

Effect of Feeding Different Levels of *Moringa oleifera* on Growth Performance and Potential Role in Muscle Proteins in Fish *Puntius altus*

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Abstract

The growth in fish farming has meant an increase in attention in terms of improving the quantity and quality of the fish. The aim of the present study is to evaluate *Moringa oleifera* as a nutritional supplement to fish, as it has demonstrated multiple biological activities. To test the beneficial effects of *M. oleifera* in fish diets, juvenile fish were separated into duplicate groups with test diets containing 0, 20 and 60 mg of *M. oleifera* leaf powder per g of fish food, respectively, for 28 days. The results were a significantly increased average body weight value ($p < 0.05$) in fish fed with a diet containing *M. oleifera*. The normal muscle cellularity correlated with an increase in the growth rate was detected in the group of fish fed with *M. oleifera* diets. Interestingly, a slight number of small muscle fibers showing strong reaction in the perimysium area were detected in fish fed with a diet containing *M. oleifera*, suggesting newly growing muscle fibers. Moreover, fish fed a diet containing *M. oleifera* leaves showed protein bands at 97 kDa, similar to those fed on *M. oleifera* leaf extract alone. These findings indicate that dietary supplementation with *M. oleifera* leaves results in significantly improved growth performance and an increase in muscle protein profile, without adverse effect to fish health. This could potentially help improve the quality of fish, as well as increase aquaculture yield.

Keywords: *Moringa oleifera*, supplement, fish, muscle, growth

Introduction

To date, the worldwide trade of fish production has been increasing [1]. In Thailand, consumer demand for fish products is increasing due to consumer perception of fish as healthy food. To sustain the high rate of growth of the aquaculture sector, an increase in fish feed production is necessary [2]. On the other hand, economically high cost diets, and high quality fish products, have led to the need to identify formulation of new feeds in cultured fish. The use of plant-derived protein sources which are less expensive and are locally available, such as soy bean [3,4], cotton seed [3,5], or rapeseed meal [3] have been evaluated due to economic and practical reasons. Therefore, developing new fish feed using medicinal plants as a natural feed supplementation in local fish farming would be useful in cultured fish, which may not have an adverse effect to human health.

Moringa oleifera, commonly known as "Drumstick tree", grows throughout the tropics and subtropics and is cultivated in all parts of Thailand. It has been used as a vegetable for cooking purposes, as well as for medicinal usage [6]. Medicinally, most parts of the herb have been applied in traditional medicine for the treatment of human diseases, as the leaves are rich in protein, carotenoids, ascorbic acid, and iron [7]. Chemical constituents, biological activities, and medicinal properties of different parts of *M.*

oleifera were previously reviewed [6-10]. High nutrients and antioxidants are common in *Moringa* spp. The leaves of *M. oleifera* have been used as a nutritional supplement and a growth promoter due to the significant presence of protein, selenium, phosphate, calcium, β -carotene and α -tocopherol [9-10]. The leaves have also been used as a cheap protein source for fodder [6]. As forage, *Moringa* spp. could be a potential bioceutical agent for use as a substitute for antibiotics in various livestock production. Particularly, positive effects on the feeding behavior in goats [11] and on the growth rate in sheep [12] have been reported. Though a broad benefit of biological activities has been reported, the mode of action of these plants when used as supplemented feed in fish has not yet been elucidated.

Puntius altus is an economically important ornamental fish and is widely cultured in Singapore and Malaysia for export [13]. It is also an important fish product in Thailand [14]. *Puntius* spp. (syn. Barbodes) has been widely cultivated due to its rapid growth and the ability to be commercially cultivated in cages and raceways, as well as in open ponds. These species are distributed widely in freshwater regions of Thailand. With the remarkable increase in fish production demand and their relatively low prices, *P. altus* is expected to become an important source of animal protein. However, there is no information regarding the effects of *M. oleifera* supplementation on growth and fish muscle changes. Therefore, this study was conducted to determine the possible benefits of *M. oleifera* leaves as supplemented feeds for fish on growth performance and muscle profiles.

