Effect of soybean and tuna gonad in the diet of juvenile bullseye puffer fish *Sphoeroides annulatus*

Efecto de la soya y gonada de atún en la dieta de los juveniles del botete Diana *Sphoeroides annulatus*

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ABSTRACT

The purpose of this study was to evaluate the utilization of different protein ingredients in practical diets for juvenile of bulleseye puffer fish *Sphoeroides annulatus*. A feeding and subsequent digestibility *in vivo* experiments were carried out to evaluate the effect of using tuna gonad (TG), extruded soybean meal (SBM_e), and solvent extracted soybean meal (SBM), as complementary protein sources to fishmeal (FM), in practical diets for puffer fish. Four diets were formulated (50.0% crude protein, 9% crude lipid and 20.1 kJ g^{-1} energy), the control diet (CRL) was elaborated with fishmeal and tuna gonad, the second diet (SBM_e) fishmeal and extruded soybean meal, the third diet (TG-SBM) included FM, tuna gonad and soybean meal, the fourth diet (SBM) fishmeal and soybean meal; a fifth diet, a commercial marine fish feed was used as a reference (REF). The response of juvenile fish (mean initial wt ± SD, 40.5 ± 1.3 g) to experimental diets and REF feed was evaluated by measuring weight gain, specific growth rate (TCE), protein efficiency ratios (PER) and feed convertion ratios (FCR) over a 70 days period. Fish fed TG-SBM diet revealed a better growth performance, however this combination of ingredients was no significantly different to the other experimental diets ($p > 0.05$). The fish fed REF feed showed a lower growth performance than the four experimental diets ($p < 0.05$). SBM and TG were digestible ingredients that were used to replace and, therefore, reduce the level of inclusion of fishmeal in the diet of bullseye puffer fish.

Key words: Digestibility, growth, protein source, soybean meal, tuna gonad.

RESUMEN

El propósito de este estudio fue evaluar la utilización de diferentes ingredientes proteínicos en dietas prácticas para juveniles del botete Diana: *Sphoeroides annulatus*. Se realizaron experimentos de alimentación y digestibilidad *in vivo* para evaluar el efecto del uso de la gónada de atún (TG), la harina de soya extruida (SBMe) y la harina de soya desgrasada (SBM), como proteínas complementarias a la harina de pescado (HP), en dietas prácticas para el botete Diana. Cuatro dietas fueron formuladas (50% PC, 9% LC y 20.1 kJ g^{-1} energía), la dieta control (CRL) fue elaborada con HP y TG, la segunda dieta (SBMe) HP y harina de soya extrudida, la tercera dieta (TG-SBM) incluyó HP, TG y harina de soya, y la cuarta dieta (SBM) se elaboró con HP y harina de soya desgrasada; un alimento comercial para peces marinos (REF) fue utilizado como referencia. La respuesta de los peces (peso promedio inicial 40.5 ± 1.3 g) a las dietas experimentales

y al alimento comercial fue evaluada mediante la tasa de crecimiento especifico (TCE), la tasa de eficencia proteica (PER) y el factor de conversión alimenticia (FCR) por un periodo de 70 días. Los peces alimentados con la dieta TG-SBM revelaron un mejor desempeño productivo, sin embargo no fue diferente a las otras dietas ($p > 0.05$). El alimento REF mostró un rendimiento productivo inferior comparado con los peces alimentados con las dietas experimentales ($p < 0.05$). La harina de soya y la gónada de atún fueron ingredientes digestibles y pueden reducir el nivel de inclusión de la HP en la alimentación del botete Diana.

Palabras clave: Crecimiento, digestibilidad, fuentes proteicas, gónada de atún, harina de soya.

