Pakistan J. Zool., pp 1-7, 2023. DOI: https://dx.doi.org/10.17582/journal.pjz/20230413200442

Effect of *Moringa oleifera* Leaf Meal on Mineral Contents of *Labeo rohita* Fingerlings

Muhammad Zubair-ul-Hassan Arsalan[1], Syed Makhdoom Hussain[2]*, Basharat Ali[3], Bilal Ahmad[2], Muhammad Qurban[1], Adan Naeem[2], Eman Naeem[2] and Tariq Rasheed[1]

[1]*Department of Life Sciences, Khawaja Fareed University of Engineering and Information Technology, Rahim Yar Khan, Pakistan*
[2]*Fish Nutrition Lab, Department of Zoology, Government College University, Faisalabad, Pakistan*
[3]*Department of Agriculture, Khawaja Fareed University of Engineering and Information Technology, Rahim Yar Khan, Pakistan*

Article Information
Received 13 April 2023
Revised 05 September 2023
Accepted 20 September 2023
Available online 07 December 2023 (early access)

Authors' Contribution
SMH: Conceptualization, funding acquisition, resources, supervision. MZHA: Writing original draft. BA and BA: Software, writing - review and editing. MQ: Data curation, writing review and editing. AN, EN and TR: writing review and editing.

Key words
***Labeo rohita, Moringa oleifera* leaf meal, Replacement, Fish meal, Mineral digestibility, Nutrients**

ABSTRACT

Fish meal, a highly digestible source of protein in aqua feed, has not been readily available to fulfill the need for sustainable aquaculture production due to its limited supply and high price. So, the aim of this study was to develop more sustainable and economically viable aquaculture production by substituting fish meal with *Moringa oleifera* leaf meal (MOLM). In this research, the mineral digestibility of *Labeo rohita* fingerlings (6.57±0.03 g) was investigated over 70 days by formulating five iso-nitrogenous diets to substitute MOLM with 0, 10, 20, 30 and 40% of fish meal. There was a random distribution of 15 fingerlings into replica tanks. Fingerlings were fed (5% body weight) twice daily. The results demonstrated that mineral digestibility was enhanced when fish meal was replaced with MOLM up to 10% (Ca, 65%; Na, 63%; K, 71%; Fe, 67%; Cu, 69%; Zn, 67%; Mn, 61%; P, 70% and Mg, 66%) compared to fingerlings fed on other replacement levels and control (Ca, 55%; Na, 52%; K, 55%; Fe, 70%; Cu, 63%; Zn, 60%; Mn, 59%; P, 64% and Mg, 64%). Mineral digestibility in *L. rohita* fingerlings was greatly improved by feeding a diet consisting of 10% MOLM level followed by 20% level. Therefore, the results of this research indicated that MOLM may successfully substitute up to 10% of the fish meal in the diet of *L. rohita* without any adverse effects. On the basis of this study, it was concluded that 10% MOLM when used as an alternative to fish meal, enhances the mineral retention in *L. rohita* fingerlings.

INTRODUCTION

Food production through aquaculture, which has been used for centuries, is currently undergoing rapid growth (Eroldoğan *et al*., 2022). The global aquaculture production has reached 122.6 million tonnes out of which, 87.5 million tonnes are aquatic fauna (FAO, 2022). One of the major Indian carp farmed extensively in Pakistan is rohu (*Labeo rohita*). This species is widely cultivated under polyculture system with other members of carp family (Cyprinidae) (Hussain *et al*., 2011).

One of the exceptional protein source in aqua feed is known as fish meal (FM) which is commonly utilized in this industry owing to its excellent digestibility, palatability and nutritionally balanced quality (Olsen and Hasan, 2012). So, FM is necessary for the aqua feed formulation. However, the demand and price of this meal has increased gradually as the aquaculture increased (Ahmad *et al*., 2021). These drawbacks compel the researchers to find the substitute for FM to meet the demand as well as to form cost effective feed for aquaculture (Caruso, 2015).

