Brazilian Journal of Biology®

ISSN 1519-6984 (Print)
ISSN 1678-4375 (Online)

Original Article

Effect of *Moringa olifera* leaves on growth and gut microbiota of Nile tilapia (*Oreochromis niloticus*)

Efeito das folhas da *Moringa olifera* no crescimento e na microbiota intestinal da tilápia do Nilo (*Oreochromis niloticus*)

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Abstract

The study was conducted to evaluate the effect of *Moringa olifera* on the growth and gut health of Tilapia (*Oreochromis niloticus*). The feed having 30% crude protein was prepared as an experimental diet with 4%, 8% and 10% *M. olifera* leaf supplementation, respectively. The control diet was devoid of *M. olifera leaves*. The 10 weeks feeding trial was carried out on 60 fish in aquaria. Fish was fed @ 3% of body weight twice a day. Diet with the high level of inclusion of *M. olifera* leaves significantly increased the growth rate, Survival Rate (SR), Specific Growth Rate (SGR) and Feed Conversion Efficiency (FCE) in all treatment groups compared to the control group. Similarly, Feed Conversion Ratio (FCR) gradually decreased and found highly-significant. To check the gut health of the Tilapia, random samples were selected and dissected. Nutrient agar was used as culture media to check the growth of bacteria. Pour Plate Method was used for viable colonies count by colony counter. Through staining method, the different bacteria such as *Escherichia coli, Salmonella*, *Shigella* and *Pseudomonas aeruginosa* were identify abundantly in the intestine of control diet fish but less number present in treatment diets groups. These results showed that *M. olifera* leaves up to 10% of dietary protein can be used for Nile tilapia for significant growth and healthy gut microbiota of fish.

Keywords: body weight gain, FCE, FCR, *Moringa olifera* leaves, Gram staining, survival rate.

Resumo

O estudo foi conduzido para avaliar o efeito da *Moringa olifera* no crescimento e saúde intestinal da tilápia (*Oreochromis niloticus*). A ração com 30% de proteína bruta foi preparada como dieta experimental com 4%, 8% e 10% de suplementação de folhas de *M. olifera*, respectivamente. A dieta controle foi desprovida de folhas de *M. olifera*. O ensaio de alimentação de 10 semanas foi realizado em 60 peixes em aquários. O peixe pesava 3% do peso corporal duas vezes ao dia. A dieta com alto nível de inclusão de folhas de *M. olifera* aumentou significativamente a taxa de crescimento, taxa de sobrevivência (SR), taxa de crescimento de sobrevivência (SGR) e eficiência de conversão alimentar (FCE) em todos os grupos de tratamento em comparação com o grupo de controle. Da mesma forma, a taxa de conversão de alimentação (FCR) diminuiu gradualmente e foi considerada altamente significativa. Para verificar a saúde intestinal da tilápia, amostras aleatórias foram selecionadas e dissecadas. O ágar nutriente foi usado como meio de cultura para verificar o crescimento das bactérias. O método da placa de Verter foi usado para a contagem de colônias viáveis por contador de colônias. Através do método de coloração, diferentes como *Escherichia coli*, *Salmonella*, *Shigella* e *Pseudomonas aeruginosa* foram identificados abundantemente no intestino de peixes da dieta controle, mas em menor número nos grupos de dieta de tratamento. Esses resultados mostraram que *M. olifera* deixa até 10% da proteína dietética e pode ser usado para tilápia do Nilo para um crescimento significativo e microbiota intestinal saudável de peixes.

Palavras-chave: ganho de peso corporal, FCE, FCR, folhas de *Moringa olifera*, coloração de Gram, taxa de sobrevivência.

