

Effects of Protein and Lipids on Growth Performance, Feed Efficiency and Survival Rate in Fingerlings of Bay Snook (*Petenia splendida*)

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Abstract: A 120-days trial with three dietary protein levels (30, 40 and 50%, respectively) and two dietary lipid concentrations (6 and 12%, respectively) was conducted to investigate the optimum dietary protein and lipid level for the growth, feed efficiency and survival rate of bay snook fingerlings, *P. splendida* (0.18±0.05 g initial body weight). A 2x3 factorial experiment in a completely randomized design with three replicates was used. Whole body amino acids and fatty acids analyses were carried out for each diet and calculated growth performance, feed efficiency and survival rate. The results showed a higher survival rate. Final Body Weight (FBW), Feed Efficiency (FE), Protein Efficiency Ratio (PER), Feed Conversion Ratio (FCR) and condition factor (K) was significantly affected by dietary protein level ($p<0.05$) and FBW, SGR, PER and K were significantly affected by dietary lipids levels ($p<0.05$). The amino acid composition in diets and whole body was similar and Saturated Fatty Acids (SFA) showed a similar trend; the Polyunsaturated Fatty Acids (PUFA) indicated that 30/12 and 50/12 diets presented high values in whole body, whereas monoenes presented a dissimilar pattern. The best growth was observed with 50/12 diets where the lipid source was corn oil.

Keywords: Amino acid, fish, nutrition, specific growth rate

INTRODUCTION

Dietary protein and lipid contents are the most important factors affecting growth performance of teleost fish and feed cost. Generally, an increase of protein and lipid levels in diet improves fish production, especially for carnivorous fish, but proportionally increases feed cost (NRC, 1993). Utilization protein and lipids for fish growth can be improved by partially replacing part of the protein and lipids in the diet. Hence, it is important to determine the proper levels of conventional and non-conventional protein sources, as well as lipid sources to reduce cost and avoid an imbalance in either non-protein energy source or level in diets (Garling and Wilson, 1976; Kim and Lee, 2005).

When fish are feeding with diets containing insufficient protein and lipids there is a reduced growth performance because of unsatisfactory digestible energy or deficiencies in essential fatty acids. The effect of

dietary lipid levels on fish growth performance varies considerably within species, size, age, diet formulation and composition, range of lipids level tested and rearing conditions. A protein sparing effect by lipid supplementation has been well demonstrated for salmonid and sea bass (Watanabe, 1982; Beamish and Medland, 1986; Dias *et al.*, 1998; Torstensen *et al.*, 2001).

The increase in dietary lipid levels may result in decreasing trend of whole body protein proportion with no apparent effect on growth performance. Page and Andrews (1973) suggested that the differences in whole-body protein are lower in fish fed high-lipid diets as a result of dilution with lipid. Similar results were reported by Wang *et al.* (2005) and Song *et al.* (2009).

The bay snook *Petenia splendida* (Günther, 1862) is one of the most important Neotropical Cichlid native specie, which is found in commercial fisheries in the Grijalva-Usumacinta River System in southeast México. Their wide acceptance as an excellent food fish and their

high demand in local markets has led to over-harvesting of wild stocks. Additionally, their artisanal fishery had diminishing in the last 20 years as result of the drastic hydrological changes in the river system and the introduction of exotic species competing for space, food and reproduction areas (Perez-Vega *et al.*, 2006).

The diet of bay snook has been fairly well documented. Miller *et al.* (2005a) indicated that this species is omnivorous with carnivorous tendency; consume prey items with high levels of proteins and lipids consisting in invertebrates and fishes. Due the increasing interest in recover natural populations of bay snook, experimental rearing have been conducted utilizing commercial steelhead rainbow trout feed with acceptable results (Pérez-Sánchez and Páramo-Delgadillo, 2008). However, there is limited data with regard to basic nutrients requirements as protein and lipid.

In order to develop efficient culture strategies, a better understanding of nutritional requirement is required. The aim of this research was to evaluate the effect of fed diets with different protein (30, 40 and 50%, respectively) and lipid (6 and 12%, respectively) levels from fish and vegetal sources, on growth performance, feed efficiency and survival rate in fingerlings of bay snook under controlled experimental conditions.

MATERIALS AND METHODS

Experimental fish and feeding trial: Bay snook fingerlings (*P. splendida*) were obtained from Agropecuaria Sciences Division, Universidad Juárez

Autónoma de Tabasco (Villahermosa, Tabasco, México) in 2010. Fingerlings were translated to Experimental Aquaculture Production Plant at the Universidad Autónoma Metropolitana Iztapalapa in México City, where this experiment was realized.