Materials and methods

Fish, tested diets, and feeding trails

Healthy *P. altus* fish were purchased from a local hatchery in Kanchanaburi, Thailand. Prior to the feeding trial, fish were acclimated under laboratory conditions for 30 days (29 ± 1 °C, total hardness 68 to 88 mg l⁻¹, and alkalinity 75 to 80 mg l⁻¹) and supplied with dechlorinated tap water under a 12 h light/dark schedule. At the start of the feeding experiment, fish were assigned into 3 aquaria, consisting of 7 fish in each aquarium. Fish in duplicate were fed their respective diets over 28 days. Group 1 served as a control and was fed only on a commercial fish diet, while groups 2 and 3 were treated with *M. oleifera* at 20 and 60 mg g⁻¹ supplemented diets; all fish were fed 2 % body weight twice daily. Throughout the experimental period, maintenance of fish was conducted according to the Mahidol University-Institutional Animal Care and Use Committee (MU-IACUC, protocol no. 210). At the end of the feeding trial, all fish were weighed and measured for length. Liver and muscle samples were collected for further analysis.

Evaluation of growth parameters

The body weight of fish was recorded at the time of initiation and completion of the experiment. Growth performance was determined and calculated as follow: Average body weight (ABW), Specific growth rate (SGR), Daily growth index (DGI), and Hepatosomatic index (HSI);

$$\begin{aligned} \text{ABW (g)} &= [\text{final weight (g)} + \text{initial weight (g)}] / 2 \\ \text{SGR (\%g day}^{-1}\text{)} &= \{[\text{final weight (g)}]^{1/3} - [\text{initial weight (g)}]^{1/3}\} \times 100 / \text{no. of days} \\ \text{DGI (\%g day}^{-1}\text{)} &= [\text{final weight (g)} - \text{initial weight (g)}] \times 100 / \text{no. of days} \\ \text{HSI (\%)} &= [\text{liver weight (g)} / \text{whole body weight (g)}] \times 100 \end{aligned}$$

One-way analysis of variance (ANOVA) was applied to determine significant differences in the results of the various groups. The results were considered significantly different at the level of $p < 0.05$. All data are presented as a mean with standard deviation. Statistical analysis was performed using the SPSS 18.0 for Windows [15].

Histological examination

For the morphological analyses, 10 % neutral buffered formalin fixed muscle samples were processed through paraffin embedding and sectioned at 5 μ m thickness. Then, all slides were stained with H&E, periodic acid Schiff's (PAS) stain for glycogen detection in the skeletal muscle [16], and trichrome

stained to distinguish muscle changes. All stain sections were observed and photographed using a Nikon DMX 1200 digital camera (Tokyo, Japan).

Fish muscle protein extraction and electrophoresis

Muscle protein fractions were prepared, as described by Laemmli (1970) with appropriate modification. Briefly, fish muscle sample (0.5×0.5×1 cm) was added in 1mL Laemmli buffer containing 4 % SDS; 20 % glycerol; 10 % β-mercaptoethanol; 0.004 % bromphenol blue; and 0.125 Tris HCl. Then, the sample was boiled for 5 min, and the supernatant collected and separated on 12 % SDS-polyacrylamide gel electrophoresis (PAGE) at a constant voltage of 198 V for 1 h. Afterward, gels were stained with Coomassie Brilliant Blue [17], de-stained until the gel background was clear, and each protein band compared.

Results and discussion

Fish growth performance

The present study showed that a supplement of different levels of *M. oleifera* had effects on feed intake which altered the growth performance of the fish ($p < 0.05$; **Table 1**). This growth implies the *M. oleifera* supplemented diets could enhance the growth of the fish. No significant differences between dietary treatments in fish specific growth rate were observed, but fish fed supplemented diets showed higher values for fish average body weight (ABW), after 28 days than fish fed the control diet. A significantly increased ABW value ($p < 0.05$) in fish fed with diet containing *M. oleifera* was observed. Similarly, several studies showed that the use of plant material as a dietary supplement improved the feed intake and growth in fish. According to Tacon *et al.* [18], supplementation of 8 g kg⁻¹ DL-methionine to a diet with 75 % of brown fish soybean meal improved the growth performance of tilapia to a level comparable to that of a fish meal diet. The significant increase in ABW in the current study could be associated with the hepatosomatic index (HSI) by the supplemented groups as a result of higher *M. oleifera* levels in the supplement than the basal feed. Furthermore, HSI is increased, suggesting that *M. oleifera* leaves may have an effect on the fish liver. As previously studied in Atlantic salmon *Salmo salar* fed soybean oil, soybean supplemented diets increased the fat composition and affected the morphology of the liver [19]. Overall, *M. oleifera* leaves appear to increase the growth of fish receiving the crude leaves as a diet supplement.

