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Kashish Bhardwaz
Department of Aquaculture,
Doon College of Agriculture and
Allied Science, Dehradun, India

Bhartendu Vimal
College of Fisheries, Kishanganj,
Bihar Animal Sciences
University, Patna, Bihar, India

Swapna Choudhary
Department of Fisheries Science,
Sri Krishna Mahila College,
Begusarai, India

Preeti Handa Kakkar
Department of Aquaculture,
Doon College of Agriculture and
Allied Science, Dehradun, India

Priyashi
BIT, Mesra, Ranchi Jharkhand,
India

Himanshu Singh
PIM Cell, Birsa Agricultural
University, Kanke, Jharkhand,
India

Corresponding Author:
Bhartendu Vimal
College of Fisheries, Kishanganj,
Bihar Animal Sciences
University, Patna, Bihar, India

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Effect of dried vegetable powder (broccoli) on growth and reproductive performance of black molly (*P. sphenops*)

Kashish Bhardwaz, Bhartendu Vimal, Swapna Choudhary, Preeti Handa Kakkar, Priyashi and Himanshu Singh

Abstract

Ornamental fish breeding and growing are an important sector of aquaculture, which shows increased demand, income and occupations within country in recent years. In this present study, the effect of broccoli powder on growth performance and fertility rate of black molly (*P. sphenops*) was assessed. 130 brooders molly were distributed into 12 glass aquarium at 10fish/tank in triplicate. Three different diets containing varying levels of broccoli powder were prepared and fed to fishes twice daily at 5% of their body weight for 60 days. The mean weight and mean length of brood fish at the beginning of the experiments were calculated to study the mean specific growth rate and the number, size and weight of spawn after breeding in T₀, T₁, T₂, and T₃ respectively. At the end of the experimental trial the mean number of spawn was 10.20±2.52, 11.28±3.59, 7.57±5.03, 10.33±3.33, the mean length of spawn was 0.75±0.04cm, 0.86±0.05cm, 0.642 ± 0.409cm, 0.853±0.044cm, the mean weight of spawn was 0.03±0.01g, 0.02±0.01g, 0.03±0.02g, 0.02±0.01g, and the mean specific growth rate (SGR) was found 0.092, 0.085, 0.097, 0.089 in T₀, T₁, T₂, and T₃ respectively. The observation made in the present investigation shows that Broccoli can act as a phytoestrogenic compound that can act as a potential as well as a natural additive to fish feed in ornamental fish industry to augment the fertility rate.

Keywords: dried vegetable powder, growth and reproductive

1. Introduction

Ornamental fish keeping is one of the most popular hobbies in the world today. There is lots of variety of ornamental fish species are found among them black molly is the most widely kept aquarium fish species. Ornamental fish reproduction and breeding has important role in increasing the fish farming industry benefits. Ornamental are from Asian origin ornamental fish species are popular known as aquarium fish [1]. Fish farmer are breed and raise many of species of aquarium fish, using a variety of technique some species will spawn after simple environmental changes have been made, such as changes in water temperature, pH, or conductivity. Other species required more advance methods including administration of hormone products by injection for induced spawning [2]. However, synthetic Furthermore, human chronic Gonadotropin (HCG), and Gonadotropin releasing hormone (GnRH) and other hormone are more expensive than plant and vegetable extract and their administration in fish is time-consuming, labor-intensive and requires specialist expert [3]. Replacement of fruits and vegetable instead of hormone could be as new approach in fish breeding. Broccoli is one of the most important fertility foods. It is an edible green plant which belongs to the cabbage family. It is a great source of vitamin C, calcium, vitamin K, omega 3 fatty acids and glucosinolates.

It reduces the risk of cancer, heart attack, stroke, allergy reaction and inflammation as well. The presence of calcium and vitamin K improves bone health and prevents osteoporosis. It is great source of omega-3 fatty acids which have been shown to help fertility by helping regulate hormone in the body. Broccoli looks firm, tight, bunched florets. Florets should be dark green, sage green or even greenish purple. Stems should not be too thick or too tough. It is reported that the dietary protein level influences the body weight in several fish species [4, 5]. Protein, lipid and carbohydrate are the major nutrients that provide energy for fish metabolism, growth and reproduction [6].