Initially, 800 fingerlings was introduced in 1,000-L recirculating circular tank and maintained during 40 days before start the experiment. During this time they were fed with steelhead rainbow trout commercial feed (47% of protein and 16% of lipids). 2x3 factorial experiment in a completely randomized design with three replicates was used. The acclimated fish (mean initial body weight 0.18±0.05 g) were placed into twelve 40-L recipients connected to a recirculation water system. Seventeen fingerlings were randomly distributed in each recipient. Fingerlings were fed with commercial steelhead rainbow trout feed during two weeks and one week before testing was fed with experimental diets. The fingerling was fed for 120 days at about 6% of body weight. Feed ration was considered enough because an insignificant amount of wastes was noted during the morning clean up. The daily ration was given in three equal proportions at 10:00, 14:00 and 18:00 h, respectively. At the beginning of the experiment and every 15 days, every fingerling was individually weighed and its length determined. Water quality was monitored along the experiment and the average±SD of water temperature was 28.9±0.24°C; dissolved oxygen 6.3±0.25 mg/L; pH 9.2±0.26; nitrite 0.01±0.00 mg/L; nitrate 8.93±0.75 mg/L and total ammonia nitrogen 0.14±0.04 mg/L, respectively.

Table 1: Composition of experimental diets (%)

Ingredients (100 g of fed)	Diets (protein/lipid)					
	30/6	30/12	40/6	40/12	50/6	50/12
Fish meal ¹⁾	26.5	26.5	35.3	35.3	30.9	57.4
Extruded soybean ²⁾	28.5	28.5	38.0	38.0	61.8	33.26
Fish oil	0.0	1.64	0.0	0.19	0.0	0.0
Corn oil	1.64	6.0	0.19	0.0	0.0	0.0
Starch	37.36	31.36	20.48	14.48	0.89	0.31
Mineral premix ³⁾	1.0	1.0	1.0	1.0	1.0	1.0
Vitamin premix ⁴⁾	1.0	1.0	1.0	1.0	1.0	1.0
Carboxyl methylcellulose	3.0	3.0	3.0	3.0	3.0	3.0
Chromic oxide	1.0	1.0	1.0	1.0	1.0	1.0
Analyzed (dry basis)						
Crude protein (%)	31.9	31.9	41.7	41.7	51.3	51.3
Fat (%)	6.0	12.10	6.1	12.0	6.3	11.9
Crude fiber (%)	1.2	0.4	0.7	0.7	1.3	0.6
Ashes (%)	10.2	10.4	12.6	12.9	22.6	14.5
NFE (%)	44.6	40.5	33.2	27.9	22.6	14.5
Gross energy (kJ)	1.757	1.927	1.796	1.938	1.848	1.932
Digestible energy (kJ)	1.368	1.537	1.451	1.600	1.542	0.651
Crude protein/gross energy	18.51	20.7	28.7	21.5	27.7	26.6
Digestible protein/digestible energy (mg protein/kJ)	23.3	20.7	28.7	26.06	33.26	31.13
Apparent digestibility of organic matter (%)	99.82	99.84	99.82	99.83	99.83	99.84

1: Fish meal premium grade, Guaymas, Sonora (protein: 56.6%; lipids: 14.6%; carbohydrates: 0.05%; NFE: 5.64%; gross energy: 20.12 kJ/g; digestible energy: 28.4 kJ/g); ²⁾ Extruded soybean (protein: 52.6%; lipids: 1.7%; carbohydrates: 3.5%; NFE: 28.1%; gross energy: 17.91 kJ/g; digestible energy: 6.70 kJ/g); 3: by Merola and Cantelino (1987); 4: by Dickson (1987)