The alternatives such as plants and animal's by-products, has been widely used in commercial aquaculture for partial replacement (Naylor *et al*., 2000; Nishshanka *et al*., 2022). One of the promising alternatives for the fish meal are the agriculture based by-products (Hussain *et al*., 2011) such as soybean meal (Ye *et al*., 2011), canola meal (Luo *et al*., 2012), cottonseed meal (Hassaan *et al*., 2019) and sunflower meal (Iqbal *et al*., 2022). Now, the researchers are looking for the unconventional sources of plant by-products i.e., seeds, leaves and other high quality protein products, to obtain eco-friendly as well as viable

* Corresponding author: drmakhdoomhussain@gcuf.edu.pk
0030-9923/2023/0001-0001 $ 9.00/0

commercial production (Abo-State *et al*., 2014). These plant-based substitutes need to be higher in protein and nutrients and lower in fibres, anti-nutritional elements, and carbohydrates. These substitutes include, corn, barley, canola and peas. The replacement of these products also depends upon their costs, palatability and accessibility for the aquaculture species (Naylor *et al*., 2009). Thus, it has been established that one of the unconventional plant-based by-products, *Moringa oleifera* leaf meal (MOLM), is the best option to replace FM.

M. oleifera's leaves, fruits, roots and flowers are used as vegetables in many countries (Siddhuraju and Becker, 2003) because they contain vitamins, phenolic acid, calcium (Ca), iron (Fe), beta-carotene and riboflavin (Shank *et al*., 2013). However, Fahey (2005) stated that Moringa leaves possess quality proteins compared to milk and egg. It contains more Ca content than milk, high K level compared with bananas, high Fe in contrast to spinach, more vitamin C than oranges and more vitamin A than that of carrots (Rag Tag, 2013). The fiber helps the body to remove some poisonous compounds and eventually stops concentration of excess cholesterol (Sodamade *et al*., 2013). The mineral makeup of MOLM is 2098 mg of Ca, 406 mg of magnesium (Mg), 1922 mg of potassium (K), 28.3 mg of Fe, 5.4 mg of zinc (Zn), and 351.1 mg of phosphorus (P) per 100 g of dry extracted leaves (Charles *et al*., 2011).

Our study's goal is to develop a more sustainable and economically viable aquaculture feed by using different levels of MOLM based diets in *L. rohita* fingerling's diet for improving mineral availability.

MATERIALS AND METHODS

This investigation was conducted to determine how different concentrations of MOLM based diets affected the mineral digestibility of *L. rohita* fingerlings. This research was done at Government College University, in Fish Nutrition Laboratory, Department of Zoology, Faisalabad. This particular area may be found at a longitude of 31.4166 degrees North and 73.0707 degrees East.

Fish and feeding trial setup

From the Government Fish Seed Hatchery, fingerlings were purchased and kept in 70 L of V-shaped water tanks. Before the trial began, they acclimatized for 15 days and soaked one to two minutes in 0.5% saline water for the prevention of pathogenic infections (Rowland and Ingram, 1991). During the trial, water quality parameters (pH, 7.5-8.5; temperature, 25-28°C; dissolve oxygen, 5.5-7.5 mg/L) and aeration were checked daily.

Preparing M. oleifera *leaf meal*

M. oleifera leaves were soaked for the removal of anti-nutritional factors and completely dried to prevent from vitamin discharge. To lower the amount of crude fiber in the feed, the leaves were threshed from the stalks. Then the leaves which were dried, crushed into powder and stored in polythene bags at room temperature.