Table 1 Body weight changes (mean ± SD) of fish fed experimental diets containing different levels of *Moringa oleifera* over 28 days.

Parameters	Diet (Supplemented with <i>M. oleifera</i> leaves per mg g ⁻¹ fish food)		
	1 (0 mg g ⁻¹ fish food)	2 (20 mg g ⁻¹ fish food)	3 (60 mg g ⁻¹ fish food)
IBW (g)	28.17±2.24	28.92±1.85	28.73±4.49
ABW (g)	43.28±12.25	59.93±10.25 ^a	50.13±4.03 ^b
SGR (%g day ⁻¹)	14.21±4.07	14.35±3.19	11.58±1.06
DGI (%g day ⁻¹)	42.63±12.21	43.04±9.56	23.65±3.03 ^{a,b}
HSI (%)	1.91±1.14	2.07±0.29	1.72±0.35

Data are mean ± SD (n = 42). Values in the same row assigned different superscript letters are significant different ($p < 0.05$). Mean initial body weight (IBW), Average body weight (ABW), Daily growth index (DGI), Specific growth rate (SGR), and Hepatosomatic index (HSI).

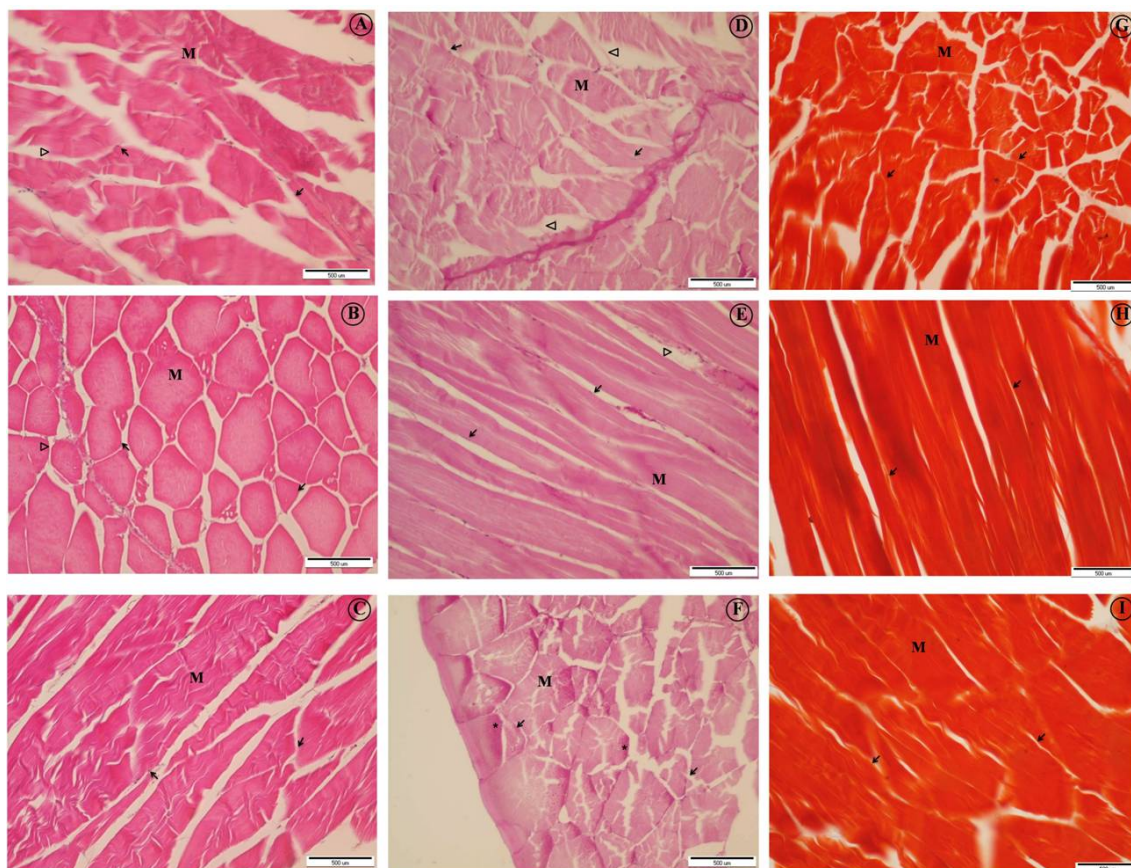


Figure 1 Representative of light micrographs of skeletal muscle of *P. altus* (A-I) showing muscle fiber (M); perimysium (Δ); myofiber nuclei (\rightarrow). A, B and C showing normal muscle morphology (H&E stain); D, E and F cross section of fish muscle stained for glycogen (PAS staining) showing difference glycogen content between small and large muscle fibers; G, H and I showing normal muscle fiber with no histopathological changes (Masson Trichrome stain). scale bar: 500 μ m.

Fish muscle histology

The influence of the diets on muscle morphology of fish is shown in **Figure 1**. Different muscle fiber types are organized in fish muscle, which differ in size and staining properties [20-22]. Fish have three types of muscle cells, as in all vertebrates. There are 3 types of fibers: red muscle, pink (intermediate) muscle: and white muscle, which forms the bulk of fish musculature [23]. This study showed normal muscle cellularity correlated with the growth rate increases. Histological examination revealed normal muscle morphology (**Figures 1A - 1C**) which comprised of several fascicles of muscle. Each fascicle was surrounded by thin connective tissue, which separated muscular bundles from one another. Several myofiber nuclei were located at the periphery of the cells (**Figure 1A**). The PAS staining is used for detection of glycogen in muscle; the glycogen will be stained purple, and nuclei will be stained blue. Interestingly, slight numbers of small muscle fibers showing strong reaction (bright magenta) in the perimysium area were detected in fish fed with *M. oleifera* diets (**Figures 1E - 1F**). This agrees with reports of dogfish, *Scyliorhinus canicula*, and thornback ray, *Raja clavata*; the red and intermediary fiber