INTRODUCTION

The bullseye puffer fish (*Sphoeroides annulatus* (Jenyns, 1842)) is considered as one of the most promising species for marine aquaculture because of its high market price; thus, it represents an attractive opportunity for the private sector from northwest Mexico. This species is found in the Gulf of California and along the eastern Pacific coast, from San Diego, CA., USA to Peru (Thomson *et al*., 1987) and it has a wide environmental tolerance because it inhabits sandy-shore-estuarine, brackish coastal lagoons and full strength salinity coastal waters. It is primarily carnivorous, feeding on crustaceans (shrimp/crabs), gastropods, bivalves, and squid (Targett, 1975; Thomson *et al*., 1987; Horinouchi *et al*., 1996). Protocols for its reproduction in captivity, egg incubation, larval rearing, juvenile culture and disease control of bullseye puffer fish are already available (Martínez-Palacios *et al*., 2002; Duncan *et al*., 2003; Fajer-Ávila *et al*. 2003; García-Ortega *et al*., 2003; Abdo-de la Parra *et al*., 2010; Rodríguez-Ibarra *et al.,* 2010). The protein and lipids requirements of this species varied between 450-550 g Kg^{-1} and 60-120 g Kg^{-1}, respectively using semi-purified diets (casein and fishmeal), (Abdo-de la Parra *et al*., 2006). Kim and Lee (2009) reported that the dietary protein requirement of juvenile tiger puffer fish was 400-450g Kg^{-1}, and the growth recorded was higher than that in other studies with bullseye puffer fish. Nevertheless, it was found by Kim and Lee (2009) that the tiger puffer fish grew slowly and reached 1 kg body weight in 17-18 months. It has been indicated by preliminary studies performed at Centro de Investigación en Alimentación y Desarrollo, A.C (CIAD). CIAD's facilities that bullseye puffer fish fed with dry feed formulated with fishmeal as main protein source plus fresh ingredients, such as tuna gonad by-product and squid, exhibited better growth than fish fed with a diet using fishmeal as the sole protein source (García-Ortega *et al*., 2002). It would appear that puffer fish have been considered slow growers (Kim & Lee, 2009) compared with the fish of the family Lutjanidae, commonly known as snappers, this specie did reveal a good growth (Benetti *et al*., 2002). Therefore, the commercial cultivation practice in Mexico depends on two factors: a) the proper identification of low cost ingredients available locally and b) the designing diets aimed at improving the growth rate. In response to the above, the availability of an underutilized marine resource, such as tuna by-products, tuna gonad or soybean meal, can be considered economically attractive potential ingredients to be included in a practical diet for feeding juvenile puffer fish during the finishing phase. The study hereby is aimed to utilize the low cost local tuna gonad and soybean meal as complementary protein to fish meal in practical diet for juvenile bullseye puffer fish.

MATERIAL AND METHODS

Experimental ingredients and diets. Based on the commercial availability, chemical composition and the amino acid profiles of the protein ingredients (Table 1). Four isonitrogenous diets (50.0%

Table 1. Chemical composition and amino acid profile of the tested protein ingredients.

Proximate analysis (% in dry matter)	FM[1]	TG[2]	SBMemMmM	SBM
Protein	66.4	63.3	49.7	48.6
Lipid	7.7	15.9	0.4	0.2
Ash	15.7	5.2	6.4	6.4
Amino Acids (g AA per 100 g of protein)				
Arg	6.42	4.81	3.44	3.59
His	3.56	2.15	1.17	1.19
Ile	5.42	2.40	2.72	2.52
Leu	6.66	5.44	3.71	3.68
Lys	6.83	5.39	2.90	3.06
Met	2.44	2.71	0.63	0.65
Phe	3.96	2.28	3.0	2.99
Ala	6.61	5.95	2.13	2.25
Asp	8.57	6.73	5.36	5.36
Glu	13.57	9.49	8.70	8.70
Gly	7.59	1.37	1.92	1.98
Ser	3.27	5.31	2.80	2.82
Thr	4.23	2.68	1.81	1.88
Tyr	3.67	1.37	1.63	1.71
Val	4.31	3.31	2.40	2.46

[1]Obtained from Selecta de Guaymas, S.A de C.V., Guaymas, Sonora, México.
[2]This byproduct is discarded by the tuna fish industry as it does not reach the market weight.

crude protein, 9.0% crude lipid and 20.1 kJ g^{-1} energy) were formulated, a control diet (CRL), a soybean diet (SBMe), a tuna gonad – soybean diet (TG-SBM) and a deffated soybean meal diet (SBM). The control diet (CRL) was selected on the basis of previous good responses obtained with juvenile bullseye puffed fed diets containing a combination of fresh tuna gonad, squid, crab meal and shrimp by-product meal (García-Ortega *et al*., 2002), the second diet (SBM_e) fishmeal and extruded soybean meal, the third diet (TG-SBM) included fishmeal, tuna gonad and soybean meal, the fourth diet (SBM) fishmeal and deffated soybean meal; a fifth commercial feed for marine fish (REF) was included as reference group, which had 49.2% crude protein, 9.4% crude lipids.

