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Partial Replacement of Fish Meal with *Spirulina pacifica* in Diets for Parrot Fish (*Oplegnathus fasciatus*)

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Abstract

An 8-week feeding trial was conducted in low temperature season to investigate the effects of fish meal (FM) replacement with spirulina on growth performance, body composition and immune response of parrot fish and dietary antioxidant capacity. Four isonitrogenous (48% crud protein) and isocaloric (17.1 MJ/kg gross energy) diets were formulated to replace FM with 0 (as control), 5, 10 and 15% spirulina (designated as Con, S5, S10 and S15, respectively) and fed to the fish (initial body weight, 57±0.1 g) to apparent satiation. At the end of the feeding trial, significantly ($P<0.05$) higher weight gain, protein efficiency ratio and feed intake and lower feed conversion ratio were observed in fish fed S5 diet compared to those fed the Con and S15 diets. Hematocrit, hemoglobin and respiratory burst activity were also significantly ($P<0.05$) increased in fish fed S5 diet. Fish fed S15 diet had significantly ($P<0.05$) higher muscle protein and lower whole-body lipid than those fed the Con diet. Spirulina supplementation in diets increased dietary polyphenols concentration and antioxidant capacity in a dose dependent manner. A second-order polynomial regression analysis shows that the optimum dietary FM protein replacement level by spirulina is approximately 7.3% for the best growth rate. The findings indicate that spirulina can replace up to 15% FM protein (26% dietary inclusion) in the presence of relatively high soybean meal contents in diets for parrot fish.

Keywords: Spirulina, fish meal replacement, growth, ımmune response, parrot fish.

Introduction

Intensive aquaculture is highly dependent to fish meal (FM) and fish oil supply from wild fisheries. FM is regarded as the best dietary protein source because it is very palatable and provides an excellent balance of essential amino acids and essential fatty acids as well as highly digestible energy (Tacon, 1993). The rapid growth of aquaculture has resulted in higher demand for FM and consequently its high price is expected to be further increased by continuous growth in its requirement (Hardy and Tacon, 2002). Furthermore, sustainability of FM production from wild fish is questionable (Naylor *et al*., 2000).

To reach a sustainable aquaculture, new alternative protein sources including cheaper plant or animal origin proteins are needed to be introduced for stable aquafeed production (Higgs *et al*., 1995). Development of FM free diets has partly been successful in some fish species (Kaushik *et al*., 1995; Watanabe *et al*., 1998; Lee *et al*., 2010). Currently, extensive researches are being performed aiming at finding sustainable substitutions for FM. It is suggested that the increased use of plant protein in fish diets can reduce the cost of FM and feeds (Lim and Lee, 2009).

A number of algal species have been used in aquaculture mainly for nutritional applications (Muller-Feuga, 2000). Microalgae play important roles in farming aquatic animals including mollusks, shrimp and fish. They are cultivated commercially as health food or as sources of bioactive chemicals such as beta-carotene (Belay *et al*., 1994; Borowitzka, 1994) for aquatic animals. Spirulina is one of the most frequently used microalgae in aquatic animal feeds due to its high contents of protein, vitamins, essential amino acids, minerals, essential fatty acids and antioxidant pigments such as carotenoids (Nakagawa and Montgomery, 2007). Positive effects of spirulina on growth, feed utilization and stress and disease resistance of cultured fish have been reported in earlier studies. Moreover, spirulina increases immune responses by promoting phagocytic and natural killer activities (Qureshi and Ali, 1996). High protein content of spirulina as well as its well-balanced amino acid profile compared with other plant protein sources

makes it as potential FM replacer in aquafeed formulation (Hanel *et al*., 2007). Spirulina was reported to replace up to 40% of FM protein in tilapia (*Oreochromis mossambicus*) diet (Olvera-Novoa *et al*., 1998) and even higher replacement of FM was possible in common carp (*Cyprinus carpio*) (Nandeesha *et al*., 1998) and Mekong giant catfish (*Pangasianodon gigas*) (Tongsiri *et al*., 2010).

