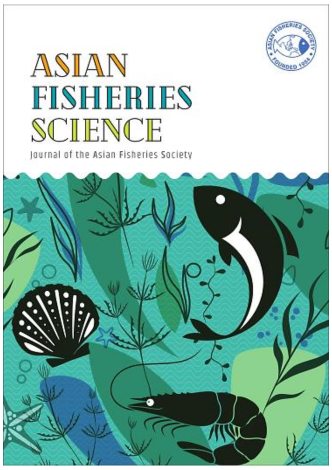

Growth Performance and Survival of Common Carp (*Cyprinus carpio* Linnaeus, 1758) Fingerlings Fed with Protease Enzyme Supplemented Diet

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Abstract

Supplementation of feed with exogenous enzymes has been proved to improvise the growth performance of various food animals by improving digestion and utilisation of the feed. This study was carried out to evaluate the effects of dietary supplementation of a protease enzyme on growth performance and survival of fingerlings of common carp (*Cyprinus carpio* Linnaeus, 1758). The indoor feeding experiment was conducted for 42 days of rearing period following complete randomized block design with four replicates for each treatment in a laboratory. Five experimental diets namely T0, T1, T2, T3 and T4 supplemented with 0.0 %, 0.1 %, 0.2 %, 0.3 % and 0.4 % of protease enzyme papain respectively were used. Maximum weight gain (191.04 %) and specific growth rate (2.54 %) were observed in fingerlings fed with T2 diet, and it was significantly higher ($P < 0.05$) from those fingerlings fed with T0, T1 and T4 diets but not significantly different ($P > 0.05$) from T3 diet. Maximum length gain (27.59 %) was observed in fingerlings fed with T2 diet, and it was significantly higher than only those fingerlings fed with T0 diet. The maximum survival (85.42 %) was recorded for fingerlings fed with T1 diet, and it was similar ($P > 0.05$) to those fingerlings fed with control and other experimental diets. The study revealed that 0.2 % dietary supplementation of exogenous papain is effective for the better growth performance in terms of length gain, weight gain and specific growth rate of *C. carpio* fingerlings.

Keywords: aquaculture, fish nutrition, fish feed additives, exogenous enzyme supplementation, papain

Introduction

Aquaculture is one of the fastest-growing food-producing sectors accounting for nearly 50 % of the world's fish production. Global fish production peaked at about 171 million metric ton in 2016 with aquaculture representing 80 million metric ton (FAO 2018). The aquaculture sector is prospering towards a reliable venture to provide nutritious and delicious food along with substantial economic returns. This expansion of aquaculture is triggering the demand for quality aquaculture feed. The feed is an integral part of commercial aquaculture accounting for up to 60 % of the total production cost (Akiyama et al. 1992; Mohanty 2001; Stankovic et al. 2011) raising concern over availability and production of cost-effective feed. The cost-effective feed production can be achieved by using alternative inexpensive feed ingredients which may degrade the quality of feed to some extent. Even feeds with a high nutrient profile may not yield favourable results if feed nutrients are not solubilised, digested or absorbed by the cultured species. Whereas the provision for efficient utilisation of feed by maximising solubility, digestibility and availability of individual feed nutrient makes it more cost-effective with the available nutrient profile.

Many researchers attempted improvisation of feed efficiency by supplementing it with growth promoters, chemo-attractants as well as exogenous enzymes to enhance acceptance, digestion and absorbance of feed ingredients. Exogenous enzymes are now extensively used as additives in the animal diet to improve anabolic as well as catabolic pathway of digestion and metabolism. Several researchers reported a positive effect of exogenous enzymes on digestibility of nutrients such as protein, carbohydrate and minerals (Drew et al. 2005; Soltan 2009; Ray et al. 2012). Results of studies on *Sparus aurata* Linnaeus, 1758 (Kolkovski et al. 1993), *Ictalurus punctatus* (Rafinesque, 1818) (Jackson et al. 1996), *Clarias batrachus* (Linnaeus, 1758) (Giri et al. 2003), *Pangasius pangasius* (Hamilton, 1822) (Debnath et al. 2005), *Macrobrachium rosenbergii* (de Man, 1879) (Patil and Singh 2009), *Ctenopharyngodon idella* (Valenciennes, 1844) (Liu et al. 2014) revealed that feed supplementation with enzymes improvises growth in culture species by increasing digestibility of nutrients and eliminating the effect of anti-nutritional factors.

