Evaluation of Invertebrates as Protein Sources in Nile Tilapia (*Oreochromis niloticus*) Diets

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Abstract

The study was conducted to approximate the composition of house fly maggots and earthworms from different substrates as well as to evaluate their suitability as protein sources in the Nile tilapia (*Oreochromis niloticus*) diets. Chicken manure, cattle manure and fermented maize were used as substrates for production household fly maggots (HFM), while chicken, cattle and rabbit manures were used as substrates for production of earthworm meal (EWM). HFMs and EWMs with the highest protein content were used to formulate practical isonitrogenous diets (30% crude protein) containing graded levels of HFM and EWM meals (25%, 30%, 35% and 40%). The diets were fed to juveniles with an average weight of 2.6g in a growth trial that lasted for eight weeks. There were significant ($p<0.05$) differences in the crude protein contents between the HFMs as well as EWMs raised on three culturing media. Chicken manure produced HFM with significantly high protein content, while cow manure did the same for EWM. Growth and feed utilization was significantly higher in fish fed diets HFM35 and EWM35. The same diets were more cost effective to produce a unit of fish. Therefore, it is recommended to include either HFM or EWM meals at 35% in practical diets containing 5% fishmeal and cotton seedcake or any similar plant protein.

Introduction

Globally, aquaculture is increasingly becoming an important source of fish protein as fish supply from capture fisheries dwindles. However, in Tanzania, aquaculture has remained mostly rural, secondary, and part-time activity taking place in small freshwater ponds within small farm holdings. Further growth has been limited by a number of factors including lack of affordable fish feeds. Feeds are expensive due to high cost of protein sources. Fish meal and oil seedcakes have high demand from terrestrial animal feed, making them scarce and unaffordable. Therefore, for aquaculture to grow, there is a need to search for affordable and locally available sources of protein. Among potential sources are invertebrates such as earthworms and housefly maggots, which are often abundantly available locally, palatable and relatively more affordable. They have short life cycle and high fecundity rate within a short period time (FAO 2012).

The study was therefore conducted to evaluate the suitability of earthworms and housefly maggots as protein sources in Nile tilapia (*O. niloticus*) diets. Specifically, the study sought to understand the following:

- Effect of different culture media on yield and proximate composition of housefly maggot and earthworm meals;
- Performance of *O. niloticus* juveniles fed diets containing different grade levels of housefly maggot and earthworm meals as protein sources; and
- Cost effectiveness of diets containing housefly maggot and earthworm meals.

MATERIALS AND METHODS

The study was conducted at the Aquaculture Research Facility belonging to the Department of Animal Science and Production at Sokoine University of Agriculture (SUA), Morogoro, Tanzania.

Effect of substrate on yield and proximate composition of housefly maggot and earthworm meal. Three substrates (fermented maize grain, cattle manure and poultry manure) in triplicates with abattoir and fish wastes as attractants were evaluated on their suitability to produce household fly maggots (HFM). Plastic buckets with a capacity of 10 L and mosquito nets were used in culturing the maggots indoors as suggested by Nzamujo (2001). The mature maggots were harvested according to Aniebo and Owen (2010). Harvested housefly maggots were blanched in 100^0C hot water and weighed to determine total weight per harvest. After completion of harvesting, the samples were oven dried at 65^0C for 48 hours and ground into a meal.

Figure 1. Culture of HFM.

Figure 2. Harvested HFM blanched in hot water.

For earthworm meal (EWM), three substrates (cattle, rabbit and poultry manures) were evaluated in triplicates. Plastic water basins with a capacity of 12 liters were used in the production of earthworms. Dried rice residues were added as bedding materials. The substrates were inoculated with adult earthworms collected from the wild and placed indoors as suggested by Nzamujo (1999). Each culture media was moistened with 1 liter of water once every week. Harvesting of earthworms was done in batches after every month when the reproduced young got matured. During harvesting, the screening method stated by El Boushy et al. (1985) and Sogbesan et al. (2006) was used, and then sorted, washed, weighed and stored at -5^{0}C until the required amount was obtained. Thereafter harvested earthworms were weighed, oven dried at 65^{0}C for 48 hours and then ground into powder.

