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Growth performance, Biochemical and antioxidative response to different feeding strategies in *Pethia conchonius*

Greeshma Thomas[1], Sreeya G. Nair.[2], Subramanian A[3]
[1]Research Scholar (19213092192031), [2]Assistant Professor, [3]Associate Professor
[1,3]Department of Zoology, S.T. Hindu College, Nagercoil, Tamil Nadu, India
[2]Department of Zoology, Sree Ayyappa College for Women, Chunkankadai, Tamil Nadu, India
Affiliated to Manonmaniam Sundaranar University, Tirunelveli.
Email id: greeshmathomas5757@gmail.com

Abstract

The current study was carried out to assess the effects of replacing fish meal with nutritional feed supplementation on the growth performance, survival, biochemical and enzymatic effects of Rosy barb, *Pethia conchonius*. A total of 180 healthy fish were divided into six feeding groups in triplicate: D1, D2, D3, D4, D5, and control. Fish were sampled at regular intervals, and growth and feed utilization indices were calculated at the end of the study. In terms of growth parameters, there was a substantial difference between the treated groups and the control group. The highest mean weight gain (1.114±0.08 g), specific growth rate (1±0.081 g/day) and survival rate (93.33%) were found in D5 while the lowest in D2 (1.86±0.106 g and 0.533±0.33 g/day). The average daily weigh gain (0.08±0.281 g) was found in D4. The treatment groups had the greatest average crude protein, carbohydrate, and fat levels of the fish when compared to the control. When beetroot and spirulina powder were added to the diet, the tissue superoxide dismutase (SOD), catalase, and reduced glutathione activities increased significantly. The D5 diet increases size, weight, net weight gain, weight gain, specific growth rate, and survival rate considerably. The inclusion of 12.5% Spirulina and 37.5% beetroot powder was shown to be sufficient to ensure Rosy barb growth as well as proximate and enzyme activity. Spirulina powder and beetroot powder can be used in fish feed as protein and carotenoid supplements to increase Rosy barb growth, survival, proximate and enzyme function.

Keywords: catalase, glutathione, *Pethia conchonius*, SOD, Spirulina

1. Introduction

Growing global demand for ornamental fish at rising prices has not been met by sustainable conservation and farming efforts, owing to increasing pressures, particularly for wild-caught fishes, as a result of overfishing, environmental degradation, or climate change (Beaugrand and Kirby, 2018; Nunez et al., 2019). According to Bilio (2007), feeding patterns are significant information in the setting of constrained aquaculture activity. Domesticated fish prefer meals combining fishmeal and live food (Li et al., 2019; Amrullah et al., 2023). Differences in type, nutrition, and a variety of other factors, on the other hand, will alter feed utilization and, ultimately, fish growth (Hien et al., 2016; Basuini et al., 2020). Natural, safe, and cost-effective food supplements must be explored as eco-friendly treatments in aquaculture to modulate the immune system (Abdel Tawwab et al. 2020; Santhosh and Umesh 2021). Vitamins are advised as dietary supplements for many aquatic animals.

Protein is the main component of fish flesh, hence protein is the key component of the fish diet. Protein is essential for the efficient functioning, development, and production of new body protein. The price of feed is determined by the proportion of protein in fish feed. Feed was found to be cost-effective if all protein in the feed was converted to body protein and only a small amount was consumed in catabolism (Gauquelin et al., 2007; Phinyo et al., 2024). For optimal growth performance, fish require a high protein level in their meal (Deng et al., 2006; Abd-elaziz et al., 2023). The relevance of feed quality and amount is critical since it has a large impact on growth rate, survival, water, and fish production. As a result, significant efforts are required to regulate the quality and quantity of crude protein in order to maintain a high fish yield (Das et al., 1991). Sound quality is an important component in feed consumption. Spirulina's high protein content percentage and well-balanced amino acid profile, when compared to other plant protein sources (Mamun et al., 2023), make it a suitable fish meal replacer in aquafeed composition. The parameters for selecting artificial feed include (1) fish acceptability, (2) effectiveness in encouraging fish growth, and (3) degree of cheapness and food availability. As a result, spirulina and beet root, which are high in protein and vitamin, were added to the diet.

