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Effects of dietary inclusion of *Spirulina* meal on growth and hematological parameters of cultured Asian sea bass, *Lates calcarifer*

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Abstract

This study was conducted to investigate the effects of dietary inclusion of *Spirulina platensis* on growth performance and hematological parameters of juvenile Asian sea bass (*Lates calcarifer*) reared in a freshwater culture system. Five experimental diets were prepared by replacing fish meal protein with the microalga at replacement levels of 5% (SP5), 10% (SP10), 20% (SP20) and 30% (SP30), and the substitution effect was compared with a control diet (Con) in which fish meal was the sole protein. Fish were stocked in net cages placed in a 150-ton tank with a stocking density of 20 fish per cage. After 8 weeks of feeding trial, the fish did not show any significant differences in growth performance but numerically higher weight gain and specific growth rate were achieved in the fish stock fed diet SP10 compared to other treatments. Feed conversion ratio and survival rate of fish were significantly affected by the inclusion of Spirulina in the diets. Except for crude ash content, whole-body proximate composition of the experimental fish was also significantly influenced by the diets. Regarding the effects of diets on blood parameters, only hematocrit, hemaglobin, HDL-c and AST were significantly affected by the inclusion of Spirulina in the diets. This study demonstrated that Spirulina could replace up to 10% of FM protein in practical diets of juvenile Asian sea bass without negative effects on growth performance. However, replacement of fish meal with Spirulina meal at 5% might be considered for commercial use considering a significant decrease in survival above this value.

Keywords: Asian seabass, Spirulina, Replacement, Fish meal

Introduction

Asian sea bass, *Lates calcarifer* (Bloch 1790), is widely cultured in Asian countries including Malaysia. It is one of the most sought-after marine fish for commercial culture because of high market potential, fast growth, euryhaline nature and easy rearing of fry to adult size in the hatchery (Copland and Grey 1986). Asian sea bass can be cultured in marine, brackish or freshwater (Harpaz et al., 2005). Because the fish is predatory, it requires high-protein diet. The use of fishmeal (FM) is estimated to be more than 50% of the variable cost in sea bass feed (Woods, 1999). The industry largely depends on FM as a major protein source in aquafeeds due to its contents of essential nutrients such as indispensable amino acids, essential fatty acids, vitamins, minerals, attractants and unknown growth factors (Zhou et al., 2004). However, for reasons of environmental compatibility efforts are being made to lessen the dependency on FM.

Spirulina is one of the most common microalgae in warm saline and alkaline waters. Its growth rate under culture conditions is fast, almost like yeasts and many bacteria (Richmond, 1988). With a short life cycle, the Spirulina can duplicate its biomass in 3-5 days and has a potential production of 25 t $ha^{-1}year^{-1}$, which is equivalent to 15 t of protein $ha^{-1}year^{-1}$(Richmond, 1988; Göhl, 1991). *Spirulina platensis* does not have cellulose cell wall. Its cells have mucopolymer murein that is easily hydrolyzed by the digestive enzymes of the fish (Beresto, 2001). It is a rich source of vitamins, essential amino acids, minerals, essential fatty acids (γ-linolenic acid), and antioxidant pigments such as carotenoids and phycocyanin (Jaime-Ceballos et al., 2006) and can serves as an excellent source of proteins and other nutrients (Nandeesha et al., 1998). It has been included in the diets for silver seabream (*Rhabdosargus sarba*) (El-Sayed, 1994); tilapia (*Oreochromis mossambicus*) (Olvera-Novoa et al., 1998), sturgeon (*Acipenser baeri*) (Palmegiano et al., 2005) and common carp (*Cyprinus carpio*) (Nandeesha et al., 1998; Ramakrishnan et al., 2008) with promising results. More research is necessary to investigate the potential of this alga in promoting growth and survival of other fish species. Thus, the present study was conducted to evaluate the use of S*pirulina platensis* as a fish meal replacement in practical diets for Asian sea bass juveniles using a simple mixture of two protein sources in a freshwater recirculating system.