reacted positively with periodic acid Schiff's staining while the white fiber showed mild coloration [24]. In addition, small muscle fibers scattered among similar diameter muscle fibers were observed in fish fed with 20 mg g⁻¹ of *M. oleifera* leaves, suggesting newly growing muscle fibers. This is consistent with previous reports on rainbow trout, of which recruitment of new fibers was directly related to growth rate, but in small juveniles [22,25]. This information was reviewed in depth by Weatherley *et al.* [26] which suggested that the presence of very small diameter fibers (< 20 µm) is considered indicative of hyperplastic growth of muscle and increase in muscle fiber number, as they are related to fish size and growth rate. Moreover, there clearly showed no muscle alteration from muscle to muscle analyzed with trichrome stain (**Figures 1G - 1I**), though an increase in distance among muscle bundles was detected in fish fed the control diet.

Protein profile in fish muscle

The change in the electrophoretic profiles of muscle in fish and aqueous extract of *M. oleifera* leaves were studied. The efficacy of *M. oleifera*-supplemented diets on the muscle proteins of fish is shown in **Figure 2**. SDS-PAGE analysis revealed different electrophoretic patterns. The muscle samples showed several protein bands, ranging from 29 to 97 kDa (**Figure 2**). The results showed that the gel pattern of actin (45 kDa) appeared to remain constant where there were differences in intensity among the feeding trails. Fish fed diets containing *M. oleifera* leaves markedly increased more in band intensity than fish fed the commercial diet alone. Note that, although actin and myosin are the major components, other proteins are also found in muscle tissue [24,26-27]. Interestingly, fish fed a diet containing *M. oleifera* leaves showed bands at 97 kDa, similar to those fed *M. oleifera* leaves extract alone. Altogether, these results suggest that a combination of *M. oleifera* affects muscle protein profiles, especially prominent proteins in these plants. *M. oleifera*-supplemented diets appear to enhance muscle growth and muscle-protein synthesis in fish.

