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Effects of moringa leaf meal (*Moringa oleifera*) on the growth performance of molly (*Poecilia sphenops*)

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Abstract
The effect of *Moringa oleifera* leaf meal on growth and development of *Poecilia sphenops* was investigated for a period of 90 days. The experiment was conducted in aquarium with recirculation facilities in the Wet Laboratory of the Faculty of Fisheries, Chattogram Veterinary and Animal Sciences University. *M. oleifera* leaf meal was substituted for fish meal at 0% (control), 5%, 15 % and 30% in the four different diets. *P. sphenops* (mean weight 0.979 ±.10 g) were randomly distributed into glass aquarium at 4 fish/aquarium in triplicate treatments and were fed twice daily at 8am to 9 am and 5 pm to 6pm for 13 weeks. The average initial weights in four treatments were 1.01g±0.10, 0.95g±0.04, 1.03±0.05g and 0.93±0.01g in T1, T2, T3, and T4 respectively. At the end of the 90 days experimental period, average final weight of the fishes of four treatments were 1.74g±0.15, 1.75±0.16g, 2.2±0.11g and 2.01±0.21g in treatments T1**,** $T_{2,}$ T_3 and T_4 respectively. In case of weight gain higher result was found in T_3 (1.17±.16) followed by T_4, T_2, and $T_{1.}$ The result showed that the fishes provided with 'Treatment-T_3 (1.40±0.69)' feed have higher growth in terms of length gain when comparing with other treatments. Results on the specific growth rate **(**% day) of *Poecilia sphenops* f**e**d on feeds containing different levels of moringa higher result was in T_4 followed by T_3, T_2 and $T_{1.}$ FCR values were also higher in T_3 treatment than other treatments. It indicated that moringa leaves used as a plant protein sources support the better growth performance (weight, weight gain, length, length gain, SGR), lower FCR value and as well as disease free molly fishes. The result concluded that moringa (*Moringa oleifera)* leaf has positive impact on the growth and development of molly fish (*Poecilia sphenops*) and substitution of fish meal with moringa is feasible without compromising growth and performance of cultured molly.

Keywords: *Poecilia sphenops*, *Moringa oleifera*, growth performance, aquarium

Introduction
Ornamental fish culture is one of the most important fields of Aquaculture. The inclusion of plant protein sources in the ration of fish requires investigation on limiting factors in the plant ingredients such as high crude fiber content and anti-nutritional factors as earlier investigations on some plants have shown that their excessive inclusion in the feed may result in slower growth rates and general poor performance of cultured fish species [1-2-3-4]. Black molly (*Poecilia sphenops)* is now most popular ornamental fish in Bangladesh. The mollies are a small-sized tropical fish that is found naturally in the warm and peaceful river of Central America. The black molly is mainly found in shallow coastal estuaries, streams, ponds and ditches, associated with heavy vegetation [7]. The black molly is easy to keep and easy to breed. They are ideal for a community aquarium. The black molly are livebearers, the female molly will produce a brood of 20 to 60 live young, some times more. *Moringa olifera* leaf is said to be an excellent source of vitamins, minerals and proteins. Earlier studies have shown that *M. oleifera* is a promising protein source for use in diet of Tilapia [9]. Another potential alternative plant protein source for fish feeds is moringa (*Moringa oleifera*), as this plant is receiving much attention because its leaves, flowers and seeds can all be used as food [9]. Moringa leaves are important due to have quality attributes that make it a potential replacement for fishmeal in aqua feeds. The leaves have high protein content (28-36%), are rich in carotenoids, ascorbic acid and iron [9]. Moringa leaf contains crude protein (CP) with about 260 g/kg of leaf, of which about 87% is true protein [10]. Essential amino acids found in moringa leaf are methionine, cysteine, tryptophan and lysine [10]. There is an abundant total amount of these essential amino acids are plenty in the leaf that can be used as animal feed [5]. Moringa can also be dried and used in the form of Moringa leaf meal (MLM), 30% substitution of *M. oleifera* leaf meal for fish meal has been recommended for the diet of Nile tilapia *Oreochromis niloticus* [8].