Materials and Methods

Experimental diets

Experimental diets were formulated to be isonitrogenous and isocaloric in terms of crude protein (45%) and gross energy (17.97 MJ/kg^{-1}). The energy value of the experimental diets was estimated on the basis of mammalian physiological fuel

value, i.e., 16.7 KJg^{-1} protein or carbohydrate and 37.7 KJg^{-1} lipid (Lee and Putnam, 1973). Five experimental diets (Table 1) were formulated to contain 0 %, 5%, 10%, 20% and 30% Spirulina meal, designated by Control, SP5, SP10, SP20, SP30, respectively. Spirulina meal was purchased from Vedan Biotechnology Corp (Taichung, Taiwan). All the ingredients were thoroughly mixed with 40% distilled water to become dough. Pellets were extruded through a meat chopper machine (Orimas TBS-200, Taiwan) in 3.0 mm diameter size, oven-dried at 40°C and stored at -20°C until use.

Table 1. Composition of the experimental diets

Ingredients	Diets				
	Con	SP5	SP10	SP20	SP30
Fish Meal[a]	59.93	56.93	53.94	47.94	41.95
Spirulina[b]	0.0	3.34	6.68	13.37	20.05
Fish Oil[c]	6.56	6.84	7.11	7.65	8.19
Vitamin mix[d]	3.00	3.00	3.00	3.00	3.00
Mineral mix[e]	2.00	2.00	2.00	2.00	2.00
Dicalcium phosphate[f]	1.00	1.00	1.00	1.00	1.00
Carboxymethyl cellulose[g]	1.50	1.50	1.50	1.50	1.50
Tapioca starch[h]	20.00	20.00	20.00	20.00	20.00
α- Cellulose[i]	6.01	5.39	4.78	3.54	2.31
Proximate composition (% DM)					
Moisture	8.30	8.20	8.40	17.15	16.64
Protein	44.55	43.88	43.25	44.47	46.90
Lipid	11.74	11.83	12.02	12.49	12.82
Ash	12.18	12.04	12.03	11.38	11.30
Energy (MJ/kg)	18.92	18,83	18.62	18.02	17.59
Major Ingredients	Moisture	Protein	Lipid	Ash	NFE[1]
Fish Meal	8.79	75.09	9.07	13.39	---
Spirulina	4.66	67.34	0.03	8.0	19.97

[a] Danish fish meal, Denmark
[b] Spirulina was provided Vedan Biotechnology Corp (Taichung, Taiwan)
[c] Dexchem Industries Sdn. Bhd, Malaysia
[d] Vitamin premix. Contained (as g kg−1): ascorbic acid, 300; inositol, 125; niacin, 50; riboflavin, 15; pyridoxine, 12; thiamin mononitrite, 15; retinyl acetate, 1.72; cholecalciferol, 0.025;menadione sodium bisulphite, 5; biotin, 0.5; folic acid, 2.5; DL-α-tocopheryl acetate, 50; vitamin B12, 0.025; calpan, 25. Dexchem Industries Sdn. Bhd, Malaysia
[e] Mineral premix. Contained (as g kg−1): calcium phosphate·H2O (MDCP), 397.65; calcium lactate, 327; ferrous sulphate·H2O, 25; magnesium sulphate·7H2O, 137; potassium chloride, 50; sodium chloride, 60; potassium iodide, 0.15; copper sulphate·5H2O, 0.785; manganese oxide, 0.8; cobalt carbonate, 0.1; zinc oxide, 1.5; sodium selenite·5H20, 0.02. Dexchem Industries Sdn. Bhd, Malaysia
[f] Merck, 64271 Darmstadt, Germany
[g] EMD Chemicals, Inc. San Diego, CA
[h] AAA Brand, Bake With Me Sdn. Bhd., Malaysia
[i] Sigma-Aldrich Corporation, USA
[1] Nitrogen Free Extracts = 100-(%Moisture+%CP+%Lipid+%Ash).