The rosy barb, also known as *Pethia conchonius* (Hamilton, 1822), is a small, wellliked freshwater cyprinid native to India and other parts of South and Southeast Asia. *P. conchonius* is another species that aquarium hobbyists' affection to keep as ornamental fish in their home aquariums due to its hardiness. The species has a higher global demand, with an export value of $6.99 - $9.99 per piece. It exhibits a wide range of environmental sensitivity, the ability to colonize disturbed environments, trophic opportunism, and quick growth rates. Given this, the current study aimed to assess the effects of feed supplementation on growth, proximate parameters, and enzyme activity in *Pethia conchonius* among six groups of fish.

2. Materials and methods

2.1. Animals and experimental condition

Pethia conchonius (Rosy barb) was purchased from a local commercial aquarium fish supplier who obtained them from Kurunthancode, Kanyakumari, Tamil Nadu, India and kept for 15 days for acclimation in the laboratory. The final experiment lasted 90 days in an aquarium (50 ml size). 180 healthy fishes were selected from the acclimatized tank and stocked in the aquarium for the experiment (average body weight of 1.31±0.05 g). Rosy barb was divided into five treatments and one control (three replicates per treatment), with each treatment containing 30 fishes (Fig. 1).

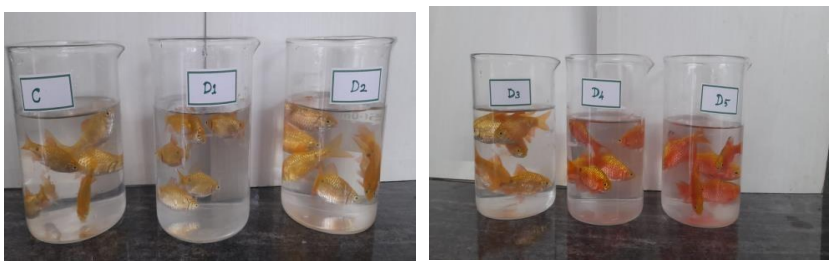

Fig. 1. Rosy barb samples used for the study; Fish fed with C- Control, D1, D2, D3, D4 and D5

2.2. Experimental diets and feeding protocol

Six different diets were formulated containing nutritional feeds with different levels of spirulina and beetroot powder for comparative study. The spirulina and beetroot powder were incorporated into the diet as: 0% (control), 0 and 50% (D1), 50 and 0% (D2), 25 and 25% (D3), 37.5 and 12.5% (D4) and 12.5 and 37.5% (D5). A diet with andwithout spirulina powder and beet root powder are used as a treatment group and control group, respectively (Table 1, Fig.

2).

The ingredients were collected from the local market. They were powdered using electric grinder and sieve through a fine mesh of 0.5 mm sieve. After taking appropriate weight of the ingredients, they were mixed thoroughly to get a homogeneous by adding the required amount of warm water and then made as dough. The dough was then placed in a pressure cooker for 15 minutes cooked and then sufficient amount of feed additives (beetroot powder and spirulina powder), vitamin and mineral mix added and then pressed through a hand palletized. The wet noodles were collected in plastic sheets and dried in Sunlight. After drying, they were stored in plastic containers for further use.

