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Evaluating the Effect of Live Brine Shrimp (*Artemia franciscana*) on Growth Performance in Ornamental Fish, *Cyprinus rubrofuscus* (Lacepede, 1803)

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

Feeding is a critical factor for both cultured and ornamental fish species. This study aimed to evaluate the effects of live brine shrimp (*Artemia franciscana*) on the growth and biochemical composition of fish. In the present study, sixty days feeding trail was conducted on the growth performance of ornamental fish, koi carp (*Cyprinus rubrofuscus*). The type of food such as live feed, nutrient-enriched prepared feed and commercial feed exerted a significant impact on the growth and body composition of fish. The specific growth rate is high in live brine shrimp fed fishes followed by prepared food. After 60 days experiment showed that the maximum length and weight of live brine shrimp fed fishes were 7.5 ± 0.9 cm and 5.3 ± 1.7 gm, respectively. The survival rates of the fish were 100 % under all feeding groups. The biochemical profiles of the fish varied significantly between the different feeding regimes. These results suggest that live feed is more effective than pelletized feed for ornamental fish culture.

Keywords: Cyprinus rubrofuscus; koi carp; Artemia franciscana; live feed; ornamental fish; growth performance.

1. INTRODUCTION

Ornamental fishkeeping is becoming a popular and stress-relieving hobby. The USA, Europe, Japan are the largest markets and for ornamental fish, with over 65% of exports coming from Asia [1]. India's trade is developing rapidly, with an estimated one million fish hobbyists and an annual growth rate of 14%. There are at least 240 types of marine ornamental fish and 226 types of freshwater ornamental fish, with about 90% of freshwater species bred in captivity [2]. India, especially West Bengal, is ideal for ornamental fish culture due to its diverse favorable species, climate. and efficient distribution. Kolkata serves as the main export center, with the surrounding districts being major fish-producing zones. Over 2000 people are involved in this trade in West Bengal, supporting livelihoods for 150 families and serving as an additional income for over 500 families. The industry is mainly focused on major carps, which make up about 80% of the total inland fish production in India [3,4].

Artificial feed plays a crucial role in fish nutrition for aquaculture production, as it provides concentrated nutrients that are not always available in natural food [5,6]. Natural food typically contains a protein content ranging from 1:1 to 1:18, while artificial feeds have certain percentage of protein content that may varies depends upon the feed type. This deficiency can be supplemented with natural food. It's important to accurately determine the protein requirements for each species and life stage, as well as to understand and provide the specific dietary protein and amino acid requirements for optimal growth and health [7-14]. Lipids (fats) are highenergy nutrients that can be utilized to partially spare protein in aquaculture feeds. Lipids have about twice the energy density of protein and carbohydrates. Lipids in fish diets, supply essential fatty acids, and serve as transporters for fat-soluble vitamins [15]. Increasing the use of lipids in fish feeds can reduce feed costs by saving protein. However, it can lead to issues like excessive fat deposition in the liver, affecting fish health and quality. Marine fish are rich in omega-3 fatty acids and are good sources for fish diets. Carbohydrates, such as starches and sugars, provide cost-effective energy in fish diets. They are included to reduce feed costs and aid in feed manufacturing [16]. Starches are useful in floating feeds and cooking starch during extrusion increases its availability to fish. Fish can use up to about 20 percent of dietary carbohydrates [17,18]. Live feeds are vital for cultured fish. Unlike formulated diets, live prey is constantly available to fish larvae in the water column. The movement of live feed triggers feeding responses in fish and is more appealing than dry-formulated diets [19].

Commercial ornamental fish breeding is a significant industry. By identifying the most effective and cost-efficient feeding options, breeders can maximize profits while maintaining the well-being of the fish [20]. The nutritional requirements of ornamental fish vary, and the choice of food can significantly influence their growth rates and overall health. А comprehensive study on the subject can help fish keepers select the most suitable diet for their fish, ensuring they reach their full potential in terms of size and coloration. Understanding the nutritional needs of ornamental fish can also have ecological implications [21]. By finding the

most appropriate diet, it's possible to reduce waste and mitigate environmental concerns. As the demand for ornamental fish continues to rise, sustainable breeding practices are essential [22]. Identifying the ideal feeding can contribute to more responsible and sustainable ornamental fishkeeping, minimizing the impact on wild populations [9,23].

