Int Aquat Res (2011) 3: 1-9
ISSN 2008-4935
Available online at www.intelaquares.com

International
Aquatic Research

Exogenous supplementation of papain as growth promoter in diet of fingerlings of *Cyprinus carpio*

Prabjeet Singh[1*], Sajid Maqsood[1], Munir Hassan Samoon[1], Vikas Phulia[2], Mohd Danish[3], Ramesh Singh Chalal[3]

[1]*Faculty of Fisheries, Shere-e-Kashmir University of Agricultural Sciences and Technology of Kashmir, India*
[2]*College of Fisheries, GADVASU, Punjab, India*
[3]*College of Fisheries, G.B.P.U.A & T, Pantnagar, India*

Received: 9 April 2010; Accepted: 11 January 2011

Abstract

The effect of different levels of papain (1%, 2% and 4%) supplemented feed in *Cyprinus carpio* on growth rate, nutrient digestibility, gross protein retention (GPR), feed conversion ratio (FCR), protein efficiency ratio (PER), apparent net protein utilization (ANPU), energy conversion efficiency (ECE%) and nitrogen retention efficiency (NRE) was studied. The fish fed on the feed supplemented with 2% papain treatment resulted in lowest feed conversion ratio (FCR), better growth rate, high protein digestibility, higher protein efficiency ratio (PER), good gross energy retention, better apparent net protein utilization (ANPU), energy conversion efficiency ECE (%) and nitrogen retention efficiency (NRE). All these parameters were lowest in case of control ($P > 0.05$). Maximum growth rate, nutrient digestibility, gross protein retention (GPR), feed conversion ratio (FCR), protein efficiency ratio (PER), apparent net protein utilization (ANPU), energy conversion efficiency (ECE %) and nitrogen retention efficiency (NRE) were seen during the 8th week of the study period and 2% papain level showed best results ($P < 0.05$). The physicochemical parameters were within the optimum range as desired for fish culture practices. Papain did not affect the water quality parameters and had no adverse effect on feeding response of fish. Thus, supplementation of papain to feed of common carp at a level of 2% resulted in better growth performance.

Keywords: *Cyprinus carpio*, Papain, Growth rate

Introduction

The expansion of global aquaculture is increasing the demand for aquaculture feed which is the prime input in fish culture practices. Generally, the selection of feed ingredients for any production system depends upon its nutritional value and cost. Protein is the vital and expensive nutrient of formulated fish feeds (De Silva et al. 1989). Both the quality and quantity of protein in fish feeds is of paramount importance in promoting fish growth for achieving marketable size of fish at an early phase.

Fish meal is used globally as dietary protein in formulated fish feeds but the major problems with use of fish meal as source of protein in the fish diet are its rising cost, uncertain availability, adulteration and variation in quality. The increasing demand, unstable supply and high price of the fish meal with the expansion of aquaculture

* Corresponding author. E-mail: prabjeet29255@yahoo.co.in. Tel: +91-7500979024.

made it necessary to search for alternative protein sources (FAO 2004; Lunger et al. 2007). Hence, there is a need to replace this ingredient partially or fully with some other suitable ingredients to reduce the production cost. This has encouraged feed manufacturers to search for cheaper alternative protein sources such as plant proteins. A number of published reports are available regarding the efficacy of plant feedstuffs as alternative protein sources in fish feeds (Hossain and Jauncey 1989). However, the presence of anti-nutritional factors within plant feed-stuffs limits their use in aqua-feeds (Tacon et al. 1990). Most of the plant origin ingredients have demerits on account of presence of anti-nutritional factors which have an adverse impact on the digestion and nutrient utilization of feed.

However, certain enzymes provide an additional powerful tool that can inactivate anti-nutritional factors and enhance the nutritive value of plant based protein in feeds. These provide a natural way to transform complex feed components into the digestible form. Endogenous enzymes found in the digestive tract of fish help to break down large organic molecules like starch, cellulose and protein into the simpler substances. Addition of exogenous enzymes in fish feeds can improve nutrient utilization, thereby reducing nutrient losses.

Exogenous enzymes have been proven to improve nutritional value of feed and decrease environmental pollution in terrestrial animals (Classen 1996). Recently, the supplementation of diets with exogenous enzymes was also known to enhance feed utilization and reduce water pollution (Kolkovski et al. 1997). Application of proteolytic enzymes to fish food has stepped into a new era of research to aquaculturists. Herbal based enzyme "papain" is a proteolytic enzyme from the cysteine proteinase family. It is derived from papaya (*Carica papaya*) leaf, unripe fruit and papaya latex, a milky fluid that oozes out of green papaya. Papaya leaves contain around 9% protein and 5.3% papain and also contain vitamin C (286 mg/100 g) and vitamin E (30 mg/100 mg) (Watt and Breyer-Brandwijk 1962). Papaya, *Carica papaya* is one of the herbal sources of proteolytic enzymes. The primary active ingredient of papaya tree is papain which is a protein cleaving enzyme and called as vegetable pepsin. The concentration of this enzyme in the unripe fruit reaches a maximum when the fruit is fully grown and decreases as the fruit ripens (Watt and Breyer-Brandwijk 1962). Thus the objective of present study was to evaluate the effect of papain supplementation on protein digestibility and growth promotion of common carp under controlled conditions.

