E-ISSN: 2347-5129
P-ISSN: 2394-0506
(ICV-Poland) Impact Value: 5.62
(GIF) Impact Factor: 0.549
IJFAS 2018; 6(3): 210-214

www.fisheriesjournal.com
Received: 28-03-2018
Accepted: 29-04-2018

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Effect of plant base digestive enzyme 'Papain' on growth, survival and behavioural response of *Cyprinus carpio*

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Abstract
The present investigation was carried out to assess the acceptability of papain and to standardize its dose for optimum growth and survival in fingerlings of *Cyprinus carpio*. The experimental fishes were fed with conventional diet mixed with three different sources of papain (i.e. latex powder, unripe fruit and green leaves of papaya) with different doses for a period of 60 days. The better results regarding growth and survival were obtained at doses i.e. feed + papain powder @ 1.0%, feed + unripe papaya fruit @ 1.25% and feed + papaya leaves @ 2.5%. During the experimentation period impact of different forms of papain on water quality as well as on behavior of fish were regularly monitored. Papain yielded no adverse effect on the water quality parameters and behavioral response of fish showing greater acceptability of papain as growth promoter.

Keywords: Papain, *Cyprinus carpio*, Proteolytic enzyme, growth, survival

1. Introduction

Due to global need for fish oil and fish meal for aquaculture [1], there is an increasing demand for more insight on the potential of alternative protein sources in aquafeeds [2]. Fish meal is a preferred animal protein source for fish diets due to its high protein quality [3]. However, due to high cost and limited availability [4] replacement of fish meal by plant protein sources is of great interest [5, 6]. Several studies have explored the effect of replacing fish meal with vegetable protein sources in diets of Atlantic salmon [7-13]. Digestibility of fish meal varies significantly and the freshness of raw material used to produce the fish meal may seriously affect the growth performance when used as ingredient in fish diets [14]. For augmenting aquaculture production various anabolic products, hormones and their analogues, antibiotics, and synthetic growth promoters are also used by fish farmers. All these artificial feed additives leave their residual effects and produce antibiotic resistant bacteria in fish, which may affect the health of consumer ultimately and resulting in decreased consumer acceptance of fish and fishery products. In view of above, many scientists are carrying out the researches on various plants and herbs to find out their efficacy as natural growth promoters.

Use of 'Papain' enzyme from papaya plant in inducement of growth is an important link in this chain. Papain is derived from the latex, unripe fruit and green leaves of papaya. The primary active ingredient of papaya tree is papain which is a protein cleaving enzyme and called as – vegetable pepsin. The leaves and unripe fruit contain a milky juice rich in proteolytic enzymes amongst which papain is most effective one [15]. The concentration of this enzyme in the unripe fruit is more and decreases as the fruit ripens [16]. Papain is allowed to act upon many kinds of proteins and involved in enhancement of growth indirectly. Papain also performs the degradation of phytate phosphorus and converts it into available form as do the phytase enzymes. Sometimes, livestock farmers and aqua farmers use phytase of microbial origin which is not easily available everywhere and not economically feasible for Indian aqua farmers. Therefore, papain may be an excellent alternative of phytase as it is a plant based, natural, cost effective source for degrading phytate. Due to these invaluable properties of papain, it can be proved as an effective feed additive. In view of above, the present study was carried out to check the feasibility of papain as a growth promoter in its natural and purified form, its acceptability by fish and effect on water quality.

2. Materials and Methods

2.1 Experimental design

Healthy fingerlings of common carp of the same age group (8±2 g, 6±2 cm) were procured from Instructional Fish Farm, College of Fisheries, Pantnagar, India. Fingerlings collected were acclimatized in circular plastic tubs in the indoor aquaculture unit of the college. Circular plastic tanks of 30 liter capacity (three tanks per treatment) were used and stocked with ten fingerlings/tank.

