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Effect of Dietary Anthocyanin on Survival and Growth Performance of Freshwater Ornamental Fishes Reared in Saline Environment

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Abstract

Objectives: The present work is to understand the influence of anthocyanin pigment (dried fruit peel powder of *Syzygium cumini*) incorporated diet on survivability, growth performance and maturation of ornamental fish species from three different families i.e., *Xiphophorus maculatus* (Platy), *Pethia conchonius* (Rosy Barb), and *Maylandia lombardoi* (Cichlid) reared in freshwater and saline medium (3ppt) for benefit in culture practice. **Methods**: 40 test fish each of three species were selected with initial average weight and distributed in 30L fiberglass aquaria as three replicas each in FW (freshwater) and SW (saltwater) with temp-25.3 $\pm$ 0.03, DO-4.8 $\pm$ 0.05 under standard aerated system. The fish were fed twice a day *viz.,* F1 (control) and F2 (0.25%), F3 (0.5%) and F4 (1% of dietary fruit peel powder of *S.cumini*) for a period of 60 days. **Findings**: 100% survivability was observed in all fish species from both FW and SW treatment, when fed with control (F1) and experimental diet (F2 to F4). F4 diet incorporated with highest concentration of anthocyanin pigment induced significant (P>0.05) improvement in BWG, SGR, FCR and CF in cichlid fish group reared in FW and when treated with SW compared to control and those fed with F2 and F3. Consequently, better growth was observed in FW fishes more so in cichlid compared to platy and rosy barb fish species. Minimum FCR values (P>0.05) were also noted in cichlid fish group fed with F4 indicating maximum utilization of feed when treated with FW compared to SW fishes followed by those fed with F3, F2 and F1. **Novelty**: The use of *S. cumini* as dietary administration of anthocyanin pigment induced good growth in freshwater fishes and limited the detrimental effects caused by stress of salinity exposure in fishes.

Keywords: *S. cumini*; BWG; SGR; FCR; CF; Ornamental fish

1 Introduction

Aquatic media controls and synchronizes growth and development of aquatic organisms. An exposure of freshwater fish to saline conditions can cause stress under natural as well as aquaculture conditions, thus affecting their metabolism that would disturb their survival, growth, feed intake and distribution in the realm [(1)]. Although fishes can acclimatize to different salinity according to their tolerance capacity and also the composition of FW consisting of carbonates and bicarbonates along with few silicates, sulphates and sodium; or SW comprising of different concentration of Na+, Mg+, Ca+ and Cl^{-} but it influences osmoregulation, metabolism, energy utilization, growth and feed intake as in *Labeo rohita* reared in FW and different concentration of saline water [(1)]. Thus, fish growth is dependent on various factors e.g., nutrients, feed ratio, salinity, digestion and microbiota, which is directly related to the assimilation of dietary compounds [(2)].

Nutrition being the essential factor for a profitable and economic ornamental fish culture practice, an alternative for synthetic pigments used as feed additives was identified from animal and plants such as, red yeast, carotenoids (beta carotene) from flowers, fruits, leaves or stem, due to their anti-stress, immunostimulatory potentials and health benefit along with growth enhancement and pigmentation thus prompting a spurt in universal demand. Several scientists have reported usage of feed additive for a nutritionally balanced diet, its effect on growth and reproduction of food and ornamental fishes because muscle nutrition, body weight and colouration depend on protein levels consumed and on the type of pigments used [(3)]. All aquatic organisms including fishes are capable in maintaining their nutritional deficiencies, if any, by selective feed intake when in natural conditions but these problems exist in confined conditions of aquarium and like and are thus dependent on artificial feed. Bano et al. [(4)] observed natural carotenoid to be more effective than synthetic one. On the other hand, Martin et al. [(5)] reported that a combination of common salt (0-3ppt) and clove oil treatment showed reduction of handling and transportation stress in *Cyprinus carp* and carotenoids incorporated feed, having antioxidant and therapeutic property stimulated tolerance capacity and immune system in aquatic organisms during harsh environments [(6)]. However, the present study on utilization of saline media in combination with dietary supplements incorporated feed for improvement in growth performance in freshwater ornamental fishes is a unique objective that does not figure in the existing research by scientists and is yet to be explored.

It is a known fact that fish growth rate is influenced by salinity of external environment by regulating osmotic balance for which more energy is expended. In extreme conditions of high or too low salinity of media compared to body fluid of fish, more energy is used (almost 10%) to regulate active ion transport during osmoregulation, thereby, less energy remains for growth [(7)].

The present study was targeted to evaluate growth performance by integrating saline media raised freshwater ornamental fishes belonging to three different families, fed with dietary supplement anthocyanin pigment from Indian blackberry, *S. cumini.* Anthocyanin is natural plant pigment belonging to the flavonoid and phenolic phytochemicals group, which is present in abundance as cyanidin, a glycosidic form of anthocyanidins whereas, delphinidin, peonidin, pelargonidin, petunidin, and malvidin are less abundant and their frequency decreases in accordance [(8)]. Similar to carotenoids consisting of biological activity like, radical scavenging and reducing properties, anthocyanins also consist of many antioxidants, anti-inflammatory, antimicrobial and anticarcinogenic properties that depends on chemical structure of anthocyanin, a cyanidin that gives fruits e.g., blue-, black-, strawberries etc. and flowers, their bright and attractive colour [(8)]. The fishes in question are in high demand locally and globally due to their shape, colouration and hardy nature and are cultured commercially for healthy growth and bright colouration in captive condition where healthy feed and good water quality is an important criterion.

