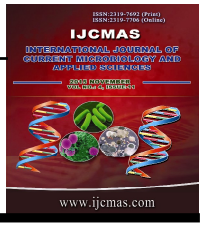
Int.J.Curr.Microbiol.App.Sci (2015) 4(11): 536-549

International Journal of Current Microbiology and Applied Sciences
ISSN: 2319-7706 **Volume 4 Number 11 (2015) pp. 536-549**
http://www.ijcmas.com



Original Research Article

Use of Effective Microorganisms (Em) in Tilapia Diets: Effects of Growth Performance and Carcass Composition

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Keywords

Effective microorganisms Tilapia, Diets, Growth, Performance

A B S T R A C T

The main purpose of this experimental study was to determine the appropriate amount of *Moringa oleifera* leaves meal (MOLM) fermented with activated effective microorganisms (EM-A) to be incorporated into the diet of fry *Oreochromis niloticus* to obtain best growth performance. Fish were fed experimental diets containing 5 differentiates of MOLM + EM-A incorporated: RI = 0% (*Moringa* + EM-A); R2 2.5% (*Moringa* + EM-A); R3 = 5% (*Moringa* + EM-A) R4 = 7.5% (*Moringa* + EM-A); R5 = 10% (*Moringa* + EM-A). The results showed that the mean weight gain (MWG), specific growth rate (SGR) and food conversion rate (FCR) were higher in fry fed the diet containing 2.5% MOLM+ EM-A followed by the fry fed 7.5% MOLM + EM-A. The daily mean weight gain (DMWG) was in favor of fry fed the diet containing 2.5% and 7.5% MOLM + EM-A. The fry fed diets with 0, 5 and 10% MOLM + EM-A presented comparable DMWG. The protein and lipids of the muscle were higher in fry fed diets containing 0 and 2.5% MOLM+ EM-A followed by other diets (5, 7.5 and 10% MOLM + EM-A). Survival rate is relatively high and ranged from 85 to 100 %. From these results, the present conclude that the diet containing 2.5% MOLM + EM meets the best growth performance of fry *Oreochromis niloticus*. Future studies may be conducted to focus on the effects of these different diets on biochemical digestion in *Oreochromis niloticus*.

Introduction

Nile tilapia (*Oreochromis niloticus*, Linnaeus 1758) is one of the most cultured fish in tropical and subtropical regions in the world. It is one of the first fish species cultured and is still the most widely cultured species of tilapia in Africa. The global production of all species increased from 1.5 million tons in 2003 to 2,500,000 tons in 2010, with a market value of more than US$ 5 billion. It is expected that the majority of this large production is attributed to Nile tilapia. In Senegal, the Tilapia production reached almost 700 metric tons (NAA, 2014).

In aquaculture systems, the increasing price of feed is considered as one of the most important factors that limit profitability, mainly caused due to the high cost of fishmeal used as a primary source of protein (Usmani *et al.,* 1997). As a result, it is then required to search for alternative protein sources for aquaculture diet. The high cost and fluctuating quality of imported fish meal have led to the need to identify alternative protein sources for use in fish feed formulations (Olvera *et al.,* 1990). The identification and utilization of non-conventional and lesser–utilized plant protein sources to replace fishmeal, either partially or totally in practical fry diets has been an area of focus in aquaculture nutrition (Hossain *et al.,* 2003).

Plant proteins are cheap and readily available, but have a certain number of antinutritional factors that limit their use as aquaculture feeds. Earlier studies have shown that, *Moringa oleifera*is a promising protein source for inclusion in fish diets at low levels (Chiseva, 2006). Numerous options had been advocated by the researchers to draw their attention to utilize non-conventional feedstuff with emphasis on leaf meals as protein substitute for fish meal in fish feeds such as *Moringa oleifera* (Richter *et al.,* 2003). *M. oleifera* constitutes huge potential source of protein, as well as an excellent source of vitamins, minerals and amino acids. It possesses some medicinal properties that could fight against malnutrition, treatment for cardiovascular diseases, anti-ulcer, anti-inflammatory, food for human consumption, reproductive health, and for other industrial purposes (Khalafalla et al 2010). *M. oleifera* is the most widely cultivated specie of the genus *Moringa*, which is the only genus in the family *Moringaceae* with high crude protein in the leaves (251 g kg-1 DM) with unimportant content of tannins and other anti- nutritive compounds (Nouala *et al.,* 2006). In Africa, Moringa is called the "Miracle tree of life", savior, and in local language "Nébédaye," which means "never die."