Table 1. Percent composition of control and experimental diets

Ingredients	**Dietary group (%)**					
	Control group		**Treatment group**			
	Control	**D1**	**D2**	**D3**	**D4**	**D5**
Fish meal	120	95	95	95	95	95
Rice bran	65	65	65	65	65	65
Ground nut oil cake	90	90	90	90	90	90
Soya bean meal	125	100	100	100	100	100
Tapioca flour	60	60	60	60	60	60
Vitamin and minerals	40	40	40	40	40	40
Beet root powder	0	50	0	25	12.5	37.5
Spirulina powder	0	0	50	25	37.5	12.5

Fig. 2. Feed formulations of nutritional feeds (Fish meal, Rice bran, Ground nut oil cake, Soya bean meal, Tapioca flour, Vitamin and minerals) with different levels of spirulina and beetroot powder0% (control), 0 and 50% (D1), 50 and 0% (D2), 25 and 25% (D3), 37.5 and 12.5%

(D4) and 12.5 and 37.5% (D5)

2.3. Growth performance

At the end of the feeding, the trial fish was weighed for calculation of percentage weight gain. A standard formula was calculated for the percentage weight gain, SGR% (specific growth rate), and ADG (Average daily gain).

$$Weightgain\ (g) = (Finalfishweight - Initialfihweight) \quad (1)$$

$$Average\ daily\ gain\ \left(ADG; \frac{g}{day}\right) = \frac{(Final\ fish\ weight - Initial\ fih\ weight)}{Days} \quad (2)$$

$$Survival\ rate\ (\%) = \frac{Fish\ stocked\ initially - Mortality}{Fish\ stocked\ initially} \times 100 \quad (3)$$

$$Specific\ growth\ rate\ \left(\frac{g}{day}\right) = \frac{(Final\ weight) - (Initial\ weight)}{Days\ of\ feeding} \times 100 \quad (4)$$

2.4. Biochemical analysis of fish

Total protein content was estimated by the method of Lowry et al. (1951). 1 ml of sample was dissolved in 1 ml of 1 N NaOH. From this 0.2 ml of the extract was taken into the test tube and 5 ml of alkaline copper solution (50 ml of 2% Na_2CO_3 and 1ml of 0.5% $CuSO_4$. $5H_2O$ in 1% sodium potassium tartrate) was added.The contents were mixed well and allowed to stand for 10 minutes. To this 0.5 ml of 50% Folin - Ciocalteau reagent (diluted with distilled water in 1:1 ratio) was added. After 30 minutes, the optical density was measured at 620 nm in a spectrophotometer against a blank. The standard graph was plotted by the method of Lowry et al. (1951) with bovine serum albumin (Sigma chemical Company, U.S.A). The values were expressed as mg/l. All the samples were taken in triplicates.

0.2 ml sample was taken and 0.2 ml of concentrated sulphuric acid was added and mixed well (Frings et al., 1972). Tubes were kept in boiling water for 10 minutes and cooled in cold water for 5 minutes. After cooling 10 ml of phosphovanillin reagent was added. The contents were mixed well and incubated at 370°C in a water bath for 15 minutes. The tubes were cooled to room temperature and the light pinkish brown colour developed was measured at 540 nm within 30 minutes against reagent blank. A set of standards with the concentrations ranging from 10 to 100 µg were run for plotting the standard graph. The total lipid concentration of the sample was calculated from the standard curve and expressed as mg/l.

Total carbohydrate content was estimated by the method of Carroll et al. (1956). Test sample was mixed with 10% Trichloro acetic acid (TCA) and centrifuged at 3000 rpm for 15 minutes. To 0.5 ml of the supernatant, 5 ml of anthrone reagent was added and boiled for 15 minutes. The tubes were cooled and absorbance measured at 620 nm in a spectrophotometer using blank, which consists 10% TCA and anthrone in the same proportion. The values were expressed as mg of glucose/l of sample.

