Statistical analysis. The water quality parameters were compared through the growth period using one-way analysis of variance (ANOVA) while the growth of the monosex *O. niloticus* was compared using Phi prime (ϕ'). All the statistical tests were carried out at $\alpha=0.05$ using Minitab Version 17 and Statgraphics Version 16.

RESULTS

For temperature, there were significant variations with time ($F_{0.5,15,80} = 4.27, p < 0.00005$). Since the p-value of the F-test is less than 0.05, there is a statistically significant difference between the mean temperatures from one level of Days to another at the 95.0% confidence level.

The dissolved oxygen concentration did not show any significance with time ($F_{0.5,15,80} = 1.31, p = 0.217$). Since the p-value of the F-test is greater than or equal to 0.05, there was no statistically significant difference between the mean DO from one level of time to another at the 95.0% confidence level.

The water pH was statistically significant in time ($F_{0.5,15,80} = 7.63, p < 0.00005$). Since the p-value of the F-test is less than 0.05, there is a statistically significant difference between the mean pH from one level of Days to another at the 95.0% confidence level.

Temperatures were low, but maintained at a range of 18.5–20°C in all the sampling weeks. Dissolved oxygen levels were at arrange of 4.8–6 mg L⁻¹, while the pH was 7.2–7.6 throughout the study (Figure 1). The range of these critical water quality parameters were within suitable range for tilapia culture.

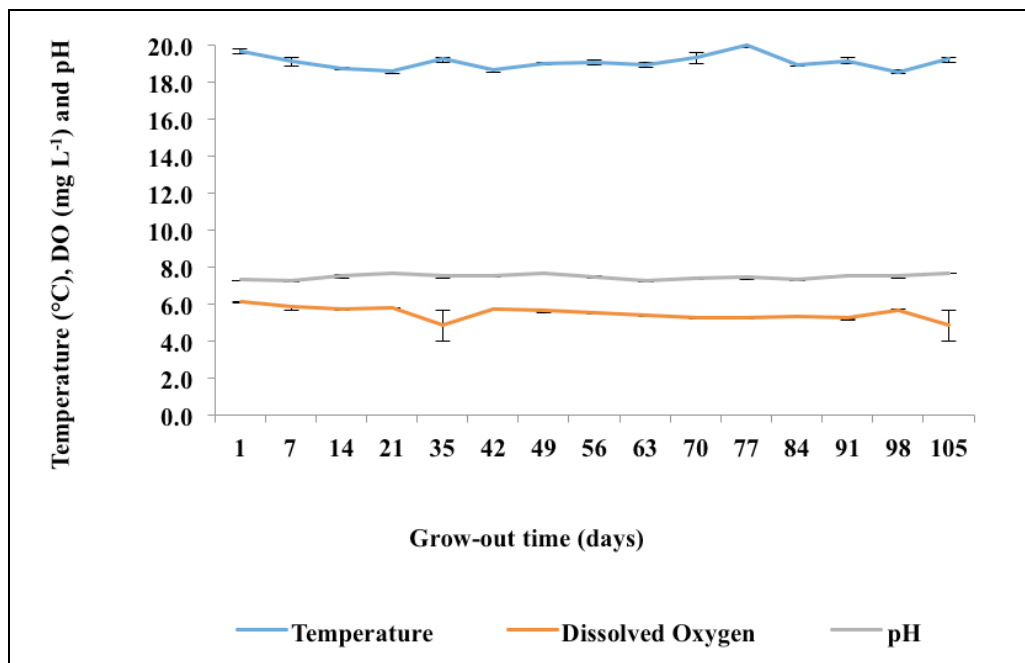


Figure 1: Water quality parameters of experimental pond at University of Eldoret fish farm.

Based on the diet formulation in this experiment, the following nutritional values were calculated based on the ingredient profiles from tropical areas on as-is basis of dry feedstuff (Table 2). The diet formulation provided a gross energy of 17.17 MJ kg⁻¹ based on 5.65 kcal g⁻¹ for protein 9.45 kcal g⁻¹ for Fat, 4.1 kcal g⁻¹ for Carbohydrate (Table 3). The conversion of kcal g⁻¹ to kJ g⁻¹ was carried out through the following relationship: 1 kcal to 4,184 kJ.

Table 2: Nutrient profiles estimates based on diet formulation in the study.