The dry ingredients were ground in a hammer mill to a particle size of 250 µm. The macro ingredients were mixed in a Hobart mixer (model AT-200) and the micro ingredients were added thereafter. Fish oil and then water were added until a homogeneous mixture was obtained. The resulting mash was passed through a meat grinder (Tor-rey ® Model 22) to produce pellets. The pellets were dried with forced air at 38 °C for 12 hours. Subsequently, the pellets were manually reduced to a size of approximately 0.5 mm using sieves to remove fine particles. The pellets were stored in labeled, sealed containers and were held at 4 °C until use. Chromic oxide (0.5%) was added as a marker to determine the ADC of protein and lipids.

Experimental conditions, fish and feeding. The experiment was performed with juvenile puffer fish, which were produced at the hatchery facilities located at CIAD, A.C., Mazatlán-Unit. The larvae were obtained by means of inducing a spawning of wild broodstock using LHRHa (Duncan *et al*., 2003) and reared according to the feeding regime for this species (Abdo-de la Parra *et al*., 2010). The fingerlings were weaned gradually as described by García-Ortega *et al*. (2003), and later fed with a control diet (CRL) until an average initial body weight of 40.5 ± 1.3 g (mean ± SD) was reached. A completely randomized experimental design with three replications for each diet (CRL, SBMe, TG-SBM, SBM and REF) was used. Groups of 20 fish were randomly stocked in a system composed of fifteen cylindrical black fiberglass tanks (volume 0.6 m^3), Each of the tanks had a central 50-mm drain covered with a 0.5-cm mesh net to prevent fish escape and to allow for cleaning of the tanks. Each tank had continuous flow-through sea water at a flow rate of 6.5 L min^{-1} and supplemental aeration. Seawater was pumped from the seashore, passed through two parallel sand filters and delivered to four 25 m^3 high density polyethylene (HDPE) head/sedimentation tanks (4 m × 15 m). From the head tanks, the seawater was pumped through a double parallel filtration system consisting of a pressured Jacuzzi sand filter (265 Lpm, 100 µm relative particle retention) and multiple cartridge filters (four 9.3 m^2 cartridge filters, 16 µm relative particle retention) within a filter room (3 m × 5 m) (Alvarez-Lajonchere *et al*., 2007).

Feed was manually supplied three times a day (0800, 1200 and 1600) until the fish were satiated. Uneaten food was collected from the bottom of the tank using a siphon 30 min after the onset of feeding and dried in an oven at 60 °C. Feed intake was calculated as the amount of feed supplied minus the amount of unconsumed feed. Dead fish were recorded and weighed. The daily environmental parameters were measured using a YSI 85-10FT. The dissolved oxygen level was maintained at 6.0 ± 0.51 mg L^{-1}. The water temperature was kept at 23.7 ± 1°C, and the salinity was maintained at 34.5 ± 0.6 g L^{-1}.

The fish were weighed every two weeks to calculate their mean body weight, the biomass present in each tank. The fish were caught with scoop nets and anesthetized with 2-phenoxyethanol (Sigma®) at a concentration of 0.3 ml L^{-1}. Then the specimens were subsequently weighed individually on a digital scale (accurate to ± 0.01 g).

The growth and feed efficiency of the fish were monitored in terms of weight gain (WG), feed intake (FI), feed conversion ratio (FCR), specific growth rate (SGR), survival (S) and protein efficiency ratio (PER).