Fish, facilities and feeding trial

The feeding trial was carried out at the hatchery facilities at Borneo Marine Research Institute of Universiti Malaysia Sabah (UMS). Asian sea bass juveniles were obtained from the hatchery. The fish were acclimatized to experimental rearing conditions and weaned to the formulated diets for two weeks with a commercial feed (Cargill Feed SDN. BHD. Ltd., Melaka, Malaysia) (45% crude protein and 8% crude lipid), prior to the feeding trials. Three hundred fish of initial body weight (BW) of 6.02 ± 0.03 g were randomly distributed into groups of 20 fish in 15 cylindrical cages, each measuring 41 cm depth and 52 cm diameter, and placed in a 150 ton FRP (fibreglass reinforced plastic) tank equipped with a water recirculation system using dechlorinated tap water and coral rubble as filter. Around 20-30% of water was exchanged daily during bottom cleaning. Water quality was maintained at a temperature of 28.12 ± 0.29 °C, dissolved oxygen (DO) at 6.80 ± 0.13 mg/L and pH at 7.89 ± 0.25. The fish were fed till apparent satiation twice a day (9:00 and 14:00 h), seven days a week, for 8 weeks. The growth of fish was measured at every 2-week interval. Feeding was terminated 24 hours prior to weighing.

Sample collection and analysis

At the beginning and the end of the feeding trial, all the fish specimens were bulk-weighed, counted, and their total and fork lengths 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. Three fish per cage (nine fish per dietary treatment) were collected for whole-body proximate analysis. Proximate composition of diets and whole-body fish were determined following the AOAC (1995) method. Crude protein was measured by using automatic Kjeltec Analyzer Unit 2300 (FOSS, Sweden) and crude lipid was determined by petroleum benzene extraction method using Soxtec™ 2043, hot extraction (FOSS Tecator, Sweden). Crude ash content was determined as the residue remaining after incineration of dry samples at 550 °C in a muffle furnace for 6 hours. Samples of the diets were assayed for gross energy (GE) by C5003 IKA adiabatic oxygen bomb calorimeter (GMBIT and Co., Dresden, Germany).

At the end of the feeding trial blood samples of six randomly selected fish from each cage (eighteen fish per treatment) were collected from the caudal vein using non-heparinized syringe for determination of hematological and biochemical parameters. For hematological examination 1 ml of fish blood was placed inside K3 EDTA tubes**.** EDTA tubes have interior walls coated with K3 EDTA. EDTA binds calcium ions and blocks the coagulation cascade (Greiner Bio-One®). Afterwards the tubes were placed in a Sysmex XT-1800i machine for hematocrit and hemoglobin measurements. For biochemical parameters 2ml of fish blood was placed inside Z Serum Sep Clot Activator tubes (Greiner Bio-One®), followed by centrifugation at 35 x 100 rpm for 10 minutes in a Kubota 2420 centrifuge and placed in a cobas® 501 analyzer (Roche Diagnostics, Indianapolis, IN) for total protein, cholesterol, cholesterol HDL, cholesterol LDL, triglyceride, alanine amino transferase and aspartate amino transferase measurements.

Another group of three fish was randomly selected from each cage for determination of hepatosomatic index (HSI) and viscerosomatic index (VSI).

Amino acid analysis
Diet samples were hydrolysed in triplicate with 6N HCl for 24 hours at 110°C. Sample was derivatized by using post column derivatizer (PCX 5200, Pickering Laboratories, USA). The reagents used for post column derivatization are *o*-Phthalaldehyde (OPA) and Thiofluor™ in pH 10.4 borate buffer (OPA diluents for Amino Acid analysis) + 30mL Brij 35® (Pickering Laboratories, USA). Chromatographic separation was done using a High Efficiency Sodium Cation-Exchange column (5µm, 4.0 x 150mm, Pickering Laboratories, USA). The column temperature is set at 53°C while the reactor temperature at 45°C. Flow rate was set at 0.4mL/minute. The HPLC system used is a system controller (SCL-10A), liquid chromatograph (LC-10AD), degasser (DGU-14A), fluorescence detector (wavelength excitation at 330nm, emission at 365nm) and an auto injector (SIL-10AD, Shimadzu, Japan). The mobile phases used were acidic buffer (Sodium eluant, pH 3.15, with 5% Silfolane), neutral buffer (Sodium eluant, pH 7.40) and Sodium column regenerant. Chromatographic peaks were integrated, identified and quantified with Class-VP Ver. 6.1 (Shimadzu) by comparing with the known standards (amino acid protein hydrolysate standard, in 0.2N sodium citrate buffer pH 2.20, 0.25 µmole mL^{-1} (Pickering Laboratories, USA). Methionine and cystine were determined from the same method of acid hydrolysis.

