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Research article

EFFECT OF *AZOLLA FILICULOIDES* ON GROWTH, COLORATION AND LEUCOCYTES COUNT IN GOLD FISH, *CARASSIUS AURATUS*

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ABSTRACT: In the present study, feeding parameters, colouration and leucocytes count were studied in gold fish, *Carassius auratus* as a different levels of *Azolla* diet. The mean body length and weight of *Carassius auratus* as increased with increase in *Azolla* levels upto a level (50 g kg^{-1}) and declined thereafter. The feed consumption, feeding rate, feed conversion rate and specific growth rate of *Carassius auratus* as increased with increase in *Azolla* levels upto a level (50 g kg^{-1}) and declined thereafter. Maximum carotenoid content was observed in fish consuming 50 g *Azolla* kg^{-1} diet in skin, fin and muscle tissues while control group elicited the low carotenoid content. Skin exhibited the maximum coloration followed by fins and muscle in all the treatments. The number of lymphocytes and monocytes were increased with an increasing of *Azolla* levels upto 50 g kg^{-1} and thereafter they declined.

Keywords: *Carassius auratus*as, *Azolla filiculoides*, growth, colouration and leucocytes count.

INTRODUCTION

Ornamental fish culture is one of the most important fields of Aquaculture. There are various factors involved in ornamental fish culture and among these quality and quantity of food, density and water hardness are the most important (James, 1998). Generally ornamental fishes are fed with live feeds (James and Sampath, 2002; 2004) but the production of live feeds does not satisfy the demand of ornamental fishes. To make the fish culture operations economically viable, use of non-conventional protein sources for feed have to be identified which could either partially or completely replace the ingredients of fish meal. Moreover, Azolla also play an important role in ornamental fish culture. The Azolla is one such non-conventional protein feed and its chemical constituents are important factors to evaluate its potential. When global fishmeal production decreases and fishmeal prices become more expensive, nutritionists seek less expensive plant protein sources such as Soybean meal, [2, 17, 14].Azolla can be untlized not only as organic fertilizer for corps but also as feed for livestock and fish [39, 40, 15] had reported that *Azolla* could be used only by replacing 25% of fishmeal in the diet of tilapia. Increased FCR due to higher feeding of *Azolla* had also reported by many workers [25] in *Etroplus suratensis*, [16] in *Clarias gariepinus*, [3] in *Oreochromus niloticus*, [7, 36, 30, 32] decribed the use of Azolla as a feed for animals, particularly for fishes. Azolla can be an inexpensive feed for tilapia grown in rice fields increased fish production has been demonstrated in integrated rice fish, Azolla production systems where azolla served as an insitu fresh food for the macro phyto phagous fish [6, 26]; Azolla as fresh feed in combination with a food level of natural feeding can be beneficial to fish production [12].There are no reports on the *Azolla* in ornamental fishes. Hence, the present investigation has been undertaken to study the different levels of *Azolla filiculoides* on the growth, coloration and leucocyes count in gold fish, *Carassius auratus*.

MATERIALS AND METHODS

Collection of experimental animal

Active and healthy experimental animal gold fish, *Carassius auratus* where procured from J.J. Aquafarm, Nagercoil and they were brought into laboratory by polythene bags containing aerated water. They were fed *ad libitum* artificial fish feed twice in a day. After 1 hour of feeding, unconsumed food was removed by pipette. The aquarium water was changed twice in a week and replenished with chlorinated free well water.

Experimental diets preparation

Feed formulation was done according to [20] and 38% basal protein diet was prepared by using ingredients like fish meal, groundnut oil cake, tapioca and wheat flour, sunflower oil (lipid source) and mineral mixture. At first dried and powdered ingredients were blended to make a homogenous mixture. Subsequently, the feed ingredients were mixed with an aliquot amount of boiled water and then cooked in steam for 15-20 minutes. The dried Azolla powder was used as a source of protein and it procured from the azolla than prepared power form. α-tocopherol acetate that was used as the source of vitamin E and L-ascorbic acid was used as vitamin C. The appropriate levels of water dissolved ascorbic acid and acetone dissolved α- tocopherol acetate were sprayed over 1kg of 38% protein diet ingredients and then uniformly mixed. The pellets (2mm size) were prepared with a hand operated pelletizer and dried in sunlight.The dried diets were stored in a refrigerator until use .The experimental feeds were prepared once in two weeks to avoid the nutrient loss [10].