Hence this study focuses on the nutrientenriched fish feed and the live feed for the growth of ornamental fish, Cyprinus rubrofuscus. C. rubrofuscus is a species belongs to the family Cyprinidae. It is commonly known as koi carp. It is omnivore and its diet consists of crustaceans, small fish, insects, worms, plants and algae. Koi carp are bottom feeders but can feed at all levels. They have a large dorsal fin, a forked caudal fin, a single anal fin, and barbels. They can reach sizes of 1 meter and 11 kilograms and some live longer than 40 years. Mature males have a rounded body and solid-colored pectoral fins near their head. This attempt is made to enhance the understanding of ornamental fish nutrition, which would be a valuable insight for both enthusiasts and the ornamental fish industry as it supports the well-being of the fish, economic sustainability, and environmental responsibility in this thriving and diverse field of aquaculture.

2. MATERIALS AND METHODS

2.1 Live Brine Shrimp Culture

For live brine shrimp culture, 1gm of artemia seeds can produce 1,80,000 nauplii. Accordingly, the number of artemia seeds required for harvesting was measured. The 35 grams of salt was added in one liter of fresh water and the alkaline pH (pH 7.5 - pH 8.5) was maintained using pH meter and sodium bicarbonate. The artemia seeds were soaked in fresh water for 30 minutes so that their outer cells would be soft for easy hatching. After 30 minutes of soaking in freshwater, filter the artemia seeds in a 100 micron mesh and introduced it into the culture tank with salt water. The culture tank was connected with the aerator. After 24 hrs, artemia seeds were hatched. The hatched larvae were filtered using a filter tube. As the fishes are freshwater, the salt water was filtered and the fishes were fed with artemia only.

2.2 Preparation of Nutrient-enriched Feed

The required quantities (Table 1) of the various components (except vitamins, minerals and fish

oil) were dried well, powdered and mixed. To this, an adequate quantity of water was added to form soft dough. The dough was then boiled for one hour in a closed water bath. After cooking the dough was cooled without removing the lid. After cooling, an adequate quantity of vitamins, minerals and fish oil was added and mixed thoroughly. Finally, it was pressed through a pelletizer having a perforated disc. The noodles were dried and broken into pieces. Care was taken so that the pellet feeds were free from moisture. The dried feed was stored for daily use in an airtight container.

Table 1. Ingredients of prepared nutrientenriched fish feed

Ingredients	Quantity
Rice bran	400 g
Boil peanuts	300 g
Dry fishes	100 g
Carrot	100 g
Beetroot	100 g
Spleen amaranth	100 g
Egg	1no.
Vitamin, mineral mix	10 g
Fish oil	10 ml

2.3 Experimental Design

A lot of the experimental fish, *C. rubrofuscus* were purchased from Tamil Nadu Dr. J. Jayalalitha Fisheries University, Parakkai, Kanniyakumari District, Tamilnadu, India, and transported safely to the laboratory in oxygenated bags. Care was taken to avoid the stress of overcrowding. They were acclimatized to the laboratory conditions in a plastic trough with water for 10 days. Fishes of the same sizes were taken to find out the effect of quality of food on the growth and body composition.

About 45 healthy young stage fishes of uniform size were taken and divided into three groups with 15 each. Three groups were named as group A, group B, and group C. Group A was fed nutrient-enriched feed, group B live feed (Artemia) and group C commercial feed (Taiyo). Each group was further subdivided into 3 groups with 5 fishes each and introduced into the troughs of uniform size, colour and shape filled with the same amount of water. Thus, the experiment was carried out in triplicates. Before the fishes were released into the experimental troughs they were weighed using balance and measured length using scale. The experimental fishes were fed daily thrice with prepared nutrient-enriched feed (Group A), Artemia (Group B), and commercial feed-Taiyo (Group C)

respectively *ad libitum*. The water was renewed once every four days. At the time of changing water, in order to undisturbed the fishes, the water in the experiment aquarium was siphoned out along with the wastes and then fresh water was added uniformly. The fishes were then fed with the concerned diet. The fish were grown for 60 days. The growth in terms of weight and body length (total and standard length) was measured on a 10-day interval throughout the experimental period of 60 days.