Figure 3. Culture of earthworms.

Figure 4. Harvested earthworms.

Yields and proximate analysis of HFM and EWM were determined to establish most suitable culture media.

Growth trial to determine growth, feed utilization and cost effectiveness of diets containing of graded levels of housefly maggot and earthworm meals as protein sources in diets of *O. niloticus* juveniles. For each meal, five isonitrogenous (30% crude protein) practical diets with 5% fish meal and graded levels of HFM and EWM were formulated as shown in Tables 1 and 2.

Table 1. Percentage inclusion levels of the ingredients in HFM Based Diets (g/100g diet).

Ingredients	Diets				
	HFM0	HFM25	HFM30	HFM 35	HFM40
FM	5.0	5.0	5.0	5.0	5.0
HFM	0.0	12.0	24.5	40.0	48.8
CSM	50.0	39.0	25.0	9.0	0.0
MM	40.0	38.5	40.5	42.0	42.2
WM	2.0	2.0	2.0	2.0	2.0
SFO	1.0	1.5	1.0	0.0	0.0
Vit/Min	2.0	2.0	2.0	2.0	2.0
TOTAL	100	100	100	100	100

Table 2. Percentage inclusion levels of the ingredients in EW Based Diets (g/100g diet).

	Diets				
Ingredients	EWM0	EWM25	EWM30	EWM45	EWM40
FM	5.0	5.0	5.00	5.0	5.0
EWM	0.0	12.0	24.0	39.8	45.0
CSM	50.0	38.0	24.5	5.0	0.0
MM	40.0	38.0	39.5	42.7	42.0
WM	2.0	2.0	2.0	2.0	2.0
SFO	1.0	3.0	3.0	3.5	4.0
Vit/Min	2.0	2.0	2.0	2.0	2.0
Total	100	100	100	100	100

D1= Diet 1, D2 = Diet 2, D3 = Diet 3, D4 = Diet 4, D5 = Diet 5, D6 = Diet 6, D7 = Diet 7, D8 = Diet 8, D9 = Diet 9, FM = Fish Meal, EWM = Earthworm meal, CSM = Cotton seed meal, MM = Maize meal, WM = Wheat meal, SFO = Sunflower oil, Min/Vit. = Minerals/Vitamin premixes

**Vitamin A 25,500,000 IU, Vitamin D3 5, 000, 000 IU, Vitamin E 5,050 IU, Vitamin B2 mg 4,750, Vitamin B6mg 2,750, Vitamin B12 mcg 11, 750, Vitamin K3 mg 4,850, CAL PAN mg 5,750, Niacinamide mg 16, 500, Vitamin C 10, 000 mg, IRON 5,250 mg, MANGANESE 12, 760 mg, COPPER 13, 250 mg, ZINC 13, 250 mg, SODIUM CHLORIDE 48, 750 mg, MAGNESIUM 12, 750 mg, POTASSIUM ACETATE 73, 750 mg, LYSINE 15,000 mg, METHIONINE 12, 000 mg, antioxidant and anticaking qsf 1 kg.

An eight-week growth trial was conducted in a recirculation system using 40-liter buckets as culture units as shown in Figures 5 and 6. Dietary treatments in triplicates were assigned randomly to 40-liter tanks each containing 14 fingerlings with an average weight of 2.6 grams. The fish were fed up to 5% of their body weights twice daily throughout experimental period.

Figure 5. Fish culture tanks.

Figure 6. Water treatment tanks.

