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Effect of Moringa, *Moringa oleifera*, leaves supplementation as a growth promoter on growth performance, body composition, and physiological profile of Nile tilapia *Oreochromis niloticus* (L.) fry

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ABSTRACT

This study was carried out to investigate the effects of dietary Moringa (*Moringa oleifera*) leaf (ML) meal on growth, feed utilization, whole body composition and physiological profile of Nile tilapia, *Oreochromis niloticus* (L.) fry.

ML was added to the tested diets (30% protein and 454.70 kcal/100 g diet) at a rate of 0.0, 0.5, 1.0, 1.5, and 2.0% (ML 0, ML 0.5, ML 1.0, ML 1.5 and ML 2.0). Fish (0.83± 0.2 g) were randomly distributed into each aquarium at a rate of 10 fish /aquarium and fed the tested diets at a feeding rate of 5% for 12 weeks.

The results indicated that fish fed diets containing 0.5 - 1.5% ML exhibited higher growth than the other treatments. On the other hand, protein efficiency ratio and protein productive value of fish fed 0.5, 1.0 and 1.5 ML were significantly ($P \leq 0.05$) higher than fish groups fed the other diets. Significant difference ($P \leq 0.05$) was found in the whole-body composition (moisture, crude protein, and ash) of fish at the end of the experiment and the best values were obtained with ML 1.0 diet. The concentrations of Hb, PCV, and total protein of fish fed 1% ML were increased significantly ($P \leq 0.05$), while the activities of all the tested liver and kidney function enzymes in serum among other ML levels were decreased significantly ($P \leq 0.05$). The present study suggested that dietary ML supplementation could improve the performance and physiological responses of Nile tilapia fry and its optimum inclusion level is 1% ML.

Key words: Moringa leaf meal, fish performance, feed efficiency, body composition, physiological profile.

INTRODUCTION

Moringa (*Moringa oleifera*) is a highly valued plant that is mostly cultivated in the tropics and subtropics. It is used for food, medication, and industrial purposes (Busani *et al*., 2011). Moringa is called “miracle vegetable" because it is both a medicinal and a functional food (Verma *et al*., 1976). Moringa yield reached 120 tones/ha/year as a leaves with a high crude protein (251g/kg DM) and negligible content of tannins (1 to 23 g/kg) and other anti-nutritive compounds (Makkar and Becker, 1996). The dried leaves had crude protein levels of 30.3% and 19 amino acids, 17 fatty acids, mineral content especially calcium (3.65%), phosphorus (0.3%) and selenium (363mg/kg) (Bennett *et al*., 2003; Aslam *et al*., 2005; Manguro and Lemmen, 2007; Group, 2009; Amaglo *et al*., 2010; Gowrishankar *et al*., 2010). Furthermore, the biological activities of moringa leaves (ML) that is hepatoprotective (Pari and Kumar, 2002), hypercholesterolemia (Mehta *et al*., 2003), antifungal (Chuang *et al*., 2007), antioxidant (Sanchez *et al*., 2006), and anti-tumor (Bharali *et al*., 2003). Moringa

leaves powder is also used as a nutritional supplement and growth promoter due to the significant presence of rotein, minerals, ß-carotene, and α-tocopherol (Foidl *et al*., 2001).

Nile tilapia (*Oreochromis niloticus*) fed with raw ML meal revealed that 10% of replacement of fishmeal-based dietary protein did not cause any adverse effect on growth performance (Richter *et al*., 2003). Bundit and Masumoto (2012) showed that the tested ML diet contains ingredients that could be used in fancy carp (*Cyprinus carpio*) diets with possibly not over up to 20 g/kg soybean protein replacement without negative effects on growth and digestibility.

There is a dearth of information on the use of ML or seed meals as fish feed ingredients. An extensive search and analyses of published data on moringa and any of its use in aquaculture were therefore carried out. Thus, the present work was carried out to investigate the dietary supplementation of graded levels (0.0, 0.5, 1.0, 1.5, and 2.0%) of Moringa leaf (ML) meal used as a growth promoter for Nile tilapia throughout examination its effect on growth performance, feed utilization, whole body composition, and physiological profile of Nile tilapia fry.

MATERIALS AND METHODS

Preparing of moringa, *Moringa oleifera* leaves:

Moringa (*M. oleifera*) was obtained from special arboretum in Alexandria Governorate. It was air dried in a clean room for about two weeks to obtain a constant weight for easy grinding. It was grounded using manual grinder to obtain right particle size for pelleted feed.

Experimental diets:

Five isonitrogenous and isocaloric diets (30 % protein and 4547 kcal GE/Kg diet) were formulated and Moringa leaf meal was included in diets at a rate of 0.0, 0.5, 1.0, 1.5, and 2.0%. Composition and proximate analysis of the experiment diets and amino acids composition are presented in Tables (1, 2 & 3).

Diet ingredients were ground and thoroughly mixed and the oil was slowly added at the same time of mixing with warm water (45°C) until the diets began to clump. Diets were processed by a California pellet mill machine and dried for 48 hours in a drying oven at 70°C. The pellet size was 0.6 mm in diameter and 2 mm in length.

Fish culture facilities:

This study has been carried out at the Wet Fish Laboratory, Department of Animal and Fish Production, Faculty of Agriculture, Alexandria University. Fish were acclimated to the experimental condition for 20 days before starting the experiments during which they fed a commercial diet. After that, fish (0.83 ± 0.2 g) were distributed at a rate of 10 fish per 100-L glass aquarium. Fish in each aquarium were fed one of the tested diets twice a day; six days a week at a rate of 5 % of their body weight for 12 weeks. A half of aquarium's water was siphoned with fish faeces and replaced by dechlorinated tap water. Every two weeks, fish per each aquarium were group-weighed by a digital scale (accurate to ± 0.001 g) and feed quantity was adjusted accordingly. Dead fish once appeared in any aquarium were recorded and removed.

