Effect of extracted phycocyanin from *Spirulina platensis*on growth parameters, colorations, digestive enzymes and body chemical compositions of Guppy fish (*Poecilia reticulata*)

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Abstract

Phycocyanin (PC) is one of the main pigments of algae *Spirulina*, which is used as a dietary supplement due to its high content of protein, vitamins, minerals and essential fatty acids. Using of extracted PC from *Spirulina platensis* was evaluated on growth, the body coloration (Skin and tissue), body chemical composition and digestive enzymes in guppy fish (*Poeciliareticulata*). 80 guppy fish with an average weight of 0.88±0.10 g were chosen. Feeding was performed on a daily basis for 3 times and fish were cultured for 45 days and period light was 12:12 (light: dark). Based on the result final weight, final length, SGR, WGR, LGR, PER and ADG has shown significant increasing when PC level was increased up to 0.15%. Phycocianin added to the diets made significantly to increase total pigments in guppy tissue. Protease activity was highly significant in different treatments (p<0.05) and extremely increased from zero up to 0.15% in fish fed with diets. PC made increase in total pigments in guppy fish when PC was between 0.05% to 0.15% and also the fish is brightly colored in skin. The present investigation reveal that, fish fed with 0.15% *Spirulina* PC elicited growth parameters and by increasing *spirulina* PC in diets of *P.reticulata* was increased digestive enzymes.

Keywords: Phycocyanin, *Poecilia reticulate* (Guppy fish), Digestive Enzymes, Spirulina.

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Introduction

Phycocyanin (PC) is one of the main pigments of algae *Spirulina*, which is used as a dietary supplement due to its high content of protein, vitamins, minerals and essential fatty acids (Cherng *et al.*, 2007; Ahsan *et al.*, 2008; Manconia *et al*., 2009; Thanh-Sang *et al*., 2013). This pigment is found in cyanobacterial and eukaryotic an algae such as Rhodophyta and Cryptomonads (Glazer and Stryer, 1983). PC is classified into three types, C-PC (obtained from cyanobacteria), R-PC (obtained from red algae) and R-PCII (obtained from Synechococcus species) (Kuddus *et al*., 2013; Wang *et al*., 2014). C-phycocyanin (C-PC) could be extracted from cyanobacterial such as *Spirulina platensis*, which has been widely used in commercial applications forthe food and cosmetic industry as a natural blue dye. Recent studies have demonstrated the hepatoprotective (Romay *et al*., 2003), anti-inflammatory (Bhat and Madyastha, 2001; Reddy *et al*., 2003; Romay *et al*., 2003) and antioxidant (Bhat and Madyastha, 2000; Estrada *et al*., 2001) properties of C-PC.PC is a protein from the phycobiliprotein (PBP) family (Patel *et al*., 2005) characterized by its intense blue color. It is a peripheral accessory light-harvesting complex called phycobilisome (PBS), which is assembled on the surface of the thylakoid membrane. Its main function is to transfer the excitation energy to the center reaction where the maximum wavelength of absorption arenear to 620 nms (De Marsacand Cohen-Bazire, 1977; Benedetti *et al*., 2006).

The commercial production of ornamental tropical fish aregaining momentum in many regions of the world. The live bearer guppy fish (*Poecilia reticulata*) isthe most popular among hobbyists because of their vibrant colors and the fact that they are easy to breed and keep (Dahlgren, 1980). The body coloration and patterns are often caused by chromatophores, which are large stars shaped pigment-containing cells located in the skin. The chromatophores are grouped into subclasses based on their color: xanthophores (yellow), erythrophores (red/orange), irido-phores (reflective/iridescent), leucophores (white), melanophores (black/brown) and cyanophores (blue). In addition to the seasonal color change, which is caused by changes in the number of chromatophores in the skin (Sugimoto, 2002), skin patterns can be modified within minutes by reflective changes in iridophores and through aggregation or dispersion of the pigment-containing organelles inside the chromatophores (Kodric-Brown, 1998; Burton, 2002; Sköld *et al*., 2002; Mähtiger *et al*., 2003). Such temporal pigment dispersal increases body pigmentation while pigment aggregation results in less body pigmentation (Fujii and Oshima, 1994, Svensson *et al*., 2005). Reduction in black body pigmentation due to melanophore pigment aggregation commonly also results in greater skin transparency (Fujii and Oshima, 1994). Long-term chromatophore pigment dispersal also stimulates pigment transfer to surrounding cells and induces chromatophore production (Sugimoto, 2002).