1. Introduction

Global fast-growing sector aquaculture recorded 114.5 million tonnes in wet weight and aquatic farmed raised fauna grew on 5.3%/year in 2001-2018 (FAO, 2020). To increase significant fish feed production to attain high fish growth (Francis et al., 2001). In last few decades the use of fishmeal in fish feed formulations due to their high profile of protein can no interminable because of overfishing, fluctuating quality and pricey (Tacon et al., 2011; Ag, 2014). Nutritionists, aqua culturist, researcher and feed industries have organized different researches and polite projects to

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Received: April 10, 2021 – Accepted: August 5, 2021

bring down the use of fishmeal (Tacon and Metian, 2013; Rana et al., 2009). To improve quality and cost effect of fish feed; substitution of fishmeal with topically cheaper plant ingredients (Tacon et al., 2006). Plant can be used as a protein source for fish feed. Conventional and considerable plant sources are soybean, groundnut, cotton seed and rapeseed meal (Jackson et al., 1982; El Sayed, 1990). However, livestock, human consumption and industrial use of these sources make them costly and put far away from the reach of fish farmers, researchers and producers of aqua feed (Fasakin et al., 1999). Therefore, for research purposes such more economical and environment-friendly protein sources are used that are obtained from the plant's leaves, seeds and other agriculture by-products (El Sayed, 1990; Siddhuraju and Becker, 2001). Animals and humans use plant products as a valuable source to maintain their health. Plants have a large number of chemical substances that contain medicinal and curative agents.

Moringa oleifera belongs to family *Moringaceae* is native to tropical and sub-tropical regions of Pakistan, India, Africa, and Arabia, the western sub-Himalayan region and Asia Minor (Mughal et al., 1999). *M. oleifera* is cultivated as vegetable (leaves, pods flowers, roasted and seed) spice mainly present in roots and have medicinal uses (Rebecca et al., 2006). These parts are used as antipyretic, antiepileptic and antihypertensive (Dahot, 1988), anti-inflammatory (Mehta et al., 2003) anti- ulcerative (Mahajan and Mehta, 2007) anti-diabetics, anti-bacterial, anti-fungal and anti-oxidant. Moreover, *M. oleifera* is fast growing and have the power to purify water. (Nikkon, 2003).

Tilapia is worldwide preferable aquaculture fish species due to rapidly growing ability, falling to family *Cichlidae*, has laterally compressed, deep bodies, like other cichlids. Their lower pharyngeal bones are fused into a single tooth-bearing structure. Beside their temperature sensitivity, Tilapia can exist and adapt wide range of conditions. Tilapia survival has been testified in brackish and mostly, enough salty water cause mortality in fresh water fish (Chapman, 2000; Lind et al., 2019). The aims of the present study were to evaluate the suitability of *M. oleifera* leaves as a partial protein replacement for fish in practical diets of Nile tilapia, based on its effects on growth performance and total bacterial count (positive or negative) on different culture media such as tryptone soya agar (TSA), Nutrient agar (NA) and Eosin methylene blue (EMB).

2. Materials and Methods

2.1. Experimental design

The present study was conducted at the hatchery, Department of Fisheries and Aquaculture, the University of Veterinary and Animal Sciences, Ravi Campus, Pattoki. The experiment was conducted in glass aquariums and the experimental fish species was tilapia (*Oreochromis niloticus*). Sixty (60) Tilapia fish, of 10 grams were collected from the pond and were placed in four different glass aquaria. Each aquarium contained 15 fish and categorized in three different treatments and one control group. The Control group was fed on a normal diet, devoid of *M. oleifera* leaves. Whereas Treatment 1 was (4%), Treatment 2 (8%) and Treatment 3 (10%) *M. oleifera* leaves supplementation. Water quality parameters; dissolved Oxygen and pH were monitored during the whole trial respectively.

2.2. Feed formulation

A feed with 30% CP was prepared by 4%, 8% and 10% *M. oleifera* leaves supplementation with different feed ingredients as shown in Table 1. The control diet was free from *M. oleifera* leaves. Fish were fed 3% of body weight twice a day during the entire trial. Feed formulation is below in Table 1.

2.3. Evaluation of growth parameters

Growth performance and diet nutrients consumption were evaluated in terms of body weight gain (BWG), length gain, daily body weight gain (DWG), feed conversion ratio (FCR), survival rate (SR) and specific growth rate (SGR).

2.4. Weight gain %

The increased body weight was figured as difference between final and initial weight by using the Formula 1:

$$Weight\ gain = Final\ body\ weight(g) - Initial\ body\ weight(g) \quad (1)$$

2.5. Feed Conversion Ratio (FCR)

Ratio of feed intake to weight gain was evaluated by the following Formula 2: (Shabir et al.,2003).

$$FCR = Feed\ intake(g) / Wet\ weight\ gain(g) \quad (2)$$

Table 1. Ingredient of fish feed with 30% crude protein (C.P).