Statistical analysis
Data was subjected to one-way ANOVA in SPSS version 11.0. Significant differences between group means were compared using Duncan´s multiple test. Data presented are means±standard deviation (SD). Data with percentage values were arcsine transformed before the ANOVA analysis. Differences were considered significant at $p<0.05$.

Results

Table 2 shows the amino acid composition of experimental diets. In general, the inclusion of Spirulina in the diets did not appreciably alter the amino acid profile of the diets. A slight reduction of methionine was observed when Spirulina inclusion was increased in the diets. Growth performance and feed utilization of fish fed the experimental diets are presented in Table 3. At the end of the eight-week feeding trial, there were no significant differences in final body weight, weight gain, specific growth rate and protein efficiency ratio of fish fed the experimental diets. Feed conversion ratio was significantly affected when fish meal was replaced with Spirulina meal at 30% replacement level. FCR for other diets ranged from 1.31-1.83 as compared to 2.97 in SP30. Similarly, the lowest feed intake was also observed in SP30. However, no significant difference was observed in protein efficiency ratio, but the highest value of PER was found in SP10. The net protein utilization was also numerically higher in fish fed SP10 diet compared to the fish offered the control diet and was also significantly higher compared to dietary treatment SP30. Survival was significantly lower in fish fed diets SP20 (60%) and SP30 (55%) compared to that of fish fed the control diet (96.67%). Table 4 shows the whole-body proximate composition and body indices of juvenile Asian seabass. Except for crude ash content, dietary treatments significantly influenced the whole body composition of the fish. Lipid content was significantly higher in fish fed diet SP5 compared to the fish fed the control diet. Increasing levels of spirulina inclusion in diets resulted in a decreasing trend in whole body lipid of the experimental fish. Hepato-somatic index was significantly higher in fish fed diets SP30 and SP20 compared to its value in the fish fed the control diet. No significant differences were found in the condition factor and viscera-somatic index of the fish fed all the experimental diets.

Table 2. Amino acid composition of experimental diets (%)

Amino acid	SP0	SP 5	SP 10	SP 20	SP 30
Aspartic acid	4.52	4.30	4.17	4.17	4.51
Threonine	1.88	1.99	1.94	1.96	2.14
Serine	2.14	2.05	2.02	2.07	2.23
Glutamic acid	6.50	6.33	6.14	6.17	6.67
Glycine	3.12	3.24	3.25	3.30	3.30
Alanine	3.15	2.99	2.96	3.05	3.28
Cystine	2.27	2.16	2.11	2.17	2.39
Methionine	1.33	1.26	1.21	1.15	1.20
Isoleucine	2.10	2.03	2.02	2.16	2.37
Leucine	3.80	3.67	3.68	3.82	4.04
Tyrosine	1.31	1.27	1.32	1.38	1.57
Phenylalanine	1.92	1.65	1.64	1.69	1.86
Lysine	4.17	4.30	4.34	4.43	4.13
Arginine	3.10	3.07	3.11	3.27	3.45

Hematocrit in fish fed diet SP30 was lowest among the dietary treatments. No significant differences were observed in hematocrit in other fish groups. Inclusion of Spirulina at 30% (SP30) also resulted in lower hemoglobin and high density lipoprotein cholesterol (HDL-c) compared to other treatments. On the other hand, aspartate amino transferase (AST) in fish fed diet SP30 was the highest among the dietary treatments. No significant differences were observed in total protein, total tryglyceride (TG), total cholesterol (T-Cho), low density lipoprotein cholesterol (LDL-c) and alanine aminotransferase (ALT) as a result of Spirulina inclusion in the diets (Table 5).

Table 3. Growth performance of Asian sea bass (*Lates calcarifer*) fed the experimental diets