Materials and methods

Fish and experimental conditions

Healthy fingerlings of common carp at the same age group (10 ± 2 g, 9 ± 2 cm) were procured from fish farm of Faculty of Fisheries, SKUAST-K, Srinagar, India. Fingerlings collected were acclimatized in FRP (Fiber Reinforced Plastic) rearing troughs (1.8 × 1.8 × 0.5 m) at the hatchery complex of the faculty for a period of two weeks. A continuous flow through of water was maintained during the experimentation period.

Twelve rearing troughs of equal size (1.8 × 1.8 × 0.5 m) were used, three troughs (replications) each for four treatments and stocked with 25 fingerlings each. Three levels of papain (1, 2 and 4%, T1 = 1% Papain, T2 = 2% Papain, T3 = 4% Papain) were used during the study on the basis of feed weight and fed to three experimental groups after mixing with the pelleted diet. One control group was maintained on pelleted feed without papain treatment. Feeding was done at the rate of 3 percent body weight daily in each experimental feeding group after ascertaining the total group weight on weekly basis. The experiment was conducted for a period of 70 days.

Table 1. Ingredient composition of experimental diets supplemented with and without papain

Ingredients	Inclusion rate
Ground nut oil cake	31%
Rice bran	26.23%
Soybean meal	15.9%
Fish meal	4.95%
Wheat flour	19.92%
Vitamin & Mineral mixture	2%

Experimental diets

Experimental feeds were prepared from the locally available feed ingredients. Feed ingredients (Table 1); groundnut oil cake (31.00%), rice bran (26.23%), soybean meal (15.90%), fish meal (4.95%) and wheat flour

(19.92%) were procured, screened and subjected to proximate analysis following standard procedure (AOAC 1995). All the ingredients were properly weighed as per their inclusion rates in the four experimental diets.

Prepared feed was mixed with chromic oxide (1%) as an indicator for determining nutrient digestibility and papain at the required level. One control feed was formulated without any papain supplementation. The dried pelleted diets were packed in air tight polythene bags and stored at -20 °C. Experimental stock in all the treatments was fed twice daily for a period of 70 days. The feeding rate was later adjusted after weighing total biomass of each group. The feed was offered in the feeding trays, which were kept at the bottom of trough and were inspected to monitor the consumption of feed, and any uneaten feed was removed daily. The papain enzyme (EC 3.4.22.2) was procured from Sigma Chemical Co. (St. Louis, MO, USA).

Sampling schedule

To record the growth parameters sampling was done during 2^{nd}, 4^{th}, 6^{th}, 8^{th} and 10^{th} week. The physicochemical parameters of the water were monitored on every alternate day to make a record of physicochemical profile.

Physiochemical parameters of water

Water temperature was recorded with the help of digital thermometer (Cooper-Atkins, USA) having range of 0-50 °C, with mark up to 0.1 °C. The pH of the water samples was measured with a pH meter (Sartorius, Germany). The dissolved oxygen content of sample water was determined by the Winkler titrimetric method (APHA 1998). The free carbon dioxide was estimated by standard titrimetric method using phenolphthalein as an indicator (APHA 1998). Alkalinity was determined titrimetrically using phenolphthalein and methyl orange as indicators (APHA 1998).

Fish growth and biochemical parameters

Growth rate

Fish specimen were weighed before stocking and final weight was recorded at each sampling. The difference between the two weights divided by time period of the experiment was used to calculate the growth rate.

Growth rate (g/day) = $W1-W_0 / T$

W_1=Fish weight at the end of study

W_0=Fish weight at start of study

T=Time interval in days.

Feed conversion ratio (FCR)

Feed conversion ratio (FCR) was calculated as the kilograms of feed provided to fish to produce one kilogram of whole fish.

Apparent net protein utilization (ANPU)

Net protein utilization provides a measure of efficiency of protein utilization. Thus, protein quality can be expressed in terms of net protein utilization.

ANPU= Weight gain/ Protein Intake.

