J Intl Cooper Agric Dev 2019; 17: 8–13

Journal of
International Cooperation for
Agricultural Development

Original

Growth and Feed Utilization of Juvenile Nile Tilapia Fed with Boiled *Moringa oleifera* Meal Diets: A Preliminary Report

Albert V. Manuel[1], A. M. Shahabuddin[1], Debasish Saha[1], Miguel H. Chele[1], Kelvin O. A. da Conceicao[1] Naoaki Tsutsui[1] and Takao Yoshimatsu[1]

[1] Laboratory of Shallow Sea Aquaculture, Graduate School of Bioresources, Mie University, Mie 514-8507, Japan

Received September 21, 2018 Accepted: December 10, 2018

Abstract. Juveniles of Nile tilapia were stocked (4.47 ± 0.26 g, eight fish per tank) and fed with test diets containing different inclusion levels of boiled *Moringa oleifera* leaf meal powder (0–30%: MLM 0, MLM 10, MLM 20, MLM 30), at 6% body weight for a total for six weeks. MLM 10 recorded the best average weight gain, specific growth rate, food conversion rate and protein efficiency ratio but there were no significance differences in all parameters with the MLM 30 diet which had the highest MLM inclusion. As there were no differences in fish growth and feed utilization between MLM 0 diet and MLM 10–30 diet, it would be possible to replace fish meal as a main dietary protein source by MLM up to 30% from 40% without any negative influence on the growth of juvenile Nile tilapia and is applicable as a practical rearing diet.

Key words: Nile tilapia, *Moringa oleifera*, growth, feed ingredient, fish meal

Introduction

Aquaculture is believed to reduce pressure on the wild-stock in fisheries and meet the needs of on-growing world food supply[1]. Fish feed is the single largest expenditure in semi-intensive and intensive fish culture operations. One major reason for its high cost is the animal protein content used in feed formulations. Animal proteins, for example, fish meal is usually the biggest proportion of commercial fish feeds. Global fish meal and fish oil production averaged 6.5 and 1.3 million metric tonnes, respectively, over the past 20 years, however, some years' productions were higher and in others lower[2]. Recently, researchers have been considering plant proteins as a substitute for animal proteins in fish feed to lower cost. For example, a study in Egypt was carried out to replace fish meal with a mixture (soyabean, cotton seed, sunflower and linseed meals) of different plant protein sources in juvenile Nile Tilapia feed and concluded that a replacement of 100% fish meal protein would have no adverse effects for up to 16 weeks old juvenile[3]. Furthermore, another study showed that cottonseed meal can partially replace fish meal as a source of protein in compound feed at a limited amount of no more than 50% for tilapia raised in recirculation systems[4]. Plant sourced proteins to replace fishmeal have successfully been carried out but there are some challenges. One being that these plant proteins need to be purchased at a certain cost which may be lower than fish meal but still carries a price to use as part of the fish feed ingredient.

Moringa oleifera, is a resilient tree that can survive in a variety of climates and substandard soils. Moringa plant is native to the sub-Himalayan regions of Northwest India and the plant thrives in many countries of Africa, Arabia,