Feeding schedule and sampling methodology

The feed components were crushed and sieved to the appropriate size (3 mm) before the feeding experiment began. These feed components were obtained from a nearby feed mill (Table I) and analyzed for chemical composition according to standard protocols (AOAC, 1995). Chromic oxide was added to the diet to obtain the digestibility. The homogeneous dough was prepared by adding water content (10-15%) and oil (5%) to the ingredients. The dough was pelletized using a lab extruder. Five iso-nitrogenous diets were prepared, with MOLM levels ranging from 0% to 40%. Fingerlings were fed 5% of their body weight two times a day. They were randomly divided into tanks with 15 fingerlings in each replication tank. Feeding trial was conducted for a total of 70 days and 5 g of feces were collected from each replicate. A system of V-shaped tanks was designed for the purpose of collecting feces. Just after a two-hour feeding session, the remaining feed was taken out of each tank. Each tank's valve was opened at two-hour interval, and the fecal material was emptied from the fecal collecting tube. Nutrient loss could be reduced by carefully collecting the fecal threads. The fecal samples were dried for further chemical analysis.

Table I. Ingredients composition (%) of test diets (TDs).

Ingredients	TD-I *MOLM (0%) (control)	TD-II MOLM (10%)	TD-III MOLM (20%)	TD-IV MOLM (30%)	TD-V MOLM (40%)
Fish meal	40	34	28	22	16
Wheat flour	34.50	30.50	26.50	22.50	18.50
Corn gluten (60%)	16.6	16.6	16.6	16.6	16.6
Fish oil	4.9	4.9	4.9	4.9	4.9
Vitamin premix	1.0	1.0	1.0	1.0	1.0
Mineral premix	1.0	1.0	1.0	1.0	1.0
Ascorbic acid	1.0	1.0	1.0	1.0	1.0
Chromic oxide	1.0	1.0	1.0	1.0	1.0
Total	100	100	100	100	100

MOLM*, *Moringa oleifera* leaf meal.

Table II. Mineral composition of MOLM based diets.

Minerals	TD-I (Control) MOLM (0%)	TD-II MOLM (10%)	TD-III MOLM (20%)	TD-IV MOLM (30%)	TD-V MOLM (40%)	PSE	p-Value
Ca	3.11	3.11	3.12	3.11	3.11	0.019	0.9969 ns
Na	0.21	0.21	0.207	0.21	0.21	0.015	0.9966 ns
K	0.25	0.24	0.25	0.24	0.24	0.018	0.9599ns
Fe	0.012	0.012	0.012	0.012	0.012	0.00026	0.9599ns
Cu	0.011	0.011	0.011	0.011	0.0109	0.00017	0.8542 ns
Zn	0.028	0.028	0.029	0.029	0.0290	0.00021	0.6185ns
Mn	0.012	0.012	0.012	0.012	0.012	0.00019	0.9966ns
P	0.57	0.57	0.57	0.57	0.57	0.0024	0.9914ns
Mg	0.0082	0.0081	0.0081	0.0081	0.0080	0.00015	0.9576ns
Al	0.022	0.021	0.021	0.023	0.021	0.0000033	0.9895ns
Cr	0.022	0.021	0.021	0.023	0.021	0.00207	0.9634ns

MOLM, *Moringa oleifera* leaf meal; Ca, calcium; Na, sodium; K, potassium; Fe, iron; Cu, copper; Zn, zinc; Mn, manganese; P, phosphorus; Mg, magnesium; Al, aluminium and Cr, chromium; PSE, pooled standard error. PSE, √MSE / n (where MSE = mean squared error). Mean within rows having different superscripts are significantly different at $p<0.05$. Data are means of three replicates (n=3).

Table III. Mineral content of feces of *L. rohita* fed MOLM based diets.