2.2 Experimental diet

Experimental diets were prepared from locally available ingredients i.e. groundnut oil cake (30%), rice bran (33%), soybean meal (25%), wheat flour (10%) and vitamin-mineral mix (2%)). All the ingredients were properly weighed as per their inclusion rate in all the experimental diets. Two different levels of papain from three different sources *viz*. papain powder @0.5% (T_1), 1.0% (T_2), unripe papaya fruit @1.25% (T_3), 2.5% (T_4) and papaya leaves @2.5% (T_5), 5.0% (T_6) were selected on the basis of papain availability from these resources and mixed with conventional feed. One control group was fed with conventional feed without any papain supplementation. Feeding was done at the rate of 4- 5 percent of body weight twice in a day for a period of 60 days.

2.3 Physico-chemical parameters

Water quality parameters in all the treatments were weekly analyzed with respect to temperature, pH, dissolved oxygen (DO), free carbon dioxide (CO_2), alkalinity and ammonia (NH_3) as per standard methods [17].

2.4 Sampling schedule

Sampling was performed at every fortnight interval, to assess the growth of fish in terms of total length and total weight. The numbers of survivors were also recorded. Behavioral aspect of the experimental fishes were keenly observed daily in terms of their swimming pattern, feeding response, changes in skin coloration and general body activity.

2.5 Fish Growth Parameters

Fish growth in terms of total length and body weight was recorded fortnightly. At the end of the study, fish growth and survival were calculated as follows-

Length weight (L-W) relationship was analyzed by the formula $W = aL^b$, where W = total weight of the fish, L = total length of the fish, a = intercept, b = regression coefficient and expressed logarithmically as: Low W = -a+b Log L.

Percent Weight gain = final weight (g) - initial weight (g)/initial weight (g) × 100

Percent Length gain = final length (cm) - initial length (cm)/initial length (cm) × 100

Specific Growth Rate (SGR) = ln final weight - ln initial weight/days of experiment × 100, where ln=natural logarithm

Percent survival = Final number of fish/Initial number of fish × 100

2.6 Statistical analysis

The data was statistically analyzed using SPSS-16 software. One way ANOVA was applied for determining significant differences among the treatments at 5% significance level ($P<0.05$).

3. Results and Discussion

3.1 Physico-chemical parameters of water

In the present study, the dissolved oxygen ranged from 5.73 to 6.30 (Table 1) in all feeding groups in agreement with optimum dissolved oxygen level of 5-7 mg/l [18]. The higher carbon di-oxide concentration imparts acidity in water and acid water reduces the appetite, growth and fish gets prone to attack of parasite and disease in acid waters. Optimum concentration of Free CO_2 recorded in all feeding groups i.e. 3.96-4.76 mg/l. Water having a pH range of 6.5-9.0 is most suitable for carp culture [19]. During complete experimentation period, pH was in optimum range (6.9-7.1) for fish growth and survival. In present study, alkalinity was recorded in the range 233-249 mg/l and ammonia value ranged from 0.238 to 0.291 in all experimental groups, falling in optimum range required for fresh water aquaculture [20]. The results indicate that papain was having no adverse impact on physico-chemical properties of water which helped the animal to be stress free resulted into better physiological performance.

Table 1: Variation in water quality parameters in fishes fed with different sources of Papain

Feeding Groups	Parameters					
	Temp. (°C)	pH	DO (mg/l)	Free CO_2 (mg/l)	Total Alkalinity (mg/l)	NH_3 (mg/l)
T_0	26.3±1.50	7.10±0.17	6.00±0.10	4.23±0.25	233±1.8	0.251
T_1	26.4±1.50	7.13±0.15	6.03±0.05	4.36±0.15	235±2.1	0.248
T_2	26.1±1.50	7.06±0.11	6.30±0.10	3.96±0.02	236±1.7	0.281
T_3	26.2±1.50	7.03±0.05	5.90±0.10	4.43±0.40	238±1.9	0.288
T_4	26.2±1.50	7.06±0.11	5.73±0.32	4.76±0.30	249±2.2	0.238
T_5	26.3±1.50	7.02±0.10	6.30±0.17	4.03±0.05	225±2.1	0.291
T_6	26.2±1.50	6.99±0.95	6.16±0.28	4.30±0.26	235±2.2	0.279