Gross protein retention

Protein content of the feed was measured using Kjeldahl method (AOAC 1990) and was calculated using 6.25 as a conversion factor. Protein content of fish muscle was calculated using Lowry method (Lowry et al. 1951) using bovine serum albumin as a standard. 500 ml dried and grinded muscle sample was taken and homogenized properly after adding 5 ml of extraction buffer. Then sample was taken in an eppendorf tube and centrifuge at 9000 rpm for 20 minute to remove the debris. The supernatant was separated and stored after measuring its volume for estimation of protein concentration.

Gross protein retention = Protein content (g) of fish at end of experiment - Protein content (g) of fish at start of experiment / Dry protein fed (g).

Protein efficiency ratio (PER)

Protein efficiency ratio (PER) is the increase in weight with respect to protein consumed. This variable was calculated for each trough from the initial mean weight and the final mean weight, as well as the amount of protein consumed.

PER= Increase in mass of animal / Mass of protein in feed.

Survival rate

The mortalities during the experimental period were monitored closely and if any was recorded. The data was recorded and used to calculate the survival rate percentage as (Final number of fish/Initial number of fish) × 100.

Energy retention efficiency (%)

Energy retention efficiency was calculated by the following formula:

ERF(%) =100× [(GE_1W_1−GE_0W_0) / (GEF × total feed intake)], where GE_0 and GE_1 are the initial and final whole body energy concentrations (J/g) of fish, W_0 and W_1 are the initial and final fish weights (g), GEF is the gross energy of food on a dry matter basis, and the total food intake was determined in grams on a dry matter basis.

The initial and final whole body energy concentrations of the fish were determined directly using an automated oxygen bomb calorimeter (IKA-Werke, Staufen, Germany). The minced whole body samples were freeze-dried for 24 h and used to determine the energy concentration, which was converted to calculate the whole body energy concentration on a wet weight basis (ECwet) using the following equation:

EC wet (J/g) = [freeze-dried sample weight (g) × energy concentration in dry basis (J/g)] / [samples weight before freeze drying (g)].

Nitrogen retention efficiency (NRE)

It is the nitrogen deposition with respect to ingested nitrogen. This variable was calculated for each treatment from the initial nitrogen content of the fish as stocked and the final nitrogen content of fish in each treatment.

NRE= [(individual mean final weight × final body nitrogen)−(initial individual mean weight × initial body nitrogen)] / nitrogen consumed.

Nitrogen content of fish was determined at the beginning of the growth trial on a sample of the fish used to stock the tanks, and at the end on four fish per tank. Fish were freeze-dried to allow proper grinding and sample homogenization, and the nitrogen content was then determined using the Kjeldahl method (Tecator 1983), calculating the final result as a percent of wet weight.

Apparent protein digestibility (%)

The digestibility of the experimental diet was carried out by indirect method using chromic oxide as marker. The experimental diets were prepared by incorporating 1% chromic oxide in the diet. The feed containing chromic oxide was fed to experimental fishes for a period of 70 days. The digestibility of dry matter was expressed as percentage.

ADC = 100 –100% markers in feed / % marker in faeces.

Chromic oxide at the rate of 1% was used as an inert marker in fish feed. The fishes were fed twice daily. The tubs were cleaned to remove any uneaten feed and the faecal matter was collected. Faeces were collected twice daily in the morning and evening by siphoning out using a small diameter plastic pipe through a mesh strainer. Faeces collected were dried in an oven at 105 ˚C to constant weight, finely ground and stored at 4 ˚C till further analysis. The chromium content of the feed and faecal matter was estimated by using Atomic Absorption Spectrometer (AOAC 1995). Wet digestion of the sample was carried out according to AOAC (1995). A sample of 2.5 g was taken in Kjeldahl flask to which nitric acid and perchloric acid was added at the ratio of 2.5:1. The sample was boiled very gently; adjusting flame as necessary, until the solution is colorless and dense white fumes appeared. Then it was cooled slightly and the volume was made up to 250 ml. This sample was filtered and stored in a plastic sample bottle. This sample was directly used to estimate the chromium content by flame ionization Atomic Absorption Spectrophotometer (AAS 4129, Electronics Corporation of India Limited) using chromium cathode lamp (357.9 nm).

Statistical analysis

Experimental data was subjected to the statistical analysis following the completely randomized design (CRD) and the variation among the treatment means was tested for the significance by analysis of variance techniques as described by Gomez (1984). Level of significance used for F and *t*-test were P= 0.05 from the table given by Fisher. The analysis was carried out by using statistical package SPSS (14).