Exogenous enzymes improve feed digestibility by break down of complex nutrients into simpler forms. Protease enzymes break down protein into peptides and amino acids. Such simpler nutrient forms are more easily digested, absorbed and utilised in the digestion process of the species resulting in better growth performance. Supplementation of feed with exogenous enzyme may develop nutritionally adequate, energetic and cost-effective feed for aqua farming. Papain is a protease enzyme which is derived from the latex, unripe fruit and green leaves of papaya. It is mainly used to break down perfectly peptide bonds in the proteins into smaller protein fragment called peptides and amino acids. Exogenous papain is one of the enzymes which can rapidly hydrolyze animal as well as plant proteins. According to Paul et al. (2013), papain possesses a very powerful digestive action, superior to pepsin and pancreatin to improve the protein utilisation from the feed. In view of the above, the present study was carried out to check the feasibility of papain supplementation in a diet as a growth promoter for fingerlings of common carp (*Cyprinus carpio* Linnaeus, 1758).

Common carp is commercially important cultivable freshwater fish species, ranked at the third position in worldwide finfish aquaculture production (FAO 2018). Common carp is preferred culture species owing to its culture traits such as wide tolerance, growth performance, market demand and omnivorous feeding habit accepting a variety of food including formulated feed. However, little work has been carried out on feed formulation of *C. carpio* and scientific study on the use of proteolytic enzymes in the diet is even lesser. Hence, the present study was designed to assess the growth performance and survival of common carp, *C. carpio* fingerlings fed with diets containing protease enzyme, papain.

Materials and Methods

Test animal

Fingerlings of *C. carpio* of 30 days old were brought and acclimatised to laboratory conditions for 15 days in 500 L plastic pool on a control diet. During the acclimatisation period, fingerlings were fed at the rate of 5 % bodyweight twice a day (0800 h and 1600 h) with the control diet. Faecal matter and remainder feed were removed from the circular plastic pool and aeration was provided throughout the acclimatisation period to avoid stress.

Diets

Control diet

Control diet containing 40 % protein was formulated as per Pearson square method (De Silva and Anderson 1995) based on previous studies on nutritional requirements of *C. carpio* fingerling (Cho et al. 2001; Stankovic et al. 2011; Ahmed Khan and Maqbool 2017). The feed was prepared by using fishmeal and groundnut oil cake as protein source feed ingredients while rice bran and wheat flour as basal source feed ingredients. The required quantity of finely powdered and sieved ingredients was precisely weighed and mixed thoroughly in a mixer. Then 350 mL of water was added to it, and further, it was mixed in a mixer to form a slurry. The slurry was steamed for 15 min and then cooled to room temperature. The cooled slurry was spread on a polythene sheet in 2 to 3 mm thick layer by using a soft brush. Then it was sun-dried for 3 h to form flakes. After sun drying the flakes were broken into small pieces of 2 to 3 mm size and stored in an airtight plastic container for future use. The control diet was denoted as T0.

Experimental diets

The experimental diets viz. T1, T2, T3 and T4 were prepared by supplementing control diet with exogenous protease enzyme-papain (Loba Chemie Pvt Ltd., Mumbai, India) at the rate of 0.1, 0.2, 0.3 and 0.4 % respectively. The enzyme was dissolved in 50 mL of distilled water and mixed thoroughly in cooled slurry of diet in a mixer. The method of preparation of the experimental diet was similar to that of the control diet. The proximate composition of control and experimental diets along with ingredients used for diets preparation are summarised in Table 1.

Experimental procedure

An indoor feeding experiment was conducted to assess the growth performance of fingerlings of *C. carpio* for 42 days of rearing period using plastic circular tubs of 30 L capacity. Plastic circular tubs were filled with 20 L of freshwater with 10 cm of freeboard and covered with net. Freshwater was sourced from the well. The experimental tubs were arranged following a completely randomized block design with four replicates for each treatment.

Fingerlings of *C. carpio* (45 days old) were randomly stocked in experimental tubs at the rate of 10 fingerlings per tub after recording initial weight and length. The fingerlings were fed with different experimental diets at the rate of 5 % of body weight

Table 1. Ingredients and proximate composition (% dry weight basis) of the control and experimental diets supplemented with exogenous protease enzyme-papain.