Corresponding author: T Yoshimatsu, e-mail: takaoyos@bio.mie-u.ac.jp

South East Asia, the Pacific and Caribbean Islands as well as South America[5, 6]. It is readily available at no cost in many of these countries and is fast growing and hardy. Moringa leaves have a high nutritional value and contain various amino acids, which are rarely found in other plants. From those practical merits, in agriculture, Moringa leaves are used to feed cattle[7], pigs[8], chickens and poultry[9]. The use of Moringa leaf meal (MLM) also might be a good choice for the aquaculture field. There are several feeding trials that have used Moringa leaf meal as a replacement to animal protein (fish meal) for fish feed in many places in the world. For example, a preliminary study by Richter et al. (2003) carried out feeding trials using Nile tilapia and results indicated that raw Moringa leaf meal can be used to substitute up to 10% of dietary protein in Nile tilapia without significant reduction in growth[10]. A study by Afuang et al. (2003) showed a comparative nutritional evaluation of raw, methanol extracted residues and methanol extracts of Moringa leaves on the growth performance and feed utilization in Nile tilapia and results showed that the solvent extracted Moringa leaf meal could be included up to 33% in tilapia diets without any negative effects on growth[11]. The solvent extraction method resulted in the inactivation and reduction of antinutrients and improved the palatability of Moringa leaf meal diets. Therefore, even though the antinutrient levels were not measured, the current experiment treated Moringa leaf by boiling and sun drying, a simple practical method for developing countries that has not been used before in treating Moringa leaves, hoping that this would also reduce negative influences by antinutrients in the test diets.

In Mozambique and most parts of Africa, tilapia farmers cannot afford expensive imported fish feed and are in dire need for cheaper feed, so ingredients that were used to prepare test diets were chosen to be practical and affordable for farmers in Mozambique. The choice of these nutrient levels, particularly protein, was intended to reflect the practical diets used in developing countries. Therefore, this preliminary experiment focuses on substituting fish meal at different inclusion levels with boiled MLM to help overcome problems like high feed cost and reduce wild stock exploitation.

Materials and Methods

Feed Formulation and Preparation

Moringa leaves were picked, boiled, dried and ground to powder form in the Instituto Superior Politécnicode Gaza (ISPG) in Chokwe, Mozambique by ISPS academic staff and sent to Mie University in Japan where feed formulation and preparation were done. A control (0%: MLM 0) and three test diets were prepared with different inclusion levels (10%: MLM 10, 20%: MLM 20, 30%: MLM 30) and analysis of their proximate compositions were carried out and is shown in Table 1.

Table 1 Feed formulation and proximate composition of MLM diets fed to *O. niloticus* during the growth trial

Ingredients (%)	Moringa Leaf	MLM 0	MLM 10	MLM 20	MLM 30
Fish Meal		40	37	33	30
Processed Moringa		0	10	20	30
Cassava Powder		20	20	20	20
Rice Bran		28	21	15	8
Fish Oil		4	4	4	4
Binder (CMC)[1]		4	4	4	4
Vitamin Premix[2]		2	2	2	2
Trace Mineral Premix[3]		2	2	2	2
Proximate Composition (%)					
Moisture	9.1	8.3	7.8	7.5	7.8
Ash	8.7	11.6	12.9	13.7	15.0
Crude Protein	26.8	33.6	32.5	32.8	30.4
Crude Lipid	8.5	7.8	9.0	9.9	11.4
Carbohydrate	46.9	38.7	37.8	36.1	35.4

[1] Carboxymethylcellulose.

[2] Vitamin Premix, diluted in cellulose, provided the following in mg. kg^{-1} diet according to the requirement of *O. niloticus*[12]: vitamin A 1.8; vitamin D_3 0.025; vitamin E 25; vitamin K 5.2; thiamine 2.5; riboflavin 6; pyridoxine 16; pantothenic acid10; niacin 121; folic acid 0.82; vitamin B_{12} 0.024; biotin 0.06; chlorine chloride 1000.

[3] Trace Mineral Mix provided by the following minerals (mg. kg^{-1} diet): zinc (as $ZnSO_4.7H_2O$) 150; iron (as $FeSO_4$. $7H_2O$) 40; manganese (as $MnSO_4$. $7H_2O$) 25; copper (as $CuCl_2$) 3; iodine (as KI) 5; cobalt (as $CoCl_2$. $6H_2O$) 0.05; selenium (as Na_2SeO_3) 0.09.

