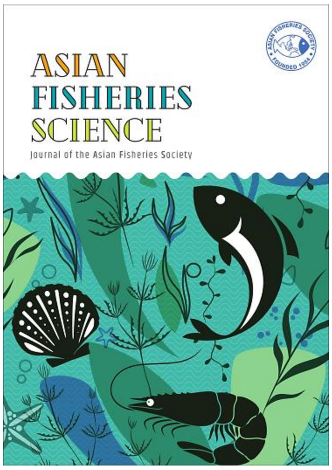

Effect of Dietary Protein on Growth, Survival and Cannibalism of Larval Striped Snakehead, *Channa striata* (Bloch, 1793)

CHIRONJIB SINGHA SAMANTA CHANDAN*, PUJA ROY, FAHIMA KHATUN, NIRMAL CHANDRA ROY
Department of Fish Biology and Genetics, Sylhet Agricultural University, Sylhet-3100, Bangladesh

*E-mail: chironjib.chandan@gmail.com | Received: 15/05/2021; Accepted: 06/09/2021

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Abstract

The available commercial feeds in Bangladesh for fish larvae, including striped snakehead, (*Channa striata* (Bloch, 1793)), lack crude protein content. Therefore, for the proper biological well-being of striped snakehead larvae, formulating a protein-rich diet is necessary. The present experiment aimed to study the effect of three formulated isocaloric diets with varying levels of protein content on growth, survival, cannibalism, and mortality in striped snakehead (29 ± 3 mg of mean weight) weaning by rearing for 4 weeks. Three experimental groups depending on the dietary crude protein (CP 35 %, CP 40 %, and CP 45 %) were done in triplicate. Growth parameters (weight and length gain, specific growth rate, feed conversion ratio, and protein efficiency ratio) indicated better growth and feed utilisation in larvae fed CP 45 %, followed by 40 % and 35 % ($P < 0.05$). However, survival was not significantly different comparing the experimental groups. The cannibalism was maximum in CP 45 %, compared to 35 % and 40 %, most probably due to higher size heterogeneity, also confirmed by the co-efficient of variation in fish size. The observed mortality (not cannibalism) was higher in the group with lower protein (CP 35%) attributable to comparatively low feed intake and utilisation. Overall, the higher dietary protein levels demonstrated comparatively higher growth, possibly indicating the importance of increased protein content in snakehead larval feed.

Keywords: fish feed, macronutrients, inland aquaculture, formulated diets, mortality

Introduction

Inland freshwater aquaculture provides economic and nutritional support for the rural people of South and Southeast Asian developing countries. Not surprisingly, the total production from inland aquaculture in 2018 was 51.43 million tons, which was 1.7 times higher than mariculture (FAO, 2020). However, several obstacles may hinder the successful aquaculture scenario; formulating fish feed with the proper level of macronutrients is one of them. Dietary protein is considered the most important factor affecting growth, survival, and feed cost (Lovell, 1989). Protein is the most important macronutrient in formulated fish diets because it provides essential amino acids, enhancing growth and health maintenance. However, protein largely affects the feed cost because it is one of the most expensive macronutrients in a fish feed (Mohseni et al., 2013). Therefore, the proper balance of protein content in a diet is crucial because a higher protein concentration can increase feed cost and increase feed waste (Kim and Lee, 2005). In contrast, the lower protein concentration in the fish diet hinders growth and other metabolic processes (Lovell, 1989).

