Pakistan J. Zool., vol. 52(5), pp 1745-1750, 2020 DOI: https://dx.doi.org/10.17582/journal.pjz/20190825090821

Effect of Substituting Soybean Meal with *Moringa oleifera* Meal on the Growth and Body Composition of *Labeo rohita* Fingerlings

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Article Information
Received 15 August 2019
Revised 12 September 2019
Accepted 25 September 2019
Available online 13 May 2020

Authors' Contribution
DM and NK planned the research. KJI, NK, AA, SAMM and CJS wrote the manuscript. SD, AH, SN and SB performed lab work. NK and KJI analysed the data statistically.

Key words
***Labeo rohita*, Growth parameters, Body composition, *Moringa oleifera*, Vital organs histology**

ABSTRACT

The present study was conducted to examine the effect of replacing soybean meal in fish diets with the cheaper alternative plant protein moringa meal (*Moringa oleifera)* on growth and body composition of *Labeo rohita* fingerlings. *L. rohita* (average weight 190.25±00g) were stocked randomly in glass aquaria for a 90 day feeding trial. Fish were fed twice daily with four different iso-nitrogenous diets at a feeding level of 3% of total biomass. The diets contained 26% crude protein in which moringa meal substituted soybean meal at 0% (control), 10%, 20% and 30%, with the groups fed these diets designated as T_0, T_1, T_2 and T_3, respectively. The highest weight gain (254.00±4.24 g) was recorded in T_3 and lowest in T_0 (80.97±17.80 g). Significant differences ($P < 0.01$) were observed in the feed conversion ratio (FCR) between T_3 (1.81±0.06) and T_1 (2.70±0.13) and specific growth rate (SGR) of T_3 (0.42±00) and T_1 (0.25±0.01). These findings indicated that *M. oleifera* meal has good potential to substitute soybean meal as a relatively cheap and good quality plant protein source without any harmful effects on fish body composition.

INTRODUCTION

Fish is an excellent source of vitamins, essential minerals, high quality protein and unsaturated fatty acid (Petricorena, 2014). The demand for fish production via aquaculture is increasing to fulfill the protein requirement in human diets across the globe. The principal input in fish production is the feed, with high prices and limited supply of fish meal, major constraints in the aqua feed industry (Gabriel *et al*., 2007). Therefore, fish culturists are currently focusing on replacement of high cost animal ingredients with natural high quality plant proteins for fish feed formulation to reduce the cost (Hashem *et al*., 2017). Plant based protein source for fish feed, especially of soybean meal (SBM), have been extensively used in aquaculture production (Storebakken *et al*., 2000; Barros *et al*., 2002). However, excess reliance on SBM may increase its price. Therefore, exploitation and consumption of other low-cost plant based protein sources for fish feed will help reduce the feed cost and with the added benefit that there is evidence that diversifying the source of plant proteins may be beneficial to the fish (Hashem *et al*., 2017).

Plants contain many bioactive compounds that are bio-degradable, ecofriendly and easily available, and can have antioxidant, antiviral and antibacterial properties (Citarasu, 2010). When used in fish nutrition these compounds can activate immune responses, enhance the secretion of digestive enzymes and stimulate the feed intake (Citarasu, 2010). In the aqua feed industry, many plants and plant products are used as functional feeds for boosting immunity, preventing infections, promoting growth, reducing stress and stimulating appetite for producing healthy fishes (Shalaby, 2004).

Many plants have been investigated for their natural potential to replace fish meal in aqua feed preparation. Amongst these *Moringa oleifera*, belonging to the family Moringaceae, is a rapidly growing plant found in tropical and subtropical areas. The leaves of *M. oleifera* are a rich source of proteins, vitamins and minerals. Hence *M. oleifera* is commonly known around the globe as "The Miracle Tree" or "Ben oil tree" due to its high nutritional value and medicinal uses (Luqman *et al*., 2012). Indeed, it

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0030-9923/2020/0005-1745 $ 9.00/0

is used for the treatment of many human health conditions including malnutrition and cardiovascular disease, and possessed diuretic properties, is hepatoprotective, has antiulcer effects and lowers cholesterol levels (Luqman *et al*., 2012).