At the start of the experiments, 50 g fish sample were collected and immediately frozen (– 20°C) and reserved for initial proximate body chemical analysis. At the end of the experiment, fish were collected from each aquarium, counted, and weighed. Then, five fish were taken from each aquarium for the proximate chemical analysis.

Fish performance and feed utilization:

Fish growth performance and feed utilization parameters were calculated according to Cho and Kaushik (1985) as following:

Average weight gain (AWG, g /fish) = [final body weight (g) - initial body weight (g)];
Average daily gain, (ADG, g /fish /day) = [AWG (g) / Experimental period (days)];
Specific growth rate (SGR, %g/day) = 100 [Ln final weight - Ln initial weight] /Experimental period (day);
Feed conversion ratio (FCR) = feed intake (g) / body weight gain (g);
Protein efficiency ratio (PER) = gain in weight (g) / protein intake in feed (g);
Protein productive value (%: PPV) =100 [protein gain in fish (g) / protein intake in feed (g)];
Energy utilization (%; EU) =100 [energy gain in fish / energy intake in feed].

Water quality parameters:

Water temperature and dissolved oxygen were measured daily using an oxygen meter (YSI Model 58, YSI Industries, and Yellow Spring Instruments, OH, USA). The pH-value was monitored twice weekly using an electronic pH meter (pH pen, Fisher Scientific, Cincinnati, OH, USA). Total ammonia, nitrite, and nitrate were measured weekly using spectrophotometer (Spectronic 601, Milton Roy Company, San Diego, CA, USA) according to APHA (1998). Total alkalinity was monitored twice weekly using the titration method of Golterman *et al*. (1978).

Proximate chemical analyses:

Samples of the experimental diets and fish were chemically analyzed to determine dry matter (DM), crude protein (CP), ether extract (EE), crude fiber (CF), and ash contents according to the methods of AOAC (2000). Nitrogen free extract (NFE) was calculated by differences, by deducting the sum of percentages of moisture, CP, EE, CF and ash from 100. Gross energy (GE) contents of the experimental diets and fish samples were calculated by using factors of 5.64, 9.44 and 4.12 kcal/g of protein, lipid and carbohydrates, respectively (NRC, 1993).

Blood parameters determination:

At the end of the experiment, fish (n = 5 of each treatment) were randomly taken and anaesthetized using 3 mL pure clove oil (dissolved in 10 mL absolute ethanol) as anesthetic material. For the hematological parameters analysis, blood samples (5-mL of whole blood at each collection) were collected from the caudal peduncle of fish in plastic heparinized vials for determination of hemoglobin concentration (Hb) using commercial colorimetric kits (Diamond Diagnostic, Egypt), and packed cell volume (PCV%) according to Stoskopf (1993). Other blood samples were collected in dried plastic tubes and were centrifuged at 3500 rpm for 15 min to obtain the blood serum for determination of total protein (Gornall *et al*., 1949), uric acid (Schultz, 1984), aspartate aminotransferase (AST) and alanine aminotransferase (ALT) (Varley *et al*., 1976) using a spectrophotometer (model 5010, Germany). Serum creatinine, urea, and uric acid were determined according to the standard methods described by Newman and Price (1999), Fawcette and Scott (1960) and Kim *et al*. (1971), respectively.

Statistical analysis:

All data were analyzed by one-way ANOVA using the statistical analysis system procedure (SAS, 2006). All percentages and ratios were transformed to arcsine values prior to

analysis (Zar, 1984). Duncan's multiple range test was used as a post hoc test to compare differences among individual means at $P \leq 0.05$.

RESULTS AND DISCUSSION

Water quality parameters in fish aquaria:

During the 12-week feeding trial, the mean values of water quality parameter (±SD) were: water temperature 28.6±0.3°C; dissolved oxygen 4.4± 0.4 mg/L; pH 7.5±0.2; total ammonia 0.023 ± 0.01 mg/L; nitrite 0.025±0.013 mg/ L; nitrate 0.8 ±0.4 mg/L, and total alkalinity 169±4.0 mg/L as $CaCO_3$. All water quality parameters herein are within the acceptable range for rearing Nile tilapia according to Abdelhakim *et al*. (2002).

The proximate composition of Moringa (*Moringa oleifera*) leaf (ML):

The potential of a feedstuff such as leaf meal in fish diets was evaluated on the basis of its proximate chemical composition, which comprises the moisture content, crude protein, crude fiber, crude fat, total ash and nitrogen free extract. The proximate composition of ML in the present investigation revealed that the crude protein content was 27.7 %, crude fat 5.20%, crude fiber 11.50%, and total ash 14.30%. These values were within the range obtained by Markker and Becker (1996). The similarities in chemical composition with the other studies may be an indication that environmental factors such as season, geographical location and stage of maturity play a minor role in determining ML nutritive value.

Moreover, chemical composition of other leaf meals such as *Leucaena leucocephala*, and *Ipomoea batatas* were studied by Adewole (2008) and Sotolu (2010). On comparing their results with that of the present study indicated that ML can be used as an animal feed as that of other leaf meals from nutritional point of view.

Growth performance:

At the end of the feeding trial, fish growth increased significantly as ML level increased up to 1.5 % after which fish growth declined at 2.0% ML (Table 4). Fish fed 0.5% - 1.5% ML exhibited significant improvement in growth than the other fish groups. No mortality was recorded in all fish groups during the experimental period suggesting that ML has no toxic effects.

ML has been widely used as an alternative protein source in fish diets and seems to be a promising protein source where it could partially replace conventional diets without any depression in growth performance of Nile tilapia (Afuang *et al*., 2003; Richter *et al*., 2003). In addition, essential amino acids (EAA) composition in ML is sulfur amino acid such as methionine, cystine, tryptophan (Makkar and Becker, 1996), as required by EAA for aquatic animals (WHO, 1985).