Ingredients	Control	Treatment 1	Treatment 2	Treatment 3
Fish meal	25	20	20	20
Soya bean meal	16	16	14	13
Maize gluten	15	16	14	13
Rice polish	40	40	40	40
Molasses	3	3	3	3
Vitamin	1	1	1	1
Moringa olifera leaves	0	04	08	10

Above feed, ingredients were used in fish feed as upper described concentrations. All treatment groups feed was inoculated with 4%, 8% and 10% *Moringa olifera* leaves. While, control diet was free from *Moringa olifera* leaves.

2.6. Specific Growth Rate (SGR)

Specific growth rate was measured by using the Formula 3:

$$SGR\ \%/day = ln(W1) - ln(W2) \times 100 / Number\ of\ days \quad (3)$$

2.7. Daily feed intake

Daily feed intake was calculated with following Formula 4 as previously described by (Helland et al., 1996)

$$Air\ -\ dry\ feed\ eaten\ (g) = (A\ XA_{DM} / 100) - (W\ x\ W_{DM} / R)\ AD, / 100 \quad (4)$$

A denoted weight of air-dry feed (g), A_{DM} for dry matter content of air-dry feed (%), W is weight of waste feed collected (g), W_{DM} for dry matter content of waste feed (%), and R for recovery of dry matter of waste feed (%)

2.8. Bacterial isolation

Three randomly chosen fish from each treatment were dissected and the intestine was removed from each sample with sterile dissecting instruments. The intestine was longitudinally opened and gently agitated for 2-3 minutes in a 9% saline solution in a flask to remove the other contents. Eppendorf tubes were filled with 900µl, 9% normal saline solution (sterilized). 100µl solution containing intestinal content was transferred to 900 µl normal saline solutions in Eppendorf tubes. This acted as 1st dilution, then 100 µl solutions from that dilution was added into 900 µl in the next tube. This resulted 100 times second dilution. Bacterial colonies were cultured by pouring and spreading 10 µl the solution in petri plate contained 20 ml media and were placed in an incubator at 37 °C for 24 to 48 hours. Colonies of bacteria were counted after incubation of 24 and 48 hours (Canberra, 1999).

2.9. Total viable count

The pour plate method was used for the estimation of viable counts. 15-20ml sterilized Tryptic soy agar was melted and then cooled at 45 °C was settled for solidification. Nutrient agar and Eosin methylene blue (EMB) were employed for the microbial count (Buchanan and Gibbons, 1974) (Formula 5).

$$Total\ Viable\ Count\ =\ Average\ no.\ of\ colonies\ \times\ Dilution\ factor \quad (5)$$

2.10. Statistical analysis

Data of growth parameters were put through one-way analysis of variance (ANOVA) (Steel et al., 1996). Means difference were evaluated by Tukey's Honestly Significant Difference Test and considered significant at P<0.05 (Snedecor and Cochran, 1991).

3. Results

3.1. Growth parameter

Growth performance and feed utilization of Tilapia is given in Table 2. Growth performance of Tilapia revealed the body weight, body length, body weight gain and daily body weight gain were significantly (P<0.05) increased in treatments T1, T2 and T3. Body weight was higher in the T3 group as compared to T2, T1, and Control group respectively. A similar pattern was observed for body length, body weight gain and daily body weight gain. In term of feed utilization, the results showed non-significant difference found in FCR, FCE, SGR, FI, SR, FCR and value of mean range cameto1.96 to 2.85. The lowest FCR with high level inclusion of *M. oleifera* leaves was observed in T3. FI increased in all groups while FCR were decreased. SGR was increased in the all-treatments groups. All fish grow normally but a high mortality rate was found in the control group although no mortality observed in T3. Progressively increased the level of *M. oleifera* leaves in the diet significantly shown the significant growth and survival rate of Tilapia and indicated the positive effect of *M. oleifera* leaves on the fish health. Similarly, Figure 1 shows the overall growth performance of the fish after using different treatments. The bar chart clearly showed the maximum weight gain and length gain in treatment 2 and treatment 3. Whereas,

Table 2. Statistical analysis of growth performance parameters and feed utilization abilities of Tilapia.