Parrot fish is an emerging aquaculture species and fisheries resource in South Korea. Its high commercial value makes it a promising aquaculture species in Asian countries. The available nutritional data on this species are very limited up to date (Wang *et al*., 2003; Nam *et al*., 2005; Shan *et al*., 2008; Kim *et al*., 2009; Lim and Lee, 2009; Bueno Galaz *et al*., 2010). Therefore, we investigated the effects of partial replacement of FM protein by spirulina on growth performance, feed utilization and innate immune response of parrot fish.

Materials and Methods

Experimental Diets

Proximate composition of the major ingredients used in this study is shown in Table 1. Four isonitrogenous (48% crude protein) and isocaloric (17.1 MJ/kg DM) experimental diets were formulated to replace FM protein with *Spirulina pacifica* powder (Cyanotech Ltd., Kailua-Kona, Hawai, USA) at levels of 0 (as control diet), 5, 10 and 15% (designated as Con, S5, S10 and S15, respectively). Formulation and proximate composition of the diets are presented in Table 2. The energy value of each diet was estimated on the basis of physiological fuel value, i.e., 16.7 KJ/g proteins or carbohydrates and 37.7 KJ/g lipids (Lee and Putnam, 1973). All dry ingredients were thoroughly mixed with distilled water. Pellets were extruded through a meat chopper machine (SMC-12, Korea) in 3.0 mm diameter size and freeze dried at –40°C for 24 h. The pellets were crushed into desirable particle sizes and stored at –20°C until used.

Table 1. Proximate composition of major ingredients used in the experimental diets (% DM)

Ingredients	Moisture	Protein	Lipid	Ash	NFE[1]
White fish meal	8.72	68.33	8.56	14.07	0.32
Soybean meal	11.68	46.91	2.52	6.54	36.44
Yeast	8.72	42.13	0.91	5.96	42.28
Spirulina	5.83	55.29	6.83	3.04	29.01

[1] Nitrogen Free Extracts = 100-(%Moisture+%CP+%Lipid+%Ash).

Experimental Fish and Feeding Trial

Parrot fish were transported from a private commercial farm (Chang-Hae Fisheries, Jeju, Korea) to Marine and Environmental Research Institute (Jeju National University, South Korea) for feeding trial. All the fish were fed a commercial diet for two weeks to be acclimated to the experimental facilities and conditions. Three hundred fish (mean body weight, 57 ± 0.1 g) were randomly distributed into twelve tanks of 100 L capacity (25 fish/tank) in a flow through system and supplied with sand filtered seawater at a flow rate of 2 L/min. Aeration was provided by a central aeration system with air stones. The feeding trial was carried out in triplicates and the fish were fed one of the experimental diets to apparent satiation twice daily (8:00 and 18:00) for eight weeks. All the fish were fasted for 24 h and anaesthetized with 0.1 ppm MS-222 prior to every handling for weighing or

Table 2. Formulation and proximate compositions of the experimental diets where fish meal was replaced by different levels of spirulina at 0, 5, 10 and 15% (% DM)

Ingredients	Diets			
	Con	S5	S10	S15
White fish meal	53.0	46.0	39.0	33.0
Soybean meal	11.0	11.0	11.0	11.0
Spirulina	0.0	9.0	18.0	26.0
Starch	10.0	8.0	6.0	4.0
Wheat flour	12.0	12.0	12.0	12.0
Yeast	2.0	2.0	2.0	2.0
Mineral mix[1]	1.0	1.0	1.0	1.0
Vitamin mix[2]	1.0	1.0	1.0	1.0
Squid liver oil	10.0	10.0	10.0	10.0
Proximate composition (% DM)				
Moisture	17.2	10.8	11.2	11.6
Protein	50.1	48.6	48.7	48.6
Lipid	15.5	14.7	14.0	14.4
Ash	10.5	10.9	11.0	11.4

[1]Mineral premix (g/kg of mixture): $MgSO_4.7H_2O$, 80.0; $NaH_2PO_4.2H_2O$, 370.0; KCl, 130.0; Ferric citrate, 40.0; $ZnSO_4.7H_2O$, 20.0; Ca-lactate, 356.5; $CuCl_2$, 0.2; $AlCl_3.6H_2O$, 0.15; $Na_2Se_2O_3$, 0.01; $MnSO_4.H_2O$, 2.0; $CoCl_2.6H_2O$, 1.0.