2.4 Growth Analysis

The experimental fishes were weighed using weighing machine and their length [both standard length (SL): from the tip of the snout to the posterior end excluding the length of the caudal (tail) fin, and total length (TL): from the tip of the snout to the tip of the longer lobe of the caudal fin] was measured periodically. The weight loss or gain i.e. growth due to different types of food intake were found out and expressed in unit weight. Growth in terms of length was also calculated by subtracting the initial length of the fish from the final length of the fish.

Growth in terms of wet body weight (weight gain) = Final weight - Initial weight

Growth in terms of body length (length gain) = Final length - Initial length

Specific growth rate (SGR) % =

 $\frac{(\textit{Final weight-Initial weight})}{\textit{Duration (days)}} \times 100$

2.5 Biochemical Analysis

After the 60th day of rearing the fishes were sacrificed and dried to measure the body composition of protein, carbohydrate and lipid. The biochemical analysis was determined using

the following Association of Official Analytical Chemists (AOAC) [24].

3. RESULTS

The experiment was performed in fresh water and the water quality was assessed before the experiment. The water quality parameters such as temperature, salinity, pH, dissolved oxygen (DO), total dissolved solid, total suspended solid, turbidity and ammonia were measured and the results were mentioned in Table 2. The present attempt clearly shows that the different feed types have an impact on the growth and biochemical content of the fish, *C. rubrofuscus* (Fig.1).

3.1 Growth Performance

The fishes fed on live feed (group B) attained maximum growth rate than those of the other feed A and C. The initial body wet weight and body length of the fish was measured in 10 days interval. The initial reading and 60th day measurements were detailed in the Table 3 and 4, respectively.

3.2 The Impact of Different Feed on the Growth

The impact of different feed on the growth of the fish, *C. rubrofuscus* fed with different types of food on the 60th day of the experiment is given in. Fig 2. In addition, the maximum growth and SGR was achieved by the live feed group (group B) compared to the other two feeds. The SGR was 0.5, 1.5 and 0.83 for the experimental groups A, B, and C respectively.

3.3 The Impact of Different Feed on the Body Composition

The proximate composition of the fish was analyzed at the end of the experiment to know ash, and crude fiber content of the fishes. The fish proximate composition was represented in the Table 5.

Table 2. Water quality parameters were performed before the experiment

Parameter	
Temperature (°C)	26.87 ± 0.94 ^a
Salinity (ppt)	0.029 ± 0.07^{a}
рН	7.69 ± 0.25^{a}
DO (mg.1 ⁻¹)	6.43 ± 0.56^{a}
Total dissolved solid (mg.L ⁻¹)	42.38 ± 0.73^{a}
Total suspended solid (mg.L ⁻¹)	14.06 ± 10.45 ^b
Turbidity (NTU)	1.00±0.93ª
Ammonia (mg.L ⁻¹)	0.05±0.02ª

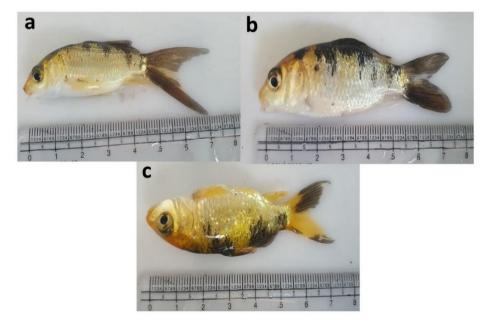
Values are presented as mean \pm SD. Means that are followed by the same letters (a, b) are not significantly different (P \leq 0.05)

Group	Initial body wet	Body Length (cm)	
-	weight (gm)	Standard length	Total length
Group A	3.6±0.5	5±0.6	6.5±0.4
Group B	4.4±1.7	5.2±0.7	7.3±0.9
Group C	3.7±0.9	5.2±0.4	7±0.7

Table 3. Initial body wet weight and body length of the fish, <i>C. rubrofuscus</i> . The value
represents an average performance of the initial individual (Mean \pm SD)

