EVALUATION OF INVERTEBRATES AS PROTEIN SOURCES IN NILE TILAPIA (*OREOCHROMIS NILOTICUS*) DIETS

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

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Objectives
The overall objective of this study is to evaluate the suitability of selected invertebrates as protein sources in Nile tilapia (*Oreochromis niloticus*) diets.
The Specific objectives are:
1. To determine the chemical composition of earthworm and maggot meals.
2. To determine the appropriate inclusion levels of earthworm and maggot meal in diets of Nile tilapia cultured in Tanzania
3. To assess growth, feed utilization and cost effectiveness of Nile tilapia diets containing invertebrate as sources of protein

Hypothesis
Growth performance of Nile tilapia (*Oreochromis niloticus*) fed diets containing invertebrate meals is not significantly different from that of those fed diet containing fishmeal.

Significance of the Study
Worldwide aquaculture production has grown significantly with its contribution to global fish supply increasing from 3.9% in 1970 to over 41.3% in 2011 and annual production amounting to 63.7 million metric tonnes valued over USD 119 billion (FAO, 2012). It is now considered as the fastest growing animal producing sector with an average growth rate of 8.8% since 1970 and has outpaced capture fisheries (1.2%) and terrestrial farmed meat production (2.8%) (FAO, 2007). This fast growth has played a key role in augmenting the dwindling catch from capture fisheries in natural water bodies.

Contrary to the global trends, aquaculture production in Africa and Tanzania in particular has remained low, despite the high demand emanating from the fact that fish account for 17.4% of total animal intake; second to Asia (25.7%) (Brummet *et al.,* 2008). In Africa capture fisheries have been exploited to their maximum and in some cases even overexploited. This has resulted in low per capita consumption of 9.1 kg/capita/year compared to global average of 18.4 kg/capita/year (FAO, 2012). African countries import in excess 4.2 million tonnes of fishery products at a net loss of more than three thousand million United State Dollars in order to cope with the demand (Brummet *et al*., 2008).

In Tanzania aquaculture has remained mostly rural, secondary and part-time activity. It mainly involves culture of tilapia and African catfish in small freshwater earthen ponds varying from 150 – 500 m^2 with irregular application of inadequate manure and feeding based on natural food and supplementation with maize bran, kitchen leftovers and green vegetables/weeds. Consequently, productivity is low, about 2,000 kg ha^{-1} yr^{-1} and culture cycle is long taking about 12 months to attain market weight (Machena and Moehl, 2000). Studies have shown that with good quality feeds it is possible to achieve yields of 10,000 kg ha^{-1} yr^{-1} and fish can attain market weight in less than 6 months (Jauncey, 1998). To realized this high yield, pond fish need to be fed with concentrate diets with 30 – 40% protein. For many decades, fishmeal and soybean have been used as the main sources of protein in fish feeds (El-Sayed, 1999; El-Saidy and Gaber, 2002). However, fish farmers in Tanzania are unable to afford good quality protein sources such as fishmeal, soybean meal and other oil cakes that can meet protein requirement required for fast growth and development of fish. Such ingredients are both costly and scarce due to high demand from other livestock sectors as well as human consumption. Attempts to use cheaper alternatives such Moringa and

Leucaena leaf meals have not been much successful due to their relatively low protein content, high fiber content, low digestibility and inherent antinutritional factors (Madalla, 2008; Mbwana 2010; Shigulu, 2012). Hence there is a need to identify and evaluate other protein sources of high quality and affordable to fish farmers.

The nutritive value of fish diet depends on quality of the protein ingredients used in diet formulation. Generally, the feed stuffs of animal origins are considered better alternative protein sources to fishmeal in formulating fish diets because of their higher protein content and the superior indispensable amino acids than that of plant origins. Such sources include invertebrates such as insects, maggots/pupae and earthworms (Omoyinmi and Olaoye, 2012; Mohanta *et al*., 2013). Merits of these protein sources include higher protein content, higher digestibility, local availability and less antinutritional factors. Moreover, these invertebrates are abundantly available and have ability to produce large biomass within a short time because of their short life cycle. Therefore, the current study is aimed at identifying and evaluating commonly available invertebrates for their suitability as protein sources in Nile tilapia (*Oreochromis niloticus*) diets.

Quanitifed Anticipated Benefits

Through this investigation feeding packages based on diets containing commonly available invertebrates as protein sources will be developed and promoted for adoption by small-scale fish farmers. It is expected that through the use of these diets the farmers will improve the productivity of Nile tilapia in their ponds and at the same time reduce the feed cost, thus increase the profitability of fish farming enterprise by selling big sized fish. The increased level of income will improve the purchasing power of the rural farmers for food products, thereby reducing risks of food insecurity at housed levels. Also their per capita consumption of fish will increase, and thus reduce the problem of malnutrition in rural areas.

Impact Indicators

- At least two diets based on invertebrates as sources of protein developed and adopted by small-scale fish farmers.
- Growth rate and body size of tilapia in farmers' ponds will be improved by 50% by the end of the project.
- Income of participating households will be increased by 30% by the end of the project.
- At least one M.Sc. Students will graduate.

Research Design

The study will be an on-station study and a completely randomized design (CRD) will be used to assign dietary treatments to the experimental units.