2.5. Enzymatic analysis of fish

Estimation of SOD activity

0.01 ml of tissue homogenate was mixed with 0.2 ml of 0.1 M EDTA, 0.1 ml of 1.5 mM NBT and phosphate buffer in a total volume of 2.65 ml. After adding 0.05 ml of

riboflavin, the absorbance of the solution was measured against distilled water at 560 nm. All the tubes were illuminated with incandescent lamp uniformly for 15 min and absorbances of the blue colour formed were measured again. Percent of inhibition was calculated after comparing absorbance of sample with the absorbance of control. The volume of the sample required to scavenge 50% of the generated superoxide anion was considered as 1 unit of enzyme activity and expressed in U/mg protein (McCord and Fridovich, 1969). **Estimation of catalase activity**

0.1 ml of tissue homogenate was mixed with 1.9 ml of the phosphate buffer. After adding 1 ml of 30 mM H_2O_2 solution in buffer, decrease in extinction was measured at 240nm, at 1 minute interval for 3 minutes (Beers and Sizer, 1952). A sample control was placed in the reference cuvette containing 0.1 ml of tissue homogenate and 2.9 ml of the buffer. Activity of catalase was calculated using the molar extinction coefficient of 43.6 cm -1 mmoles of H2O2 decomposed/min/mg protein or (U/mg protein). **Determination of tissue reduced glutathione (GSH)**

Reduced glutathione in the tissue was determined according to the method of Moron et al. (1979). 0.5 ml of the tissue homogenate was mixed with 0.1 ml of 25 % TCA, kept on ice for few minutes. These were then subjected to centrifugation at 3000 g for few minutes to settle the precipitate. 0.3 ml of the supernatant was mixed with 0.7 ml of 0.2 M sodium phosphate buffer (pH 8) and 2 ml of 0.6 mM DTNB (prepared in 0.2 M buffer, pH 8). The yellow color obtained was measured after 10 min at 412 nm against a blank which contained 0.1 ml of 5% TCA in place of the supernatant. A standard graph was prepared using different concentrations (10-50 n moles) of GSH in 0.3 ml of 5 % TCA. The GSH content was calculated with the help of this standard graph and expressed as n mols /mg protein or U/mg protein.

2.6. Statistical analysis

One-way ANOVA was used to evaluate the effect of the feed on the growth, proximate composition and enzyme activity in fish. Differences were considered significant at p-value <0.05. Statistical analysis was carried out with SPSS14.

3. Results and Discussion

The fundamental difficulty in aquaculture is managing the relationship between fast growth rate and feed usage. Fish nutrition is an important consideration in this industry since feed costs are weighed against total yield (Craig and Helfrich, 2002). Food additives are one of the ways that can stimulate fish growth and development while also enhancing fish health by increasing resilience to infections and stress. The study was designed to evaluate the effect of diets on growth metrics, proximate composition, and enzyme analysis in fish *P. conchonius*.

In the current study, different quantities of beetroot powder and spirulina were introduced to a fish diet to evaluate the prospective impacts on Rosy barb development and feed utilization. The addition of beetroot powder and spirulina to the meal proved to have a good effect on fish growth. Significantly highest (P <0.005) final mean weight was found in D5 (2.46±0.056) while lowest in D2 (1.86±0.106). The net weight gain was recorded

significantly (P <0.005) in D5 (1.114±0.081) while lowest in D2 (0.628±0.369). The average daily gain was found to be significantly highest (P <0.005) in D4 (0.08±0.281) followed by D5 (0.01±0.081), D3 (0.007±0.261) and lowest in D1, D2 and control (0.005±0.323, 0.005±0.369 and 0.005±0.0009 respectively). The specific growth rate was also found to be significantly highest in D5 (P <0.005). In this study, increased growth was observed in *Pethia conchonius* in the treatment group D5, which contained a large amount of beetroot powder, when compared to the treatment groups D2, D3, D4, D5, and control. D5 had a high net weight and specific growth rate. Fish fed a carotenoid-rich diet perform better in terms of growth, probably due to improved metabolism and nutritional utilization (Amar et al., 2001; Naverian et al., 2014). Various compounds found in this plant have been shown to promote growth (Goyal et al., 2015; Amiri et al., 2019; Wang et al., 2020). Beetroot powder contains a high concentration of carotenoids (alpha, beta-carotene, and lutein) and vitamin E, which function as antioxidants and may have played a part in Rosy barb survival.