Table 2: Amino acids and fatty acids compositions of the experimental diets

Amino acid (g AA/100 g protein)	Diets					
	30/6	30/12	40/6	40/12	50/6	50/12
Asp	10.17	8.79	9.19	9.39	10.05	10.43
Glu	17.83	15.05	15.06	15.98	16.46	16.86
Ser	4.27	3.62	4.32	4.07	4.35	3.85
His	3.05	2.57	2.77	2.81	2.60	3.13
Gly	7.05	5.87	3.85	5.59	6.22	7.47
Thr	3.76	3.34	5.76	4.28	4.24	3.59
Arg	8.29	6.91	6.74	7.31	7.41	7.65
Ala	6.43	5.49	5.26	5.72	4.99	6.06
Tyr	4.17	3.18	3.15	3.50	5.47	3.83
Met	2.36	2.05	1.90	2.10	1.96	0.57
Val	5.60	4.84	4.68	5.04	5.22	5.98
Phe+ala	5.24	4.45	4.44	4.71	4.95	5.25
Ile	5.54	4.67	4.71	4.98	4.51	5.67
Leu	9.13	7.62	7.69	8.15	7.96	8.95
Lys	8.35	7.07	6.74	7.39	6.88	8.19
Fatty acids (% 100 g)						
13:0	0.00	0.00	0.00	0.00	0.00	0.00
14:0	0.56	1.10	0.40	0.8	0.30	0.60
15:0	0.08	0.20	0.10	0.10	0.00	0.10
16:0	0.04	0.10	1.50	3.10	1.20	2.30
18:0	0.71	0.71	1.40	0.90	0.30	0.70
21:0	0.79	1.60	0.50	1.00	0.40	0.70
23:0	0.0	0.0	0.0	0.0	0.0	0.0
Total saturated	2.18	4.40	2.90	5.90	2.20	4.40
17:1	0.11	0.20	0.10	0.20	0.10	0.10
18:1n-9	0.08	0.20	1.00	2.00	0.30	0.70
18:3	0.46	0.90	0.10	0.20	1.10	2.30
20:1	0.18	0.40	0.10	0.20	0.10	0.10
24:1	0.97	1.90	0.60	1.20	0.40	0.90
Total monoenes	1.80	3.60	1.90	3.80	2.00	4.10
16:1	0.00	0.00	0.40	0.80	0.30	0.60
18:1	0.49	1.00	0.20	0.50	0.90	1.80
18:2-6	1.38	2.80	0.40	0.90	0.50	1.10
20:4	0.12	0.20	0.10	0.20	0.10	0.10
Total polyunsaturated	1.99	4.00	1.10	2.90	1.80	3.60

Experimental diets: Ingredient and nutrient contents of the experimental diets are presented in Table 1. Six practical diets were formulated and prepared to contain three protein levels (30, 40 and 50%, respectively) using a proportion of 50% fish meal and 50% extruded soybean and two lipid levels for each protein (6 and 12%, respectively) with Fish Oil (FO) and/or Corn Oil (CO). In two experimental diets (30/12 and 40/12) were included FO and in all diets different proportions of CO. The gross energy ratio (Protein/Energy) ranged from 18.1 to 27.7; Nitrogen-Free Extracts (NFE) varied from 14.5 to 44.6% in diets (Table 1). The experimental diets contents of amino acids and fatty acids are shown in Table 2.

For diet preparation, ingredients were grounded into fine powder and then mixed in a food mixer (Hobar, Troy, OH, USA) for 30 min. First, dry ingredients and vitamin and minerals mixtures were added and then oil was poured into dry mixture. Subsequently, a previous starch gelatinized was incorporated and 100 mL of water was added to the mixture, which was blended for another 5 min and passed through in a meat grinder having 5 mm.

The pellets were dried in a force air-drying oven for 24 h at 55±5°C. After drying, diets were sieved to obtain proper pellet size and stored at -20°C.

From each experimental diet, 30 g of sample was separated to conduct the proximate chemical analysis according to the methods AOAC (1995). Gross energy was estimated using the following coefficients 23.6 kJ/g for crude protein, 39.5 kJ/g for crude fiber and 17.2 kJ/g for carbohydrates (NRC, 1993). Content of digestible energy in diets was calculated with the digestibility coefficients of 20.9, 35.1 and 11.0 for crude protein, lipids and carbohydrates respectively (Hillestad *et al.*, 1999). Protein/gross Energy (P/E) and crude Protein Digestible/Energy (PD/E) ratios were calculated according to Lupatsch *et al.* (2001, 2002), Skalli *et al.* (2004) and Portz *et al.* (2008). The digestibility of chromic oxide was analyzed according to the methodology described by Furukawa and Tsukahara (1966) and De Silva and Anderson (1995). Apparent Digestibility of Organic Matter (ADOM) was calculated with Eq. (1):

$$ADOM = (100 - (CR_{feed} \times CR_{feces})) \quad (1)$$

where, CR_{feed} and CR_{feces} are Chromic oxide (%) in feed and feces, respectively.