The improved growth of fish fed ML-containing diets may be related to the presence of 19 amino acids, 17 fatty acids, vitamins and minerals especially phosphorus (0.3%) and selenium (363 mg/kg) in dried ML. Those compounds may positively affect the physiological functions resulting in improved fish growth. On the other hand, the retarded growth by fish fed 2.0 % ML may be due to the presence of anti-nutrients such as phenol, tannins, phylates, and saponins (Richter *et al*., 2003). Also, in most cases, the inclusion of high level of plant proteins in fish diets has resulted in reduced growth and poor feed efficiency. This might be due to the presence of toxic substances or antinutritional factors (Siddhuraju *et al*., 2000; Francis *et al*., 2001). In this context, Afuang *et al*. (2003) reported that Nile tilapia fed a diet containing moringa methanol extract at the level of 102 g/kg of feed caused a 17.5% reduction in body mass gain when compared to fish fed the control diet.

Feed and nutrient utilization:

Feed intake increased as ML increased in fish diets; meanwhile the optimum FCR was obtain at 1.0 % ML (1.69; Table 5). Moreover, highest values of PER, PPV, and EU were obtained in fish fed 1% ML-containing diet (2.01, 34.9 %, and 21.63 %, respectively). In the present study, low dietary levels of methionine caused a suppressed growth and feed utilization. Methionine content of the experimental diets supplemented with ML gradually increased at 1.342 and 1.453 % compared to the control diet (1.292%). The high crude protein content of levels (23% in DM), the high proportion of this protein potentially available in the intestine (Makkar and Becker, 1996), the presence of adequate amounts of EAA (higher than levels present in FAO reference protein), and low of levels of anti-nutrients indicate their high nutritional quality. The high pepsin soluble nitrogen (82-91 %) and the low acid detergent insoluble protein (1.0 - 2.0 %) values for the meal suggest that most of the protein in the meal is available to most animal (Makkar and Becker, 1996). However, EAA composition in ML is sulfur amino acid such as methionine, cysteine, and tryptophan, which should be used as supplements only (Goff and Gatlin, 2004).

Whole body compositions:

The proximate composition was significantly different ($P \leq 0.05$) among the five experimental fish groups (Table 6). The highest contents of crude protein, total lipids, and energy contents, and the lowest ash contents were obtained in the whole body of fish fed 1.0% ML. Dry matter ranged from 28.83 to 28.44 %, while that of initial fish was 23.25 %. Crude protein and lipid ranged from 59.87 to 58.08 % and from 23.6 to 23.2 %, respectively. Fish fed 2.0 % ML showed the highest ash content (18.75 %), while fish fed 1.0 % ML showed the lowest value (16.50 %). Energy contents varied from 560.73 to 546.29 (kcal / 100 g), which were also significantly different ($P \leq 0.05$) among fish groups.

In the present study, neither growth nor feed efficiency parameters were affected significantly by supplementation of high ML levels. The low growth at high ML inclusion levels may be due to the levels of cell wall constituents (neutral detergent fiber and acid detergent fiber) as detected by Richter *et al*. (2003). Shiau (1997) reported the importance of nutrient absorption dependency on the time for which nutrients are in contact with the absorptive epithelium. Dietary fiber apparently influences the movement of nutrients along the gastrointestinal tract and significantly affects nutrient absorption. Another exacerbating effect might be a change in enzyme activity, possibly through adsorption or immobilization of enzymes by dietary fiber. It has also been shown that fiber can bind nutrients like fat, protein (Shah *et al*., 1982; Ward and Reichert, 1986) and minerals (Ward and Reichert, 1986), and reduce their bioavailability. In this respect, De Silva and Gunasekera (1989) suggested that the acceptability of the diet, particularly changes in texture and taste, was negatively affected by the increasing incorporation of plant materials in Nile tilapia fry feed. Similarly, Afuang *et al*. (2003) reported that Nile tilapia fed a diet containing ML- methanol extract at the level of 102 g/kg of feed caused a 17.5% reduction in body mass gain when compared to fish fed the control diet.

Hematological and serum biochemical parameters:

Hematological and serum biochemical parameters have become attractive and useful for monitoring environmental quality, and the health condition of aquatic organisms (Kori-Siakpere and Ubogu, 2008; Olufayo, 2009). Data of hematological and serum biochemical parameters of Nile tilapia fry fed different levels of ML are shown in Table (7). It was noticed that dietary supplementation of ML up to 1% led to significant ($P \leq 0.05$) decreases

of Hb, PCV, and total protein among all fish groups, while the highest level of ML (2%) led to significant ($P \leq 0.05$) increases of AST, ALT, creatinine, urea, and uric acid among all fish groups. Generally, fish fed 1% ML had the highest ($P \leq 0.05$) concentrations of Hb, PCV, and total protein and the lowest ($P \leq 0.05$) values of AST, ALT, creatinine, urea, and uric acid among all fish groups, including the control group (ML0.0).

Haematological components of blood are also valuable in monitoring feed toxicity especially with feed constituents that affect the formation of blood in cultured fish (Oyawoye and Ogunkunle, 1998). According to the present findings, the PCV and Hb reduced as the ML levels in the diet increased up to 1%. The PCV range in this study was 21.00 to 32.00%; it is within the range of 20 to 50% as reported by Pietse *et al*. (1981) and rarely exeeds 50% (Clark *et al*., 1976; Etim, *et al*., 1999). These reductions of PCV and Hb in the present study may be due to the high level of saponins, total phenolics and phytic acid, which were detected in ML (Richter *et al*., 2003). Reduction in the concentration of PCV in the blood usually suggests the presence of toxic factor which has adverse effect on blood formation (Oyawoye and Ogunkunle, 1998) or detected to anemic condition in fish (Blaxhall and Daisley, 1973). Further, the reduction in Hb concentrations could imply that diets having higher ML levels contained low quality diet resulting to poor transportation of oxygen from the respiratory organs to the peripheral tissue (Robert *et al*., 2000).