Feed intake, feed costs, body weight measurements were used to calculate growth, nutrient utilization and cost effectiveness as follows:

- Average daily gain (ADG)

$$ADG = \frac{\text{Final body weight - Initial body weight(g)}}{\text{Time (day)}}$$

- Metabolic Growth Rates (MGR)

$$MGR = \frac{\text{(Final body weight - Initial body weight)}}{[\{(\text{Initial body weight}/1000)^{0.8} + (\text{Final body weight}/1000)^{0.8}\}/2]/\text{trial days}}$$

- Percentage Survival (PS)

$$PS = \frac{\text{Final number of fish at harvest} \times 100}{\text{Initial number at stocking}}$$

- Protein Efficiency Ratio (PER)

$$PER = \frac{\text{Body weight gain (g)}}{\text{Crude protein intake (g)}}$$

- Feed Conversion Rate (FCR)

$$FCR = \frac{\text{Feed supplied (g)}}{\text{Body weight gain (g)}}$$

- Daily Feed Intake (DFI)

$$FI = \frac{\text{Feed Supplied (g)}}{\text{Time (day)}}$$

- Cost effectiveness

$$FI = FCR \times \text{Price of feed per kg}$$

The main hypothesis tested was “there is no significant difference between treatment means.” One-way analysis of variance (ANOVA) was used to determine differences between treatment means which were deemed significant at $P<0.05$.

The model used was $Y_{ij} = \mu + T_i + \varepsilon_{ij}$
Where,

$i = 1, 2... t_i$
$j = 1, 2... r_j$
Y_{ij} = Observed value (yields, nutritional composition of experimental meals, fish growth performance and feed utilization)
μ = General means (yields, growth performance and feed utilization)
T_i = the effects of culturing media and dietary treatment
ε_{ij} = Error factors

Tukey's honest significant difference test was used for mean separation where significant differences existed.

RESULTS

Effect of substrate on proximate composition of housefly maggot and earthworm meals. Housefly maggots from chicken manure had significantly high protein content while that from cow manure had significantly low protein content (Table 3).

Table 3. Proximate Composition of House Fly Maggots Produced from Different Substrates (Means±SE)

Item	Culturing media		
	Chicken Manure	Fermented Maize	Cow Manure
Dry Matter	97.52 ± 0.47^a	96.42 ± 1.47^a	96.71 ± 0.08^a
Crude Protein	48.55 ± 0.81^a	42.63 ± 0.23^b	40.43 ± 0.21^c
Crude Fibre	5.71 ± 0.25^a	5.02 ± 0.25^a	6.00 ± 0.25^a
Ether Extract	19.07 ± 0.46^c	20.40 ± 0.42^c	20.00 ± 0.06^c
Ash	11.13 ± 0.23^a	10.70 ± 0.48^a	18.47 ± 0.19^b

Means with different superscript letters within a row are significantly ($p < 0.05$) different.

Protein content of earthworms produced from cow manure was significantly higher than either of chicken manure or rabbit manure (Table 4).

Table 4. Proximate Composition of Earthworms Produced from Different Substrates (Means±SE)

Items (%)	Culturing media		
	Cow Manure	Chicken Manure	Rabbit Manure
Dry Matter	95.02 ± 0.96^a	97.20 ± 0.47^a	95.26 ± 0.67^a
Crude Protein	48.61 ± 0.18^a	40.83 ± 0.43^b	39.80 ± 0.41^b
Ether Extract	6.80 ± 0.49^c	5.60 ± 0.22^c	5.23 ± 0.12^c
Ash	28.60 ± 0.11^b	28.77 ± 0.48^b	29.77 ± 0.10^b

*Earthworm meal used in the formulation of fish diet
Means with different superscript letters within a row are significantly ($p < 0.05$) different.

Proximate composition of other ingredients used in formulating experimental diets is shown in Table 5.

Table 5. Proximate Composition and Gross Energy Content of Other Feed Ingredients Used in Formulation of Treatment Diets.

	Ingredients			
Item	**FM**	**MM**	**WM**	**CSM**
Dry matter	98.96	88.01	96.9	97.50
Crude Protein	69.20	10.5	11.74	41.60
Ether Extract	10.28	3.60	1.80	8.5
Crude Fibre	1.0	2.3	-	14.37
Ash	22.76	1.30	1.91	6.70
NFE[1]	2.38	84.30	79.15	23.34
GE(Kcal/g)	19.99	17.93	17.10	18.88

[1] NFE=Nitrogen free extract + fiber, (NFE) = 100 - (% protein + % fat + % ash).