Analysis of 15 Amino Acids (AA) to diets and whole body were effectuated according with the techniques described by Vázquez-Ortiz *et al.* (1995) in a liquid chromatography microprocessor (Varian model 5000) coupled to fluorescent detector with deuterium lamp and connected to Vista 401 data collector. Separation of Essential Amino Acids (EAA) was carried out with a spherical diameter column of 10 cmx4.6 mm I.D. (Microsorb HPLC) and a 30 cmx4.6 mm I.D column (Lichrosorb RP-C18, 5 μ m) was used for determination and separation of non-essential AA.

Extraction of fatty acids in experimental diets and whole body were carried out as described in Folch *et al.* (1957). Fatty acids methyl esters were separated and quantified by gas-liquid chromatography (Hewlett Packard Series 6890 equipped with a flame ionizer detector and self-sampler 6890 with a capacity for 100 vials) using a 30 m x 0.25 mm capillary column (DB-23) with Helium gas as carrier.

Growth performance and fed efficiency: The following indicators were calculated: Final Body Weight (FBW); Specific Growth Rate (SGR in %/day) = 100 x (Ln final weight (g) – Ln initial weight (g) /days of the experiment); Feed Conversion Rate (FCR) = feed intake (g)/weight gain (g); Feed Efficiency (FE) = weight gain (g)/Feed intake (g); Protein Efficiency Ratio (PER) = weight gain (g) /dietary crude protein intake (g); Fulton’s condition index (K) = (body weight/total length³) x100 and survival (% survival transformed to arc sin), according with Ergün *et al.* (2010).

Statistical analysis: Data were subjected to a two-way ANOVA to test the effect of dietary protein and lipids

levels on fish performance. The data were subjected to analyses of variance and multiple comparisons of means by Tukey’s test (Puri and Mullen, 1980) in order to determine significant differences among factors ($p < 0.05$) using the statistical program SPSS Version 17.0 (SPSS, Michigan Avenue, Chicago, IL, USA).

RESULTS

Growth performance: FBW, FE, PER, FCR and K (Table 3) of fingerlings were significantly affected and improved by dietary protein level ($p < 0.05$). A linear correlation was observed between Protein/Energy ratio (P/E) and experimental diets ($Y = 1.6886x + 17.14$; $R^2 = 0.8109$; $p < 0.05$). The highest survival rate (97.1%) was obtained in experimental diet with a 30/12 ratio and lowest (76.5%) in diet having 40/12 at the end of the feeding trial.

The best growth results of FBW and SGR were observed in fish fed with 50% protein and 12% lipid diet displaying a significant difference ($p < 0.05$) with the other diets. Diets 30/12 and 50/12 produced the best productive values in terms of increase of mean of body weight with time. FBW, SGR, PER and K were significantly affected by dietary lipids level ($p < 0.05$). The highest values of FBW, SGR, FE, PER were observed in diet 50/12. The lowest feed conversion ratio was detected in diet 50/12 and the highest in diet 40/12, whereas the highest condition factor (K) was observed in diets 30/6 and 50/6.

Body composition: The results of amino acids and fatty acids in whole body composition are given in Table 4 and total amino acids composition in diets and whole body is showed in Fig. 1.

The pattern of AA content was similar in both cases showing higher values of glutamic acid and arginine in diets and glutamic acid and glycine in whole body.

The fatty acids composition of diets and whole body are shown in Fig. 2. The Total Saturated Fatty Acids

Table 3: Growth performances, feed efficiency and condition factor of bay snook fingerling, in diets containing various protein and levels for 120 days

Dietary protein (%)	Dietary lipid (%)	Final body weight (g/fish)	SGR (%/day)	Feed efficiency	Protein efficiency ratio	Feed conversion ratio	Condition factor
30	6	0.625±0.030 ^a	1.16±0.056 ^{ab}	0.95±0.063 ^a	1.46±0.097 ^b	1.17±0.065 ^a	1.3±0.056 ^c
	12	0.784±0.025 ^b	1.32±0.045 ^b	0.99±0.044 ^{ab}	1.96±0.087 ^c	1.07±0.055 ^a	1.1±0.023 ^{ab}
40	6	0.636±0.044 ^a	1.08±0.077 ^a	0.84±0.085 ^a	1.09±0.111 ^a	1.62±0.230 ^a	1.1±0.017 ^{ab}
	12	0.656±0.050 ^{ab}	1.09±0.099 ^a	0.83±0.096 ^a	1.15±0.132 ^a	2.40±0.629 ^b	1.0±0.037 ^{ab}
50	6	0.627±0.038 ^a	1.05±0.065 ^a	0.81±0.076 ^a	0.84±0.079 ^a	1.46±0.115 ^a	1.2±0.051 ^{bc}
	12	0.928±0.072 ^c	1.57±0.082 ^c	1.21±0.117 ^b	1.51±0.145 ^b	1.03±0.082 ^a	1.1±0.014 ^{ab}
Two-way ANOVA		$p < 0.05$	$p < 0.08$	$p < 0.10$	$p < 0.05$	$p < 0.05$	$p < 0.05$
Protein		$p < 0.05$	$p < 0.05$	$p < 0.32$	$p < 0.05$	$p < 0.67$	$p < 0.05$
Lipid		$p < 0.13$	$p < 0.05$	$p < 0.05$	$p < 0.24$	$p < 0.05$	$p < 0.13$
Interaction		$p < 0.05$	$p < 0.05$	$p < 0.05$	$p < 0.05$	$p < 0.05$	$p < 0.05$