Parameter	Nutritive value	Parameter	Nutritive value
DM%	90.72	Cholesterol%	0.12
Ash%	8.03	Astaxanthin (mg kg ⁻¹)	804.05
GE MJ kg ⁻¹	17.17	Arginine%	2.53
DE MJ kg ⁻¹	9.67	Histidine%	0.81
CP%	30.15	Isoleucine%	1.04
Dig CP%	22.91	Leucine%	2.24
Lipid%	5.53	Lysine%	1.96
Fiber%	6.87	Methionine%	0.95
LOA (18:2n-6)%	0.72	M+C%	2.27
LNA (18:3n-3)%	0.03	Phenylalanine%	1.57
ARA (20:4n-6)%	0.02	P+T%	2.62
EPA (20:5n-3)%	0.64	Threonine%	1.21
DHA (22:6n-3)%	0.40	Tryptophan%	0.30
Total n-3%	1.07	Valine%	1.34
Total n-6%	0.75	Ca%	1.44
n3:n6	1.43	Available P%	1.14
Total phospholipids%	1.77		

The proximate analysis of all the diet composition before EAA supplementation for crude protein (CP%), Carbohydrates (%) and Fats (%) showed that the ingredients gave relatively consistent with nutrient profile for raising tilapia and were also close to the values used for formulation. Dry matter (DM) was 92%, CP was 29.6%, nondigestible CP was 9%, lipids was 6.3%, and ash was 10%.

The growth of monosex *O. niloticus* on the four test diets exhibited superior performance on Diet 2 which had lysine supplement, as compared to the commercial Diet 4 at the least end. The control and methionine/lysine test diets (Diet 1 and 3) had an average performance (Figure 2).

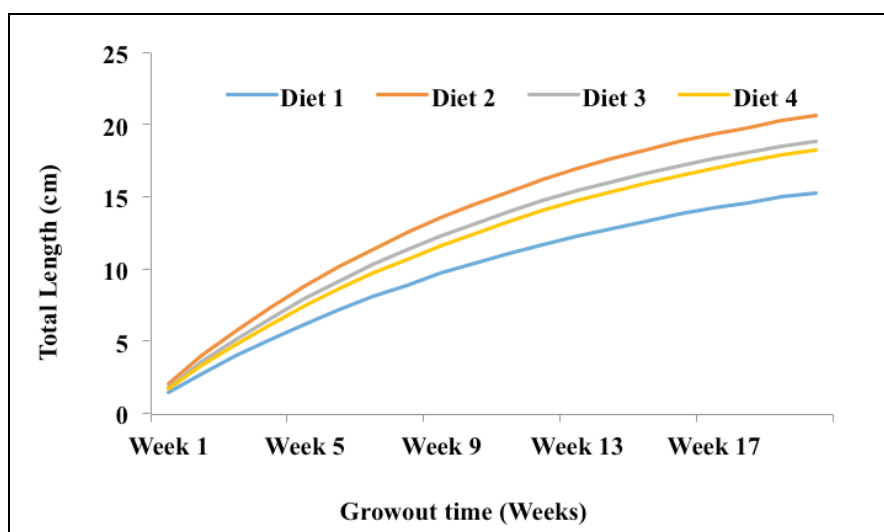


Figure 2: Growth of mono-sex *O. niloticus* over 105 days on the four test diets.

An index of growth performance, phi prime (ϕ') was calculated for each diet for the diets based on the asymptotic length (L_{∞}) and growth curvature (K), to show once again that lysine supplemented Diet 2 had better growth performance than the other diets (Figure 3).