The different inclusion levels of HFM and EWM had effect on proximate composition of the diets as shown in Tables 6 and 7, respectively. For instance, crude fiber decreased as inclusion levels of HFM and EWM increased in the diet. On the other hand, ash content increased with increasing inclusion levels of HFM and EWM.

Table 6. Proximate Composition and Gross Energy of Diets Containing Graded Levels of HFM Fed to *Oreochromis niloticus* Juveniles.

	Diets				
Item	**HFM0**	**HFM25**	**HFM30**	**HFM35**	**HFM40**
Dry Matter	93.55	92.71	91.48	90.02	93.07
Crude Protein	30.39	30.19	30.18	30.20	30.25
Ether Extract	8.49	10.42	10.50	10.80	11.90
Crude Fibre	8.17	7.20	5.89	4.45	4.43
Ash	5.03	6.52	7.95	9.80	11.12
Nitrogen Free Extract	46.86	43.99	43.41	42.19	38.84
Gross Energy	18.35	18.07	18.34	18.77	18.85

Table 7. Proximate Composition and Gross Energy of Diets Containing Graded Levels of EWM Fed to *Oreochromis niloticus* Juveniles.

	Diets				
Item	**EWM0**	**EWM25**	**EWM30**	**EWM35**	**EWM40**
Dry Matter	93.55	95.79	95.79	92.5	91.9
Crude Protein	29.59	30.31	30.31	29.58	29.80
Ether Extract	8.49	10.34	9.34	9.53	9.84
Crude Fibre	8.17	7.76	5.76	4.69	4.22
Ash	5.03	7.90	7.90	11.80	12.96
Nitrogen Free Extract	46.86	41.61	41.61	40.32	39.10
Gross Energy	18.35	17.21	17.21	16.30	16.07

Nile tilapia fed the different diets gained weights throughout the experiment period as shown in Figures 7 and 8. Fish fed diets containing 25%–35% HFM gained more weight compared to those fed diet control and 40% HFM diets. The same trend was observed in fish fed diets containing EWM but with much lower weight gain in fish fed the control diet and 40% EWM diets.