Till date studies have been conducted on protective and therapeutic influence of consumption of anthocyanin for treatment of many common disease in humans and rat claiming that antioxidant activities of anthocyanins and their metabolites prevent tissue damage by scavenging of ROS and stimulation of the synthesis or activity of antioxidant enzymes [(8)] but reports regarding anthocyanin as feed additives for ornamental fish reared in salt water is lacking. Therefore, with this background a pioneer work was carried out to investigate better understanding of nutritional benefit and antioxidant effect of an indigenous anthocyanin pigment from locally available, blackberry fruit peel as a dietary supplement on survival and growth pattern (BWG, SGR, FCR & CF) of important FW ornamental fishes e.g., black wagtail platy, rosy barb and cichlid from three different families reared in freshwater and salt water (3ppt) for a period of 60 days. The antioxidant effect of pigment was mostly determined on growth of osmotic stressed fishes reared in SW.

2 Methodology

2.1 Experimental fish and design

Juveniles of about 8 weeks old, belonging to family Poecillidae (black wagtail platy-*Xiphophorus maculatus)*, Cyprinidae (rosy barb-*Pethia conchonius)* and Cichlidae (kenyi cichlid– *Maylandia lombardoi)*, of uniform size were brought to laboratory from Fisheries Research and Ornamental fish breeding and Information Centre (Inland), Hesaraghatta, Bangalore, Karnataka in airtight polythene bags half-filled with oxygenated water. They were maintained in 50 liters of pre-washed aquarium with aerated fresh dechlorinated tap water after quarantining in std. 0.1% $KMnO_4$ solution (1-2 dips) for two weeks and fed with control feed (F1) twice a day under laboratory condition. Water temperature was maintained at 24-26°C and photoperiod at 12/12 cycle. The healthy fishes aged about two and half months with mean initial body weight and body length viz., black wagtail platy (0.33 $\pm$ 0.03g; 2.64 $\pm$ 0.07cm), rosy barb (1.18 $\pm$ 0.05g; 4.85 $\pm$ 0.05cm) and kenyi cichlid (0.19 $\pm$ 0.07g; 1.71 $\pm$ 0.02cm) respectively were selected for experimental purpose. They were stocked @15 nos./tank randomly distributed and divided into four groups (one control and 3 experimental group II, III & IV) with three replicates each in 30-liter capacity of fiber-glass aquaria filled with FW and SW (3ppt). The fishes were starved for 24h prior to the experiment and were fed twice a day (9.00am & 6.00pm) during the experiment. The control fish group I were fed with customized formulated feed F1 and experimental group II, III and IV with F2, F3 and F4 (formulated feed F1 with dietary supplement of *S. cumini*).

The physico-chemical parameters of water from control and experimental aquariums were analyzed on alternate days. Temperature was measured by mercury silver glass thermometer; dissolved oxygen, free ammonia, total hardness and total alkalinity was analyzed by following standard method of APHA [9] and pH by digital pH meter, Eu-Tech, throughout the experimental period. Healthy and disease-free fishes were selected from the stock for further experiments.

2.2 Experimental feed formulation

The formulated feed (F1) was prepared with fish meal, wheat flour, groundnut oil cake, vegetable oil, rice bran, vitamin and mineral mix as represented in Table 1. The above ingredients were dried and ground to powder form, sieved and mixed proportionately in the laboratory to form dough kneaded with water and oil. Pellets (2mm) of 20μg were dried in hot air oven (40°C) to reduce the moisture level.

The experimental feed prepared was formulated with Indian blackberry, *Syzygium cumini*, an indigenous fruit with medicinal properties (anti-inflammatory, anti-hyperglycemic, cardioprotective and antioxidant), traditionally used as herbal medicines that was obtained from local fruit vendors/market. The total anthocyanin pigment content, was determined from fruit peel of blackberry by drying it in oven at 40 °C. It was ground to flour in a grinder and a solution was prepared by addition of 50g of this powdered blackberry peel with 100 mL of 1:1 ethanol/ acidified water (1% HCl) mixture. This solution was subjected to pH differential method as described by AOAC [10] spectrophotometrically. The dietary supplement experimental feed was prepared by mixing fruit peel of *S. cumini* with the formulated feed F1 at the rate of 2.5g/1000g (F2), 5g/1000g (F3) and 10g/1000g (F4) [2].

2.3 Proximate composition analysis

The formulated feed, dried blackberry fruit peel and experimental feed were subjected to proximate composition by using standard methods [10] that is represented in Table 1. Crude protein was analysed by Kjeldahl method (2100-Auto-analyzer, Foss, Hillerod, Denmark); crude lipid by Soxtec System HT6, Foss, Hillerod, Denmark; moisture (oven drying at 105°C for 24hr for a stable weight); total Ash content (combustion at 550°C for 12 hr); crude fibre i.e., Neutral Detergent Fibre (NDF) and Acid Detergent Fiber (ADF) [10].