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The main scope of this study was evaluated of using extracted PC from *Spirulina platensis* on growth, the body coloration (Skin and tissue), body chemical composition and digestive enzymes in guppy fish (*Poecilia reticulata*). PC powder was added into artificial feed as a supplemental dietary.

Materials and methods

Spirulina algae cultivation and phycocianin (PC) extraction

Algae cultivation (*Spirulina platensis*) was conducted in 10-liter containers and temperature 35°C, with a 24-hour exposure of lighting and aeration. Zarrouk (1966) was used as medium. *Spirulina* centrifuged with Sigma 8K in 5000 rpm for 5 minutes. The algae was dried in freeze dry and PC was extracted by water with amount of 50 g in 1-liter in deionize water for 48 h (the best result from pre-tests). Then, the PC was freeze dried and the blue powder was used. PC purity is evaluated based on the absorbance ratio A620/ A280. The absorbance at 620 and 280 nm according to PC and total protein, respectively (Patil *et al*., 2006). PC is categorized food grade when A620/ A280 is ≤0.7, reagent grade when A620/A280 is between 0.7 and 3.9and analytical grade when A620/A280 is ≥4.0 (Patil *et al*., 2006; Antelo *et al*., 2010; Kuddus *et al*., 2013). Equation is used to determine the categorized concentration (mg/ mL) in crude extracts (Bennett and Bogorad, 1973; Patel *et al*., 2005; Silveira *et al*., 2008; Antelo *et al*., 2010).

$$PC = \frac{(OD_{620} - 0.474OD_{652})}{5.34}$$

Preparation of guppy fish (Poecilia reticulata)

80 guppy fishes with an average weight of 0.88±0.10 g was purchased. Randomly, they were distributed in 8 aquarium 50-liters and were fed for 7 days with commercial food contained 4% fats and 30% protein. During rearing, physicochemical parameters were set in the optimum range (pH 7-7.2, Temperature 23-24°C and gentle aeration). Feeding was performed on a daily basis for 3 times and satiation (Oliviotto *et al*., 2006). Fish were cultured for 45 days and period light was 12:12 (light:dark), also all aquariums filtered by central filtration.

Preparation of diets

Four diets were prepared to consist of PC added at 0, 0.05, 0.10 and 0.15 percent in diets. The ingredients used for the preparation of diets as followed (Table 1).

The biochemical composition of body

Total protein, lipid and ash contents

The total lipid content (ether extraction) was determined using a soxhelet assay. The dry cells were washed with ether (100%) for six hours. The ash was measured by burning the weighed samples of an electric furnace at 550°C for six hours. Crude protein (N × 6.25) was determined by the Kjeldahl method after an acid digestion using an auto-Kjeldahl System (AOAC, 2005).

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Table 1: The combination of dietary ingredients used in study.