Fish Sampling and Experimental Setup

Juveniles of Nile tilapia *Oreochromis niloticus* were sampled from the wild stock in Nikko river, Aichi Prefecture, Japan and then transferred to Mie University where they were kept for a week to acclimatize. Following acclimatization, fish weighing an average weight of 4.47 ± 0.26 g were distributed (eight fish per tank) into twelve 10 L-volume tanks (three tanks per treatment) in a closed recirculating system set indoor. Fish were fed with test diets containing different levels of MLM at 6% (wet basis) body weight for a total of six weeks at temperature, 25 ± 2ºC. In a two-week interval, individual weights were measured until the end of the experiment where all fish were sacrificed and sent for whole body composition proximate analysis (Table 3).

Growth Performance and Utilization

Growth and feed utilization were determined in terms of average daily weight gain (ADWG), specific growth rate (SGR), feed conversion ratio (FCR), protein efficiency ratio (PER) as follows:

ADWG (g fish^{-1} day^{-1}) =
final weight – initial weight/ number of days

SGR (% day^{-1}) = 100 × (ln [final body weight] – ln [initial body weight])/ no. of days

FCR = feed intake / live weight gain

PER = live weight gain/ crude protein intake

Whole Body Composition

After the sixth week of rearing, fish were sacrificed and sent for a whole-body proximate analysis to determine the amount of crude protein, crude lipid, ash, moisture and carbohydrates and results expressed as a percentage of live weight.

Statistical Analysis

Statistical analysis (SPSS vers. 16. IBM®, New York, USA) was carried out using Tukey's test after one-way analysis of variance (ANOVA) to determine differences among treatment groups which were deemed significant at $P<0.05$.

Results

Growth Performance and Feed Utilization

Body weight gain during the six weeks of growth trial is shown in the Fig. 1. Fish fed actively on the prepared test diets but not at a consistent rate and this resulted in a low weight gain.

Moringa leaf has high levels of crude protein (26.8% in the present study) and previous studies have proved this to be similar, for example, a study showed that Moringa leaf had a crude protein content of 31–35%[13], 25%[10], and 25.4%[11]. By adding MLM with different inclusion levels, test diets including 30.4–33.6% crude protein were formulated in the laboratory (Table 1).

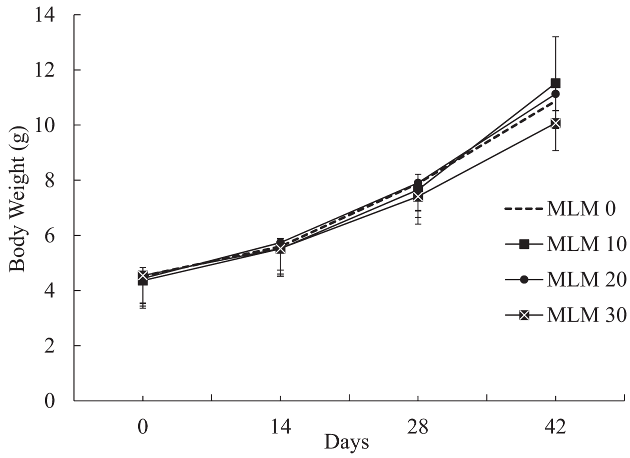

Fig. 1. Change in body weight of *O. niloticus* over a period of six weeks after been fed with different levels of MLM diets.

The overall performance of our feeding trial is summarized in Table 2 together with the nutrient utilization. Although, fish fed with the MLM 10 diet clearly showed the best performance in average daily weight gain (ADWG) of 1.78 ± 0.78 g fish^{-1} day^{-1}, specific growth rate (SGR) of 0.48 ± 0.07% day^{-1}, feed conversion rate (FCR) of 2.79 ± 0.61, and protein efficiency ratio (PER) of 1.20 ± 0.23, there were no significant difference in comparison to other diets, mainly due to the varied weight gain values and limitation of individual number used in each treatment.

Whole Body Composition

As for the effect of MLM supplementation on proximate body compositions of juvenile Tilapia, crude protein and lipid contents were measured 14.1% and 1.2%, respectively in initial fish body. After 6 weeks feeding, the values of 18.8% and 3.6% were noted in MLM 0 group and 16.7–19.2% and 3.0–3.3% were obtained in each MLM supplemented groups.

