Journal of Entomology and Zoology Studies

Available online at www.entomoljournal.com

E-ISSN: 2320-7078
P-ISSN: 2349-6800
www.entomoljournal.com
JEZS 2020; 8(6): 260-265

Received: 13-08-2020
Accepted: 02-10-2020

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Studies on the effects of different dietary protein levels on the growth and survival of *Channa striatus* fry to fingerling stage

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Abstract
The importance of the present study, to evaluate to identification of the effective level of protein in the diet plays the role on growth and survival of *Channa striatus* fry. The experiment was conducted in wet laboratory of College of Fisheries Science for a period of 56 days. The fry was reared with four semi-purified diets containing crude protein percentage of CP 40%, CP 45% and CP 50% and control were estimated for the protein requirement of *Channa striatus* fry (initial weight 0.5 ± 0.01g and length 3.0 ± 0.1cm) in triplicate set. The fry was reared in 12 fibre tanks (including control treatment) of 54 litre capacities (size 0.6x0.3x0.3 m) and at a stocking density of 15 fry per tank and fed @ 8–5% of body weight. The AWG was significantly high in diet CP 45% (6.11±0.07) in comparison with other treatments and control. The SGR were found significantly (p<0.05) higher in diet CP 45% (1.31 ± 0.01) than other treatments. The FCR were significantly low in diet CP 45% (1.52 ± 0.01) followed by CP 40%, CP 50% and lowest in control. The diet CP 45% showed significantly (p<0.05) high survival levels (75.5%) when compared to other treatment diets after 60 days of rearing. The study revealed that the protein requirement of *C. striatus* fry is CP 45% and the fry could be reared to fingerling size on formulated diets.

Keywords: AWG, *Channa striatus*, crude protein, FCR, SGR, survival

Introduction
Striped Murrel, *Channa striatus* is a freshwater fish that belongs to the family Channidae. It is one of the favourite freshwater fishes in Asia-Pacific countries and Africa [1]. *Channa striatus* is the state fish of Telangana State and locally called as 'Korrameenu'. Among the other fishes, it is regarded as an excellent table fish in Telangana and Andhra Pradesh states [2]. In their native range, many snakeheads are commonly used in aquaculture and are highly valued as food fish [3, 4] Cited this species as the most economically important member of the snakeheads. Most investigations on this species were commonly found on nutritional values [5]. Young murrels also used as aquarium fish because of their bright orange body colour [6].
The fish is well known for its taste, high nutritive value, recuperative and medicinal qualities. *Channa striatus* contains all the essential amino acids for wound healing and pain reduction from arthritis particularly glycine as well as high contents of arachidonic acid (AA) and polyunsaturated fatty acids (PUFAs) that can promote prostaglandin and thromboxin synthesis [7]. Therefore, amino acid composition of *Channa striatus* suggests that the fish is an excellent source of dietary protein for human [8]. Murrel fingerlings are used for giving medicine every year in the month of June on the eve of Mrigasira Karthi day for dispensation to asthma patients (Fish Medicine) that gather from all over the country in Hyderabad City since long back. The International Union for Conservation of Nature (IUCN) status of striped murrel in Telangana State and Andhra Pradesh was found to be in 'Least Concern (LC)' category [9] where as in Bangladesh this species is acknowledged as an endangered fish [10].
Murrels and Catfishes are the second most important group of freshwater fishes of commercial importance in A.P. Murrels constitute the most common and dominant group of air breathing freshwater fishes in the Andhra Pradesh. About 28 to 30 *Channa* species have been reported in the global scenario with 8 to 10 species in India [11]. There are several species of murrels belonging to the genus Channa (syn. Ophiocephalus), but only four types of murrel species are

available in A.P. viz *Channa striatus*, *C. punctatus*, *C. marulius* and *C. gatchua*. Among these, one species, namely *Channa striatus* enjoys a good deal of popularity as food fish in many parts of A.P. along with [12]. Among freshwater fishes marketed in India, air breathing fishes constitute 13% [13]. There is some information available on the life history of *Channa striatus*, but there is not enough information available about the nutrient requirements of this fish except for some reports on protein requirements [14]. Presently, striped murrel developed into a major cultivable fish and became economically important in both culture and capture fisheries. It is known from the study of [15] that *Channa striatus* could be cultured in cages and fences.