Minerals	TD-I (Control) MOLM (0%)	TD-II MOLM (10%)	TD-III MOLM (20%)	TD-IV MOLM (30%)	TD-V MOLM (40%)	PSE	p-Value
Ca	1.45^{c}	1.18^{d}	1.38^{c}	1.63^{b}	1.90^{a}	0.035	0.0000***
Na	0.11^{ab}	0.08^{b}	0.09^{ab}	0.12^{ab}	0.13^{a}	0.0086	0.0138 *
K	0.12^{ab}	0.08^{c}	0.10^{bc}	0.11^{abc}	0.15^{a}	0.00019	0.0012**
Fe	0.0030^{c}	0.0040^{c}	0.0050^{c}	0.0050^{b}	0.0070^{a}	0.000000077	0.0000***
Cu	0.0040^{bc}	0.0030^{c}	0.0040^{b}	0.0050^{a}	0.0060^{a}	0.00014	0.0000***
Zn	0.010^{b}	0.010^{c}	0.0090^{c}	0.010^{b}	0.010^{a}	0.00034	0.0000***
Mn	0.0060^{a}	0.0050^{d}	0.0050^{cd}	0.0060^{b}	0.0070^{a}	0.00014	0.0000***
P	0.27^{c}	0.18^{d}	0.25^{c}	0.31^{b}	0.37^{a}	0.0078	0.0000***
Mg	0.0030^{c}	0.0020^{cd}	0.0020^{d}	0.0030^{b}	0.0040^{a}	0.000086	0.0000***
Al	0.00010^{bc}	0.000090^{c}	0.00010^{b}	0.00010^{a}	0.00010^{a}	0.0000029	0.0000***
Cr	0.010^{a}	0.010^{a}	0.0090^{a}	0.010^{a}	0.010^{a}	0.00098	0.1758ns

For abbreviations and statistical details, see Table II.

Minerals analysis of feces and feed

The digestion of the feed and feces samples was done by boiling nitric acid and perchloric acid (2:1) (AOAC, 1995). Atomic absorption spectrophotometer was used to assess mineral content after adequate dilutions. Calorimetric analysis of phosphorus at 350nm (UV/VIS spectrophotometer) was performed. Chromic oxide was added to the diet to obtain the digestibility of feed and feces. It was determined at the absorbance of 370 nm following oxidation through perchloric acid via Spectrophotometer (Divakaran *et al*., 2002).

Statistical analysis

The mineral content of the experimental diets was evaluated using One-way Analysis of Variance (Steel *et al*., 1996). Tukey's Honestly Significant Difference Test was used to compare means, and differences were significant when $p<0.05$ (Snedecor and Cochran, 1991). These analyses were performed using Co-Stat software (Version 6.303, CA, 93940 USA, Monterey, PMB 320).

RESULTS

Tables II and III show the analyzed mineral composition (%) of MOLM based diets and feces, respectively. The results of analyzed composition of minerals in *L. rohita* fed MOLM based diets are presented in Table IV. Digestibility of minerals such as Ca, Mg, Na, P, K, Mn, Cu, Zn, Cr and Al showed that different levels

Table IV. Mineral content of muscle of *L. rohita* fed MOLM based diets.

Minerals	TD-I (Control) MOLM (0%)	TD-II MOLM (10%)	TD-III MOLM (20%)	TD-IV MOLM (30%)	TD-V OLM (40%)	PSE
Ca	55.82±0.82[c]	65.12±0.63[a]	60.28±0.40[b]	53.88±0.34[c]	46.23±0.95[d]	0.38
Na	52.51±0.70[c]	63.56±0.65[a]	59.00±0.92[b]	50.55±0.90[c]	44.69±0.45[d]	0.43
K	55.95±0.65[d]	71.76±0.60[a]	66.46±0.87[b]	59.78±0.64[c]	47.46±0.83[e]	0.42
Fe	70.53±0.91[a]	67.14±0.96[b]	62,85±0.91[c]	59.70±0.81[d]	51.42±0.91[e]	0.52
Cu	63.15±0.92[b]	69.85±0.29[a]	60.71±0.89[c]	55.90±0.43[d]	51.46±0.07[e]	0.35
Zn	60.47±0.97[b]	67.96±0.80[a]	69.30±0.75[a]	59.41±0.87[b]	44.67±0.47[c]	0.45
Mn	59.97±0.54[c]	61.87±0.84[a]	58.92±0.99[b]	50.14±0.32[d]	43.73±0.85[e]	0.43
P	54.65±0.81[c]	70.08±0.66[a]	60.36±0.48[b]	51.74±0.84[d]	43.13±0.74[e]	0.41
Mg	64.66±0.43[b]	66..41±0.98[b]	71.68±0.66[a]	58.97±0.34[c]	48.04±0.72[d]	0.38
Al	48.42±0.99[b]	56.51±0.50[a]	49.72±0.80[b]	40.60±0.99[c]	36.64±0.82[d]	0.48
Cr	52.15±0.54[c]	57.55±0.57[b]	62.72±0.87[a]	55.89±0.96[b]	46.41±0.88[d]	0.45