Broccoli is good source of omega-3 fatty acid, shown to help fertility by regulation of hormone within body, increase cervical mucus promote ovulation. The mollies are a small-sized tropical fish that is found naturally in the warm and peaceful rivers of Central America. Today, mollies are extremely popular fish to be kept in the community of an artificial aquarium, all around the world. Boschung [7] observed that mature male anal fin molly can be distinguished by the presence of a gonopodium, a modification of the anal fin into a rod like copulatory organ that is used for internal fertilization. Afzal [8], Suggested Two available ovulation inducing preparation Gonadotropin release hormone analogues with dopamine receptor antagonists viz. Ovaprim-C and Ovatide were tested in Pakistan on bighead carp *Aristichthys nobilis*. Hence the present study was undertaken to study the effect of broccoli vegetable powder on reproductive and growth performance of black molly.

Results and Discussions

Breeding behavior

The breeding behavior behavior of *P. sphenops* Courtship behavior was observed in both male and female they fishes took parallel position to one another and moved closely. In

most cases males followed females. While following the females the male usually remained at the tail region of the female. Sometime, they also took position at 90° angle to one another while head of male touched the abdomen of the female. While swimming very close, the male thrust the female at her tail region with his head. Following thrusting, both male and female performed mouth to mouth collision. After the collision they again started to follow each other.

Length and weight of *P. sphenops*

Growth of fish was measured on weekly basis for 60 days. Fish were taken out and length and weight was measured from each aquarium. The same procedure was repeated for each treatment and average length and weight was calculated on weekly basis. Length of black molly at each week interval for 60 days of culture period is shown. The initial average length of fish in T₀, T₁, T₂, and T₃ were 4.52cm, 4.54cm, 4.55cm, and 4.57cm respectively (Fig.1). The final average length of fish in treatment T₀, T₁, T₂, and T₃ were 4.61cm, 4.62cm, 4.64cm, and 4.65cm respectively. Weight of black molly at each week interval for 60 days of culture period is shown. The initial average weight of fish in T₀, T₁, T₂, and T₃ were 1.0gm, 1.15gm, 1.27gm, 1.4gm respectively (Fig.2). The final average weight of fish in treatment T₀, T₁, T₂, and T₃ were 1.72gm, 1.85gm, 2.0g, and 2.15gm respectively. Statistically there was no significant effect of treatments on length and weight increment of black molly in initial two weeks. The mean length of brood fish at the beginning of the experiment were followed by 4.52cm, 4.54cm, 4.55cm, 4.57cm in T₀, T₁, T₂ and T₃ respectively. The mean weight of brood fish at the beginning of the experiment were followed by 1.0gm, 1.15gm, 1.27gm, 1.4gm in T₀, T₁, T₂, T₃ respectively. Maclaren *et al.* (2006) [9] studies have shown that females prefer larger males when presented with several mates simultaneously.

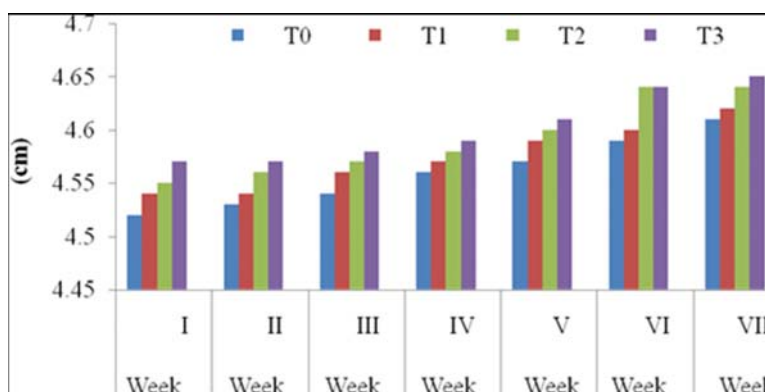


Fig. 1: Length increment (cm) of *P. sphenops* in different treatments during culture period

