

Use of *Moringa oleifera* Leaves and Maggots as Protein Sources in Complete Replacement for Fish Meal in Nile tilapia (*Oreochromis niloticus*) Diets

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Abstract

For *Oreochromis niloticus* rearing, a feeding test was carried out for 42 days on fingerlings fish with an average initial weight of 4.6 g. Five experimental isoproteic and isoenergetic diets (30.44±1% protein; 19.64±0.23 kJ. g⁻¹) were formulated, including one (D1) control containing fish meal, and meet the essential amino acid requirements of *Oreochromis niloticus* fingerlings. In triplicate, fish meal was completely replaced by ratios of 3:5, 1:2, 2:5, 1:3 between *Moringa oleifera* leaves and maggots respectively for feed D2, D3, D4 and D5. At the end, significant differences (P<0.05) were observed on the growth and feed utilization performances between the control diet (D1) and the other (D2 to D5) diets without fish meal. These have been reduced in diets without fish meal. The survival and feed utilization were significantly affected by the ratios between *Moringa oleifera* leaves and maggot meals in the diets tested with the best results obtained with D2 diet (ratio 3:5). Protein levels in the carcass fed with experimental diets were high (D2 and D5). Maggots and *Moringa oleifera* leaves mixture can totally replace fish meal in *Oreochromis niloticus* diets but technological treatments, phytase addition and attractants could improve zootechnical performance.

Introduction

In the world, aquaculture is one of the rapid production systems for animal protein (Sanchez-Lozano, Martínez-Llorens, Tomás-Vidal, & Jover Cerdá, 2009). The continuing boom in aquaculture such as Tilapia culture (FAO, 2012) has increased the demand for feed and therefore the materials that make it up. The choice of the alternative sources of proteins is justified by the reduction in the principal raw materials (fish meal and fish oils) resulting from the natural reserves.

Fish meal and fish oils have always been used predominantly as raw materials in aquafeeds, especially in fish (Médale, Le Boucher, Dupont-Nivet, Quillet,

Aubin, & Anserat, 2013). These raw materials, less and less available these recent years (FAO, 2012), contain all the nutrients not only to meet the nutritional requirements of fish but also to enrich their flesh with n-3 polyunsaturated long-chain fatty acids, beneficial for human health. In order to meet the demand, alternative plant and animal raw materials are used. Several studies were focused on the replacement of fish meal with alternative sources of protein in fish feed (Kaushik, Coves, Dutto, & Blanc, 2004).

The total replacement of fishmeal with alternative protein sources may affect the fish growth performance (Espe, Lemme, Petri, & El-Mowafi, 2006; Médale *et al.*, 2013) because these alternative sources didn't have the

same palatability and the same nutritional values than fish meal. Watanabe, Verakunpiriya, Watanabe, Viswanath and Satoh (1998) have shown the possibility to completely replace fish meal of the feed in rainbow trout, without altering their growth, by using a mixture of different products getting from animal and vegetable origin.

Moringa oleifera, from the Moringaceae family, is a rapidly growing plant widely available in tropical and subtropical areas with great economic importance for food and medical industry (Becker & Makkar, 1999; Djissou, Vodounnou, Tossavi, Toguyeni, & Fiogbe, 2016a). Its leaves are rich in proteins, vitamins, carotenoids, ascorbic acid and iron (Djissou *et al.*, 2016a). *M. oleifera* leaves were used as a protein source in partial replacement of fish meal in the diet of Nile tilapia (Richter, Siddhuraju, & Becker, 2003). Maggots (*Musca domestica*) are larvae of flies that are also an alternative source of animal proteins (Tegua, Mpoame, & Okourou, 2002), rich in nutrients (Odesanya, Ajayi, Agbaogun, & Okuneye, 2011), especially essential amino acids (Adesina, 2012). Maggot meal was tested in the feeding of the fish *Oreochromis niloticus* (Ajani, Nwanna, & Musa, 2004; Ogunji, Kloas, Wirth, Schulz, & Rennert, 2008; Djissou *et al.*, 2016a) and catfish (Sogbesan, Ajuonu, Musa, & Adewole, 2006) with different encouraging results. These alternative sources of protein have shown their potential for use in feed and also in reducing the cost of production.