The biological indicators were calculated as follows:

WG = final mean weight (g) – initial weight (g)

FI = $\sum_i n$ [(total feed consumption (g))/(No. of fish)]/number of days

FCR = feed intake (g)/weight gain (g)

SGR = [Ln (final weight-initial weight)/number of days] × 100

S = (Final number/initial number) × 100

PER = weight gain/protein intake

Chemical analysis. Ten randomly chosen fish were sampled from the initial population to determine the starting carcass composition. To analyze the final composition, two fish were selected at random from each tank for a total sample size of six fish per treatment group. The fish samples, ingredients and diets were homogenized and dried at 105 °C for 24 hours prior to chemical analysis. The determination of the moisture, protein, fat and ash levels in the ingredients, diets and carcasses was conducted according standard methods (AOAC, 1984). Crude protein was determined as N × 6.25 by a Kjeldhal analysis with a Kjeltec 1030 analyser (Foss Tecator AB, Sweden). The fat content was analyzed using a micro Soxhlet. Moisture was determined using a Craft stove, and the ash by calcination of the samples in a muffle (Felissa®).

The amino acid contents of the ingredients and diets were determined using samples (1.0 mg) that had been hydrolyzed with 6 N HCl for 6 h. Sodium thioglycolate was added to the samples to prevent oxidation. The samples of the ingredients and diets

were suspended in sodium citrate buffer (pH 2.2), derivatizated with o-phthaldialdehyde (OPA), and injected (10 µL) into a Varian 9012 HPLC apparatus equipped with a fluorescent detector with a 340-380 nm excitation filter and 460 nm emission filter. Amino acid standards were used and α-aminobutyric acid was added as an internal standard. The solvent flow rate was 1.5 mL/min at 25-29 °C. The amino acids were all eluted within 20 min, and the column was equilibrated for 10 min with the starting solvent (Vázquez-Ortiz *et al.*, 1995). Chromic oxide content of the feed and faecal samples was estimated using the acid digestion technique (Furukawa & Tsukahara, 1966).

Digestibility determination. A subsequent digestibility experiment was carried out after the feeding trial to measure the crude protein apparent digestibility coefficients (ADCs) for each of the TG, SBM, TG-SBM, SBMe experimental diets and commercial feed. The bullseye puffer fish were produced in CIAD (Duncan, 2003). The fish were distributed at stocking density of 15 fish (initial mean body 79 ± 3.1 g) per tank among fiftteen indoor 80-L cylindro conical fecal collection tanks fitted with faeces settling columns. The ambiental conditions were similar to the feeding trial. Each tank was supplied with aerated seawater at a rate of 5 L min^{-1} under natural lighting conditions.

For the first 15 days after stocking, feeding was done daily to apparent satiation at 0900 and 1300 h with the assigned diets to adapt the organisms to feeding and handling practices. One hour after each feeding, the rearing tanks were scrubbed and cleaned to remove uneaten feed and fecal residues. After this adaptation period, the faeces were collected daily at 3-4h between the morning and afternoon feedings. No fish mortality or disease signs occurred during the acclimation and experimental period. Fresh and intact collected faeces were carefully rinsed with distilled water, centrifuged (3200 rpm at 6 °C for 20 min) and frozen daily at –20 °C. After freeze drying, the feaces were analyzed for chromic oxide, dry matter, crude protein, crude lipids, amino acids and energy. Daily fecal samples from each tank were pooled until sufficient weight was available for chemical analysis.

Water parameters during this experiment remained similar to those described in the feeding trial. Apparent digestibility coefficients (ADCs) of protein and lipids of the experimental diets were calculated according to Hardy and Barrows (2002).

ADC nutrient (%) = 100-(% Cr_2O_3 in feed/% Cr_2O_3 in feces) (% nutrient in feces/%nutrient in feed) × 100

Data analysis. Data for each parameter was tested for normality and homoscedasticity. One-way analysis of variance (ANOVA) was performed with diet as the independent variable. Tukey´s test was used as a post-how test to determine significant differences among dietary treatment groups with a significance level of 5% (Zar, 1984). All of the statistical procedures were performed with $\alpha = 0.05$, using the SigmaPlot 12.0 software package.

RESULTS

All experimental diets were close to isonitrogenous (50.7-51.9% CP) and isoenergetic (20-20.4 kJ g^{-1}) (Table 2). The essential amino acid (EAA) profiles of the diets with extrudida or deffated SBM revealed lower levels of methionine, compared to levels in the diet TG-SBM or amino acids profile determined in the muscle content of the *Sphoeroides annulatus* (Table 3).

