Moringa oleifera (Lam) LEAF: A RARE PROTEIN SOURCE FOR NILE TILAPIA (*Oreochromis niloticus*) FINGERLINGS

NWANKPA K.I.
*Department of Fishery and Aquatic Resources Management, Michael Okpara University of Agriculture, Umudike, Umuahia, P.M.B 7267, Abia state, Nigeria.
*Corresponding Author: kingsleynwankpa123@yahoo.com +2348064165673

ABSTRACT
The study examined the effects of the varying levels of Moringa oleifera leaf meal diets on the growth performance, survival rate and nutrient utilization of O.niloticus fingerlings for a period of 90 days. Moringa oleifera leaf meal diets substituted soybean at 0 %(control), 5%, 10% and 15%. A total number of 180 O.niloticus fingerlings of mean weight 2.43± 0.22g were randomly distributed into twelve transparent plastic tanks at 15 fish per tank in triplicate treatment and fed twice daily for 90 days. The mean weight gained (MWG), specific growth rate (SGR), average daily gain (ADG),survival rate (SR),feed intake(FI),feed conversion ratio(FCR),protein efficiency ratio(PER),protein gain (PG),protein production value (PPV) and carcass chemical composition(CCC) were calculated. The results obtained from this study showed that M.oleifera leaf meal can be used to substitute soybean up to 10% level in tilapia feed formulation without any probable effect on the growth parameters and nutrient utilization. Furthermore, the higher survival rate of fish fed M.oleifera leaf meal brings to bear the efficacy of the abundant minerals and antimicrobial properties inherent in the leaf, which is readily available and affordable than the highly competed for and expensive soybean.

Keywords: Moringa leaf, tilapia, growth performance, feed utilization

INTRODUCTION

We live in a world of ever increasing population. The world population is estimated to reach a stunning height of 9.8 billion people by 2050(FAO, 2014).The herculean task of feeding this enormous population while safeguarding our natural resources for the future generation would be quite overwhelming. Aquaculture and Fisheries are the two realistic and key vehicles for the actualization of the 2030 agenda for sustainable development, considering that many of the World's poorest people depend on fish to meet their basic needs (FAO, 2015). Fish provides about 6.7% of all protein consumed by humans and provides not only high value protein but also a wide range of essential minerals (zinc, calcium and iron) and polyunsaturated omega -3-fatty acid (FAO, 2012).The developing countries contribute about 54% of the total world export sell of fish, making aquaculture a viable and sustainable source of foreign exchange (FAO, 2010; 2012b).

The choice of species for aquaculture is critical in ensuring sustainable fish supply and profit, while conserving wild fish stocks. According to G.H.Yue etal., (2016), fish that do not need fish protein or less fish protein in their feeds will be the future fish for aquaculture. Tilapia is mainly an herbivorous animal and farmed in more than 120 countries. In 2015, its global production increased rapidly above 5.6 million tons (FAO, 2012a; G.H.Yue etal., 2016). More than 475 million pounds of tilapia is consumed in the United States which qualifies tilapia as a perfect industry fish (FAO, 2012a).Fitzsimmons (2000) was optimistic that tilapia (food fish) will surpass production of the carp in aquaculture during the new millennium. Fish continues to be one of the most traded communities worldwide.

Nigeria is the second largest producer of farm –raised tilapias in Africa after Egypt (El-Sayed, 2007; Fagbenro etal., 2010). The country has a vast amount of natural resources, a great diversity of high-valued indigenous fish species, thousands of hectares of irrigated land, man-made ponds and numerous wetlands and ditches. If these resources can be properly exploited and made productive through aquaculture; the benefit would be beyond the country's expectations.

Fish makes up about 41% of the total zoo-protein consumed by Nigerians, thus indicating a significant preference for fish in the country. Nigeria spent over N100 billion on the importation of frozen fish in 2010(NANTS, 2014). The estimated annual fish demand in Nigeria was about 2.66 million as against the annual domestic production of about 0.78 million, resulting in a supply lapses of about 1.8 million tons (NANTS, 2014). This colossal shipping-in of iced preserved fish into the country has ranked Nigeria first in Africa with respect to frozen fish import (NANTS, 2014; FMARD, 2014).If this amount of money is judiciously invested in the aquaculture industries yearly, the feedback would be tremendously overwhelming and consequently boost the countries chances of being a major world aquaculture and aquatic product exporter and also secure job and food security for its citizenry.