The purpose of this study was to determine the effect of total replacement of fish meal by mixing varied proportions of leaves of *M. oleifera* and maggots (*Musca domestica*) on the growth of Nile tilapia fingerlings (*Oreochromis niloticus*).

Materials and Methods

Experimental Procedure

This study was carried out at the research station on the diversification of pisciculture of Abomey-Calavi University, Benin. The ingredients used are: cottonseed meal, wheat bran, soybean (roasted), brewer's yeast, palm oil and starch produced locally. The fish meal used was that made in Ghana. Leaf meal of *Moringa oleifera* and maggot meal (*Musca domestica*) were obtained according to the production method of Hèdji, Houinato, Yehouenou, Sobakin and Fiogbe (2014) and Djissou, Tossavi, Vodounnou, Toguyeni and Fiogbé (2015) respectively. These protein sources (*M. oleifera* leaves and maggots) have been used to completely replace fish meal in the fish feed *O. niloticus*. The synthesized methionine was used in the event of a deficiency to meet the requirements of *O. niloticus*.

Based on the protein content of the ingredients and their essential amino acid profile (Table 1), four experimental diets (D2, D3, D4 and D5) were formulated with different levels (3: 5, 1: 2, 2: 5, 1: 3 respectively) of maggot meal and *M. oleifera* flour as a complete replacement for fishmeal. These ratios were chosen to satisfy the protein and essential amino acid requirements of *O. niloticus* fingerlings. The control diet D1 was formulated with an incorporation rate of fishmeal in the feed (40%). All the formulated diets were isonitrogenous and isoenergetic (30.44±1% protein and 19.64±0.23 kJ. g⁻¹). These experimental diets were formulated for the feeding of Tilapia (*O. niloticus*) in the pre-growth phase (Table 1).

Table 1. Formulation and composition of experimental diets (g 100g⁻¹ dry matter)

Ingredients	D1	D2	D3	D4	D5
Moringa oleifera	0.0	14.0	12.0	10.0	8.0
Fish meal	40.0	0.0	0.0	0.0	0.0
Soybean meal	20.0	20.0	20.0	20.0	20.0
Brewer's yeast	10.0	10.0	10.0	10.0	10.0
Cottonseed meal	20.0	17.6	18.8	20.0	20.2
Wheat bran	5.0	5.0	5.0	5.0	5.0
Maggot meal	0.0	23.4	24.2	25.0	25.8
Palm oil	3.0	3.0	3.0	3.0	3.0
Vitamines mix ^a	1.0	1.0	1.0	1.0	1.0
Mineral mix ^b	1.0	1.0	1.0	1.0	1.0
Starch	0.0	4.0	4.0	4.0	5.0
Methionine	0.0	1.0	1.0	1.0	1.0
Ratio*	-	3 :5	1 :2	2 :5	1 :3

*ratio between *M. oleifera* leaves and maggot meal

^a Vitamin premix contains (g 100 g⁻¹ of premix): ascorbic acid, 50.0; D-calcium pantothenate, 5.0; choline chloride, 100.0; inositol, 5.0; menadione, 2.0; niacin, 5.0; pyridoxine HCl, 1.0; riboflavin, 3.0; thiamin HCl, 0.5; DL-alpha-tocopherol acetate (250 IU g⁻¹), 8.0; vitamin A acetate (20,000 IU g⁻¹), 5.0; vitamin micro-mix, 10.0; cellulose, 805.5. Vitamin micro-mix contains (g kg⁻¹ of micro-mix): biotin, 0.5; cholecalciferol (1µg = 40 IU), 0.02; folic acid, 1.8; vitamin B₁₂, 0.02; cellulose, 97.66.

^b Mineral premix contains (g kg⁻¹ of premix): calcium phosphate (monobasic) monohydrate, 136.0; calcium lactate pentahydrate, 348.49; ferrous sulfate heptahydrate, 5.0; magnesium sulfate heptahydrate, 132.0; potassium phosphate (dibasic), 240.0; sodium phosphate (monobasic) monohydrate, 88.0; sodium chloride, 45.0; aluminum chloride hexahydrate, 0.15; potassium iodide, 0.15; cupric sulfate pentahydrate, 0.50; manganese sulfate monohydrate, 0.70; cobalt chloride hexahydrate, 1.0; zinc sulfate heptahydrate, 3.0; sodium selenite, 0.011.