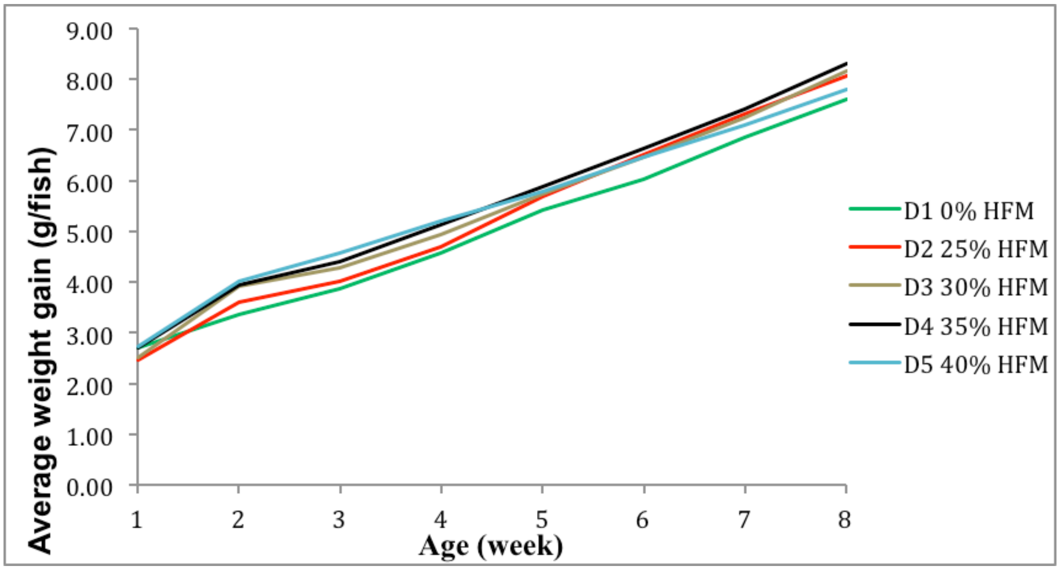

Figure 7. Change in weight of *O. niloticus* fed HFM-based diets**.**

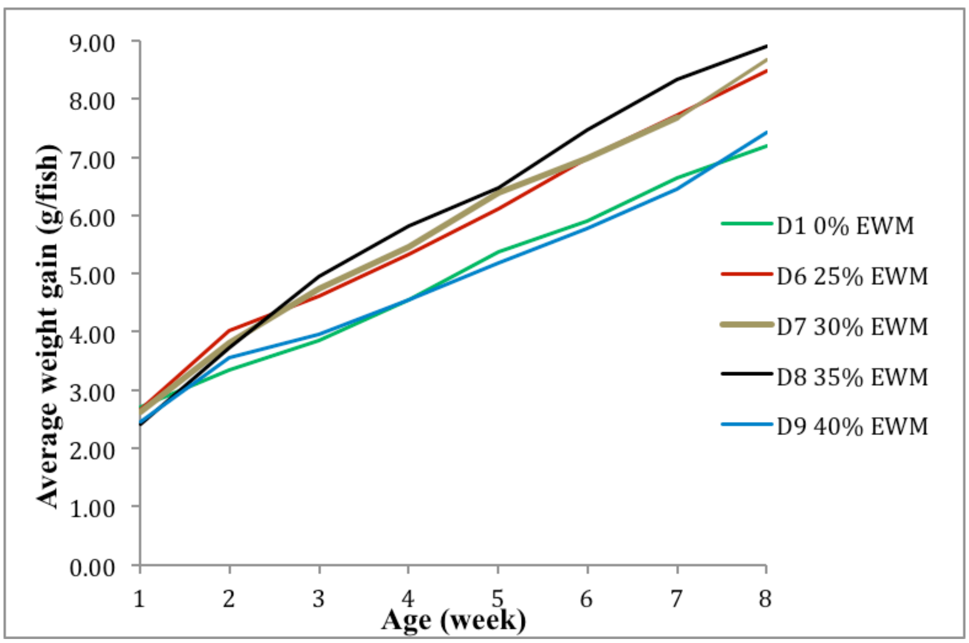

Figure 8. Change in weight of *O. niloticus* fed EWM diets.

Growth, nutrient utilization and survival of fish fed diets containing graded levels of HFM are shown in Table 8. Different dietary treatments had no significant effect on final weight and weight gain. However, average daily gain and feed intake differed significantly with fish fed diet HFM35, which performed significantly higher. Daily feed intake differed significantly with the highest values observed in fish fed HFM35. Similarly, fish fed diet HFM35 had significantly higher FCR and PER.

Table 8. Growth, nutrient utilization and survival of *O. niloticus* fed diets containing graded levels of HFM (Mean ± SD, n=3)

	Diets				
Parameter	**HFM0**	**HFM25**	**HFM30**	**HFM35**	**HFM40**
IW (g)	2.31±0.18^{a}	2.48±0.11^{a}	2.54±0.12^{a}	2.72±0.09^{a}	2.68±0.03^{a}
FW (g)	7.71±0.11^{a}	8.09±0.45^{a}	8.19±0.30^{a}	8.33±0.20^{a}	7.49±0.32^{a}
BWG (g)	5.40±1.21^{a}	5.61±1.32^{a}	5.65±1.20^{a}	5.81±1.31^{a}	5.61±1.20^{a}
ADG (g)	0.115±0.0^{e}	0.117±0.0^{d}	0.119±0.0^{c}	0.125±0.1^{a}	0.122±0.02^{b}
DFI (g)	0.59±0.03^{c}	0.64±0.05^{b}	0.65±0.01^{b}	0. 69±0.04^{a}	0.50±0.01^{d}
FCR	2.65±0.14^{b}	2.18±0.16^{b}	2.17±0.16^{b}	1.87±0.05^{a}	1.89±0.02^{a}
MGR	1.32±0.03^{c}	1.38±0.01^{ab}	1.42±0.04^{b}	1.48±0.03^{a}	1.42±0.05^{b}
PER	0.029±0.002^{a}	0.029±0.002^{a}	0.029±0.001^{a}	0.030±0.002^{a}	0.030±0.003^{a}
Surv (%)	88.09±1.2^{b}	97.62±0.3^{a}	97.62±0.3^{a}	95.24±0.6^{a}	97.62±0.3^{a}
Cost Effectiveness (Tshs/Kg)	3071	2995	2949	2485	2511