Striped snakehead, (*Channa striata* (Bloch, 1793)), is carnivorous, obligate air breather (Wee and Tacon, 1982), hardy, can withstand hypoxia, high stocking rates, and a higher level of ammonia (Sampath, 1985). It is widely distributed in Asia and Africa and is considered a valuable food in Asian countries probably because of its preferable flesh characteristics- firm, white, and fewer bones. Several studies also reported snakehead fish extract containing potential antioxidants (e.g., albumin) and pain-relieving effects like morphine (Hidayati et al., 2018). It is cultured commercially in Asian countries, including Taiwan, Thailand, China, Philippines, Vietnam, Cambodia, Indonesia, Malaysia, and India (Wee and Tacon, 1982; Aliyu-Paiko et al., 2010). The snakehead contributes 1.88 % of the total fish produced in Bangladesh (DoF, 2018). The traditional culture practices of striped snakehead in Bangladesh include collecting fry from natural waters. However, the commercialisation of culturing snakeheads in some countries, like Bangladesh, has not been possible due to a lack of seed supply and knowledge of their breeding techniques in captive conditions (Muntaziana et al., 2013). The higher mortality in fry affecting seed supply in hatcheries can result from improper feed (Curnow et al., 2006; War and Haniffa, 2011). Striped snakehead, as carnivorous fish, requires comparatively more dietary protein for their growth and survival, resembling the nutrient content of natural live feeds. However, there is no commercial feed specific to the striped snakehead fry in Bangladesh, and available fry feeds contain low crude protein content. Intraspecific predation is also observed in the earlier stages of snakeheads (Baras and Jobling, 2002; Hseu et al., 2004; Roy et al., 2020), which can reduce the survival of seeds. Few studies assessed the growth of juvenile striped snakehead with different levels of dietary protein, where the higher level of growth was obtained by feeding snakehead with a diet containing 45 % to 52 % of crude protein (Wee and Tacon, 1982; Wee, 1983; Mohanty and Samantaray, 1996; Aliyu-Paiko et al., 2010). However, no studies discussed larval growth, survival, mortality, and cannibalism with different crude protein levels.

Most commercial feeds (Hatchery and nursery) of Bangladesh contained 35 %–38 % crude protein content, which is hypothesised as one of the main reasons for reduced growth and increased mortality. Therefore, the objectives of this experiment include assessing growth and survival with different levels of dietary protein in larvae of striped snakehead.

Materials and Methods

Experimental diets

Three isocaloric experimental diets, using local fishmeal, wheat flour, cellulose, binder, corn oil, vegetable oil, vitamin, and mineral mix as the ingredients, were prepared. The crude protein levels of 35 %, 40 %, and 45 % were assigned as P35, P40, and P45, respectively (Table 1). The local fishmeal served as the main source of protein. Both vegetable oil and corn oil were used as different ingredients because vegetable oil is comparatively expensive. Despite being cheaper, corn oil can boost the digestibility and immune system of fish. However, there is no evidence of impaired growth because of corn oil (Apraku et al., 2019). The gross energy was estimated based on the calculation of Pike and Brown (1967) (Table 2). The ingredients were mixed and grounded with an electric blender to make powder. Then the powder was mixed

Table 1. Formulation of experimental diets (%) for the larvae of striped snakehead *Channa striata*.

Component	P35	P40	P45
Fish meal	38	51.33	66.05
Wheat flour	34	25.66	15.94
Cellulose[1]	22	17	12
Binder	1	1	1
Corn oil	2	2	2
Vegetable oil	2	2	2
Vitamin[2] and mineral[3] premix	1	1	1

[1]Carboxymethyl cellulose.

[2]To supply per 100 g diet: vitamin A - 10000 IU, vitamin D_3 - 2000 IU, vitamin E - 150 mg, vitamin K_3 - 4 mg, vitamin B_1 - 15 mg, vitamin B_2 - 20 mg, vitamin B_6 - 10 mg, vitamin B_{12} - 0.03 mg, pantothenic acid - 40 mg, ascorbic acid - 150 mg, nicotinic acid - 100 mg, folic acid - 3 mg, biotin - 0.5 mg.

[3]To supply per 100 g diet: iron - 50 mg, copper - 3 mg, manganese - 20 mg, zinc - 30 mg, iodine - 1 mg, cobalt - 0.1 mg, selenium - 0.1 mg.

Table 2. Proximate composition (%) of experimental diets for the larvae of striped snakehead *Channa striata*.