Table 1. Specification of Refined (CCS) Iodized Salt under BIS: IS 7224:2006

Sl. No.	Characteristic	Requirement (percent by mass, on dry basis)
1.	Moisture	Max 0.50
2.	Water insoluble matter	Max 1.00
3.	Chloride content (as NaCl)	Min 98.50
4.	Matter soluble in water other than NaCl	Max 1.00
5.	Calcium (as Ca)	Max 0.15
6.	Magnesium (as Mg)	Max 0.10
7.	Sulphate (as SO_4)	Max 0.60
8.	Iodine content (ppm) Manufacturer's level Distribution channel including retail level	20-30; 15-30
9.	Alkalinity (as Na_2CO_3)	Max 0.15
10.	Iron (as Fe) (ppm)	Max 50.00

2.4 Standardization of Salinity Concentration

LC50 of saline water prepared with refined iodized salt was determined by following standard methods[9]. Platy, rosy barb and cichlid, were reared in saline media for 96h and a salinity tolerance bioassay was conducted by preparing different concentrations of SW, ranging from 1 to 4ppt for the conduct of experiment. No mortality was recorded in the fishes treated with 1 to 3ppt of salinity at 96h. Although fishes showed stress and changes in behaviour, death was not recorded among any of the three fish species till 3 ppt. 50% mortality and fading of colouration was recorded at 3.25ppt, 75% at 3.5 ppt and almost all fishes died at 4ppt. The data obtained from salinity tolerance was used to establish the required concentration for the conduct of experiments. Thus, the water salinity was maintained at 3 ppt in all the tanks.

Salinity is measured in milligrams per liter (equivalent to parts per million, ppm) and in grams per liter (equivalent to parts per thousand, ppt) in freshwater, brackish and seawater. It is defined as the total concentrations of all ions in water such as calcium, magnesium, sodium, potassium, bicarbonate, chloride and sulfate and not only the concentration of sodium chloride in water. Table 2 represents the composition of Refined Iodized salt provided and managed by Salt Commissioners office (SCO), Ministry of Commerce and industry, Govt. of India and Rajasthan State Unit, Jaipur (June 2024).

Table 2. Ingredients of formulated feed and proximate composition of feed ingredients: formulated feed F1 (Control), F2, F3 and F4 incorporated with 0.25 g, 0.5 g & 1 g of 100 g^{-1} dried fruit peel powder of *S. cumini* respectively

Sl. No.	Ingredients	Feed 1 (F1) mg/g	Feed 2 (F1+ 0.25 g100 g^{-1})	Feed 3 (F1+ 0.5 g100 g^{-1})	Feed 4 (F1+ 1 g100 g^{-1})	*S. cumini* fruit peel powder mg/g
1	Fish meal	30	30	30	30	-
2	Groundnut oil cake	22	22	22	22	-
3	Wheat flour	15	15	15	15	-
4	Rice bran	22	22	22	22	-
5	Vegetable oil (ml)	3ml	3ml	3ml	3ml	-
6	Vitamin and mineral mix* (Maxirich forte)	4	4	4	4	-
	Proximate Composition	F1	F2	F3	F4	
7	Total lipidis	5.02 ± 0.01	7.23 ± 0.37	7.35 ± 0.40	7.40 ± 0.44	2.43 ± 0.43
8	Crude Protein	32.2 ± 0.04	38 ± 0.27	39 ± 0.29	40 ± 0.33	7.8 ± 0.29
9	Crude Fiber	1.14 ± 0.02	17.28 ± 0.35	17.32 ± 0.33	17.38 ± 0.38	16.24 ± 0.36
10	Total Ash	17.28 ± 0.06	19.75 ± 0.43	20 ± 0.49	23 ± 0.53	6.84 ± 0.47
11	Moisture	11.18 ± 0.04	21.17 ± 0.17	21.22 ± 0.25	21.27 ± 0.20	10.9 ± 0.16
12	Total Carbohydrates (TCHO)	45.5 ± 0.68	127.67 ± 0.83	127.82 ± 1.03	127.97 ± 1.3	82.93 ± 0.58
13	Organic Matter	82.72 ± 0.82	175.58 ± 1.07	175.62 ± 1.04	175.78 ± 1.7	93.16 ± 0.95
14	Nitrogen Free Extract (NFE)	50.03 ± 0.02	107.03 ± 0.78	107.09 ± 0.55	107.16 ± 0.98	76.09 ± 0.64
15	Neutral Detergent Fiber (NDF)	19.19 ± 0.45	94.01 ± 0.60	94.07 ± 0.85	94.11 ± 0.90	74.92 ± 0.45
16	Acid Detergent Fiber (ADF)	21.56 ± 0.34	68.08 ± 1.03	68.14 ± 1.09	68.04 ± 1.12	46.48 ± 0.78
17	Dry Matter	88.51 ± 0.15	177.41 ± 0.85	177.56 ± 0.34	177.61 ± 0.63	89.10 ± 0.48
18	Mineral Content	2.02 ± 0.14	6.25 ± 0.32	6.29 ± 0.35	6.28 ± 0.38	4.26 ± 0.24
19	Anthocyanins (mg/g)	Nil	84.32 ± 2.2	84.32 ± 2.2	84.32 ± 2.2	84.32 ± 2.2
20	Polyphenols	Nil	68.35 ± 0.023	68.35 ± 0.023	68.35 ± 0.023	94.91 ± 0.45

Note: *Vitamins and mineral mix (mg/100g feed): (Vitamin A 1600 IU; Vitamin D3 100 IU; Vitamin E Acetate 5 IU; Energy 7.457 Kcal; Protein 0.00253 g; Fat 0.824 g; Carbohydrate 0.01.4 g; Calcium 75 mg; Phosphorous 58 mg; Vitamin C 25 mg;