Discussion

The rearing results obtained in the present experiment suggested that an inclusion of MLM up till 30% would be possible without any negative influence on feed intake and fish growth. One factor that limits the high inclusions of MLM in fish diets is the presence of antinutrients. According to a previous study, 30% inclusion of Moringa leaf caused a depression in growth and this could

Table 2 Growth performance and nutrient utilization of *O. niloticus* fed with MLM diets

	MLM 0	MLM10	MLM 20	MLM 30
Initial Body Weight (g)	4.53 ± 0.44^a	4.36 ± 0.10^a	4.44 ± 0.13^a	4.54 ± 0.29^a
Final Body Weight (g)	10.88 ± 0.60^b	11.52 ± 1.68^b	11.13 ± 0.43^b	10.07 ± 0.46^b
ADWG (g fish-1 day^{-1})	0.79 ± 0.07^c	1.78 ± 0.78^c	0.96 ± 0.52^c	0.72 ± 0.01^c
SGR (% day^{-1})	0.45 ± 0.02^d	0.48 ± 0.07^d	0.47 ± 0.04^d	0.41 ± 0.01^d
FCR	2.94 ± 0.16^e	2.79 ± 0.61^e	2.84 ± 0.26^e	3.26 ± 0.83^e
PER	1.01 ± 0.55^f	1.20 ± 0.23^f	1.09 ± 0.11^f	1.01 ± 0.61^f

Mean ± SE (n=3) in each column followed by different letters ($P<0.05$) indicates a significant difference. AWG: Average daily weight gain, SGR: specific growth rate, FCR: feed conversion rate, and PER: protein efficiency ratio.

Table 3 Whole body proximate composition of *O. niloticus* fed test diets before and after the experiment (% fresh weight)

	Initial	MLM 0	MLM 10	MLM 20	MLM 30
Moisture	81.2	74.3	77	74.9	74.9
Crude Protein	14.1	18.8	16.7	19.2	18.9
Crude Lipid	1.2	3.6	3.1	3.0	3.3
Ash	3.2	3.2	2.4	2.8	2.8
Carbohydrate	0.3	0.1	0.8	0.1	0.1

likely be attributed to several factors, most importantly, antinutrients[7]. However, this problem could be solved by drying and/or grinding of moringa leaves before aqueous extraction that would improve the removal of soluble antinutrients[13]. The study further mentions that rejection was prominent in (45%: MLM 45) and (60%: MLM 60) fish diets, so therefore it is safe to say that an inclusion of (30%: MLM 30) would have no negative effect on fish intake and growth.

The best FCR value of 2.79 ± 0.61, obtained from fish fed with the MLM 10 diet in the current experiment was considered to be quite high, however, FCR values reported in a previous study showed that an inclusion of 30% aqueous extracted MLM in fish diets recorded a FCR of 4.97 ± 0.37[13] and in comparison, to the current experiment, a 30% MLM inclusion in fish diet recorded a much lower FCR value of 3.26 ± 0.83. The experiment was conducted over months of October to December and room temperatures ranged from 18–25°C but heaters were placed in the recirculating tanks to control water temperature. The optimum range of culturing *O. niloticus* is from 26°C to 29°C[14] and it appears that low temperatures may have affected FCR. A study carried out in winter reported FCRs ranging from 3.3–4.7[15] for different sizes of *O. niloticus* reared during winter and compared to the current experiment, FCR values ranged from 2.79–3.26. Therefore, to obtain a much better FCR value with the same experimental design, a study with more individuals per treatment, bigger culture tanks, an effective antinutrient removal technique, and a temperature of 28 ± 1°C, would provide an optimum culture condition which would maximize feed in-take and conversion, resulting in obtaining a reasonable FCR value. If all these parameters are achieved and a low FCR value is obtained, MLM substitution instead of fish meal could be carried out in developing countries to reduce fish feed cost and encourage more farmers to tap into the fish farming business. Encouraging farmers to begin fish farming businesses would not only boost the local economy but be a favorable protein provider which could help tackle the issue of malnutrition faced in African countries.