Location

The study will be conducted at the Aquaculture Research Farm of Sokoine University of Agriculture in Morogoro, Tanzania.

Methods

The following activities will be undertaken to achieve the stated objectives.

Activity 1: Collection and culturing of Invertebrates with Potential for Use in Aquafeeds

Two invertebrates; earthworms (*Eudrilus eugeniale*) and maggots (*Musca domestica*) will be used as protein sources in Nile tilapia diets. Earthworms will be collected and cultured on mixtures of sand, cattle dung and saw dusts while maggots will be cultured on the mixture pig dung, pig hairs, chicken droppings and feathers in order to generate large quantities.

Activity 2: Biochemical Analysis of Selected Invertebrates
Proximate analysis (moisture, crude protein, crude lipid, crude fiber, ash and nitrogen-free extracts) will be performed according to standard methods (AOAC, 1990). Samples of fishmeal, earthworm meal and maggot meal will be analyzed for amino acid (Antoine *et al*., 1999) and fatty acid (Teng and Made Gowda, 1993) profiles using the HPLC method.

Activity 3: Growth Trials to Evaluate the Effects of Invertebrate Meal on Growth Performance of Nile Tilapia
Two growth trials will be undertaken as follows:
Sub – Activity 3.1 Short term growth Trials
The trial will be undertaken for a period of 60 days to determine optimum inclusion levels of earthworm and maggot meals in the diets of Nile tilapia. Ten diets will be formulated to evaluate the effects of replacing fishmeal with earthworm and maggot meals. Other ingredients that will be used to formulate the diets are hominy meal, wheat flour, vitamin premix and mineral premix. Diet 1 will serve as a control diet and it will be based on fish as the sole source of protein. Diet 2, Diet 3 and Diet 4 will be based on earthworm meal, maggot meal and mixture of moringa meal and sunflower seed cake as the only sources of protein in the diets, respectively. In diet 5, diet 6 and diet 7 fishmeal will be replaced by 50% with earthworm meal, maggot meal and mixture of moringa meal and sunflower seed cake, respectively. In diet 8, diet 9 and diet 10 fishmeal will be replaced by 75% with earthworm meal, maggot meal and mixture of moringa meal and sunflower seed cake, respectively. All diets will be formulated to contain 30% crude protein and 10% crude lipid. Each of the dietary treatment will be assigned randomly to experimental units and replicated three times. The experimental units will be comprised of buckets with capacity of 30 liter connected to a recirculation system. Ten 10 Nile tilapia fingerlings of approximately 3 - 5 g will be cultured in each bucket. The fish will be fed the respective experimental diets to apparent satiation. Body weight and feed intake will be determined on weekly basis. Growth performance and feed utilization will be determined using the following parameters: Average Daily Weight Gain, Specific Growth Rate, Feed Conversion Ratio, Protein Efficiency Ratio, Protein Productive Value. In addition, whole body composition of fish samples will be determined using proximate analysis scheme before and after the experiment.

Sub – Activity 3.2 Long term growth Trial
The best diets (based on earthworm and maggot meals as sources of protein) from the short-term trial will be used in the long-term growth trial to ascertain the benefits of using those diets in comparison to the fishmeal as protein sources. All the diets will be formulated to contain 30% protein and 10% lipid and the fish will be fed at 5% of their body for a period of 180 days. Fish will be stocked at a density of $3/m^2$ in outdoor concrete tanks with capacity of 4.5 m^2. Growth, feed utilization as well as cost effectiveness will be determined. Cost effectiveness will be determined through gross margin analysis and feed cost to produce a kilogram of fish. In both trials, water quality parameters such as oxygen, pH, total ammonia nitrogen and nitrate will be monitored to ensure that they are within acceptable limits.

Activity 4: Data Analysis
One way ANOVA will be used to analyze the data to test the effects of the diets on body weight gain, growth rate, feed conversion ratio and Protein Efficiency Ratio. Initial body weight of the fish will be used as a covariate to adjust the variation in initial body weight. Where significant difference in treatment means exist the LSD test will be applied to establish which means are actually significantly different.

Deliverables
- Outputs will include reports and peer-reviewed publications that will be made available to policy makers. These will be available online and in print.
- A workshop will be offered to stakeholders to present findings from the study.Findings from the investigation will be presented at regional and international professional conferences.

Schedule (1 July 2013 – 30 September 2015)

Activities	**2013/2014**				**2014/2015**			
	Q1	**Q2**	**Q3**	**Q4**	**Q1**	**Q2**	**Q3**	**Q4**
Objective 1: To determine the chemical composition of earthworm and maggot meals								
Activity 1.1: *Collection and culturing of Invertebrates with Potential for Use in Aquafeeds*								
Activity 1.2: *Biochemical Analysis of Selected Invertebrates*								
Activity 1.3: *Data analysis and report writing discussion*								
Objective 2: To determine the appropriate inclusion levels of earthworm and maggot meal in diets of Nile tilapia cultured in Tanzania								
Activity 2.1: Conduct short-term feeding trials for 60 days								
Activity 2.2: Data analysis and report writing								
Objective 3: To assess growth, feed utilization and cost effectiveness of Nile tilapia diets containing invertebrate as sources of protein								
Activity 3.1: Conduct long-term feeding trial for 180 days								
Activity 3.2: Data analysis and report writing survey								
Final Report Writing								