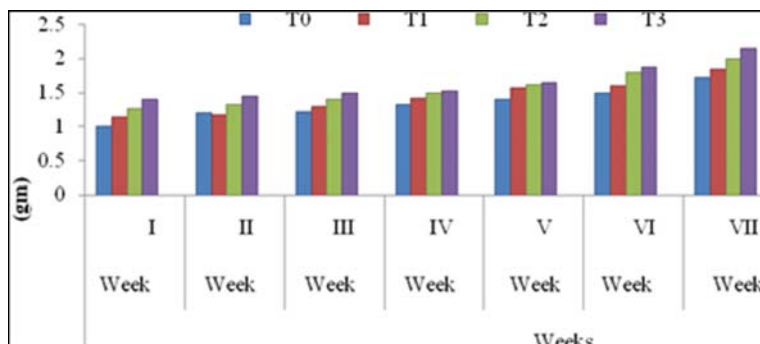


Fig. 2: Weight increment (gm) of *P. sphenops* in different treatment tanks during culture period

Number of spawn, length and weight

The mean number of spawn at the beginning of the experiment was 10.20 ± 2.52 , 11.28 ± 3.59 , 7.57 ± 5.03 and 10.33 ± 3.33 in T₀, T₁, T₂, and T₃ respectively (Fig.3). Females gives birth to up to 141 young, but usual brood size is 6 to 36 individuals; the number of young increases with the size of the mother^[10]. Zedler^[11] observed that females brood several times throughout the year, producing batches of up to 140 fry measuring about 8.7 mm each. Growth rates in female juveniles and adjust usually exceed those in males. The mean length of spawn at the beginning of the experiment were 0.75 ± 0.04 cm, 0.86 ± 0.05 cm, 0.642 ± 0.409 cm and 0.853 ± 0.044

cm in T₀, T₁, T₂, T₃ respectively (Fig.4). The mean weight of spawn fish at the beginning of the experiment were 0.03 ± 0.01 g, 0.02 ± 0.01 g, 0.03 ± 0.02 g and 0.02 ± 0.01 g in T₀, T₁, T₂, and T₃ respectively (Fig. 5). Brown^[12] indicated that Genistein as a phytoestrogen has effect on reproductive endpoints in a female fighting fish Bettasplenden. Phytoestrogens increased vitellogenin synthesis in Tilapia primary hepatocytes^[13]. Similar study also reported by Turkerand Takemura^[14] that plant extract such as fennel, fenugreek, aniseed, safflower, flaxseed, liquorice, pomegranate and soybean were considerably less potent than estradiol.

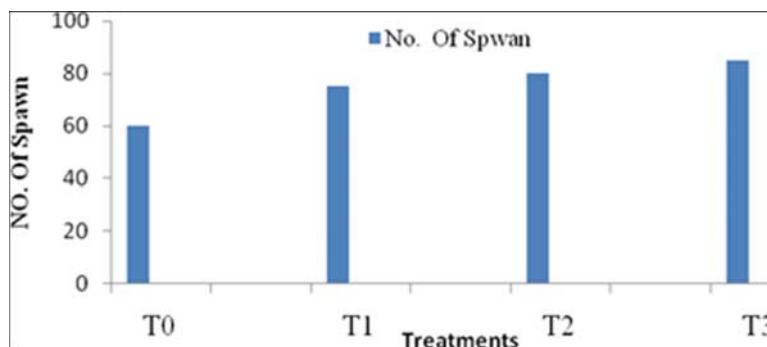


Fig. 3: Number of spawn in different treatment

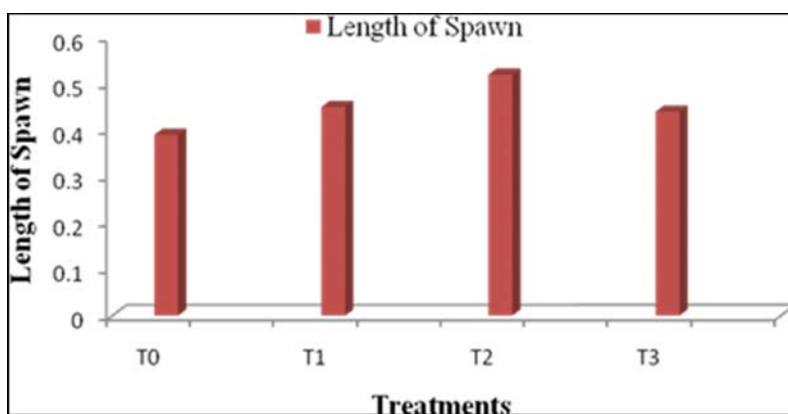


Fig. 4: Length of spawn in different treatments.