The challenge of aquaculture in Nigeria is overwhelming but top on the list is the high cost of aqua-feed. According to Fagbenro etal., (2005), Nigeria's aquaculture industries consumed an estimated 35,570 tons of feed in 2000, consequently leading to high cost of feed materials. Soybean is the major source of protein in tilapia feed formulation next to fishmeal and blood meal but the competitive demand for soybean from other sectors of agriculture therefore makes the search for alternative source for fish protein imperative. It is needful to focus on readily available and less expensive protein sources to replace soybeans. Plants –derived compounds such as alkaloids, flavonoids, phenolics, steroids and several minerals have been reported to promote various animal activities such as growth promotion, appetite stimulation, immune booster and antimicrobial promoter in fish culture (Citarasu, 2010).

There are very few plants that can favourably compete with *Moringa oleifera* plant in terms of its all encompassing efficacy and potency in human and animal health and also ecological remediation (Fahey, 2005).*Moringa oleifera* (Lam.), a multipurpose plant belongs to the Moringaceae family. All its parts can be consumed and used for a variety of purposes. This is because of its impressive range of nutritional and medicinal values (Bukar etal., 2010). The protein content of its leaves is high (20 – 35%) on a dry weight and most important is that the protein is of high quality, having significant quantities of all the essential amino acids (Foild etal., 2008; Ogbe and Afikku, 2011). Phytochemical analysis of Moringa leaves indicates that it contains a wealth of essential disease preventing chemicals which makes it suitable to be included in human and animal diets (Krishnaiah etal., 2009; Nweze etal., 2014).Therefore, considering all that *Moringa oleifera* leaf is endowed with, it can be used in place of soybean either partially or completely as protein source for tilapia feed preparation.

MATERIALS AND METHODS

Experimental site and facilities

The study was carried out at the wet laboratory of Department of Fishery and Aquatic Resources Management in Michael Okpara University of Agriculture Umudike, Abia state, Nigeria. The Wet laboratory is equipped with aeration pumps and a flow through system, containing aquaria of 60 liters carrying capacity, with adequate water supply from the Department's borehole.

Feedstuff

Moringa leaves were harvested fresh from a private moringa farm in Port-Harcourt, Rivers state, shade dried and milled into fine particles and used for feed formulation. Other components of the diets were purchased from the local market in Rumuokoro, Port-Harcourt, Rivers state, Nigeria.

Feed formulation

Prior to the feed formulation, the proximate analysis and qualitative phytochemicals analysis of the dried leaves was carried out and presented in table 1 and 2 .The ingredients for the feed formulation were manually mixed together in proportions determined by Pearson square method. Four thirty-five percent (35%) crude protein isonitrogenous and isoenergetic diets were formulated to replace soybean with 0%, 5%, 10% and 15% moringa leaf inclusions. The mixed feed ingredients were pelleted and grinded into small particles using a grinding machine and air dried. The air dried feed were then packed into plastic containers and stored safely.

Table 1: Proximate Composition (g/100g) of Dried *Moringa oleifera* Leaves Meal.

Nutritional Analysis	Proximate Composition (g/100g)
Moisture	5.38 ± 1.60
Dry matter	94.62 ± 0.66
Crude protein	23.60 ± 0.22
Crude lipid	2.62 ± 0.38
Crude fiber	7.33 ± 0.43
Ash	8.29 ± 0.81
NFE	52.78 ± 1.22
Energy value (Kcal/100kg)	3291 kcal/kg

NFE = Nitrogen–Free Extract = 100 – (Crude protein + Crude lipid + Crude fiber + Moisture content + Ash).

Table 2: Qualitative Phytochemicals Analysis of *Moringa oleifera* Leaves Extract

Phytochemicals	Aqueous Extract	Ether Extract	Ethanol Extract
Saponins	+	+	+
Flavonoids	+	+	+
Tannins	+	+	+
Alkanoids	+	-	+
Reducing sugar	+	-	-
Glycosides	+	+	+
Anthroquinones	+	+	+
Triterpenes	+	+	+
Phenols	-	+	+

KEY = -: Not present +: Present

Fish

Two hundred *Oreochromis niloticus* fingerlings of average weight of 2.4±0.5g were purchased from a tilapia farm in Umuahia, Abia state and transported to the Wet laboratory at Department of Fisheries and Aquatic Resources Management in Michael Okpara University of Agriculture Umudike, Umuahia, Abia state, Nigeria. The fish were feed commercial feed and allowed to acclimatize for two weeks

Experimental Design and feeding trials

A total of 180 fish were randomly assigned in triplicates into 12 tanks of 15 fish per tank. The fish were fed twice at 9am and 4 pm daily at 5 % body weight for 12 weeks.