For abbreviations and statistical details, see Table II.

of MOLM based diets were non-significant in *L. rohita* fingerlings. Analyzed mineral composition of diets, feces and digestibility showed that minimum amount of minerals such as K (0.08%), Ca (1.18%), Mg (0.002%), P (0.18%), Na (0.08%), Cu (0.003%), Mn (0.005%), Al (0.00009%), Cr (0.01%) and Zn (0.01%) were excreted through feces at a 10% level followed by 20% level of MOLM replacement in comparison with control diet and remaining levels of MOLM. Digestibility coefficient values showed that test diet-II (10% level of MOLM based diet) proved best for fingerlings when compared to control and other replacement levels of MOLM based diets. The digestibility values of Na, Cu, Ca, Zn, Mn, P, K and Al were 63.56%, 69.85%, 65.12%, 67.96%, 61.87%, 70.08%, 71.76% and 56.51%, respectively, at 10% MOLM level which were substantially significant ($p<0.05$) when compared to other levels of MOLM based diets and control diet. However, Mg (71.68%) and Cr (62.72%) showed best digestibility values at 20% MOLM based test diet as compared to control and other reference diets and are substantially different ($p<0.05$) from all other experimental diets. The Fe digestibility (70.53%) recorded at control level was higher than other levels of MOLM based test diets. According to the findings, best mineral digestibility values were obtained at 10% MOLM replacement level.

DISCUSSION

The main challenges aquaculture is facing these days is to provide feed that have nutritionally balanced ingredients. One of the most promising sources of protein for various aqua-feeds is *M. oleifera* (Hussain *et al*., 2017). MOLM is regarded as a unique substitute for fish meal because of its nutritious amino acid profile (tryptophan, methionine, cysteine and lysine) and its availability around the globe (Tagwireyi *et al*., 2018). Moreover, it has remarkable mineral content i.e., calcium, potassium, magnesium, zinc, iron and phosphorus (Kou *et al*., 2018).

In our study, mineral composition of all MOLM based test diets were equal. According to our results, 10% MOLM based diet showed less minerals discharge than control and other levels of MOLM based test diet whereas the maximum mineral excretion was noted in 40% MOLM based level which clearly showed that 10% MOLM based level is better substitute for fish meal. Different researchers partially or entirely replace fish meal with various plants and their by-products (Daniel, 2018) such as soybean meal (Ye *et al*., 2011), canola meal (Luo *et al*., 2012), cotton seed meal (Hassaan *et al*., 2019) and sunflower meal (Iqbal *et al*., 2022). However, the variable results have been observed with different sources of plants as a substitute.