Biochemical biomarkers like glucose, protein and enzymes are regularly used as an indicator of the general state of health and early warning of stress in fish under stressful conditions (Abou El-Naga *et al*., 2005; Osman *et al*., 2010). The significant decreased level of serum total protein in the present study by increasing level of dietary ML may be due to their degradation and also to their possible utilization for metabolic purposes. Similar observation was also given for *Channa punctatus* exposed to latices of *Euphorbia royleana* and *Jatropha gossypifolia* (Singh and Singh, 2002). In addition, in the current findings, there were significant increased ($P \leq 0.05$) in AST, ALT, creatinine, urea, and uric acid among all fish groups, as the level of ML increased up to 2.0% in the diet. These increase especially when fish fed 2.0% ML (ML2.0) are suggestive of hepatic and renal cellular damage leading to their leakage into circulation system (Mousa *et al*., 2008). Also, these results might be due to the presence of toxic substances or antinutritional factors in ML (Siddhuraju *et al*., 2000; Francis *et al*., 2001).

The high activities of ALT and AST enzymes in blood indicate organ dysfunction in aquatic organisms during stress condition (Gabriel and George, 2005). Similarly with the obtained results herein, Gabriel *et al*. (2009) noted elevation of both AST and ALT in different organs of catfish hybrid exposed to aqueous extracts from *Lepidagathis alopecuroides* leaves and suggested that the elevation may be due to disturbances in the Kreb's cycle; the elevation in ALT indicates hepatic damage caused by this plant extracts. In a recent study, Kavitha *et al*. (2012) indicated that seed extract of *M. oleifera* has significant effect on hematological and plasma biochemical parameters of *Cyprinus carpio.*

Finally, it could be noted that increasing levels of dietary addition of ML led to impaired growth and feed efficiency, carcass composition parameters, besides their drastically effects on the hematological and serum biochemical parameters of Nile tilapia fry. However, dietary 1% ML gave the highest significant growth performance and feed utilization, highest contents of crude protein, total lipids, and energy contents in fish body, as well as significantly improved the physiological responses for *O. niloticus* fry among all experimental groups. Thus, its use as a food supplement for human and animals, and most of its parts of moringa are widely used for many medical applications.

Conclusion:

It was concluded that dietary Moringa (*M. oleifera*) leaf could positively affect growth performance, feed utilization, and whole body composition of Nile tilapia fry. Thus, *M. oleifera* leaf could possibly add to fish diets up to 1.0%.

REFERENCES

Abdelhakim, N.F.; Baker, M.N. and Soltan, M.A. (2002). Aquatic Environment for Fish culture. Cairo, (ISBN: 977 – 298 – 228 – 5).

Abou El-Naga, E.H.; El-Moselhy, K.M. and Hamed, M.A. (2005). Toxicity of cadmium and copper and their effect on some biochemical parameters of marine fish *Mugil seheli*. Egypt. J. Aquat. Res., 31: 60–71.

Adewole, M.A. (2008). Potentials of sweet potato (*Ipomoea batatas*) leaf meal as dietary ingredient for *Tilapia zillii* fingerling. Pak. J. Nutr.,7: 44-449.

Afuang, W.; Siddhuraju, P. and 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 (*O. niloticus*). Aquacult. Res., 34: 1147–1159.

Amaglo, N.K.; Bennett, R.N.; Lo Curto, R.B.; Rosa, E.A.; LoTurco, V.; Giuffrid, A.; Lo Curto, A.; Crea, F. and Timpo, G.M. (2010). Profiling selected phytochemicals and nutrients in different tissues of the multipurpose tree (*M. oleifera* L.), grown in Ghana. Food Chem., 122: 1047–1054.

AOAC (2000). Association of Official Analytical Chemists, Official methods of analysis, 17th Ed. Washington, DC., USA.

APHA (1998). Standard Methods for the Examination of Water and Wastewater, 20th edition. American Public Health Association, Washington, D.C.

Aslam, M.; Anwar, F.; Nadeem, R.; Rashid, U.; Kazi, T.G. and Nadeem, M. (2005). Mineral composition of *M. oleifera* leaves and pods from different regions of Punjab, Pakistan. Asian J. Plant Sci., 4: 417–421.

Bennett, R.N.; Mellon, F.A. and Foidl, N. (2003). Profiling glucosinolates and phenolics in vegetative and reproductive tissues of the multi-purpose trees *Moringa oleifera* L (horseradish tree) and *Moringa stenopetala* L. J. Agric. Food Chem., 51: 3546–3553.

Bharali, R.; Tabassum. J. and Azad, M. (2003). Chemomodulatory effect of (*M. oleifera* Lam). On hepatic carcinogen metabolizing enzymes, antioxidant parameters and skin papillomagenesis in mice. Asian Pacific J. Cancer Prevention, 4: 131-139.

Blaxhall, P.C. and Daisley K.W. (1973). Routine haematological methods for use with fish blood. J. Fish. Biol., 5:771-781.

Bundit, Y. and Masumoto, T. (2012). Replacing moringa leaf (*M. oleifera*) partially by protein replacement in soybean meal of fancy carp (*C. carpio*) Songklanakarin. J. Sci. Technol., 34:479-485.

Busani, M.; Masika, P.J.; Hugo, A. and Muchenje, V. (2011). Nutritional characterization of Moringa (*Moringa oleifera*) leaves. Afr. J. Biotechnol., 10: 12925–12933.

Cho, C. Y. and Kaushik, S. J. (1985). Effect of protein intake on metabolizable and net energy values of fish diets. In: Nutrition and Feeding in Fish. (ed.) Cowey C.B., Mackie A.M. and Bell J.G., 95-117.

