Global Veterinaria 13 (1): 105-111, 2014
ISSN 1992-6197

DOI: 10.5829/idosi.gv.2014.13.01.84228

Evaluation of Feeding Raw Moringa (*Moringa oleifera* Lam.) Leaves Meal in Nile Tilapia Fingerlings (*Oreochromis niloticus*) Diets

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Abstract: Moringa is one of promising plant protein sources for aquaculture. The fluctuating results surrounding the optimal feeding level in fish diets were the motivation behind this study. A 75-day feeding trail was conducted to evaluate the effect of feeding different levels of raw moringa (*Moringa oleifera* Lam.) leaves meal (0%,8%,10% and 12%) on growth performance, feed utilization and carcass composition of Nile tilapia (*Oreochromis niloticus*) fingerlings diets. All the diets were isonitrogenous (30% CP kg^{-1}) and isoenergetic (4936.65k.cal/kg) diets. Two hundred and forty fish were fed diets in twelve aquaria (each 60x40x30cm) with four treatments (three aquaria/treatment) and twenty fish (3.25g) per aquarium. All Fish were fed diets three times daily at 4% feeding level of total biomass. Different dietary inclusion levels of moringa numerically improved growth performance, feed utilization parameters and carcass composition. However, no significant differences were observed between the treatments and the control, except in the crude protein CP which indicate enhancement in 10% comparing with other treatments or control. So, these results suggest that raw moringa leaves meal might be used up to 8% level of dietary saya protein in Nile tilapia (*Oreochromis niloticus*) fingerlings diets without negative effect on growth performance, nutrient utilization and carcass composition.

Key words: *Moringa oleifera* · Growth Performance · Feed Utilization · Nile Tilapia

INTRODUCTION

Aquaculture is capable of increasing the total production and fulfilling the high demand for fish protein [1]. The growth of aquaculture in Egypt has steadily increased over the past few years [2] consist of about 74% of total fish production [3]. To sustain such a high rate of growth, a matching increase in fish feed production is imperative [4]. On the other hand, the high cost and fluctuating quality of fish meal as well as its uncertain availability have led to the need to identify alternative protein sources for fish feeds [5], considerable emphasis has been focused on the use of conventional protein sources, soybean [6], pea meal and canola [7], plant protein [8], rapeseed [9] and Guar [10]. However, their scarcity and competition from other sectors for such conventional crops for livestock and human consumption as well as industrial use make their costs is too high and put them far beyond the reach of fish farmers or producers of aqua feeds [11]. Therefore, in order to attain a more economically, sustainable, environmentally, friendly and viable production, research interest has been directed towards the evaluation and use of unconventional protein sources.

Moringa oleifera (Moringaceae) is a cosmopolitan tree which grows in many tropical and subtropical countries under stressful environmental conditions and requires lower external energy subsidies showing uncountable folk uses due to its various nutritional and pharmacological applications [12]. This plant has excellent ability to adapt to poor soils and dry climates [13]. *Moringa oleifera* can be an alternative to some leguminous seeds as a source of high quality protein, oil, antioxidant compounds and a way to treat water in rural areas where appropriate water resources are not available [14]. The leaves of *Moringa oleifera* are rich in carotenoids, minerals, ascorbic acid and iron. Earlier studies have shown that, *Moringa olifera* is consider as promising protein source for inclusion in fish diets at low levels [15, 16]. The fluctuating results of the optimum level

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of Moringa leaves meal (MLM) as partial replacement of total dietary protein in practical diets for Nile tilapia led us to study the effect of using different levels of raw Moringa leaves meal on growth performance, feed utilization and carcass composition of Nile tilapia (*Oreochromis niloticus*) fingerlings diets.

MATERIALS AND METHODS

Experimental Set up and Fish: Nile tilapia (*Oreochromis niloticus*) fingerlings (3.25g) were used in this study provided from private farm in Kafr El-Shigh governorate, Egypt. All the fingerlings were transferred to fish lab in National Research Center, Dokki, Giza, Egypt, in 200 L tanks to be acclimatized for one week. Two hundred and forty fingerlings were randomly selected and divided into twelve aquaria (each 60 x 40 x 30cm) with four treatments (three aquaria/treatment) and twenty fish (3.25g) per aquarium.