Diet composition	Levels of PC in the diet (%)			
	Control	**0.05**	**0.10**	**0.15**
Fish meal	45.5	45.5	45.5	45.5
Soy-meal	30	30	30	30
Wheat	12	12	12	12
Starch	5	5	5	5
Vitamin premix[1]	0.5	0.5	0.5	0.5
Mineral premix[2]	0.5	0.5	0.5	0.5
Fish oil	6.5	6.5	6.5	6.5
PC	0	0.05	0.10	0.15
The biochemical composition of diets (%)				
Dry matter	91.16	91.26	91.64	91.73
Protein	41.50	41.68	41.82	41.99
Ether extraction	12.80	12.70	12.67	12.60
Ash	5.10	5.10	5.10	5.10
Digestible Energy (Kcal/g)	3.98	3.98	3.99	4.00

[1]In terms of Kg: vitamin A, 10,000 IU; vitamin D3, 2,000 IU; vitamin E, 100 mg; vitamin K, 20 mg; vitamin B_1, 400 mg; vitamin B_2, 40 mg. vitamin B_6 20 mg; vitamin B_{12}, 0.04 mg; biotin, 0.2 mg; choline chloride, 1200 mg; folic acid, 10 mg; inositol, 200 mg; niacin, 200 mg; pantothenic calcium, 100mg.

[2]In terms of Kg: $MgS0_4$. $2H_20$, 127.5; KCl, 50.0; NaCl, 60; $CaHPO_4$. $2H_2O$, 727.8; $FeSO_4$. $7H_2O$, 25.0; $ZnSO_4$. $7H_2O$, 5.5; $CuSO_4$. $5H_2O$, 0.785; $MnSO_4$. $4H_2O$, 2.54; $CoSO_4$. $4H_2O$, 0.478; Ca $(IO_3)_2$. $6H_2O$, 0.295; $CrCl_3$. $6H_2O$, 0.128.

Total carbohydrate content

First, 100 mgs of algae was weighed into a boiling tube and 5 ml 2.5 N HCl was added to it. Then, the samples were hydrolyzed to simple sugars by keeping it in a boiling water bath for three hours and then cooled to room temperature. After that, it was neutralized with solid Na2CO3 until the effervescence ceased. Next, the volume was taken up to 100 mlsand centrifuged (5000 rpm for 5 min). Then, 0.5 mlsof the supernatant was collected and taken up to 1 mL with distilled water. Then, 4 mlsof 0.2% anthroned reagents was added to it and it was heated for 8 minutes in a boiling water bath. The sample cooled rapidly and turned from the green to dark greens colors at 630 nms. The standard solution were prepared at a concentration of 100 µ.mls-1 glucose (Hedge and Hofreiter, 1962).

Calculation of Digestive Enzymes

For enzyme analysis, the intestine was washed with cold deionized water to remove as much mucus as possible and were then homogenized in cold sodium phosphate buffers (0.1 M, at pH 7.0, and 4 ºC) by a ratio of 1:9 (m/v) (Liu *et al.*, 2008). The homogenate was centrifuged at 4 ºC at 10000 g for 30 minutes. The soluble protein content ofthe enzyme extract was measured by Lowry method (Lowry *et al.*, 1951). α-Amylase was determined by starch-hydrolysis method according to Robyt and Whelan (1968). The enzymatic reaction mixture consisted of 2% (w/v) starch solution (0.125 mL), 0.1 M citrate–phosphate buffer at pH 7.5 (0.125 mL) and a digestive extract (0.05 mL). The reaction mixture was incubated for 1 hour at 37 ºC. Absorbance was determined at 600 nm. Maltose was used as a standard and the activity unit of a-amylase was defined as the quantity of