Nutrient content	P35	P40	P45
Moisture	7.9 ± 0.1	7.97 ± 0.2	7.99 ± 0.1
Crude protein	35.1 ± 0.2^{c}	40.0 ± 0.1^{b}	45.2 ± 0.3^{a}
Crude lipid	7.7 ± 0.4	8.1 ± 0.1	8.5 ± 0.5
Ash	9.9 ± 0.6^{b}	12.4 ± 1.6^{ab}	15.2 ± 0.5^{a}
Gross energy[1] (kcal.100^{-1} g)	430.5 ± 4.1	430.7 ± 1.5	430.3 ± 5.6

Values expressed as mean ± SD, n = 3. Different superscripts in the same row show significant differences ($P < 0.05$).

[1]Calculated on an estimated 5.7 kcal.g^{-1} protein; 9.5 kcal.g^{-1} lipid; 4.0 kcal.g^{-1} carbohydrate.

with an appropriate amount of corn oil, vegetable oil, and water (30 % of dry ingredients). Then the moist mixture was pelleted by using a kitchen-type pellet machine to make appropriate sized (0.3 mm in diameter) feed pellets. The pellets were then air and sun-dried first to reduce the water content for 6 h. Then the feeds were packed separately in sealed plastic bags. These were blasted frozen (-18 °C) to maintain the feed quality and the binding and palatability performance simultaneously. Feeds were stored at -18 °C until use.

Experimental design and procedure

The larvae of *C. striata* were brought from a nearby commercial hatchery and acclimated in an aerated tank for a week. Faecal wastes and debris were removed daily from the respective tank. On the first day of acclimatisation, no water change, and feed supply were performed. However, for the rest of the week, larvae were fed twice with cladoceran zooplankton (*Moina* sp., captured from nearby plankton culture pond) up to apparent satiation. From the second to the sixth day, 20 % of the water was renewed daily from the tank. On the last day of acclimatisation, the water of the tank was completely renewed. Then, 50 larvae of striped snakehead (29 ± 3 mg mean weight and 15.8 ± 0.02 mm mean total length, n = 10 per replication) were stocked randomly in each tank (9 tanks in total, 200 L capacity). The tanks were continuously aerated and the water volume in each tank was 150 L. Each experimental diet (P35, P40, and P45) was assigned to three tanks (each experimental group was triplicated). The experiment was conducted for 4 weeks. Fish were fed twice a day (09:00 and 16:00) at the rate of 10 % body weight. Any uneaten feed was collected through siphoning immediately to record the actual amount of feed intake. The amount of feed was balanced weekly according to the average body mass. The water temperature was maintained at 25.2–26.9 °C. Dissolved oxygen (DO), pH, and concentration of ammonia were also monitored throughout the experiment and varied from 6.3–6.6 mg.L^{-1}, 7.3–7.7, and 0.003–0.005 mg.L^{-1}, respectively. The water temperature, DO, pH, and concentration of ammonia were measured once daily by using a thermometer (Hydroponic Solutions, Australia), DO meter (Hydroponic Solutions, Australia), pH meter (Hydroponic Solutions, Australia), and ammonia test kit (Aquasol, USA), respectively. Fish were reared under natural photoperiod, and about 40 % of the total tank water was changed weekly through siphoning to maintain suitable water quality. Tanks were monitored five times daily to count the observed mortality and cannibalism. The total length and weight of 10 fish per replication were also recorded before and after the feeding trial period. The phenomenon was considered as observed mortality if any dead fish was found intact without any visual sign of being attacked. Whereas the death was considered as cannibalism if the deceased fish body was partially consumed, body with a clear sign of being attacked, and any fish that was unaccounted for during the daily counts.

Sample analysis and calculations

Feed ingredients and experimental diets were done in triplicate for their proximate composition analysis. Moisture, crude protein, crude lipid, and ash content were determined following AOAC (1997) methods. In short, the crude protein was determined from the total nitrogen (crude protein = N × 6.25) by Kjeldahl method; crude lipid was determined by extracting the sample with petroleum ether in a Soxhlet apparatus for 8 h. The diet samples were dried to a constant weight at 105 °C for 6 h to determine moisture content; ash content was determined by heating the sample in a muffle furnace for 5 h at 550 °C.

