Bulletin of Pure and Applied Sciences
Zoology (Animal Science), Vol.43A, No.1,
January-June 2024: P.49-63

Print version ISSN 0970 0765
Online version ISSN 2320 3188
DOI: https://doi.org/10.52710/bpas-zoo.24

Original Research Article *Content Available online at: https://www.bpasjournals.com/*

Influence of Plant Additive, *S. Cumini* on Growth and Colouration of *X. Maculatus*, *P. Conchonius*, and *M. Lombardoi*

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Received on 8.01.2024
Revised on 28.03.2024
Accepted on 18.04.2024

ABSTRACT:
Effect of dietary fruit peel powder (*Syzygium cumini*-wild Indian blackberry) incorporated formulated feed as a natural anthocyanin source was undertaken to assess survivability, growth and colouration on *Xiphophorus maculatus* (Platy), *Pethia conchonius* (Rosy Barb), and *Maylandia lombardoi* (Cichlid). Four types of feed, one control (F1) and three experimental feed were formulated as F2-25mg10^{-1}g, F3-50mg10^{-1}g and F4-100mg10^{-1}g dietary fruit peel powder of *S.cumini* fed twice a day for a period of 60 days. Ten fish per aquaria weighing 0.33g (platy), 1.18g (barb) and 0.18g (cichlid) were distributed in 30L fiberglass aquaria in triplicate with standard aerated system (temp-25.3±0.03, DO-4.8±0.05). 100% survivability and a steady rise in the body weight (BW) of all fishes fed with F1 to F4 was recorded on weekly basis. Enhancement was noted in growth parameters (BWG, SGR and CF) for cichlid fish group when fed with F4 compared to other feeds and fish species. The feed conversion ratio (FCR) was lowest in cichlid fish group due to high feed utilization ($P>0.05$). The levels of total anthocyanin content (TAC) were higher in rosy barb than platy fish and minimum in cichlid fish group. The study suggested that F4 feed with highest anthocyanin pigment (100mg10^{-1}g) consisting dried *S. cumini* fruit peel incorporated feed induced better growth in cichlid fish and bright pigmentation in rosy barb. This indicated that feed utilization is species specific. Synthetic pigments in fish feed can be replaced by anthocyanin pigment incorporated feed which can be applied as diet supplement feed in aquaculture practices (a pioneer study).

Keywords:
Anthocyanin, *Syzygium cumini,* Body Weight Gain, Specific Growth Rate, Condition Factor, Feed Conversion Ratio, Colouration

How to cite this article: Srividya. A and Zutshi B. (2024). Influence of Plant Additive, *S. cumini* on Growth and Colouration of *X. maculatus*, *P. monchonius*, and *M. mombardoi*. *Bulletin of Pure and Applied Sciences-Zoology*, 43A (1), 49-63.

INTRODUCTION

Attractive, colourful and unique body characteristics of ornamental fishes are accountable for causing an expansion of global ornamental fish trade from 2022 (USD 5.88 billion) with a compound annual growth rate (CAGR) of 8.5% in 2023 to 2030 (Grand View Research, 2023). Nutrition with respect to fish feed is the most essential element and expensive input for good growth and colour of different types of ornamental fish in aquaculture (Kaur and Shah, 2017). Synthetic pigments have been used as additive in fish feed commercially and in aquaculture practices which are harmful to the environment and fish in general. Therefore, to reduce such effects, pigments from natural plant source are recommended for healthy growth and bright coloration. Many scientists have focused their studies on growth improvement and colouration of ornamental fish by using natural carotenoid sources as supplementary feed for example, red pepper on rainbow trout for growth performance (*Oncorhynchus mykiss*) (Jha et al, 2012), dried flower petal in salmonids for pigmentation (Torrissen et al, 1989) and improvement in reproduction performance of fish (Vilchez et al, 2011; Watanabe and Vassallo-Agius, 2003). Like carotenoids, anthocyanins are known to have anti-inflammatory (Youdim et al, 2002), anti-carcinogenic (Fimognari et al, 2004) and additionally neuroprotective properties and arterial vasomotion effects (Colantuoni et al, 1991) with dietary antioxidants which play a role to decrease the risks of many human degenerative diseases (Wang et al, 1998). Nevertheless, there are some reports regarding effect of anthocyanin on rats and mice exposed to cadmium (Kowalczyk et al, 2003), dietary anthocyanin rich purple corn on obesity and hyperglycemia in mice (Tsuda et al, 2003) and amelioration of mulberry extract supplement on arthritic rats (Kim and Park, 2006).The presence of anthocyanin pigment in fruits and plant sources, such as blueberries, blackberries, Saskatoon berries etc., comprise of elevated levels of antioxidants when extracted occur as a heterogeneous mixture of numerous types of molecules consisting of various chemical structures of anthocyanidins in glycosidic forms which give fruits and flowers their bright colours (Fang, 2014; Khoo et al, 2017). In nature, around 653 anthocyanins have been recorded involving six anthocyanidins (aglycones) (He and Guisti, 2010) found ubiquitously distributed *viz.*, delphinidin, cyanidin, peonidin, petunidin, malvidin and pelargonidin.

