Trends in Fisheries Research
An International Peer-Reviewed Journal
UGC Approved Journal No: 63495
www.sciencejournal.in

IMPACT OF *ANANAS COMOSUS* EXTRACT SUPPLEMENTATION ON THE GROWTH AND BIOCHEMICAL PROFILE OF *CYPRINUS CARPIO* FINGERLINGS

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ABSTRACT
Administration of exogenous enzymes in the early stages of fish will improve the uptake and utilization of nutrients. Taking this into consideration, the present study was conducted to assess the proteolytic activity of pineapple (*Ananas comosus)* extracts, and the impact of its dietary supplementation on the growth and biochemical profile of common carp, *Cyprinus carpio* fingerlings. The results of the proteolytic activity test registered maximum with the peel, followed by core> flesh> crown. The experimental fingerlings were fed with pineapple peel extract (PPE) supplemented diet (with extract: feed ratio of 1:2) for 30 days and the control were fed with basal diet (without supplementation). The results revealed that all the growth parameters considered, except Length Gain showed a significant improvement when compared to the control. The biochemical analysis of the fingerlings showed a significant increase in protein and amino acid content and an insignificant difference in the carbohydrate content in the experimental fingerlings when compared to the control. Thus, PPE supplemented fingerlings showed an improved growth performance and, dietary supplementation of PPE could potentially improve the aquaculture of *C. carpio* fingerlings.

KEYWORDS: Common carp, enzyme, dietary supplementation, pineapple, proteolytic.

INTRODUCTION

India has made rapid progress in the fisheries sector and has emerged as the second largest fish producer globally (Bais, 2018). Common carp, *Cyprinus carpio,* Linnaeus, 1758 has been cultivated globally, is omnivorous; with a wide range of feed acceptance and adaptability to the environment (Rahman, 2015). Most feed formulations for carnivorous and omnivorous fishes involve the use of expensive fish meal and fish oil as the primary or sole dietary source of proteins and are limited in supply (Tacon, 1997). Many studies have been conducted on finding alternative sources for dietary protein in feed formulations, and some well-known alternatives from plant sources are soybean meal, corn gluten meal, mustard oilcake, linseed, sesame meal, plant proteins supplemented with lysine etc (Daniel, 2018).

Effective utilization of dietary protein source by fish is an important criterion for aquaculture, especially at hatchery level, because the levels of the proteolytic enzymes are lower in fish fingerlings than in adults (Govoni *et al*., 1986). Thus, the basal diet supplementation with exogenous enzymes can improve nutrient utilization, assimilation (Kolkovski, 2001) and increase protein digestibility (Yigit *et al.*, 2016) in fish. Various studies have been conducted on the dietary supplementation of exogenous enzymes in fishes (Castillo and Gatlin, 2015; Adeoye *et al*., 2016; Choi *et al*., 2016; Yigit *et al*., 2016; Rachmawati and Samidjan, 2018; Subandiyono *et al*., 2018; Tewari *et al*., 2018; Yuangsoi *et al*., 2018) and prawns (Saydmohammed *et al*., 2013; Patil and Singh, 2014) to study their effect on growth, feed utilization, intestinal health etc. However, these studies have assessed the impact of enzyme supplementation on growth parameters alone, but the effects on the biochemical profile have not been well studied and are highly warranted.

Pineapple, *Ananas comosus* (L.) Merr. is widely cultivated in many tropical countries across the globe. It contains phenolics, citric acid, malic acid, vitamin A, vitamin B, is high in dietary fiber, demonstrates high antioxidant properties and contains a proteolytic enzyme, bromelain (Martínez *et al*., 2012; Hossain *et al*., 2015). Various parts of the pineapple plant contain different concentrations of bromelain (Ketnawa *et al.*, 2012), and bromelain has many therapeutic and commercial applications.

Hence, the present study was conducted to evaluate the impact of the pineapple peel extract (PPE) supplementation on the growth and biochemical profile of *C. carpio* fingerlings, along with the proteolytic activity of the various parts of the pineapple fruit. This approach could also effectively utilize pineapple peel wastes that are produced during pineapple processing.

MATERIALS AND METHODS

Preparation of crude extract

The ripe *A. comosus* fruit, used for the study, was purchased from a local market in Bengaluru and the different parts such as peel, core, crown, and flesh were separated, weighed and homogenized with ice-cold 0.1M sodium acetate-acetic acid buffer (pH 5) in the ratio of 1:1(w:v). The homogenates were filtered through a muslin cloth and centrifuged at 10,000 × g for 20 minutes at 4˚C (Krishnan and Gokulakrishnan, 2015; Yuangsoi *et al.*, 2018). The supernatants of the respective parts were considered as the 'crude extracts' and used for further study.