The *M. oleifera* leaves contain a high proportion of crude protein, varying from 25% to 32% (Makkar and Becker, 1996; Soliva *et al*., 2005). This protein is easily digestible due to the presence of a low content of acid detergent insoluble protein (1 to 2%) and high content of pepsin soluble protein (82 to 91%) (Makkar and Becker, 1996). The leaves of *M. oleifera* have been reported previously to be an excellent fish meal substitute in the diet of north African catfish *Clarias gariepinus* and roho labeo *Labeo rohita* fingerlings up to a 10% inclusion level (Ozovehe *et al*., 2013; Ezekiel *et al*., 2016; Arsalan *et al*., 2016; Mehdi *et al*., 2016). Furthermore, seed meal of M. *oleifera* has also been explored as a good alternative plant based protein feed in Nile tilapia (*Oreochromis niloticus*) fingerlings (Hashem *et al*., 2017). With this in mind the present study was designed to evaluate the potential of *M. oleifera* meal to replace soybean meal in diets for *L. rohita* and also to analyze its effects on growth, body composition, liver and gut health of *L. rohita* fingerlings.

MATERIALS AND METHODS

Study site, experimental fish and procedure

The experiment was conducted at Fish Seed Hatchery of the Department of Fisheries and Aquaculture, University of Veterinary and Animal Sciences (UVAS), Ravi Campus, Pattoki. The fish *L. rohita* (average weight 190.25±00g) were collected from fish ponds at UVAS Ravi Campus Pattoki, Punjab, Pakistan and stocked randomly in 8 aquaria (60.96 × 55.88 × 40.64 cm) at15 fish/aquarium. Before stocking fish net body weight and length were measured and recorded. The feeding trial lasted for 90 days.

Collection of Moringa oleifera *and processing*

M. oleifera leaves were collected from Bahawalpur and washed with freshwater to remove all contaminants, properly drained and dried under shade for 1 week. Thereafter, dried leaves were crushed into a fine powder with an electric grinder and stored in opaque, air tight plastic zipper packets.

Fish feed formulation

Four types of experimental feeds containing 26% crude protein were formulated (Table I), with differing inclusion levels of *M. oleifera* meal, with other nutrients from soybean meal, fish meal, rice polish, wheat bran, sunflower meal and nutrimix. The *M. oleifera* meal was incorporated into each diet to give levels of 0% (control), 10%, 20% and 30%, with the fish fed these diets designated as T_0, T_1, T_2 and T_3, respectively. Experimental diets were analyzed using proximate analyses. All analyses followed the procedure described by AOAC (2006). Feed ingredients were mixed thoroughly and a sufficient amount of water was added for smooth pelleting. Pellets (2mm) were then formed using a local pelleting machine and stored in the air tight packaging until use.

Feeding protocol

The *L. rohita* fingerlings were fed twice a day at 7:30 - 8:30 AM and 2:30 - 3:30 PM at a rate of 3% of total body weight per day. Feed quantity was recalculated every fortnight.

Determination of growth parameters of fish

Growth parameters of fish, such as initial weight and length, were measured before stocking. At the end of trial other parameters such as final weight, feed conversion ratio (FCR), percentage weight gain, net gain in weight and specific growth rate (SGR) were also recorded.

Physico-chemical parameters of water

All physico-chemical parameters of aquarium water such as dissolved oxygen (DO), temperature, salinity, electrical conductivity, pH and total dissolved solids (TDS) were recorded on a daily basis using a dissolved oxygen meter and multi meter, except nitrates which were recorded on a fortnightly basis using HANNA Nitrate Test Kit HI3874.

Statistical analysis

The results of body composition, growth and physico-chemical parameters were analyzed using one way analysis of variance (ANOVA) using statistical software SAS version 9.1.

RESULTS

Statistically significant ($P \leq 0.05$) differences were recorded in growth parameters of all treatment groups (Table II). The maximum weight gain (254.00±4.24g) was observed in T_3 followed by T_1 (127.50±9.19g), T_2 (198.50±20.50g) and control T_0 (80.97±17.80g). Highest length increase (85.10±0.98cm) was found in T_2, followed by T_0 (78.10±1.83cm), T_1 (75.95±4.59cm), and T_3 (74.20±3.95cm). Significant differences ($P < 0.01$) were observed in the feed conversion ratio (FCR) between T_3 (1.81±0.06) and T_1 (2.70±0.13) and specific growth rate (SGR %) of T_3 (0.42±00) and T1 (0.25±0.01). The lowest (0.25±0.01) specific growth rate was recorded in fish fed

Table I. Fish feed formulation replacing soybean with *Moringa oleifera* meal.