A recent study by Monica et al (2019) evaluated the effects of dietary incorporation of co-stabilized anthocyanin extracts on swordtail fishes fed with from beetroot peels and red cabbage waste, having anti-microbial properties; Vanegas-Espinoza et al (2019) studied fantail goldfish fed microencapsulated anthocyanins from roselle and its flour which revealed improved growth and pigmentation (Pérez-Escalante et al, 2012). But there is no further information published on dietary anthocyanin effects on food and other ornamental fishes viz., rosy barb (Order-Cypriniformes, Family-Cyprinidae), black wagtail platy (Order-Cyprinodontiformes, Family-Poeciliidae) and kenyi cichlid (Order-Perciformes, Family-Cichlidae).Thus, taking into account the popularity of the above-mentioned ornamental fish among the hobbyists for its body shape and colour, different concentrations (0.25%, 0.5% and 1%) of dried and powdered peel of *Syzygium cumini* (blackberry) was used as dietary supplement to assess their survivability, growth performance (BWG, SGR, FCR & CF) and colouration for a period of 60 days.

MATERIALS AND METHOD

Collection of fruits of *Syzygium cumini*

Syzygium cumini, a blackberry, belonging to Phylum: Spermatophyta; Subphylum: Angiospermae; Class: Magnoliopsida; Order: Myrtales; Family: Myrtaceae was purchased from local fruit vendors/Hopcoms and was identified by a Professor from the Department of Botany, Bangalore University. It is a medicinal plant traditionally used in herbal medicines due to its anti-hyperglycemic, anti-inflammatory, cardioprotective, and antioxidant properties.

Preparation of formulated diet

The formulated feed (F1) was prepared using fish meal, groundnut oil cake, wheat flour, rice bran, vegetable oil, vitamin and mineral mix purchased from a local market in Bangalore. The above ingredients were dried, powered in a grinder and sieved and mixed proportionately (table 2) in the laboratory blender and kneaded with sufficient water and oil to form 2mm pellets of 20µg. It was then dried in hot air oven (40 °C) to reduce the moisture level (Square method by Hardy, 1980). They were kept in refrigerator for daily use. The three concentrations of dietary supplement experimental feed (F2, F3 and F4) were prepared using the following proportions. F2 (F1+2.5g/1000g), F3 (F1+5g/1000g) and F4 (F1+10g/1000gof fruit peel of *S. cumini*).

Proximate composition

Proximate composition of F1, F2, F3, F4 and *S. cumini* fruit peel powder was analyzed for the presence of various compounds including pigment anthocyanin as represented in table 3. 50 g of dried and powdered blackberry peel was added to 100 mL of 1:1 ethanol/ acidified water (1% HCl) mixture. The said solution was mixed thoroughly and the total anthocyanin amount was determined spectrophotometrically by using pH differential method as described by AOAC (2005). Crude protein was assessed by Kjeldahl method (2100-Auto-analyzer, Foss, Hillerod, Denmark); Crude lipid by ether extraction method using a Soxtec System HT (Soxtec System HT6, Foss, Hillerod, Denmark), Moisture contentby oven drying at 105°C for 24hr in oven for a stable weight; Total Ash content by combustion at 550°C for 12 hr; Crude fibre with respect to Neutral Detergent Fibre (NDF) (AOAC, 2012, Method No. 2002:04) and Acid Detergent Fiber(ADF) Levels (AOAC, 2012, Method No.973:18).

Experimental fish and design

75 numbers of each of the experimental fishes species viz., black wagtail platy-*Xiphophorus maculatus*, rosy barb-*Pethia conchonius* and kenyi cichlid– *Maylandia lombardoi* belonging to family Poecillidae, Cyprinidae and Cichlidae respectively, were obtained from Fisheries Research and Information Centre (Inland), Hesaraghatta, Bangalore, Karnataka. Live and healthy juvenile fishes of 8-10 weeks with similar body weight and length were selected and brought to the laboratory in air-tight polythene bags half-filled with oxygenated water. They were quarantined by giving bath (1-2 dips) in std. 0.1% $KMnO_4$ solution, transferred into a pre-washed and dried aquarium with 50 liters of water capacity and acclimatized in laboratory conditions for a period of two weeks. The fishes were fed with formulated feed (control) during the acclimatization period.10-15% of the water was siphoned off along with faecal matter and replaced with fresh dechlorinated tap water every alternate day. For further experiments, healthy and disease-free fishes were selected from the stock acclimatized fishes in lab.

Experimental procedures

After habituation, the selected test fishes were divided into four groups (one control and 3 experimental group I, II&III) with three replicates each in 30-liter fiber-glass aquaria. Each aquaria was stocked with 15 juveniles for a period of 60 days with mean initial body weight and body length of black wagtail platy (0.33 ± 0.03g; 2.64 ± 0.07cm), of rosy barb (1.19 ± 0.05g; 4.85 ± 0.05cm) and kenyi cichlid (0.19 ± 0.07g; 1.71 ± 0.02cm) respectively. The fishes were starved for 24 h prior to the experiment and were fed twice a day (9.00 am & 6.00 pm) during the experiment. The control fish group were fed with customized formulated feed F1 (without addition of dried fruit peel powder of *S. cumini*), experimental group I were fed with F2, group II was fed with F3 and group III were fed with F4. Daily rations were adjusted every week according to fish body weight (% BW/day) i.e., for initial 15 days 15%, next 30 days 10% and remaining 15 days 5% of the BW.