The separated aquaria system was subjected to a photoperiod of 12 hour light /12 hour dark. Culture aquaria were continuously provided with aeration through an air compressor. Aquaria were cleaned every morning, before the first feeding and the feces were removed, water was replaced by 10% of new fresh, dechlorinated water daily. Water quality parameters, including temperature (T) pH, dissolved oxygen (Do), total ammonia (NH_3) and nitrites (NO_2) were monitored weekly. The average values of these parameters throughout the study were, T= 27±0.5°C, pH= 7.03±0.38, Do= 6.8±0.63 mg^{-1}, NH_3= 0.4±0.1 mg^{-1} and NO_2 = 0.2±0.02mg^{-1}. The fingerlings were starved for 2 days before the start of experiment while during the experimental period; the fingerlings were fed at feeding level 4% of total body weight 6 day a week at three equal installments.

Fish weighed in bulk every 2 weeks intervals to adjust the new feeding levels. Before starting the experiments, 100 fish from the stock were killed and analyzed for initial body composition. At the end of the experimental period (75 day), all fish were weighed and analyzed for whole body composition.

Diet Formation: *Moringa oleifera* leaves, obtained from private farm in El Behira Govenorate were freshly harvested and immediately dried on site were finely ground in a laboratory mill and used for feed formulation and analysis. Other components of the diets were perched from local market in Egypt. Prior to feed formulation, the dry matter composition of dried moringa leaves meal (MLM) is presented in Table 1.

Table 1: Dry matter composition of dried Moringa leaves meal (DM basis)

Item	Moringa %
Moisture	7.22
DM	92.78
CP	30.57
EE	9.49
CF	10.87
Ash	10.13
NFE	38.94

Four isonitrogenous and isoenergetic diets were formulated to include 30% total dietary protein and isoenergetic (4936.65 k.cal/kg). Moringa leaves meal was included in the diets at levels 0%, 8%, 10% and 12% of total dietary protein (Table 2).

Evaluation of Growth Parameters: Growth performance and diet nutrient utilization were analyzed in terms of body weight gain (BWG), feed intake (FI) feed conversion ratio (FCR) and specific growth rate (SGR), protein efficiency ratio (PER), protein productive value (PPV), fat productive value (FPV) and energy utilization the following formula were used.

Average daily gain [17] was calculated according to the following equation:

$$ADG = (W1 - W0) /T$$

Where:
W0 Initial body weight (g).
W1 Final body weight (g).
T Experimental period (days).
Specific growth rate [18]
SGR (%/day) = (ln W1 - ln W0) / T × 100
Where: ln = Natural logarithm
Feed conversion ratio [19]
FCR = Feed intake (g) / weight gain (g)
Protein efficiency ratio [20]
PER (%) = weight gain (g) / protein intake (g) × 100
Protein productive value [21]
PPV (%) = Retained protein (g) / protein intake (g) × 100
Fat productive value:
FPV (%) = Retained fat (g) / fat intake (g) × 100
EU (%) = Retained energy (kcal) / energy intake (kcal) × 100

Statistical Analysis: All data were subjected to one-way ANOVA using SPSS [22] 8.0, released version and the significance of the difference between means were tested using Duncan [23] at 5% level. Differences were seemed to be significant at $P< 0.05$. Values are expressed as means ± standard deviation.

Table 2: Formulation and proximate analysis of the experimental diets Moringa leaves meal (MLM)

	Percentage of components in experimental diets			
Ingredient	MLM (0%)	MLM (8%)	MLM (10%)	MLM (12%)
Corn	43.0	43.0	43.0	43.0
Soya	20.1	16.0	15.0	13.7
FM	24.0	24.0	24.0	24.0
Bran	7.0	2.4	1.2	0.3
Oil	5.5	5.5	5.5	5.5
Ascorbic acid	0.15	0.15	0.15	0.15
Moringa (MLM)	-	8.7	10.9	13.1
Premix*	0.25	0.25	0.25	0.25
Total	100	100	100	100
	Determined analyzed composition (dry matter basis)			
DM	92.63	94.56	95.60	93.11
CP	34.55	31.25	34.05	32.30
EE	11.56	11.15	11.29	10.82
Ash	5.24	4.91	5.78	5.99
Total carbohydrate (%)	48.65	52.69	48.88	50.89
Methionin**	0.69	0.69	0.69	0.69
Lysine**	1.98	1.98	1.96	1.95

* Each 3-kg quantity of the mineral and vitamin mixture contains the following: vitamin A, 10,000,000 IU; vitamin D3, 2,500,000 IU; vitamin E, 10,000 mg; vitamin K, 1,000 mg; vitamin B1, 1,000 mg; vitamin B2, 5,000 mg; vitamin B6, 1,500 mg; vitamin B12, 10 mg; niacin, 30,000 mg; pantothenic acid, 10,000 mg; folic acid, 1,000 mg; biotin, 50 mg; iodine, 300 mg; iron, 30,000 mg; manganese, 60,000 mg; copper, 4,000 mg; cobalt,100 mg; selenium, 100 mg; zinc, 50,000 mg; and calcium carbonate, 3,000 mg.