Diets	Con	SP5	SP10	SP20	SP30
Initial Body Weight (g)	6.02 ± 0.02	6.02 ±0.01	6.02 ± 0.01	5.98 ± 0.03	6.05 ± 0.004
Final Body Weight (g)	15.15 ± 2.53	17.71 ± 1.17	18.17 ± 1.94	16.05 ± 4.21	12.64 ± 3.24
Weight gain (%)[a]	151.48 ± 41.46	194.24 ± 19,28	201.65 ± 32,33	168.37 ± 71.10	109.10 ± 53.72
Specific growth rate (% d^{-1})[b]	1.60 ± 0.31	1.89 ± 0.11	1.93 ± 0.18	1.68 ± 0.51	1.26 ± 0.44
Feed conversion ratio[e]	1.83 ± 0.29[ab]	1.66 ± 0.24[ab]	1.61 ± 0.04[a]	1.31 ± 0.86[a]	2.97 ± 1.23[b]
Feed intake (g)[f]	0.27 ± 0.04[ab]	0.30 ± 0.01[b]	0.29 ± 0.05[b]	0.25 ± 0.01[ab]	0.21 ± 0.04[a]
Protein efficiency ratio[c]	1.33 ± 0.13	1.60 ± 0.07	1.74 ± 0.05	1.62 ± 0.36	1.14 ± 0.21
Net protein utilization[d]	18.46 ± 1.48[ab]	21.64 ± 1.54[ab]	26.90 ± 1.20[b]	19.02 ± 5.29[ab]	20.00 ± 3.58[a]
Survival (%)	96.67 ± 2.89[c]	83.33 ± 10.41[bc]	73.33 ± 7.64[ab]	55.00 ± 13.23[a]	60.00 ± 18.03[a]

Table 4. Whole-body composition (% wet weight basis) and body indices of juvenile Asian sea bass fed experimental diets

Parameters	Initial	Diet group				
		Con	SP5	SP10	SP20	SP30
Moisture	73.81 ± 0.82[ab]	73.80 ± 0.94[ab]	72.81 ± 1.13[a]	73.92 ± 0.58[ab]	76.04 ± 0.44[c]	75.25 ± 1.13[bc]
Crude Protein	17.66 ± 0.09[e]	15.77 ± 0.60[bc]	15.56 ± 0.38[ab]	16.62 ± 0.47[d]	15.08 ± 0.22[a]	16.40 ± 0.04[cd]
Crude Lipid	3.49 ± 0.39[ab]	5.03 ± 0.53[c]	6.12 ± 0.81[d]	4.37 ± 0.34[bc]	2.91 ± 0.20[a]	2.62 ± 0.87[a]
Ash	4.35 ± 0.01	4.55 ± 0.25	4.36 ± 0.17	4.36 ± 0.26	4.66 ± 0.23	4.72 ± 0.61
Condition factor [a]		1.44 ± 0.10	1.38 ± 0.13	1.46 ± 0.04	1.36 ± 0.10	1.36 ± 0.02
Hepato-somatic index[b]		0.82 ± 0.10[a]	1.11 ± 0.22[ab]	1.27 ± 0.12[ab]	1.35 ± 0.18[b]	1.53 ± 0.47[b]
Vicero-somatic index[c]		5.10 ± 1.10	5.80 ± 0.20	5.82 ± 0.39	5.26 ± 0.71	5.85 ± 1.42

Values are mean ± standard deviation. Values in the same row with different letters are significantly different (*P < 0.05*)
[a]Condition factor = fish weight (g) x 100/fish length (cm)[3].
[b]Hepatosomatic index = 100 x (liver weight/body weight).
[c]Vicerosomatic index = 100 x (Vicera weight/body weight).
*Values are mean ± standard deviation. Values in the same row with different letters are significantly different (*P <0.05*).

Table 5. Blood parameters of juvenile Asian sea bass (*Lates calcarifer*) fed the experimental diets

Diets	Con	SP5	SP10	SP20	SP30
Hematocrit (%)	40.33 ± 3.06[ab]	44.00 ± 4.00[b]	46.00 ± 4.58[b]	32.00 ± 12.77[ab]	22.00 ± 18.36[a]
Hemoglobin (g dL^{-1})	6.40 ± 0.30[b]	6.50 ± 0.52[b]	6.87 ± 0.75[b]	5.73 ± 1.85[ab]	3.87 ± 1.85[a]
Total protein (g dL^{-1})	3.30 ± 0.60	3.47 ± 0.21	3.53 ± 0.21	3.47 ± 0.06	3.43 ± 0.12
TG (mg dL^{-1})[a]	271.00 ± 54.53	183.00 ± 35.00	148.33 ± 26.50	285.33 ± 123.62	262.33 ± 71.59
T-Cho (mg dL^{-1})[b]	294.67 ± 68.16	307.67 ± 4.62	317.67 ± 10.97	283.00 ± 15.72	258.67 ± 13.28
HDL-c (mg dL^{-1})[c]	123.33 ± 20.60[ab]	151.67 ± 15.63[bc]	171.00 ± 22.52[c]	128.33 ± 17.04[ab]	114.00 ± 19.97[a]
LDL-c (mg dL^{-1})[d]	117.00 ± 38.11	118.00 ± 9.54	117.00 ± 16.09	97.33 ± 2.31	91.33 ± 17.90
ALT (U L^{-1})[e]	16.67 ± 12.22	9.00 ± 1.73	9.00 ± 1.73	32.33 ± 24.50	18.33 ± 5.69
AST (U L^{-1})[f]	71.00 ± 48.45[ab]	37.67 ± 8.08[a]	39.33 ± 10.69[a]	60.33 ± 29.37[ab]	99.67 ± 33.08[b]