In recent years, there have been considerable interests in using some probiotic microorganisms and organic acids as alternative to the usage of antibiotics in feed. Probiotics are viable microorganisms and supportive substances that, once ingested by humans and animals, produce beneficial physiological effects by assisting in the establishment of an intestinal population which is profitable to the host's entity and antagonistic to harmful bacteria.

Probiotics have been directly supplied through feed, in some cases using binders for stabilization (Kolndadacha *et al.,* 2011). Supplementing feed with probiotics is common in aquaculture. Furthermore, probiotics have been directly applied to culture ponds in view to improve the quality of water and the survival of cultured animals. The effectiveness of probiotics can be explained by bio-increase or biocontrol devices through which the microbial ecology of the water and sediment is improved (Rengpipat, 2005). Supplementing the diet fish with probiotics can reduce the usage of antibiotics and synthetic chemicals in the feed (Fuller, 1989). Consequently, the addition of probiotics to fish diets has become widespread on aquaculture farms. The application of probiotics results in reduced feed costs, which plays an important role in determining practices. Probiotics are used to stimulate aquatic animal growth of aquaculture. Interestingly, previous research findings have shown that the beneficial effects of probiotics can manifest as enhanced feed utilization of cultured aquatic animals through the supplementation of digestive enzymes,

improved feed efficiency and higher growth, the prevention of intestinal disorders and the pre-digestion of anti- nutritional factors present in mixed feed (Suzer *et al.,* 2008). The thorough function (utility) of probiotics in the improvement of growth and feed utilization in fish was noticed as related to the improvement of nutrient digestibility. However, using higher concentrations of probiotic does not always conduct to improved growth performance.

Probiotics have been identified as live microorganisms colonized in the intestine and/or animal originated microbial supplements could be effected on fish health by improving the intestinal microbial balance (Wang *et al.,* 2008). Nowadays, probiotics in aquaculture are commonly supplemented to control diseases and also rise up feeding and husbandry parameters. The useful effects of these probiotics as nutrient supplementary additive include relatively higher growth rates of feed efficiency, also prevention of intestinal disorders and pre digestion of anti-nutritional factors as well present in ingredients. So, recent studies focused on probiotics are mainly related to the resistance of some aquatic pathogens. However, a few studies have been carried out based on growth performances to incorporate effective microorganism effects in tilapia.

Effective Microorganisms, or EM is one of the most popular microbial technologies now used worldwide and EM products have been on the market since 1983 in Japan. EM is safe to use because they are harmless and they are 100% organic, not chemically synthesized. In addition, EM is not a drug (Ismah, 2011). EM consists of many different kinds of effective and disease-suppressing microorganisms. Each of these effective microorganisms has specific task. EM consists of the following five families of microorganisms: Lactic acid bacteria: such bacteria are differentiated by their powerful sterilizing properties. They suppress harmful microorganisms and encourage quick breakdown of organic substances. Moreover, they can suppress reproduction (development) of *Fusarium*, harmful fungus. Yeasts**:** manufacture anti-microbial and useful substances for plant growth. Their metabolites are food for other bacteria such as lactic acid and actinomycete groups. Actinomycetes: suppress harmful fungi and bacteria and can live together with photosynthetic bacteria. Photosynthetic bacteria: such bacteria play the leading role in EM's activity.

They synthesize useful substances from secretions of roots, organic matter and/or harmful gases (e.g. hydrogen sulphide) through the use of sunlight and heat of soil as sources of energy. They contribute to the better usage of sunlight or, in other words, better photosynthesis.

Metabolites developed by the above cited microorganisms are directly absorbed into plants. In addition, these bacteria increase the number of other bacteria and act as nitrogen binders. Fungi that bring about fermentation these quickely break down the organic substances. This eliminates suppresses smell and prevents damage that could be caused by harmful insects**.** EM comes in a liquid form and consists of naturally occurring beneficial microorganisms.

The objective of this study is indeed to evaluate effects of *Moringa oleifera* leaves meal (MOLM) fermented with active effective microorganisms (EM-A) on the growth performance, survival and carcass composition of tilapia (*Oreochromis niloticus*).