** Tilapia requirement of methionine and lysine are 0.4 and 1.4, respectively, According to NRC [24].

RESULTS AND DISCUSSION

No signs of disease were observed neither in the control nor the treatments groups. No rejection of feed until the end of the experiment was noticed and the acceptability of the diets was similar in the control and the treatments.

Growth performance in terms of body weight gain (WG), specific growth rate (SGR) and average daily gain (ADG) are presented in Table 3. The results obtained in the present study show that the performance of the fish fed with higher levels of moringa (10% and 12%) was inferior to those of fish fed with the control diet and the diet containing 8% leaves meal (diets 1 and 2).

Ozovehe [25] concluded that, *Moringa oleifera* leaf meal could be substituted fish meal up to 10% level in catfish (*Claries gariepinus*) diets without any negative effects on the growth performance and feed efficiency. The toxicological test also showed that 10% substitution rate of *M. oleifera* leaf meal in catfish diet would not have any adverse effect on the blood and serum enzymes. Francis *et al*. [26] used Moringa leaf meal in *Tilapia nilotica* feeds indicate growth reducing effects at high levels of inclusion of raw leaf meal. Richter *et al*. [27] reported that, the replacement of fish meal in a diet for tilapia with moringa leaf meal beyond a 10% level (Replacement of total dietary protein) produced poor growth and lowered the feed utilization efficiency. Afuang *et al*. [28] found that extraction of the anti nutrients/anti metabolites components of raw leaf meal by 8% methanol extraction permitted a higher incorporation of moringa leaf meal up to 33% in tilapia diets without any adverse effects on growth. Dongmeza *et al*. [29] found that different anti nutrients present in moringa leaves generally affect the fish growth and inhibit it.

Table 4 indicate that feed intake and the nutrient utilization parameters showed no significant difference were observed among treatments with different MLM levels.

Chemical composition of the studied fish carcass is given in Table 5. Different inclusion levels of MLM did not affect any of carcass parameters except cp which increased significantly between treatments while ash content declined with increasing dietary content.

Table 3: Performance of Tilapia (*O. niloticus*) fed Moringa leaves meal (MLM) containing experimental diets

Treat MLM	Initial wt.	Final wt.	WG	SGR(%/d)	ADG (g/d)	SR
0%	3.25	14.07[a]	10.82 [a]	1.95 [a]	0.14 [a]	91.66
		±0.36	±0.36	±0.03	± 0.01	± 6.66
8%	3.25	13.27 [a]	10.02 [a]	1.87 [a]	0.13 [a]	90.00
		±1.33	±1.33	±0.12	±0.02	±6.00
10%	3.25	11.80[a]	8.53[a]	1.71[a]	0.12[a]	87.50
		±0.69	±0.71	±0.08	±0.01	±4.33
12%	3.25	12.89[a]	9.59[a]	1.81[a]	0.13[a]	78.33
		±0.86	±0.08	±0.08	±0.01	±11.67

SR: Survival rate, WG: Weight gain

a, b Means in the same column within each factor with different superscripts letters are significantly different (P<0.05).

Table 4: Feed consumption, feed conversion ratio and nutrients utilization of Tilapia (*O. niloticus)* fed MLM experimental diets

Treat.	Feed consumption (g)	FCR	PER	PPV%	FPV%	EU%
0%	22.69[a]	2.10[a]	1.38[a]	18.37[a]	24.9[a]	13.20[a]
	±0.19	±0.05	0.035	±0.90	±1.05	±0.56
8%	22.26 [a]	2.26 [a]	1.43 [a]	19.09 [a]	21.04 [a]	11.63 [a]
	±1.48	±0.16	±1.11	±1.47	±1.87	±0.90
10%	21.40 [a]	2.55 [a]	1.18 [a]	17.79 [a]	19.01 [a]	11.22 [a]
	±0.51	±0.28	±1.18	±1.39	±1.19	±0.76
12%	21.28 [a]	2.24 [a]	1.39 [a]	19.68 [a]	24.43 [a]	12.97 [a]
	±0.82	±0.12	±0.07	1.85	±3.63	±1.50

a, b Means in the same column within each factor with different superscripts letters are significantly different (P<0.05).