For the development of any aquaculture operation, one of the main problems is the availability of seed. Indian fish farmers are unable to culture murrels due to non-availability of seed as well as feed. Larviculture of murrels is a tough task, since they are carnivorous, piscivorous and cannibalistic. They can easily consume more than half of their length and high cannibalism occurs in juveniles [16]. The fingerlings of the murrels are available in rivers, reservoirs, perennial tanks and other derelict water bodies [17]. Optimum growth of aquatic animals largely depends on food quality [18] provided during the weaning period of larviculture [19].

The status of tropical aqua feeds and feed management has been reviewed by various authors [20, 21]. Most of these reviews are aimed on feed and feeding management at the grow-out stage, as there is high feed cost during this period. However, it is important to conducted trials on feeding management in tropical aquaculture since from the larval stages, as feed management practices at the grow-out stage will be governed by the early nutrition and feeding history of the fish. Therefore, the aim of the present study was to evaluate the dietary protein requirement of the striped murrel fry so that a nutritionally balanced diet could be developed for raising fingerling in captivity in India.

Material and methods:

Procurement of Experimental Fry and their acclimatization:

The experiment was conducted in wet laboratory of College of Fisheries Science, Muthukur, Nellore for a period of 56 days. The fry of Channa striatus were collected from the creek namely Kolleru Lake in Eluru Town, West Godavari District of Andhra Pradesh state. The fry was transported to the experimental site through the train in an Aluminium tin which is a specialized can for murrel seed distribution. The fish has Air-Breathing organs could survive for long distances and the fry were observed active and free swimming. As the fry reached/brought to the experiment site, they were transferred to the Wet Laboratory and placed for 24 hours in a circular tank consisting of clean ground water.

Fish fry collected from the wild are often infected with parasites and need to develop suitable treatments to enhance survival rates. Hence, fry was dipped in 1 ppm $KMnO_4$ solution for 30 seconds and were carefully released into a 0.60 m diameter circular, FRP holding tank, with about 0.48 m^3 of chlorine-free ground water. Then the fry was transferred to circular reservoir tank (of the same size as previous) with the clean, de-chlorinated ground water free from suspended solids and sand particles. The water was kept static, and adequate aeration was provided with two air stones (HANA-200). The fry was not fed and allowed to acclimate for 24 hours prior to the commencement of the feed experiment.

Weaning Period

The weaning period was for 4 days before starting the experiment. In this period fry of *Channa striatus* were trained to accept Formulated feed consequently. The initial diet of 100% egg white was given as crushed particles for the first day, followed by 75% egg white and 25% Formulated Feed (FF) on the 2nd day, 50% egg white and 50% Formulated Feed on the 3rd day and 25% egg white and 75% Formulated Feed on the 4th day. Fish were fed to satiation four times a day during daylight hours at 07:00, 11:00, 15:00, and 18:00 hrs. Thereafter the fishes fed twice a day with the formulated pelleted diet throughout the experiment.