Materials and Methods

Culture conditions

Tilapia fry were taken from the hatchery area in Richard Toll, Saint-Louis, Senegal. After that, they were acclimated to laboratory conditions for two weeks during which period they were fed with commercial diet imported from China. At the beginning of the experiment, 100 tilapia fry were randomly divided into five different groups with two replicates (i.e. areas/water basins) containing 10 fish/each. Fish were kept in 10 glass tanks (50 x 40 x 30 cm) containing (50 L).The initial mean weight was 4.1 ± 0.6 g in 10 glass tanks. Each aquarium was part of a closed re-circulating system maintained at 28 ±1°C. An air stone continuously well ventilates each aquarium. All aquaria were cleaned up every day in mornings and afternoons by siphoning off accumulated waste materials.

Fish were then fed with 10% of body weight per day and gradually decreased to 4% per day. Each diet was fed twice a day at 08 h 00 (a.m.) and 5 h 00 (p.m.) for 8 weeks to duplicate groups of fish. On the other hand, each group of fish was weighed in the beginning and every two weeks and the amount of diet fed was adjusted, accordingly. No feed was given on the weighing days to prevent stress. A photoperiod of 12 h light, 12 h dark (08 h 00 (a.m)–8 h 00 (p.m.) was used, while fluorescent ceiling lights supplied the illumination. After 8 weeks of feeding, fish were taken out from each treatment; the dorsal muscle tissue of each was dissected and used for carcass composition analysis purposes.

Production of effective microorganisms

Making EM-A (called Activated-EM) is realized by mixing EM-1 purchased from TeraGanix, Inc, North American distributor for EM Technology® products, under license from EMRO USA Effective Microorganisms in Tucson, Arizona with molasses (which must be sugar cane and not sugar beet molasses), waste food and rice bran allowing them to ferment in a sealed bokashi bin for 7 days in room temperature.

Raw materials

Moringa oleifera leaves used were bought from the local market. And then, they (viz. leaves) were rinsed in clean water to remove dirt and dried by spreading out thinly on a concrete floor under a shady area to prevent the loss of vitamins. After this step, they were also covered with mosquito net to protect them from pests and other contaminants with constant mixing. After 3 days of drying, the leaves became brittle and were ground into powder using mortar and pestle and later sifted to remove the remaining stems. The clean dried powder was stored in airtight container and kept at room temperature. After the analysis of its gross protein content, which is equal to 25.84%, was achieved. Then the dried powder underwent 48 hours of fermentation with EM-A at ration of 100g of dried powder of *Moringa oleifera* leaves: 100 ml of EM-A).

Fishmeal (CP: 56.05%) used for the purpose was purchased from the "Nouvelle Sociétéd'Assistanceetd'Approvisonnement" (NSAAP) (New Society Assistance and Supply) (NSAAP) located in Sacré Coeur 3 (district), Dakar Sénégal. Carboxymethyl-cellulose used as binder, vitamins and minerals premix was purchase from AquavetcompayinTivaouane, Thiès, while Fish oil was bought from AFRIC AZOTE, Dakar Senegal and wheat flour (PB: 9.5) bought from the local market.

Diet preparation

Five iso-nitrogenous (approximately 35% crude protein) and iso-lipidic (approximately 5% crude lipid) experimental diets were formulated to contain same ingredients but different rates (R1: 0%, R2: 2.5%, R3: 5%, R4: R5 7.5% and 10%) of *Moringa oleifera* rate + EM-A fermented for 48 hours. The main animal protein source was fishmeal at 56.05% of crude protein. The remnant comes from various ratios of wheat flour and *Moringa oleifera* (Never die) leaves meal. They were grounded into mill and passed as particles through no. 40 (425µm) mesh sieve. Mineral and vitamin premix were purchased from Aquavet feed Company, Thiès, Senegal. After all, ingredients were thoroughly mixed, and appropriate quantity of water provided (30% for 100 g of mixed ingredients). Diets were supplemented with 5% of fish oil (Table 1). Dough was passed through an extruder to produce spaghetti and dried at 37°C for two days. So, the concerned dried diet was packaged into plastic bag and stored frozen until its usage.

The experimental diets and samples of the dorsal muscle were analyzed for proximate composition based on AOAC (1984) methods. Crude protein was determined with a Kjeltec system 1002 (Tecator). Crude lipid was determined by chloroform-methanol (2:1, v/v) extraction method (Folch *et al.*, 1957). Crude fiber was determined by the Fibertec system M 1020 hot extractor (FOSS Tecator). Ash and moisture were determined with conventional methods using muffle furnace at 505°C and an oven at 105°C.