Parameters	Control	Treatment 1	Treatment 2	Treatment 3	P value
Body weight	25.55±15.36a	27.80±16.63bcd	38.33±26.39cbd	45.21±30.16dbc	0.000*
Body length	12.45±7.54acd	13.40±7.90bcd	17.04±11.94cab	16.2±9.29dab	0.003*
Body weight gain	15.43±16.80acd	17.23±17.07bcd	38.33±26.39cab	45.21±30.16dab	0.000*
Body length gain	12.45±7.54acd	13.40±7.90bcd	17.04±11.94cab	16.2±9.29dab	0.000*
D.W.G.	1.02±1.12acd	1.14±1.13bcd	1.67±1.65cab	1.96±1.95dab	0.003*
F. I.	331.25±258.13a	226.62±135.50b	384.72±123.84c	376.14±256.72d	0.628
F.C.R.	2.85±1.34abd	1.47±0.63ba	1.75±0.12c	1.52±0.06da	0.080
F.C.E.	0.40±0.14ab	0.80±0.42ba	0.57±0.50c	0.70±0.14d	0.149
S.G.R.	0.97±0.65a	0.12±1.00b	1.42±1.15c	1.59±1.30d	0.848
S.R.	88.00±14.30acd	95.80±3.83b	98.00±3.13ca	100.00±0.00da	0.096

D.W.G. = Daily Body weight Gain; F.I. = Feed intake; F.C.R. = Feed conversion ratio; F.C.E. = Feed conversion efficiency; S.G.R. = Survival Growth Rate; S.R. = Survival Rate. *Shows significant results among treatments at 5% significance level. [ab]the mean values with different case letters shows significantly different from each other (DMRT).

weight gain and length gain in control and treatment 1 can be seen as identical. The graph shows that the SR of fish in Treatment 3 remains approximately a hundred percent while others were less.

3.2. Bacterial growth

Microbiota in the intestine of sampled fish from each treatment were examined and compared with the control group. The microbiota of the control group was different from treatments. There were present maximum numbers of disease-causing bacteria in the intestine of the control group compared to the treatments.

In the present study *Escherichia coli*, *Pseudomonas aeruginosa*, *Shigella* and *Salmonella*e were identified in large amounts in the control group as compared to the treatments that contain a different concentration of *M. oleifera* leaves. There was 80% growth of bacteria in the control group that is devoid of *M. oleifera* leaves and resistant to control group but in the treatments that contain *M. oleifera* there were minimum no of bacteria because *M. oleifera* inhibit the growth of bacteria listed in Table 3.

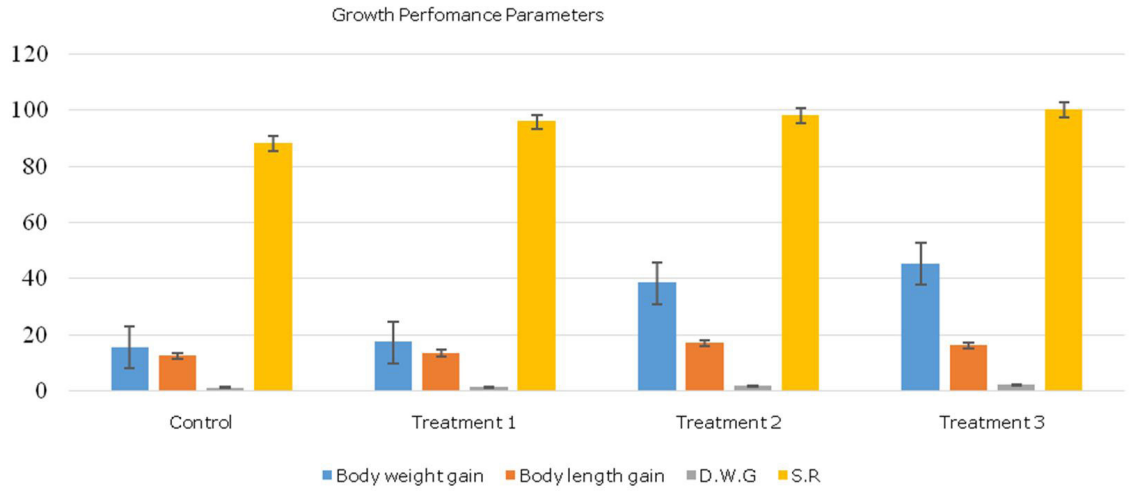

Figure 1. Growth performance parameters.