Ingredients	Control			10 % Moringa meal			20% Moringa meal		30% Moringa meal	
	Crude protein (CP) % of ingredients	Inclu-sion level	Contri-bution of CP %	Crude protein	Inclu-sion level	Contri-bution of CP %	Inclusion level	Contribu-tion of CP %	Inclusion level	Contribu-tion of CP %
Fish meal	52.72	4	2.1	52.72	7	3.7	8	4.2	11	5.8
Rice polish	15.1	29.5	4.5	15.1	22	3.3	15	2.3	9	1.4
Wheat bran	16.4	23	3.8	16.4	21	3.4	13	2.1	10	1.6
Sunflower meal	30.23	4	1.2	30.23	5.5	1.6	19.5	5.9	25.5	7.7
Soybean meal	43.09	35	15.1	43.09	30	13	20	8.6	10	4.3
Moringa meal	--	--	--	18	10	1.8	20	3.6	30	5.4
Vitamins		4	0		4	0	4	0	4	0
Molasses		0.5	0		0.5	0	0.5	0	0.5	0
Total		100	26.63		100	26.8	100	26.7	100	26.2

Table II. Effect of varying levels of *Moringa oleifera* meal in fish diets on the growth parameters of *Labeo rohita.*

Parameters	Control	T_1	T_2	T_3
Weight gain(g)	80.97±17.80^d	127.50±9.19^c	198.50±20.50^b	254.00±4.24^a
Increase in length(cm)	78.10±1.83^a	75.95±4.59^a	85.10±0.98^b	74.20±3.95^a
% weight gain	23.89±5.35^d	67.09±4.82^c	102.00±14.14^b	140.00±1.41^a
FCR	2.38±0.43^a	2.70±0.13^a	1.96±0.18^b	1.81±0.06^b
SGR	0.28±0.06^d	0.25±0.01^c	0.33±0.03^b	0.42±00^a

T_1, 10 % Moringa meal; T_2, 20 % Moringa meal; T_3, 30 % Moringa meal.

Table III. Proximate analysis of *Labeo rohita* fed on different *Moringa oleifera* meal diets.

Parameters	Control	T_1	T_2	T_3
Crude protein %	59.41±0.79^b	61.82±0.10^a	60.26±0.28^b	62.45±0.15^a
Crude fat%	5.70±0.05^d	6.53±0.04^c	8.67±0.03^a	7.43±0.04^b
Ash%	6.80±0.08^a	4.60±0.28^b	4.73±0.12^b	4.66±0.16^b
Moisture%	5.90±0.84^a	5.60±0.56^a	4.50±0.70^a	4.45±0.07^a
Dry matter%	94.10±0.84^{ab}	93.40±0.56^b	95.50±0.70^a	95.45±0.07^a

For abbreviations, see Table II.

Table IV. Physico-chemical analysis of aquarium water cultured *Labeo rohita* under various treatments.

Parameters	Control	T_1	T_2	T_3
Temperature (°C)	22.83±2.70^a	22.38±2.54^a	21.97±2.91^a	22.28±3.13^a
DO (mg/L)	5.81±0.29^a	5.72±0.91^a	5.60±0.80^a	5.52± 0.87^a
pH	8.02±0.30^a	8.09±0.33^a	8.11±0.34^a	8.01±0.35^a
EC (µS/cm)	2468.22±912.80^a	2410.90±679.63^a	2640.86±965.67^a	2402.11±1043.90^a
TDS (mg/L)	1215.42±488.30^a	1194.58±292.47^a	1390.66±639.81^a	1188.05±509.47^a
Salinity (mg/L)	0.90±0.02^a	0.89±0.02^a	0.90±0.02^a	0.89±0.02^a

For abbreviations, see Table II.

10% of *M. oleifera* meal and was significantly different to the control group (T_0) while highest one was recorded in fish feed carrying 30% of *M. oleifera* meal.

Proximate analysis of the fish fed on the four diets formulated for the experimental trial is presented in Table III. A significantly higher proportion of crude protein (62.45±0.15%) was observed in T_3 that was notably different from the control group (T_0). The maximum fat content (8.67±0.03%) was found in T_2 exhibiting significantly greater content than T_1 (6.53±0.04%) and control T_0 (5.70±0.05%) fish. Ash content in T_0 (6.80±0.08%) was significantly higher than all other diets. No difference in moisture content was found in fish fed the different diets. The dry matter of the fish sampled in T_2 and T_3 showed significantly higher values ($P < 0.05$) than T_1.

Physico-chemical parameters of the water exhibited non-significant results in all treatments (Table IV). A favorable temperature (21-22°C) was recorded in all the treatment groups. The DO remained in the favorable range throughout the experiment (5.72±0.91mg/L, 5.60±0.80mg/L, 5.52± 0.87mg/L and 5.81±0.29mg/L for T_1, T_2, T_3 and T_0 groups respectively). During the whole experimental period the pH was stable at 8 in all the treatments. The maximum electrical conductivity (2640.86±965.67µS/cm) was observed in T_2. No significant differences were found in salinity, TDS and nitrates among all the treatments (Table IV).