Determination of proteolytic activity

The proteolytic activity of the crude extracts of different parts of the pineapple was assayed according to Cupp- Enyard (2008) with slight modifications where 1% BSA was used as a substrate (Thazeem *et al*., 2017).

Collection and rearing of fingerlings

Fingerlings of common carp, *C. carpio*, measuring 3.3±0.1cm and weighing 0.46±0.02g, were purchased from The Fisheries Research and Information Centre, Hesaraghatta, Karnataka and acclimated to the laboratory conditions for a week in 25L capacity tanks. During acclimatization, the fingerlings were fed *ad libitum* with commercial basal diet, once a day, at 3% of their body weight.

Feed preparation and experimental design

The commercially available feed was used as a basal diet for the present study and was treated as the control diet. For the preparation of the experimental diet, PPE was chosen (see further) and was prepared by mixing the PPE to the ground basal diet in a ratio of 1:2 and made into pellets. These pellets were air- dried and used for feeding trial experiment.

After acclimatization, the fingerlings were randomly distributed into experimental and control groups and were stocked in 25L capacity plastic tubs at a stocking density of 2 fingerlings L^{-1}. Each tank contained 40 fingerlings that were fed with the respective diets, for 30 days, with three replicates. The physicochemical parameters of the water of all the groups, such as hardness (106±3 mg L^{-1}), temperature (23.69±0.45 °C), pH (7.89±0.45), and dissolved oxygen content (4.19±0.45 mg L^{-1}) were maintained throughout the experimental period. Every ten days, sampling was done for the analysis of feed consumption, biochemical profile and growth parameters.

Proximate Analysis

The control and the experimental feeds were subjected to a proximate analysis. The crude fiber, crude protein, crude lipid, moisture, ash, and nitrogen-free extract were experimentally determined using standard methods (Kaur, 2015) and were tabulated (Table 1).

Table 1 Proximate composition of the control and experimental diets.

Proximate Composition (%)	Control diet	Experimental diet
Moisture	9.32±0.78	9.75±0.85
Crude protein	20.53±0.85	21.90±0.76
Crude lipid	9.27±0.38	9.50±0.68
Crude fibre	15.82±0.65	17.57±0.78
Ash	12.73±0.25	13.75±0.43
Nitrogen-free extract	32.33	27.53

Growth Parameters
To evaluate growth performance, fingerlings from both the groups were selected randomly every 10 days and the growth performance was assessed by calculating the following growth parameters:

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

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

$$\text{Feed Conversion Ratio (FCR)} = \frac{\text{Feed intake (g)}}{\text{Weight Gain (g)}}$$

$$\text{Feed Efficiency Ratio (FER)} = \frac{1}{\text{Feed Conversion Ratio}}$$

$$\text{Specific Growth Rate (SGR)(\% day}^{-1}) = \frac{(\ln \text{final weight} - \ln \text{initial weight})}{\text{number of days}} \times 100$$

$$\text{Protein Efficiency Ratio (PER)} = \frac{\text{Weight gain (g)}}{\text{Protein intake (g)}}$$

$$\text{Average Daily Gain (ADG)(g day}^{-1}) = \frac{\text{Weight gain (g)}}{\text{Total duration of the experiment(in days)}}$$

Biochemistry
The entire organism was homogenized and the biochemical profile of both the groups of fingerlings was assessed once in 10 days for total protein (Lowry *et al*., 1951), total carbohydrate (Ludwig and Goldberg, 1956) and free amino acid content (Sharma and Sangha, 2009).

Statistical Analysis
All the data of growth performance and biochemistry were analyzed by one-way ANOVA using MS Excel 2007 software keeping a significance level of $P<0.05$.

RESULTS AND DISCUSSION

Proteolytic activity of the pineapple parts
Proteolytic enzymes catalyze the hydrolysis of proteins and peptides. Plant-based proteases like ficin, papain and bromelain are well known for their proteolytic activity, that have many diverse functions in the medicinal and food industry (González-Rábade *et al*., 2011). Pineapple waste portions included the peel, core, stem and crown, accounted for 29–40 %, 9–10 %, 2–5% and 2–4 % of the weight of the fruit respectively (Ketnawa *et al*., 2012). In the present study, among the various parts of the pineapple assayed for proteolytic activity, the peel exhibited a significantly higher activity (137.50±16.50 units mL^{-1}), followed by core (86.16±22.22 units mL^{-1}) >flesh (80.66±15.87 units mL^{-1}) >crown (77.00±10.83 units mL^{-1}) (Fig. 1).