Table 4. The weight and length of the fish, <i>C. rubrofuscus</i> fed on different types of food on the
60 th day of experiment. The value represents an average growth performance of fish (Mean ±
S.D)

Group	Weight of the fish (gm)	Body Length (cm)	
		Standard length	Total length
Group A	3.9 ± 0.6	5.3 ± 0.5	7 ± 0.7
Group B	5.3 ± 1.7	5.8 ± 0.7	7.5 ± 0.9
Group C	4.2 ± 1	5.5 ± 0.4	7.2 ± 0.6



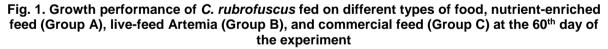


Table 5. Proximate composition of fishes feed with different feeds at the 60 th day of
experiment. The value represents an average growth performance of fish (Mean ± S.D)

Proximate analysis	Group A	Group B	Group C
Ash	15.25 ± 0.07	10.15 ± 0.05	12.80 ± 0.06
Crude fiber	3.59 ± 0.09	3.67 ± 0.03	3.92 ± 0.04

3.4 Protein Content

The protein content of the whole body of the fish in the experimental groups is presented in Table 6. The results revealed that the protein content in the fish fed group B is high comparatively higher to other groups. The fishes fed with commercial feed also showed high protein content compared to prepared feed. The values of protein in fish group A, B and C was 8.8 ± 1.1 mgL⁻¹, 10.1 ± 0.4 mgL⁻¹, 9.8 ± 0.3 mgL⁻¹ respectively.

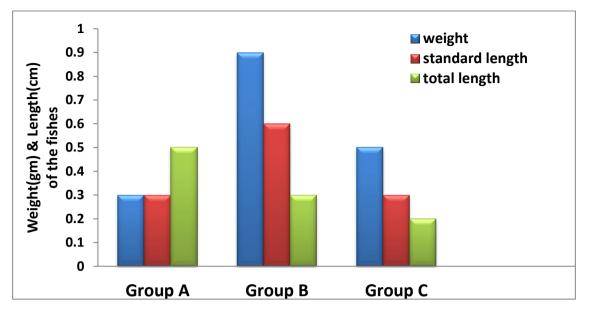


Fig. 2. The impact of feed on the growth of the fishes during the experimental 60 days

Table 6. The protein, carbohydrate, and fat composition of the experimental fish after 60 days	j,
of experiment. The value represents an average performance of fish (Mean ± S.D)	

Group	Protein (mgL ⁻¹)	Carbohydrate (mgL ⁻¹)	Fat (mgL ⁻¹)
A	8.8±1.1	4.6±0.4	0.8±0.2
В	10.1±0.4	1.2±0.8	0.4±0.05
С	9.8±0.3	3.4±0.7	0.3±0.2

3.5 Carbohydrate Content

The carbohydrate content of the fish in the experimental group A, B and C are presented in Table 6. The results showed that the carbohydrate level of the group fed with prepared feed (group A) was greater than other groups. The values of carbohydrates in the group A, B, and C were 4.6 ± 0.4 mgL⁻¹, 1.2 ± 0.8 mgL⁻¹, and 3.4 ± 0.7 mgL⁻¹ respectively.

3.6 Fat Content

The fat content of the whole body of the fish in the experiment groups is presented in Table 6. The results showed that the fish fed with prepared feed (group A) showed higher fat content than the other groups. The value of fat in fish group A, B and C was 0.8 ± 0.2 mgL⁻¹, 0.4 ± 0.05 mgL⁻¹, and 0.3 ± 0.2 mgL⁻¹ respectively.

4. DISCUSSION

The primary aim of fish farming is to maximize survival and growth at minimum cost [25]. In general, the fishes display diversified food spectrum and feeding strategy, the growth of culture fishes is influenced by many factors. In order to grow and survive, fishes require adequate nutrition. Food is the major source of input energy into the physiological machinery of the organisms. The nutritional value of the food is an important aspect to be evaluated to analyze the influence of food on growth. Thus, the nutritional composition may influence the growth i.e., the composition of carbohydrate, fat, and protein content of the food. Meanwhile, the experiment was conducted for sixty days to analyze the average growth attained within this particular period [10]. The growth performance as well as the biochemical composition results was in agreement with the study of Khan and Magbool [26], who performed an 8-week feeding trial to study the effects of dietary protein levels on the growth, feed utilization and haematobiochemical parameters of mirror carp, C. specularis.