The raw ingredients are finely ground and sieved through 355 µm mesh. For each feed, the ingredients were weighed and blended until a homogeneous powder was added to which the palm oil was added. Water was then added in a proportion of 50% of the dry matter so as to obtain malleable dough. This paste was passed through a chopper (Moulinex HV8) and gave small filaments 1.5 mm in diameter. The manufactured feeds are dried in the sun at a temperature of 30°C during 24h and then broken to the desired size before being placed in storage boxes for packaging (temperature -18°C) until distribution.

These feeds were tested on 750 Tilapia fingerlings (*O. niloticus*), with an initial average weight of 4.6 g. These fish were distributed in 15 tanks of 150L of useful volume, ie 50 fish per tank, thus forming five treatments in triplicate each corresponding to a diet. The feeding trial was conducted during 42 days (6 weeks) in recirculated system including 15 tanks in cement containing each 0.5 m³ of water supplied by a drilling and a compressor (FIAC, axair 100L 2CV 10B 230 V) at a flow rate of 3L min⁻¹.

These fish were fed with experimental diets four times a day (8 AM, 11 AM, 2 PM and 5 PM). The daily ration was fixed at 5% according to reports of Fiogbé and Kestemont (2003). In each tank, the rate of water turnover is 3 L. minute⁻¹. Each tank is covered half of its surface with a fence to avoid the direct penetration of the solar rays which favors great variations of the temperature of the water but also the development of chlorophyllian algae. The water quality parameters taken every three days and remained throughout the trial within the acceptable range reported for the rearing of Nile tilapia (Djissou, Ochiai, Koshio & Fiogbé, 2017). These parameters were 5.37±0.5; 4.98±0.31 mg L⁻¹ and 27.8±0.4°C respectively for pH, dissolved oxygen and temperature in ponds. In each control fishery (every 7 days), the fish fingerlings are counted and weighed per tank to determine the biomass and adjust the ration.

Biochemical Analyzes

The biochemical analyzes (proteins, dry matter, ash) were carried out in triplicate according to the AOAC (1990) standard methods and involved ingredients, experimental feeds, homogenized carcasses of whole fish taken randomly at the beginning of experience. These analyzes were also carried out on the homogenized carcasses of 10 whole fish taken randomly after 3 days of the end of the experiment in each of the 15 experimental tanks, ie 30 fish per diet. The crude protein is assayed with the kjeldahl method. The material is determined by measuring the loss of weight after drying for 24 hours in an oven at 105°C. The ash is determined after the incineration of the samples at 550°C for 12 hours. Analysis of the amino acids in the ingredients was carried out by high performance liquid

chromatography (HPLC, Waters 474, Milford, MA, USA). These analyzes were carried out according to the method described by Bosch, Alegria and Farré (2006). The alkaloids and tannins were determined by the spectrophotometric method described by Nyinawamziza (2007) and Aganda and Mosase (2001) after extraction with the organic solvent, whereas gossypol was determined according to the method described by Imorou Toko, Fiogbe and Kestemont (2008).

Growth Performance and Feed Efficiency

Growth performances and diet nutrient utilization were analyzed using the feed conversion ratio (FCR), specific growth rate (SGR), protein efficiency ratio (PER), survival rate (SR) and protein productive value (PPV). These parameters were calculated using the following formula:

$$FCR = \frac{\text{dry matter feed intake (g)}}{\text{body mass gain (g)}}$$

$$SGR (\%) = 100 \times \frac{(\ln[FBW] - \ln[IBW])}{\text{number of days}}$$

Where IBW and FBW are Initial Body Weight and Final Body Weight

$$PER = \frac{\text{wet body mass gain}}{\text{protein intake}}$$

$$SR (\%) = 100 \times \frac{\text{final number of fish}}{\text{initial number of fish}}$$

$$PPV = \frac{\text{Body protein gain}}{\text{protein intake}}$$

Statistical Analysis

Data obtained from the experiment were subjected to one-way analysis of variance after verifying the normality and the homogeneity of variance using the statistical software Statviews (version 5.01). Least-Significant-Difference test of Fisher was used to compare differences among individual means. Treatment effects were considered significant at $P < 0.05$.