Results and discussion

Growth Parameter

Growth parameters of common carp fed on different levels of papain supplemented feed are presented in Table 2. Lowest FCR value of 2.07 ($P < 0.05$) was obtained in T_2 treatment group in which papain was supplemented at a level of 2%. Exogenous application of enzyme resulted in improvement in FCR when compared to control (Fig. 1). The reason may be attributed to fast metabolism in fish fed on papain supplemented feed which in turn resulted in better FCR. Papain is a protease enzyme that hydrolyzes proteins to short peptides in diet, which is the key factor to increase protein digestibility and fast absorption, and helps to increase growth factors (Wong et al. 1996).

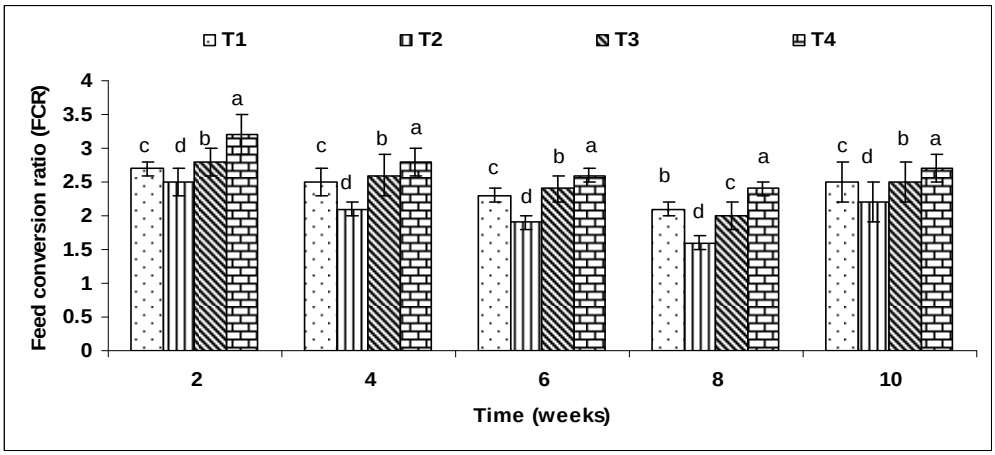

Fig. 1. Weekly variation in the feed conversion ratio (FCR) of common carp fed on diet containing different levels of papain during the experimental trail of 70 days. Data represents mean±SEM. Bars having same letters on the given sampling time differ significantly ($P < 0.05$).

Highest growth rate was also found in common carp feed supplemented with 2% papain (Fig. 2). Among the entire feeding groups, better growth rate was found in treatments receiving papain as compared to control treatment ($P < 0.05$) in which feed was given without any enzyme supplementation. The reduced growth rate in control treatment could be due to the presence of anti-nutritional factors in feed (Kakade et al. 1973) which in turn have an adverse impact on growth performance and availability of various dietary nutrients (Spinelli et al. 1983, Richardson et al. 1985). The suppressed growth performance in fish fed on control diet may be attributed to the presence of anti-nutritional factors in soybean meal (Kakade et al. 1973).

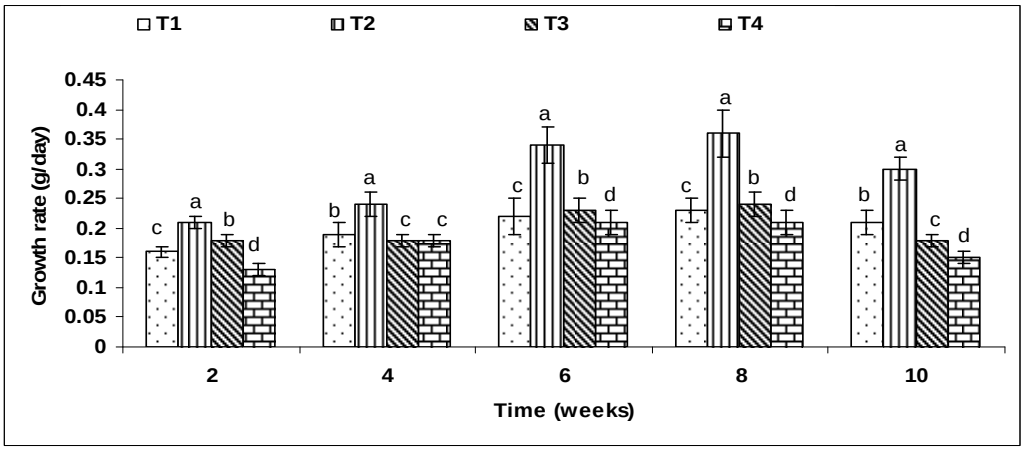

Fig. 2. Weekly variation in the growth rate (g/day) of common carp fed on diet containing different levels of papain during the experimental trail of 70 days. Data represents mean±SEM. Bars having same letters on the given sampling time differ significantly ($P < 0.05$).