Materials and Methods
The research was carried out to observe the effect of different dietary levels of moringa leaf meal on growth performance of molly fish (*Poecilia sphenops*). As moringa leaf meal is widely found in Bangladesh and the ornamental fish industry is contributing a good share into national economy we decided to have a test of moringa

leaf meal over molly fish (*Poecilia sphenops*). In this experiment molly fry were reared and maintained in the recirculatory system for 3 months by providing different dietary levels of moringa leaf meal to observe the growth performance of molly fish (*Poecilia sphenops).* The experimental system consists of 12 rectangular glass aquaria each of size (22 X 18 X 28.5 mm) containing about 4 liter of water for three (3) replications and four (4) treatments. There were another two glass aquarium for conditioning and stocking of fish. An adequate level of oxygen in each aquarium was maintained through artificial aeration using aerators and effective filtration facility also provided. We provided electric motor which provides both aeration and filtration facility. Syphoned water was taken to the biological filtter. For the study of the effects of moringa on the growth performance of molly, 12 aquariums were divided into four groups containing 3 aquariums in each group. These four groups corresponded to four experimental treatments (T1, T2, T3 and T4) and each treatment group had three (30 replication. We chose this method to get ensured about proper efficient dose of Moringa leaf meal on a better growth of molly fish (*Poecilia sphenops*). Each aquarium stocked with 4 fish. Feed with four different levels of moringa (*Moringa olifera)* 0% (served as control), 5%, 15%, and 30% feed were administered for studying the growth of fish. Before stocking, weight of every individual fish was taken. To make a feed formulation chart 'Karl Pearson Feed Formulation Method' was followed. Inclusion level was determined by 'Karl Pearson Formula'.

Fig 1: (a) *Moringa oleifera* (b) *Poecilia sphenops*

Table 1: Layout of the experiment showing the distribution of 'molly' fishes in tank and the applied treatments

	Treatment	**T_1**	**T_2**	**T_3**	**T_4**	**Total**
Replication	Moringa feed dose	(0%)	(5%)	(15%)	(30%)	Fish
	R_1	4	4	4	4	16
	R_2	4	4	4	4	16
	R_3	4	4	4	4	16
	Total	12	12	12	12	48

Table 2: Composition of experimental diets and their amount of inclusion

Ingredients Name	**Treatment 0**	**Treatment 1**	**Treatment 2**	**Treatment 3**
Fish meal	43.75g	41.56g	37.187g	30.625g
Mustard oil cake	34.37g	34.37g	34.37g	34.37g
Rice bran	31.25g	31.25g	31.25g	31.25g
Wheat flour	13.75g	13.75g	13.75g	13.75g
Vitamin	1.25g	1.25g	1.25g	1.25g
Salt	0.625g	0.625g	0.625g	0.625g
Moringa	0g	2.187g	6.5625g	13.125g

Table 3: Proximate composition of experimental diets

Treatments	**Crude protein (%)**	**Crude Lipid (%)**	**Fibre (%)**	**Ash (%)**	**Moisture (%)**
T_1	30.74	6.62	0.20	16.682	14.131
T_2	30.33	6.62	0.50	18.264	12.058
T_3	29.17	6.64	1.5	18.54	17.23
T_4	26.25	6.66	2.1	22.14	15.30

The feed which were applied in the aquariums as treatment for 'molly' was prepared in 'Wet Lab' of Chittagong Veterinary and Animal Sciences University by adding appropriate amount of Moringa. 'Fish meal' served as the animal protein source whereas 'Mustard oil cake',' rice bran', and 'wheat bran' served as plant protein source. Mustard oil cake was the main source of crude fat in the feed. 'Rice bran', 'wheat bran' and 'wheat flour provided maximum carbohydrate content to the feed. A fixed amount of the experimental diet was weighted out for each aquarium of fish each week. Handling methods were followed during feeding fish in aquariums. Each

time a small amount of feed was dropped in to the aquarium. Feeding rate and feeding frequency were adjusted by their body weight. Dry powdered feed were fed with fish in 2 times in a day.

Parameters studied for growth performance of fish

1. Length gain (cm) = Mean final length- Mean initial length
2. Weight gain (g) = Mean final weight- Mean initial weight
3. Specific growth rate, $\text{SGR (\% day)} = \frac{log_e W_2 - log_e W_1}{T_2 - T_1} \times 100$ (after Brown, 1957)

Where, W_1= the initial live body weight (g) at time T_1 (day)
W_2= the final live body weight (g) at time T_2 (day)

4. $\text{FCR} = \frac{\text{Total amount of feed given (g)}}{\text{Total weight gain (g)}}$

Results and discussion

After completion of research work, highest weight was found in T_3 (1.17±.16) treated fish followed by the T_4, T_2, and T_1 treated fish. The present work represents that fish fed without moringa leaf show lower weight than the fish fed with moringa supplementation at 15% (T_3) which clearly indicates that moringa leaf meal has great effects on fish growth (Figure 2)**.** From week (1-3) showed lower growth rate and lower weight gain due to adjustment in new habitat. Then the week 4 to week 11 showed higher weight gain in all experimental fish in which T_3 showed higher weight gain when comparing other treated fish (Figure 2). The replacement of moringa in conventional diets did not affect the growth performance of Nile tilapia (*Oreochromis niloticus*) [5].