Means with different superscript letters within a row are significantly (p <0.05) different.
IW = Initial body weight, FW = Final body weight, BWG = Body weight gain, ADG = Average daily gain,D FI = Feed intake, FCR = Feed conversion ratio, MGR = Metabolic growth rate, PER = Protein efficiency ratio, Surv = Percentage survival

Growth, nutrient utilization and survival of fish fed diets containing graded levels of EWM are shown in Table 9. Fish fed diets EWM25, 30 and 35 had significantly higher final body weights. Significantly higher body weight gain and average daily gain were observed in fish fed diet EWM35 while the least performance was observed in fish fed diets EWM0 and EWM40. Feed intake was significantly higher in fish fed diets EWM30 and EWM35 while the least intake was observed in fish fed diet EWM40. A significantly low feed conversion ratio was observed in diets EWM30 and EWM35 while that of diet EWM40 was significantly higher.

Table 9. Growth performance and nutrient utilization of *O. niloticus* fed diets containing various inclusion levels of EWM (Mean ± SD, n=3)

Parameter	Diets				
	EWM0	EWM25	EWM30	EWM35	EWM40
IW	2.31±0.18[a]	2.68±0.05[a]	2.63±0.05[a]	2.42±0.08[a]	2.46±0.28[a]
FW	7.71±0.11[b]	8.50±0.28[a]	8.69±0.06[a]	8.92±0.21[a]	7.85±0.58[b]
BWG	5.40±0.01[c]	5.82±0.01[b]	6.06±0.01[b]	6.50±0.01[a]	5.39±0.01[c]
ADG	0.12±0.01[d]	0.13±0.01[c]	0.14±0.01[b]	0.15±0.01[a]	0.12±0.01[d]
DFI	0.59±0.03[b]	0.61±0.02[ab]	0.69±0.04[a]	0.71±0.08[a]	0.42±0.10[c]
FCR	2.65±0.14[d]	2.22±0.07[b]	2.10±0.09[a]	1.85±0.31[a]	2.43±0.04[c]
MGR	1.21±0.03[c]	1.47±0.01[b]	1.52±0.04[a]	1.57±0.01[a]	1.13±0.08[c]
PER	0.02±0.02[c]	0.02±0.01[c]	0.03±0.01[b]	0.04±0.01[a]	0.03±0.01[b]
Surv (%)	88.09±1.2[b]	97.62±0.3[a]	97.62±0.3[a]	97.62±0.3[a]	88.09±0.6[b]
Cost Effectiveness (Tshs/Kg)	3071	3150	2980	2653	3521

Means with different superscript letters within a row are significantly (p <0.05) different.
IW= Initial body weight, FW = Final body weight, BWG = Body weight gain, ADG = Average daily gain,D FI = Feed intake, FCR = Feed conversion ratio, MGR = Metabolic growth rate, PER = Protein efficiency ratio, Surv = Percentage survival

CONCLUSION

- Chicken manure was found to be the best substrate for production of housefly maggots in terms of yield and protein content. On the other hand, cow manure was found to be the best substrate for producing earthworm in the same context;
- Fish fed diets HFM35 and EWM35 had a superior overall performance. Thus, inclusion of the invertebrate meals at 35% in practical diets containing 5% fish meal and cotton seedcake or any similar plant protein is recommended. Of the two, the HFM is best; and
- Diets HFM35 and EWM35 are more cost effective in producing a unit of fish.

Therefore, it is recommended to include either HFM or EWM at 35% in practical diets containing 5% fish meal and cotton seedcake or any similar plant protein.

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