Chuang, P.H.; Lee, C.W.; Chou, J.Y.; Murugan, M.; Shieh, B.J. and Chen, H.M. (2007). Antifungal activity of crude extracts and essential oils of (*Moringa oliefera*). Biores. Technol., 98: 232-236.

Clark, S.; Whitmore, D.H. and McMahon, R.F. (1976) . Consideration of blood parameters of large mouth bass, *Micropterus salmoides*. J. Fish. Biol., 14:147-158.

De Silva, S.S. and Gunasekera, R.M. (1989). Effect of dietary protein level and amount of plant ingredient (*Phaseolus aureus*) incorporated into the diets on consumption, growth performance and carcass composition in *Oreochromis niloticus* fry. Aquaculture, 80:121–133.

Dongmeza, E.; Siddhuraju, P.; Francis, G. and Becker, K. (2006). Effects of dehydrated methanol extracts of moringa (*Moringa oleifera*) leaves and three of its fractions on growth performance and feed nutrient assimilation in Nile tilapia (*Oreochromis niloticus* (L.). Aquaculture, 261: 407–422.

Etim, L.; Lebo, P. and King, R. (1999). The dynamics of an exploited population of Siluroid Catfish (*Schilbe intermedius* Ruppell, 1832) in the Cross River, Nigeria. Fish. Res., 40: 295–307.

Fawcette, J.K. and Scott, J.E. (1960). Practical Clinical Biochemistry. 4th Edn., Arnold Harold Varley, India, 119-122.

Foidl, N.; Makkar, H. and Becker, K. (2001). The Potential of *Moringa oleifera* for agricultural and industrial uses. What development potential for Moringa products? October 20 th- November 2nd 2001. Dar Es Salaam.

Francis, G.; Makkar, H.P. and Becker, K. (2001). Antinutritional factors present in plant-derived alternate fish feed ingredients and their effects in fish. Aquaculture, 199: 197–227.

Gabriel, U.U. and George, A.D. (2005). Plasma enzymes in *Clarias gariepinus* exposed to chronic levels of round up (glyphosate). Environ. Ecol., 23:271–276.

Gabriel, U.U.; Obomanu, F.G. and Edori, O.S. (2009). Haematology, plasma enzymes and organ indices of *Clarias gariepinus* after intramuscular injection with aqueous leaves extracts of *Lepidagathis alopecuroides*. Afr. J. Biochem. Res., 3:312–316.

Goff, J.B. and Gatlin, D.M. (2004). Evaluation of different sulfur amino acid compounds in the diet of red drum, *Sciaenops ocellatus*, and sparing value of cystine for methionine. Aquaculture, 241: 465–477.

Golterman, H.L.;Clymo, R.S. and Ohnstad, M.A. (1978). Methods of physical and chemical analysis of fresh waters. Blackwell Scientific Publications, Oxford, 214pp.

Gornall, A.G., Bardawill, C.J. and David, M. M. (1949). Determination of serum proteins by means of the biuret reaction. J. Biol. Chem., 177:751-66.

Gowrishankar, R.; Kumar, M.; Menon, V.; Divi, S.M.; Saravanan, M.; Magu- dapathy, P.; Panigrahi, B.K.; Nair, K.G. and Venkataramaniah, K. (2010). Trace element studies on *Tinospora cordifolia* (Menispermaceae), *Ocimumsanctum* (Lamiaceae), *Moringa oleifera* (Moringaceae) and *Phyllanthus niruri* (Euphorbiaceae) using PIXE. Biol. Trace Elem. Res., 133:357–363.

Group, E. (2009). The health benefits of antioxidants. Healthy foods, natural health, organic living. Global Healing Centre. New York.

Kavitha, C.; Ramesh, M.; Kumaran, S.S. and Lakshmi, S.A. (2012). Toxicity of *Moringa oleifera* seed extract on some hematological and biochemical profiles in a freshwater fish, *Cyprinus carpio*. Experimental and Toxicologic Pathol., 64: 681–687.

Kim, E.K.; Waddell, L.D.; Sunderland, M.L. and Logan, J.E. (1971). Observations on Diagnostic Kits for the determination of uric acid. Clin Biochem., 4:279-286.

Kori-Siakpere, O. and Ubogu, E.O. (2008). Sublethal haematological effects of zinc on the freshwater fish, *Heteroclarias* sp. (Osteichthyes: Clariidae). Afr. J. Biotechnol., 7: 2068–73.

Makkar, H. and Becker, K. (1996). Nutritional value and antinutritional components of whole and ethanol extracted (*Moringa oliefera*) leaves. Animal feed Sci. Technol., 63: 211-228.

Manguro, L.O. and Lemmen, P. (2007). Phenolics of *Moringa oleifera* leaves. Nat. Prod. Res., 21: 56–68.

Mehta, K.; Balaraman, R.; Amin, A.H.; Bafna, P.A. and Gulati, O.D. (2003). Effect of fruits of (*Moringa oliefera*) on the lipid profile of normal and hypercholesterolaemic rabbits. J. Ethnopharmacol., 86: 191–195.

Mousa, M.A.; El-Ashram, A.M. and Hamed, M. (2008). Effects of Neem leaf extract on freshwater fishes and zooplankton community. 8th International Symposium on Tilapia in aquaculture. The central laboratory for Aquaculture Research, Cairo, Egypt. 12th – 14th October, 2008.

Newman, D.J. and Price, C.P. (1999). Renal Function and Nitrogen Metabolites. In: Tietz Textbook of Clinical Chemistry, Burtis, C.A. and E.R. Ashwood (Eds.). 3rd Edn., W.B. Saunders Co., Philadelphia, ISBN-13, 1204-1270.

NRC: National Research Council (1993) Nutrient Requirements of Warm Water Fishes and Shellfishes. National Academy Press Washington, D.C. 1993.

Olufayo, M.O. (2009). Hematological characteristics of *Clarias gariepinus* (Burchell 1822) juveniles exposed to Derris elliptical root powder. Afr. J. Food Agric. Nutr. Sci., 9:920–33.