[2]Vitamin premix (g/kg of mixture):L-ascorbic acid monophosphate, 100.0; DL-αtocopheryl acetate, 20.0; thiamin hydrochloride, 4.0; riboflavin, 4.4; pyridoxine hydrochloride, 4.0; niacin, 30.0; D-pantothenic acid hemicalcium salt, 14.5; myo-inositol, 40.0; D-biotin, 0.2; folic acid, 0.48; menadione, 0.2; retinyl acetate, 1.0; cholecalficerol, 0.05; cyanocobalamin, 0.01.

blood sampling. Rearing water temperature during the experiment varied from 12 to 16 ºC and the photoperiod was fixed on a 12 h light:12h dark cycle. Dissolved oxygen, pH, nitrate and salinity of rearing water were measured at every week and their average values ranged at 6.5±0.3 mg/L, 7.9±0.4, 6.3±0.5 mg/L and 33.4±0.1 g/L, respectively.

Sample Collection and Analyses

At the beginning and the end of the feeding trial, all fish were bulk-weighed, counted and their total and fork length were measured for calculation of weight gain (WG), specific growth rate (SGR), protein efficiency ratio (PER), feed conversion ratio (FCR), condition factor (CF) and survival. Five fish per tank (fifteen fish per dietary treatment) were collected for whole-body and muscle proximate composition. Analysis of moisture and ash content were performed by the standard procedures (AOAC, 1995). Crude protein was measured by using automatic Kjeltec Analyzer Unit 2300 (FOSS, Sweden) and crude lipid was determined using Soxhlet Extraction System C-SH6 (Korea).

At the end of the feeding period blood samples of three randomly selected fish from each tank (nine fish per treatment) were collected through the caudal vein using heparinized syringe for determination of hematological and immunological parameters. The same fish were used for determination of organosomatic indices including hepatosomatic index (HSI) and viscerosomatic index (VSI). Hematocrit was determined by microhematocrit method (Brown, 1980) and the hemoglobin concentration was determined using an automated blood analyzer (SLIM, SEAC Inc., Florence, Italy). The oxidative radical production by phagocytes during respiratory burst was measured by nitro blue tetrazolium (NBT) assay described by Anderson and Siwicki (1995). Briefly, blood and NBT (0.2%) (NBT; Sigma, St. Louis, MO, USA) were mixed in equal proportion (1:1), incubated for 30 min at room temperature, then 50 µl was taken out and dispensed into glass tubes. Then, 1 ml of dimethylformamide (Sigma, USA) was added and centrifuged at 2000×g for 5 min. Finally, the optical density of supernatant was measured at 540 nm using spectrophotometer (Genesys 10 UV, Rochester, NY, USA). Dimethylformamide was used as blank.

Total polyphenol concentration of the experimental diets was measured by a colorimetric method described by Skerget *et al*. (2005) with some modifications. Briefly, 1 g of each diet was extracted with 250 ml methanol for 2 h at 40 ºC. The extracts were cooled and filtered through a 0.45 µm syringe filter (Whatman Inc., Clifton, NJ). Folin-Ciocalteu reagent (0.2 N) (Sigma) of 2.5 ml was added to the filtered solution and incubated for 5 min at room temperature. Then 2 ml of Na_2CO_3 solution (75 g/L) was added and the mixture was incubated for 5 min at 50 ºC. After being cooled, the absorbance of the samples was read at 760 nm using a spectrophotometer. The results were expressed as gram of gallic acid per kg of dry diet.

Antioxidant capacity of experimental diets was determined using 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging assay as described by Brand-Williams (1995) with some modifications. For this, 2 g of the diets were homogenized in 20 ml aqueous methanol (80%) and kept at room temperature for 10 min. The homogenates were centrifuged (5000 rpm, 4 ºC, 10 min) and filtered through a 0.45 nm syringe filter (Whatman Inc., Clifton, NJ) prior to the assay. Then, 100 µl of filtered extract was added to 1.5 ml cuvette and 900 µl DPPH methanol solution (100 µM) was added to obtain a final volume of 1 ml. The absorbance of the mixture was recorded at 517 nm with 1 min intervals for 10 min using a spectrophotometer. The antioxidant capacity of the extract against the DPPH radicals was calculated as percent inhibition:

$$\text{Percent inhibition} = [(A_0 - A_s) / A_0] \times 100$$

where A_0 and A_s are the absorbance of sample after 0 and s min, respectively.