Biochemical analysis

The experimental diets and samples of dorsal muscle (in the beginning and after the experiment) were analyzed for proximate composition using standard methods of AOAC (1984).

Fatty acids analysis

Lipid was extracted from feed samples by homogenization in chloroform / methanol (2:1, v/v) containing 0.01% butylated hydroxytoluene (BHT) as antioxidant, according to the methods of Folch *et al.* (1957). Fatty acid methyl esters were prepared by acid-catalyzed transmethylation of total lipids (Shantha and Ackman, 1990). The FA composition was analyzed by a gas chromatograph (Auto System XL Perkin Elmer) using a 30 x 0.25 mm capillary column (FID detector CP- 2380 Supelco, Bellefonte, USA). The conditions of the method were: carrier gas, helium; flame ionization detection temperature, 260°C; split rate: 1 / 50, oven temperature programmed to rise from 120°C / 2 min to 220°C/ 15 min at a rate of 5°C /min; injector temperature, 240°C. The identification of the individual methyl esters was achieved by comparison of their retention times with commercial standards (Sigma, St. Louis, MO, USA).

Aminoacids analysis

The amino acid compositions of experimental diets were analyzed following acid hydrolysis using an automatic amino acid analyzer (Hitachi 835-50, Tokyo, Japan) equipped with a column for physiological fluid analysis by a professional laboratory.

Growth parameters

Growth response parameters were calculated as follows: Mean Weight gain (MWG, %) = 100* [(final mean body weight - initial mean body weight)/ initial mean body weight];

Specific Growth Rate (SGR, % /day) = 100* [(In Wt- In Wi) /T], where Wt is the weight of fish at time t, Wi is the weight of fish at time 0 and T is the rearing period in days; Feed Conversion Rate (FCR) = total dry feed fed g/ fish / total wet weight gain g/ fish. Survival rate (%) = 100* (number of fish which survived/initial number of fish).

Water Quality Measurement

Water temperature and dissolved oxygen were measured each following day using YSI Model 58 oxygen meter (Yellow spring Instrument, Yellow Spring, OH, USA)

Statistical analysis

The obtained data were entered and calculated with Microsoft Excel. Analysis of these data was performed with the Statistical Analysis System (SAS-PC) (Joyner, 1985) and subject to one way analysis of variance (ANOVA). Duncan's test was used to compare the significant differences between treatments. The treatment effects were considered significant at P< 5%.

Results and Discussion

Comparison of the diet composition

The chemical composition of the diets is shown in table 2. A slight variation in proximate composition was observed among diets. The dry matter, crude fiber and Ash content slightly increased with an increase in fermented MOLM with EM-A. However, gross energy, digestible energy, digestible protein and lipid content slightly decrease with an increase in fermented MOLM with EM-A. Crude protein was marginally different between the fermented MLM with EM-A diets.

The biochemical composition of processed diets is shown in table 3. In general, aqueous extraction led to reduction of amino acid content in particular histidine, lysine and methionine as compared with NRC (2011).

The results in table 4 showed that all diets have no significant difference in quantitative requirements for essential fatty acids EPA, DHA and ARA. However, the total n-3 is more important than the total n-6 in all diets. The total phospholipid content of diets slightly decrease with an increase of fermented *Moringa* leaves meal with EM-A.

Water Quality Control

The water quality parameters monitored were with tolerable limits for tilapia. Water temperature varied from 27.1 to 29.6°C and the dissolved oxygen from 7 to 8 mg/l.

The growth performance, survival and feed utilization of tilapia fingerlings fed experimental diets for 8 weeks days are presented in table 5.

The fish fed R2 (2.5%) and R4 (7.5%) diets gained weight significantly higher ($P < 0.05$) than those fed with R1 (control), R3 (5%) and R5 (10%) diets. The best weight gain was obtained in those fish fed with diet R4. There was no significant difference ($P>0.05$) in mean weight gain between fish fed R1, R3 and R5 diets. The SGR of fish fed with the test diets followed the same general pattern as the mean weight gain. Fish fed on R2 and R4 had the best food conversion rate (FCR) that showed significant difference ($P<0.05$) with the FCR of fish fed with the control diet R1. The best FCR was observed in fish fed diet R2. The fish fed R1 and R3 diets had the lowest FCR and showed no significant difference ($P>0.05$) with the R5 diet. Overall survival rate obtained in the experiment was satisfactory and varies from 85% to 100% being 95% for R1, 85% for

R2, 100 for R3, 90% for R4 and 95% for R5. The best survival rate was observed with fish fed with the diet R3.