PER was best recorded in fish fed with MLM 10 diet but there were no significant differences to other MLM diets. PER also might have been affected by the suboptimal temperature during the rearing period. PER decreased with decreasing weight of fish and protein level and this was the same case in an experiment by Richter et al. (2003)[10].

The level of whole-body moisture as shown in Table 3 was higher in fish fed with MLM 10, MLM 20 and MLM 30 diets compared to the control MLM 0 group. This agreed with results of a reported study which had similar lose in whole body lipid content and energy of common carp fed with plant-based protein, Mucuna[16]. Furthermore, another study that studied factors affecting the proximate composition of cultured fishes observed that body moisture and body lipid were inversely related[17], which was the case in this experiment.

The results of the present study showed that MLM 10 diet recorded the best ADWG, SGR, FCR and PER but the results were not that statistically different from the MLM 30 diet. Due to these experimental results, a 30%

inclusion of MLM in fish diets replacing fish meal would have no interference in the growth and performance of juvenile Nile tilapia. The use of Moringa leaf as a dietary source in fish diets is limited due to the presence of anti-nutritional factors, particularly saponins and to a lesser extent tannin, phytic acid and hydrogen cyanide[18]. The present study processed Moringa leaves by boiling and this may have led to a reduction of anti-nutrients due to the leaching of soluble fractions in water. To clarify this point, more detailed experiments and biochemical analyses from various viewpoints would be necessary in future. Also in the next step, larger scale rearing experiments would be indispensable to establish the usage of *boiled Moringa* meal as a cheap and sustainable feed source for fish.

Acknowledgement

This experiment was carried out as one part of a Japan International Cooperation Agency (JICA) project (2016, BOP Business Survey Project in Mozambique) carried out under an academic MOU between ISPG and Mie university. We would like to thank JICA for its financial support and ISPG in Mozambique for preparing the key ingredient for this laboratory-level feeding experiment, Moringa leaf meal. We acknowledge that the country of origin of the material is Mozambique and that the country's biodiversity had been managed by various legislations, in particular the Convention on Biological Diversity and the Nagoya Protocol which need to be adhered to.