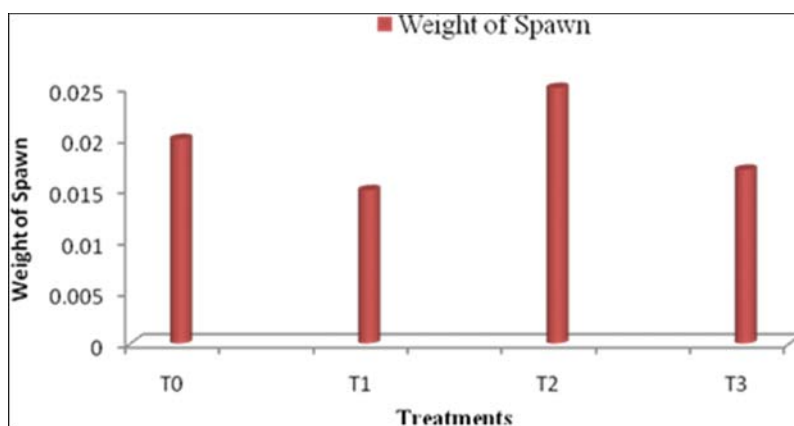


Fig. 5: Weight of spawn in different treatments.

Specific growth rate (SGR):-

The mean Specific Growth rate (SGR) at the end of the experiment was 0.092, 0.085, 0.097, and 0.089 in T₀, T₁, T₂, and T₃ respectively. The highest (0.097) was found in T₂, while the lowest (0.085) was in T₁. The mean FCR at the end

of the experiment were 5%, 10%, 15% in T₁, T₂ and T₃ respectively (Fig. 6). Generally, the commercial feeds contain a minimum of 12% fishmeal and additional animal by-products, which probably ensures them against serious fatty acid deficiencies^[15].

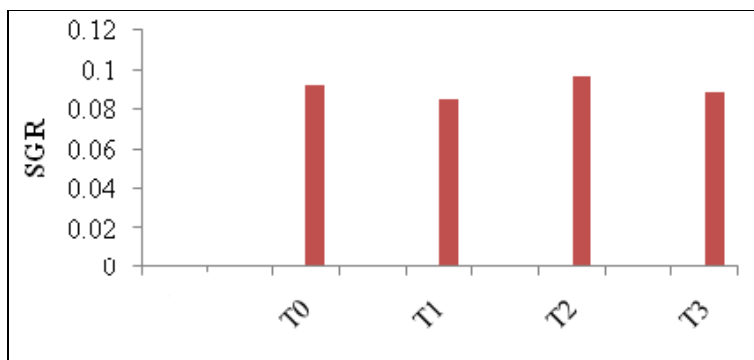


Fig. 6: Specific growth rate in black molly in different treatment tanks.

Feed conversion ratio (FCR)

The observation of mean weight gain obtain in various treatment have been presented. The highest feed conversion ratio was observed in treatment T₃ after 60 days of experiment which was significantly different from T₀, T₁, and T₂. The best result for feed conversion ratio in T₁ (5%), T₂ (10%), T₃ (15%) and T₀.

Survival rate of black molly fish:-

Survival rate of was measured for each treatment for 60 days. To determine survival of black molly in each aquaria daily observation was made and dead fishes were removed from each aquarium and their numbers were recorded. The average survival rate 100.00%, 90.00%, 80.00%, 80.00% was observed in treatments T₀, T₁, T₂ and T₃ respectively.

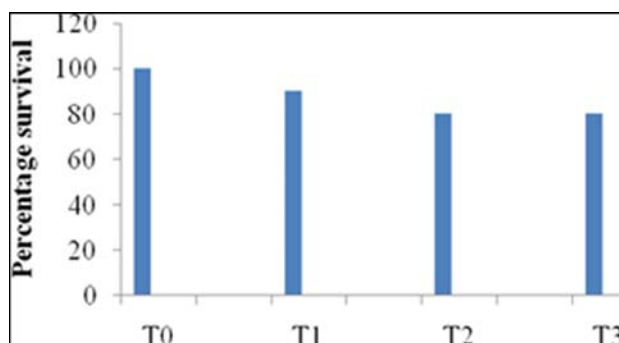


Fig. 7: Survival rate (%) of *P. sphenops* in different treatments during culture period