Table 2. Growth parameters of *Pethia conchonius* among different treatments

Parameters	Control	D1	D2	D3	D4	D5	*P* value
Initial weight	1.35±0.039	1.28±0.064	1.40±0.248	1.28±0.056	1.228±0.017	1.34±0.072	*P* <0.005
Final weight	1.98±0.071	1.96±0.382	1.86±0.106	2.06±0.213	2.14±0.276	2.46±0.056*	
Net weight	0.63±0.092	0.672±0.32	0.628±0.36	0.786±0.261	0.917±0.281	1.114±0.08*	
ADG	0.005±0.0009	0.005±0.32	0.005±0.36	0.007±0.261	0.08±0.281*	0.01±0.08	
SGR	0.566±0.09	0.533±0.28	0.533±0.33	0.733±0.262	0.9±0.282	1±0.081*	

ADG- Average daily gain; SGR- Specific growth rate

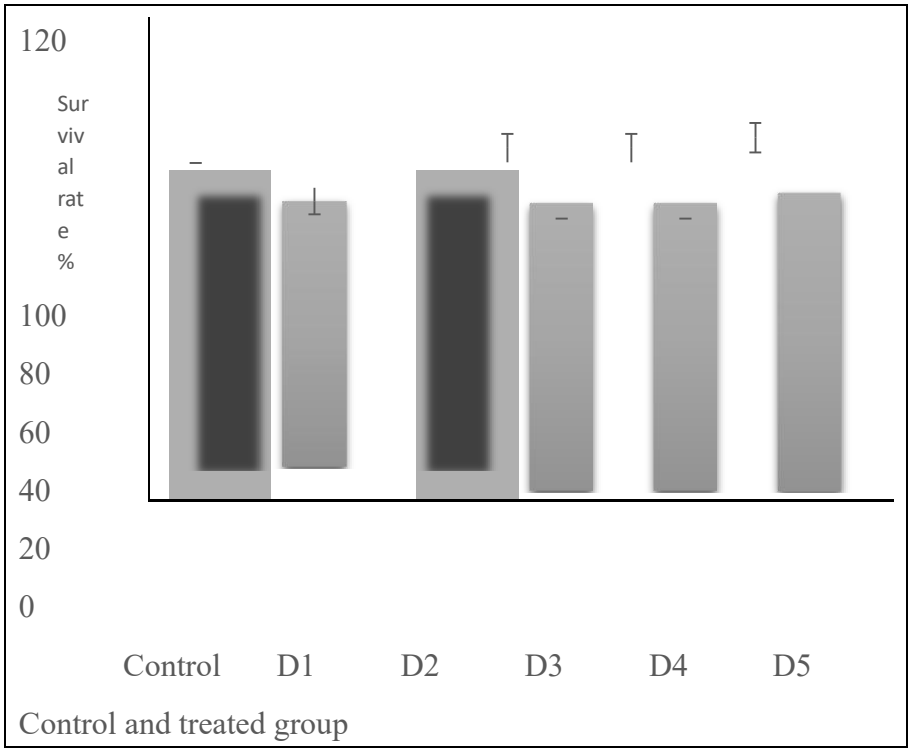

Fig. 1. Survival rate of *Pethia conchonius* fed with different percentage of feed

The nutritional profile is formed when the proximate components of the fish flesh combine to provide a first indication regarding the fish's commercial criteria that are required

for food regulations (Marichamy et al., 2012). The research of fish protein, fat, and carbohydrate concentrations is important from a variety of perspectives for consumers, manufacturers, and scientists. Aside from determining the nutritional content of fish, such research can help with better processing and preservation (Mridha et al., 2005) and regular assessment of the physiological status of fish from a fisheries standpoint (Cui and Wootton, 2011).