Analysis of water quality parameters

The physico-chemical characteristics of water used for control and experimental aquarium were monitored weekly. 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 (2005) and pH (digital pH meter, Eu-Tech) for all the control and experimental aquariums throughout the experiment period.

Analysis of growth performance

The growth parameters such as, survival rate (SR), body weight gain (BWG), body length gain (BLG) and specific growth rate (SGR) was analyzed following the standard formula by Zhu et al, (2014a) and feed conversion ratio (FCR) and condition factor (CF) was analyzed by Bailey et al, (2003).

(1) Survival rate (SR %) = (Final fish number - Initial fish number)/Initial fish number *100

(2) Body Weight gain (BWG %) = (final body weight - initial body weight) / Initial body weight *100

(3) Body Length gain (BLG%) = (Final body length - Initial body length)/Initial body length*100

(4) Specific growth rate (SGR% d^{-1}) = $[Log_n(W_2) - Log_n(W_1)]$ / time (days) *100

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

(5) Feed conversion ratio (FCR) = Feed delivered to group (g) / Live biomass gain of that group (g)

(6) Condition factor (CF%) = (W/L^3) *100

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

Analyses of Total Monomeric anthocyanin pigment content (TMAC)

TMAC of fish body tissue was analysed by pH Differential Method (AOAC, 2005). Monomeric anthocyanin pigments showed change in colour with a change in pH. Control and experimental test fish tissue sample was diluted with pH 1.0 buffer and another sample with pH 4.5 buffer in the ratio of 1:4. Both samples pH1 and pH4.5 were read at 520 and 700 nm with UV spectrophotometer. The diluted test samples are read versus a blank cell filled with distilled water. Absorbance was measured within 20-30 min of preparation after centrifugation of the test samples

Calculations: The anthocyanin pigment concentration (as cyanidin-3-glucoside equivalents) was calculated using the following equation:

(7) Anthocyanin pigment (cyanidin-3-glucoside equivalents, mg/L) =

$(A * MW * DF * 10^3) / (\varepsilon * l)$

where A = (A520nm – A700nm) pH 1.0 – (A520nm – A700nm) pH 4.5;

MW (molecular weight) = 449.2 g/mol for cyanidin-3-glucoside (cyd-3-glu);

DF = dilution factor established in D;

10^3 = factor for conversion from g to mg.

ε = 26 900 molar extinction coefficient, in L & mol $^{-1}$& cm^{-1} for cyd-3-glu;

and l = pathlength in cm;

Results are reported as monomeric anthocyanins, expressed as cyanidin-3-glucoside equivalents in µg/g

Data sampling and Statistical Analysis

The statistical analysis of data of control and experimental groups is expressed in the form of Mean and standard error (± SEM) and tabulated by using Two-way analysis of variance (ANOVA) and post hoc Bonferroni test and the least significant differences was used to compare means ($P<0.05$) using GraphPad Prism 5.1. 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.

RESULTS

Physico-chemical parameters of water

Physico-chemical parameters of water from control and experimental tank analyzed on weekly basis throughout the experimental period for 60 days showed water temperature ranging from 24ºC to 26ºC, dissolved oxygen (DO) 5.5 ± 0.08 mg/l, free ammonia 0.37 ± 0.04 mg/l, total alkalinity 136 ± 0.3mg/l, total hardness 247 ± 0.8mg/l and pH at 7.2 ± 0.04. The water parameters were within BIS limits of freshwater (table 1).

Table 1: Physico-chemical parameters of water sampled from control and experimental tanks

Parameters	BIS: 2012	Control and Experimental tank
Temperature (°C)	24 -26	24 -26
D.O (mg/l)	4.5-6.5	5.5
NH_3 (mg/l)	≥0.5	0.37
Total alkalinity ($mgCaCO_3L^{-1}$)	200-600	136
Total hardness (mg $CaCO_3L^{-1}$)	200-600	247
pH	6.5-8.5	7.2

Table 2: Ingredients of formulated feed in grams

Sl. No.	Formulated Feed (F1) Ingredients	(g 100g-1 feed)
1	Fish meal	**30**
2	Groundnut oil cake	**22**
3	Wheat flour	**15**
4	Rice bran	**22**
5	Vegetable oil (ml)	**3**
6	Vitamin and mineral mix* (Maxirich forte)	**4**

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).

Table 3: Proximate composition of feed ingredients: formulated feed F1 (Control), F2, F3 and F4 incorporated with 0.25g, 0.5g & 1g of $100g^{-1}$ dried fruit peel powder of *S. cumini* respectively.

