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Effect of *Acanthus ilicifolius* Linn. As feed ingredient in the growth performance of *Poecilia sphenops*

Saliny Aiyer*, KG Manju
Research Division, Department of Zoology, Mar Ivanios College, Trivandrum, Kerala, India

Abstract
This study exploits the threshold of the mangrove *Acanthus ilicifolius*, sea holly, as an ingredient of food of *Poecilia sphenops*, black molly. In various recent studies, *Acanthus ilicifolius* have been proved to be of high medicinal property though it is being used as traditional medicine among the tribes since very ancient times. The powder and silage form of the leaf substituted 25% of fish meal in the fish feed in two tests separately. The growth indicators such as Specific Growth Rate, Feed Conversion Ratio, and Condition factor were calculated and recorded after 45 days of observation. Specific Growth Rate, Feed Efficiency Ratio and Protein Efficiency ratio were significantly ($p<0.05$) greater in control group than experimental feed group. Biochemical indicators like fish tissue protein and glycogen were also examined at the end of the experiment and found to be greater in experimental feeds at significance level 1%. Protein was highest among the fish fed with leaf silage diet and Glycogen was highest among fish fed with leaf powder diet. Both leaf powder and leaf silage diet proved to be successful as feed ingredient.

Keywords: *Acanthus ilicifolius, Poecilia sphenops*, growth indicators, Microbial silage, *Staphylococcus aureus*

Introduction
One fourth of the world's food demand is met by aquaculture, nevertheless, demanding the aquaculture industry to increase its production in a sustainable way to satiate increasing food demand of ever growing human population. Apart from providing food, aquaculture also contributes widely in pleasing the aesthetic sense by displaying various forms and colors of ornamental fishes apart from aiding financially. Viviparous fishes such as Moly, Guppy and Oviparous fishes like Koi carp, Zebra fish etc. contribute to the piscine diversity and are the most sought after ornamental fishes all over the world [1].

Since last two decades' production of fish meal has remained constant, that is about 20%, but recently there has been a change in the trend as the demand for fish meal by aquaculture has risen, to about 60-70%. This clearly indicates the restrained availability of fish meal has eventually paved way for their high prices [2]. Hence, pressure has been build up in exploring innovative ways of discovering alternative feed ingredients that can replace fish meal, and are environmental friendly, easily accessible and economical [3].

Various studies have been successfully done in this regard using animal by-products and plant products as feed ingredients [4, 5, 6, 7, 8, 9, 10]. *P. sphenops* fed with garlic, *Allium sativum* supplemented diet was found to show enhancement in growth performance and body composition [11]. In another study involving the comparative study of effect of animal based waste products and agro based products as feed on *Poecilia latipinna*, showed that animal based products are more efficient in promoting growth and nutrition utilization and also cost efficient [12].

In a feed study in fries of *Poecilia sphenops* using live feeds, proved that former are more efficient feed when compared to pelletized feed, in the success of survival rate and growth performance [1]. Formulating artificial diets by substituting fish meal with ingredients of plant origin that supports the aquaculture industry to meet the increasing demand for affordable, safe and high quality aquaculture products is crucial [13]. Apple mangrove extract, *Sonneratia caseolaris*, supplemented in the diet of tiger shrimp provided with the positive results in various responses [14]. A study by Nagarajan proved that using mangrove leaf extracts as feed additive have potential to control the bacterial infections observed in ornamental fishes [15]. Through numerous studies on the characteristic medicinal properties of *A. illicifolius* has been studied, no concrete evidence till now is put forward, regarding its use as a potential feed ingredient in aquaculture.

However, this study intends to outline the efficiency of *Acanthus ilicifolius* as feed ingredient by partially replacing fish meal in powdered and silage form, to explore its influence on the overall performance of ornamental fish *Poecilia sphenops* with special stress on the growth performance.

Materials and Method
Site of Study
Research Lab at the Department of Zoology, Mar Ivanios College, Trivandrum was chosen for conducting the study.