DISCUSSION

Fish demand is continuously rising globally due to its nutritious and healthy characteristics. The aquaculture industry is rising globally as the fastest growing fish producing sector (Ottinger *et al*., 2016). However, this industry is facing a lot of challenges to provide nutritionally balanced feed to fish. Increasing demands, high prices and limited supply of fish meal turn the attention of fish culturists to replace costly fish feed ingredients with alternatives which are cheaper and that are easily, locally and widely available. The chief protein source ingredient in fish feed is fishmeal. Plant derived protein is therefore a good potential substitute and the leaves of *M. oleifera* are a rich source of plant based protein (30%) (Arsalan *et al*., 2016). Hence we have investigated the use of moringa leaf meal, at different inclusion levels, in fish diets.

In the present study the maximum weight gain was observed in T_3, followed by T_1, T_2 and T_0. Highest length increase was seen in T_2, followed by T_1, T_3 and control groups. Yuangsoi and Matsumoto (2012) showed that when soybean meal was replaced with *M. oleifera* leaf meal (MOLM) it affected the growth performance and fish (fancy carp) digestibility. Hence, MOLM could be replaced not up to 20% of plant protein in soybean. Continuous rise in the replacement of fish meal (animal protein) with *M. oleifera* leaf meal (MOLM) could slow down the growth of aquaculture (Richter *et al*., 2003). Ritcher *et al*. (2003) observation was further supported by El-Nadi (2015) when it was shown that growth performance of *O. niloticus* decreased with the increase of *M. oleifera* plant meals in the diet. Further, substitution of 55% MOL meal for soybean meal awarded the best growth performance (less than 7.34%) and feed nutrient utilization in *O. niloticus* was explored by Tiamiyu *et al*. (2016). While the present study shows contradictory results to those of Ozovehe *et al*. (2013). These authors reported that growth and feed consumption of fish, *Clarias gariepinus* decreased with increasing proportion of MOLM in fish feed. Afuang *et al*. (2003) described that solvent extracted MOLM could replace 30% fishmeal in the feed of *O. niloticus*. Mehdi *et al*. (2016) studied the effect of *M. oleifera* on the growth of *Labeo rohita* and found that 10% inclusion of this plant is better for the growth of this species, beyond this it has negative effect which may be due to presence of anti-nutritional factors or amino acid imbalance. However, the results of the present study showed that increase in *M. oleifera* meal replacing soybean meal increases the *L. rohita* growth.

The products derived from *M. oleifera* have been explored as important ingredients for practical feed of *L. rohita*. The findings of the present study revealed that MOLM could be used as a protein substitute up to inclusion levels of 20-30% in diets for growing *L. rohita.* The findings of our study are broadly in agreement with that of Ncha *et al*. (2015) who demonstrated that fish fed 0% MOLM diet exhibited a peak value of 6.59 and lowest value of 5.86 in fish given a 60% MOLM diet. Karina *et al*. (2015) replaced soybean meal with MOLM to give levels of 0%, 8%, 16%, 24% and 32 %, and found highest SGR in fish fed a diet with 32 % MOLM (1.45 ± 0.09%). However, the lowest FCR was seen in the control group (7.50± 1.10), while the highest value was found in fish given the 32% inclusion (9.96±0.13). Similarly, increment in the FCR values were also recorded in Nile tilapia with the inclusion increment of moringa leaves in diet which is an indication of presence some anti-nutrients (Richter *et al*., 2003).

The crude fat content was found to increase with increasing moringa meal up to 20%. This data agrees with results from Mehdi *et al*. (2016) where increase in fat was observed in fish being fed up to 20% moringa meal in *L. rohita*. Further, the present research supports the results of Ganzon-Naret (2014) where an increased proportion of crude fat was found on addition of MOLM in Asian sea bass (*Lates calcarifer)* diets. Our results differ from other experiments, reported the highest value of crude fat in different fish species using 10% MOLM in diets (Olaniyi *et al*., 2013; Thiam *et al*., 2015; Arsalan *et al*., 2016).

In the present study, all physico-chemical parameters of water exhibited no significant differences between the treatment groups.

CONCLUSION

In conclusion, the current research shows that *M. oleifera* meal could be used safely for promoting the growth of fish without any harmful effects on body composition of *L. rohita*. Our results show that *M. oleifera* meal inclusion up to 20-30% is suitable to replace soybean meal in fish diets.

Conflict of interest

The authors declare that they have no conflict of interest.

Ethical approval

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

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