Experimental Section

Fish and experimental design

Black molly an omnivorous ornamental fish was chosen for the present study as the experimental animal. The fishes were collected from a fishery from at Rajpur road, Dehradun and brought in polythene bags filled with oxygen. The length of 4-5 cm approximately was selected for the study. The female fishes which have completed 10 days after the previous breeding were chosen. The broods fishes were transferred in previously prepared tank by hand scoop net to avoid any kind of injury. Nursing of the brooder was done with by provided with a commercial pelleted diet twice a day at the rate of 10% of their body weight. Total 3 treatments were made with different percentage of T₁ - Diet with 5%, T₂ - Diet with 10% and T₃ - Diet with 15% powder of Broccoli all the sample were compared with control diet T₀ - Control diet without powder of Broccoli. The whole experiment was conducted in controlled condition in the wet lab, experiment conducted of a group with triplicate (4T₃3R=12), 12 equal size (1x1x1 feet) glass aquarium. Each aquarium stocked with 10 fish of black molly. The fishes were fed with the 5% body weight per day for 60 days. The growth parameters in the term of length and weight measured weekly. 30% protein diet was prepared by Pearson's square method.

Preparation of experimental diet

Fresh 1kg Broccoli was purchased from a local vegetable market, Dehradun Uttarakhand India and it was dried at room

temp with thin cover for one week. After fully drying it was grounded using manual grinder. The grounded powder was kept in air tight box in laboratory condition for further use. For the Incorporation into fish diet at 0%, 5%, 10%, & 15% were used with other feed ingredients. All the feed ingredients procured and dried properly before preparation of the experimental diet. The dried ingredients were weighed individually and mixed well using grinder. The quantity of individual ingredients required to formulate a kg of diet was worked out using Pearson's square method to balance protein and energy levels. All the weighed ingredients were mixed thoroughly in a mixture grinder and oil was added to the dry ingredients. Subsequently all the ingredients were hand mixed to ensure homogenous mixing followed by addition of required quantity of boiled water and hand kneaded to form dough. The dough thus prepared was sterilized in an autoclave at 121 PSI for 15 mins sterilized dough then cooled under room temperature. After proper cooling, required quantity of weighed vitamin and mineral premix was added, mixed properly by hand kneading to prevent immobilization of vitamin and mineral premix, which were further palletized by using a hand pelletizer to form experimental feed pellets. The pellets thus formed were oven dried at 100°C. Finally, the dried pellets were powdered to approximate sizes before feeding to the experimental animals.

Estimation of proximate composition of formulated feed:

The proximate composition of three experimental diets and

one control were analyses following the procedures recommended by AOAC (1975) [17]. The parameters were analyzed moisture, crude protein, crude fat and Ash contents.

Table 1: Composition of experimental feed

Ingredients	(%)Composition of ingredients
Rice bran	18.25
Mustard oilcake	31.25
Soybean meal	31.25
Wheat flour	18.25
Agrimim forte	1.0
Total	100

Table 2: Proximate composition of experimental feed

Content	Percentage
Ash	7.0
Crude protein	25.0
Crude fat	7.50
Crude fiber	6.0
Mustard	11

Evaluation of growth and reproductive parameters

Growth parameters analysis

The growth parameters in the term of length and weight of the fingerlings measured forth nightly. The amount of feed adjusts accordingly weight. At the end of 60 day, specific growth rate (SGR), feed conversion ratio (FCR) were calculated.

Mean weight gain (MWG)

MWG = Final mean weight-Initial mean weight

Mean length gain (MLG)

MLG = Final mean length-Initial mean length

Specific growth rate (SGR)

$$SGR(\%) = \frac{\log(\text{finalweight}) - [\log(\text{initialweight})]}{t(\text{Timeintervalindays})} \times 100$$

Feed conversion ratio (FCR)

Feed conversion ratio is the ratio between mass of food consumed dry weight and increase in mass of animal produced.

FCR = Dry weight of feed given in gm/Wet weight gain in gm

Survival rate

The survival rate of each treatment was calculated by counting each species survived at the end of the experiment. The survival rate was estimated by the following formula:

$$\text{Survival rate} = \frac{\text{No. of fish harvested}}{\text{No. of fish stocked}}$$

Statistical Analysis

The data obtained was subjected to the statistical analysis following the CRD and the variation among the treatment means was tested for the significance by analysis of variance techniques as described by Gomez [18]. Level of significance used was P=0.05 from the table given by fisher. The critical difference between treatment means and the interaction between days and treatment have been worked out.

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