Ingredients and proximate composition	Experimental diets				
	T0	T1	T2	T3	T4
Ingredients (g)					
Fish meal	37.50	37.50	37.50	37.50	37.50
Groundnut oil cake	37.50	37.50	37.50	37.50	37.50
Wheat flour	12.50	12.50	12.50	12.50	12.50
Rice bran	12.50	12.50	12.50	12.50	12.50
Papain	0.00	0.10	0.20	0.30	0.40
Proximate composition (%)					
Moisture	10.9	11.55	12.87	11.25	12.53
Crude protein	38.83	39.88	40.52	39.3	40.27
Crude lipid	7.08	6.92	7.69	7.51	7.29
Ash	6.13	6.85	7.13	7.08	6.25
Carbohydrate	37.06	34.80	31.79	34.86	33.66

and the ration was given twice a day at 0800 h and 1600 h. Aeration was provided in each tub to avoid stress of the experimental animals. Unconsumed feed and faecal matter were removed daily at 0700 h along with 25 % of siphoning of water from each tub and then refilling with the same quantity of freshwater.

Water parameters

Water quality parameters such as temperature, pH, hardness, alkalinity and dissolved oxygen were weekly assessed following standard methods of the American Public Health Association (APHA 1998).

Growth and survival

The length and weight of all the fingerlings were measured weekly and recorded during 42 days of experiment along with the number of living individuals. Individual wet weight and length were determined to the nearest 0.01 mg and 1 mm respectively after blotting the excess moisture with the help of blotting paper.

Growth performance indicators namely weight gain, length gain, specific growth rate (SGR) and survival rate, exhibited by fingerlings fed with experimental diets were calculated using the following mathematical formulae:

$$\text{Weight gain (\%)} = \frac{\text{Final weight} - \text{Initial weight}}{\text{Initial weight}} \times 100$$

$$\text{Length gain (\%)} = \frac{\text{Final length} - \text{Initial length}}{\text{Initial length}} \times 100$$

$$\text{Specific growth rate (SGR)} = \frac{\text{Ln (Final weight)} - \text{Ln (Initial weight)}}{\text{Rearing period (Days)}} \times 100$$

$$\text{Survival (\%)} = \frac{\text{Final count}}{\text{Initial count}} \times 100$$

Proximate composition analysis

The proximate composition components such as moisture, crude protein, ether extract and ash of each diet were determined by the standard methods of Association of Official Analytical Chemist (AOAC 1990). Total carbohydrate was calculated by remainder method i.e. total carbohydrate % = 100 - (Moisture % + Crude protein % + Ether extract % + Ash %) (Table 1).

Statistical analysis

Average values of length, weight, specific growth rate and survival were calculated for each replicate of every treatment along with associated standard error (SE) values. Variations in the average values of length, weight, specific growth rate and survival of experimental treatments were tested using one way ANOVA at 0.05 level of significance. Student-Newman-Keuls test was used for comparison of variation among mean values after the revelation of significant variations in ANOVA (Zar 1974).

Results

Growth analysis

The measurements of average initial length and weight, final length and weight, weight gain (%), length gain (%), specific growth rate (%) as well as survival (%) of *C. carpio* fingerlings fed with experimental diets for 42 days of rearing period are summarised in Table 2.

Table 2. Initial length and weight, final length and weight, length gain, weight gain, specific growth rate and survival of *Cyprinus carpio* fingerlings fed with control and experimental diets supplemented with exogenous protease enzyme-papain for 42 days of rearing period.

Growth parameters	Experimental diets				
	T0	T1	T2	T3	T4
Initial length (cm)	2.88 ± 0.04	2.83 ± 0.04	2.74 ± 0.02	2.74 ± 0.04	2.79 ± 0.03
Final length (cm)	3.25 ± 0.03	3.48 ± 0.07	3.50 ± 0.07	3.47 ± 0.06	3.49 ± 0.05
Initial weight (g)	0.24 ± 0.01	0.23 ± 0.02	0.20 ± 0.01	0.21 ± 0.02	0.22 ± 0.01
Final weight (g)	0.53 ± 0.02	0.55 ± 0.04	0.58 ± 0.04	0.57 ± 0.03	0.56 ± 0.03
Length gain (%)	13.09 ± 1.29 [a]	22.78 ± 0.82 [b]	27.59 ± 2.67 [b]	26.58 ± 1.42 [b]	25.29 ± 1.01 [b]
Weight gain (%)	126.08 ± 1.77 [a]	146.08 ± 8.22 [b]	191.04 ± 1.57 [c]	173.96 ± 8.31 [cd]	159.49 ± 7.52 [bd]
Specific growth rate (%)	1.94± 0.02 [a]	2.14 ± 0.08 [b]	2.54 ± 0.01 [c]	2.40 ± 0.07 [cd]	2.27 ± 0.07 [bd]
Survival (%)	77.08 ± 10.96 [a]	85.42 ± 3.99 [a]	60.42 ± 3.99 [a]	62.50 ± 12.03 [a]	75.00 ± 3.40 [a]