Statistical Analysis

The data are presented as mean ±SD. All the data were subjected to one-way ANOVA in SPSS version 11.0. The significant differences between group means were compared using Tukey's HSD test. The percentage data were arcsine transformed before the ANOVA analysis. Differences were considered significant at $P<0.05$. The optimum dietary FM replacement level with spirulina was estimated using a second-order polynomial regression analysis based on weight gain.

Results

The results of growth performance, feed utilization and survival by the supplementation of spirulina for FM replacement are provided in Table 3. Significantly ($P<0.05$) higher growth performance and protein efficiency ratio and lower feed conversion ratio were observed in fish fed S5 diet compared to those fed the Con diet. However, higher replacement levels did not produce further improvement in fish growth; nevertheless, the values were numerically higher than those of the fish fed the Con diet. Among the organosomatic indices (Table 3), the VSI was only influenced and the highest value was obtained in the group fed S15 diet. Fish survival rate was 100% regardless of dietary treatment.

Significantly ($P<0.05$) higher hematocrit and hemoglobin values and NBT activity were detected in fish fed S5 diet compared to those of the fish fed the Con diet (Table 4). Whole-body composition analysis

Table 3. Growth performance and organosomatic indices of parrot fish (*Oplegnathus fasciatus*) fed the experimental diets where fish meal was replaced by different levels of spirulina at 0, 5, 10 and 15% for eight weeks

Diets	Con	S5	S10	S15
IBW (g)[1]	56.91±0.05	56.96±0.25	57.14±0.12	57.07±0.23
FBW (g)[2]	83.47±2.10^{a}	89.44±0.24^{b}	86.67±0.83^{ab}	84.03±3.07^{a}
AWG[3]	26.60±2.06^{a}	32.50±0.35^{b}	29.50±0.77^{ab}	27.00±2.90^{a}
SGR (%)[4]	0.68±0.04^{a}	0.81±0.01^{b}	0.74±0.02^{ab}	0.69±0.06^{a}
PER[5]	0.91±0.05^{a}	1.04±0.01^{b}	0.92±0.02^{a}	0.91±0.08^{a}
FCR[6]	2.25±0.12^{b}	1.98±0.01^{a}	2.23±0.04^{b}	2.27±0.18^{b}
CF[7]	2.19±0.05	2.20±0.08	2.22±0.19	2.32±0.29
HSI[8]	2.29±0.27	1.94±0.31	2.66±0.52	2.48±0.22
VSI[9]	2.53±0.37^{a}	3.13±0.64^{ab}	2.53±0.34^{a}	3.53±0.08^{b}
Survival (%)	100	100	100	100

Values are presented as mean ±SD. Values in the same row having different superscript letters are significantly different ($P < 0.05$). The lack of superscript letter indicates no significant differences among treatments.
[1]IBW (g): Initial body weight
[2]FBW (g): Final body weight
[3]Absolute weight gain (g/fish) = (mean individual final body weight – mean individual initial body weight).
[4] Specific growth rate (%) = [(ln final body weight - ln initial body weight)/days] × 100
[5] Protein efficiency ratio = wet weight gain/ total protein given.
[6] Feed conversion ratio = dry feed fed/wet weight gain.
[7] Condition factor = (body weight/fork length^{3}) × 100
[8]Hepatosomatic index = (liver weight/body weight) × 100
[9]Viscerosomatic index = (viscera weight/body weight) × 100

Table 4. Hematological parameters of parrot fish (*Oplegnathus fasciatus*) fed the experimental diets where fish meal was replaced by different levels of spirulina at 0, 5, 10 and 15% for eight weeks

Diets	Con	S5	S10	S15
Hematocrit (%)	41.2±0.14^{a}	47.1±3.99^{b}	45.6±4.73^{ab}	44.1±0.80^{ab}
Hemoglobin (g/dl)	10.1±0.53^{a}	12.5±0.58^{b}	11.7±1.06^{ab}	11.8±0.63^{ab}
NBT activity	1.00±0.08^{a}	1.24±0.12^{b}	1.18±0.16^{ab}	1.16±0.10^{ab}

Values are presented as mean ±SD. Values in the same row having different superscript letters are significantly different ($P<0.05$).