Experiment Design

The experiment was conducted for the effect of different dietary protein percentages (CP 40%, CP 45% and CP 50%) fed to *Channa striatus* fry. Feed trial was conducted in triplicate set with three variable diets in 12 fibre tanks of 54 litre capacities (size 0.6x0.3x0.3 m) kept indoor in Wet Laboratory, Department of Aquaculture, College of Fishery Science, Muthukur, Nellore. The tanks were filled with 50 litre ground water and covered with green house netting to avoid entry of light into the experimental tanks, escaping of fishes and also to prevent insects and organisms entering into the tank (as the fishes are carnivorous preying on insects and other organisms may alter the feeding programme and growth of experimental fishes). A household tube light was placed at the ceiling in order to avoid direct penetration of light into the tank. A 12hr/12hr light and darkness maintained in the laboratory throughout the experiment. Fifteen fries (mean length 3.00 ± 0.1cm) of test fish were stocked in each of the nine designated tanks. The average initial weight (approximately 0.5±0.01 g) of stocked fry of each of the tank was recorded before placing them in the tank which did not differ significantly among three treatments. Feed was given twice a day (10.00 and 16.00 hrs) initially @ 8% of average body weight for first week and then adjusted to 7% of average body weight for the next two weeks and then to 5% of average body weight for the rest of the period. Fish were considered satiated when they stopped consuming or searching food and hide at the corners of the tank. Thus, on weekly sampling feeding rates were adjusted accordingly. The tanks were cleaned on everyday morning by scrapping the dead algae attached, with the help of scrapper. The tanks were siphoned off everyday morning by siphoning tube to reduce the nitrogenous waste accumulated as debris and faecal matters and 50% tank water was replaced with fresh chlorine-free ground water. Aeration was not provided to the tanks directly but instead already aerated water from the reservoir tank was pumped to the experimental tanks.

Experiment diets and feeding programme

Experimental diets were prepared in the Nutrition Laboratory of Department of Aquaculture, College of Fishery Science, Muthukur, Nellore. The experimental diets were prepared separately by using Pearson Square Method and then by adding the known quantities of different ingredients for the experiment effect of different dietary protein percentages (Table 2) fed to *Channa striatus*. All selected ingredients were powdered and sieved to get fine particles of uniform size. Ingredients were then weighed according to the formulation and made homogenous mixture by adding fresh water at the rate of 30 ml per every 100g of feed and finally made into dough. The dough was then cooked in a closed

aluminium container for about 25 min and then cooled. The resulting dough processed in a hand pelletizer to make 1 mm diameter pellets consequently. The prepared feeds were stored in airtight glass containers and kept in dark and cool place. Formulations and protein percentages of the diets is given in the following tables (Table 1 and 2).

Table 1: Composition of ingredients in experimental diets (Percentage)

Ingredients	Control (TC)	Crude Protein 40%	Crude Protein 45%	Crude Protein 50%
Soy flour	25	0	0	0
Tapioca flour	20	20	15	10
Wheat flour	20	15	13	8
Rice flour	20	0	0	0
Rice bran	10	0	0	0
Fish oil	2.5	1	1	1
Vitamin/Mineral mix	2.5	1	1	1
Fish meal	0	60	70	80

Table 2: Protein percentage (dry basis) of the experimental diets.

Ingredients	Crude Protein
Control	17.0
CP 40%	39.87
CP 45%	44.90
CP 50%	49.95

Water Quality Parameters

The water pH, Temperature, dissolved oxygen (DO), Total Alkalinity and Total Hardness were measured on daily basis. All the water quality parameters were measured at 10:00 Am by the following methods (Table 3).

Table 3: Water quality parameters

Parameter	Method
pH	Universal indicator method
Temperature	Thermometer
Dissolved oxygen	Titrimetric Winkler's method [22]
Total alkalinity	Titrimetric method [22]
Total hardness	Titrimetric method [22]

Growth Parameters

During the experimental period fishes were subjected to growth assessment at weekly interval. The growth parameters such as Average body weight gain (AWG), specific growth rate (SGR) and food conversion ratio (FCR) were observed during the experimental period.

Average Weight Gain (g) = Final body weight – Initial body weight

$$\text{Specific Growth Rate (\%)} = \frac{\ln(\text{Final Weight}) - \ln(\text{Initial Weight})}{\text{Experimental period in days}} \times 100$$

$$\text{Food Conversion Ratio} = \frac{\text{Feed intake on a dry matter basis}}{\text{Weight gain on wet weight basis}}$$

$$\text{Survival Rate (\%)} = \frac{\text{Total number of shrimps survived}}{\text{Total number of shrimps stocked}} \times 100$$

Statistical Analysis:

Statistical Analysis were performed by using Web Agri Stat Package (WASP) version 2.0. The data obtained on Growth, Weight Gain, Food Conversion Ratio (FCR) and Survival was statistically analysed by applying Randomized Block Design (RBD) of two-way classification.