Juvenile puffer fish responded well to all the experimental diets. Survival was high (88.3-98.3%) in all treatments and did not differ ($p > 0.05$) amongst treatments (Table 4).

The best results for weight gain (WG), feed intake (FI) and specific growth rate (SGR) was the diet (TG-SBM) with inclusion level of SBM and tuna gonad, but this result was not significantly different from that obtained with the diet CRL diet, neither with SBM and SBM_e diets (Table 4). Fish fed the CRL, SBM and SBM_e diets showed a significantly higher growth perfomance and feed utilization than fish fed the commercial feed (REF). The highest protein efficiency ratio was obtained with the TG-SBM diet but this was not significantly different from PER values from the other treatments ($p < 0.05$).

The ADC of protein for the CRL, SBMe, TG-SBM, SBM and REF diets evaluated in the digestibility experiment ranged from 84.6% to 87.9% (Table 4). The SBMe; SBM-TG dietary treatments were significantly higher than the SBM and REF diet. The ADC of lipids for diets was affected ($p < 0.05$) by tuna gonad inclusion.

The final carcass composition varied considerably among treatments (Table 5). Fish fed diet SBM-TG, SBM and CRL had a higher ($p < 0.05$) crude protein content and a lower ash and moisture than those in the other diets. Fish fed diet SBMe and REF diet had similar protein and moisture contents ($p > 0.05$). However, animals fed SBM and REF diets had a higher lipid content ($p < 0.05$) than those in other diets.

DISCUSSION

The results of this study demonstrate that the combination of soybean meal and tuna gonad as protein sources in practical diets for juvenile juvenile bullseye puffer improve the fish performance, chemical composition, and digestibility for protein and lipids.

The bullseye puffer fed TG-SBM diet reached a singnicantly higher growth rate than fish fed on the other experimental diets, these finding indicate that juvenile bullseye puffer can utilize deffated or extruded SBM suplemented with a fresh and palatable ingredient such as squid or TG, without detriment to short-term growth.

Methionine is often the limiting amino acid in SBM ingredient (Hertrampf & Piedad-Pascual, 2000), and methionine deficiencies in soy products have been observed in other studies (Chong *et*

Table 2. Ingredient and chemical composition of the experimental diets.

	Diet			
Ingredients (g Kg^{-1} wet.wt.)	TG	SBMe	TG-SBM	SBM
FM sardine	332	424	318	424
TG	157	—	158	—
SBM_e	—	252	—	—
SBM	—	—	197	246
Squid	161	—	—	—
Fish oil sardine	1.2	14	12.8	28.2
Crab byproduct meal	20	20	20	20
Shrimp byproduct meal	150	188	188	188
Squid liver meal	20	20	20	20
Dextrine	91.8	15	19.2	6.8
Soy lecithin	10	10	10	10
Vitamin premix	15	15	15	15
Mineral premix	15	15	15	15
Vitamin C L-ascorbyl-2-polyphosphate 25% Active	2	2	2	2
Carboximetilcelulosa	20	20	20	20
Chromic oxide	5	5	5	5
Chemical composition (% DM)				
Crude protein	51.9	50.7	51.7	51.3
Crude lipid	8.9	9.5	9.7	9.3
Ash	10.8	12.4	12.2	12.8
NFE*	28.4	27.2	26.4	26.6
GE (MJ 100 g^{-1})**	20.0	20.4	20.1	20.3

NFE*= (crude protein + crude lipid + ash).
GE**= Gross energy (MJ 100 g^{-1}) calculated using the factor 2.34 MJ 100 g^{-1} , 3.92 MJ 100 g^{-1}, and 1.72 100 g^{-1} protein, fat and carbohidrate, respectively (Goddard 1996).

Table 3. Amino Acid composition of experimental diets (g AA per 100 g of protein).