In this investigation, the protein contents of the fishes in this study are within the range of 154.346±0.005 to 430.35±0.12 mg/l. The maximum protein value was reported in D5 group fishes (430.35±0.12 mg/l) followed by D4 (428.053±0.008 mg/l) and minimum protein value were reported in control (154.346±0.005 mg/l). Next to protein, notable carbohydrate content was observed in D5 (64.896±0.422 mg/l) followed by D4 (62.56±0.364 mg/l), D3 (60.21±0.063 mg/l), D2 (59.133±0.005 mg/l), D1 (56.866±1.305 mg/l) and lowest amount of carbohydrate was noted in control (22.236±0.06 mg/l). The amount of lipid content was high in all treated groups, when compared to control (Table 3).

There was no current research on pigment inclusion in fish feed and its influence on muscle composition in *P. conchonius*. The current investigation revealed a substantial difference in the crude protein, carbohydrate, and fat content of *P. conchonius*. When compared to control diet fed fishes, the muscle biochemical composition of test fishes fed experimental diets (D5, D4, D3, D2 and D1) was high. The improved feed intake could be attributed to increased fish appetite, which leads to greater feed intake and thus improved growth. variations in protein, carbohydrate, and lipid contents in fish bodies, on the other hand, may be associated with variations in their synthesis, deposition rate in muscle, and/or different growth rate (Abdel Tawwab et al., 2006; Jasmine and Somnath, 2016). According to Oyelese (2006), feed intake has a large influence on the proximate composition of fish since these animals ingest a wide variety of foods and use them to gain the necessary nutrients for optimal growth and development. The availability of food varies throughout the year, resulting in variations in various bodily constituents (Habshy, 1973). It has been discovered that during periods of heavy or normal meals, the protein content of the muscles rises slowly at first, followed by a rapid increase in fat content.

Table 3. Proximate composition of control and experimental fishes

Parameters	**Control**	**D1**	**D2**	**D3**	**D4**	**D5**	***P* value**
Carbohydrate (mg/l)	22.236±0.06	56.866±1.305	59.133±0.005	60.21±0.063	62.56±0.364	64.896±0.422	*P* <0.005
Protein (mg/l)	154.346±0.005	424.103±0.06	424.426±0.00	424.706±1.074	428.053±0.008	430.35±0.12	
Lipid (mg/l)	2.026±0.003	10.42±0.017	10.426±0.015	10.506±0.005	10.633±0.071	10.653±0.187	

SOD (Superoxide dismutase assay), CAT (Catalase assay), and Glutathione reduced assay antioxidant enzymes are considered the first line of antioxidant defense and have been used as sensitive indicators of oxidative stress (Jiang et al., 2009). SOD is thought to be the first enzyme responsible for scavenging ROS and protecting cells from free radical damage by catalyzing the dismutation of the superoxide anion O_2 to molecular oxygen and H_2O_2, thereby halting the radical chain reaction launched by O_2 (Chien et al., 2003; Yu et al., 2024). Following that, CAT or GPX degrade H_2O_2 to molecular oxygen and H_2O. Increased SOD and CAT enzyme activity indicates an increase in antioxidant potential in an attempt to catalyze oxygen radicals and avoid oxidative damage. The results of the enzyme activity are represented in Fig. 2. The Glutathione reducedactivity was observed significantly highest (P <0.005) in D5 (480.11±0.008) than the other treatments and control. Superoxide dismutase activity was also observed significantly highest (P <0.005) in treatment D5 (6.466±0.385) compared to other treatments. In treatment D5 (2.22±0.008), Catalase activity was observed significantly highest (P <0.005) compared to other treatments and control. The enzyme activity is increased when beet root and spirulina powder are mixed with natural feed.