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enzymes that produced 1mmol of maltose ml-1 min-1.Lipase activity was determined by the evaluation of the degradation of triacylglycerols, diacylglycerols, and monoacylglycerols to free fatty acids following the method of Metin and Akpinar (2000). For the emulsion, a 1% solution topolyvinyl alcohol (PVA) in distilled water was used. Then 5 mls of 0.1 N HCl was added, heating to 75–85ºC for 1 hour, followed by cooling, filtering, and adjusting pH to 8.0 with 0.1 N NaOH. To an aliquot of the above solution, virgin olive oil was added to a substrate concentration of 0.1 M. The mixture was emulsified for 5 minutes. The reaction mixture composed of a PVA solution-emulsified substrate (1 mL), McIlvaine buffer at pH 8 (0.5 mL), and digestive extract (0.5 mL). The McIl-vaine's buffer was prepared for 0.1 M citric acid and 0.2 M bisodium phosphate. The reaction mixture was incubated for 4 hours at 37° which 3 mL of a 1:1 ethanol–acetone solution was added to stop the reaction and break the emulsion. A few drops of 1% phenolphthalein in ethanol were added to the reaction mixture and titrated with 0.01 M NaOH. For the blank tubes, the same procedure werefollowed but with boiled enzyme. One unit of lipase activity was defined as the hydrolysis of 1.0 micro-equivalents of fatty acids from triacylglycerols in 1 hour at pH 7.7 and 37°C. Total proteolytic activity was measured using the casein hydrolysis method by Walter (1984). The assay was conducted using a wide range of pH values. The buffers used were 0.1 M KCl–HCl (pH=1.5), 0.2 M glycine–HCl (pH= 3.0), 0.1 M citrate–0.2 M phosphate (pHs 4.0 and 7.0), 0.1 M Tris–HCl (pHs 8.5 and 9.0) and 0.1 M glycine–NaOH (pH= 10.0), at 25°C. Enzyme reaction mixtures consisted of 1% (w/v). Casein in water (0.25 mL), buffer (0.25 mL) and enzyme sample (0.1 mL) were incubated for 1 hour at 37 ºC. The reaction was stopped by adding 0.6 mls of 8% w/v trichloroacetic acid. After holding for 1 hour at 28 ºC, samples were centrifuged at 1800g for 10 minutes and the absorbance of the supernatant recorded at 280 nm. Tyrosine was used as standard and one unit of enzyme activity was defined as the amount of enzyme needed to catalyze the formation of 1 mg of tyrosine per 1 minute.

Total pigments

To measure the total pigment, 1 g of tissue was gained. After placing in 10 ml of bottle (with screw cup), 2.5 g sodium sulfate anhydrate was added to the samples and filled with pure acetone up to 10 ml. The samples were kept at -20 ° C for 48 hours to extract pigments. Then centrifuge (5000 rpm for 5 minutes) and read in the wave length of200-800 nm and the highest absorption was used for calculation of total pigments (Olson, 1979).

Total pigments (mg/g) = {optimum absorption ÷ [0.25 × sample weight (g)]} × 10

Measure the color

For measuring the color used HunterLab. The L * index was used to express the brightness, a * indexes for the expression of red and green background and b * index for the expression of yellow-blue dimension (Park, 2005).

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Growth Parameters

The characteristics of the culturing period included Specific Growth Rate (SGR), Weight Growth Rate (WGR), Length Growth Rate (LGR), Average Daily Growth Rate (ADG), Protein efficiency ratio (PER) and Condition Factor (CF) were calculated based on the following equations (Promya and Chitment, 2011).

- SGR (%/day) = [(LnWt - LnWi) / T] ×100
- WGR (%) = [(Wt – Wi/ Wi)] ×100
- LGR (%) = [(Lt – Li / Li)] ×100
- ADG (g/ day) = (Wt – Wi)/T
- CF = Wt × Lt -3 × 100
-PER = (Wt-Wi)/Dp

Where Wt and Lt are respectively final weight and length, Wi and Li are respectively initial the mean weight and length of fish, Dp is dry protein intake and T is the length of the period.

Statistical Analysis

The results were analyzed using a standard one-way analysis of variance (ANOVA) using SPSS (version 22). Kolmogo-rov–Smirnov and Bartlettl's tests were applied to check the normality and homogeneity of variances. To compare data obtained from treatments was used of Tukey test for5% significance level. Excel 2013 was used for diagramming.