Water quality parameters

The physico-chemical parameters (temperature, pH and dissolved oxygen) of the experimental water were monitored weekly. The tanks were washed every morning before first feeding and the faeces removed. The average values of temperature, pH and dissolve oxygen concentration throughout the study period were T = 27 ± 0.73 ^{o}C, pH = 7.02 ± 0.26, DO = 6.8 ± 36 mgl^{-1}. Before, the experimental diets (MLM) were administered; the fishes were starved for two days and fed at feeding level 5% of the body biomass for 90 days.

Evaluation of Growth Parameters:

The following growth performance and diet nutrient utilization were analyzed, calculated and recorded for each treatment and control every three weeks for twelve weeks: Growth body weight gain (BWG), specific growth rate (SGR), average daily gain (ADG), survival rate (SR), feed intake (FI), Feed conversion ratio (FCR) protein efficiency ratio (PER), protein gain (PG) and protein productive value (PPV). All the parameters above were performed according to the standard AOAC (2009) methods and the calculation was according to the following equations:

BWG (g) = Wf minus Wi)

SGR (% /day) = (In final wt - In initial wt)/days x100

FCR = total feed intake (g)/total wet weight gained (g)

ADG (g) = (final wt – initial wt)/period of experiment

SR (%) = 100x (final fish number)/(initial fish number).

FCR = Feed intake (g) / weight gain (g)

PER (%) = weight gain (g) / protein intake (g) × 100

PPV (%) = Retained protein (g) / protein intake (g) × 100

Where:

Wt = weight

Wf = final weight

Wi = initial weight

In = natural logarithm

Statistical analysis

All data generated were subjected to one-way analysis of variance (ANOVA) using SPSS 13.0 package (SPSS,2007) and the significance of the difference between mean were tested using Duncan's Multiple Range Descriptive Test (Duncan,1955) at 5% level. Differences were significant at P>0.05.

RESULTS

Table 3: Ingredient Composition of the Experimental Diet (*Moringa oleifera* leave meal) used

	Protein Replacement in Soybean Meal by Moringa Leaves (g/100).			
Ingredients	**Diet 1: 0%**	**Diet 2: 5%**	**Diet 3: 10%**	**Diet 4: 15%**
Moringa oleifera leaf	-	3.65	7.29	10.93
Toasted soybeans	41.4	37.75	34.11	30.47
Wheat bran	27.20	27.20	27.20	27.20
Fish meal	20.70	20.70	20.70	20.70
Palm oil	5.00	5.00	5.00	5.00
Premix (lysine)	0.50	0.50	0.50	0.50
Vitamin C	0.10	0.10	0.10	0.10
Methionine	0.10	0.10	0.10	0.10
Binder	5.00	5.00	5.00	5.00
Total	100.00	100.00	100.00	100.00

Table 4: Proximate Composition (g/100) of the Experimental Diets fed to *O.niloticus.*

	0% MLM Diet 1	**5% MLM Diet 2**	**10% MLM Diet 3**	**15% MLM Diet 4**
Moisture content	7.28 ± 0.24^{a}	7.28 ± 0.33^{a}	7.26 ± 0.30^{a}	7.25 ± 0.27^{a}
Crude protein	34.87 ± 0.67^{a}	34.68 ± 0.41^{a}	34.63 ± 0.56^{a}	34.55 ± 0.27^{a}
Crude lipid	6.3 ± 0.05^{a}	6.18 ± 0.17^{a}	5.45 ± 0.07^{ab}	4.98 ± 0.03^{b}
Crude fiber	7.12 ± 0.12^{b}	7.36 ± 0.25^{b}	8.52 ± 0.27^{a}	8.98 ± 0.23^{a}
Total Ash	6.87 ± 0.07^{a}	7.28 ± 0.03^{a}	7.50 ± 0.04^{a}	7.78 ± 0.02^{a}
NFE	37.56^{a}	37.21^{a}	36.54^{a}	36.46^{a}