Fish and Experimental Setup
Fish were collected from nearby fish farm. They were acclimatized for two weeks in their ambient temperature and pH. It was strictly noted that the temperature remained at 28± 2ºC and well aerated throughout the study. The fish tanks were maintained mild saline in order to avoid fungal attacks. The experiment was carried out in triplicates and each tank contained 10 fish for 45 days. 20% water from the tank was everyday replaced by fresh water so that dissolved oxygen concentration and pH was maintained along with preventing

accumulation of nitrites, nitrates and ammonia.

Experimental food formulation

Leaves of *A. ilicifolius* were collected from Mangalavanam area (9º59'13" North and 76º16'26" East) of Ernakulam district in Kerala. The plant material was identified as *Acanthus ilicifolius* L.by Curator of Department of Botany, University of Kerala, Kariavattom. The voucher specimen is kept in the Herbarium of Department of Botany, University of Kerala, Kariavattom with Voucher number KUBH-6026. The leaves were washed and shade dried for a period of two weeks. Dried leaves were finely powdered and stored at 4ºC. Fresh leaves were transformed to silage under sterilized condition using *Staphylococcus aureus* as the mediator [16]. The experimental diet was prepared by replacing 25% fish meal protein with powdered (T1) or silage (T2) *Acanthus ilicifolius*. The control diet and experimental diet were prepared using usual composition as suggested in Hardy Square Method [17]. The dietary composition is as follows:

Table 1: Table showing the percentage composition of each ingredient in three different diets

Ingredient composition (g)	Leaf powder diet T1 (%)	Leaf silage diet T2 (%)	Control
Fish Meal	10.53	10.53	14.04
Acanthus ilicifolius	3.51	3.51	NIL
Rice bran	3.1	3.12	3.2
Tapioca powder	2.84	2.84	2.84
Groundnut oil cake	9.72	9.72	9.72

Experimental design

The experiment was conducted in triplicates. The fish were fed at the rate of 5% of their body weight between 10-11 am every day, before feeding all the fecal matters were collected and dried. Once the fish were satiated, the unused feed was collected from the bottom of the tank through siphoning separately and oven dried. The weight and length of the fish were measured in the beginning and at intervals of 7 days till the end of the study, in order to avoid stress.

Water Analysis

Water from all the tanks was replaced 20% everyday with fresh water so as to maintain pH and remove waste materials. Parameters like temperature were measured using mercuric thermometer and pH using pHTestr 10 and dissolved oxygen following the protocol given in APHA [18].

Proximate analysis

The protein content of the feed and fish tissue was examined using Lowry's method [19]. Glycogen estimation was done following the protocol suggested by Seifter [20], Growth performances of the fish was observed and calculated every week. The growth parameters observed are weight gain (%), Feed conversion ratio (FCR), Specific Growth Rate (SGR), Protein efficiency ratio (PER) as proposed in the method of Gomes [21] and Aliyu-Paiko [22].

Growth parameters

Every seventh day of a week, the following growth parameters were observed and assessed following their standard formulas:

Percentage weight gain

Percentage weight gain = (final weight – initial weight) / initial weight x 100

Specific growth rate (SGR)

Specific growth rate = [Ln (final weight) - Ln (initial weight)] / Experimental period in days x 100

Feed Conversion Ratio

Feed Conversion Ratio (FCR) = Feed given (dry weight) / Body weight gain (wet weight)

Protein Efficiency Ratio (PER)

PER = Fish weight gain (g) / Protein intake (g, dry weight basis)

Survival Percentage

Survival percentage was calculated by the end of the experiment by observing the fish harvested at the end of the experiment and fish stocked at the beginning of the experiment

Survival % = [(Initial no. of fish - Final no. of fish) / Initial no. of fish) x 100

Feed efficiency

Feed efficiency was calculated as the percentage reciprocal of FCR.