Aminoacids (%/100 g of protein)		Diets		
	Muscle[1]	CRL	SBMe	TG-SBM	SBM
Alanine	*2.93*	3.67	3.97	4.52	3.97
Arginine*	*1.72*	3.32	4.38	4.57	4.38
Aspartic acid	*4.79*	4.51	6.41	6.62	6.40
Glutamic acid	*6.89*	6.86	10.26	10.28	10.25
Glycine	*2.76*	2.86	3.79	3.21	3.79
Histidine*	*0.95*	1.71	2.60	2.54	2.59
Isoleucine*	*1.96*	2.10	3.21	3.07	3.21
Leucine*	*3.56*	3.56	4.59	4.88	4.58
Lysine*	*3.77*	3.60	4.40	4.72	4.39
Methionine*	*1.52*	1.48	1.35	1.70	1.34
Phenylalanine*	*1.46*	1.88	3.23	3.06	3.22
Serine	*2.21*	2.40	2.85	2.73	2.84
Threonine*	*1.99*	2.07	2.68	2.31	2.67
Tyrosine	*2.00*	1.56	2.28	1.88	2.28
Valine*	*2.15*	2.35	3.21	2.80	3.21

[1] = Mean amount of amino acid in muscle of *S. annulatus*. * = Essential amino acids. Tryptophan was not determined.

Table 4. Growth performance and feed utilization of juvenile bullseye puffer fed four practical diets and a REF diet during a 70 days trial (n = 3).

Experimental diets					
Parameter	CRL	SBMe	TG-SBM	SBM	REF
Final weight (g)	78.7 ± 7.6^{ab}	80.2 ± 1.5^{ab}	82.6 ± 2.5^{a}	75.1 ± 2.7^{b}	57.7 ± 3.1^{c}
SGR (% d^{-1})	0.94 ± 0.1^{a}	0.97 ± 0.03^{a}	0.98 ± 0.04^{a}	0.88 ± 0.05^{a}	0.50 ± 0.08^{b}
FI (g fish^{-1})	83.6 ± 5.9^{a}	87.8 ± 1.9^{a}	81.5 ± 2.7^{a}	78.8 ± 5.8^{a}	45.9 ± 4.8^{b}
FCR	2.2 ± 0.4^{a}	2.2 ± 0.1^{a}	1.9 ± 0.1^{a}	2.3 ± 0.1^{a}	2.7 ± .6^{b}
PER	0.92 ± 0.3	0.89 ± 0.06	1.0 ± 0.06	0.86 ± 0.1	0.77 ± 0.19
ADC protein (%)	86.6 ± 0.4^{a}	87.5 ± 0.2^{a}	87.9 ± 0.4^{a}	85.4 ± 0.01^{b}	85.3 ± 0.2^{b}
ADC lipid (%)	96.4 ± 0.6^{a}	84.8 ± 0.8^{b}	96.2 ± 0.2^{a}	80.1 ± 0.1^{c}	84.1 ± 0.1^{b}
Survival (%)	93.3 ± 7.6^{a}	98.3 ± 2.9^{a}	96.7 ± 2.9^{a}	96.7 ± 5.8^{a}	88.3 ± 5.8^{a}

Mean values ± standard deviation in the same row with different superscript are significantly different ($p < 0.05$).

Table 5. Carcass composition of puffer fish *S. annulatus* fed experimental diets.

Parameter[1]	Initial	Final				
		CRL	SBMe	TG-SBM	SBM	REF
Moisture (%)	77.6 ± 0.3	72.7 ± 0.3^{b}	74.7 ± 0.9^{a}	72.1 ± 0.1^{b}	71.74 ± 0.7^{b}	73.4 ± 0.3^{b}
Protein (%)	14.0 ± 0.3	17.6 ± 0.6^{a}	16.3 ± 0.3^{b}	18.2 ± 0.3^{a}	17.7 ± 0.5^{a}	16. ± 0.7^{b}
Lipid (%)	6.6 ± 0.1	7.4 ± 0.1^{b}	7.0 ± 0.1^{b}	7.6 ± 0.4^{b}	8.6 ± 0.1^{a}	8.8 ± 0.1^{a}
Ash (%)	1.8 ± 0.1	2.3 ± 0.1^{a}	2.0 ± 0.03^{bc}	2.1 ± 0.1^{b}	2.0 ± 0.2^{bc}	1.8 ± 0.0^{c}

Mean values ± standard deviation in the same row with different superscript are significantly different ($p < 0.05$).

al., 2003; Chou *et al.*, 2004). In this study, supplementing the diets containing SBM with tuna gonad did prevent lower weight gain compared to fish fed the SBM diet, suggesting that the combination of both ingredients could meet the amino acid requirements of bullseye puffer.