Different superscripts in the same row indicate significant difference (p<0.05)

Table 5: Growth performance, survival parameters and feed utilization of *O.niloticus fed Moringa oleifera* leaf meal diet

Parameters	Diet 1 0% MLM	Diet 2 5% MLM	Diet 3 10% MLM	Diet 4 15% MLM
Initial mean weight(g)	2.44 ± 10.22^{a}	2.43 ± 10.20^{a}	2.44 ± 10.17^{a}	2.43 ± 10.32^{a}
Final mean weight(g)	14.92 ± 1.56^{a}	14.57 ± 1.33^{a}	13.01 ± 1.95^{b}	12.17 ± 2.02^{c}
Mean weight gain(g)	12.48 ± 1.34^{a}	12.14 ± 1.13^{a}	10.57 ± 1.72^{b}	9.74 ± 1.92^{c}
SGR	2.01 ± 0.02^{a}	1.99 ± 0.01^{a}	1.86 ± 0.02^{a}	1.79 ± 0.03^{a}
ADG	0.14^{a}	0.13^{a}	0.12^{a}	0.11^{a}
Survival rate (%)	97.87^{a}	98.56^{a}	99.33^{a}	98.56^{a}
Feed intake(g)/fish	29.76^{a}	29.52^{a}	28.36^{ab}	27.84^{b}
FCR	2.38^{a}	2.43^{a}	2.68a	2.86a
PER	1.20^{a}	1.18^{a}	1.06^{a}	1.00^{a}
PG	0.12^{a}	0.11^{a}	0.11^{a}	0.11^{a}
PPV	0.34^{a}	0.32^{a}	0.32^{a}	0.34^{a}

Different superscripts in the same row indicate significant difference (p<0.05)

Table 6: Carcass Chemical Composition of *O.niloticus* fed *M.oleifera* leaf meal diets

	Moisture content %	Crude protein%	Crude fat %	Ash %	Dry matter %	Crude fiber %
Initial	2.78	51.32	8.24	9.92	97.22	7.43
Diet 1	4.47[a]	53.42[a]	8.65[a]	10.16[b]	95.83[a]	7.50[b]
Diet 2	4.38[a]	53.39[a]	8.61[a]	10.24[ab]	95.52[a]	7.82[a]
Diet 3	4.72[a]	53.12[a]	8.15[b]	10.33[a]	96.18[a]	7.96[a]
Diet 4	4.65[a]	52.45[a]	8.15[b]	10.37[a]	95.75[a]	7.98[a]

Different superscripts in the same row indicate significant difference (p<0.05)

Table 1.shows the proximate analysis of the *M.oleifera* leaves used in the feed formulation. The percentage of crude protein, crude lipid, crude fiber and crude ash obtained in this study are 23.6 ±0.22%, 2.65±0.38%, 7.33 ±0.43% and 8.29 ±0.81.

Table 2 shows the qualitative analysis of the photochemical inherent in *M.oleifera* leaf using different extraction methods. The results show that *M.oleifera* leaf contains a good number of phytochemicals of great importance to the health and growth of fish.

Table 3 show the ingredient composition of the experimental diets and percentage replacement of soybean with *M.oleifera* leaf in each of the diets. Diet 1 is the control containing 0% MLM, while diet 2,3 and 4 contain 5%,10% and 15% MLM respectively.

Table 4 shows the percentage composition of the experimental diets. The results indicate there was no significant difference (p<0.05) in the crude protein, ash content, moisture and NFE of the experimental diets but there was significant difference in the crude lipid and fiber content among the diets. Diet 1 (0% MLM) had the highest crude lipid of 6.3 ± 0.05% and the lowest crude fiber of 7.12 ± 0.12 % while diet 4 (15% MLM) had the lowest crude lipid of 4.98 ± 0.03% and highest crude fiber of 8.98 ± 0.23. It can be observed from the results that crude lipid decreased while crude fiber increased as the MLM increased.

Table 5 shows the results of the growth response, survival rate and nutrient utilization of fish fed with the experimental diets. The fish fed the control diet (0% MLM) gained 12.48±1.34 g which is the highest weight gained, but not significantly different (p>0.05) from the weight gained by fish fed 5% MLM diet which gained 12.14 ± 1.13g .The fish fed 10% MLM diet gained 10.57± 1.72g while the fish fed 15% MLM diet had the lowest weight gain of 9.74 ±1.92g which are significantly different (p<0.05) from the weight gained by fish fed 0% and 5% MLM diet. The fish fed 10% MLM diet had the highest survival percentage of 99.33 while the fish fed 0% MLM diet had the lowest survival percentage of 97.87 but there was no significant difference (p>0.05) between the diets.