Carcass composition data at the beginning and the end of the experiment are presented in table 6. There was no significant difference for the carcass organic matter content between fish fed with different diets and initial fish. Contrary for carcass dry matter content, significant differences ($P<0.05$) were observed. The dry matter contents of the carcass of fish subject to R2 diet (94.77%) and R3 (93.29%) did not have significant difference ($P>0.05$) with those of initial fish's carcass (93.13%). Dry matter contents of the carcass of fish fed with R1 diets (89.93%), R4 (89.90%) and R5 (88.71%) were lower than those of initial fish (93.13 %). Significant differences ($P<0.05$) were observed in body composition of protein of fish fed with the different types of diets. The body protein content was significantly higher in initial fish (82%), followed by those fed diets with R5 (80.53%) and R2 (75.52%).

While, the carcass protein content of fish fed with diets R1 (71.96%), R4 (71.02%) and R3 (70.82%) are lower than that of initial fish. Regarding lipid, there is significant difference between initial fish (22.63%) and fish fed with diets R1 (16.85%), R2 (16.93%), R3 (26, 62%) and R5 (27.97%). The highest lipid carcass content was presented by fish fed with R5 and R3 diets. There were no significant differences of lipid carcass composition of tilapia fed with R4 diet (23.42%) initial fish.

Water Quality

The water quality parameters during the present study are in the ranges recommended optimum values of tilapia. Temperature values (27.1 to 29.6°C) recorded in this experiment are similar to those (26–30°C) reported by Mélard (1999). Enda and Boyd (1997) found that temperature between 28 and 32 ° **C** is optimal for the growth of tilapia. The dissolved oxygen content in the present experiment was between 7 and 8 suitable to make tilapia grow up. These results are in line with those of Kestmont *et al.* (1989) and Mélard (1999) who reported that dissolved oxygen content greater than 3 mg / l is the optimum for good growth of tilapia. According to Mélard (1999), growth performance of fry *O. niloticus* are dependent on thermal conditions and availability of dissolved oxygen in the rearing environment earlier than distributed diets.

Survival

Our study, regardless the feed distributed, survival rate is relatively high and ranged from 85 to 100 %. According to Sumi *et al.* (2011), the survival rate above 80% is excellent in nursery and those obtained in the range above, we can consider that our results are within the accepted standard**.**

The feed conversion ratio (FCR)

In this survey, fish fed R2 (2,5% MOLM+EM-A) and R4 (7,5% MOLM+EM-A) had the best food conversion rate (FCR) that showed significant difference ($P<0.05$) with the FCR of the one fed with the control diet R1 (0% MOLM+EM-A).

Fish fed R1 and R3 (5% MOLM+EM-A) diets had the lowest FCR and showed no significant difference ($P>0.05$) with the R5 (10% MOLM+EM-A) diet. Huang *et al.* (1999) reported that the FCR of shrimp where experimentation ponds were added with EM decreased by 9.4%. In contrast,

Niang (2013) showed that the FCR of fry tilapia fed with diet containing 30% crude protein and supplemented with 100 g of EM-Awas 0.29.

Mean Weight gain (MWG) and specific growth rate (SGR)

The study of utilization of *Moringa* (*Moringa oleifera* Lam.) leaf meal fermented with EM-A on growth performance and survival rate in tilapia was conducted. Diets were prepared by supplementing with fermented *Moringa* leaf at R1: 0%, R2: 2.5%, R3: 5%, R4: R5 7.5% and 10% all diets contained 35 % crude protein. Fish fed R4 diets gave the best weight gain as compared with other diets. These results are consistent with those of Huang *et al.* (1999). The latter, after raising for 45 days Chinese carp using EM as food additive, showed that EM can obviously promote the growth of *Cyprinus carpio* Linnaeus, 1758.Moreover, moringa (*Moringa oleifera*Lam.) leaf was used in hybrid catfish (*Clarias macrocephalus x Clarias gariepinus)* diets at 0, 5, 10 and 15%, all diets contained 35 % crude protein. The result showed that the highest weight gain of fish nursed at 5% of moringa leave meal in diet was 51.05±0.76 g and followed by 0, 10 and 15 % of moringa leave meal in diet were 50.72±3.84, 47.82±2.46 and 44.89±7.13 g, respectively. This contradicts the study, which shows that *M. oleifera*leaf meals (MOLM) were incorporated in sea bass diets at 0 % (T1); 10 % (T2); 20 % (T3) and 30 % (T4). The highest percentage weight gain (%) had been observed in sea bass fed with control diet (0 % MOLF) than fish fed 10, 20 and 30 % MOLM diets (Erlinda S. Ganzon-Naret, 2014). Furthermore, Afuang *et al.* (2003) showed no effects of dietary supplement of methanol-extracted leaf meal containing 11, 22 and 33 g kg-1 found on the growth of Nile tilapia (*Oreochromis niloticus* L.).