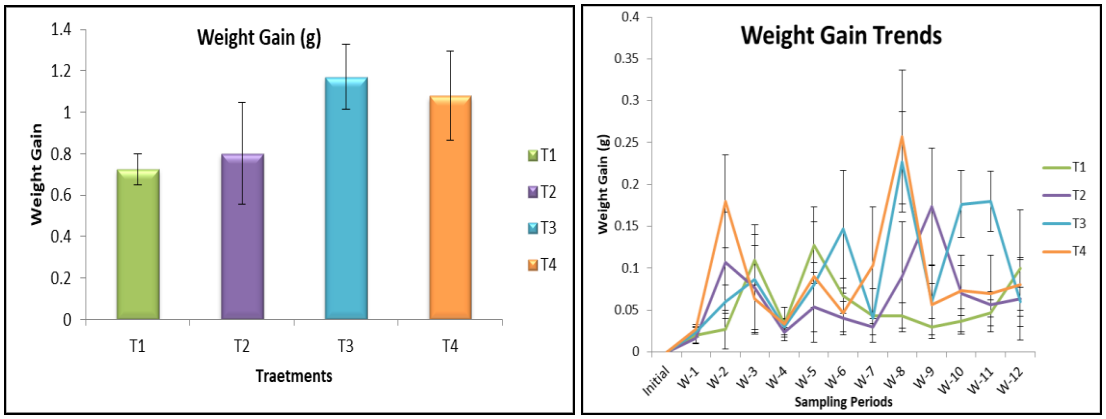

Fig 2: Weight gain of *Poecilia sphenops* reared under different dietary levels of moringa Vertical bars

The initial average length of fish in each treatment such T1, T2, T3 and T4 were 3.9±0.10cm, 3.8±0.15cm, 3.7±0.10 cm and 3.7±0.05cm. In final sampling, it showed that the average length of each treatment such as T1, T2, T3 and T4 were 4.7±0.05cm, 4.8±0.25cm, 5.1±0.10cm and 5.1±0.15 cm respectively. The data showed that the fishes provided with 'Treatment-T3 (1.40±0.69)' feed have higher growth in terms of length gain when comparing with other treatments. The result showed that the fishes provided with 'Treatment-T3 (1.40±0.69)' feed have higher growth in terms of length gain when comparing with other treatments (Figure 3).

Results on the specific growth rate *(*SGR percent/day) of *Poecilia sphenops* fish fed on feeds containing different levels of *Moringa oleifera* has been shown in Figure 4.

In result of specific growth rate higher result was in T_4 followed by T_3, T_2 and T_1. The highest SGR were observed in the fish fed with moringa leaf supplemented diets at 30% (T4), which were significantly higher than other groups. These results agree with [2] who found that diets supplemented with methanol-extracted moringa leaf meal containing 11, 22 and 33 g kg-1 did not affect the growth of Nile tilapia (*Oreochromis niloticus*). Using raw moringa leaf in the diets for tilapia showed that the fish meal protein replacement at rate of 10% did not affect the growth performance [11].

The present experiment was conducted to investigate the effects of different levels of dietary moringa leaves in the diet of molly fish (*Poecilia sphenops*) on the growth performance. The results presented for *Poecilia sphenops*, indicate that there exists a positive impact on the growth performance of molly (*Poecilia sphenops).* Experimental fish showed best FCR in treatment T_3 (3.26±0.47*)* in 15% moringa treated feed, whereas in other treatments were 5.22±0.55, 5.03±0.59 and 3.59±0.78 in T_1, T_2 and T_3 respectively (Figure 5). There was a positive effect of moringa was observed on growth performance of molly (*Poecilia sphenops.* A detailed study concluded that feeding frequency of two times per day was suitable for rearing of *C. batrachus* [6].