This suggests that papain supplementation in the diet may be effective in reducing either anti-nutritional factors or adverse consequences of phytate from plant origin ingredients of feed, which is supported by the finding of Liu (1997). The reduction of phytate-protein complexes in the gut increases nutrient availability (Liebert and Portz 2005). The improvement in growth rate of fish after papain supplementation is consistent with other studies where fish were fed either phytase supplemented diets (Jackson et al. 1996) or phytase pretreated ingredients (Vielma et al. 2002). Growth rate or net weight gain of red tilapia (De Silva et al. 1991) was correlated to dietary protein consumption irrespective of dietary lipid content. Thus it is clear from the present study that papain increased the availability of proteins by its proteolytic activity and thus contributed to the growth performance of common carp (*Cyprinus carpio*).

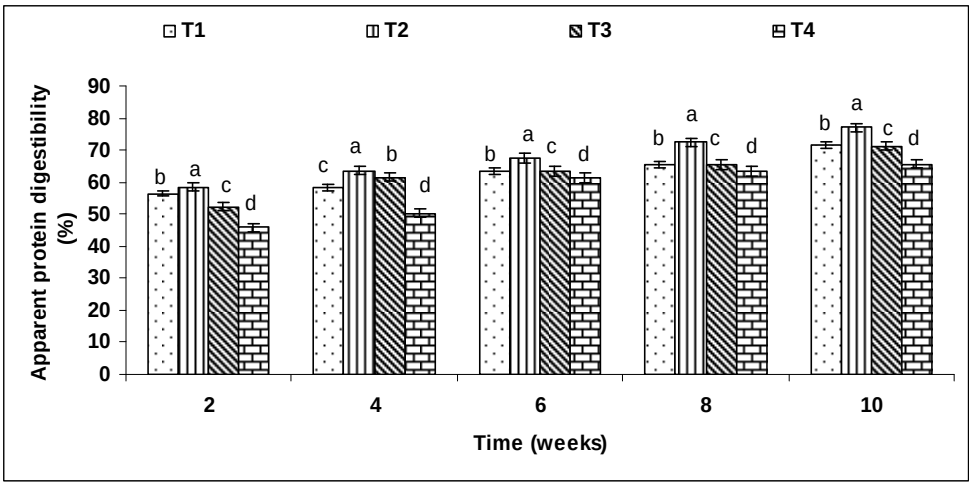

Fig. 3. Weekly variation in the apparent protein digestibility (%) of common carp fed on diet containing different levels of papain during the experimental trail of 70 days. Data represents mean±SEM. Bars having same letters on the given sampling time differ significantly ($P < 0.05$).

As clear from Fig. 3, highest apparent protein digestibility was found in T_2 group and the lowest was found on control group ($P < 0.05$). The feed supplemented with papain showed higher protein digestibility values when mixed with papain showed high digestibility values as compared to control feed where no enzyme supplementation was done ($P < 0.05$). This may be due to the more extensive hydrolysis of protein which was caused by papain with results shown by Kimmel and Smith (1957). Dabrowski and Glogowski (1977) reported that addition of proteolytic enzyme exogenously to fish show increase in protein content.

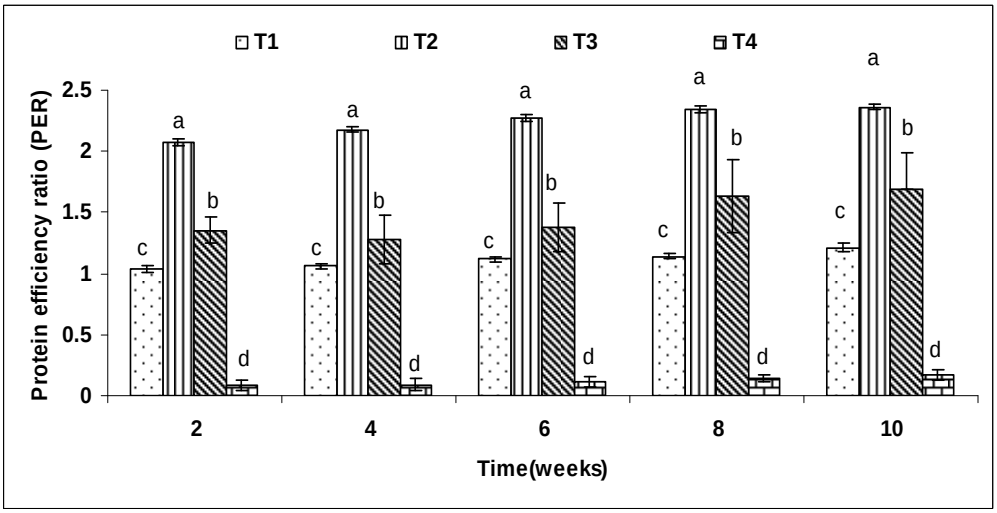

Fig. 4. Weekly variation in the protein efficiency ratio of common carp fed on diet containing different levels of papain during the experimental trail of 70 days. Data represents mean±SEM. Bars having same letters on the given sampling time differ significantly ($P < 0.05$).