Moringa leaf can partially replace conventional diets without any depression in growth performance of Nile tilapia (*Oreochromis niloticus* L.). For instance, Tagwireyi *et al.* (2008) reported that the results of this study indicated that up to 10% inclusion of steam heated moringa leaves can be recommended for *Nile tilapia.* Richter *et al.* (2003) reported that using moringa (*Moringa oleifera* Lam.) as an alternative protein source for Nile tilapia (L.) at 10%, 20% and 30% for 7 weeks was found that Nile tilapia fed with 10% of moringa in diet was the best growth performance, but Nile tilapia was fed with moringa from 20 and 30% of moringa leave meal in diet, the growth performance was reduced. Afuang *et al.* (2003) reported that the utilization of moringa (*Moringa oleifera* Lam.) leaf on growth performance and feed utilization in Nile tilapia (*Oreochromis niloticus*L.) at 13, 27 and 40% were found that Nile tilapia fed with 13% of moringa leave meal in diet reduced growth performance. A number of studies showed that *M. oleifera* leaf meal could be used to substitute FM up to 10 % level in *Clarias gariepinus* (Ozovehe, 2013) and this is also in accordancewith findings obtained by Tagwireyi *et al.* (2008) for *Oreochromis niloticus*fry without any negative effects (side effects) on the growth and feed efficiency.

The SGR of fish fed with test diets followed the same general pattern as the mean weight gain. For the specific growth rate, we achieved the best values in the R4 diet (2.27) and R2 (2.23), while the lowest SGR were observed in fish fed diets R1, R3 and R5. The specific growth rate carp groups fed diets containing 2%, 4% and 6% of EM increased of 0.6%, 9.2% and 16.0%,

respectively in comparison with the control (Huang *et al.*, 1999). According to Niang 2013, the specific growth rate of fish fed diet containing EM (1.78g/day) was higher than that of fishfed with the control diet (0.61 g / day). The specific growth rates were 6.88±0.19, 6.90±0.03, 6.73±0.17 and 6.51±0.35 %/day, respectively. Average daily gains reached 1.44±0.11, 1.45±0.02, 1.36±0.07 and 1.31±0.17%/day at 0, 5, 10 and 15%, all diets contained 35 % crude protein (Phommanivong and Doolgindachbaporn).

Table.1 Composition of the experimental diets for tilapia (*Oreochromis niloticus*)

	Treatments				
Ingredients	**R1**	**R2**	**R3**	**R4**	**R5**
Fish meal	57.2	56.3	55.5	54.6	53.7
MOLM +EM-A	0	2.5	5	7.5	10
Wheatmeal	30.8	29.2	27.5	25.9	24.3
Cellulose	5	5	5	5	5
Fish oil	5	5	5	5	5
Vit mix[a]	1	1	1	1	1
Minmix[b]	1	1	1	1	1

[a] = vit A 250000 UI; vit D3 250000UI; vit E 5000mg; vit B1 100mg; vit B2 400mg; vit B3(pp) 1000mg; vit B5 pantode Ca2000mg; vit B6 300mg; vit K3 1000g; vit C 5000mg; H biotin 15mg; choline 100g; anti-oxydant (BHT), crushed and calcined attapulgite qs 1000mg;

[b] = phosphorus 7%; calcium 17%; sodium 1.5%; potassium 4,6%; magnesium 7,5%; manganese 738mg; zinc 3000mg; iron 4000mg; copper 750mg; iodine 5mg; cobalt 208mg; calcined and ground attapulgite qs 1000g; fluorine 1.5% (approximately).