Nicotinamide 15 mg; Magnesium 3 mg; Potassium 2 mg; Vitamin B1 1 mg; Vitamin B2 1mg; Calcium Pantothenate 1 mg; Vitamin B6 0.5mg; Manganese 0.5 mg; Zinc 0.5 mg; Folic acid 50 mcg; Vitamin B12 0.5 mcg; Copper 0.45 mg; molybdenum 0.1 mg; Iodine 0.075 mg); Values are expressed as mean $\pm$ SE

2.5 Analysis of growth performance

The growth parameters were analyzed following the standard formula for survival rate (SR), body weight gain (BWG), body length gain (BLG) a specific growth rate (SGR), and feed conversion ratio (FCR), condition factor (CF) was analyzed.[2]

- Survival rate (SR %) = (Final fish number - Initial fish number)/Initial fish number *100
- Body weight gain (BWG %) = (final body weight-initial body weight)/ Initial body weight *100
- Body length gain (BLG%) = (Final body length - Initial body length) /Initial body length*100
- Specific growth rate (SGR% d^{-1}) = [$\text{Log}_n(W_2)$ - $\text{Log}_n(W_1)$]/time (days) *100

Where, W_1 and W_2 indicate the initial and final weight (g), respectively.

- Feed conversion ratio (FCR) = Feed delivered to group (g) / Live biomass gain of that group (g)
- Condition factor (CF%) = (W/L^3) *100

Where, W= wet body weight (g) and L = standard body length (cm)

2.6 Data sampling and Statistical Analysis

The statistical analysis of data is expressed in the form of Mean and standard error ($\pm$ SEM) Prior to analysis, raw data were diagnosed for normality of distribution and homogeneity of variance with Kolmogorov-Smirnov test and Levene's test. One way ANOVA and Tukey tests were used to compare the data of growth parameters on different types of feed among the same species. A probability level of 0.05 was used for rejection of the null hypothesis. Two-way ANOVA and Bonferroni tests were performed using growth parameters of three species of fishes. Since no homogeneity of variances was achieved, the non-parametric Kruskal–Wallis followed by Mann–Whitney test was used (Zar, 1996) to test the results obtained for BWG and SGR, CF, FCR in both freshwater and saline water conditions. Minimum significance level was set at $p < 0.05$ in all cases. using GraphPad Prism 5.1. Polynomial regression tests were conducted using both linear and non-linear response models with measured R^2values and p-values Superscripts a, b, c and d indicate statistical mean differences at P<0.0001, P<0.001, P<0.01 and P<0.05 respectively.

3 Results and Discussion

3.1 Physico-chemical parameters of water

Physico-chemical parameters of freshwater and salt water (3ppt) from control and experimental tank showed dissolved oxygen (DO) 5.5 $\pm$ 0.08 mg/l, free ammonia 0.37 $\pm$ 0.04 mg/l, total alkalinity 136 $\pm$ 0.3mg/l, total hardness 280 $\pm$ 0.8mg/l and pH at 7.2 $\pm$ 0.04. The water parameters were within BIS limits of freshwater. The fishes were found to withstand and tolerate a narrow range of salinity of 1-3 ppt. Although there was 100% survivability of all three species of fish in 1-3ppt at 96hrs of exposure but with increase in saline levels, a gradual reduction in their activity and survivability was observed. At 3.25 ppt, fading of colouration was recorded with 50% mortality; at 3.5 to 4ppt, 75-100% of fishes showed reduced swimming activity, further discoloration and mortality. Similarly, in tilapia fish, with increase in salinity from 10 to 20ppt, a passive swimming activity and resting at the corner of aquarium was noted[11]. A decrease in survivability in *C. carpio* with increase in saline levels of 15 to 20ppt was also reported by Dawood et al[12]. The tolerance of all species of ornamental fishes to SW (0-3ppt) in the present study corroborates with those of Tonny et al.[13] who observed 100% survivability and tolerance capacity of *H. fossilis* fingerlings exposed to 0-4 ppt of brine solution. Contrasting to these results, reports of Valente et al.[14] showed high saline concentration (15ppt) as tolerance capacity of an ornamental fish, platy- a Poecillid, although decrease in blood glucose levels was noted on exposure for 96hr.

3.2 Behavioural studies

Our experiments regarding behaviour of all species of ornamental fishes reared in FW was normal and active whereas those in SW(3-4ppt) exhibited stressful behaviour e.g., increased respiration rate, fast to slow swimming movements, gasping near the water surface, poor appetite and variation in colour caused by osmoregulatory disturbances but there was zero mortality. Foyle et al.[15] observed altered physiological conditions in *C. carpio* when exposed to hypersaline media causing oxidative stress resulting in high mortality that correlated with the capability of fish to acclimatize to any medium depending on its osmoregulatory capacity of gills. Gills are the main organ to maintain homeostasis by active uptake of ions in FW fishes since, the adaptation to osmotic stress in saline media involves a cascade of ion-regulatory and histological adjustments that can be activated by elevated water hardness[16]. Tonny et al.[13] observed a constant growth of *H. fossilis* in FW along with low saline conditions (6%) and attributed the result to the presence of almost equal osmotic pressure in both environments thus minimal energy spent on osmoregulation. Whereas a decrease in growth rate was recorded in high salinity of water (18%) that he credited to increased oxygen consumption for higher energy requirement so as to maintain homeostasis which would otherwise affect the physiology of fish[7]. But there are no scientific reports published on the use of anthocyanin pigment from blackberry fruit peel on growth performance of ornamental fishes raised in FW or SW as mentioned in this study. Exposure of fishes to saline condition from 3ppt onwards to 4ppt showed discoloration and 100% mortality. The salinity tolerance test proved that all the three fish species were tolerant to 3ppt of saline condition, but the stress of salinity increased with increase in saline levels and time of exposure.