The results for the specific growth rate (SGR) showed that fish fed with 0% MLM diet had the highest value of 2.01 and the lowest value of 1.79 was recorded in fish fed 15% MLM diet .There was no significant difference (p>0.05) between the diets as indicated in Table 5. It can be observed that the fish fed the control diet consumed more feed (29.76g).The fish fed 15% MLM diet consumed least feed(27.84g). The fish fed 5% and 10% MLM diet consumed 29.52g and 28.36g respectively. There was significant difference (p>0.05) in the feed consumed by the fish fed 0% MLM diet and 15% MLM diet. There was no statistical significant difference (p>0.05) in the feed conversion ratio (FCR) in the fish fed the experimental diets. The protein efficiency ratio (PER), protein gain (PG) and protein production value (PPV) followed the same pattern as feed conversion ratio (FCR) of the fish fed the experimental diets.

The chemical composition of the studied fish carcass is presented in Table 6.The results shows that there was decrease in crude protein and crude fat and increase in crude ash and fiber of the fish carcass as MLM inclusion increased.

DISCUSSION

The crude protein content of MLM used in this study is similar to 23.56% and 23.50% reported by Adewumi (2016) and Ochang etal., (2015) respectively. In their work, Hanan etal., (2014) and Odura etal., (2008) reported 30.57% and 27.51% respectively, which is a higher protein percentage. However, Kakengi etal., (2003) reported 22.5%, a lower protein percentage. These differences can be attributed to climatic conditions, age of plant, nature of soil on which the plant is grown and processing techniques (Adewumi, 2016).

In this study, the increase in MLM diet increased the survival rate of the fish indicating that the experimental diets did not have any adverse effect on the health of the fish. However, the growth performance and nutrient utilization decreased as the percentage of MLM increased. This negative trend can be attributed to several factors. Ozovehe, etal (2013) has reported that the decrease in growth performance was due to decrease in feed intake. Hanan etal., (2014) has reported inferior growth parameters in fish fed with higher MLM inclusion as compared to those fish fed lower MLM inclusion. The presence of metabolite inhibitors with bitter taste such as saponins and tannins could be responsible for the low consumption of the feed (Francis etal., 2001; Makkar, 2003; Afuang etal., 2003; Dangmeza etal., 2010). Generally, digestibility of plant material decreases with decrease in moisture content and this could inhibit fish utilization of other nutrients contained in the diet (Dongmeze etal., 2010; Bundit etal, 2014). Adewumi (2012) has reported that fiber increases bulkiness and a feeling of fullness, resulting in reduced feed consumption and decrease in growth parameters. Increase in dietary fiber decreases gastrointestinal tract passage time, diet digestibility, nutrient absorption, enzyme activities and available energy for protein synthesis (Hossain and Jauncey, 1993; Anderson etal., 1984; Hilton etal., 2000; Shiau etal., 1988; Thompson, 1993). All these conditions named above inadvertently contribute to the decreased growth indices and nutrient efficiency reported in this study. According to El-sayed (1999) many plant protein sources contain high level of phytic acid which binds with divalent minerals such as Ca, P, Zn, Mg and Fe to form water –insoluble salts rendering the minerals unavailable for use in the body. Many recent studies have suggested that the inclusion of phytase in tilapia diets can be an effective tool in reducing phytic acid activity, improve utilization of plant protein sources and reduce the effects of antinutritional factors. These could eventually lead to improved growth and nutrient utilization in tilapia (Hossain & Jauncey, 1993; El-sayed., 1999; Richter, 2003).

CONCLUSION

In conclusion, the results obtained from this study showed that *M.oleifera* leaf meal can be used to substitute soybean up to 10% level in tilapia feed formulation without any adverse effect on the growth parameters and nutrient utilization. Furthermore, the higher survival rate of fish fed *M.oleifera* leaf meal brings to bear the efficacy of abundant mineral and antimicrobial properties inherent in the leaf which is readily available and affordable than the highly competed for and expensive soybean.

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