Sl. No.	Proximate composition	Feed 1 (F1) mg/g	Feed 2 (F1+ $0.25g100g^{-1}$)	Feed 3 (F1+ $0.5g100g^{-1}$)	Feed 4 (F1+ $1g100g^{-1}$)	*S. cumini* fruit peel powder mg/g
1	Fat	5.02 ± 0.01	7.23 ± 0.37	7.35 ± 0.40	7.40 ± 0.44	2.43 ± 0.43
2	Crude Protein	32.2 ± 0.04	38 ± 0.27	39 ± 0.29	40 ± 0.33	7.8 ± 0.29
3	Crude Fiber	1.14 ± 0.02	17.28 ± 0.35	17.32 ± 0.33	17.38 ± 0.38	16.24 ± 0.36
4	Total Ash	17.28 ± 0.06	19.75 ± 0.43	20 ± 0.49	23 ± 0.53	6.84 ± 0.47
5	Moisture	11.18 ± 0.04	21.17 ± 0.17	21.22 ± 0.25	21.27 ± 0.20	10.9 ± 0.16
6	Total Carbohydrates (TCHO)	45.5± 0.68	127.67 ± 0.83	127.82 ± 1.03	127.97 ± 1.3	82.93 ± 0.58
7	Organic Matter	82.72 ± 0.82	175.58 ± 1.07	175.62 ± 1.04	175.78 ± 1.7	93.16 ± 0.95
8	Nitrogen Free Extract (NFE)	50.03 ± 0.02	107.03 ± 0.78	107.09 ± 0.55	107.16 ± 0.98	76.09 ± 0.64
9	Neutral Detergent Fiber (NDF)	19.19 ± 0.45	94.01 ± 0.60	94.07 ± 0.85	94.11 ± 0.90	74.92 ± 0.45
10	Acid Detergent Fiber (ADF)	21.56 ± 0.34	68.08 ± 1.03	68.14 ± 1.09	68.04 ± 1.12	46.48 ± 0.78

11	Dry Matter	88.51 ± 0.15	177.41 ± 0.85	177.56 ± 0.34	177.61 ± 0.63	89.10 ± 0.48
12	Mineral Content	2.02 ± 0.14	6.25 ± 0.32	6.29 ± 0.35	6.28 ± 0.38	4.26 ± 0.24
13	Anthocyanins (mg/g)	Nil	84.32 ± 2.2	84.32 ± 2.2	84.32 ± 2.2	84.32 ± 2.2
14	Polyphenols	Nil	68.35 ± 0.023	68.35 ± 0.023	68.35 ± 0.023	94.91 ± 0.45

Values are expressed as mean ± SE

Growth performance

The survival percentage was 100% in all three species of control as well as in experimental fish groups when fed with F1, F2, F3 and F4 feed during 60 days of the experimental period as represented in table 4, 5 and 6. The growth performance of the *M. lombardoi* (kenyi cichlid), *P. conchonius* (rosy barb) and *X. maculatus* (black wagtail platy) analyzed according to their survivability, BWG, BLG, SGR, FCR, and CF for 60 days of the experiment is represented in table 4,5 and 6. The control and experimental group of all the three fish species showed 100% survivability when fed with F1, F2, F3 and F4 respectively. The results with respect to body weight and length gain showed significantly higher BWG (531.58 ± 1.32) and BLG (64.91 ± 0.67) (P<0.01) in cichlid fish group when compared to rosy barb and black wagtail platy (BWG –79.66 ± 0.16, BLG –12.99± 0.14 and BWG –124.24 ± 1.71, BLG –24.53 ± 0.55) respectively(table 4,5 & 6) when fed with F4.Although similar trend of BWG and BLG was observed in the fish group fed with F3 and F2 but the values were significantly (P<0.01) less than those fed with F4. The control group of fish fed with F1 (without incorporation of dietary supplement of anthocyanin from *S. cumini*) showed comparatively minimum improvement in their body weight and length gain.

Table 4: Growth performance including survival rate (SR), initial body weight (IBW), initial body length (IBL), final body weight (FBW), final body length (FBL), body weight gain (BWG), body length gain (BLG), specific growth rate (SGR), feed conversion ratio (FCR), condition factor (CF) of *M. lambordoi* fed with four different feeds F1, F2, F3 and F4

Sl. No.	Parameters	F1	F2	F3	F4
1	**SR%**	100	100	100	100
2	**IBW(g)**	0.18 ± 0.09	0.19 ± 0.02	0.19 ± 0.02	0.18 ± 0.01
3	**IBL(cm)**	1.71 ± 0.05	1.71 ± 0.06	1.71 ± 0.03	1.71 ± 0.02
4	**FBW(g)**	0.72 ± 0.04	0.88 ± 0.09	1.00 ± 0.04	1.19 ±0.14
5	**FBL(cm)**	2.40 ± 0.13	2.55 ± 0.09	2.70 ± 0.07	2.82 ± 0.15
6	**BWG%**	300 ± 1.11^{a}	363.16 ± 1.19^{b}	426.32 ± 0.85^{c}	531.58 ± 1.32^{d}
7	**BLG%**	40.35 ± 0.87^{b}	49.12 ± 0.98^{a}	57.89 ± 0.72^{a}	64.91 ± 0.67^{b}
8	**SGR%**	0.92 ± 0.02^{a}	0.99 ± 0.03^{b}	1.02 ± 0.03^{a}	1.09 ± 0.03^{a}
9	**FCR**	1.18 ± 0.03^{a}	1.14 ± 0.02^{c}	1.10 ± 0.02^{a}	1.06 ± 0.04^{a}
10	**CF%**	1.27 ± 0.0.03^{a}	1.73 ± 0.04^{d}	1.87 ± 0.03^{a}	2.08 ± 0.03^{a}

Values are expressed as mean ± SE. Significance was calculated by one-way ANOVA and post-hoc test was done with Tukey's multiple comparison (P<0.05). The statistical mean difference is indicated as P<0.0001^{a}, P<0.001^{b} and P<0.01^{c} respectively.