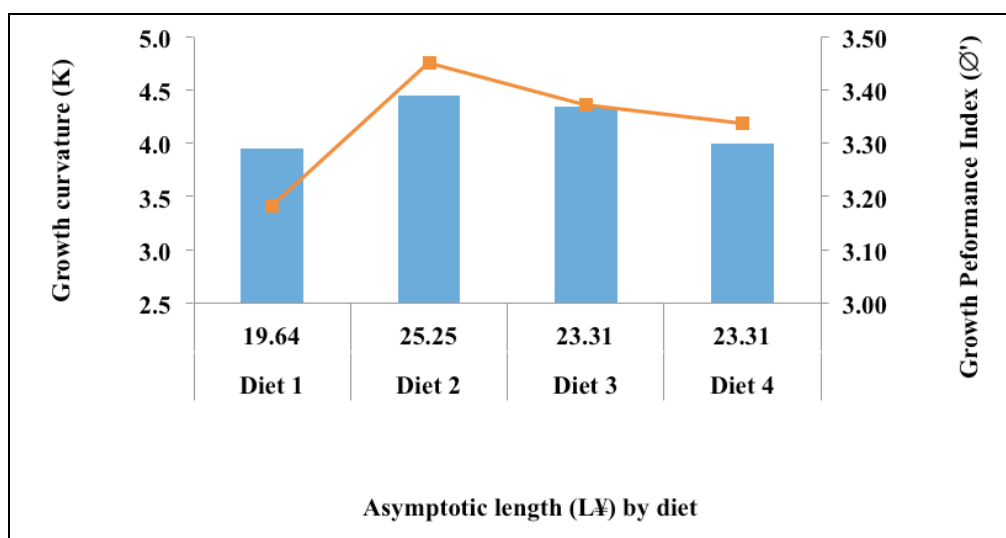


Figure 3: Growth performance, phi prime (ϕ') of mono-sex *O. niloticus* on the four test diets.

The performance of the feeds also showed better final average weight for the lysine supplemented diet of 201.8 g, as compared to the methionine+lysine diet at 196 g and the non EAA supplemented diet at 182.2 g. The commercial diet performed least with the final average weight being 174.7 g. All the other performance indicators; BWG, DWG, SGR, FCR and PER showed that the lysine supplemented diet was better than all the other diets (Table 3).

Table 3: Performance indicators of test diets on monosex *O. niloticus* in hapas for 105 days.

Performance indicators	Diet 1	Diet 2	Diet 3	Diet 4
Initial average weight (g)	0.72	0.69	0.74	0.69
Final average weight (g)	196.00	201.81	182.19	174.66
Weight of dry feed (g)	250.00	250.00	250.00	250.00
BWG	271.22	289.79	245.53	253.60
DWG	1.86	1.92	1.73	1.66
SGR	5.34	5.40	5.25	5.28
FCR	1.28	1.24	1.38	1.44
PER	2.60	2.68	2.42	2.32

DISCUSSION

Most commercial tilapia feeds in East Africa region have achieved poor performance due to a number of reasons (Munguti et al. 2012) while there has been a worldwide increasing effort to supplement many fish diets with EAAs and other additives in order to improve diet quality and enhance growth (Ketola 1983, Ruscoe et al. 2005, Tacon and Metian 2008, Nunes et al. 2014). The cost, availability, and quality of tilapia fish feed still poses a challenge to many small-scale farmers in East Africa.

One possible solution to this feed challenge is for fish farmers to formulate their own feeds in the farm so as to guarantee the desired quality. However, on-farm formulated tilapia feeds may also not solve the feed scarcity and feed quality problems due to the nutritionally unbalanced composition. This experimental study has shown that the feed formulation, processing and nutritional balancing can be achieved through supplementing the diets with EAAs, as these are often the limiting factors in tilapia feed performance.

Our study has shown that nutritional balancing of tilapia fish feeds can provide up to 17.17 MJ kg⁻¹ of energy to fish and at the same time produce high protein efficiency ratio of up to 2.7. The FCR values of 1.44 to 1.24 obtained in this study are close to the ones reported in the literature for extruded diets (1.88, 2.1, 1.5, and 1.7 kg feed for each kg gain in weight).

CONCLUSION

The study concludes that:

- Practical feeds for tilapia can be formulated using locally available protein sources at the farm level and supplementing the diets with EAA to produce a nutritionally balance diet for tilapia;
- Only limiting EAAs require supplementing in the on-farm diet formulations;
- Supplementing practical tilapia diets with lysine at a rate of 24.5 g per kg⁻¹ improves the growth performance of monosex *O. niloticus* in hapas; and
- On-farm practical diets of tilapia can produce superior DWG, SGR, FCR, and PER as compared to commercial feeds in the market.

QUANTIFIED ECONOMIC BENEFITS

The experimentation and testing of the diets have shown that:

- The technology can be implemented by farmers at the farm level with minimal investment in a

- hammer mill and pelletizing equipment;
- Farmers can now purchase EAA and supplement their formulated on-farm diets with recommended quantities;
 - About 600 farmers in Uasin Gishu, 300 farmers in Vihiga County, and 400 farmers in Kakamega counties stand to benefit from this initiative through the University of Eldoret extension initiatives;
 - Four undergraduate students and three postgraduate students are likely to undertake research projects on feed formulation, processing, and nutrition, with emphasis in EAA profiles; and
 - This new knowledge was applied at the University of Eldoret hatchery which is under construction to produce and supply an estimated 1.2 million tilapia fingerlings to over 600 fish farmers in Uasin Gishu County.

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