In our study, maximum mineral digestibility was also recorded in 10% MOLM based diet than all other test diets and control. Digestibility data of minerals like Ca, Mg, Na, P, K, Mn, Cu, Zn, Cr and Al showed significant differences ($p<0.05$) when rohu fingerlings fed different levels of MOLM based diets. MOLM contains tannins, saponins, and lignins, which are anti-nutritional components. High concentration of these anti-nutrients might negatively impact the assimilation of trace elements and delay the digestion of proteins where as their low concentration is utilized as energy source. Our study is in line with Adeshina *et al*. (2018), in which 30% of MOLM was proved to be the option to replace soybean meal in *Cyprinus carpio*. Digestibility data showed that diet-II which was the 10% level of MOLM based test diet, proved best option and showed maximum apparent digestibility coefficient values compared to other replacement levels of MOLM based test diets and control diet. Nowadays, feed additives have been utilized and are being used with MOLM by various researchers to enhance the efficiency of plant by-products (MOLM) and inhibit their negative effects. According to Sarfraz *et al*. (2023), 35% MOLM supplemented with phytase (600 FTU kg^{-1}) improved the mineral digestibility of *Oreochromis niloticus.* Variations in outcomes are a result of different species, age, feeding habitats and environmental conditions.

CONCLUSION

It was deduced that 10% MOLM level is ideal replacement level that improves the mineral digestibility in *L. rohita* fingerlings. It was also concluded that MOLM based plant by product's addition by replacing the fish meal is very beneficial in development of affordable and environment friendly aqua-feed.

ACKNOWLEDGEMENT

The authors are thankful to Higher Education Commission, Pakistan for funding Department of Zoology, Government College University, Faisalabad for provision of facilities for this research.

Funding

The authors are grateful to HEC Islamabad, Pakistan for its consistent assistance by providing Project # 5649/ Punjab/NRPU/RandD/HEC/2016 and Project # 20-4892/ NRPU/R and D/HEC/14/1145 to be able to manage this research work.

IRB approval

All applicable institutional, national and international guidelines for the care and use of animals were followed.

Ethical statement

All the procedures and methods used in this study followed the ethical guidelines provided by Government College University Faisalabad.

Statement of conflict of interest

The authors have declared no conflict of interest.

REFERENCES

Abo-State, H., Hammouda, Y., El-Nadi, A. and AboZaid, H., 2014. Evaluation of feeding raw moringa (*Moringa oleifera* Lam.) leaves meal in Nile tilapia fingerlings (*Oreochromis niloticus*) diets. *Glob. Vet.,* **13:** 105-111.

Adeshina, I., Sani, R.A., Adewale, Y.A., Tiamiyu, L.O. and Umma, S.B., 2018. Effects of dietary *Moringa oleifera* leaf meal as a replacement for soybean meal on growth, body composition and health status in *Cyprinus carpio* juveniles. *Croatian J. Fish*., **76:** 174-182. https://doi.org/10.2478/cjf-2018-0021

Ahmad, B., Hussain, S.M., Ali, S., Arsalan, M., Tabassum, S. and Sharif, A., 2021. Efficacy of acidified phytase supplemented cottonseed meal based diets on growth performance and proximate composition of *Labeo rohita* fingerlings. *Braz. J. Biol.,* **83:** e247791. https://doi.org/10.1590/1519-6984.247791

Allan, G.L. and Rowland, S.J., 1992. Development of an experimental diet for silver perch *(Bidynus bidyanus)*. *Austasia Aquac.,* **6:** 39-40.

AOAC (Association of Official Analytical Chemists), 1995. *Official methods of analysis. 15th Ed*. Association of Official Analytical Chemists, Washington, D.C. USA., pp. 1094.

Caruso, G., 2015. Use of plant products as candidate fish meal substitutes: An emerging issue in aquaculture productions. *Fish. Aquacult. J.,* **6:** e123.

Charles, W.Y., Marce, D.B., Aly, S., Philippe, A.N. and Sabadenedyo, A.T., 2011. Determination of chemical composition and nutritional values of *Moringa oleifera* leaves. *Pak. J. Nutr.,* **10:** 264-268. https://doi.org/10.3923/pjn.2011.264.268

Daniel, N., 2018. A review on replacing fish meal in aqua feeds using plant protein sources. *Int. J. Fish. aquat. Stud.,* **6:** 164-179.