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Feeding experiment

One hundred active 30 days old juveniles of *Carassius auratus* (mean body length: 31.6±2.10 mm; mean body weight: 1.13±0.01g) were collected and divided into 5 groups, corresponding to five levels of *Azolla* diets (0,25,50,100 and 200 g kg^{-1} diet) each groups consisting of 10 individuals. They were stocked in a circular cement tank (75 lit capacity) containing 75 lit throughout the experiment. Triplicates were maintained for each experimental diet. The hydrobiological parameters like temperature, pH and dissolved oxygen were 29±1°C; 6.8±0.05 and 3.17±0.12 ml^{-1} respectively. The tank were drained once a day and replenished with freshwater to remove accumulated faeces from the bottom.

Feeding

The different weight groups of *Carassius auratus* were fed on weighed quantities of experimental diet *ad libitum* twice in a day at 0900 and 1600 hours for a period of 1 hour each. The unconsumed food remaining in the aquarium after the feeding time was collected with a pipette causing least disturbance to the fish. Water content of the food sample was estimated by drying a known weight at 80°C. To estimate the growth of the fish at the commencement of and termination of the experiment, "Sacrifice method" (Maynard and loosli, 1962) was followed. At the end of the experiment, the test animal was waited to estimate the growth of the animals. All weighing were made in an electrical digital balance too an accuracy of l mg. Before beginning the experiment, total body length (mm) and weight of the fish in each aquarium were measured. Every 10 days interval to measure the mean body length (mm) and weight of the fish in each aquarium. Total length of the 5 individuals was determined using a graph sheet and the average was taken as mean body length (mm). Mean body weight (g) was calculated by wet weight of test animals divided by total number of animals in the aquarium at that time.

$$\text{Mean body weight (g)} = \frac{\text{Wet weight of fish (g)}}{\text{No. of fish in the aquarium}}$$

Food consumption was estimated gravimetrically in terms of dry weight, subtracting the weight of unconsumed food from that of food offered.

Production (or) conversion was estimated as the difference between the dry weight of the fish at the commencement and at the termination of the experiment. Rates of feeding and conversion were calculated by dividing the respective quantities by the product of initial wet weight of the fish and duration (day) of the experiment. The rates were expressed as mg g^{-1} live fish day^{-1}. Gross conversion efficiently was calculated as the quantum of production to consumption.

$$\text{Feeding rate} = \frac{\text{Total food consumed (mg)}}{\text{Initial live weight of the fish (g)} \times \text{Number of days}}$$

Feed consumed = Total feed consumed (mg) – Unconsumed feed(mg)

Gain in weight was calculated as the difference between the wet weight at the beginning of the experiment and that on the day of calculation. Specific growth rate (SGR) was calculated according to the following expression.

$$\text{Specific growth rate} = \frac{W_2 - W_1}{t} \times 100$$

W_2 = Final wet weight of fish
W_1 = Initial wet weight of fish
t = time

Feed conversion ratio (FCR) was calculated by relating the feed consumption to gain in wet weight of fish.

$$\text{Feed conversion ratio (FCR)} = \frac{\text{Feed consumed (g)}}{\text{Wet weight gained (g)}}$$

Series: 2

Total carotenoids in the fins, skin and muscle of *C. auratus* were analysed following the method Bjerkeng (1992). Blood smear preparation was done according to James *et al* (2006).

RESULTS

The mean body length and weight of *Carassius auratus* increased with increase in *Azolla* levels up to a level (50 g kg^{-1}) and declined thereafter. The mean body length of experimental fish fed *Azolla* levels at the 0, 25, 50, 100 and 200 g kg^{-1} diets was 43.3, 46.2, 56.3, 52.2 and 43.9 mm respectively only on day 30. The mean body weight of experimental fish fed *Azolla* levels at the 0, 25, 50, 100 and 200 g kg^{-1} diets was 1.94, 2.16, 2.68, 2.32 and 1.96 g wet weight of fish respectively only on day 30.

Fish fed with 50 g *Azolla* kg^{-1} diet exhibited the maximum feed consumption. The feed consumption of experimental fish fed *Azolla* levels at the 0, 25, 50, 100 and 200 g kg^{-1} diets was 13.60, 15.10, 19.00, 16.50 and 13.70 g dry matter respectively only on day 30.