Table 5: Growth performance including survival rate (SR), initial body weight (IBW), initial body length (IBL), final body weight (FBW), final body length (FBL), body weight gain (BWG), body length gain (BLG), specific growth rate (SGR), feed conversion ratio (FCR), condition factor (CF) of *P. Conchonius* fed with four different feeds F1, F2, F3 and F4

Sl. No.	Parameters	F1	F2	F3	F4
1	**SR%**	100	100	100	100
2	**IBW(g)**	1.18 ± 0.03	1.19 ± 0.05	1.19 ± 0.04	1.18 ± 0.07
3	**IBL(cm)**	4.85 ± 0.09	4.85 ± 0.03	4.85 ± 0.08	4.85 ± 0.04
4	**FBW(g)**	1.72 ± 0.02	1.88 ± 0.05	2.00 ± 0.04	2.12 ± 0.07
5	**FBL(cm)**	5.20 ± 0.05	5.34 ± 0.07	5.42 ± 0.02	5.48 ± 0.05
6	**BWG%**	45.76 ± 0.42^{c}	57.98 ± 0.24^{c}	68.06 ± 0.14^{b}	79.66 ± 0.16^{a}
7	**BLG%**	7.21 ± 0.11^{a}	10.10 ± 0.12^{a}	11.75 ± 0.18^{a}	12.99 ± 0.14^{a}
8	**SGR%**	0.46 ± 0.02^{a}	0.54 ± 0.03^{a}	0.56 ± 0.03^{a}	0.60 ± 0.03^{a}
9	**FCR**	1.99 ± 0.004^{a}	1.86 ± 0.03^{c}	1.80 ± 0.04^{a}	1.71 ± 0.04^{a}
10	**CF%**	0.86 ± 0.04^{a}	1.07 ± 0.03^{b}	1.24 ± 0.04^{a}	1.41 ± 0.04^{a}

Values are expressed as mean ± SE. Significance was calculated by one-way ANOVA and post-hoc test was done with Tukey's multiple comparison ($P<0.05$). The statistical mean difference is indicated as $P<0.0001^{a}$, $P<0.001^{b}$ and $P<0.01^{c}$ respectively

Table 6: Growth performance including survival rate (SR), initial body weight (IBW), initial body length (IBL), final body weight (FBW), final body length (FBL), body weight gain (BWG), body length gain (BLG), specific growth rate (SGR), feed conversion ratio (FCR), condition factor (CF) of *X. maculatus* fed with four different feeds F1, F2, F3 and F4

Sl. No.	Parameters	F1	F2	F3	F4
1	**SR%**	100	100	100	100
2	**IBW(g)**	0.33 ± 0.05	0.33 ± 0.08	0.33 ± 0.04	0.33 ± 0.06
3	**IBL(cm)**	2.64 ± 0.09	2.64 ± 0.10	2.65 ± 0.07	2.65 ± 0.08
4	**FBW(g)**	0.55 ± 0.02	0.64 ± 0.06	0.70 ± 0.07	0.74 ± 0.04
5	**FBL(cm)**	3.08 ± 0.08	3.16 ± 0.12	3.22 ± 0.04	3.3 ± 0.06
6	**BWG%**	66.67 ± 1.00^{a}	93.94 ± 0.58^{a}	112.12 ± 1.52^{b}	124.24 ± 1.71^{a}
7	**BLG%**	16.67 ± 0.11^{b}	19.70 ± 0.43^{a}	21.50 ± 0.63^{a}	24.53 ± 0.55^{b}
8	**SGR%**	0.63 ± 0.03^{a}	0.7 ± 0.02^{a}	0.75 ± 0.03^{a}	0.79 ± 0.03^{a}
9	**FCR**	1.56 ± 0.04^{a}	1.42 ± 0.03^{c}	1.32 ± 0.05^{a}	1.24 ± 0.06^{a}
10	**CF%**	1.26 ± 0.03^{a}	1.38 ± 0.04^{b}	1.60 ± 0.04^{a}	1.79 ± 0.05^{a}

Values are expressed as mean ± SE. Significance was calculated by one-way ANOVA and post-hoc test was done with Tukey's multiple comparison ($P<0.05$). The statistical mean difference is indicated as $P<0.0001^{a}$, $P<0.001^{b}$ and $P<0.01^{c}$ respectively.

Specific growth rate and condition factor analyzed with respect to body weight and length showed significant rise ($P<0.01$) (1.09 ± 0.03; 2.08 ± 0.03) in cichlid fish group fed with F4 when compared to fishes fed with F3 & F2 and control group fed with F1for a period of 60 days as represented in table 4, 5 and 6. Platy and rosy barb group of fish followed the similar trend showing enhanced values of SGR and CF when fed with F4, (followed by F3 and F2) but it was significantly ($P<0.01$) less than those of cichlid fish groups (fig.3 & fig.4). 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. Thus, growth rate in the three fish species varied and cichlid fish group showed better response to feed with higher concentration of dietary supplement relative to platy and rosy barb fishes.