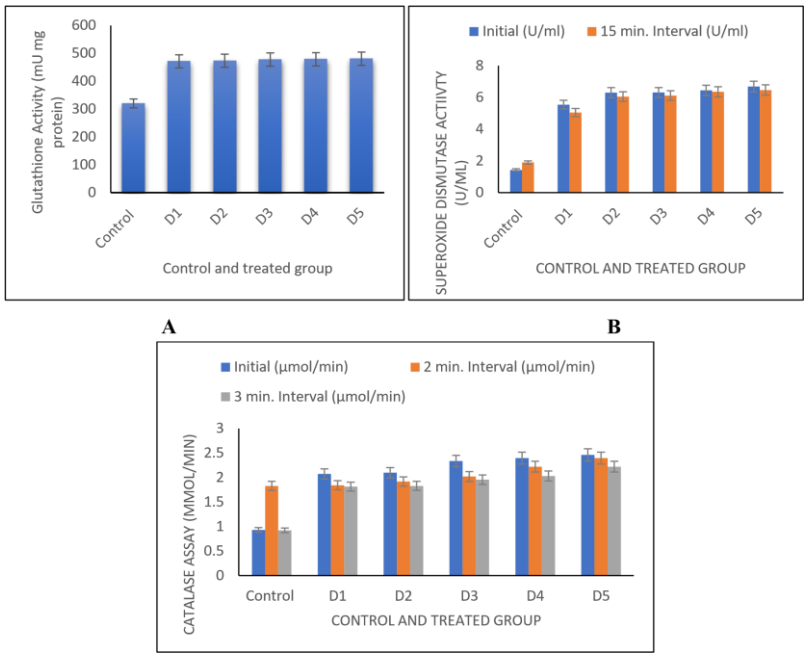

Fig. 2. Enzymatic assay of fish *Pethia conchonius* fed with different feeds A. Glutathione reduced assay; B. Superoxide dismutase assay; C. Catalaseassay

Beetroot supplementation may be an effective technique for boosting endogenous antioxidant defenses and protecting cellular components from oxidative damage. The biological environment of a cell is regarded to be in a state of redox balance under normal metabolic conditions, or in other words, an equilibrium exists between reducing (antioxidants) and oxidising (pro-oxidants) agents (Kohen and Nyska, 2002). Beetroot is a very high source of antioxidant chemicals. Several in vitro investigations have shown that betalain pigments, in particular, protect cellular components from oxidative stress (Teoriere et al., 2008). Kanner et al. (2001) found that two betalain metabolites (betanin and betanidin)

reduced linoleate damage caused by cytochrome C oxidase and lipid membrane oxidation caused by H_2O_2-activated metmyoglobin and free iron (AA-Fe). The scientists also discovered that betanin, the most abundant betalain present in beetroot (300-600 mgkg-1), was the most effective lipid peroxidation inhibitor. The remarkable antioxidant activity of betanin believed to be due to its excellent electron donating capability and ability to defuse highly reactive radicals targeting cell membranes (Kanner et al. 2001). However, as previously stated, betalains are not the only antioxidant molecules found in beetroot. Beetroot includes a number of highly bioactive phenolics, including rutin, epicatechin, and caffeic acid, all of which are known to be effective antioxidants (Manach et al., 2005). Following the oxidative stress, the beetroot extracts appeared to retain endogenous antioxidant activity (reduced glutathione, glutathione peroxidase, and catalase enzymes) at normal cellular quantities. The authors hypothesized that in response to *in vivo* cellular damage, beetroot may exhibit indirect antioxidant effects that operate to up regulate antioxidant defense pathways (Vulic et al., 2014; Clifford et al., 2015).

4. Conclusion

This study offers an update at the usage of beetroot and spirulina powder in the diet of the Rosy barb in order to optimize diet compositions. The results showed that adding beetroot and spirulina powder to fish meals increased their survival index. Beetroot and spirulina powder supplementation enhanced specific growth rate, proximate composition, and enzyme activity. For optimum Rosy barb growth and survival, we propose D5 diet which contains

37.5% beetroot powder and 12.5% spirulina powder in the fish diet.

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