Osman, A.G.; Koutb, M. and Sayed, A.E. (2010). Use of hematological parameters to assess the efficiency of quince (*Cydonia oblonga* Miller) leaf extract in alleviation of the effect of ultravioleta radiation on African catfish *Clarias gariepinus* (Burchell, 1822). J. Photochem. Photobiol. Biology, 99:1–8.

Oyawoye, E.O. and Ogunkunle, M. (1998). Physiological and biochemical effects of raw Jack beans on broilers. Proc. Ann. Conf. Nig. Soc. Anim. Prod., 23:141-142.

Pari, L. and Kumar, N.A. (2002). Hepatoprotective activity of (*Moringa oliefera*) on antitubercular drug-induced liver damage in rats. J. Medic. Food, 5: 171–177.

Pietse, J.J.; Smith, G.L.; van Viiet, K.J.; Schoobe, H.J. and Hattingh, J. (1981). Some blood parameters of the Chinese grass carp, *Ctenopharygodon idella* (Valenciennes). South Afr. J. Zool., 16:124–126.

Richter, N.; Siddhruraju, A. and Becker, K. (2003). Evaluation of nutritional quality of moringa (*Moringa oleifera* Lam.) leaves as alternative protein source for Tilapia (*Oreochromis niloticus* L.). Aquaculture, 217:599-611.

Robert, K.M.; Daryl, K.G.; Peter, A.M. and Victor, W.R. (2000): Lipid Transport and Storage. In: Harper's Biochemistry. McGraw-Hill, USA. 25th edition. Pp: 278-289.

Sanchez, N.R.; Stig, L. and Inger, L. (2006). Biomass production and chemical composition of (*Moringa oliefera*) under different management regimes in Nicaragua. Agrofores Sys., 66: 231–242.

Santiago, C.B. and Lovell, R.T. (1988). Amino acid requirement for growth of Nile tilapia. J. Nutr., 118: 1540-1546.

SAS (2006). SAS procedure user's guide. SAS Institute Inc., Cary, NC, USA.

Schultz, A.(1984). Uric acid. Clin. Chem. The C.V. Mosby Co. St. Louis. Toronto. Princeton, 1261-1266.

Shah, N.; Atallah, M.T.; Mahoney, P.R. and Pellet, P.L. (1982). Effect of dietary fiber components on fecal nitrogen excretion and protein utilisation in growing rats. J. Nutr., 112: 658– 666.

Shiau, S.Y. (1997). Utilisation of carbohydrates in warmwater fish—with particular reference to tilapia, *Oreochromis niloticus* × *O. aureus*. Aquaculture, 151: 79–96.

Siddhuraju, P.; Becker, K. and Makkar, H.P. (2000). Studies on the nutritional composition and antinutritional factors of three different germplasm seed materials of an under-utilised tropical legume, *Mucuna pruriens* var. *utilis*. J. Agric. Food Chem., 48: 6048–6060.

Singh, D. and Singh, A. (2002). Biochemical alteration in freshwater fish *Channa punctatus* due to latices of *Euphorbia royleana* and *Jatropha gossypifolia*. Environ Toxicol Pharmacol., 12:129–136.

Sotolu, A.O. (2010). Growth performance of *Clarias gariepinus* (Burchell, 1822) fed varying inclusions of *Leucaena luecocephala* seed meal. Tropicultura, 28:168-172.

Stoskopf, M.K. (1993). Fish medicine. WB Saunders Company, Philadelphia, USA.

Varley, H.; Gowenlok, A.H. and Bel, M.C. (1976). Practical Biochemistry. Heinmann, London, UK., pp: 389-391.

Verma, S.C.; Bannerji, R.; Mirra, G. and Nigam, S.K. (1976). Nutritional value of Moringa. Curr. Sci., 45: 769-770.

Ward, A.T. and Reichert, R.D. (1986). Comparison of effect of cell wall and hull fibre from canola and soybean on the bioavailability for rats of minerals, protein and lipid. J. Nutr., 116: 233– 241.

World Health Organization (1985). Energy and protein requirements. Report of a Joined FAO/WHO/UNU Expert Consultation Meeting Series, n. 724, Geneva, Switzerland.

Zar, J.H. (1984). Biostatistician Analysis. Prentice Hall, Englewood Cliffs, New Jersey, USA.

Table 1: Chemical composition (g/100 g) **of Moringa Leaf Meal (MLM)**

Parameters	MLM
Proximate composition (%)	
Dry matter	93.40
Crude protein	27.70
Crude lipid	5.20
Crude fiber	11.50
Ash	14.30
Nitrogen free extract	41.30
Gross energy (kJ g^{-1})	375.81
Amino acid composition (%)	
Arginine	13.25
Histidine	6.13
Isoleucine	8.25
Leucine	9.50
Lysine	13.25
Methionine	3.5
Phenylalanine	13.88
Threonine	11.88
Valine	10.63
Tryptophan	4.25
Cystine	3.50

Table 2: Composition and Proximate analysis (%) of experimental diets with different Levels of Moringa leaf meal (MLM).

Parameters	Experimental diets[1]				
	D 1	D 2	D 3	D 4	D 5
Ingredient (g 100g^{-1})					
Fish meal (FM)	23	23	23	23	23
Soybean meal (SBM)	23	23	23	23	23
Moringa leaf meal (MLM	-	0.5	1.00	1.50	2.00
Corn gluten meal	2	2	2	2	2
Wheat bran	7	7	7	7	7
Yellow corn	40	39.5	39.00	38.50	38.00
Corn oil	3	3	3	3	3
Vita.[2] and Min.[3] mix	2	2	2	2	2
Proximate analysis (%) on DM basis					
Dry matter	85.91	85.93	86.96	85.98	86.00
Crude Protein	29.72	29.82	29.93	30.02	30.13
Ether Extract	6.54	6.55	6.55	6.56	6.56
Ash	5.38	5.38	5.34	5.31	5.30
Crude Fiber	3.80	3.84	3.88	3.93	3.97
Nitrogen free extract	54.56	54.41	54.30	54.18	54.04
Gross energy (kJ g-1)	454.51	454.55	454.72	454.83	454.87

[1]Diet 1 (control diet without MLM); diet 2, 3, 4 and 5 contained 0.5, 1.0, 1.5 and 2.0 % Moringa leaf meal, respectively.