Fish fed with 50 g *Azolla* kg^{-1} diet exhibited the maximum feeding rate. The feeding rate of experimental fish fed *Azolla* levels at the 0, 25, 50, 100 and 200 g kg^{-1} diets was 83.95, 86.28, 95.47, 90.16 and 84.56 mg g^{-1} live fish day^{-1} respectively only on day 30 (Fig.1). Fish fed with 50 g *Azolla* kg^{-1} diet exhibited the maximum specific growth rate. The specific growth rate of experimental fish fed *Azolla* levels at the 0, 25, 50, 100 and 200 g kg^{-1} diets was 2.1, 2.5, 3.4, 2.8 and 2.0 respectively only on day 10 (Fig.1). The feed conversion rate (FCR) of experimental fish fed *Azolla* levels at the 0, 25, 50, 100 and 200 g kg^{-1} diets was 3.90, 3.40, 2.64, 3.10 and 4.15 respectively only on day 10 (Fig.1). Feeding rate and specific growth rate (SGR) were increased with increase in rearing period from 0 to 30 days in all treatments (Fig 1).

Total carotenoid content in skin and fins of *C. auratus* was increased with increase in rearing period and *Azolla* levels upto a middle level and declined thereafter. But total carotenoid content in muscle of *C. auratus* was decreased with increase in rearing period. Maximum carotenoid content was observed in fish consuming 50 g *Azolla* kg^{-1} diet in skin, fin and muscle tissues while control group elicited the low carotenoid content. Skin exhibited the maximum coloration followed by fins and muscle in all the treatments (Fig. 2). The number of lymphocytes and monocytes were increased with an increasing of *Azolla* levels upto 50 g kg^{-1} and thereafter they declined (Fig. 3). Similar result was obtained for thrombocyte. However, neutrophil and basophil showed the opposite trend.

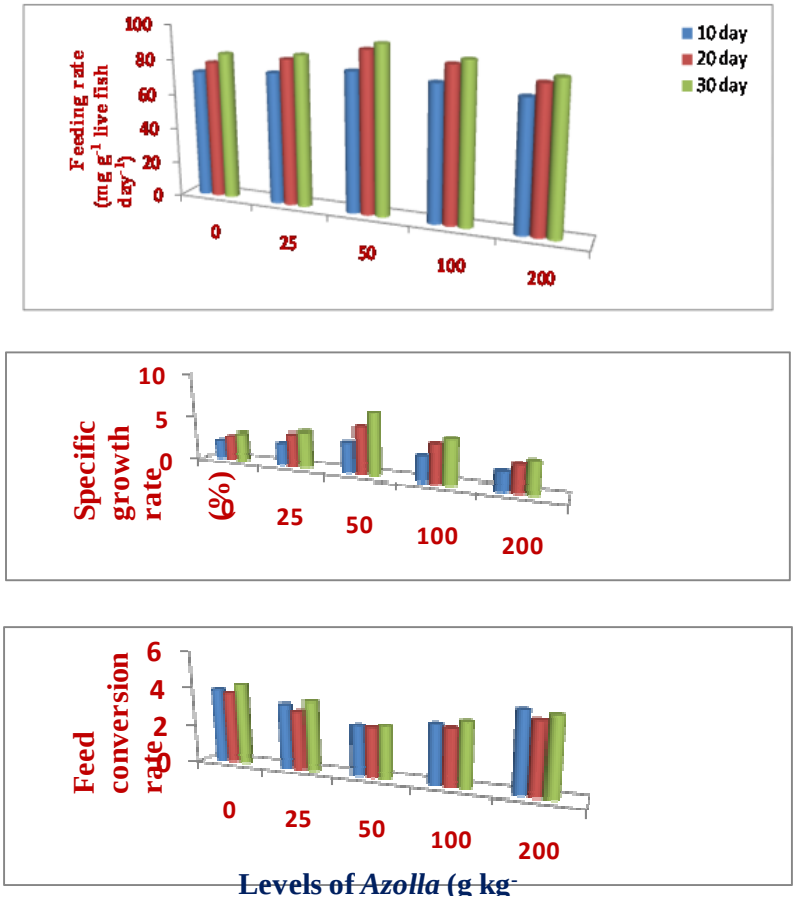

Fig.1. Effect of different levels of *Azolla* on feeding rate,specific growth rate and feed conversion rate as a function of rearing period in *C.auratus*.