Table 3. Bacterial susceptibility in experimental fish at concentration of 20 mL/100µl.

	Escherichia coli	***Pseudomonas aeruginosa***	***Shigelae***	***Salmonella***
Control	1010	5000	1098	4000
	789	1090	1000	3598
	554	567	967	769
	551	430	890	679
	432	234	760	560
Treatment 1	21	09	657	67
	54	05	651	51
	51	03	545	21
	41	00	530	10
	01	00	521	00
Treatment 2	16	10	78	45
	12	00	67	43
	05	00	43	20
	00	00	21	21
	00	00	09	10
Treatment 3	08	08	00	08
	02	05	00	05
	00	03	00	02
	00	00	00	02
	00	00	00	00

4. Discussions

Earlier studies evaluated significantly the effect of *Moringa olifera* (*M. oleifera*) on growth parameters (Ozovehe, 2013; Ahmed et al., 2014). Those significant results recognized that *Moringa* is most effective source of protein, crude fibers, and fats (Francis et al., 2001). Recent study conducted in Egypt revealed that *Moringa* leaf meal improves the growth rate of Nile tilapia (Elabd et al., 2019). Puycha et al. (2017) concluded that *M. oleifera* leaf @ 100 g/kg demonstrate significant growth. Another study reported the significant growth rate of tilapia fed with *M. oleifera* supplements (Tiimub et al., 2020). Protein extracted from *M. oleifera* seed at 400 mg kg^{-1} used as growth promoter in Tilapia (Stadtlander et al., 2013). Aqueous extract of *M. oleifera* is used as growth accentuate for Tilapia (Shourbela et al., 2020)

In a previous study *M. olifera* examined as a better antibacterial effect against Gram-positive bacteria (*S. aureus and E. faecalis*) than Gram-negative strains (*E. coli, Salmonella, P. aeruginosa, V. parahaemolyticus and A. caviae*) (Peixoto et al., 2011). *Moringa* seed contains pterygospermin, moringine, 4-(α-L-rhamnosyloxy)-phenylacetonitrile glycosides and 4-(α-L-rhamnosyloxy) benzylisothiocyanate and constituents inhibit the growth of *Streptococcus, E. coli, Shigella* and *Pseudomonas aeruginosa* (Jeon et al., 2014). Another study was conducted on *Moringa* seed extract to observed the *Salmonella enteritidis* cultures to be vulnerable to water that is treated by *Moringa* seed extract and results showed that the extract is not able to inhibit the growth of *salmonella* (Madsen et al., 1987). In 2010 study held by Oluduro et al. (2010), use *M. oleifera,* seeds showed strong antibacterial activity against *Pseudomonas aeruginosa, E. coli, S. aureus, Penicillium sclerotigenum* and *Cladosporium cladosporioides*. At Sao *Paulo* study was held by Viera et al. (2010), evaluated the effect of *M. oleifera* against Gram Positive and Gram-negative bacteria. All the previous studies showed a close resemblance with the present study.

5. Conclusion

In conclusion, the results obtained from the present study recommended *Moringa oleifara* leaves could replace with or used with fish meal in Nile tilapia (*O. niloticus*) feed as a growth enhancer. It is suggested that *M. olifera* leaves added in cultured fish diet had the best growth performance parameters with minimum cost and maximize profit. *M. olifera* leaves inhibit the growth of various disease-causing bacteria such as *E. coli, P. aeruginosa*, *Shigella* and *Salmonella* in the intestine of fish. The presence of minerals and vitamins in *M. olifera* leaves help to enhance the immune system and cure a myriad of diseases. The antimicrobial factors found to present in *M. olifera* leaves is responsible for antimicrobial properties.

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