corrected model

Values (mean±S.E. of two replications) in the same column not sharing a common superscript letter are significantly different ($p < 0.05$)

Table 4: Whole body composition of amino acids and fatty acids of bay snook fingerling

Amino acid (g AA/100 g protein)	Diets					
	30/6	30/12	40/6	40/12	50/6	50/12
Asp	8.66	8.93	10.08	10.29	9.52	9.49
Glu	13.78	14.20	15.88	15.23	14.82	14.09
Ser	2.47	2.54	3.07	3.11	2.85	3.00
His	1.50	1.56	2.42	2.08	2.39	2.20
Gly	13.35	13.75	11.07	8.88	10.08	7.55
Thr	5.04	5.19	4.36	5.20	3.88	5.02
Arg	7.77	8.01	7.18	8.53	7.01	6.22
Ala	7.36	7.58	6.63	6.90	6.05	5.62
Tyr	4.01	4.13	4.55	4.79	3.76	4.60
Met	2.26	2.33	2.65	2.72	2.62	2.26
Val	4.74	4.88	4.52	5.18	4.46	4.45
Phe+ala	4.00	4.12	4.26	4.55	4.15	4.25
Ile	5.14	5.58	4.28	5.58	4.57	5.52
Leu	7.70	7.94	7.71	8.54	7.19	8.44
Lys	10.27	10.58	6.64	11.03	8.18	9.81
Fatty acids (% 100 g)						
13:0	0.00	0.20	0.00	0.00	0.00	0.30
14:0	0.38	0.80	0.50	1.00	2.10	0.80
15:0	0.00	0.00	0.00	0.00	0.40	0.00
16:0	1.70	3.00	2.20	4.50	0.00	3.10
18:0	0.71	1.20	0.90	1.80	1.70	1.20
21:0	0.00	0.00	0.00	0.00	0.70	0.00
23:0	0.27	0.50	0.10	0.30	0.00	0.30
Total saturated	3.06	5.70	3.70	7.60	0.30	5.70
17:1	0.00	0.00	0.00	0.00	3.10	0.00
18:1n-9	1.71	0.00	1.30	2.60	0.00	0.00
18:2	0.00	0.60	0.00	0.00	1.70	0.60
18:3	0.00	0.00	0.00	0.00	0.00	0.00
20:1	0.00	0.00	0.00	0.00	0.00	0.00
24:1	0.00	0.00	0.00	0.00	0.00	0.00
Total monoenes	1.71	0.60	1.30	2.60	1.70	0.60
16:1	0.32	0.60	0.40	0.30	0.30	0.60
18:1	0.29	5.10	0.30	0.80	0.30	5.20
18:2n-6	0.57	0.50	0.30	0.60	0.60	0.50
20:4	0.00	0.00	0.00	0.70	0.00	0.00
Total polyunsaturated	1.18	6.20	1.00	0.00	1.20	6.30