Divakaran, S., Leonard, G.O. and Lan, P.F., 2002. Note on the methods for determination of chromic oxide in shrimp feeds. *J. Agric. Fd. Chem.,* **50:** 464-467. https://doi.org/10.1021/jf011112s

Eroldoğan, O.T., Glencross, B., Novoveska, L., Gaudêncio, S.P., Rinkevich, B., Varese, G.C. and Rotter, A., 2022. From the sea to aquafeed: A perspective overview. *Rev. Aquac*. **2022:** 1-30.

Fahey, J.W., 2005. *Moringa oleifera*: A review of the medical evidence for its nutritional, therapeutic, and prophylactic properties. Part 1. *Trees Life J.,* **1:** 1-15.

FAO, 2022. *The world fisheries and aquaculture towards blue transformation*. Food and Agriculture Organization of the United Nations, Rome.

Hassaan, M.S., El-Sayed, A.I.M., Soltan, M.A., Iraqi, M.M., Goda, A.M., Davies, S.J. and Ramadan, H.A., 2019. Partial dietary fish meal replacement with cotton seed meal and supplementation with exogenous protease alters growth, feed performance, hematological indices and associated gene expression markers (GH, IGF-I) for Nile tilapia, *Oreochromis niloticus*. *Aquaculture,* **503:** 282-292. https://doi.org/10.1016/j.aquaculture.2019.01.009

Hussain, M., Hussain, S.M., Iqbal, R., Javid, A., Shahzad, M.M., Arsalan, M.Z. and Riaz, D., 2017. Effect of citric acid acidified *Moringa oleifera* seed meal-based diet on nutrients digestibility and growth performance of *Cirrhinus mrigala* fingerlings. *Int. J. Agric. Biol.,* **19:** 719-725. https://doi.org/10.17957/IJAB/15.0346

Hussain, S.M., Javed, M., Javid, A., Javid, T. and Hussain, N., 2011. Growth responses of *Catla catla, Labeo rohita* and *Cirrhina mrigala* during chronic exposure of iron. *Pak. J. agric. Sci.,* **48:** 225-230.

Iqbal, M., Yaqub, A. and Ayub, M., 2022. Effects of partial and full dietary substitution of fish meal and soybean meal by sunflower meal on growth performance, feed consumption, body indices, serum chemistry and intestine morphology of *Oreochromis niloticus*. *Turk. J. Fish. aquat. Sci.,* **22:** TRJFAS20784. https://doi.org/10.4194/TRJFAS20784

Kou, X., Li, B., Olayanju, J.B., Drake, J.M. and Chen, N., 2018. Nutraceutical or pharmacological potential of *Moringa oleifera Lam. Nutrient,* **10:** 343. https://doi.org/10.3390/nu10030343

Lovell, R.T., 1989. *Nutrition and feeding of fish*. Van Nostrand-Reinhold, New York, pp. 260. https://doi.org/10.1007/978-1-4757-1174-5

Luo, Z., Liu, C.X. and Wen, H., 2012. Effect of dietary fish meal replacement by canola meal on growth performance and hepatic intermediary metabolism of genetically improved farmed tilapia strain of Nile tilapia, *Oreochromis niloticus*, reared in fresh water. *J. World Aquacult. Soc.,* **43:** 670-678. https://doi.org/10.1111/j.1749-7345.2012.00601.x

Naylor, R.L., Goldburg, R.J., Primavera, J.H., Kautsky, N., Beveridge, M.C.M., Clay, J., Folke, C., Lubchenco, J., Mooney, H. and Troell, M., 2000. Effect of aquaculture on world fish supplies. *Nature,* **405:** 1017-1024. https://doi.org/10.1038/35016500