Statistical analysis showed that the proteolytic activity among the various parts was insignificant, other than peel ($P<0.05$). The proteolytic activity of the various parts of *A. comosus* is dependent on the amount of bromelain present in it (Pavan *et al*., 2012). High concentrations of bromelain have been reported in the crowns and peels of various cultivars of pineapple (Ketnawa *et al*., 2012; Omotoyinbo and Sanni, 2017; Benefo and Ofosu, 2018). But in our study, the peel showed the highest proteolytic activity, and this could be attributed to varietal differences, differences in soil conditions, and climatic conditions during the growth of pineapples (Benefo and Ofosu, 2018). With this result in consideration, the tissue with the highest proteolytic activity (peel) was used for supplementation with the basal diet for experimental group fingerlings. This also served as a constructive use of the large amount of pineapple wastes generated.

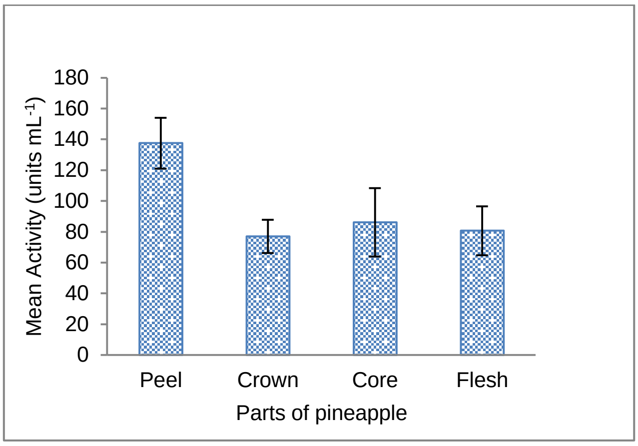

Fig.1 Proteolytic activity of the different parts of *A. comosus.*

Growth Parameters

The growth of fishes is sensitive to a number of factors such as the feed quality and composition, physicochemical parameters of water, stocking densities, etc. Growth studies are important in aquaculture because they provide estimates of feed consumption, stock sizes, and mortalities and can be used as an index of growth performance of fish populations (Divu *et al.*, 2012).

Exogenous supplementation of proteases with fish diets has been shown to improve the growth performance (Choi *et al*., 2016; Tewari *et al*., 2018; Rachmawati and Samidjan, 2018) and immunity (Choi *et al*., 2016) in many fishes. Our results were consistent with these studies, where PPE supplemented fingerlings showed improved growth performances but, statistically insignificant survival rate between the groups ($P > 0.05$) was observed (Fig. 2). This was found to be consistent with the results of Subandiyono *et al*. (2018) with Java barb, *Puntius javanicus.* After 30 days of the feeding trial, all the growth parameters such as WG (74.13±11.96 %), FER (1.92±0.31), SGR (0.80±0.10) and PER (4.35±0.67) were increased significantly in experimental fingerlings compared to control fingerlings ($P<0.05$), whereas FCR (0.53±0.09) decreased significantly (Table 2). Improvement in the growth parameters in the experimental group could be attributed to the proteolytic activity of bromelain present in PPE, which enhanced the uptake of nutrients. These results were found to be consistent with those obtained when pineapple wastes were incorporated into the diets of Nile tilapia (*Oreochromis niloticus*) (Yuangsoi *et al*., 2018).

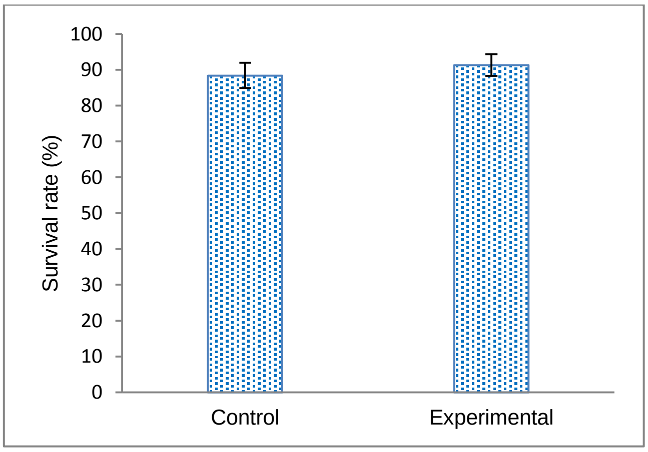

Figure2. Survival rate of *C. carpio* fingerlings fed with control and experimental diets