The exogenous proteolytic enzymes, originating from invertebrate food organisms, play role in fish digestion in addition to activation of fish's own enzymes (Janearik 1964, cited in Dabrowski and Glogowski 1977). Thus it is clear from the digestibility values recorded in the common carp fed on papain supplemented diet (2%) that exogenous enzymes play a considerable role in fish digestion process and added enzymes advantageously influence fish growth and food utilization. Improvement in protein digestibility of feed by papain supplementation has been reported in other studies (Spinelli et al. 1983). This may also be attributed to the activity of papain to dephosphorylate the phytic acid and phytate phosphorus to increase the phosphorus availability (Lanari et al. 1998).

Table 2 . Weekly variation in growth parameters of common carp fed on a diet containing different levels of Papain for a trail of 70 days. Data represents mean±SD. Values in same row having same superscript does not differ significantly ($P > 0.05$).

Parameters	T1	T2	T3	T4
FCR	2.43 ± 0.1^{a}	2.07 ± 0.2^{b}	2.48 ± 0.21^{c}	2.75 ± 0.1^{d}
Growth rate (g/day)	0.20 ± 0.01^{a}	0.29 ± 0.02^{b}	0.20 ± 0.03^{c}	0.18 ± 0.01^{d}
APD%	63.06 ± 1^{a}	67.80 ± 1.5^{b}	62.18 ± 1.3^{c}	57.34 ± 1.2^{d}
PER	1.11 ± 0.01^{a}	2.24 ± 0.02^{b}	1.46 ± 0.03^{c}	0.12 ± 0.01^{d}
GPR%	24.96 ± 1.2^{a}	27.45 ± 1.2^{b}	24.93 ± 1.3^{c}	24.18 ± 1.2^{d}
ANPU%	32.64 ± 2.1^{a}	39.67 ± 2.1^{b}	29.30 ± 1.8^{c}	26.29 ± 1.76^{d}
ECE%	34 ± 1.5^{a}	36.8 ± 1.66^{b}	35 ± 1.8^{c}	32 ± 1.7^{d}
NRE%	0.28 ± 0.01^{a}	0.32 ± 0.01^{b}	0.26 ± 0.02^{c}	0.20 ± 0.01^{d}
SR%	99 ± 1^{a}	99 ± 1^{b}	98 ± 1^{c}	97 ± 1^{d}

Protein efficiency ratio is a measure to show as to how well the protein sources in the diet could provide essential amino acid requirement of the fish. Hence, the PER in the range of 1.03-2.3 (g/g) could favour fat deposition in common carp and this finding is in agreement with the work done by Desilva and Anderson (1995). PER ranged from 0.12-2.24 which is in close agreement with the work done by Siddiqui et al. (1988) (Table 2). During the whole experiment there was an increase in protein efficiency ratio in all treatments (Fig. 4) and it was always higher than the control treatment which could be due to the exogenous application of vegetable pepsin.

Gross protein Retention was found in the range of 24.18-27.45% (Table 2). However, the highest GPR was recorded in T_2 feeding group ($P < 0.05$). Exogenous application of enzyme in fish feed breaks the anti-nutritional factors present in plant origin food stuffs, hence making more protein available to fish which in turn results in better protein retention by the fish. The Gross Protein Retention (GPR) values recorded in the present study are in agreement with those of Jana et al. (2006) who recorded a GPR value in the range of 28-31.05% in milk fish *Chanos chanos*.

The data on apparent net protein (%) reveals that the highest and lowest ANPU values were shown by T_2 and control treatments, respectively ($P < 0.05$) (Table 2). Similar results were found by Siddhuraju and Becker (2000). The reason for higher ANPU in case of papain supplemented feed when compared to control treatment can be ascribed due to action of papain against anti-nutritional compounds in feed and therefore, more amino acid availability to the experimental fish. Papain may cause extensive hydrolysis of proteins thus making more availability of amino acids. The values of energy retention efficiency obtained in the present study are also in close agreement with those of results found by Kim et al. (2004). There was a small difference in the nitrogen retention efficiencies in different feeding groups, although the highest retention values were shown by T_2 feeding group ($P < 0.05$).

In the present study the dissolved oxygen content ranged from 7.15-10.26 ppm (Table 3) and thus favorable for fast growth of fish and in agreement with the optimum dissolved oxygen level of 5-7 ppm as proved by (Jhingran 1982). The recorded temperature in all treatments was in the range of 14-22 ˚C and suitable for survival of common carp as it a hardy species and can live in versatile culture conditions (Stickney 1979). Optimum concentration of free Co_2 0.93-1.71 was recorded in all feeding groups (Boyd 1979). In T_1, T_2 and T_3 (0.93-1.71), the lower values of free Co_2 were recorded as compared to T_4 (2.3).