Values within the same row with the same superscript are not significantly different at 0.05 level of significance.

Maximum length gain (27.59 %), weight gain (191.04 %) and specific growth rate (2.54 %) were recorded for fingerlings fed with T2 diet and maximum survival (85.42 %) was recorded for fingerlings fed with T1 diet. One way ANOVA revealed a significant difference (P < 0.05) among the experimental diets with respect to length gain, weight gain and specific growth rate for fingerlings of *C. carpio*. Student Newman Keuls test revealed that length gain of fingerlings fed with T2 diet was not significantly different (P > 0.05) from those fingerlings fed with T1, T3 and T4 diets whereas it was significantly higher (P < 0.05) than those fingerlings fed with T0 diet.

Weight gain of fingerlings fed with T2 diet was significantly higher from those fingerlings fed with T0, T1 and T4 diets (P < 0.05) but it was not significantly different (P > 0.05) from those fingerlings fed with T3 diet. The specific growth rate of fingerlings fed with T2 diet was not significantly different (P > 0.05) from those fingerlings fed with T3 diet but significantly higher (P < 0.05) from those fingerlings fed with T0, T1 and T4 diets.

Survival

In the present study survival of fingerlings of *C. carpio* fed with papain supplemented experimental diets were similar (P > 0.05) to those fingerlings fed with control diet without supplementation of papain.

Water quality

During the experimental period, water temperature ranged between 24.2 and 25.5 °C, pH varied from 7.64 to 7.96, the dissolved oxygen varied between 5.9 and 6.3 mg.L^{-1}, total alkalinity varied from 209 to 231 mg.L^{-1} and the hardness was in the range of 352 to 375 mg.L^{-1}. Water quality parameters such as temperature, dissolved oxygen and pH were within permissible limit while total alkalinity and hardness were slightly higher than the permissible limit but these water quality parameters did not show any remarkable variation during 42 days of the rearing period (Table 3).

Discussion

The result of the present study revealed significant improvements in growth-related parameters such as length gain, weight gain and SGR of fingerlings of common carp fed with a diet supplemented with exogenous papain. The results also showed that significant growth performance (weight gain and SGR) of fingerlings of *C. carpio* increased with an increased level of exogenous papain supplementation up to 0.2 % after which it showed decreased growth performance with further increased level of enzyme supplementation. Kolkovski et al. (1993) also reported better growth performance in the larvae of gilthead seabream (*Sparus aurata* Linnaeus, 1758) fed with microdiet incorporated with commercial pancreatic enzymes. Enhanced growth performance in terms of weight gain, SGR, FCR and PER as well as improved body composition along with nutrient utilisation was observed in rohu (*Labeo rohita* (Hamilton, 1822)) fingerlings by dietary incorporation of thermostable ∝-amylase (Ghosh et al. 2001). Bogut et al. (1995) also observed similar results by addition of a multi-enzyme preparation containing amylase, protease, β-gluconase, β-glucosidase and cellulose at the rate 1.5 kg of enzymes per ton of feed. They reported a significant influenced in the daily and total weight gain as well as the nutritional parameters in the trial group of common carp, *C. carpio* fingerlings fed with feed containing a multi-enzyme as compared to the control group fed with feed containing no enzymes.

Table 3. Water quality parameters during 42 days of rearing period for the fingerlings of *Cyprinus carpio* fed with control and experimental diets supplemented with exogenous protease enzyme-papain.