3.3 Survivability and growth

The present work was designed to explore the combined effects of dietary supplementary feed using anthocyanin pigment on growth parameters of FW ornamental fishes and those reared in SW showing salinity stress caused due to saline media (3ppt). The results indicated comparatively slower growth rate in SW raised fish than FW ones and had diminished productivity and colouration when fed with F1 (formulated feed without pigment). Interestingly, fishes of experimental group from FW and SW, fed with diet supplemented with anthocyanin feed (F2, F3 & F4) revealed 100% survivability and better growth than those fed with F1. This can be attributed to anthocyanin pigment having antioxidant and anti-carcinogenic properties that might have induced reduction of stress due to osmoregulatory compensation, improving immunity, inducing normal behaviour and growth in SW raised freshwater fishes. The present observation is in agreement with several studies reporting that pigments such as carotenoid when incorporated in fish feed improved growth, reduced oxidative stress levels, improved immune system but fishes are unable to biosynthesize carotenoids and are dependent on dietary carotenoid content for growth and colouration. Mohana et al.[17] reported a decrease in growth in *Catla catla* raised in the range 3-9ppt salinity since hyperosmotic water caused stress initiated production of stress hormone, cortisol in large quantity and degradation of osmoregulatory system that might have reduced growth in fish.

In the current study, survival rate of all FW and SW fish groups did not show any marked difference within the treatment and control groups but growth performance with respect to body weight gain, specific growth rate and feed utilization efficiency showed significant improvement in fish fed with anthocyanin supplemented diets F2, F3 and F4 more so in those fed with F4 compared to those fed with formulated diet, F1 (Figure 1, 2 & 3). Polynomial regression for two factors (growth parameter i.e., SGR, FCR and CF vs body weight) for 3 fish species (*Maylandia lombardoi, Pethia conchonius, Xiphophorus maculatus*) exposed to freshwater and saltwater conditions was evaluated. The effects of FW and SW (3ppt) within each level of inclusion of *S. cumini* revealed following: R^2 value of growth parameter with respect to BWG in fishes reared in FW and SW when fed with F2 to F4 showed good growth in all fish species compared to those fed with F1. Fishes fed with F4 feed had maximum R^2 values followed by those fed F3, F2 and F1. Cichlid fish group showed the maximum range of R^2 values within each level of inclusions of *S.cumini* followed by platy and rosy barb. Thus, the effects of saline water decreased with increase in *S. cumini* levels (**Suppl. Table 1**). The results of the impact of *S. cumini* within each level of water salinity i.e., FW & SW revealed good interaction in fishes reared in FW when fed with F1 to F4 feed. Platy with maximum R^2 value showed better growth due to favourable interaction with feed consisting of *S. cumini* in FW was closely followed by cichlid but rosy barb showed minimum interaction due to lower range of R^2 value whereas those reared in SW revealed cichlid with better interaction with maximum R^2 value compared to those of platy and minimum interaction was observed in rosy barb (**Suppl. Table 2**).

[Data are shown as mean ± SE. Significance by two-way ANOVA and post-hoc test – Bonferroni (P<0.01) using GraphPad Prism 5.1. Scripts A, B and C indicate statistical mean differences at P<0.0001, 0.001 and 0.01 respectively.]

[Data are shown as mean ± SE. Significance by two-way ANOVA and post-hoc test – Bonferroni (P<0.01) using GraphPad Prism 5.1. Scripts A, B and C indicate statistical mean differences at P<0.0001, 0.001 and 0.01 respectively.]

[Data are shown as mean ± SE. Significance by two-way ANOVA and post-hoc test – Bonferroni (P<0.01) using GraphPad Prism 5.1. Scripts A, B and C indicate statistical mean differences at P<0.0001, 0.001 and 0.01 respectively.]

Nevertheless, improvement in survivability and growth in SW treated fish compared to its control ones, can be attributed to antioxidant and anti-carcinogenic and hepato-protective[8] properties of anthocyanin pigment that might have caused reduction of stress by osmoregulatory compensation, probably improving immunity and thus inducing normal behaviour and

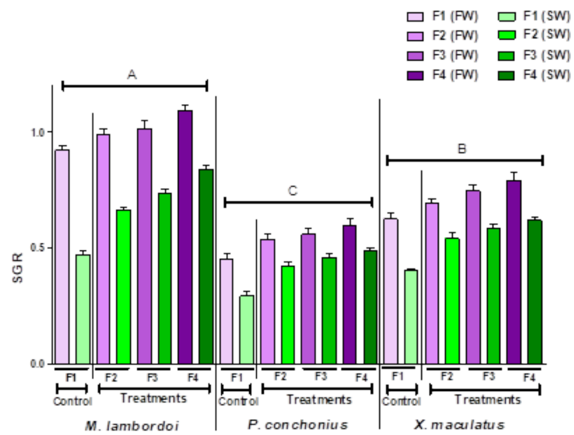

Fig 1. Specific growth rate (SGR) of *M. lambordoi*, *P. conchonius* & *X. maculatus* fed with different diets *viz.,* F1, F2, F3, F4 for the experimental period of 60 days