Result

Spirulina composition

Analysis showed that *spirulinas* powders contains 63.2% protein, 6.4% fat, 16.3% carbohydrates and 8.3% ash and 5.7 percent moisture. The PC extracted showed 2.6 mg/ml concentrations that it considered as analytical grade.

The effects of different levels of PC in diets on growth parameters of Guppy fish

Final weight, final length, SGR, WGR, LGR, PER and ADG has shown significant increasing when PC level was increased up to 0.15% (p<0.05), but there was significant differences betweenthe level of 0.15% PC to zero percent for CF factor ofdifferent treatments. (p<0.05) (Table 2).

The effects of different levels of PC in diets on pigments and colors of Guppy fish

Phycocianin added to the diets made significantly to increase total pigments in guppy tissue and about 2-fold increased pigments compared to control fish (p<0.05). The L *, a* and b* indexes showed lowest in 0.05%, 0.15% and 0.15% PC, respectively (p<0.05) (Table 3).

The effects of different levels of PC in diets on body biochemical composition of Guppy fish

Moisture of body showed no significant difference between the treatments (p>0.05). Male and female protein increased with rising *spirulina* (p<0.05). Crude protein increased significantly while PC raised up to 0.15% in diets.

On the other hand, with increasing PC in diets, the lipid and carbohydrate contents decreased (p<0.05). Although, there was no significant differences between the ashes (p>0.05) (Table 4).

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Table 2: Growth parameters of Guppy fish (*poeciliareticulata*) fed with different levels of PC for 6 weeks

parameters	Level of PC in the diets (%)			
	Control	**0.05**	**0.10**	**0.15**
Initial Weight (g)	0.88±0.13	0.88±0.13	0.88±0.13	0.88±0.13
Final Weight (g)	1.09±0.04[a]	1.36±0.40[b]	1.49±0.30[b]	1.95±0.21[c]
Initial Length (cm)	2.44±0.24	2.44±0.24	2.44±0.24	2.44±0.24
Final Length (cm)	3.00±0.41[a]	3.56±0.86[b]	3.90±0.51[b]	4.50±0.20[c]
SGR (% / day)	9.37±0.12[a]	31.48±1.03[b]	42.46±3.61[c]	67.79±0.86[d]
WGR (%)	24.86±0.16[a]	55.76±1.61[b]	73.89±6.28[c]	123.95±1.93[d]
LGR (%)	25.41±3.47[a]	54.51±12.17[b]	56.15±5.21[b]	87.70±4.63[c]
PER	0.55±0.08[a]	0.84±0.12[b]	0.96±0.05[c]	1.13±0.09[d]
ADG (g / day)	0.005±0.000[a]	0.011±0.000[b]	0.014±0.001[c]	0.024±0.000[d]
CF	3.83±0.31[b]	2.59±0.58[a]	2.77±0.37[ab]	2.05±0.13[a]

Different letters indicate significant differences at the level of 5 percent.

Table 3: Total pigments and LAB of Guppy fish (*poeciliareticulata*) fed with different levels of PC for 6 weeks

Color metric	Level of PC in the diets (%)			
	Control	**0.05**	**0.10**	**0.15**
Total Pigments (mg / g wet weight)	7.97±0.31a	9.20±0.64b	9.91±0.42b	15.57±0.33c
L *	82.0±1.41a	67.0±10.51b	80.5±4.95a	91.3±6.01a
a *	6.75±1.06a	6.01±4.25a	5.30±3.75a	0.71±.0.50b
b *	17.68±12.50a	2.12±1.50b	14.14±1.00a	1.06±0.75c

Different letters indicate significant differences at the level of 5 percent.

Table 4: The biochemical composition of Guppy fish (*poeciliareticulata*) fed with different levels of PC for 6 weeks.