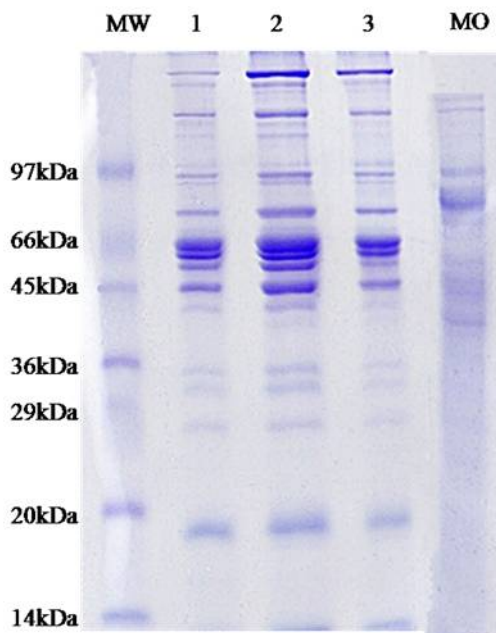


Figure 2 Electrophoretic (SDS-PAGE) pattern of *P. altus* muscle protein extracts. Bands detected in all samples are numbered and the corresponding molecular weights are shown. Lane 1 = group1; Lane 2 = group2; Lane 3 = group3; MO = aqueous extract of *M. oleifera* leaves; MW = molecular weight standard.

Conclusions

Dietary supplementation with *M. oleifera* leaves has resulted in improved growth performance and increased muscle protein profile without adverse effect to fish health. The best results were observed in fish fed 20 mg g⁻¹ of *M. oleifera*-supplemented diet. These findings indicate that *M. oleifera* leaves can be used as a diet supplement to increase the growth and protein components in muscle of the fish. Moreover, using *M. oleifera* leaves as a dietary supplement to fish may contribute to the reduction of feed cost to fish farmers.