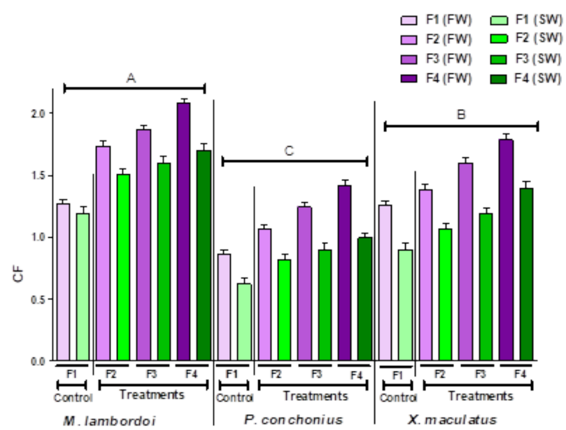

Fig 2. Condition factor (CF) of *M. lambordoi*, *P. conchonius* & *X. maculatus* fed with different diets *viz.,* F1, F2, F3, F4 for the experimental period of 60 days

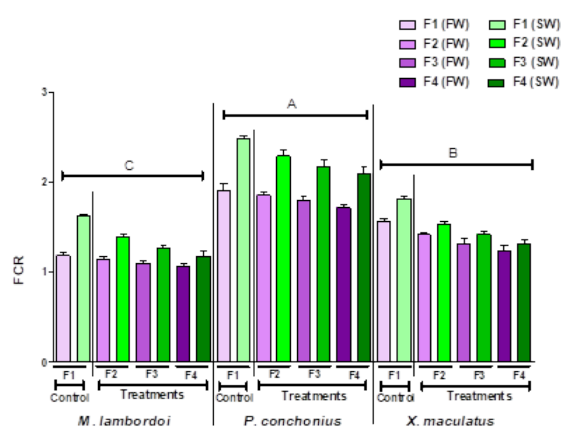

Fig 3. Feed conversion ratio (FCR) of *M. lambordoi*, *P. conchonius* & *X. maculatus* fed with different diets *viz.,* F1, F2, F3, F4 for the experimental period of 60 days

growth. These results are in agreement with reports by Banoet al. [4] in giant gourami *(Trichogaster fasciata);* Vanegas-Espinoza et al. [18] in *Carassius auratus* fed with low levels (D150 & D300) of extract from *Hibiscus sabdariffa*, a dietary anthocyanin supplement; Mousumi [19] in *Danio rerio* where carotenoid incorporated feed enhanced their survival and growth performance like weight gain and specific growth rate, food intake and utilization of nutrients when compared to control and Diab et al. [20] in red tilapia on improvement of survival, growth performance and stress resistance by using carotenoid pigment as feed additive.

Cichlid fishes displayed significant (P<0.01) elevated growth with higher BWG (531.58 $\pm$ 1.32), BLG (64.91 $\pm$ 0.67), SGR (1.09 $\pm$ 0.03) and CF (2.08 $\pm$ 0.03) reared in FW with negligible salinity and fed with F4 diet, when compared to those of rosy barb and black wagtail platy BWG = 79.66 $\pm$ 0.16, BLG=12.99 $\pm$ 0.14, SGR=0.60 $\pm$ 0.03, CF=1.41 $\pm$ 0.04 and BWG=124.24 $\pm$ 1.71, BLG=24.53 $\pm$ 0.55, SGR=0.79 $\pm$ 0.05, CF=1.79 $\pm$ 0.05) respectively. Similar trend was noted in cichlid fish group of SW (3ppt) that also attained highest growth when fed F4 (BWG=410.33 $\pm$ 1.89, BLG=56.50 $\pm$ 0.87, SGR=0.84 $\pm$ 0.05; CF=1.70 $\pm$ 0.05) compared to black wagtail platy and rosy barb (BWG=106.01 $\pm$ 1.27, BLG=22.18 $\pm$ 0.65, SGR=0.62 $\pm$ 0.04, CF=1.43 $\pm$ 0.05 and BWG=54.15 $\pm$ 0.36, BLG=12.08 $\pm$ 0.65, SGR=0.49 $\pm$ 0.03, CF=1.02 $\pm$ 0.04)) respectively. However, the saline conditions caused stress to all fish species that probably resulted in decrease in their growth parameters compared to those of FW fishes. Reduction in BWG, SGR and CF in all fish species exposed to 0-4ppt SW in the present study was also reported by Sarma et al. [1] in *L. rohita* exposed to 4.5‰ salinity for 90 days whereas 2.5‰ was desired salinity; by Debroy et al. [21] in *Anabas testudineus* within 0 to 6 ppt with best results at 3ppt but a striking decrease at high salinity (9-12ppt) and by Prananingtyas et al. [7], in catfish reared at 3-6ppt salinity compared to FW.

Previous studies have shown improved growth and FCR in FW fishes when reared in both fresh water and low salinity environments but with increase in salinity levels a decrease in growth was reported [1]. In the present study, cichlid fish group of freshwater condition showed minimum FCR values (1.06 $\pm$ 0.04) when fed with F4, when compared with those fed with F3 followed by F2 and F1 (1.18 $\pm$ 0.02, 1.14 $\pm$ 0.02 and 1.16 $\pm$ 0.002 respectively) (Figure 3) and also compared to fishes raised in saline media (1.20 $\pm$ 0.08). Subsequently, F4 fed black wagtail platy and rosy barb in SW also showed less FCR values compared to control ones but was significantly (P<0.01) more (1.30 $\pm$ 0.05 and 1.38 $\pm$ 0.06 respectively) than cichlid fish groups which resulted in reduced growth. Similarly, the FCR values of rosy barb and black wagtail platy of SW was on the higher side when fed with F3 and F2 that showed decrease in growth compared to those in FW conditions and cichlid fish group (Figure 3). Thus, it can be assumed that formulated diet provided the main nutritional requirements, but additional nutrients might have been provided by incorporated anthocyanin in feed F3 and F4. The results proved that high salinity induces stress in fish in SW and the energy from feed is utilized to regulate osmoregulation.