FE = 1/FCR %

Condition factor

Condition factor, K = [Weight/ (Length) ^{3}] ×100

Result and Discussion

The result of the growth and biochemical parameters of *Poecilia sphenops* after feeding them with *Acanthus ilicifolius* in different forms replacing 25% of fishmeal protein from their diet, were observed and calculated successfully. The growth performance exhibited by Black molly showed that inclusion of *A. ilicifolius* in their diet results in the enhancement of their performance and survival when compared with control diet. The water parameters like pH, temperature and dissolved oxygen were found to be within the optimum range for the survival of fish.

Table 2: Table Showing Water Analysis during the Experiment

	Leaf powder diet	Leaf silage diet	Control diet
Temperature	29 ±1.8	29 ± 1.2	29 ± 1.6
pH	7.1 ± 0.4	7.1 ± 0.2	7.2± 0.1
Dissolved oxygen(mg/l)	6.8 ± 0.41	6.9 ± 0.55	6.7 ± 0.23

The values represented as Mean ± SD

Table 3: Table showing proximate composition of different feeds

	Protein	Carbohydrate	Fibre
Leaf powder diet	35%	12%	1.02%
Leaf silage diet	34.9%	15%	1.06%
Control diet	34.7%	19%	0.92%

Table 4: Table showing the biochemical performance of the fish after being fed with *Acanthus ilicifolius* inclusion diet

Sample (muscle tissue)	Protein (µ gm/ml)	Glycogen (µ gm/ml)
Leaf powder diet	968.33±0.35 [a]	0.1192±0.005 [a]
Silage diet	1090.41±0.14 [b]	0.09±0.001 [b]
Control	955±0.07 [a]	0.1118±0.006 [a b]

Confidence level at 99% (level of significance $p \leq 0.01$). Values with same superscript indicate that there is no significant difference among them. The values represented as Mean ± SD

Table 5: Table showing various growth parameters of fish observed during the experimental study

Growth Parameters	Leaf powder diet	Leaf silage diet	Control
Initial weight (gm)	2.11 ± 0.56	2.6 ± 0.32	1.28 ± 0.27
Final weight	2.65 ± 0.14	3.36 ± 0.73	2.1 ± 0.26
Change in weight	0.54 ± 0.12 [a]	0.76 ± 0.09 [b]	0.82 ± 0.19 [b]
Weight gain %	25.59	29.23	64.06
Initial length	5.2 ± 0.5	5.09 ± 0.9	4.5 ± 0.75
Final length	5.73 ± 0.44	5.78 ± 0.72	5.35 ± 0.63
Change in length	0.53 ± 0.07 [a]	0.69 ± 0.11 [b]	0.85 ± 0.16 [c]
Length gain %	10.2	13.55	18.88
SGR %/ day	0.22 ± 0.005 [a]	0.249 ± 0.005 [b]	0.478 ± 0.004 [c]
FCR	9.69 ± 0.04 [a]	7.95 ± 0.10 [b]	4.23 ± 0.05 [c]
Survival %	97.66 ± 2.08 [a]	96 ± 1 [a]	90.33 ± 1.5 [c]
PER	0.437 ± 0.03 [a]	0.516 ± 0.05 [a]	0.8512 ± 0.05 [b]
Feed efficiency (%)	10.3	12.5	23.8
Initial condition factor	1.50	1.97	1.40
Final condition factor	1.40	1.74	1.37

The level of confidence evaluated at 95% (Level of significance, $p \leq 0.05$). Values with same superscript indicate that there is no significant difference between them. The values represented as Mean ± SD.