Tropical ornamental fish are generally sensitive to poor water quality [11]. pH requirements for tropical ornamental fish culture should be from 7.2-7.8 [12]. In this study pH range was 7.1 to 7.8 which the above report is within close range to that. However, values for DO ranged from 5-7.5 ppm. This finding indicated that black molly fry were quite tolerant to low dissolved oxygen in the culture water. Black molly is a eurythermal species

and it can adapt to higher water temperature [13]. Whereby, black molly was able to tolerate temperature ranged between 24-28°C. So considering the result mentioned above it is evident that moringa had a positive impact on the growth and maturation of molly fish (*Poecilia sphenops*).

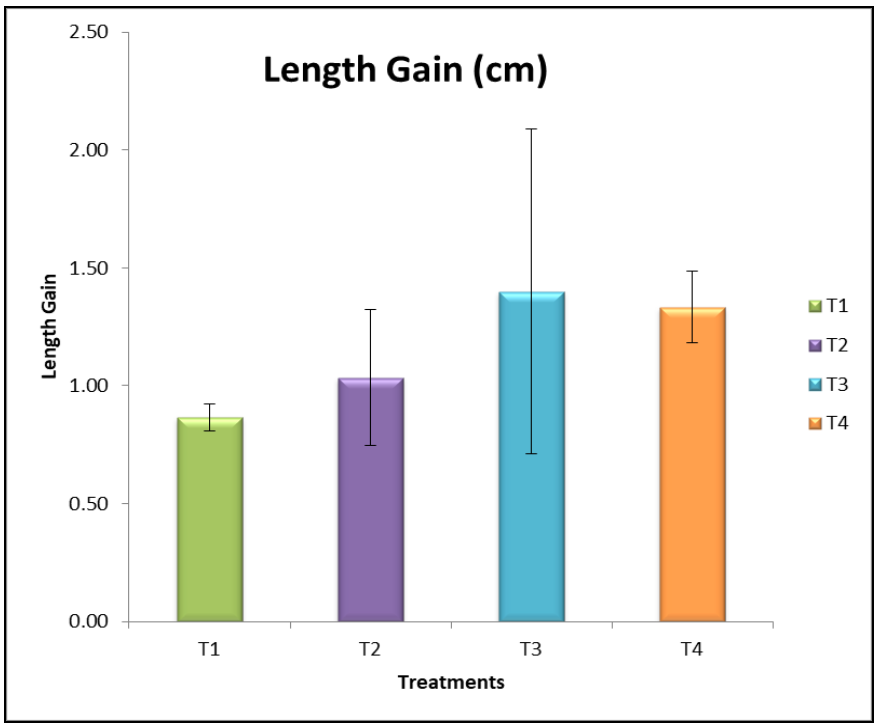

Fig 3: Length gain of *Poecilia sphenops* reared under different dietary levels of moringa Vertical bars

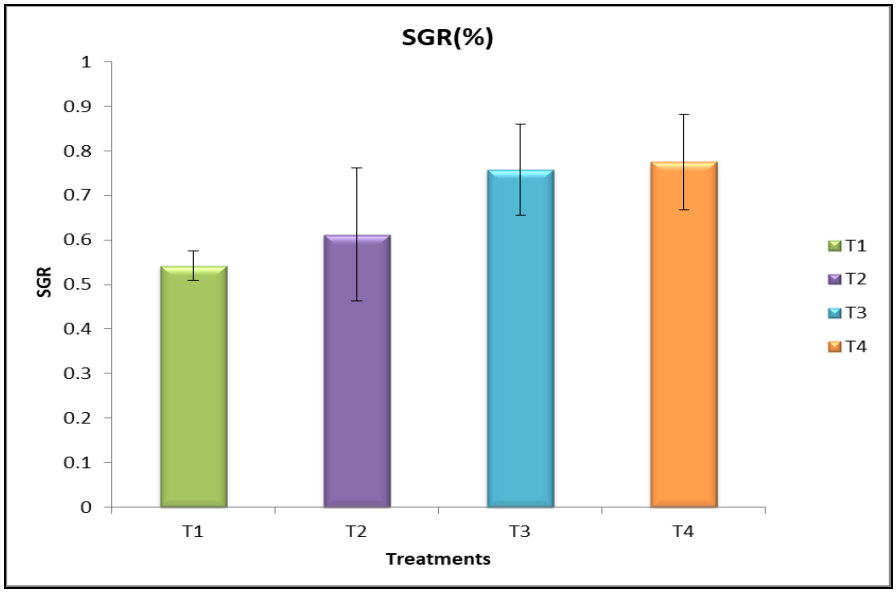

Fig 4: Specific growth rate of *Poecilia sphenops* reared under different dietary levels of moringa Vertical bars