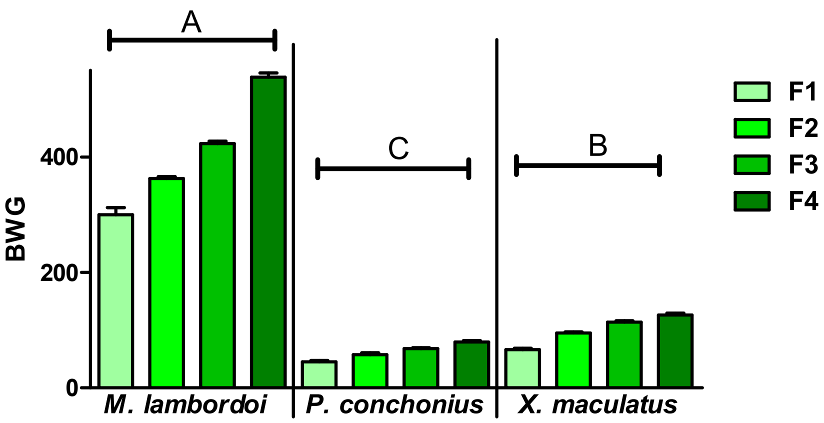

Figure 1: Body weight gain (BWG) of *M. lambordoi*, *P. conchonius* & *X. maculatus* fed with different diets *viz.*, F1, F2, F3, F4 for the experimental period of 60 days

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.

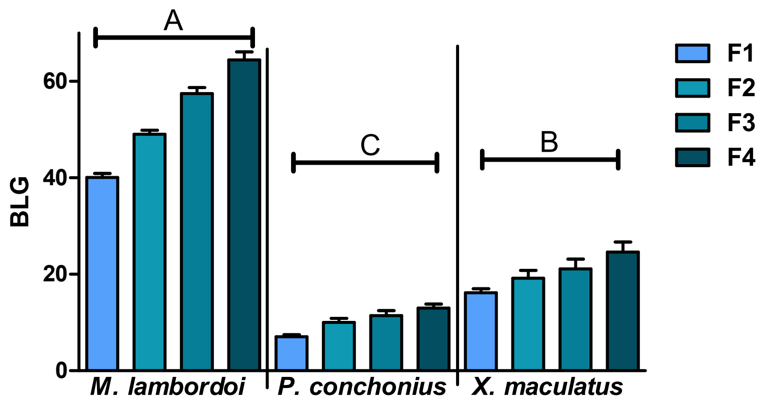

Figure 2: Body length gain (BLG) of *M. lambordoi*, *P. conchonius* & *X. maculatus* fed with different diets *viz.*, F1, F2, F3, F4 for the experimental period of 60 days

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.

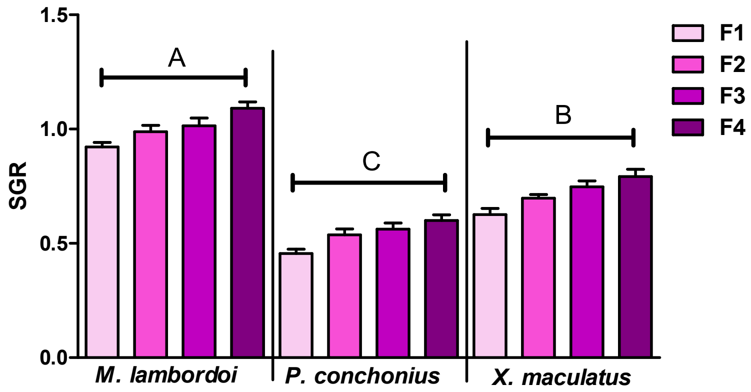

Figure 3: Specific growth rate (SGR) of *M. lambordoi*, *P. conchonius* & *X. maculatus* fed with different diets *viz.*, F1, F2, F3, F4 for the experimental period of 60 days

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.

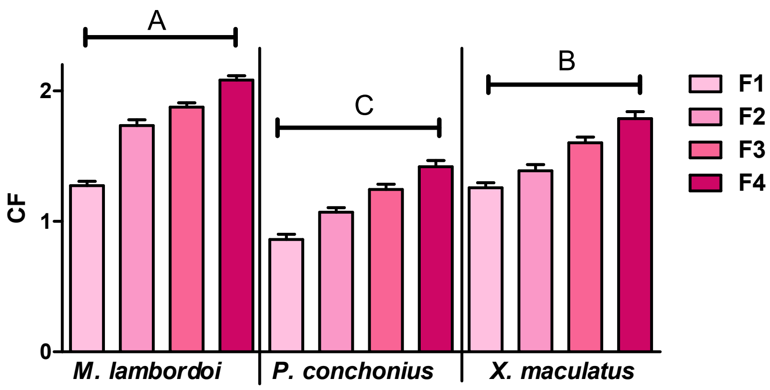

Figure 4: Condition factor (CF) of *M. lambordoi*, *P. conchonius* & *X. maculatus* fed with different diets *viz.*, F1, F2, F3, F4 for the experimental period of 60 days

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.