Results

The protein contents and the essential amino acid composition of the feed ingredients are shown in Table 2. The maggots and brewer's yeast have high crude protein content and an essential amino acid composition comparable to that of fish meal. Table 3 provides information on the biochemical composition of experimental isoproteic diets after analysis with crude protein content of 30.44±1%. The essential amino acid composition of the experimental diets is consistent with the essential amino acid requirements of Nile tilapia.

Table 2. Composition of essential amino acids (EAA), crude protein (CP) and crude lipid of the main ingredients (g100g⁻¹ dry matter)

EAA	<i>Moringa oleifera</i>	Fish meal	Soybean meal	Brewer's yeast	Cottonseed meal	Wheat bran	Maggot
Threonine	0.62	2.31	0.76	2.40	0.45	0.31	2.09
Valine	0.57	2.77	0.56	2.80	0.50	0.25	1.91
Methionine	0.24	1.94	0.24	0.80	0.20	0.06	1.82
Isoleucine	0.38	2.45	0.52	2.30	2.50	0.13	3.05
Leucine	1.19	3.79	1.72	3.50	0.95	0.50	6.35
Phenylalanine	1.00	3.74	1.36	2.10	1.10	0.50	3.53
Histidine	0.43	1.75	0.64	1.20	0.70	0.44	3.01
Tryptophan	0.29	0.57	0.32	0.70	0.40	0.25	3.17
Lysine	0.67	4.22	1.20	3.80	0.50	0.44	4.23
Arginine	1.05	3.43	2.04	2.60	2.15	0.94	6.06
CP	27.7	66.2	32.4	50	30.3	14.7	54.6
CL	9.9	13.2	11.1	10.6	7.0	1.7	29.82

Table 3. Proximate nutritional (% dry matter) and amino acid composition (g 100g⁻¹ of diet) of experimental diets after analysis*

Parameters	D1	D2	D3	D4	D5	<i>O. niloticus</i> requirement**
Ash (%)	11.82	7.90	7.83	7.63	8.14	-
Crude protein (%)	31.44	29.74	29.85	29.51	29.44	-
Crude lipid (%)	13.1	14.0	14.2	14.5	14.9	-
Dry matter (%)	91.53	85.26	90.64	87.02	85.97	-
Gross Energy (kJ g ⁻¹)	19.76	19.41	19.76	19.59	19.87	-
P/E ratio***	15.91	15.32	14.09	14.55	14.81	-
<i>Essentials Amino Acids (g.100g⁻¹DM)</i>						
Threonine	1.4	1.1	1.1	1.1	1.1	1.05 – 1.1
Valine	1.6	1.0	1.0	1.0	1.0	0.78 – 1.5
Methionine	0.9	1.6	1.6	1.6	1.7	0.75 – 1.0
Isoleucine	1.8	1.5	1.6	1.6	1.7	0.87 – 1.0
Leucine	2.4	2.5	2.6	2.6	2.6	0.95 – 1.9
Phenylalanine	2.2	1.7	1.7	1.7	1.7	1.05 – 1.6
Histidine	1.1	1.2	1.2	1.2	1.2	0.48 – 1.0
Tryptophan	0.5	1.0	1.0	1.0	1.1	0.28 – 0.3
Lysine	2.4	1.8	1.8	1.9	1.9	1.43 – 1.6
Arginine	2.5	2.7	2.7	2.8	2.8	1.18 – 1.2
<i>Antinutritional factors</i>						
Alkaloids	trace	trace	trace	trace	trace	-
Tannins	0.1	0.2	0.2	0.4	0.3	-
Gossypol	0.18	0.14	0.15	0.18	0.19	-

* Number of sampling analysis (n = 3)

**NRC (1993, 2011)

***P/E = Protein to energy ratio in mg protein kJ⁻¹ gross energy.

Tannins have relatively high levels in diets without fish meal (D2 to D5) compared to diet D1 with fishmeal. Alkaloids are practically absent in all diets with gossypol levels of 0.14-0.19% (D1-D5).