Table 5. Proximate composition of whole-body and muscle of parrot fish (*Oplegnathus fasciatus*) fed the experimental diets where fish meal was replaced by different levels of spirulina at 0, 5, 10 and 15% for eight weeks

	Diets			
	Con	S5	S10	S15
Whole-body (%)				
Protein	17.2±0.4	17.2±0.5	17.0±0.2	17.1±0.4
Lipid	9.2±0.4^{b}	9.1±0.2^{ab}	8.9±1.4^{ab}	7.4±1.2^{a}
Ash	4.6±0.3	4.5±0.2	4.5±0.4	4.5±0.3
Moisture	67.5±0.3	67.3±0.6	67.4±1.1	68.5±1.9
Muscle (%)				
Protein	19.6±0.5^{a}	20.7±0.3^{ab}	20.5±1.1^{ab}	21.1±0.3^{b}
Lipid	2.3±0.6	2.7±0.3	2.6±0.4	2.5±0.6
Ash	1.44±0.03	1.44±0.03	1.44±0.12	1.42±0.03
Moisture	75.1±0.8	74.5±1.0	74.1±0.8	74.9±0.2

Values are presented as mean ± SD. Values in the same row having different superscript letters are significantly different ($P<0.05$). The lack of superscript letter indicates no significant differences among treatments.

revealed a significant ($P<0.05$) reduction of lipid content in fish fed S15 diet (Table 5). Also, the group of fish fed S15 diet showed significantly ($P < 0.05$) higher muscle protein content than the Con group.

Dietary polyphenol concentration was positively correlated with spirulina replacement level and significantly ($P<0.05$) higher concentrations were detected in S10 and S15 diets (Figure 1a). Also, antioxidant activity of the experimental diets was increased in a dose dependent manner and significantly ($P<0.05$) higher activities were found in S10 and S15 diets (Figure 1b). A second-order polynomial regression analysis on fish growth showed that the optimum dietary FM protein replacement level by spirulina is approximately 7.3% (Figure 2).

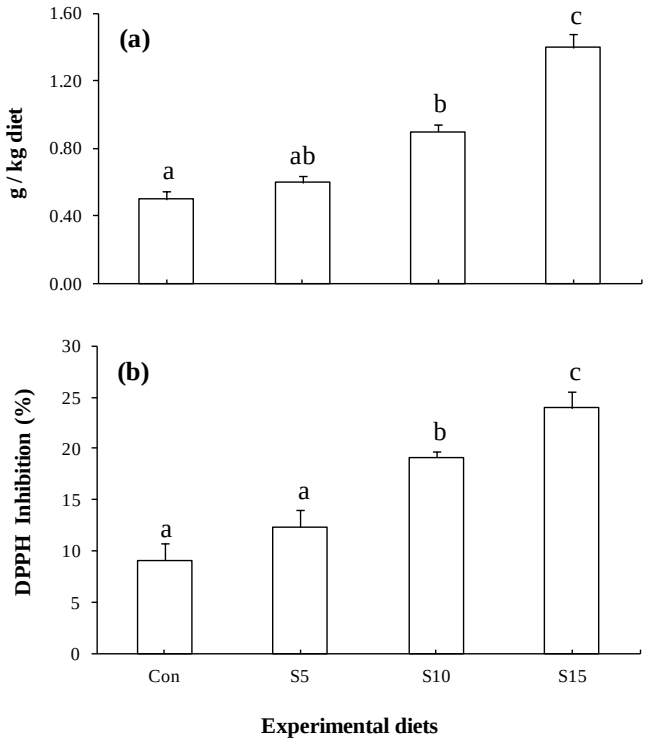

Figure 1. Total polyphenolic compounds concentration (a) and antioxidant capacity of the experimental diets, where fish meal was replaced by different levels of spirulina at 0, 5, 10 and 15% (b). Values are mean of three replicates per treatment. Bars with different letters are significantly different ($P<0.05$).