There are evidences that carotenoid pigment played an important role in improving the growth performance with respect to BWG, BLG, SGR and CF (in terms of mean body weight and length) in fishes. Similar observations were also observed in the present work, in three families of fishes fed with anthocyanin pigment from *S. cumini* fruit peel incorporated feed (F3 and F4), more so in cichlid fish than cyprinid and poeciliid. Our results are also in agreement with those of Zutshi and Rekha who had reported an increase in growth of *Xiphophorussp*. compared to *Poecilia sp*., due to high concentration of carotenoid supplemented feed from *L. camara* [2] and Saifuddin et al. [3] in *X. helleri* when fed with china rose, marigold flower and carrot meal supplemented feed showed significant improvement in BWG and SGR values compared to the control groups. The findings of Tekale et al. [22] regarding anthocyanin activity showed that chalcones, the derivative of anthocyanin act as precursors for various activities of the liver and glycyrrhetinic acid, another derivative of anthocyanins regulates the enzyme 11β-HSD2, to control cortisol levels [23] which in turn helps in osmoregulation and growth in fishes [24]. Thereby it is assumed that the two derivatives of anthocyanin when present in fish, protects it from stress and boosts osmoregulation and growth.

The hypothesis as to how salinity affects energy budget in fish during high or low saline conditions in the external environment compared to fish body fluid (depending on fresh water or marine fish), depends on expenditure of more energy to regulate osmotic balance is widely accepted. Although in these environments most of the energy (10%) is used for ion transport (osmoregulation) via gills resulting minimum energy for growth [7] but Esmaeili [25] reported that maximum feed is converted to energy by FW fish which is saved to be utilized in growth parameters. Most of the studies have been conducted to estimate the effect of salinity on growth of food fish barring a few studies on aquarium fish [4], [14]. Sarma et al. [1] reported a descending trend in growth of *Labeo rohita* exposed from 0 to 4.5ppt salinity and result was attributed to expenditure of energy during osmotic regulation caused by changes in saline levels. Thus, in high saline conditions, food energy is utilized due to increase in osmoregulation activity [7] and osmoregulation is influenced by physiological factors since fishes have complex relationship in osmoregulation and energy metabolism. Therefore, in the present work an anthocyanin pigment that consists of antioxidants was incorporated in the basic formulated nutritious diet to compensate for salinity stress and improvement in growth was observed in all ornamental fishes even though they were exposed to saline conditions. Vanegas-Espinoza et al. [18] used microencapsulation as carrier for anthocyanins while preparation of fish diet and observed enhancement in growth of fantail goldfish reared in freshwater.

Although a few authors had previously reported contrastive results with no significant effect of carotenoid pigment supplementary diet on food fishes and few ornamental fishes. Such controversial results were assumed to be associated with type of digestion, its enzymes and availability of microbiota, that affects the acclimatization or absorption of dietary compounds. Lim et al.[6] found that interaction of pigment with dietary components might interfere with the digestibility of nutrients resulting in reduction in growth in fishes.

Anthocyanin pigment incorporated feed plays a crucial role to reduce stress as it is precursors for various activities in liver due to presence of antioxidants and aids in controlling the release cortisol which in turn facilitates osmoregulation to balance homeostasis and growth is promoted in SW fish. The plant pigment (carotenoid) incorporated diets fed *X. helleri*[3] and carrot meal to Zebrafish, *Danio rerio*[19] resulted in improved FCR that enhanced growth. Lim et al.[6] in support of our results further proved natural carotenoids incorporated diet posed as growth promoters which in turn improve immunity and productivity of the fish by improving their body weight in addition to food conversion ratio and feed efficiency. Thus, cichlid fish group fed with F4 containing higher concentration of dietary supplement (anthocyanin) showed rise in SGR and CF and maximum feed utilization (low levels of FCR) relative to platy and rosy barb fishes. Control fish groups of all three species showed significantly ($P<0.01$) less growth when fed with formulated feed F1 in absence of dietary anthocyanin pigment supplement. The experiment on SW exposed fishes would benefit during aquaculture studies on their survivability, behavior, and growth.

4 Conclusion

The findings of present study indicate significant increase and positive effect on different growth parameters (BWG, SGR, CF and FCR) in popular ornamental fishes belonging to three different families reared in freshwater and saline media at a tolerance capacity of 3ppt suggesting that fishes have the capacity to utilize pigment anthocyanin from peel of Indian blackberry fruit. The dietary supplement feed F4 at 10g/1000g was found to be most effective in producing required increase in SGR and CF due to maximum utilization of feed (minimum FCR values) in cichlid fish group that was closely followed by platy and rosy barb without any negative effect on their survivability or ill health. Although such effect was found more in the fishes reared in FW compared to salt water, but the pigment did assist the fishes in saline media to overcome the salinity stress and their growth performance showed improvement. Hence, anthocyanin pigment extract maybe required to be used in aquaculture industry that may prove to be beneficial for health and general welfare of ornamental fishes. Thus, this research gives rise to new opportunities for the aquaculture industry to investigate extraction of anthocyanin pigments commercially from blackberry and utilize it for improvement in growth performance of edible, therapeutic value-added fishes and popular ornamental fishes having high commercial demand could be further explored.