Weeks	Water temperature (°C)	Dissolved oxygen ($mg.L^{-1}$)	Total alkalinity ($mg.L^{-1}$)	Hardness ($mg.L^{-1}$)	pH
1	24.2 ± 0.4	6.3 ± 0.08	209 ± 3.46	352 ± 10.94	7.64 ± 0.03
2	24.8 ± 0.3	6.2 ± 0.05	215 ± 3.25	361 ± 13.50	7.80 ± 0.02
3	25.4 ± 0.3	6.2 ± 0.09	220 ± 4.07	367 ± 11.72	7.75 ± 0.03
4	25.1 ± 0.4	6.1 ± 0.08	217 ± 3.05	375 ± 12.85	7.67 ± 0.03
5	25.0 ± 0.3	6.0 ± 0.07	229 ± 3.67	369 ± 11.42	7.72 ± 0.02
6	25.1 ± 0.3	6.2 ± 0.09	226 ± 4.41	372 ± 12.03	7.75 ± 0.02
7	25.5 ± 0.2	5.9 ± 0.06	231 ± 5.03	375 ± 09.30	7.96 ± 0.03

Moreover, Jackson et al. (1996) also demonstrated that the use of microbial phytase in a diet was effective in improving bioavailability of phytate phosphorus to channel catfish without affecting its survival.

Kumar et al. (2006) delineated the positive effect of supplementation of exogenous α-amylase in gelatinised and non-gelatinized corn to improve the apparent dry matter digestibility and digestive enzyme activity in juveniles of *L. rohita*. Yildirim and Turan (2010 a and b) indicated that a multi-enzyme complex (fungal xylanase, β-glucanase, pentosonase, α-amylase, hemicellulase, pectinase, cellulase, and cellubiase) supplementation can significantly improve growth performance and feed utilization in juvenile tilapia (*Oreochromis aureus* Steindachner, 1864) and African catfish (*Clarias gariepinus* Burchell, 1822). Improved weight gain, specific growth rate, protein efficiency ratio and food conversion ratio for grass carp, *C. idella* ($P < 0.05$) were observed by supplementing phytase at different levels in the diet along with different application methods by Liu et al. (2014). Adeoye et al. (2016) also concluded that supplementation of the diet with a combination of enzymes and probiotic was capable of improving Nile tilapia (*Oreochromis niloticus* Linnaeus, 1758) growth and intestinal morphology without deleterious effect on the intestinal microbial composition.

Manush et al. (2013) reported that synergistic effect of vitamin C and enzyme papain on growth performance in the post-larvae of freshwater giant prawn, *M. rosenbergii* were better than a diet containing only vitamin C or enzyme papain. Patil and Singh (2014) also reported better growth and feed utilisation for the post-larvae of freshwater giant prawn, *M. rosenbergii* fed with diet supplemented with 1 % of individual papain.

Supplementation of the exogenous enzyme not only improves growth performance but also improve water quality in the culture tank. Mo et al. (2016) found that better growth and improved water quality ($P < 0.05$) in culture tank by lowering the concentration of ammonia and nitrite in three marine fishes when fed with papain supplemented diet.

Few researchers showed no significant effect or negative impact on growth performance in fish when fed with diet containing enzymes. Similar studies on Rainbow trout (*Salmo salar* Linnaeus, 1758) parr showed no significant effect on the consumption-growth relationship when fed with diet containing supplementary enzyme α-amylase (Carter et al. 1992). Kazerani and Shahsavani (2011) reported that supplementation of feed with exogenous a multi-enzyme feed supplement, Endofeed W was not only ineffective in improving the growth and feed conversion rates, but also it may even exert negative effects with higher doses in common carp, *C. carpio*.

Observation in the present study showed no significant ($P > 0.05$) effect on the survival of fingerlings of common carp fed with a diet supplemented with exogenous papain. Similar results were observed in tilapia, African catfish (Yildirim and Turan 2010 a and b) and freshwater giant prawn (Patil and Singh 2009; 2014; Manush et al. 2013). Thus, the findings of previous research are in agreement with the outcome of present work. In future, the exogenous feed enzymes will play a key role in the aquafeed industry and required further research with multiple enzyme activities with probiotic combination rather than individual enzyme for better understanding and development of nutritious and cost-effective aquaculture feed..

Conclusion

The results of the present study concluded that 0.2 % supplementation of exogenous papain in a diet can improve the growth performance in terms of length gain, weight gain and specific growth rate of fingerlings of *C. carpio* as compared to those fingerlings fed with control diet without supplementation of papain. Survival of fingerlings of *C. carpio* fed with a diet supplemented with exogenous papain was similar to those of fingerlings fed with control diet without supplementation of papain.

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