Shaun et al. [27] reported artemia as a sustainably cultured live feed for ornamental fish with immunostimulant properties when bioencapsulated with spirulina *Arthrospria platensis*. Sustainable in-house cultures of bioenriched live feeds for ornamental fish could

enhance the fish's development, nutrition and welfare by improving immunity and reducing costs. Artemia is an economical easilv harvestable feed with its nutritional profile highly dependent on its diet which is easily manipulated through bioencapsulation. They evaluated the effects of two types of commonly used feeds: Artemia spirulina and yeast, and analyzed how they affect the growth, feed conversion ratio (FCR), survival rate, immunity, and color intensity of ornamental fish, compared to a commercial pellet (control) diet. Fish growth is essentially indeterminate, to the extent it is difficult to establish the ultimate maximum body size of individuals as they live concluded that response to dietary supplements result from the combined effect of both simulations of food intake and the improved metabolic efficiency.

The purpose of this study was to investigate growth performance and the body composition of koi carp, fed with three different feeds namely, the prepared feed (group A), the live feed artemia (group B), and the commercial feed (group C). Growth in terms of weight gain was highest in fish fed with feed B which contained the live feed. The development of fish in cultural conditions depends upon the availability of essential nutrients in the diet, digestibility, and its effect on the meat of fish. These parameters are considered the basic growth of the fish [28]. An increase in weight was recorded in fishes fed with three types of food. This shows that more of the experimental diets contain growth factors. This observation is in agreement with the finding of Sogbedan et al. 2006 [8]. There is a welldefined relationship between the rate of growth and the amount of food consumed.

The present study concentrates on the estimation of protein, carbohydrate and lipid levels in the experimental fish in order to know the effect of different feed. Marked variation in the level of protein, lipid and carbohydrate was observed in koi carp. Total protein was high in group B, fish fed with live feed. The results of the present study showed that live feed improved fish growth and protein content of the body. Our findings were in line with the results of Bharti et al. [16], who reported that fish meal could be successfully substituted with other feed ingredients. Protein is the most important constituent in living tissues, which is of considerable metabolic and structural value. Therefore, any change in this constituent indicates the stress inflicted on the metabolic functions required for maintaining a healthy

physiological state. In this work the protein level is high in the fish (C. rubrofuscus) which feed on the live feed (group B). Lipids are organic substances insoluble in water, but soluble in organic solvents. They form important dietary constituents because of their high calorific value and fat- soluble vitamins and essential fatty acids contained in them. They are present in the cytoplasm as well as the cell membrane and also in the specialized areas of the body as deposits of fat. The lipid level in the fish (*C. rubrofuscus*) is observed maximum in group feed on the prepared feed (group A). Carbohydrates are basic substances of protoplasm and involved in the storage and release of energy. They are defined chemically as aldehyde or ketone derivatives of the higher polyhydric alcohols or as compounds which yield these derivatives on hvdrolysis. Glucose, fructose, mannose, sucrose, galactose, maltose, lactose, and glycogen are the important carbohydrates in the animal cells. The carbohydrate level is observed maximum in the fish (C. rubrofuscus) feed on the prepared feed (group A) [29].

The chemical composition of fish is affected by environmental conditions and there are considerable differences in the chemical composition of reared and wild populations [30]. From the above discussion, it is evident that the dietary constituents affect the biochemical nature of fish tissues and growth performance.

5. CONCLUSION

The present study concludes that the rearing of *C. rubrofuscus* with different feed types influences the growth performance. The fish showed significant growth with the live feed, artemia compared to other man-made feeds. Similarly, the protein was high in the case of artemia fed fishes. Hence, this live feed provision might support fast growth with less investment.

DISCLAIMER (ARTIFICIAL INTELLIGENCE)

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc.) and text-to-image generators have been used during writing or editing of manuscripts.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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