Results and Discussion

Water quality parameters

The physic-chemical parameters of water were in optimal range in all the treatment tanks during the experimental period presented in table 4. The minimum and maximum average values of pH, Temperature, Dissolved Oxygen, Total Alkalinity and Total Hardness were observed at every sampling (weekly data provided) in all the experimental tanks for *Channa striatus* fed with different dietary protein percentages.

Table 4: The water quality parameters value ranges during experiment period.

Parameter	Range
pH	7.40±0.06-7.64±0.06
Temperature ^{0}C	26.0±0.03 – 28.70±0.01
DO (ppm)	5.70±0.06 – 6.60±0.04
Total Alkalinity (mg/L)	158.33±1.53–163.67±1.53
Total Hardness (mg/L)	210.67±1.53 – 216.33±0.58

Table 5: The Average Weight Gain (g) (AWG), SGR and FCR of *Channa striatus* fry.

Parameter	Control	CP 40%	CP 45%	CP 50%
Initial Weight (g)	0.50±0.01	0.50±0.02	0.50±0.01	0.50±0.03
Final Weight (g)	1.93±0.10	5.24±0.13	6.61±0.12	5.86±0.08
AWG	1.43±0.02	4.74±0.08	6.11±0.07	5.36±0.03
SGR	0.25±0.01	1.12±0.01	1.31±0.01	1.21±0.01
FCR	3.15±0.01	1.50±0.01	1.21±0.01	1.36±0.01
Survival (%)	40.0	62.20	75.50	68.90

* CP = Crude Protein 40%, 45%, 50%

AWG, SGR and FCR

In the present study, the data on growth performance of fry fish in all the experimental tanks were observed at weekly intervals. The protein percentage included in the diet showed significant ($p < 0.05$) difference among the treatments in Average Body Weight gain (g) (AWG), Specific Growth Rate (SGR) and Feed Conversion Ratio (FCR) (table 5 and fig. 1). Overall after 60 days, among all treatments, the highest average body weight gain of 6.11±0.07g in treatment CP 45%, followed by CP 50% (5.36±0.03 g), CP 40% (4.74±0.08 g) and the lowest average body weight gain of 1.43±0.02 g in control, the highest Specific Growth Rate was recorded 1.31±0.01 in CP 45% and lowest was recorded 0.25±0.01 in control group and the highest feed conversion ratio value of 3.15±0.01 in control group and lowest value of 1.21±0.01in treatment CP 45%.

The minimum or the maximum amount of protein needed per animal per day is the protein requirement [23]. Protein requirements changes with biotic, abiotic factors and dietary characteristics (e.g., protein quality, energy: protein ratio).

The protein requirement of any organism needs, not only to satisfy the organism's need for substrates, for the maintenance and growth, but also, to activate the various processes associated with the growth [24]. The protein requirement has been defined more adequately as the amount of protein required per animal biomass per day [25].

It has been also reported that some freshwater fish species were exclusively reared on artificial diets from the beginning of exogenous feeding [26, 27]. However, inclusion of excess protein in the diet is undesirable because some of it could be catabolized for energy production [28]. This is not cost effective because it increases the cost of protein relative to energy and could also result in increased nitrogenous wastes in culture systems [29]. Therefore, the amount of protein included in the diet of fish is a vital consideration, to promote feed efficiency and growth performance. Utilization of commercial pellet feed is now popular, especially for carnivorous fish, in order to reduce the dependence on trash fish, feeding cost and environmental impact. Studies on replacement of trash fish by formulated feed in several species demonstrated better growth rates and more profit [30]. replaced trash fish with formulated diets in cage culture of red drum (*Sciaenops ocellata*) and Japanese seabass (*Lateolabrax japonicus*) and concluded that fish consuming formulated diet (43% crude protein) with 35% soybean meal showed better growth and less feeding cost than fish fed trash fish.