Naylor, R.L., Hardy, R.W., Bureau, D.P., Chiu, A., Elliott, M., Farrell, A.P., Forster, A.P., Gatlin, I., Goldburg, D.M., Hua, R.J. and Nichols, P.D., 2009. Feeding aquaculture in an era of finite resources. *Proc. natl. Acad. Sci.,* **106:** 15103-15110. https://doi.org/10.1073/pnas.0905235106

Nishshanka, K.M., Radampola, K. and Bulugahapitiya, V., 2022. Effects of partial replacement of dietary fishmeal using plant-protein sources on the growth performance, coloration and liver histology of guppy fry (*Poecilia reticulata*) in outdoor farming conditions. *J. appl. Aquacult.,* **34:** 715-733. https://doi.org/10.1080/10454438.2021.1886216

Olsen, R.L. and Hasan, M.R., 2012. A limited supply of fishmeal: Impact on future increases in global aquaculture production. *Trends Fd. Sci. Technol.,* **27:** 120-128. https://doi.org/10.1016/j.tifs.2012.06.003

Rag Tag Research Geeks, 2013. *The moringa tree: A nutritional and medicinal powerhouse*. http://ragtagresearchgeeks.com/themoringatreeanutritional-andmedicinalpowerhouse/

Rowland, S.J. and Ingram, B.A., 1991. Diseases of Australian native freshwater fishes, with particular emphasis on the ectoparasitic and fungal diseases of Murray cod (*Maccullochella peeli*), golden perch (*Macquaria ambigua*) and silver perch (*Bidyanus bidyanus*). *Fish. Bull.,* 4, NSW Agriculture and Fisheries, Sydney.

Sarfraz, Q., Hussain, S.M., Sharif, A., Selamoglu, Z., Bashir, F. and Ahsan, S., 2023. Potential of phytase supplemented *Moringa oleifera* leaf meal based diet on mineral digestibility of *Oreochromis niloticus* fingerlings. *J. Sur. Fish. Sci*., pp. 65-77. https://doi.org/10.18331/SFS2020.6.2.8

Shank, L.P., Riyathong, T., Lee, V.S. and Dheeranupattana, S., 2013. Peroxidase activity in native and callus culture of *Moringa oleifera* Lam. *J. med. Biol. Eng.,* **2:** 163-167. https://doi.org/10.12720/jomb.2.3.163-167

Siddhuraju, P. and Becker, K., 2003. Antioxidant properties of various solvent extracts of total phenolic constituents from three different agroclimatic origins of drumstick tree (*Moringa oleifera* Lam.) leaves. *J. Agric. Fd. Chem.* **51:** 2144-2155. https://doi.org/10.1021/jf020444+

Snedecor, G.W. and Cochran, W.G., 1991. *Statistical methods, 8th Ed.* Iowa State University Press, Americans. USA, pp. 503.

Sodamade, A., Bolaji, O.S. and Adeboye, O.O., 2013. Proximate analysis, mineral contents and functional properties of *Moringa oleifera* leaf protein concentrate. *IOSR J. appl. Chem.,* **4:** 47-51. https://doi.org/10.9790/5736-0464751

Steel, R.G.D., Torrie, J.H. and Dickey, D.A., 1996. *Principles and procedures of statistics (3rd ed.).*

McGraw Hill International Book Co. Inc., New York. USA, pp. 336-352.

Tagwireyi, T., Mupangwa, J.F., Jepsen, J. and Mwera, P., 2018. The effect of feeding heat treated *Moringa oleifera* (lam) leaf meal on the growth performance of *Oreochromis niloticus* (Lam) fry. *S. Afr. J. Educ. Sci. Technol.,* **2:** 50-56. https://doi.org/10.4314/sajest.v2i2.39816

Ye, J., Liu, X., Wang, Z. and Wang, K., 2011. Effect of partial fish meal replacement by soybean meal on the growth performance and biochemical indices of juvenile Japanese flounder *Paralichthys olivaceus*. *Aquacul. Int.,* **19:** 143-153. https://doi.org/10.1007/s10499-010-9348-1