[a] TG: triglyceride.
[b] T-Cho: total cholesterol.
[c] HDL-c: high density lipoprotein cholesterol.
[d] LDL-c: low density lipoprotein cholesterol.
[e] ALT : alanine aminotransferase, unit per liter (U L^{-1}) is the amount of enzyme which oxidizes 1 μmol L^{-1} of NADH per minute.
[f] AST : aspartate aminotransferase
* Values are means ± SE of triplicate groups. Within a row, means with the same letters are not significantly different (P> 0.05).

Discussion

Spirulina has been successfully utilized alone or in combination with other protein sources to replace fish meal. It has been used to replace fish meal in the diet of *Cyprinus carpio* (Nandesha et al., 1998), *Acipenser baeri* (Palmegiano et al., 2005) and *Oreochromis mossambicus* (Olvera-Novoa et al., 1998), with successful replacement values of 100, 60 and 40%, respectively. The variation in results might be attributed to different Spirulina strains utilized or the variations in the ability of fish species to utilize the algae meal. The growth of Asian sea bass fed the experimental diets did not differ significantly (*P>0.05*) in terms of WG, SGR and PER. Similarly, no significant effects have been reported on growth performance in common carp, *Cyprinus carpio* (Nandeesha et al., 1998) and catla *Catla catla* (Nandeesha et al., 2001). However, in this experiment the inclusion of spirulina in diet improved FCR with the exception of dietary treatment SP30. Similar trend was also observed for the NPU values. Survival rate of the fish was significantly affected as a result of cannibalism and low feed intake of fish offered high inclusion level of Spirulina in diet. Low feed intake is directly proportional to the protein intake, which in turn affects growth rate (Phumee et al., 2011). Observations made by Qin et al. (2004) also revealed that the cannibalism in Asian sea bass culture caused severe losses during the early stages of development particularly before fish reach a length of about 10 cm. An individual Asian sea bass cannibal can swallow a sibling with a body length of 70% or less than its own. According to Appelbaum and Arockiaraj (2010), sibling cannibalism in juvenile Asian sea bass could begin when the body length of a cannibal reaches about one and half times that of the prey fish. Also, cannibalism may occur in spite of *ad libitum* feeding.

Olvera-Novoa et al. (1998) reported low feed intake in tilapia when offered diets which contained increasing level of spirulina. This was attributed to increase in particle hardness and low palatability resulting from high spirulina content in the diet. Higher feed intake values obtained in diets SP5 and SP10 were reflected in improvement in growth, feed efficiency, health and immune parameters in fish groups fed by these two diets. On the other hand, fish fed diet SP30 (with the highest inclusion level of Spirulina) suffered impairment of growth and feed efficiency parameters as well as health condition as expressed by significantly lower values of hemoglobin and and hematocrit as compared to the fish fed the control diet. SP30 also exhibited a potential liver damage expressed by the numerically higher values for aspartate amino transferase (AST) compared to the fish fed the control diet and significantly higher in comparison with the fish fed the lowest inclusion level of spirulina SP5. The cellulose walls of Spirulina are made of digestible mucoproteins (Richmond, 1988) which have a digestibility of 83-84% (Santillán, 1979). However, little information is available on the digestibility of Spirulina in fish diets. Olvera-Novoa et al. (1998) reported a reduction in digestibility with an increase of Spirulina inclusion in diets for tilapia (82.55, 81.92, 69.85, 78.17, 46.00, 45.45% apparent digestibility with an inclusion of 0, 20, 40, 60, 80, 100% of Spirulina in diets, respectively). Ekpo and Bender (1989) reported a digestibility of 62% for a mixture of Cyanophyta consumed by *Oreochromis niloticus* in ponds. In this experiment, digestibility was not measured but reduced digestibility as the level of Spirulina increased is expected, especially at 30% inclusion level.