Table.2 Proximate analysis of experimental diets fed tilapia (*Oreochromis niloticus*)

Composition	R1	R2	R3	R4	R5
Dry Matter%	92.02	92.07	92.13	92.18	92.23
Ash%	1.32	1.43	1.53	1.63	1.74
Gross Energy (MJ/kg)	7.18	6.92	6.64	6.38	6.12
Digestible Energy (MJ/kg)	5.54	5.36	5.17	4.99	4.81
Crude Protein %	35.30	35.28	35.31	35.29	35.27
Digestible Protein %	2.97	2.83	2.67	2.52	2.38
Lipid%	11.57	11.54	11.51	11.47	11.44
Fiber%	0.60	0.78	0.96	1.14	1.31

Table.3 Profile of essential fatty acids of the experimental diets fed *O. niloticus*

Aminoacids	**NRC 2011**	**R1**	**R2**	**R3**	**R4**	**R5**
Arginine	1.2	2.3	2.2	2.1	2.1	2.0
Histidine	1.0	0.8	0.7	0.7	0.7	0.6
Isoleucine	1.0	1.4	1.4	1.3	1.2	1.2
Leucine	1.9	2.7	2.6	2.5	2.4	2.3

Lysine	1.6	1.4	1.3	1.3	1.2	1.2
Methionine	0.7	0.5	0.5	0.5	0.5	0.5
Met+Cys	1.0	1.4	1.4	1.3	1.3	1.2
Phenylalanine	1.1	1.9	1.8	1.7	1.7	1.6
Phe+Tyr	1.6	3.1	2.9	2.8	2.7	2.6
Threonine	1.1	1.2	1.2	1.1	1.1	1.0
Tryptophane	0.3	0.4	0.4	0.4	0.4	0.4
Valine	1.5	1.9	1.8	1.7	1.6	1.6

Table.4 Profile of essential Fatty acids of the diets fed *O. niloticus*

Fattyacids	R1	R2	R3	R4	R5
LA (18 :2n-6)	2.5	2.4	2.4	2.4	2.3
LNA (18 :3n-3)	0.5	0.5	0.5	0.5	0.5
ARA (20 :4n-6)	0.3	0.3	0.3	0.3	0.3
EPA (20 :5n-3)	3.8	3.8	3.8	3.8	3.8
DHA (22 :6n-3)	4.9	4.9	4.9	4.9	4.9
Total n-3	9.2	9.2	9.2	9.1	9.1
Total n-6	2.8	2.7	2.7	2.7	2.6
N3:n6	33.2	33.5	33.9	34.3	34.7
Total phospholipid	7.5	7.3	7.2	7.1	6.9
Cholesterol	0.3	0.3	0.3	0.3	0.3

Table.5 Initial and final mean weight, mean weight gain, SGR, FCR and survival of the tilapia (*Oreochromis niloticus*)

	Treatments				
	R1	**R2**	**R3**	**R4**	**R5**
Initial mean weight g/fish	4.13	4.12	4.14	4.12	4.13
Final mean weight g/fish	13.76	14.35	13.65	14.71	13.37
Mean weight gain g/fish	9.63	10.23	9.51	10.59	9.24
Meanweight gain (%)	233.55	248.62	229.99	257.13	223.66
SGR	2.15	2.23	2.13	2.27	2.10
FCR	2.42	2.11	2.42	2.17	2.39
Survival (%)	95	85	100	90	85

Table.6 Carcass composition of tilapia *O. niloticus*

	Treatments					
Composition	**Initial fish**	**R1**	**R2**	**R3**	**R4**	**R5**
Dry matter (%)	93.13	89.93	94.77	93.29	89.90	88.71
Organicmatter (%)	99.70	99.60	99.70	99.60	99.60	99.60
Protein (%)	82.00	71.96	75.52	70.82	71.02	80.53
Lipid (%MS)	22.63	16.85	16.93	26.62	23.42	27.97

Diets for growth trial were formulated in such a way that processed Moringa leaves meal (MLM) provided at 0, 15, 30, 45 and 60g 100g-1 of total dietary crude protein. The results showed the specific growth rate (2.73, 2.18, 1.44, 1.44 and 0.97 % day^{-1}) and an increase in feed conversion ratio (2.26, 3.11, 4.97, 4.34 and 7.27) respectively. This was, in a large scale, due to the saponins and other anti-nutritional factors which affected palatability (Madalla *et al.*, 2013)