3.2 Daily observation of feeding response and behavior

During the study period, behavioral responses of experimental fishes reveal the greater acceptability of papain (Table 2). No abnormality was reported in behavior and skin coloration of *C. carpio,* when fed with crude (papaya fruit and leaves) and pure (powder form) papain supplemented diet. Maximum feeding response was observed in group T_5, followed by T_2 and T_3. At the same time highest movement and activity was reported in same groups attributed to maximum feeding response.

Table 2: Behavioral responses of *Cyprinus carpio* fingerlings fed with different sources of papain

Feeding Groups	Feeding response	Skin coloration	Movement & activity
T_0	Optimum	Normal	Normal
T_1	Optimum	Normal	Normal
T_2	More than optimum	Normal	Fast & more active
T_3	Optimum	Normal	Normal
T_4	Less than optimum	Normal	Less than normal
T_5	More than optimum	Normal	Fast movement & active
T_6	Optimum	Normal	Normal

3.3 Growth parameters

L-W relationship gives information on the condition and growth patterns of fish that is presented in table 3 for present study. Fish are said to exhibit isometric growth when length increases in equal proportions with body weight for constant specific gravity. The value of regression co-efficient (b) for isometric growth is '3' and values greater or lesser than '3' indicate allometric growth [21].

Table 3: Matrix of length-weight relationship of *C. carpio* fed with different sources of papain

Feeding Groups	Experimental Period	
	Initial	Final
T_0	Log w=-3.23 + 2.12 Log L (r= 0.961)	Log w=-3.40 +2.41 Log L (r= 0.977)
T_1	Log w=-4.10 + 2.58 Log L (r= 0.971)	Log w=-3.77 + 2.58 Log L (r= 0.979)
T_2	Log w=-4.85 + 2.41 Log L (r= 0.975)	Log w=-4.58 + 3.05 Log L (r= 0.981)
T_3	Log w=-3.77 + 2.79 Log L (r= 0.965)	Log w=-2.72 + 2.99 Log L (r= 0.996)
T_4	Log w=-3.89 + 2.26 Log L (r= 0.981)	Log w=-3.81 + 2.62 Log L (r= 0.988)
T_5	Log w=-4.99 + 3.71 Log L (r= 0.981)	Log w=-4.26 + 2.87 Log L (r= 0.963)
T_6	Log w=-3.68 + 2.15 Log L (r= 0.979)	Log w=-3.45 + 2.43 Log L (r= 0.982)

In present study, the value of 'b' ranged from 2.41-3.05 showing pattern of near isometric growth. The correlation coefficient values are near to + 1, ranged from 0.961 to 0.998 in all experimental feeding groups showing positive correlation between length and weight increment. Analysis of L-W relationship yielded no abnormality in growth pattern in all feeding groups fed with papain. These results indicate acceptability of papain as feed supplement without any adverse effect on general growth pattern of fish.

Table 4: Growth parameters of fingerlings of *C. carpio* fed with different sources of Papain

Feeding groups	Growth parameters			
	% Length gain	% Weight gain	SGR	Survival (%)
T_0	$8.99^a±1.11$	$37.75^a±1.06$	$1.51^a±0.05$	70
T_1	$12.80^b±1.27$	$51.25^b±1.76$	$1.92^a±0.03$	80
T_2	$13.31^b±1.55$	$59.89^c±1.61$	$2.20^b±0.01$	80
T_3	$12.22^b±1.91$	$51.02^b±2.81$	$2.01^a±0.03$	70
T_4	$10.13^a±2.33$	$48.90^b±2.68$	$1.98^a±0.05$	70
T_5	$14.21^c±1.31$	$64.42^d±1.11$	$2.35^b±0.06$	80
T_6	$14.12^c±1.50$	$57.11^c±1.40$	$2.12^b±0.02$	70

Values with same superscript are not significantly different.