Table 4: Growth performance of cultured molly

Treatment	Weight	Length	Condition factor	Specific growth rate
Control (T_1) (0% Moringa)	1.736667±.17 (1.54-1.85)	4.766667±.06^b (4.7- 4.8)	1.600599±.10 (1.48-1.67)	.602730±.03 (.5696-.62933.12)
T_2 (05% Moringa)	1.75±.23 (1.55 - 2.01)	4.833333±.25^b (4.6 - 5.1)	1.545272±.04 (1.51-1.59)	.673093±.16 (.4981-.8211)
T_3 (15% Moringa)	2.196667±.11 (2.07- 2.29)	5.1±.1^b (5- 5.2)	1.655248±.03 (1.63-1.68)	.845201±.11 (.7229-.9250)
T_4 (30% Moringa)	2.01±.22 (1.77- 2.19)	5.1±.1^b (5- 5.2)	1.51±.12 (1.42-1.65)	.851947±.12 (.7151-.9398)
Level of significance	0.05	0.05	0.247	0.069

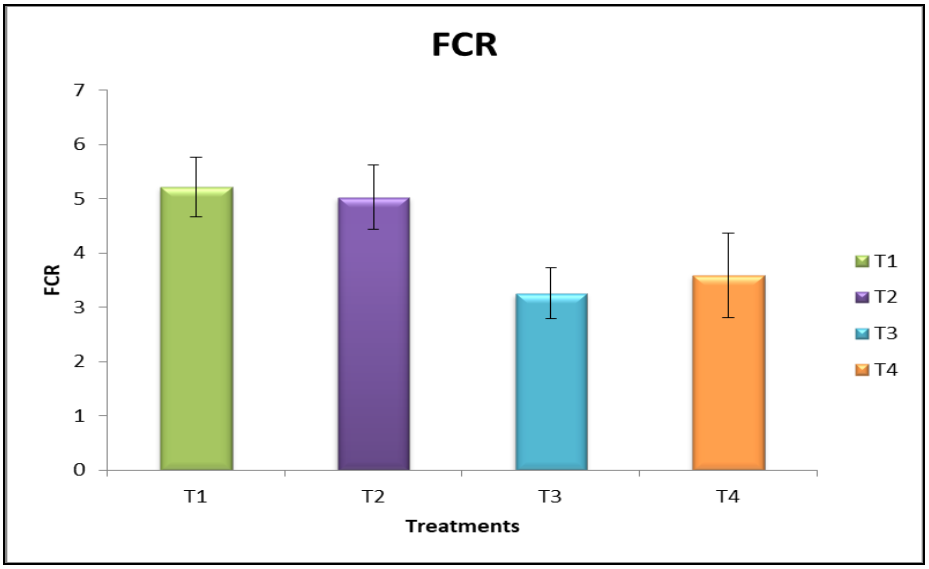

Fig 5: FCR value *Poecilia sphenops* under different dietary levels of moringa Vertical bars

Table 5: Water quality parameters

Treatments	P^H	DO (dissolved oxygen) (ppm)	Temperature (℃)
T_1	7.5-8.5	5-7	24-27.8
T_2	7.2-8.0	6-7.2	24-27
T_3	7.5-8.2	5.5-7.5	25-28
T_4	7.0-8.0	5-7.5	23-28

Conclusion

The Moringa plant is one of the cheapest close substitutes being a plant feed stuff, it has a nutrient profile that is close to what is obtainable in fishmeal and it can also be made available in quantity that can support the aquaculture industry. With these investigations revealing that 15% substitution rate of *M. oleifera* leaf meal for fishmeal in molly fish (*Poecilia sphenops*) feed produces highest result. In conclusion, this study indicated that moringa leaves used as a plant protein sources support the better growth performance (weight, weight gain, length, length gain, SGR), lower FCR value and as well as disease free molly fishes and having no adverse toxicological effect on the growth performance and development, fish feed can be produced at a relatively cheaper cost and as thus profit of fish farmers can be increased.

Disclosure statement

Conflict of Interest: The authors declare that there are no conflicts of interest.

Compliance with Ethical Standards: This article does not contain any studies involving human or animal subjects.

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