In the present study, fish were reared in a freshwater recirculating system which is a common practice for commercial purposes. A large percentage of dietary salt in commercial feeds for carnivorous fish originates from the fish meal component of the diet (Murray and Andrews 1979). It is, therefore, important to take this into account when replacing fish meal with various plant-derived meals, which are not as rich in salt. Asian sea bass is very similar to other carnivorous fish in its nutritional requirements (Catacutan and Coloso, 1995; Catacutan and Coloso, 1997). The poor performance of fish fed the highest inclusion of Spirulina in the present study might suggest that the rearing of a marine fish such as Asian sea bass in a freshwater environment and at the same time, using diets in which fishmeal is replaced with plant protein sources could face a lack of some minerals needed for the passive outward flux of ions such as Na^+ and Cl^- from the fish to the external medium. This must be overcome by active uptake of ions (e.g., Na^+, Cl^-, K^+, and Ca^{2+}) from the water and/or from the diet (Smith et al., 1989; Schmidt-Nielsen, 1997). The diet constitutes an important source of salts that can satisfy the osmoregulation requirements of fish maintained in freshwater where energy otherwise mobilized for osmoregulation is spared and becomes available for supporting growth (Zaugg et al., 1983; Gatlin et al., 1992). Previous study done on Asian sea bass showed that the addition of salt to the diet which resulted in a significant improvement in feed conversion ratio (FCR) had no effect on carcass composition of fish, although alkaline phosphatase, lactase, and, to some extent, leucine amino peptidase activity were enhanced, with most pronounced effect exhibited in the pyloric caeca (Harpaz et al. 2005). The contribution of salt to fish nutrition can be explained by a better enzymatic activity due to absorption mechanism of the end products, including glucose and amino acids. Since the glucose and most of the amino acid absorption depends on the Na^+/K^+ ATPase pump (Klein et al., 1998), and higher concentration of Na^+ in the lumen might lead to a better absorption of carbohydrates and amino acids (De la Fuente et al., 1997). It is also interesting to note that the amino acid profile of the diets based on Spirulina did not significantly differ from the control diet, suggesting that the experimental diets were able to supply the necessary essential amino acids to the cultured fish.

In this study, fish fed SP20 and SP30 diets showed significantly higher HSI value than fish fed the control diet. However, different levels of Spirulina in the diets did not produce any significant difference in HSI of *Acipenser baeri* and *Oplegnathus fasciatus* (Palmegiano et al., 2005; Kim et al., 2013). The results of proximate composition analysis revealed significant increase in whole body protein content in fish fed SP10 diet. With the exception of the dietary treatment SP20, values were similar to those found for silver seabream (El-Sayed, 1994). On the contrary, Nandeesha et al. (1998), Palmegiano et al. (2005), and Tongsiri e al. (2010) did not find any significant differences in carcass composition of *Cyprinus carpio*, *Acipenser baeri* and *Pangasianodon gigas*. In the present study, fish fed SP20 and SP30 diets showed significantly lower whole-body lipid content than the fish fed the control diet. These results are in agreement with Mustafa et al. (1994) who found that dietary Spirulina supplementation was associated with a significant decrease in lipid level with a compensatory increase in moisture content. Similarly, in striped jack (*Pseudocaranx dentex*) it was found that the inclusion of 5% Spirulina in diets resulted in depression of body lipid and improved growth rates (Liao et al., 1990; Watanabe et al., 1990).

Conclusion

In conclusion, *Spirulina plantensis* meal can be used as a source of protein in the diets for Asian sea bass cultured in freshwater environment. Diet SP10 showed higher values for growth performance, feed efficiency and the selected blood parameters. However, the results suggest that 5% fishmeal replacement by dietary inclusion of Spirulina meal might be optimum and a safe level for commercial use for the juvenile Asian sea bass.

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