References

- [1] Food and Agriculture Organization of the United Nations (FAO) (2009) FISHSTAT Plus. Universal software for fishery statistical time series, Available at: <http://www.fao.org/fi/statist/FISOFT/FISHPLUS.asp>, accessed August 2014.
- [2] G Francis, PS Makkar and K Becker. Antinutritional factors present in plant-derived alternate fish feed ingredients and their effects in fish. *Aquaculture* 2001; **199**, 197-227.
- [3] AJ Jackson, BS Capper and AJ Matty. Evaluation of some plant proteins in complete diets for tilapia, *Sarotherodon mossambicus*. *Aquaculture* 1982; **27**, 97-109.
- [4] SOE Sadiku and K Jauncey. Soybean flour-poultry meat meal blends as dietary protein source in practical diets of *Oreochromis niloticus* and *Clarias gariepinus*. *Asian Fish Sci.* 1995; **8**, 159-68.
- [5] AFM El-Sayed. Long term evaluation of cotton seed meal as a protein source for Nile tilapia *Oreochromis niloticus*. *Aquaculture* 1990; **84**, 315-20.
- [6] H Makkar and K Becker. Nutritional value and antinutritional components of whole and ethanol extracted *Moringa oleifera* leaves. *Anim. Feed Sci. Tech.* 1996; **63**, 211-28.
- [7] DI Sánchez-Machado, J López-Cervantes and NJR Vázquez. High-performance liquid chromatography method to measure α - and γ -tocopherol in leaves, flowers and fresh beans from *Moringa oleifera*. *J. Chromatogr. A* 2006; **1105**, 111-4.
- [8] WOK Grabow, JL Slabert, WSG Morgan, SAA Jahn. Toxicity and mutagenicity evaluation of water coagulated with *Moringa oleifera* seeds preparations using fish, protozoan, bacterial, enzyme and Ames Salmonella assays. *J. Am. Water Works Assoc.* 1985; **76**, 319-21.
- [9] N Foidl, HPS Makkar and K Becker. *The Potential of Moringa Oleifera for Agricultural and Industrial Uses*. In: LJ Fuglie (ed.). *The Miracle Tree: The Multiple Attributes of Moringa*. Wageningen, Netherlands, 2001, p. 45-76.
- [10] P Ferreira, D Farias, J Oliveira and A Carvalho. *Moringa oleifera*: Bioactive compounds and nutritional potential. *Revista de Nutrição* 2008; **21**, 431-7.
- [11] L Manh, N Dung and T Ngoi. Introduction and evaluation of *Moringa oleifera* for biomass production and as feed for goats in the Mekong Delta. *Livestock Research for Rural Development* 17, Available at: <http://www.lrrd.org/lrrd17/9/manh17104.htm>, accessed August 2014.
- [12] SH Ben and HPS Makkar. Defatted *Moringa oleifera* seed meal as a feed additive for sheep. *Anim Feed Sci Tech.* 2009; **150**, 27-33.
- [13] A Elangovan and KF Shim. The influence of replacing fish meal partially in the diet with soybean meal on growth and body composition of juvenile tin foil barb (*Barbodes altus*). *Aquaculture* 2000; **189**, 133-44.
- [14] FE Dierberg and W Kiattisimkul. Issues, impacts, and implications of shrimp aquaculture in Thailand. *Environ. Manag.* 1996; **20**, 649-66.
- [15] S Sirimongkolvorakul, W Jiraungkoorskul, T Tansatit, N Preyavichyapugdee, P Kosai and K Uakulwarawat. Influence of *Moringa oleifera* on histopathological changes due to lead-toxicity in red-tail tinfoil barb, *Puntius altus*. *Fresenius Environ. Bull.* 2013; **22**, 1946-50.
- [16] GT Gurr. *Biological Staining Method*. 7th ed. George T. Gurr Ltd., London, 1963, p. 1-166.
- [17] UK Laemmli. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature* 1970; **227**, 680-5.

- [18] AGJ Tacon. Feed ingredients for warm water fish: Fish meal and other processed feedstuffs. FAO Fisheries Circular No.856. FAO, Rome, 1993, p. 1-64.
- [19] B Ruyter, C Moya-Falcón, G Rosenlund and A Vegusdal. Fat content and morphology of liver and intestine of Atlantic salmon (*Salmo salar*): Effects of temperature and dietary soybean oil. *Aquaculture* 2006; **252**, 441-52.
- [20] RE Burke, DN Levine, FE Zajac, P Tsairis and WK Engel. Mammalian motor units: physiological-histochemical correlation in three types in cat gastrocnemius. *Science* 1971; **174**, 709-12.
- [21] J Lannergreen and JFK Hoh. Myosin isoenzymes in single muscle fibres of *Xenopus laevis*: Analysis of five different functional types. *Proc. Biol. Sci.* 1984; **222**, 401-8.
- [22] N Chayen, A Freundlich and JM Squire. Comparative histochemistry of a flatfish fin muscle and of other vertebrate muscles used for ultrastructural studies. *J. Muscle Res. Cell Motil.* 1987; **4**, 358-71.
- [23] Q Bone. On the function of the two types of myotomal muscle fibres in elasmobranch fish. *J. Mar. Biol. Assoc. UK* 1966; **46**, 321-49.
- [24] MT Thebaulta, L Izemb, JP Leroyc, E Gobinc, G Charriera and GP Raffin. AMP-deaminase in elasmobranch fish: A comparative histochemical and enzymatic study. *Comp. Biochem. Physiol. B* 2005; **141**, 472-9.
- [25] AH Weatherley, HS Gill and AF Lobo. Recruitment and maximal diameter of axial muscle fibres in teleost and their relationship to somatic growth and ultimate size. *J. Fish Biol.* 1988; **33**, 851-9.
- [26] AH Weatherley and HS Gill. *The biology of Fish Growth*. Academic Press, London, 1987, p. 1-443.
- [27] IM Herman. Actin isoforms. *Curr. Opi. Cell Biol.* 1993; **5**, 48-55.