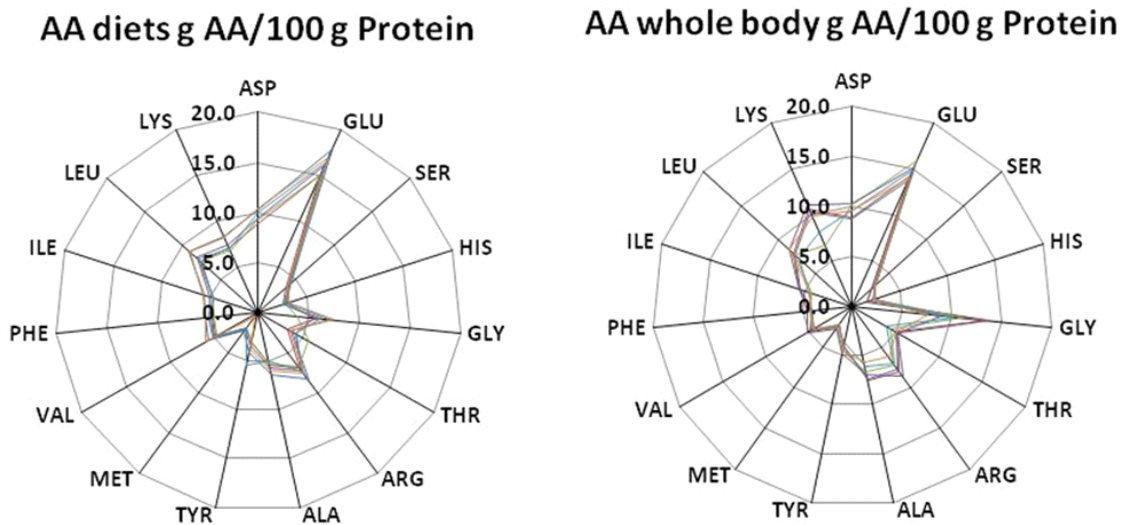


Fig. 1: Amino acids composition of experimental diets and whole body of *P. splendida* fingerling AA: amino acids

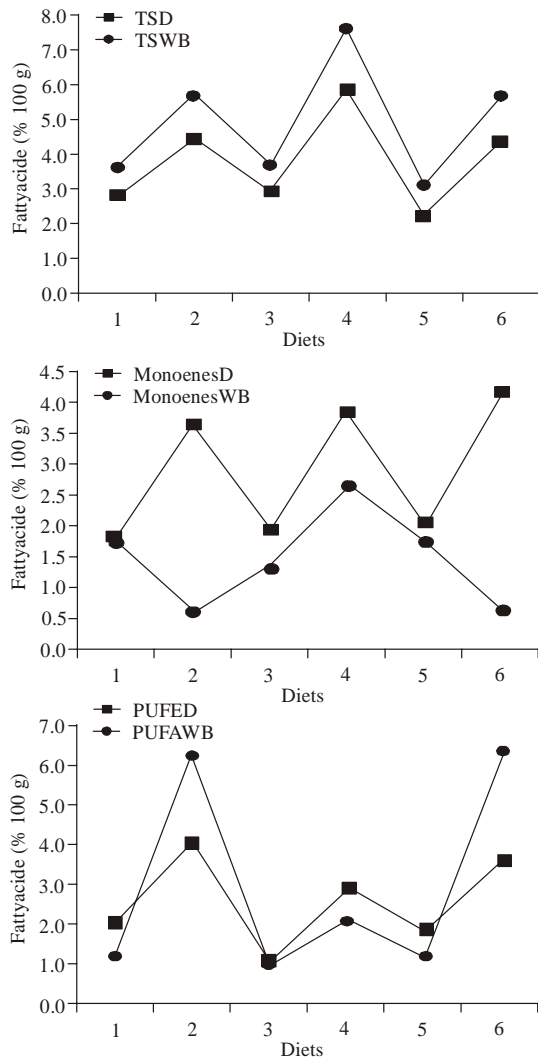


Fig. 2: Average values of Total Saturated fatty acids (TS), Polyunsaturated Fatty Acids (PUFA) and monoenes in experimental Diets (D) and Whole Body (WB) of *P. splendida* fingerling

(TSFA) indicated a similar trend in both cases, but the quantity increased in whole body; the Polyunsaturated Fatty Acids (PUFA) showed that diets 30/12 and 50/12 in whole body presented higher values compared with the other diets. The monoenes presented a dissimilar pattern where diets 30/12 and 50/12 in whole bodies presented lower values.

DISCUSSION

Growth performance and protein and lipids requirement: The bay snook fingerlings maintained a trend of weight increase along the 120 days trial. However, higher increase was observed at 90 days in diets