The optimum protein level for juvenile snakehead based on daily weight gain and tissue protein deposition was estimated to be about 52% in the diet when herring meal was used as a protein source. The estimated protein requirement of snakehead is similar to that obtained for other carnivorous fish species; grouper [31] and puffer fish [32] respectively.

Normally fish size/weight increases with the increase in the protein percentage to a maximum and then starts decreasing in growth even though the protein percentage further increased. In other words, we cannot increase the fish growth certain percentage and there is always an optimum protein percentage beyond which there will decrease or in other words weight decrease of fish. In the present study the optimum protein percentage is around 45% dietary protein. This is like the findings of [13] at the protein content of the diets 44.0% (dry matter) gave good growth performance results for C. *striatus* fry rearing. This is similar to the results reported by [33, 34], where in two separate studies, *C. striatus* fingerling performed best at 43% protein levels. Better growth performance has also been recorded with a diet containing the same level of dietary protein 45%. However, this was not corroborated with the work of [35] who obtained highest growth performances in *C. striata* fry fed formulated diet containing 55% protein fed at the rate of 10% body weight/day. Similar observations have also been made in case of juvenile *C. striata* [36]. The diet containing 49.72% protein in the feed was well suited for better growth of *C. striatus*. Growth and survival of larval snakehead (*C. striatus*) fed different diets has been reported by [37]. But in the present study the feeding rate was only 5% body weight/day.

According to [38], Chicken liver diet with 42.54% crude protein gave better growth, survival, FCR and SGR which is similar in the present study. In aquaculture, a feed with FCR of 1:1 and 1: 2 are considered as efficient [39]. In our present study, a FCR value of 1.21 obtained when *C. striatus* fry was fed at 5% body weight with protein of 45% and correlate with [40].

[33] Observed depressive growth above protein percentage of 55% at 8-5% body weight. But in the present study, better growth and survival was observed for crude protein 45% rather than crude protein 50% at 5% body weight. Feed the fish more than the optimum waste food, increase FCR, and deteriorate water quality [41]. [13] Stated the protein requirement of *Channa striatus* as 43% and fingerling 36% which is correlate with our present study.

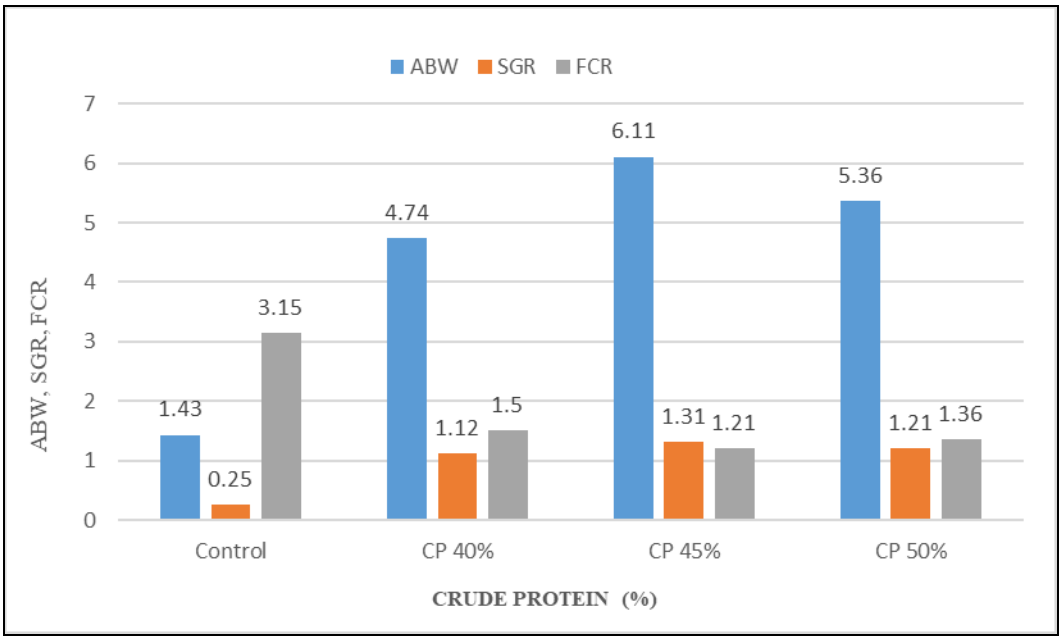

Fig 1: The Average Weight Gain (g) (AWG), SGR and FCR of *Channa striatus* fry.