Body composition (%)	Level of PC in the diets (%)			
	Control	**0.05**	**0.10**	**0.15**
Moisture (%)	64.16±0.54a	64.32±0.41a	65.08±0.35a	64.95±0.52a
Crude protein (%) (DW)	44.34±1.21a	46.94±1.24b	48.64±1.21c	51.25±1.54d
Lipid (%) (DW)	19.84±1.20c	18.68±1.08c	17.15±1.31b	15.72±1.38a
Ash (%) (DW)	8.47±1.65a	8.85±1.59a	8.15±1.09a	8.59±1.19a
Total Carbohydrate (%) (DW)	26.19±0.95b	24.19±1.05b	25.01±1.05b	23.15±1.08a

Different letters indicate significant differences at the level of 5 percent.

The effects of different levels of PC in diets on digestive enzymes of Guppy fish

Lipase activity has significantly decreased from increasing PC (p<0.05). Amylase activity showed a significant decrease of rising in PC (p<0.05). Although protease activity was highly significant in different treatments (p<0.05) and extremely increased from zero up to 0.15% in fish fed with diets (Table 5).

Table 5: Digestive enzymes of Guppy fish (*poeciliareticulata*) fed with diets containing different levels of PC for 6 weeks

Parameters	Level of PC in diets (%)			
	Control	**0.05**	**0.10**	**0.15**
Lipase activity (µg per mg soluble protein)	5.42±0.03c	5.40±0.02c	5.37±0.02b	5.33±0.01a
Amylase activity (µg per mg soluble protein)	4.07±0.10c	3.87±0.12b	3.84±0.07b	3.59±0.10a
Protease activity (µg per mg soluble protein)	271.66±5.17a	290.87±3.62b	309.12±2.13c	364.53±5.52d

Different letters indicate significant differences at the level of 5 percent.

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Discussion

The present investigation reveal that, fish fed with 0.15% *Spirulina* PC elicited growth parameters (mean body length and weight, SGR, WGR, LGR a, PER and ADG); it may be due to the high amount of PC and growth stimulatory effect of *Spirulina* PC in the diet. *Spirulina* has been identified as a potential protein source ofanimal feeds. It contains high protein and many essential amino acids, gamma linolenic acid, beta carotene and phycocyanin pigments, vitamins and minerals in large quantities. Scaria *et al*. (2000) found that ornamental fishes guppy (*Poecilia reticulata*) and platy (*Xiphophorus maculatus*) consumed maximum amount of *Spirulina* substituted feed than those fed with mushroom and azolla. Maximum growth rate was found in fishes fed with *Spirulina* diet than non-*Spirulina* diets (Daniel and Kumuthakalavalli, 1991; Okada *et al*., 1991). Aravindan *et al*. (2001) reported that dietary E-carotene contents (10-30 mg $100g^{-1}$) increased the specific growth rate (in terms of mean body length and weight) as compared to non-E-carotene diet ofgold fish *Carassius auratus*.

PC made increase in total pigments in guppy fish when PC was between 0.05% to 0.15% and also the fish is brightly colored in skin. Even though the red swordtail fish is brightly colored, dietary substitution of *Spirulina* significantly further enhanced the coloration in the fins and skin. The increase in carotenoid contents in skin, fins and muscle of *X. helleri* in relation to dietary carotenoid content of *Spirulina* diets demonstrates that, the fish has capacity to utilize it efficiently (James *et al*., 2006). Similar observations in the muscle of trout and salmon have been made by a few authors earlier (Storebakken *et al*., 1987; Bjerkeng *et al*., 1990). A dose-dependent carotenoid content has been reported in the muscle of Arctic char and salmon (Bjerkeng *et al*., 1990; Ando *et al*., 1994; Halten *et al*., 1997; Wathne *et al*., 1998). Paripatananont *et al*. (1991) found that 36-37 mg astaxanthin kg^{-1} diets produced maximum coloration in the goldfish, *C. auratus* and the coloration was stable even after 2 months. They also reported that feeding astaxanthin could be a suitable way for gold fish producers to stimulate color among fish grown in an algae free environment. In ornamental fishes, (unlike salmon and Arctic char) the pigmentation was highly found only in the skin and fins and this might be due to acquiring, digesting, utilizing dietary carotenoids and transporting more directly to the skin and fins rather than storing in muscle (Aravindan *et al*., 2001). The low carotenoid contents in the muscle of *X. helleri*, indicates that the assimilated carotene is directly transported to the skin and fins to provide necessary pigmentation (James *et al*., 2006). Also in guppy fish in this study beside high total pigments in tissue, there was highly color in skin. According to Schiedt *et al*. (1985) this was achieved by establishing reductive metabolic pathways tomuscle to the skin and fins. In salmon, Arctic char and trout, the pigmentation of integument and fins occurs only during sexual maturation and a reduction in the muscle carotenoid is an