Growth parameters in terms of percent length and weight gain, SGR and survival of *C. carpio* fingerlings fed with control and experimental diets are presented in Table 4. The percent weight and length gain is significantly higher in all papain fed groups than that of control with maximum value in T_5, followed by other papain fed groups and minimum in control. The highest growth in T_5 might be attributed to higher feeding responsiveness towards leaf mixed diet and its digestibility. The results regarding better performance of papain fed groups are in concurrence with the study, where *Cyprinus carpio* fed with papain digested feed resulted in significantly better growth, than their counterparts fed without papain digestion [22]. This indicates the efficacy of papain in growth enhancement as being a proteolytic enzyme it transforms protein into peptones, which are more easily absorbed into blood stream, thus facilitating protein assimilation subsequently resulted into growth increment. Moreover, this protease may be an effective and eco-friendly substitute of animal protein. Increased proteolytic activity was observed in common carp fed with diet containing bovine trypsin and stated that there was a new approach to use the proteolytic enzymes to fish diets for better growth [23]. In the present study, feed supplemented with papain resulted in higher SGR as compare to control. The SGR results are in accordance with percent weight gain (T5>T2>T6>T3>T1>T4). Significant higher growth rate was recorded in common carp fingerlings receiving feed mixed with papain @ 2% as compared to control treatment (P<0.05) in which feed was given without any enzyme supplementation [24]. Better growth performance of common carp was also reported when fed with synthetic feed mixed with 3% papain and synthetic feed mixed with 10% papaya leaf attributed to increased protein digestion due to papain [25]. Diet supplementation with 0.1% of papain resulted in better growth of post larvae of *Macrobrachium rosenbergii* [26]. In another study, incorporation of proteolytic enzyme, papain in diets of *M. rosenbergii* post larval stage resulted in an increase in growth performance as well as elevation in protease activity [27]. Nile tilapia, *Oreochromis niloticus* L. yielded better results in terms of percent weight gain and SGR in diets supplemented with crude papain in the form of papaya leaf [28]. Another study revealed that application of papain in the diet has the significant effect on growth performance, feed utilization and survival rate of *keureling* fish (*Tor tambra*) @27.50 mg kg^{-1} [29]. A combination of hydrolysed feather meal and freshwater shrimp meal mixed with papaya leaf meal (papain) yielded highest growth performance in Nile tilapia (*Oreochromis niloticus* L.) comparison to their counterparts not mixed with papain [30].

The reduced growth rate in control treatment may also be attributed to the presence of anti-nutritional factors (phytate etc) in plant based diet [31], which in turn have an adverse impact on growth performance and availability of various dietary nutrients. The papain supplemented diet may be effective in reducing either anti-nutritional factors or adverse consequences of phytate from plant origin ingredients of feed [32, 33]. Thus it is clear from the present study that fingerlings of *C. carpio* fed with papain supplemented diet showed better growth performance.

4. Conclusion

It can be concluded that supplementation of different forms of papain had no adverse effects on the feeding and behavioral response of *C. carpio*, resulted in better growth than control diet. From the results of the present study, it is stated that groups fed with papain powder @1% (T_2), unripe papaya fruit @1.25% (T_3) and papaya leaf @2.5% (T_5) yielded better results as compare to their counterparts. Thus it is recommended that efficacy of papain as a growth promoter might be explored being a cost effective, easily available and eco-friendly package for augmenting aquaculture production.

5. Acknowledgements

The authors are grateful to Head, Department of Fisheries Resource Management and Dean, College of Fisheries, Pantnagar for providing necessary facilities for this investigation.

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