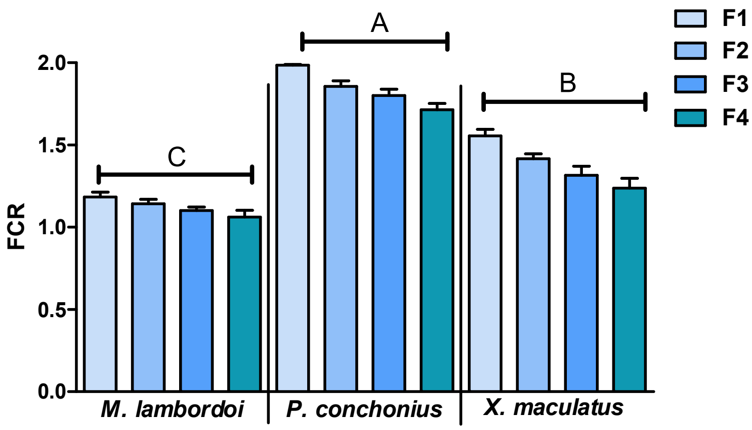

Figure 5: Feed conversion ratio (FCR) of *M. lambordoi*, *P. conchonius* & *X. maculatus* fed with different diets *viz.*, F1, F2, F3, F4 for the experimental period of 60 days

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.

FCR values were minimum (1.06 ± 0.04) in cichlid fish group fed F4 when compared with those fed with F3 followed by F2 and F1 (1.18 ± 0.02, 1.14 ± 0.02 and 1.16 ± 0.002 respectively). F4 feed fed platy and rosy barb also showed less values of FCR, but was significantly ($P<0.01$) more (platy-1.24 ± 0.06; barb-1.71 ± 0.04) than those recorded in cichlid fish groups. The F1 fed control groups of three fish species showed maximum FCR values and minimum improvement in growth. Thus, the above data revealed good growth performance due to better utilization of feed by experimental cichlid fish compared to platy and rosy barb fish group when fed with F4 which was enriched with 1% fruit peel powder of *S. cumini*. Although fish species fed with F3 and F2 also showed an increase in growth parameters compared to those of control but it was relatively less than cichlid.

The results obtained on pigmentation of the three fish species viz., cichlid, rosy barb and platy with respect to total anthocyanin content/levels (TAC) when fed with formulated feed F1 and experimental feed enriched with blackberry fruit peel F2, F3 and F4 are represented in fig.6.The total anthocyanin content (TAC) was maximum (1.62µg/g) in rosy barb group of fishes when fed the F4 feed for 60 days. Although similar trend was noted in anthocyanin content of body tissues of platy and cichlid fish group when fed with F4 (1.36 µg/g and 0.95 µg/g resp.) followed by F3 (1.27µg/g and 0.91µg/g) and F2 (1.12µg/g and 0.83µg/g) respectively but the TAC values were comparatively less than those of rosy barb (F3 - 1.39µg/g; F2 – 1.06µg/g). In contrast, the TAC of control fish groups of all the three fish species fed with F1 showed minimum (cichlid - 0.33µg/g; rosy barb – 0.54 µg/g and platy – 0.5µg/g) values relative to the ones fed with F2, F3 and F4. The above results indicated that the effect of different concentrations of dried fruit peel powder from *S.cumini* used as an additive for fish feed (F2-F4) was species specific since it induced improvement in growth performance in cichlid fish group and pigmentation enrichment in rosy barb after 60 days of feeding especially with F4.

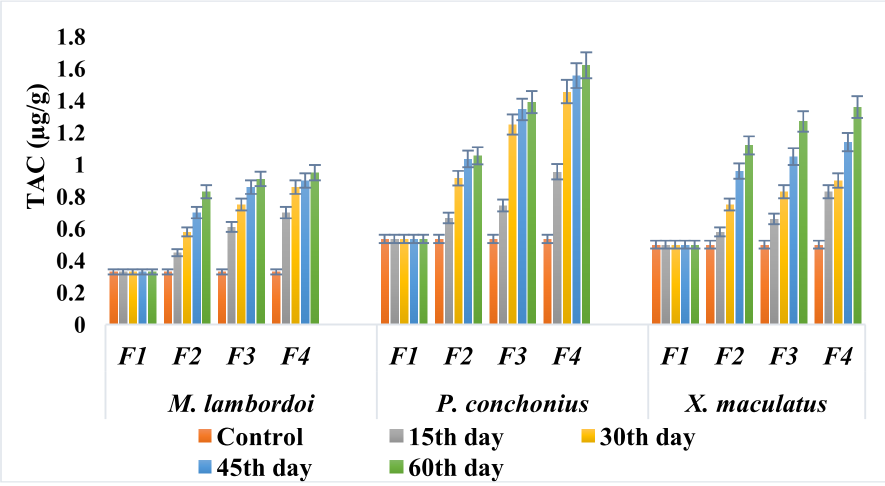

Figure 6: Total anthocyanin content (TAC) of *M. lambordoi*, *P. conchonius* & *X. maculatus* fed with different diets *viz.*, F1, F2, F3, F4 for the experimental period of 60 days

DISCUSSION

Information regarding nutritional requirements for good survivability, healthy growth and pigmentation of the fishes are inadequate or based on the information from species of food and ornamental fishes fed with carotenoid as a pigment supplement in aquaculture. Although it is well known that carotenoids when incorporated with feed induced growth and maturation, enhanced coloration, promoted resistance to stress, so as to improve the immune system and other body functions in the aquatic organisms (Petit et al, 1991; Olson, 1993; Menassueta et al, 1994) but survivability of test fishes was below 100%. Arulvasu et al, (2013) recorded only 96.6% survival rate in *Xiphophorus helleri* fed with rose petal extract (2000mg/kg) and 76.6% in control group when fed with diet without rose petal extract whereas Jain (2015) had found 100% survivability in Koi carp fed with diets incorporated with 3, 5 and 7% carrot meal. Whereas in the present study, related to anthocyanin pigment from three concentrations (0.25%, 0.5% & 1%) of dried fruit peel powder of *S. cumini* incorporated formulated feed has proved 100% survival rate with good growth in the all three fish species (kenyi cichlid, blackwag tail platy and rosy barb) groups fed for 60 days.