In this study, the amino acid content of the experimental diets showed reduction of histidine, lysine and méthionine as compared with NRC, 2011. Methionine generally limits amino acid and methionine deficiency, which frequently causes reduced growth (Jackson *et al.*, 1982; Gaber, 2006). This indicates that methionine deficiency may be one of the reasons responsible for the lower growth performance and poorer diet utilization of the groups fed on the diets supplemented with moringa leaves. Similar to a study report of low dietary levels of methionine, growth of juvenile hybrid striped bass and increased mortality has been shown (Keembiyehetty and Gatlin, 1993). The slight difference observed may be due to various moringa + EM rates used, various components, formulation methods, different environmental conditions, the level of food intake and the experimental period.

Some authors using different animals have demonstrated the importance of EM in growth. Li *et al.* (1997) showed an increase in the average weight per hen for the EM group was 9.12 kg against 8.03 kg for the control. The weight of the report food consumption of eggs weight was 2.52 for the EM group and 2.64 for the control. They also showed that the average body weight of the four groups namely G1 (control), G2 (EM solution), G3 (Bokashi) and G4 (both solution EM Bokashi +) in 54 days of experience reached 1492 g, 1812 g, 1788 g and 1860 g, respectively. The net weight gain of chickens in G2 (EM solution), G3 (Bokashi) and G4 (both solutions EM Bokashi +) on the G1 control was 30.5; 24.6 and 30.5%, respectively. Finally, Niang (2013), showed that those fed with food made with MS have the absolute best average weight gain (70.73%), followed by the group fed GE (54.15%) and lower is noticed in fish fed the control diet (20.00%).

Dahal (1997) showed that the goats weight gain was more pronounced in the two treatment groups. Net weight gains G1 (control), G2 (Bokashi 5%) and G3 (Bokashi 10%) reached 2.2 kg; 3.64 kg and 5.27 kg, respectively.

Carcass composition

There was no significant difference for the carcass organic matter content between fish fed with different diets and initial fish**.** By cons for carcass dry matter content, significant difference ($P<0.05$) was observed. The dry matter contents of the carcass of fish subject to R2 diet (94.77%) and R3 (93.29%) did not have any significant difference ($P>0.05$) with those of the carcass of initial fish (93.13%). The dry matter contents of the carcass of fish fed with R1 diets (89.93%), R4 (89.90%) and R5 (88.71%) were lower than those of initial fish (93.13 %).

Significant difference ($P<0.05$) was then observed in body composition of protein of fish fed on the different types of diets. The body protein content was significantly higher in initial fish (82%), followed by those fed diets with R5 (80.53%) and R2 (75.52%). While, the carcass protein content of fish fed with diets R1 (71.96%), R4 (71.02%) and R3 (70.82%) is lower than that of initial fish.Such results are not

consistent with those reported by Niang (2013) who reported that the protein content is higher in fish fed EM than initial fish.

Regarding the fat, there is significant difference between initial fish (22.63%) and those fed with diets R1 (16.85%), R2 (16.93%), R3 (26, 62%) and R5 (27.97%). Fish fed with R5 and R3 diets presented the highest fat carcass content. There was no significant difference of fat carcass composition of tilapia fed with R4 diet (23.42%) and initial fish. Contrary to our results, Niang (2013, personal communication) reported that there is no significant difference in lipid body content of initial fish and that of the group fed with EM. The highest value was observed in the control group.

In conclusion, the main objective of this survey was to identify which of food stuffs made from local inputs remain zootechnically interesting. This attempt to resolve the problem of the lack of fish feed available and fish farmers contributes to the development of aquaculture. Following this investigation, encouraging results, although preliminary, were obtained. Given growth performance and feed efficiency, we can, in the current state of knowledge, consider the R4diet as the most promising one. However, we must recognize that the successful R4 diet still accused of nutrient deficiencies, this diet is far from balanced. Thus, it would be interesting to deepen the work performed by determining the profile of essential amino acids (EAA) of experimental diets and fish flesh after the test. Future studies may also address the effect of different treatments on biochemical digestion.

Acknowledgements

Our indebtedness to the National Agency of Aquaculture (NAA) for the supply of fish, vitamin and mineral premix. We would highly appreciate the assistance of staff members of ENSA (EcoleNationale des Sciences Agronomiques) laboratory. We would like to highly appreciate Mr. RenéNdiéroFALL's contribution for his critical review on this manuscript.

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