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indication that the carotenoids are mobilized directly to the integument and fins from the muscle during that season.

High density lipoproteins have been demonstrated to be responsible for the carotenoid transport from muscle to integument in salmon (Ando and Hatano, 1988). Moreover, several abiotic and biotic factors are also expected to influence the ingestion, mobilization and metabolism of carotenoids like other feed constituents (Halten *et al*., 1996). It is likely that, a similar mechanism operates in *P. reticulata* also.

By increasing spirulina PC in diets of *P. reticulata* was increased digestive enzymes so that lipase in each treatment showed a significant increase with others. Amylase in the digestive tract was significant decrease that could be due to low levels of carbohydrate in *spirulina* PC. Decreasing lipase in the digestive tract up to 0.15% *spirulina* PC showed significant differences because PC very rich in protein and pigments. The present study also revealed that supplementation of 0.15% *Spirulina* PC increased the chosen gut enzyme activities at all (amylase, protease and lipase) while the control diet (without PC) reduced the enzyme activities in *P. reticulata*. Nandeesha *et al*. (1998) found that higher levels of *Spirulina* (60-100%) supplementation reduced the intestinal protease and lipase in *Cyprinuscarpio* and it supports the observations made in the present study. Field beans and groundnut leaf meals increased the amylase activity in the foregut and midgut; prawn head meal and chicken intestine diets showed an elevated amylase secretion in the foregut but decreased gradually in the mid and hind guts of *Labeorohita* (Sethuramalingam and Haniffa, 2002). They also reported that prawn head and chicken intestine meals produced the higher secretion of protease in the midgut of *L. rohita*. Red swordtail fish exhibited the maximum lipase activity in hindgut of all the treatments. On the contrary, lipase activity was high in midgut followed by hindgut in cultivable fishes (Sethuramalingam and Haniffa, 2002).

In terms of biochemical composition, moisture and ash in *P. reticulata* were not affected by *spirulina* PC. *P. reticulata* protein content was significant differences when PC increased in diets up to 0.15%. Without taking into account the different levels of *spirulina* PC, the results of this study's is consistent with findings Nandeesha *et al.* (1998) on common carp, El-Sayed (1994) on silver sea bream and Chou and Shiau (1996) on tilapia (*Oreochromis* sp.). With the increasing levels of *spirulina* PC, body fat reduced significantly. These results are similar to Kim *et al*. (2013) reveled effects replacement of fish meal with *spirulina* in *Oplegnathus fasciatus* in levels of 5%, 10% and 15% that fat was significantly reduced in 15%. In general, the use of plant resources in the diets reduces the amount of body fat. Unlike the results of this study, Mustafa *et al*. (1994) on sea bream and Puwastien *et al*. (1999) on tilapia observed significant difference in body fat feeding algal sources. Polyphenol compounds are other antioxidant compounds in plants (Balasundram *et al*., 2006). Kim *et al*. (2013) known the reason

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for reduced the body fat fish, increasing concentrations of polyphenols in diet with increasing levels of *spirulina* that diet was positively correlated with antioxidant capacity.

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