Growth and survival parameters were calculated by using the following formula for survival, observed mortality, cannibalism, specific growth rate, protein efficiency ratio, and feed intake (Roy et al., 2020); condition factor (Fulton, 1904); feed conversion ratio, and protein efficiency ratio (Aliyu-Paiko et al., 2010):

$$\text{Survival (\%)} = \frac{\text{no. of fish survived}}{\text{no. of fish stocked}} \times 100$$

$$\text{Observed mortality (\%)} = \frac{\text{no. of fish found dead with no sign of cannibalism}}{\text{no. of fish stocked}} \times 100$$

$$\text{Cannibalism (\%)} = \frac{\text{no. of death due to cannibalism}}{\text{no. of fish stocked}} \times 100$$

$$\text{Specific growth rate (\% day}^{-1}) = \frac{\ln \text{ final weight} - \ln \text{ initial weight}}{\text{Number of rearing days}} \times 100$$

$$\text{Feed conversion ratio} = \frac{\text{Dry feed fed (g)}}{\text{Wet weight gain (g)}}$$

$$\text{Protein efficiency ratio} = \frac{\text{Weight gain (g)}}{\text{Total protein intake (g)}}$$

$$\text{Condition factor (\%)} = \frac{\text{Average weight (g)}}{(\text{Average total length in cm})^3} \times 100$$

$$\text{Feed intake (g.fish}^{-1}) = \frac{\text{Total feed intake (g)}}{\text{Number of fish}}$$

Statistical analysis

Statistical analysis of data was performed in R (version 3.6.0, Team, 2018) using R studio (version 1.2.1335). Shapiro-Wilk normality test and Bartlett test of homogeneity were run before the analysis of variance. For the proximate composition of feed, growth, and survival data, one-way ANOVA was performed to compare the average total effect of different experimental groups, followed by the HSD Tukey as the *post hoc* test ($P < 0.05$). One-way repeated measure ANOVA was conducted for comparing the changes in feed intake, cannibalism, and observed mortality in different rearing weeks, followed by the Bonferroni as the *post hoc* test ($P < 0.05$). The coefficient of variation from total length and weight was also calculated. The correlation among different parameters was measured by using Pearson correlation ($P < 0.05$).

Results

Survival, observed mortality, and cannibalism

After the completion of 28 days study period, the average survival (%) was not significantly different (One-way ANOVA, $P > 0.05$), however, the observed mortality (%) and cannibalism (%) were significantly different (One-way ANOVA, $P < 0.05$). The observed mortality was higher in P35 (30.7 ± 1.2 %) compared to P45 (25.3 ± 1.2 %, HSD Tukey, $P < 0.05$), still no significant variation with P40. While average cannibalism (%) was comparatively higher in P45 (13.3 ± 2.3 %) than P35 (6.0 ± 2.0 %) and P40 (7.3 ± 2.3 %), respectively (HSD Tukey, $P < 0.05$; Fig. 1a). The observed mortality (%) had significant differences in different weeks (one-way repeated measure ANOVA, $P < 0.05$), however, there was no significant effect of the different experimental groups and the effect of the weeks and group interaction. The observed mortality was significantly different in different weeks, reduced with the rearing week (Bonferroni, $P < 0.05$; Fig. 1b). There was a weekly and interactive effect of the weeks and group variations in cannibalism (one-way repeated measure ANOVA, $P < 0.05$). No cannibalism was observed in the first week of the rearing period. The cannibalism (%) in P45 was significantly higher compared to P35 and P40 from the third to the fourth week of rearing (Bonferroni, $P < 0.05$). Cannibalism in all groups in the fourth week was higher than in the first and second weeks of rearing (Bonferroni, $P < 0.05$; Fig. 1c).