Table 3. Weekly variation in the physicochemical parameters (mean±SD) during the experimental period of 70 days

Parameters	T1	T2	T3	T4
Temperature (˚C)	$18.12 \pm 1^{a*}$	18.03 ± 1.1^{b}	18.4 ± 1.1^{c}	18.48 ± 1.3^{d}
pH	6.9 ± 1^{a}	6.79 ± 1.1^{b}	6.83 ± 1.2^{c}	7.05 ± 1.3^{d}
Dissolved oxygen (mg/l)	8.08 ± 1.3^{a}	7.79 ± 1.2^{b}	8.51 ± 1.1^{c}	8.46 ± 1^{d}
Free carbon–dioxide (mg/l)	0.93 ± 0.01^{a}	$1.19 \pm 0.02^{\mathbf{b}}$	1.71 ± 0.01^{c}	2.3 ± 0.01^{d}
Total Alkalinity (mg/l)	233.9 ± 2.1^{a}	235.3 ± 2.2^{b}	236.9 ± 2.4^{c}	238.9 ± 2.3^{d}

*Values in the same row having different supercripts does not differ significantly ($P > 0.05$).

Thus papain had no adverse impact on water quality in terms of free Co_2. In the present investigation, alkalinity was recorded in the range of 221-247 mg/l. Higher values of alkalinity (> 300 mg/l) have been reported to cause eutrophication (Pant et al. 1979). In all treatments, the alkalinity was > 100mg/l and < 250 mg/l, thus within the optimum range. During complete experimentation period, pH was in the range of 6.5-7.5 in all feeding groups and thus suitable for fish growth and survival. Therefore, it is clear that addition of papain had no effect on water quality in terms of pH (Swingle 1976).

Conclusion

From the results of this study, it can be concluded that adding of papain to the feed will result in efficient protein metabolism by hydrolyzing of proteins and forming short peptides in diet, leading to an increase increase in digestibility and fast absorption of proteins, which in tern helps to increase growth factors. Hence, papain can be added at the level of 2% to the feed to enhance overall growth and production of common carp. Papain is an ecofriendly growth promoting agent and has no deteriorating effect on the aquatic environment. It is recommended that the efficacy of papain should be explored for other species, and papaya leaves or its natural extract may be used instead of commercial papain to establish its cost effective application.

Acknowledgement

The research assistance provided by SKUAST-K, Srinagar, India to carry this work is duly acknowledged.

References

AOAC 1990. Official Methods of Analysis, 15th ed. Association of Official Analytical Chemists, Arlington, VA.

AOAC 1995. In: Cunniff PA (ed.), Official methods of analysis of the Association of Official Analytical Chemists, vol 1,16th ed. AOAC International, Arlington, USA.

APHA 1998. In: Clesceri, LS; Greenberg AE and Ealton AD (eds). Standard methods for the examination of water and waste water, 20th edition. American Public Health Association, American Water Works Association, Water Environment Federation, Washington DC.