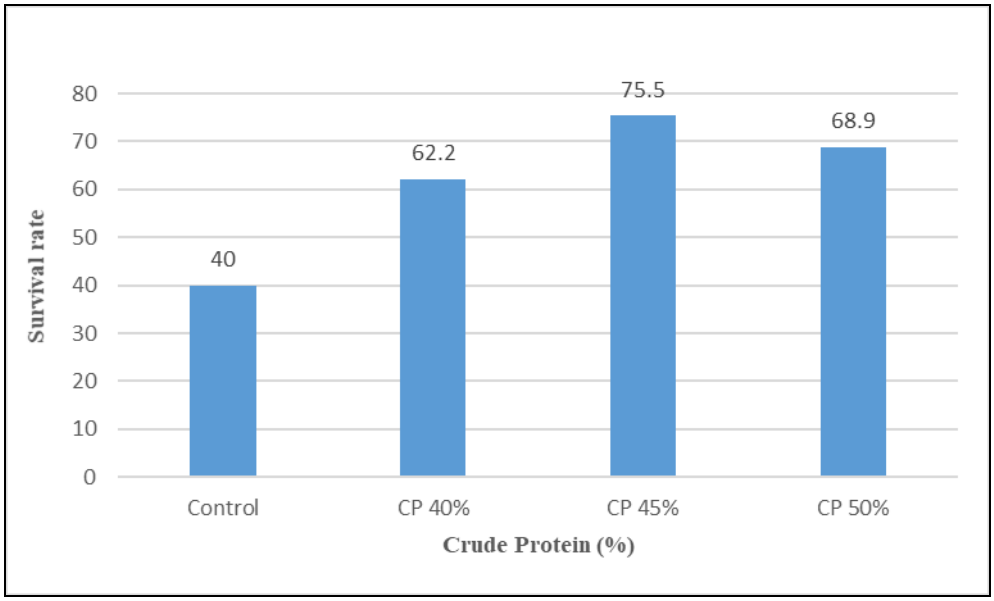

Fig 2: The survival rate of *Channa striatus* fry.

Survival Rate

The survival rate of *Channa striatus* fed on different dietary protein percentages throughout the period of experiment showed significantly ($p<0.05$) higher survival rate was for Crude protein 45% (75.5%) followed by CP 50% (68.98%), CP 40% (62.20%) and lowest survival in control (40%) showed in table 5.

In fishes, cannibalism is usually associated with heterogeneous size variation, lack of food, high density, lack of refuge area and light condition. Among these variables, size variation and unsuitable food are considered the primary causes of cannibalism. Cannibalism was reported in most larval rearing especially under artificial diet [42]. The optimum dietary protein levels reduce the tendency of cannibalism. In the present study no cannibalism occurred. [43] Also have reported that when snakeheads begin feeding on formulated feed, the progressive size variation as fish grows does not necessarily provoke cannibalism when an adequate amount of suitable food is available. [44, 45] observed that *C. striatus* in all treatments of feed application rates was found to cannibalize most small individuals and all treatments had a few large individuals at the end. The low survival rate may be related to snakehead behaviour [42]. This situation did not arise in the present study due to short-term experiment and higher survival rate was obtained in CP 45% (75.5%) when camper to the other treatments. However, no greater differences in minimum and maximum size.

Conclusion

Results of the present study indicated CP 45% level of protein supplementation is optimal for the growth and survival of *Channa striatus* fry among all other treatments. Further, it is evident from different studies that, the level of protein supplementation is species-specific.

Acknowledgement

The authors sincerely thanks to Sri Venkateswara Veterinary University, Tirupati for extending facilities to carry out this work. The Sri Venkateswara Veterinary University, P.G. Fellowship granted to the first author, is gratefully acknowledged.

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