Table 4 shows information on growth performance and feed utilization. Experimental feeds were well accepted by fish without observation of feed rejection during feeding throughout the experiment. There was no significant difference ($P > 0.05$) in the amount of feed ingested between fishes fed diet D1 and fishes fed D2 to D5. However, fish fed diets based on *M. oleifera* leaves and maggots consumed less food than those fed

on the control diet (D1) with fish meal. The survival rate was high (96.67-98.67%) and was not significantly affected by the complete replacement of fishmeal.

A significant reduction was observed in the final average weight of fish fed diets D2 to D5 compared to fish fed the control diet D1. The same observation was made with the specific growth rate (SGR) where a decreasing trend is noted from D1 to D5. FI followed the same trend as SGR. As for feed utilization performance (Table 4), feed conversion ratio (FCR) values increased with D2 to D5 ($P < 0.05$), with the *M. oleifera* / maggot ratio increasing. The same trend is observed with the

protein efficiency ratio (PER) and the amount of protein intake (PI) with the exception of the diet D5 where the PI is not significantly different from that obtained with the control diet D1. The protein productive values (VPP) are significantly different from one diet to another, except for diets D3 and D4, where the difference is not significant ($P>0.05$).

Approximate compositions of the whole body of *O. niloticus* fingerlings at the beginning and at the end of the experiment were presented in Table 5. These compositions were clearly affected by diet composition. There was a significant difference in the protein content of fish carcasses between diets except for diets D3 and D4. The dry matter content of carcasses was significantly lower in fish fed diet D2 than those fed on other diets. The ash content did not vary significantly between diets except diet D4.

Discussion

Throughout the trial, water quality in all diets remained within the margin of tolerance required for Nile tilapia (Ballarin & Hatton, 1979). Our work showed a significant difference between fish fed on the control diet (D1) with fish meal and those fed on diets without fish meal (D2 to D5) with all parameters of growth and feed utilization, except for protein intake (PI) in diet D5. This can be explained by the high level of protein obtained after analysis in the control diet (D1) compared to other diets without fish meal. These results confirm the work of Médale *et al.*, (2013) who asserted that for optimal efficiency of production performance, protein should represent 30% of the dry matter in the diet for fish of lower trophic level, such as carp and tilapia, which are more effective at producing Energy from food

carbohydrates (NRC, 2011). Similar results (total replacement of fishmeal) were observed in the Nile tilapia fry by Djissou *et al.*, (2016a) with a mixture of leaf maggots *Azolla filiculoides-Dialum guineense* and Fasakin, Balogun and Fagbenro (2001) with *Azolla africana*. These findings are contrary to work on other species such as *Pagrus major* (Kader *et al.*, 2012) using dehulled soybean and attractants, *Clarias gariepinus* (Djissou, Adjahouinou, Koshio, & Fiogbé, 2016b) using maggots and earthworms. This difference in performance is due to the nature of the fish diet and the digestibility of the feed. Indeed, digestibility is high in fish (NRC, 2011) whether high or low trophic level. Reduced growth performance and low feed intake in fish fed diets without fish meal are due to the lack of attractants capable of improving dietary intake (Espe *et al.*, 2006) and presence of antinutritional factors (Dias *et al.*, 2005). Burel and Médale (2014) asserted that plants contain substances that can negatively impact appetite, digestion, nutrient absorption and metabolism. Furthermore, Richter *et al.*, (2003) showed during their work not only that there is a relative presence of a high amount of phenols, tannins, saponins and phytic acid in the leaves of *M. oleifera* but also that the growth of *O. niloticus* is used for a use of more than 10% of *M. oleifera* leaves as food proteins in replacement of fishmeal. The presence of antinutritional factors such as alkaloids (trace), gossypol and tannins in relative proportions hinders the digestibility of proteins. According to Francis, Makkar and Becker (2001), alkaloids would affect the appetite while tannins and gossypol inhibit digestive enzymes. This is in agreement with the performances recorded during this experiment where feed intake does not vary significantly from one diet to another, on the one hand, but varies significantly

Table 4. Growth performance and feed utilization of *Oreochromis niloticus* fingerlings fed with the experimental diets