Growth parameters

By analysing the growth data, final weight (g), final total length (cm), length gain (%), specific growth rate (%), and feed intake ($g.fish^{-1}$) were significantly higher in P45 followed by P40 and P35 (HSD Tukey, $P < 0.05$; Table 3). The feed conversion ratio (FCR) was lower in P45 (1.07 ± 0.03) that was followed by P40 (1.35 ± 0.04) and P35 (1.68 ± 0.13, HSD Tukey, $P < 0.05$). For the weight gain (%) and protein efficiency ratio, the value was significantly higher in P45 (HSD Tukey, $P < 0.05$), however, there was no significant variation between P35 and P40 for these two parameters. There was no variation in the condition factor (%) among the experimental groups (Table 3). The coefficient of variations in final total length and weight were

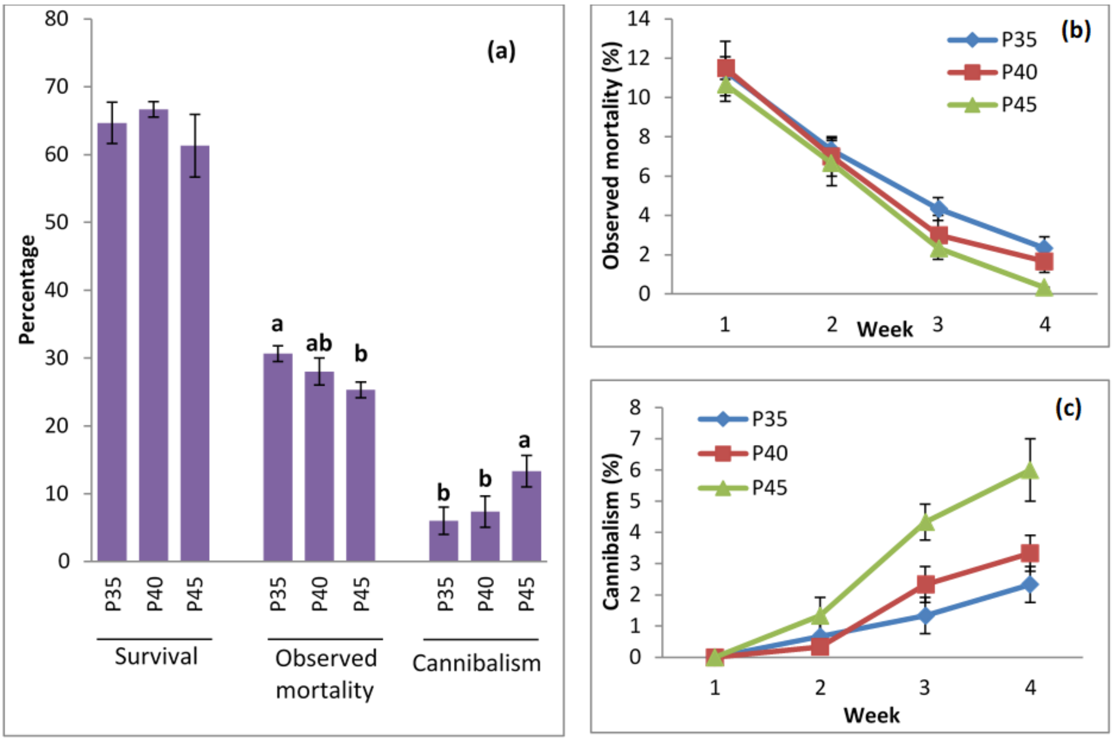

Fig. 1. (a) Average survival (%), observed mortality (%) and cannibalism (%) of *Channa striata* in different experimental groups (mean ± SD, different superscripts indicate significant differences), (b) Observed mortality (%) (mean ± SD) in different rearing weeks and, (c) Cannibalism (%) (mean ± SD) in different rearing weeks.

Table 3: Growth parameters of *Channa striata* in different experimental groups.