Table 2 Growth Parameters of *C. carpio* fingerlings fed with control and experimental diets

Growth Parameters	Control group	Experimental group
WG (%)	20.41±2.68	74.13±11.97
LG (%)	14.06±4.32	20.74±9.04
SGR (% day^{-1})	0.27±0.03	0.80±0.10
FCR	1.91±0.25	0.53±0.09
FER	0.53±0.07	1.93±0.31
PER	1.24±0.13	4.35±0.67
ADG (g day^{-1})	0.0032±0.0003	0.012±0.0016

Biochemistry

The biochemical composition of the fish body depends on the intrinsic biology and the environmental conditions of the fish (Herawati *et al*., 2018). It is well known that the biochemical profile is an indicator of the growth performance of fishes. In the present study, the biochemical profile of the *C. carpio* fingerlings fed with basal and PPE supplemented diets was assessed. Protein is the most essential nutrient for the growth and maintenance of fishes, and is utilized preferentially to lipids and carbohydrates (Ahmed and Maqbool, 2016; Kim *et al*., 2012). On the initial day, the protein content of the control and experimental fishes was found to be 35.40±3.75 mg g^{-1} of body weight and 34.40±2.60mg g^{-1} of body weight respectively, and increased in the experimental fishes (66.96± 2.00 mg g^{-1} of body weight) after 30 days, when an improved growth performance was also seen (Fig. 3).

Amino acids play a key role in fish nutrition, metabolism, and growth (Li *et al*., 2009). The free amino acid content changed to 25.68±1.75 mg g^{-1} body weight and 44.32±2.71 mg g^{-1} body weight for the control and experimental groups, after 30 days (Fig. 4). The increase in protein and free amino acid content can be attributed to the enhanced digestion of proteins into amino acids, brought about by bromelain present in the PPE, hence resulting in a significant increase in the size of the PPE supplemented fishes. This result was found to be consistent with another study with European eels, *Anguilla anguilla*, where the amino acid content in the fishes was found to increase with an increase in size (Gómez-Limia *et al*., 2019).

Despite being the least expensive source of energy, carbohydrates are vital for growth, and provide precursors to some amino acids and nucleic acids and their dietary inclusion is known to improve feed utilization in fishes (Krogdahl *et al*. 2005; Prabhu *et al*., 2017). After the experimental duration of 30 days during our experiment, the carbohydrate content of control and experimental groups was found to be 39.85±1.91 mg g^{-1} body weight and 41.42±1.55 mg g^{-1} body weight respectively (Fig. 5).

The statistical analysis revealed that the biochemical profile of the PPE supplemented *C. carpio* fingerlings showed a significant ($P< 0.05$) increase in the total protein and free amino acid content and an insignificant variation in the total carbohydrate content when compared to the control fingerlings, at the end of the experimental period of 30 days. As the growth performance of PPE supplemented *C. carpio* fingerlings improved, the biochemical profile also improved.

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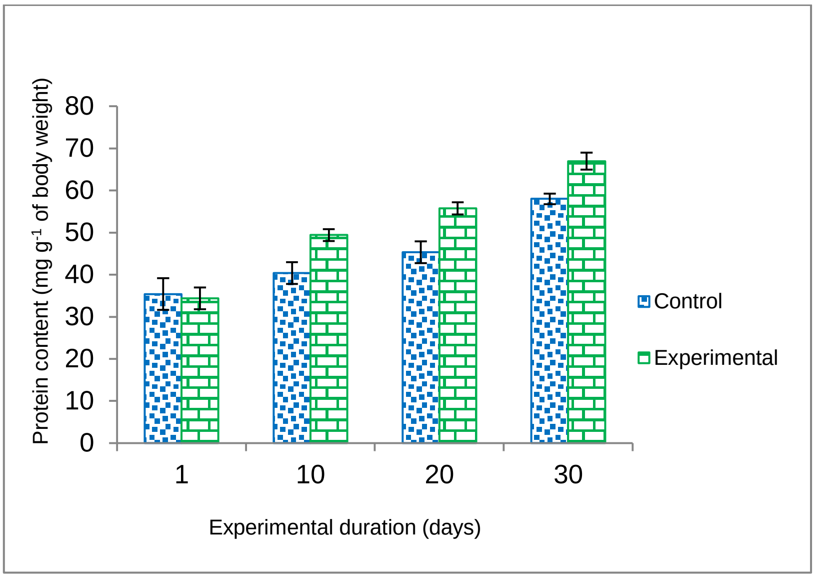