Table 5: Carcass chemical composition and energy content of tilapia (*O. niloticus*) fed MLM experimental diets (on DM basis)

Treat	Moist%	CP%	EE%	Ash%
At start	76.89	51.21	24.98	23.50
At the end				
0%	75.72 [a]	53.0[b]	24.44 [a]	14.73 [a]
	±0.82	±0.63	±0.52	±0.40
8%	76.34 [a]	54.33[ab]	22.5[a]	14.60[a]
	±0.20	±1.01	±0.45	±0.83
10%	75.58[a]	57.90[a]	22.32[a]	16.30[a]
	±0.45	±0.35	±0.72	±0.40
12%	75.62[a]	55.12[ab]	23.64[a]	14.93[a]
	±1.05	±1.95	±1.02	±0.23

a, b Means in the same column within each factor with different superscripts letters are significantly different (P<0.05).

Different inclusion levels of MLM in tilapia diets up to 8% did not negatively affect the growth performance, which in agreement with results of Tagwireyi *et al.* [30] who demonstrate that 10% inclusion level of steam heated Moringa leafs in fry meal yielded good growth performance and in agreement with the results of Ozovehe [25] which showed that Moringa leaves meal could be substituted with fish meal up to 10% level in *Calaries gariepinus* diets without any negative effects on the growth of feed efficiency.

Our results may be attributed to the presence of the anti nutritional factors. (ANFs; Saponin, Phytate, Tannic acid and Crude fiber) of MLM examined levels. In this connection Ozovehe [25] found that saponins increased growth of tilapia in low dietary levels (150 mg^{-1}) but with increasing its amount, a negative effect was recorded. Saponins well known of its effect as a surface - active component on the biological membrane by which the permeability of the intestinal mucosal cells is increased and the active nutrient transport hindered Johnson *et al.*

[31]. As well, Phytate could reduce the bioavailability of minerals and protein digestibility by the formation of phytic acid - protein complexes. That complex damages the pyloric ceacum and depressing the absorption of nutrients [26].

It has been reported that 5-6 of phytic acid/kg diet can impair the growth of rainbow trout [32], common carp [33] and Nile tilapia [27].

Tannic acid levels may also cause growth desperation as reported by Al Owafeir [34] who observed growth reduction in tilapia fed low levels (0.27%) of tannic-acid. In contrast, Becker and Makkar [35] reported that 2% inclusion of (Condensed tannis) in tilapia diet had no adverse effect on growth, whereas, similar levels of hydrolysable tannins (Tannic acid) reduced the feed acceptability after 4 weeks of feeding.

The presence of higher concentration of total phenolic substances is known to reduce the protein digestibility and amino acid availability through binding protein in form of phenolics-protein and/or phenolic-protein enzyme complexes.

In our study, different experimental diets showed acceptable levels of crud fiber which, led to avoiding growth retardation. One the other hand, High crud levels of fiber in fish diets in presence of aforementioned antinutrients caused retardation in weight gain and EU% as reported by Anderson *et al.* [36] who reported a drastic reduction in growth, PER and whole body fat of Nile tilapia fed more than 10% cellulose. Hilton *et al.* [37] also noticed a similar reduction in growth performance of rainbow trout fed high fiber diet. Shiau *et al.* [38] observed a significant decrease in lipid content of Nile Tilapia fed diets containing 6%, 10% and 14% of carboxyl methyl cellulose (CMC) as compared to those fed 2% CMC. This phenomenon was associated with a decrease in gut passage time and diet digestibility [37].

Furthermore, Shiau [39] reported the importance of nutrient absorption dependency on the time for which nutrients are in contact with absorptive epithelium. Dietary fiber apparently influences the movement of nutrients along the gastrointestinal tract and significantly affects nutrient absorption. Another effect might be a change in enzyme activity, possibly through adsorption or immobilization of enzymes by dietary fiber. It has been shown that fiber can bind nutrients like fat, protein, minerals and reduce their bioavailability Richter *et al.* [27]. The same authors mentioned too, A higher level of moringa inclusion would therefore probably have reduced the energy available needed for protein synthesis and led to lower growth performance and nutrient utilization.

CONCLUSION

Finding new plant protein sources is a challenge for aquaculture industry. When we compared raw moringa and processed moringa we find such evidence: Although processing procedures for moringa such as heating, soaking, drying and boiling, improved palatability for fish. Some nutrients within the plant cells lost during the process treatment such as the precipitation of polyphenolic and other phytochemical compounds which might depress fish growth. Meanwhile, boiling induces the formation of colloidal starches as a result, reduces the amount of available glycoprotein for fish.

Our study suggest that raw Moringa leaves meal can be used in fingerlings Nile tilapia diets up to 8% without adverse effect on growth performance, feed utilization and carcass analysis as an eco friendly new plant protein source with favorable amino acid profile which exist in wide and ready availability throughout tropical and subtropical region like Egypt.

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