Group	ITL	IW	FTL	FW	LG	WG	SGR	FCR	PER	CF	FI
P35	1.57 ± 0.01	0.03 ± 0.0	3.65 ± 0.09^{c}	0.15 ± 0.01^{c}	132.1 ± 5.5^{c}	455.0 ± 81.4^{b}	5.9 ± 0.5^{c}	1.68 ± 0.13^{a}	1.71 ± 0.13^{b}	0.30 ± 0.01	0.21 ± 0.06^{c}
P40	1.58 ± 0.01	0.03 ± 0.01	4.41 ± 0.06^{b}	0.28 ± 0.04^{b}	178.4 ± 3.6^{b}	765.1 ± 101.8^{b}	7.4 ± 0.4^{b}	1.35 ± 0.04^{b}	1.86 ± 0.06^{b}	0.33 ± 0.04	0.34 ± 0.06^{b}
P45	1.59 ± 0.01	0.03 ± 0.0	5.24 ± 0.21^{a}	0.48 ± 0.08^{a}	229.1 ± 12.3^{a}	1631.5 ± 296.7^{a}	9.8 ± 0.6^{a}	1.07 ± 0.03^{c}	2.08 ± 0.05^{a}	0.33 ± 0.02	0.48 ± 0.08^{a}

Values expressed as mean ± SD, n = 3. Different superscripts in the same column show significant differences ($P < 0.05$). ITL: initial total length (cm); IW: initial weight (g); FTL: final total length (cm); FW: final weight (g); LG: length gained (%); WG: weight gained (%); SGR: specific growth rate (% day^{-1}); FCR: feed conversion ratio; PER: protein efficiency ratio; CF: condition factor (%); FI: feed intake (g.fish^{-1} 28 days^{-1}).

significantly higher in P45 (20.18 ± 2.05 % for total length and 28.81 ± 2.05 % for weight) compared to P35 (12.88 ± 1.99 % for total length and 15.02 ± 1.76 % for weight), and P40 (13.79 ± 2.0 % for total length and 15.18 ± 2.41 % for weight, HSD Tukey, $P < 0.05$). The feed intake was significantly different considering both rearing week and group (one-way repeated measure ANOVA, $P < 0.05$). During the first and second rearing week, feed intake (g.fish^{-1} week^{-1}) in different experimental groups was not significantly different. However, feed intake in the third and fourth rearing week was significantly higher in P45, followed by P35 and P40 (Bonferroni, $P < 0.05$; Fig. 2).

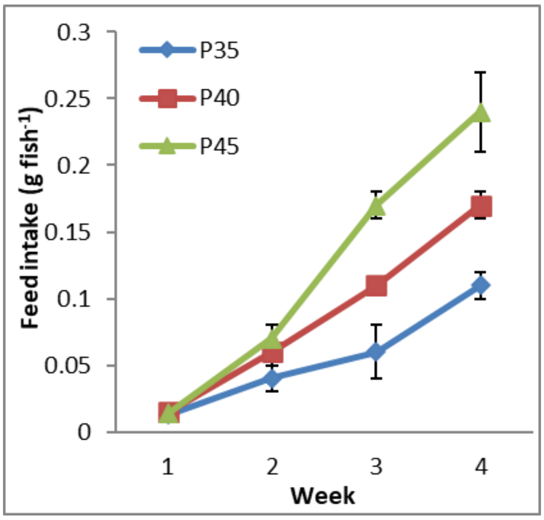

Fig. 2. Feed intake per fish in different rearing weeks. Values expressed as mean ± SD.