[2]Vitamin mixture/kg premix containing the following: 3300IU vitamin A, vitamin D3, 410 IU vitamin E,2660, mg vitamin B1,133mg vitamin B2,580 mg vitamin B6 ,410 mg vitamin B12- 50 mg biotin , 9330 mg Colin chloride, 4000mg vitamin C, 2660 mg Inositol, 330 mg para -amino benzoic acid, 9330 mg niacin, 26.60 mg pantothenic acid.

[3]Mineral mixture/kg premix containing the following 325 mg Manganese, 200mg Iron, 25 mg Copper, 5 mg Iodine, 5mg Cobalt.

[4]GE (Gross Energy): gross energy calculated as 5.64, 9.44 and 4.12 Kcal per gram of protein, lipid and carbohydrate, respectively after (NRC, 1993).

Table 3: Amino acid composition of experimental diets and amino acid requirements of Nile tilapia, *Oreochromis niloticus*, L., (%).

Indispensable amino acid	Required[1]	Experimental diets				
		D1	D2	D3	D4	D5
Arginine	1.60	1.63	1.695	1.76	1.824	1.889
Histidine	0.65	0.653	0.683	0.712	0.742	1.236
Isoleucine	1.18	1.047	1.088	1.128	1.168	1.48
Leucine	1.29	1.783	1.876	1.97	2.063	2.368
Lysine	1.95	1.554	1.619	1.684	1.75	1.909
Methionine	1.02	1.292	1.309	1.325	1.342	1.453
Phenylalanine	1.43	1.316	1.384	1.451	1.519	1.681
Threonine	1.43	1.171	1.363	1.331	1.389	1.542
Valine	1.06	1.398	1.449	1.501	1.552	1.698

[1]From Santiago and Lovell (1988).

Table 4: Effect of supplementation of MLM on growth performance of Nile tilapia, *O. niloticus* L., fry.

Diets No.*	Body weight		weight gain (g/fish)	Average daily gain (ADG) (g/fish/day)	Specific growth rate (SGR) (%/day)
	Initial (g/fish)	Final (g/fish)			
D 1	0.82±0.01	16.42^{c}±0.06	15.6^{c}±0.04	0.19^{b}±0.01	3.6^{b}±0.01
D 2	0.82±0.01	16.82^{c}±0.08	16.0^{c}±0.08	0.19^{b}±0.01	3.6^{b}±0.01
D 3	0.82±0.01	19.75^{a}±0.05	18.9^{a}±0.05	0.22^{a}±0.01	3.79^{a}±0.01
D 4	0.82±0.01	17.42^{b}±0.06	16.6^{b}±0.06	0.19^{b}±0.01	3.6^{b}±0.01
D 5	0.81±0.01	15.24^{d}±0.07	14.3^{d}±0.07	0.17^{c}±0.01	3.5^{c}±0.01
F	0.36(NS)	1999.4**	2372.2**	2369.6**	7378.7**
EMS	0.000733	0.00417	0.0055	0.0000005	0.0000996

*Diet 1 (control diet without MLM);
diet 2, 3, 4 and 5 contained 0.50, 1.0, 1.5 and 2.0% MLM, respectively.
**Means followed by different letters in the same column differ significantly (P≤0.01).

Table 5: Effect of supplementation of MLM on feed and nutrient utilization parameter of Nile tilapia, *O. niloticus*, fry.

Diet No.*	Feed intake (gm/fish)	Food conversion ratio (FCR)	Protein utilization		Energy utilization (EU%)
			Protein efficiency ratio (PER)	Protein productive value (PPV)	
D 1	30.77±0.1^{d}	1.97 ±0.01^{c}	1.71±0.01^{bc}	29.0±0.1^{b}	17.92±0.1^{b}
D 2	31.04±0.1^{cd}	1.94±0.01^{c}	1.73±0.01^{b}	29.5±0.2^{b}	18.32±0.2^{b}
D 3	31.57±0.1^{c}	1.69±0.01^{d}	2.01±0.01^{a}	34.9±0.1^{a}	21.63±0.2^{a}
D 4	41.50±0.4^{b}	2.50±0.01^{b}	1.33±0.01^{bc}	22.5±0.3^{c}	14.0±0.2^{c}
D 5	44.76±0.2^{a}	3.11±0.01^{a}	1.07±0.01^{c}	17.9±0.2^{d}	11.18±0.1^{d}
F	98185**	36075.1**	3000.7**	3879.2**	17306**
EMS	0.0137	0.000027	0.000013	0.0364	0.0287

*Diet 1 (control diet without MLM);
diet 2, 3, 4 and 5 contained 0.5, 1.0, 1.5 & 2.0% MLM, respectively.
**Means followed by different letters in the same column differ significantly (P≤0.01).

Table 6: Effect of supplementation of MLM on carcass composition of Nile tilapia, *O. niloticus*, fry (On dry matter basis).