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Supplementary Tables

Table 1. Polynomial regression for two factors (growth parameter vs body weight for 3 fish species (*Maylandia lombardoi*, *Pethia conchonius, Xiphophorus maculatus*) exposed to freshwater and saltwater conditions when fed with *S. cumini* incorporated feed

Growth parameters	Feeds	Freshwater			Saltwater		
		Fish species					
		M. lombardoi	*X. maculatus*	*P. conchonius*	*M. lombardoi*	*X. maculatus*	*P. conchonius*
SGR:BW	F1	R^2= 0.60 P<=0.01	R^2= 0.5 P<=0.05	R^2=0.52 P<=0.04	R^2=0.53 P<=0.03	R^2=0.45 P<=0.05	R^2=0.38 P<=0.04
	F2	R^2=0.77 P<=0.03	R^2=0.64 P<=0.01	R^2=0.63 P<=0.04	R^2=0.61 P<=0.05	R^2=0.53 P<=0.01	R^2=0.41 P<=0.03
	F3	R^2=0.88 P<=0.01	R^2=0.75 P<=0.03	R^2=0.71 P<=0.02	R^2=0.68 P<=0.07	R^2=0.59 P<=0.03	R^2=0.48 P<=0.02
	F4	R^2=0.96 P<=0.05	R^2=0.89 P<=0.05	R^2=0.86 P<=0.01	R^2=0.76 P<=0.04	R^2=0.64 P<=0.05	R^2=0.55 P<=0.01
FCR:BW	F1	R^2=0.51 P<=0.02	R^2=0.49 P<=0.05	R^2=0.45 P<=0.01	R^2=0.44 P<=0.02	R^2=0.41 P<=0.01	R^2=0.38 P<=0.02
	F2	R^2=0.60 P<=0.01	R^2=0.57 P<=0.01	R^2=0.51 P<=0.02	R^2=0.53 P<=0.01	R^2=0.48 P<=0.03	R^2=0.43 P<=0.02
	F3	R^2=0.67 P<=0.02	R^2=0.64 P<=0.03	R^2=0.61 P<=0.02	R^2=0.62 P<=0.02	R^2=0.55 P<=0.01	R^2=0.49 P<=0.01
	F4	R^2=0.75 P<=0.01	R^2=0.72 P<=0.01	R^2=0.71 P<=0.01	R^2=0.70 P<=0.01	R^2=0.62 P<=0.01	R^2=0.54 P<=0.01
CF:BW	F1	R^2=0.62 P<=0.01	R^2=0.58 P<=0.02	R^2=0.55 P<=0.02	R^2=0.56 P<=0.01	R^2=0.48 P<=0.02	R^2=0.42 P<=0.02
	F2	R^2=0.70 P<=0.03	R^2=0.66 P<=0.04	R^2=0.63 P<=0.07	R^2=0.62 P<=0.03	R^2=0.52 P<=0.04	R^2=0.46 P<=0.07
	F3	R^2=0.84 P<=0.02	R^2=0.75 P<=0.01	R^2=0.70 P<=0.03	R^2=0.69 P<=0.02	R^2=0.60 P<=0.01	R^2=0.51 P<=0.03
	F4	R^2=0.92 P<=0.05	R^2=0.83 P<=0.05	R^2=0.79 P<=0.04	R^2=0.74 P<=0.05	R^2=0.66 P<=0.05	R^2=0.54 P<=0.04

Table 2. Polynomial regression for two factors (average of the growth parameter vs body weight for 3 fish species (*Maylandia lombardoi*, *Pethia conchonius, Xiphophorus maculatus*) exposed to freshwater and saltwater conditions when fed with *S. cumini* incorporated feed

Growth parameters	Feeds	Freshwater			Saltwater		
		Fish species					
		M. lombardoi	*X. maculatus*	*P. conchonius*	*M. lombardoi*	*X. maculatus*	*P. conchonius*
SGR:BW	Avg. of (F1, F2, F3, F4)	$y=2.1096x^2 - 1.8595x + 1.327$ $R^2=0.95$	$y=4.2118x^2 - 3.3213x + 1.2867$ $R^2=0.99$	$y=-0.1586x^2 + 1.0395x - 0.9207$ $R^2=0.81$	$y=1.3075x - 0.1302R^2=0.99$	$y=2.0865x - 0.5356R^2=0.87$	$y=0.349x - 0.3039R^2 = 0.7004$
FCR:BW		$y=-0.4176x + 1.3852$ $R^2 = 0.91$	$y=-3.1991x + 3.0376$ $R^2= 0.97$	$y=-0.5488x + 2.9354$ $R^2=0.91$	$y=-1.629x + 2.3829R^2=0.9795$	$y=-4.826x + 4.0178R^2=0.92$	$y=-0.9799x + 4.2865R^2=0.95$
CF:BW		$y =3.0504x - 0.1647$ $R^2= 0.99$	$y= 4.8858x - 1.0706$ $R^2= 0.97$	$y=1.1643x - 1.2525$ $R^2=0.96$	$y=1.934x + 0.3236R^2=0.98$	$y=4.4862x - 1.1853R^2=0.96$	$y=0.769x - 0.7538R^2=0.90$