with 30/12 and 50/12 ratio, being the latter the highest. This experimental diet presented highest values of FBW, SGR, FE and low FCR, showing that fingerling bay snook require a dietary protein level of 50 and 12% of lipids, P/E of 26.6 and digestible crude protein/digestible energy ratio of 31.1 mg protein/kJ. This diet allows for a good growth and high survival rate (76.5 to 94.1%, respectively). This result is in agreement with the optimum P/E ratio for optimum growth of several species of teleost fish that range from 19 to 27 (NRC, 1993). Comparatively, the requirements of fingerlings bay snook are similar to those reported in other carnivorous fishes ranging between 32 to 50% of protein and 7 to 17% of lipids such as in the mangrove red snapper, *Lutjanus argentimaculatus* (Catacutan *et al.*, 2001); freshwater catfish, *Mystus montanus* (Raj *et al.*, 2007); flounder, *Paralichthys olivaceus* (Lee *et al.*, 2008); red snapper, *Pagrus pagrus* (Schuchardt *et al.*, 2008); largemouth bass, *Micropterus salmoides* (Bright *et al.*, 2007), striped bass hybrid, *Morone chrysops* x *M. saxatilis* (Wester *et al.*, 1995); Florida pompano, *Trachinotus carolinus* (Lazo *et al.*, 1998) and red snapper, *Lutjanus campechanus* (Miller *et al.*, 2005b).

The estimation of protein and lipids requirements of fish can be affected by factors such as rearing conditions, stage of growth and sources of protein, but more significantly by dietary energy content based on a quantitative determination (Wilson, 1989). In other species of teleost fishes such as grouper *Epinephelus malabaricus* protein level depend on the energy contained in the diet; the protein requirement can be as high as 50% (18 to 20 MJ/kg). Diets with a P/E from 24 to 24.4 mg/kg but with different protein concentrations displayed dissimilar growth rate in the grouper (Shiau and Lan, 1996; Chen and Tsai, 1994). The different growth response to protein-lipid levels in diets is probably due to dietary non-protein level (De Silva *et al.*, 1991; Company *et al.*, 1999; McGoogan and Gatlin, 1999). The optimum diet for bay snook fingerling is similar to grouper because both are carnivorous. Thoman *et al.* (1999), Chou *et al.* (2001) and Catacutan *et al.* (2001) registered a relationship between the body weight increase and the protein and lipids added to experimental diets, with different species such as red drum *Scianops ocellatus*, cobia *Rachycentron canadum* and mangrove red snapper *Lutjanus argentimaculatus*. For red drum, the apparent consume rate was influenced by fish body weight and not by energy content in diet and is recommended a diet with 44% of protein and 9.2% of lipids. The cobia instead, required a diet with 44.5% of protein and 5.76% of lipids and the mangrove red snapper required 40% of protein and a P/E of 23.3 mg protein/kJ. According with the reported data bay snook fingerling requires high values of protein and lipids and a P/E ratio similar of the previously mentioned species. Lipid indicated protein-sparing effect of lipid agreeing with other studies (Vergara *et al.*, 1996;

Harpaz *et al.*, 1999; Lee *et al.*, 2002) and also was observed a high relationship between P/E and the content of protein and lipids in the different experimental diets. Thus, it is important to provide an adequate level and ratio of dietary protein and lipids in order to reduce catabolism of protein energy. Other studies showing that lipid content of fish fed high-energy diets is higher than that of fish fed low-energy diets (Hillestad and Johnsen, 1994; Catacutan and Coloso, 1995; Peres and Oliva-Teles, 1999; Kim and Lee, 2005). Despite of the origin of protein and lipids resources, high lipid diets especially from CO improved fish performance and feed utilization compared to low lipid diets when proper protein is provided.

Complete analysis of lipid requirement in different development stages is convenient to find out the maximum percentage of dietary lipids without affect immunology respond but achieving an adequate growth and survival rates. Furthermore, the protein and lipids sources must be investigated in order to reduce feed cost without altering growing and survival. The substitution of 50% of fishmeal in this experiment or the use of vegetal oils as CO can be an interesting alternative in other experimental diets.

Amino acid and fatty acids: Deficiency of some EAA does have effects on fish by reducing growth rate, increasing feed conversion ratio, diminishing appetite, provoking mortality and morphological abnormalities, in addition to the presence of nutritional diseases such as lordosis, scoliosis and cataracts (Kloppel and Post, 1975; Poston *et al.*, 1977). The requirements of EAA have been studied in different teleost fishes and varied according with species, feed habits, age and energy content in diet. Ngamsnae *et al.* (1999) estimated the EAA requirements in silver perch *Bidayanus bidayanus* and reported values of 2.7 of arginine, 1.03 of histidine, 1.84 of isoleucine, 3.61 of lysine, 1.50 of methionine, 2.22 of phenylalanine, 2.2 of treonine and 2.9 g/100 g of valine, respectively. The results of the AA content in diets and whole body showed consistent values without wide variations; however, values were higher than those reported for silver perch. The arginine and phenyl alanine requirement were more than two fold higher than those reported in other species such as rainbow trout, salmon and tilapia (Akiyama, 1987; Kaushick *et al.*, 1988; Santiago and Lovell, 1988), therefore meaning high values requirement of EAA for bay snook fingerling diets. Nevertheless, it is noteworthy that high levels of AA should have repercussions in fed ingest, deformities in dorsal spine, cataracts formation and increase of calcium and magnesium levels in liver (Tibaldi *et al.*, 1993; Tulli *et al.*, 1997). In this experiment pathological syndromes no were observed, bay snook fingerlings accepted well experimental diets and waste fed was negligible.