The inclusion level of 20% in the present study seems to be appropriate; there were no differences in the feed consumption among experimental dietary treatments. Similar level of SBM supplemented with mussels was reported for tiger puffer *Takifugu rubripes* (Temminck & Schlegel, 1850) (Ukawa *et al.*, 1996; Kikuchi & Furuta, 2009). It has been demonstrated by the aceptable growth performance observed in the fish fed with a TG-SBM diet that soybean meal protein can be utilized for growth, as long as the diet is attractive enough to be consumed by the bullseye puffer fish. The effects of possible combinations of protein sources on fish performances have also been recently investigated (Aoki *et al.*, 1998). Fish grew better with a combination of a vegetable and animal protein combination.

Thus, it is suggested by these results that a more balanced amino acid composition of TG-SBM, compared to other experimental dietary treatments, is an effective ingredient to adjust the essential amino acids profile required by this carnivorous species; additionally, the growth and feed utilization improved.

In agreement with the present results, it has been recently reported by Kikuchi and Furuta (2009) that the tiger puffer fish fed with SBM-based diets supplemented with increasing levels of blue mussels extracts did not reveal any differences in growth between control and experimental diets, with diets containing up to 20% of the deffated SBM. It was stated by the author that the inclusion level of mussel extracts had a positive effect on feed consumption and growth that was corroborated in our study with the inclusion of SBM and Tuna gonad by-products.

On the other hand, the FM inclusion in the diets performed in this study was in the order of 32%; nevertheless, the best growth was obtained with the protein fraction of the TG-SBM diet and the best lipid digestibility was revealed by this diet, probably due to the optimum content of highly unsaturated fatty acids (HUFA) fatty acids of n-3 series, in contrast to the SBM diet, which was less digestible and, as a consequence, probably less efficient due to an n-3 series fatty acids defficiency. It could be infered, that the high FM inclusion level in the diet of this species is crucial in order to meet the aminoacids and trace elements requirements for this carnivorous species because, in studies performed on the Japanese species globe fish, *Takifugu rubripes*, the high FM inclusion level used in experimental diets is quite remarkable and, as a result, improved growth rates have been reported (SGR of 2.5% d^{-1})

(Kim & Lee, 2009). It was revealed by the digestibility determinations that TG-SBM may be a good mixture of protein sources for *Sphoeroides annulatus*, given that the digestible protein of this diet was correlated to growth and feed utilization; according to Dias *et al.* (2005), the nutritional value of a protein feedstuff is primarily based on its essential amino acids content and bioavailability and it is strongly related with growth. All dietary treatments had crude protein digestibility values greater than 85%, which is in line with Robaina *et al.* (1995), who published similar protein digestibility (>80%) for gilthead seabream *Sparus aurata* fed with soybean-based diets. Similarly, Berge *et al.* (1999), and Day *et al.* (2000) found comparable nitrogen digestibility values (>80%) for Atlantic halibut and turbot fed diet with soybean protein concentrate. Likewise, Kaushik *et al.* (2004) published superior results (>90%) of protein digestibility for European seabass fed diets with plant protein. It was shown by the values of diets lipid digestibility that the long chain highly unsaturated fatty acids composition, which are esential in the diet of puffer fish (Kanazawa *et al.*, 1979), was improved by the TG inclusion. A high value of fatty acids content series 20:5n-6 EPA = 14.83 %; 22:6n-3 DHA = 9.65% of total fat was revealed by the analysis previously determined in the lab located at CIAD's facilities.

Nevertheless, further research is required to obtain optimal growth of puffer fish, based upon the assessment of specific nutrients through ingredients digestibility and amino acids availability. The ability of the animal to digest and utilize nutrients from a conventional mixture of ingredients is demonstrated by the results obtained in the study; although, the slow growth revealed by the species makes it necessary to look forward to partially mitigate the high costs of feeding and long term profitable cultivation of bullseye puffer fish.

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