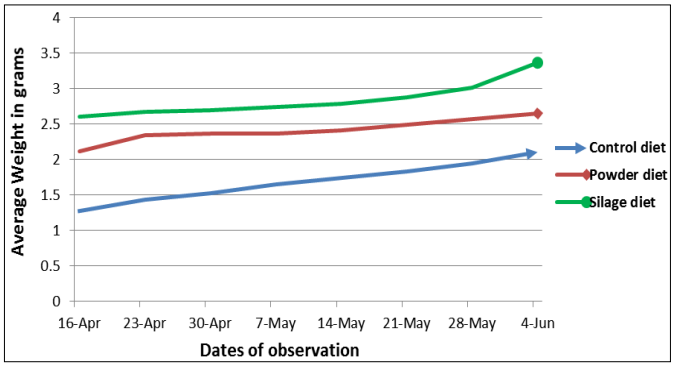

Fig 1: Line chart showing the trend of average weight gain of experimental and control diet fish during the period of study

The weight gain percentage was lower in experimental feeds, with leaf powder feed making the least change (25.59%). There was remarkable weight gain in fish fed with control feed (64.06%) which may be more palatable. Similarly, gain in length was also highest in control group. Specific growth rate value among fish was found to be highest in control diet (0.478± 0.004) and lowest in leaf powder diet (0.22 ± 0.005) which was significantly ($p < 0.05\%$) higher to the silage diet (0.249 ± 0.05). The feed conversion ratio in the fish fed with leaf powder diet was found to be highest, 9.69 ± 0.04 and that of control group found to be lowest (4.233 ± 0.05) and in silage feed, value was 7.955 ± 0.09. PER was shown to be highest in control group, that is, 0.851 ± 0.05, however there was no significant difference in the PER values of silage and leaf powder diet, 0.516 ± 0.05 and 0.437 ± 0.03 respectively. The condition factor monitors the feeding intensity and growth rates apart from contributing to the management of fish and maintenance of optimum ecosystem [23] [24].The final condition factor was found to depreciate from initial; nevertheless the depreciation was lowest among control fish followed by leaf powder diet which preceded silage diet. This difference in the condition factor may be attributed to the lack of added vitamins and minerals in the experimental diet, which the fish normally avail from natural conditions [25]. The muscle protein was found to be greatest in fish fed with silage (1090.41 µg/ml ± 0.14), followed by the fish fed with leaf powder (968.33 µg/ml ± 0.35) and lowest for fish fed with control diet (955 µg/ml ± 0.07). This indicates protein in the feed in which silage of *Acanthus ilicifolius* was included were more available to the fish for metabolism than in the leaf powder form. The fish fed with silage meal was found to have lowest muscle glycogen (0.09048 mg/100ml ± 0.02) preceded by fish fed with control diet (0.11178 µg/100ml ± 0.02) and the fish fed with leaf powder meal found to have highest glycogen concentration (0.11907 µg/100ml ± 0.05). There was no significant difference between the survival percentage among fish of leaf powder diet (97.6 ± 2.08) and silage diet (96 ± 1), though survival percentage was significantly low among control fish (90.3 ± 1.5).

Conclusion

This present study clearly depicts that there is no negative influence of *Acanthus ilicifolius* imparted on the growth and biochemical performance of Black molly. The muscle protein and muscle glycogen was found to be highest in fish fed with leaf silage feed and fish fed with leaf powder feed, respectively. The cellulose fiber present in leaf may interfere with the absorption of protein by the fish from the feed, hence reduced tissue protein content in fish fed with leaf powder diet

when compared to the silage and control diet fish. Silage diet exhibited an enhanced tissue protein in fish, which may be due to microbial activity that mediated the conversion of leaf substance into more palatable form for the fish. However, fish meal substituted by *A. ilicifolius* leaf transformed into silage is found to be promising through this study, therefore, exhibiting an increase in biochemical performance of the fish tissue from the experimental diet fishes. Specific growth rate, feed efficiency rate and protein efficiency ratio were highest in control fish whereas, feed conversion ratio was highest in fish fed with experimental diet with leaf powder (9.69 ± 0.04) and silage feed (7.95 ± 0.10). Most important promising aspect of the *Acanthus ilicifolius* included diet was the enhanced survival rate of the fish which might be because of *A. ilicifolius's* potential to increase immunity in the fish and fight pathogenic diseases among fish. Therefore, this study shows that including *Acanthus ilicifolius* by partially replacing the fish meal in the diet of *Poecilia sphenops* can enhance its overall performance.

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