Discussion

Fish, like other vertebrates, require more protein during their early life stages. Therefore, better growth performance and nutrient utilisation in fish larvae can be achieved with the feed containing a reasonably higher dietary protein level depending on the species (NRC, 1993). Thus, in this experiment, almost all the growth parameters, including weight gain (WG), length gain (LG), and specific growth rate (SGR), were higher in striped snakehead larvae fed with a diet containing 45 % crude protein (CP), followed by the 40 % and 35 % of CP group. This growth variation was probably because of the higher requirement of amino acids during development stages achieved through dietary protein (NRC, 1993; Sagada et al., 2017). The findings of this experiment were in line with other studies where higher protein content resulted in higher growth in the early stages of striped snakehead (Wee and Tacon, 1982; Aliyu-Paiko et al., 2010). The higher growth can also result from higher feed intake and utilisation in the 45 % CP group, followed by 40 % and 35 % CP. The significant positive correlations of growth parameters (WG, LG, and SGR) with feed intake and protein efficiency ratio (PER) support these hypotheses (Pearson correlation, $P < 0.05$). The feed conversion ratio (FCR) and PER in this experiment were also better in the group fed with 45 % CP, followed by 40 % and 35 % CP. These findings probably refer to the better protein digestibility and utilisation in the larvae group fed with higher protein content. Similarly, several studies documented that increased level of dietary protein, obviously up to an extent, improved PER and FCR in juvenile snakehead because of increased apparent protein digestibility, protein retention, tissue protein deposition, and apparent net protein utilisation (Wee and Tacon, 1982; Wee, 1983; Mohanty and Samantaray, 1996; Sagada et al., 2017). Therefore, a higher level of dietary protein than 45 % may result in higher growth in snakehead larvae (as observed in juveniles by Mohanty and Samantaray, 1996), which requires further studies.

Average survival among the experimental groups was not significantly different. Apart from the nutritional requirement, other factors, e.g., feed types and low feeding frequency (twice a day in this experiment), can also have some effects on the survival in this experiment (Cho et al., 2003; Xie et al., 2011; Campelo et al., 2019; Roy et al., 2020). However, the observed mortality (not cannibalism) was higher in the group fed with 35 % crude protein, compared to 45 %, indicating better health and fitness in the group provided with a higher protein level. As the diets were isocaloric, the observed mortality can also be related to higher feed intake in the group fed with 45 % crude protein followed by 40 % and 35 % (Starvation; Table 3). Observed mortality correlated negatively with feed intake (Pearson correlation, $P < 0.05$). Eguia et al. (2000) also observed a similar kind of feed intake-related observed mortality for larvae of river catfish, *Hemibagrus nemurus* (Valenciennes, 1840). The snakehead larvae, in this experiment, seemed reluctant to feed on the experimental diets initially and habituated gradually to the experimental diets. Feeding quantity and response (promptness and competition for feed) from larvae increased with the rearing weeks. As several individuals seem to have difficulties in getting accustomed to the formulated diet first, partial replacement of live feed with artificial feed may help to reduce observed mortality (Hien et al., 2017; Roy et al., 2020)

A higher proportion of cannibalism was noticed in higher crude protein-fed larvae. These significant differences in cannibalism among different groups and weeks were probably due to the size heterogeneity (Roy et al., 2020). Different cannibalistic fish species may start exhibiting cannibalism at a different age or size, and the intensity of cannibalism can also vary in different stages of life (Baras and Jobling, 2002). Cannibalism is more likely to increase during domestication because the supplied diets can create heterogeneity in size (Hecht and Pienaar, 1993). In this experiment, there was no cannibalism recorded in the first week of rearing, but the intensity of cannibalism increased with the rearing period. The larvae fed with 45 % crude protein had a higher coefficient of variation in size (both body length and weight) than the larvae fed with 35 % and 40 %, which indicates that the higher size heterogeneity can elevate cannibalism in the striped snakehead. Low feeding frequency, as mentioned before, can also be another reason for the cannibalism in snakehead. While size variations in a batch and low feeding frequency are potential reasons for cannibalism, frequent feeding and regular sorting/grading may help to reduce deaths due to cannibalism (Cho et al., 2003; Xie et al., 2011; Szczepkowski et al., 2011; Baras et al., 2013).

Conclusion

This experiment revealed that dietary protein has significant effects on the growth and observed mortality of larval striped snakehead *Channa striata*. Therefore, feeds containing a low level of crude protein, as is currently supplied in snakehead aquaculture of Bangladesh, can be detrimental to larvae quantity and quality. The present study elucidated that a higher striped snakehead larvae growth can be obtained with feed containing 45 % crude protein (CP), followed by 40 % and 35 %. Though the observed mortality was higher in CP 35 % fed snakehead larvae, dietary protein content alone does not necessarily affect the number of survival because the cannibalism was higher in CP 45 % fed larvae.

Conflict of interest: The authors declare that they have no conflict of interest.

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