In contrast to the present result, Pérez-Escalante et al, (2012) reported only 90% of survival rate in control and 96.6% in experimental groups of goldfish fed with 40, 80and 160mg of anthocyanin extract from flour of Roselle calyx/kg diet. Even though carotenoids are vital nutrients and play an important role in growth, metabolism, reproduction and colouration of ornamental and food fish e.g., enhanced growth of *Puntius sophore* fed with 10% *Spirulina platensis* (Bagre et al, 2012) and increment in SGR values in Koi carp fed with 180mg kg^{-1} of marigold meal (Swian et al, 2014) but the total amount (g) carotenoid utilized as additive is huge and the availability of sources of carotenoid is relatively less compared to that of anthocyanin pigment source. Many studies (Amar et al, 2001; Gomes et al, 2002; Xu et al, 2006; Yi et al, 2014) have also reported contrasting results with insignificant effect of carotenoid pigment supplemented diet on growth rate of ornamental fish proving Anthocyanin pigment to be a better option.

Recent reports showed that micronutrients along with fibre and Vitamin C, present in anthocyanin pigment can boost digestion and improve immunity which in turn played a significant role in healthy growth of fishes (Petre, 2019), in Nile tilapia when fed with 40mg/kg and 80mg/kg dietary anthocyanin from blackberry (Yilmaz, 2019) and in goldfish when fed with D150 and D300 of anthocyanin encapsulated diet from hibiscus flower when compared to control and higher doses (160mg/kg & D450) respectively

(Vanegas-Espinoza et al, 2019). These findings are in agreement with present studies on improved growth performance of kenyi cichlid, blackwag tail platy and rosy barb fish groups when fed with F2, F3 and F4 supplemented with 0.25%, 0.5% and 1% respectively of *S. cumini* fruit peel powder containing anthocyanin pigment. In contrast to the results by Vanegas-Espinoza et al, (2019), the present work showed a significant increase ($p<0.01$) in growth performance (BWG, SGR and CF) of all fish species when fed with high concentration (F4-1%)of anthocyanin pigment incorporated feed compared with other experimental diets with low concentration (F2 &F3).

In the present study, enhanced growth in F4 fed experimental cichlid fish species with significantly less ($P<0.01$) FCR levels and minimum growth in platy and rosy barb and those fed with control diet may depend upon the interactions of different concentrations of anthocyanin pigment with digestibility of nutrients (Earle, 1995; Sales and Janssens, 2003). Such variation in growth parameters of Cichlid fish group with other fish species as platy and rosy barb may also be due to dissimilarities in digestion, metabolism and microbiota of fish as suggested by Bordenave et al (2014) and Jakobek (2015). It also indicated that anthocyanin pigment functions in a different way in each species and is species specific. Simultaneously, in rosy barb fish group, maximum levels of TAC were noted, that might have generated due to utilization of anthocyanin pigment from feed towards skin colouration and not towards growth. Yanar et al, (2008) had reported similar observation in gold fish fed with high supplementation levels of alfalfa, a carotenoid diet source with decrease in their growth rate but an increase in skin pigmentation. Such controversial results might be due to inhibitory effect of pigment on digestive enzymes which in turn alters the nutrients digestibility or may be related to the assimilation of dietary compounds. The rising cost and carcinogenic effect of synthetic pigments present in commercial fish feed has led to gain interest towards easily available green plant, flowers and darkly coloured fruit based anthocyanin compounds from Cassava leaves, wild berry, elderberry, hibiscus flower, beetroot etc which has been effective regarding growth performance and colouration in *Carassius auratus, Nile tilapia, X. helleri* etc as reported by Gurung et al, (2018); Monica et al, (2019); Yilmaz, (2019); Vanegas-Espinoza et al, (2019).The present work fills the lacuna and is a pioneer and preliminary study on utilizing anthocyanin pigment from dried fruit peel of *S.cumini* in fish feed for good growth and colouration of commercial ornamental fish, kenyi cichlid, blackwag tail platy and rosy barb.

CONCLUSION

We recommend the implementation of anthocyanin pigment from commonly available sources as *S. cumini* in fish feed for aquaculture of ornamental and food fishes for their healthy, disease-free growth along with bright coloration of the fishes which in turn influences the market price of the fishes.

Acknowledgement
Authors are thankful to Department of Zoology, Bangalore University for providing continuous support and lab facility during course of study.

Conflicts of Interest/Competing Interests:
The authors have No conflict of interest and no relevant financial or non-financial interests to disclose.

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