Despite of the recent advances with doses-respond in a wide interval of EAA, further research is needed to know the effects of EAA in fishes. The correct inclusion of EAA to supply nitrogen and the lost implications in the organism must be investigated to precise the requirements of bay snook fingerling towards high quality and low cost commercial feeds including no-conventional protein resources. In this study, two combined sources of protein as fishmeal and soybean extruded showed that served in a wide range the requirements of protein and EAA for bay snook fingerling.

The diet deficiency of fatty acids can affect the immune respond in channel catfish, *Ictalurus punctatus*, rainbow trout, *Oncorhynchus mykiss* and Pacific salmon *Salmo salar* (Fracalossi and Lovell, 1994; Kiron *et al.*, 1995; Thompson *et al.*, 1996). A mix of 1:1 of FO and CO can provide enough fatty acids to prevent the deficiency of any fatty acids that can affect the immune respond. The experimental diets analysis showed that diet 40/12 presented higher values of SFA, especially palmitic acid (16:0) followed by diet 50/12. In contrast, in whole body analysis the results indicated that diet 40/12 presented higher values of palmitic acid, followed by diets 30/12 and 50/12, being in these diets where were observed the higher growth rates.

Previous studies have suggested that some species like Atlantic salmon can grow efficiently when fed diets with high dietary fat and low protein content (Azevedo *et al.*, 2004a, b). A significant difference between the dietary treatments was show on growth due to oil source. Specifically, the replacement of 60% of the dietary FO by Rapeseed Oil (RO) resulted in higher final weight and previous studies on Atlantic salmon have concluded that the partial replacement of FO or other vegetable oils has no negative effect on growth and FCR (Torstensen *et al.*, 2004, 2005; Turchini *et al.*, 2009). In these previous studies, the authors suggested that the increased growth was due to higher digestibility of the vegetable oils, fatty acids, resulting in better utilization of the dietary oil for energy by the fish. The effect on fatty acids digestibility may increase the chain length and unsaturation index (Karalazos *et al.*, 2011).

Both higher oil levels and CO inclusion could also have enhanced the protein sparing effect. Previous studies have also suggested a positive effect of increased dietary lipids content on protein retention and hence, on protein sparing (Karalazos *et al.*, 2007). Information about the effects that dietary vegetable oils inclusion may have on protein sparing and the mechanisms involved is scarce. In this study, the CO inclusion promoted the growth of bay snook fingerling, especially in diets containing 50% of protein, but an interesting effect was observed in diet containing 30% of protein and 12% of lipid from CO and

FO. Thus, it is possible reduce the protein levels combining 50% of fishmeal and 50% of extruded soybean with 12% of lipids of CO, reducing the feed costs in this species.

Some studies had demonstrated that low levels of Saturated Fatty Acids (SFA) are easily digested by fish and the pattern exhibited between the SFA content in experimental diets and whole body, was similar being the diet with 40% of protein and 12% of lipid derivate of CO and FO, which presented higher levels of SFA. The levels of SFA present in vegetables oils such as Rapeseed (RO) and CO have been reported by Karalazos *et al.* (2011). As well, SFA levels in diets influenced the digestibility of PUFA (Menoyo *et al.*, 2003). The same result was detected in the whole body composition where diets 30/12 and 50/12 that presented higher levels of PUFA. Differences of SFA, PUFA and monoenes were observed between the sources of lipids including in experimental diets. The best growth was observed in diet 50/12 where the oil source was only CO. The protein level had a significant effect on the CO diets, presenting higher PUFA values. Taking into account, that SFA and monoenes, largely 16:0 (palmitic acid) and 18:1n-9 (elaidic acid) is preferred substrates for β -oxidation; the significant increase of CO apparently influenced positively the energy utilization and consequently enhanced the growth of fish. It should be noted that CO diets had a much higher (approximately 4-fold) content of 18:1n-9 compared with the other diets.

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