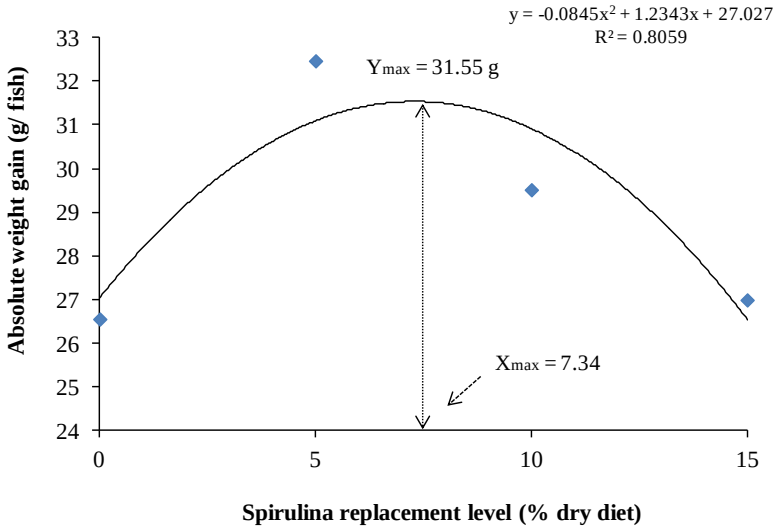

Figure 2. Second-order polynomial regression analysis based on absolute weight gain of parrot fish (*Oplegnathus fasciatus*) to dietary FM replacement by different levels of spirulina.

Discussion

In the present study, the fish readily accepted the experimental diets, however, all fish groups exhibited relatively low growth performance due to low rearing water temperature during the feeding trial. The results showed improvement of fish growth by replacing 5% FM protein with spirulina, while higher substitution levels could not provide further enhancement. Similarly, Tongsiri *et al*. (2010) showed that replacement of 5% FM with spirulina resulted in the best growth performance of *P. gigas*, but higher replacement levels lowered the fish weight gain. Olvera-Novoa *et al*. (1998) reported that spirulina can replace up to 20% of FM protein in diets for *O. mossambicus*, but reduced growth and feed utilization were observed at higher replacement levels. Other studies suggested that dietary FM can be replaced up

to 100 and 60% by spirulina in diets for *C. carpio* and Siberian sturgeon (*Acipenser baeri*), respectively, without any adverse effects (Nandeesha *et al*., 1998; Palmegiano *et al*., 2005).

Growth enhancement effect of spirulina is attributed to its role in nutrient digestibility and its high contents of several nutrients, such as vitamins and minerals (Abdel-Tawwab and Ahmad, 2009). On the other hand, negative effects of high dietary inclusion levels of spirulina on fish growth can be resulted from reduced phosphorous availability and decreased feed palatability (Olvera-Novoa *et al*., 1998). The variations in spirulina effects on fish growth performance are ascribed to different nutrient content of spirulina species used in the studies (Nandeesha *et al*., 1998). Nandeesha *et al*. (2001) found that FM can be totally replaced with spirulina in diets for rohu carp (*Labeo rohita)* and even significantly higher growth can be obtained compared to the use of FM as the sole protein source, whereas no significant effect was observed on growth performance of catla (*Catla catla*) by the same spirulina supplemented diets. Such differences in growth response of *L. rohita* and *C. catla* to dietary spirulina clearly show that the growth response of fishes to spirulina is likely to be species-specific. The other significant factor that affects the results of spirulina administration is the composition of experimental diets in which spirulina is incorporated (Takeuchi *et al*., 2002). In the present study, FM and soybean meal were used as dietary protein sources which resembles to that of the study by Tongsiri *et al*. (2010) where the best performance was obtained by replacing 5% of FM with spirulina. No further increment in fish growth at higher replacement levels of spirulina over 5% FM seems to be as a result of reduced phosphorous availability due to a relatively high supplementation of soybean meal in diets, while in the other studies FM was used as the sole protein source for spirulina supplementation (Nandeesha *et al*., 1998; 2001; Olvera-Novoa *et al*., 1998; Palmegiano *et al*., 2005).