Diet No.*	Dry Matter %	Crude Protein	Ether Extract	Ash	Energy Content
At start	**23.25**	**55.65**	**20.65**	**23.7**	**509.565**
D 1	28.68±0.08^{bc}	58.60±^{b}	23.4±0.3	17.95±0.5^{c}	551.75±2.65^{b}
D 2	28.76±0.13^{ab}	58.70 ^{b}	23.6±0.3	17.76±0.12^{c}	553.38±3.23^{b}
D 3	28.83±0.08^{a}	59.87±^{a}	23.6±0.4	16.50±0.16^{d}	560.73±4.12a
D 4	28.58±0.06^{cd}	58.3±^{c}	23.4±0.2^{c}	18.26±0.14^{b}	549.33±4.9c
D 5	28.44±0.06^{d}	58.08±^{d}	23.2±0.1	18.75±0.15^{a}	546.29±3.58^{d}
F	9.91**	17.13**	1.43(NS)	116.68**	6.19**
EMS	0.00712	0.0842	0.0759	0.017	14.2

*Diet 1 (control diet without MLM);
diet 2, 3, 4 and 5 contained 0.50, 1.0, 1.5 and 2.0% MLM, respectively.
**Means followed by different letters in the same column differ significantly (P<0.01).

Table 7: Effect of supplementation of MLM on blood hematological and biochemical parameters of Nile tilapia (*O. niloticus*) fry.

Diet No	HB[2] (g/dl)	HT3 (%)	PCV[4] %	Total protein (g/dl)	Hepatic function		Kidney function		
					AST[5] (U/L)	ALT[6] (U/L)	Creatinine (Mg/dl)	Urea (mg/dl)	Uric Acid (mg/dl
D 1	5.61^{b}	25.3^{d}	20.12^{d}	4.76^{c}	37.19	26.39^{b}	0.43^{ab}	4.54^{bc}	1.84^{b}
D 2	5.87^{a}	25.67^{c}	21.12^{c}	4.82^{c}	36.46^{c}	25.99^{c}	0.40^{bc}	4.60^{ab}	1.78^{bc}
D 3	5.93^{a}	26.44^{a}	23.12^{a}	5.42^{a}	35.54^{d}	25.35^{d}	0.34^{c}	4.22^{c}	1.65^{d}
D 4	5.70^{b}	26.10^{b}	22.54	4.97^{b}	37.08^{b}	25.40^{d}	0.39^{bc}	4.35^{bc}	1.73^{cd}
D 5	5.44^{c}	24.86^{e}	16.56^{e}	3.765^{d}	39.66^{a}	28.95^{a}	0.48^{a}	4.93^{a}	19.65^{a}
F EMS									

[1]Diet 1(control diet without MLM); diet 2, 3, 4 and 5 contained 0.50, 1.0, 1.5 and 2.0% MLM, respectively. [2]HB=Hemoglobin,/dl [3]HT=Hematocrit,% [4]PCV=Packed cell value
[5]ALT = aspartate aminotransferase [6]AST = Alanine aminotransferase

تأثير اضافة اوراق المورينجا كمحفز للنمو على اداء النمو والكفاءة الغذائية لاسماك البلطى النيلى

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المستخلص

أجريت دراسة نمو لأثني عشر أسبوع لأختبار تأثير أضافة مسحوق أوراق المورينجا (ML) الى علائق أسماك البلطى النيلى وتأثير ذلك على إداء النمو، وكفاءة الأستفادة من الغذاء ومحتوى جسم الأسماك وعلى المعايير الفسيولوجية . وتم توزيع مائة وخمسين سمكة بلطى نيلى وحيد الجنس (متوسط وزن 0.83, جرام / سمكة) بشكل عشوائي فى خمسة عشر حوض زجاجى (ثلاثة أحواض / معاملة) بواقع (10 سمكة / حوض) . وتم أضافة أوراق المورينجا الى خمسة علائق على النحو التالي 0.5٪، 1٪، 1.5٪، و 2٪ فى العليقة رقم ، 2 و 3 و 4 و 5 على التوالي.أما العليقة القياسية رقم 1 بدون أستخدام أوراق المورينجا. بعد فترة التغذية أظهرت النتائج أن معدل الزيادة فى وزن أسماك البلطى النيلى (WG) المغذاه على العليقة رقم 2 ،3 ، 4 كانت أعلى معنويا من أسماك البلطى المغذاه على العليقة رقم 5 والعليقة القياسية رقم 1. معدل النمو النوعي (SGR%/ day) أخذ نفس أتجاه معدل الزيادة فى الوزن لأسماك البلطى النيلى. كما أن كفاءة الاستفادة من البروتين للأسماك التى غذيت على العليقة القياسية رقم 1 ، والعليقة رقم 2 المحتوية على 0.5 ML% والعليقة رقم 3 المحتوية على 1.5 % MLM كانت أعلى بكثير وكانت الفروق معنوية ($P \leq 0.05$) فى الأسماك المغذاة على العليقة رقم 3 والعليقة رقم 5. كما ظهرت فروق ذات معنوية عالية ($P \leq 0.05$) لكفاءة المحتجز من البروتين للأسماك المغذاه على العلائق المحتوية على 1، 0.5 ، 1.5 % ML على الترتيب .كما وجدت فروق معنوية ($p \leq 0.01$) في محتوى جسم أسماك البلطى من (الرطوبة، البروتين الخام والرماد والألياف) في نهاية التجربة. وأوضحت النتائج عدم وجود فروق معنوية عند المستوى 0.5 ، 1.5 % بالمقارنة بالكنترول وكان أفضل اضافة لاوراق المورنجا عند مستوى 1% في العلائق. الأسماك المغذاة على 1% من أوراق المورينجا أدت إلى زيادة معنوية للهيموجلوبين والهيماتوكريت والبروتين الكلى للسيرم ، بينما أدت نفس المعاملة إلى انخفاض معنوى لنشاط أنزيمات وظائف الكبد والكلي فى السيرم مقارنة بالمستويات الأخري من أوراق المورينجا. وتشير الدراسة إلى أن أستخدام أوراق نبات المورنجا لها تأثير إيجابيا على أداء النمو، وكفاءة الأستفادة من الغذاء ومحتوى الجسم و الاستجابات الفسيولوجية لزريعة أسماك البلطى النيلى.