Boyd CE. 1979. Water Quality in Warm water Fish Ponds**.** Agriculture Experiment Station, Auburn, Alabama. pp. 359.
Classen HL. 1996. Cereal grain starch and exogenous enzymes in poultry diets. Anim Feed Sci Technol 62: 21–27.
Dabrowski K, Glogowski K.1977. A study of application of proteolytic enzymes to fish food. Aquaculture 12: 249-360.
De Silva SS. 1989. Digestibility estimations of natural and artificial diets, in Fish Nutrition Research in Asia (ed. S. S De Silva), Asian Fisheries Society Manila, Manila, pp. 36-45.
De Silva SS, Gunasekara RM, Shim KF. 1991. Interactions of varying dietary protein and lipid levels in young red tilapia: evidence of protein sparing. Aquaculture 95: 305-318.
FAO 2004. State of World Fish Nutrition in Practice. INRA, Paris, 459-468. Fisheries and Aquaculture 2004. FAO, Rome, Italy.
Gomez KA, Gomez AA. 1984. Statistical procedure for Agricultural Research (2nd ed). John Wiley and Sons, Inc., New York.
Hossain MA, Jauncey K. 1989. Nutritional evaluation of some Bangladeshi oil seed meals as partial substitutes for fish meal in the diets of common carp, *Cyprinus carpio* L. Aqua Fish Manag 20: 255-268.
Jackson LS, Li MH, Robinson EH. 1996. Use of microbial phytase in channel catfish *Ictalurus punctatus* diets to improve utilization of phytate phosphorus. J World Aqua Soc 27: 309-313.
Jana S, Garg SI, Berman U, Arasu A, Patia B. 2006. Effect of varying dietary protein levels on growth and production of *Chanos chanos*. Aqua Int 14(5): 479-498.
Jhingran AG. 1988. Reservoir fisheries in India. J Ind Fish Assoc 18: 251-273.
Kakade ML, Hoffa DE, Liener IE. 1973. Contribution of trypsin inhibitors to the deleterious effects of unheated soybeans fed to rats. J Nutr 103: 1172-1077.
Kimmel JR, Smith EL. 1957. The properties of Papain. Adv Enzymol 19: 267-334.
Kolkovski S, Tandler A, Izquierdo MS. 1997. Effects of live food and dietary digestive enzymes on the efficiency of microdiets for seabass (*Dicentrarchus labrax*) larvae. Aquaculture 148: 313-322.
Lanari D, Agaro E, Turri C. 1998. Use of nonlinear regression to evaluate the effects of phytase enzyme treatment of plant protein diets for rainbow trout (*Oncorhynchus mykiss*). Aquaculture 161: 345-356.
Liebert F, Portz L. 2005. Nutrient utilization of Nile Tilapia, *Oreochromis niloticus* fed plant based low phosphorus diets supplemented with graded levels of different sources of microbial phytase. Aquaculture 248: 111-119.
Liu K. 1997. Chemistry and nutritional value of soybean components. In: Soybeans:chemistry, technology, and utilization. Chapman & Hall, New York, pp. 25-113.
Lowry OH, Rosebrough NJ, Farr AL, Randall RJ. 1951. Protein measurement with Folin phenol reagent. J Biol Chem 193: 256-275.
Lunger AN, McLean E, Craig SR. 2007. The effects of organic protein supplementation upon growth, feed conversion and texture quality parameters in juvenile cobia (*Rachycentron canadum*). Aquaculture 264: 342–352.
Pant MC, Sharma P, Gupta PK. 1979. Trophic status of two lakes of Kumaon. In: Proc. of Symposium on Environment of Biology, Muzaffarnagar, India, pp. 201- 205.
Richardson NL, Higgs DA, Beames RM, McBride JR. 1985. Influence of dietary calcium, phosphorus, zinc and sodium phytate level on cataract incidence, growth and histopathology in juvenile chinook salmon (*Oncorhynchus tsawytscha)*. J Nutr 115: 553–567.
Siddhuraju P, Becker K. 2000. Preliminary nutritional evaluation of Mucuna seed meal (*Mucuna pruriens* var. *utilis)* in common carp (*Cyprinus carpio* L.): an assessment by growth performance and feed utilization. Aquaculture 196: 105-123.
Siddiqui AQ, Howlader MS, Adam AA. 1988. Effect of dietary protein levels on growth, feed conversion and protein utilization in fry and young Nile tilapia, *Oreochromis niloticus***.** Aquaculture 70: 63-73.
Spinelli J, Houle CR, Wekell JC. 1983. The effect of phytates on the growth of rainbow trout (*Salmo gairdneri*) fed purified diets containing varying quantities of calcium and magnesium. Aquaculture 30: 71-83.
Stickney RR. 1979. Principles of Warmwater Aquaculutre. John Wiley & Sons, Inc. New York, pp. 375.
Swingle HS. 1976. Standardization of chemical analyses for waters and pond muds. FAO Fisheries Report, 44(4): 397-421.
Tacon AGJ. 1990. Standard methods for the nutrition and feeding of farmed fish and shrimp. In: Nutritive Sources and Composition, vol. 2. Argent Laboratories Press, Redmond, WA, pp. 129.
Tecator 1983. Fat Extraction on Feeds with the Soxtec System HT— The Influence of Sample Preparation and Extraction Media. Application note AN 67/83 (1983.06.13). Soxtec System HT Manual Tecator, AB Sweden.
Vielma J, Ruohonen K, Peisker M. 2002. Dephytination of two soy proteins increases phosphorus and protein utilization by rainbow trout, *Oncorhynchus mykiss***.** Aquaculture 204: 145-156.
Watt JM, Breyer-Brandwijk MG. 1962. The medicinal and poisonous plants of Southern and Eastern Africa, being an account of their medicinal and other uses, chemical composition, pharmaceutical effects and toxicology in man and animal, 2nd edn. Edinburgh: E&S Livingstone Ltd.
Wong MH, Tang LY, Kwok FSL. 1996. The use of enzyme digested soyaben residue for feeding common carp. J Biol, Hong Kong Bapist University, Kowloon Tong, Hong Kong, 9: 418-42.