Fig.3 Protein content of *C. carpio* fingerlings fed with control and experimental diets

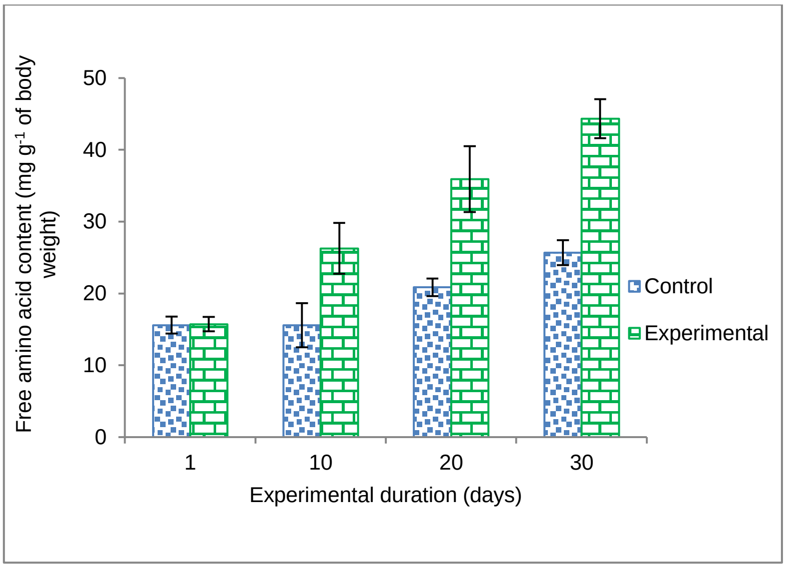

Fig.4.Free amino acid content of *C. carpio* fingerlings fed with control and experimental diets

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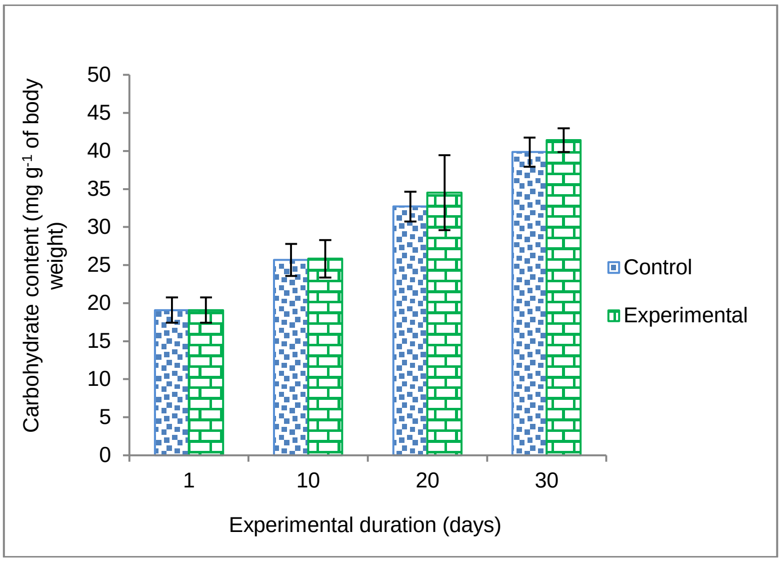

Fig.5 Carbohydrate content of *C. carpio* fingerlings fed with control and experimental diets

In conclusion, the growth parameters (except LG) and protein and amino acid content showed a significant improvement in the fingerlings fed with PPE supplemented diet when compared to the fingerlings fed with basal diet. Thus, the dietary supplementation of PPE could promote the effective utilization of pineapple wastes, and also boost the growth performance and biochemical profile of the fingerlings of the common carp, *C. carpio.*

ACKNOWLEDGEMENTS
We would like to thank the Department of Life Sciences, CHRIST (Deemed to be University), Bengaluru for providing all the facilities to carry out this research and Central Institute of Brackishwater Aquaculture (CIBA), Chennai for helping us with the proximate analysis of the fish diet.

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