Parameters	D1	D2	D3	D4	D5
FBW (g)	11.55±0.08 ^a	9.52±0.27 ^b	9.40±0.29 ^{bc}	8.91±0.09 ^c	8.99±0.08 ^{bc}
FCR	1.20±0.05 ^a	1.47±0.07 ^b	1.62±0.07 ^{bd}	1.73±0.07 ^{cd}	1.87±0.05 ^c
SGR (%/day)	2.57±0.06 ^a	2.05±0.07 ^b	1.94±0.05 ^b	1.87±0.07 ^b	1.89±0.03 ^b
FI (g)	0.59±0.11	0.48±0.02	0.49±0.21	0.45±0.16	0.47±0.13
PI	2.92±0.05 ^a	2.54±0.08 ^b	2.67±0.11 ^{bc}	2.63±0.02 ^b	2.88±0.05 ^{ac}
PER	0.92±0.05 ^a	0.76±0.04 ^b	0.67±0.05 ^{bc}	0.64±0.04 ^{bc}	0.59±0.03 ^c
PPV	2.18±0.27 ^a	1.74±0.11 ^b	0.95±0.09 ^c	0.86±0.02 ^c	1.48±0.03 ^d
SR (%)	98±1.16	98.67±1.33	96.67±3.33	98.67±1.33	98.67±1.33

Mean ± SD values in the same line followed by the same superscript are not significantly different ($P>0.05$).

Table 5. Proximate composition of *Oreochromis niloticus* fingerlings fed experimental diets

Composition (% DM)	Initial	D1	D2	D3	D4	D5
Ash	35.16	23.59±0.13 ^a	22.03±0.08 ^b	22.89±0.19 ^a	20.98±0.16 ^c	22.42±0.07 ^b
Protein	35.32	41.68±0.84 ^a	39.74±1.11 ^b	37.88±1.16 ^c	37.58±0.89 ^c	39.58±0.48 ^b
Dry matter	88.74	87.94±1.21 ^{ac}	86.26±0.89 ^b	88.64±1.02 ^a	88.29±0.53 ^a	87.59±0.74 ^c

Mean ± SD values in the same line followed by the same superscript are not significantly different ($P>0.05$).

with the use of proteins in diets without fish meal on the other hand. Moreover, these results confirm the work of Jackson, Copper and Matty (1982) who found that an approximate rate of 2.4% of tannins caused growth reduction in fish of the genus *Tilapia* and *Labeo rohita*. In addition, tannins are known to interact with other antinutritional factors (Francis *et al.*, 2001).

However, the ratio between animal proteins / plant proteins has, at *Tilapia*, a significant role on the performances of growth which increase with the share of animal proteins (Azaza, Mensi, Abdelmouleh, & Kraiem, 2005). This assertion is in contrast with the growth performance recorded when the proportion of animal proteins is low (D2 with the ratio 3:5). This could be explained by the quality of the test diets tested.

Essential amino acid (EAA) compositions of maggots, *M. oleifera* leaves and fish meals are different. The EAA content of the maggots is in more than 50% of the cases higher than the fish meal when EAA of *M. oleifera* are in lower proportions. Except for methionine and tryptophan, the control diet D1 is higher in EAA than diets without fish meal. The quality of a protein source depends not only on the amount of protein but also on the essential amino acid composition and bioavailability (Cai & Burtle, 1996). The poor performance recorded with diets without fish meal can be explained by the digestibility due to the presence of tannins and gossypol in the diets without fish meal, corollary of the bioavailability of the AAE. The variety of antinutritional factors in protein sources, such as plants, their potential interactions and the sensitivity of fish must be taken into account in the formulation of aquaculture feed.

Several studies (Francis *et al.*, 2001) have shown that antinutritional factors can be reduced by heat treatment, soaking, germination and fermentation. But particular attention must be paid to these methods, which sometimes have unfortunate adverse effects on the nutritional quality of the raw material. This is the case of heat treatment which can change the chemical nature and decrease the protein quality and carbohydrates (van der Poel, 1989 in Francis *et al.*, 2001).

Growth and feed utilization performance during the complete replacement of fish meal with a mixture of maggot and *M. oleifera* meals can be improved in the feeding of *O. niloticus*. The use of food additives, phytase and a treatment (thermal) preliminary to the raw materials (vegetable) would increase the appetibility and digestibility of unconventional sources of protein used to optimize the bioavailability of amino acids and thus growth fishes.

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