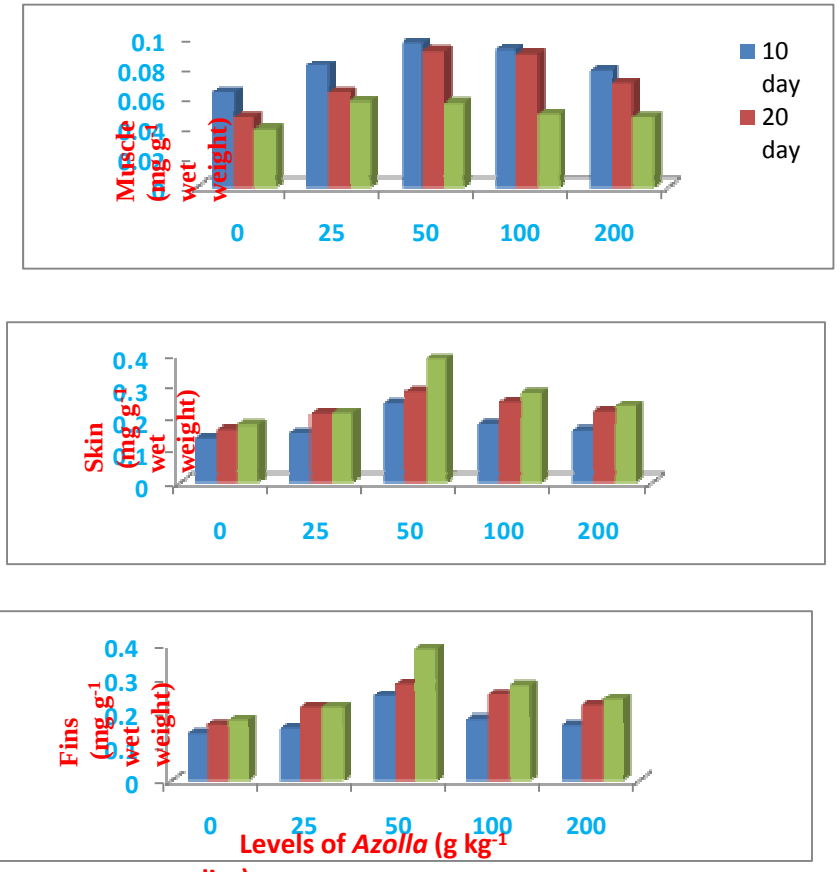

Fig.2. Effect of feeding different levels of *Azolla* (g kg^{-1} diet) on carotenoid contents (mg 100 mg^{-1} wet tissue) in muscle, skin and fins of gold fish, *C.auratus* as a function of time. Each value is the mean ($\overline{X}$ ±SD) of three estimations.

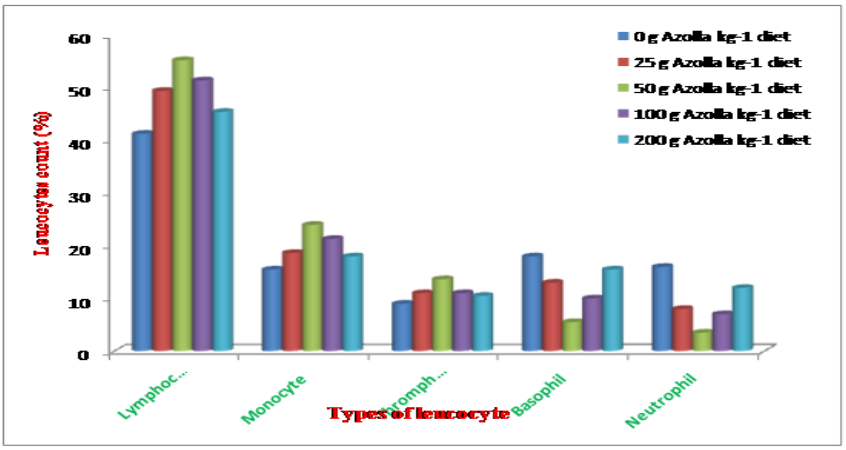

Fig. 3. Effect of different levels of *Azolla* diet on leucocytes count in *Carassius auratus*.