References

1. Naylor RL, Goldburg RJ, Mooney H, Beveridge M, Clay J, Folke C, Kautsky N, Lubchenco J, Primavera J, Williams M. (1998) Nature's subsidies to shrimp and salmon farming. Science 282: 883-884.
2. Hardy RW. (2010) Utilization of plant proteins in fish diets: effects of global demand and supplies of fishmeal. Aquaculture Research 41: 770-776.
3. El-Saidy DMSD, Gaber MMA. (2003) Replacement of fish meal with a mixture of different plant protein sources in juvenile Nile tilapia, *Oreochromis niloticus* (L.) diets. Aquaculture Research 34: 1119-1127.
4. Mbahinzireki GB, Dabrowski K, Lee KJ, El-Saidy D, Wisner ER. (2001) Growth, feed utilization and body composition of tilapia (*Oreochromis niloticus* sp.) fed with cottonseed meal-based diets in a recirculating system. Aquaculture Nutrition 7: 189-200.
5. Foidl N, Makkar HPS, Becker K. (2001) The potential of *Moringa oleifera* for agricultural and industrial uses. In: Fuglie LJ (eds.), The Miracle Tree: the multiple uses of Moringa. CTA, Wageningen, The Netherlands: 45-76.
6. Isaac N. (2012) Reaping the gains of R&D in *Moringa oleifera*. http://leadership.ng/nga/articles/31240/2012/07/31/reaping_gains_rd_moringa_oleifera.html.
7. Sánchez NR, Spörndly E, Ledin I. (2005) Effect of feeding different levels of foliage of *Moringa oleifera* to creole dairy cows on intake, digestibility, milk production and composition. Live Stock Science 101: 24-31.
8. Ly J, Samkol P, Preston TR. (2001). Nutritional evaluation of tropical leaves for pigs: Pepsin/pancreatin digestibility of thirteen plant species. Livestock Research for Rural Development 13: 5. http://www.lrrd.org/lrrd13/5/ly135.htm.
9. Olugbemi T, Mutayoba S, Lekule FP. (2010) Effect of moringa (*Moringa oleifera*) inclusion in cassava-based diets fed to broiler chickens. International Journal of Poultry Science 9: 363-367.
10. Richter NS, Siddhuraju P, Becker K. (2003) Evaluation of nutritional quality of moringa (*Moringa oleifera* Lam.) leaves as an alternative protein source for Nile tilapia (*Oreochromis niloticus*). Aquaculture 217: 599-611.
11. Afuang WS, Siddhuraju P, Becker K. (2003) Comparative nutritional evaluation of raw, methanol extracted residues and methanol extracts of moringa (*Moringa oleifera* Lam.) leaves on growth performance and feed utilization in Nile tilapia (*Oreochromis niloticus* L.). Aquaculture Research 34: 1147-1159.
12. Shiau S, Lin Y. (2006) Vitamin requirements of Tilapia—A review. In: Sàurez LEC, Marie DR, Salazar MT, López MGN, Cavazos DAVC, Ortega ACPCAG (eds.), VIII International Symposium on Aquaculture Nutrition. Mazatlán, Mexico: 15-17.
13. Madalla NA. (2013) Evaluation of aqueous extracted moringa leaf meal as a protein source for Nile tilapia juveniles. Tanzania Journal for Agricultural Sciences 12: 53-64.
14. Chervinski J. (1982) Environmental physiology of tilapias. In: Pullin RSV & Lowe-McConnell, RH (eds.), ICLARM, Manilla, Philippines: 119-126.
15. Siddiqui AQ, Howlader MS. (1991) Growth of Nile tilapia, *Oreochromis niloticus* (L.), in response to winter feeding. Journal of Aquaculture in the Tropics 6: 153-156.
16. Siddhuraju P, Becker K. (2001) Preliminary nutritional evaluation of mucuna seed meal (*Mucuna pruriens var. utilis*) in common carp (*Cyprinus carpio* L.): an assessment by growth performance and feed utilization. Aquaculture 196: 105-123.
17. Shearer K. (1994) Factors affecting the proximate composition of cultured fishes with emphasis on salmonids. Aquaculture 119: 63-88.
18. Francis G, Makkar HPS, Becker K. (2001) Antinutritional factors present in plant-derived alternate fish feed ingredients and their effects in fish. Aquaculture 199: 197-227.

モリンガ飼料を与えた際のナイルティラピアの成長と飼料利用効率（予報）

Albert V. Manuel, A. M. Shahabuddin, Debasish Saha, Miguel H. Chele, Kelvin O. A. da Conceicao,
筒井　直昭，吉松　隆夫

三重大学大学院生物資源学研究科 浅海増殖学研究室

要旨

ナイルティラピア幼魚（4.47 ± 0.26 g）を各水槽に８尾ずつ収容し，煮沸による熱処理を施したモリンガ *Moringa oleifera* 葉体の乾燥粉砕物（MLM）を０－30％添加した試験飼料を体重の６％分６週間の試験期間中毎日給餌して，成長，生残，飼料成分の利用効率，体成分に与える影響を予備的に検討した。その結果，MLMを10％添加した試験区で最高の平均増重，比増殖率および飼料成分の利用効率が得られたが，MLM無添加，あるいは20-30％添加した区との間で統計的有意差は認められず，また給餌試験終了後の魚体の一般分析値にも大きな差異はなかった。本実験により，ナイルティラピア幼魚に対して，MLMを30％添加することにより飼料タンパク質源である魚粉を10％分削減することが可能であることが本試験で分かった。

キーワード：ティラピア、モリンガ、成長、飼料成分、魚粉