In this study, fish fed S15 diet showed significantly higher VSI value than fish fed the Con diet and this confirmed the results of the study with *A. baeri* where higher VSI values were obtained by replacement of 40 or 60% of FM with spirulina (Palmegiano *et al*., 2005). The results of proximate composition analyses revealed significant increase of muscle protein content in fish fed S15 diet. However, different levels of spirulina in diets could not produce any significant differences in carcass or flesh protein content of *C. carpio*, *A. baeri* and *P. gigas* (Nandeesha *et al*., 1998; Palmegiano *et al*., 2005; Tongsiri *et al*., 2010). In the present study, fish fed S15 diet showed significantly lower whole-body lipid content than fish fed the Con diet. Nandeesha *et al*. (1998) found significant decreases in whole-body lipid of *C. carpio* fed spirulina containing diets, whereas a significant increase was reported for *L. rohita* by spirulina administration (Nandeesha *et al*., 2001). Spirulina effects on body protein and lipid contents are associated with their synthesis and accumulation rate in muscle as well as growth rate of the organism (Abdel-Tawwab *et al*., 2006; Abdel-Tawwab and Ahmad, 2009). Inconsistent results of spirulina administration on fish body lipid content have been reported due to different spirulina species on fat deposition (Nandeesha *et al*., 2001). In the present study, significantly higher polyphenol concentration was detected in S10 and S15 diets. Regarding the hypolipidemic activity of polyphenols (Perona *et al*., 2006; Yang *et al*., 2007), the significant decrease of whole-body lipid in fish fed S15 diet was at least partially due to the higher concentration of polyphenols in the diet.

The antioxidant capacity of spirulina is well established with its high contents of different bioactive materials including phytopigments (Bhat and Madyastha, 2000; Wang *et al*., 2007; Bermejo *et al*., 2008). Polyphenolic compounds are of the other principal antioxidants in plants (Moure *et al*., 2001; Balasundram *et al*., 2006), which exert such effects through the chelation of redox-active metals as well as the acceptance of electrons from reactive oxygen species (Afanas'ev *et al*., 1989; Khokhar and Apenten, 2003). In this study, increased dietary polyphenol concentration by an increment in spirulina inclusion was positively correlated with antioxidant capacity of the diets. According to the enhancement of dietary antioxidant capacity by spirulina, its inclusion in diets would protect fish from tissue damage by inhibition of the formations of reactive oxygen derivatives.

Spirulina has long been recognized as a potential immunostimulant. It augments components of mucosal and systemic immune system through the activation of non-specific immune system. Its aqueous extract was reported to influence the immune system by enhancement of phagocytic activity and stimulation of NK cells (Ravi *et al*., 2010). The immunomodulatory activity of spirulina is ascribed to its C-phycocyanin content (Vonshak, 1997). Recent studies showed that polyphenolic compounds possess protective effects on immune system (Aquilano *et al*., 2008; Franova *et al*., 2010). The results of NBT activity analysis in the present study confirmed such positive effects on non-specific immune response of fish. Similarly, Abdel-Tawwab and Ahmad (2009) showed significant increases of NBT activity in Nile tilapia (*Oreochromis niloticus*) fed spirulina containing diets. However, lower NBT activities were detected at higher replacement levels of spirulina in this study probably indicating an immunosuppressive effect (Sakai, 1999). This finding is in agreement with that of the study by Andrews *et al*. (2011) where lower NBT activities were obtained at higher levels of immunostimulants. In the present study, higher hematocrit and hemoglobin levels were observed in fish fed spirulina containing diets indicating better

fish health condition.

In conclusion, the beneficial effects of spirulina supplementation for FM replacement were demonstrated on growth performance, feed utilization and non-specific immune response of parrot fish. The optimum FM replacement level by spirulina was estimated at approximately 7.0% in diets for parrot fish based on fish growth.

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