DISCUSSION

The present investigation reveals that, fish fed with 50 g *Azolla* kg^{-1} diet elicited the maximum feeding and growth parameters (mean body length and mean body weight, feed consumption, specific growth rate and feed conversion rate). [37] report that Azolla as a protein supplement to enhanced the bioenergetics parameters like feeding rate and growth in Tilapia. [21] found that 45% animal protein or plant protein significantly enhanced the feed consumption and growth rate in *X. helleri,* Vasudhevan (2008) found that 30% Spirulina diet significantly enhanced the feed consumption, feeding rate, specific growth rate and feed conversion rate in *Carassius auratus.* [41] found that different types of food quality on growth in Koi carp, *Cyprinus carpio var.koi*, which supports the observations made in the present study. Increased FCR due to higher feeding of Azolla had also reported by many workers [25] in *Etroplus suratensis*, [16] in *Clarias gariepinus*, [3] *Oreochromus niloticus*, [7, 34] found that ornamental fishes guppy (*Poecilia reticulata*) and platy (*Xiphophorus maculatus*) consumed maximum amount of *Spirulina* substituted feed than those fed with mushroom and azolla. [33] that high feed intake, body weight gain and specific growth rate in fish silver seabream (*Rhabdosargus sarba*) which consumed *Spirulina* substituted diet than those consuming soybean meal and chicken offal meal. Maximum growth rate was found in fishes fed with *Spirulina* diet than non-*Spirulina* diets [13, 28, 27] reported that, 5% supplementation of *Spirulina* resulted the higher body weight gain in nibbler, *Girella punctatus*. The high FCR value at post-spawning was probably as a result of larger proportion of the feed being utilized for maintenance (Brett and Groves, 1979) and spawning of eggs.

Even though the gold fish is brightly colored, dietary substitution of *Azolla* significantly further enhanced the coloration in the fins and skin. The increase in carotenoid content in skin, fins and muscle of *C. auratus* in relation to dietary carotenoid content of *Azolla* diets demonstrates that, the fish has capacity to utilize it efficiently. Similar observations in the muscle of trout and salmon have been made by a few authors earlier [38, 8]. A dose-dependent carotenoid content has been reported in the muscle of Arctic char and salmon [9, 18, 29] found that 36-37 mg astaxanthin kg^{-1} diet produced maximum coloration in goldfish, *C. auratus* and the coloration was stable even after 2 months. They also reported that feeding astaxanthin could be a suitable, way for goldfish producers to stimulate color among fish grown in an algae free environment. In ornamental fishes, (unlike salmon and Arctic char) the pigmentation was highly found only in the skin and fins and this might be due to acquiring, digesting, utilizing dietary carotenoids and transporting more directly to the skin and fins rather than storing in muscle [1]. The low carotenoid content in the muscle of *C. auratus*, indicates that the assimilated carotene is directly transported to the skin and fins to provide necessary pigmentation. According to [35] this was achieved by establishing reductive metabolic pathways from muscle to the skin and fins. In salmon, Arctic char and trout, the pigmentation of integument and fins occurs only during sexual maturation and a reduction in the muscle carotenoid is an indication that the carotenoids are mobilized directly to the integument and fins from the muscle during that season. High density lipoproteins have been demonstrated to be responsible for the carotenoid transport from muscle to integument in salmon (Ando and Hatano, 1988). Moreover, several abiotic and biotic factors are also expected to influence the ingestion, mobilization and metabolism of carotenoids like other feed constituents [19]. It is likely that, a similar mechanism operates in *C. auratus* also. Lymphocytes and monocytes were the leucocytes types with positive response to the addition of *Azolla* in the diet. [23] found that, *Xiphophorus helleri* fed with 8% *Spirulina* enhanced the lymphocyte and monocyte populations among leucocytes which inturn increased the resistance. The powder form and cell extracts of *Spirulina* were found to enhance immunity in *Ictaluras punctatus* by increasing phagocytic activity [31] α – and β – chitosan were used as immunomodulatory feed additive in carp nutrition [43] which supports the findings of present study. Based on the result of the present investigation, it was observed that, 50 g *Azolla* kg^{-1} diet produced more growth, coloration and immune resistance in gold fish, *C. auratus* then other treatments. Hence 50g *Azolla* kg^{-1} diet is considered as optimum level to maximise the growth, coloration and leucocytes count in *C. auratus*. *Azolla* could be used as nutrition and disease resistance feed additive in gold fish nutrition.

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