Use of different types of soybean meal as alternative protein sources for juvenile Pacific bluefin tuna, *Thunnus orientalis*

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The Pacific bluefin tuna (PBT) is a highly prized fish in demand all over the world.[1] Demand for PBT has increased sharply and is being fuelled by advances in cage culture technology of this species.[2] At present, the cage culture of PBT depends entirely on wild caught juveniles, however the supply and availability of wild juveniles has been steadily declining over the last few decades posing serious problems for the future sustainability of the PBT industry.[3,4] In order to sustain the development of this industry it is necessary to improve the technologies supporting production of hatchery reared seedlings. Of major importance is development of artificial diets for seedlings that provide adequate nutrition for growth and development. Therefore, we recently conducted a series of experiments in which we successfully formulated diets for juvenile PBT using enzyme treated fish meal (EFM).[5,6] We have also shown that EFM can be replaced by 20% soybean meal (Biswas et al. unpublished data). These encouraging results drove our laboratory to investigate the use of other soybean meal products in diets for juvenile PBT. Therefore, the aim of this study was to verify whether soybean meal (SM), soya protein concentrates (SPC) or full fat soybean meal(FFS) could be used to partially EFM in the diet of juvenile PBT without hampering their growth performance.

Materials and methods

Four experimental diets were formulated using EFM; a control diet in which EFM was the primary protein source and 3 other diets that replaced 20% of the EFM in the control diet with the same amount of SM, SPC or FFS, respectively (Table 1). These diets were fed in triplicate to 25 day old PBT (stocking weight 0.49 g) to apparent satiety, 6 times daily (05:30, 08:00, 11:00, 14:00, 1600 and 18:00 h) for 12 days under continuous light. Each of the experiment tanks (15 m^3) was stocked with 300 juvenile PBT and each tank was provided with filtered seawater that was introduced at a flow rate of 30 l min^{-1} during the feeding trial.

The temperature and DO of the experiment tanks was maintained around 27.5°C and 6.5 mg l^{-1}, respectively. Initial and final fish samples were taken for comparisons of whole body proximate composition. All samples were kept at -80°C until analyzed. Data were analyzed by one-way analysis of variance

Table 1. Dietary formula and proxumate composition of experimental diets

	Diets			
	EFM	SM	SPC	FFS
Fish meal	65.84	45.84	40.84	49.84
Soybean meal	0.00	20.00	20.00	20.00
Fish oil	8.00	8.00	13.00	4.00
α- starch	8.00	8.00	8.00	8.00
Vitamin mixture [1]	5.00	5.00	5.00	5.00
Mineral mixture [2]	5.00	5.00	5.00	5.00
Soybean lecithin	1.50	1.50	1.50	1.50
Cellulose	4.00	4.00	4.00	4.00
Wheat gluten	2.50	2.50	2.50	2.50
APM (ppm)	1200	1200	1200	1200
Vitamin E (ppm)	400	400	400	400
Proximate analysis (% of dry matter basis)				
Crude protein	57.77	50.75	57.58	52.46
Crude lipid	16.30	14.61	18.94	14.98
Crude ash	8.15	7.98	6.32	8.09
Crude sugar	10.99	16.96	11.53	15.53
Energy (kJ/g)	22.89	22.55	23.61	22.30

[1] Halver, 1957 (without AsA)
[2] Halver, 1957

(ANOVA) using the Statistical Package for the Social Sciences (SPSS) program for Windows (v. 12.0, Chicago, IL, USA). Data were expressed as the means ± standard error of mean (SE) of triplicates. When the factor was detected to be significant, the means among the treatments were compared using Tukey's test of multiple comparison with a 95% level of significance.

Results and discussion

The growth performance of juvenile PBT fed the four experimental diets is presented in Table 2. Final mean body weight was significantly higher in PBT fed the control diet (EFM), similar for PBT fed the SPC and SM diets and lowest in PBT fed the FFS diet. A similar statistical response was recorded for relative weight gain (%) and specific growth rate (SGR; Table 2).

There was no significant difference between the feed intake of PBT fed the EFM, SM or SPC diets; however feed intake on the FSS diet was significantly lower. Feed efficiency was best in PBT juveniles fed the control

Table 2. Growth performance of fish fed the experimental diets

Parameters	Initial	Diets			
		EFM	SM	SPC	FFS
Initial body weight (g)	0.49				
Initial body length (cm)	3.56				
Final body weight (g)		3.0±0.0^a	2.5±0.1^b	2.6±0.1^b	1.9±0.2^c
Final body length (cm)		6.6±0.3^a	6.2±0.1^b	6.2±0.1^b	5.6±0.1^c
Weight gain (%)		389.1±12.3^a	308.8±1.3^b	333.9±14.5^b	129.2±30.3^c
SGR (%)		15.2±0.1^a	13.6±0.2^b	14.1±0.2^b	11.2±0.7^c
Feed consumed (g)*		478.7±41.2^a	460.8±21.5^a	510.2±10.7^a	287.1±14.8^b
FCE (%)*		91.5±1.3^a	77.1±2.0^b	74.3±0.8^{ab}	41.3±7.6^c
CF		1.1±0.0	1.1±0.0	1.1±0.0	1.1±0.0
Survival rate (%)		56.7±4.2^a	57.4±1.9^a	60.1±3.3^a	28.0±6.1^b

Values in a raw with different letters are significantly different (P <0.05).
* Dry basis

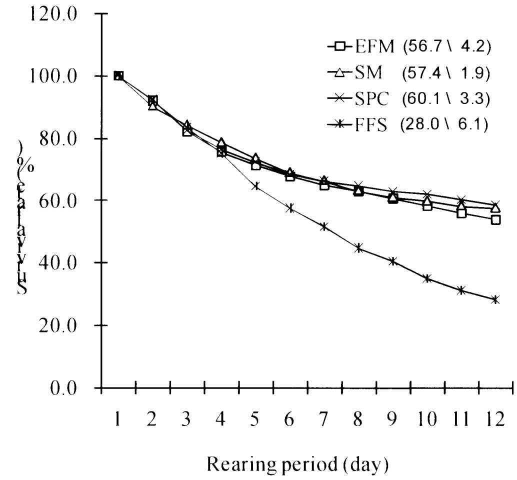

Fig. 1. Changes in survival rate of Pacific bluefin tuna (PBT) *Thunnus orientalis* juvenile fed the different test diets. Data are expressed as the mean of the results of duplicate tanks, and values indicate means±SE (n=2).

diet (EFM) and worst in PBT fed the dietcontaining FFS (Table 2). Although the condition factor was similar among the experimental groups, the survival rate for PBT fed the FFS diet was significantly lower (Fig. 1). The whole body proximate composition of PBT sampled at the beginning and end of the feeding trial is presented in Table 3, however only data on the harvest composition of PBT has been compared. A similar moisture content was observed among the treatments, however; lipid content was significantly higher (P<0.05) in PBT fed diet EFM, SM and SPC than diet FFS. Protein

Table 3. Carcass proximate composition

Ingredients	Initial	EFM	SM	SPC	FFS
Moisture (%)	79.8±0.2	81.0±0.3[a]	80.7±0.8[ab]	79.4±0.5[b]	80.4±0.5[ab]
Crude protein (%)	13.1±0.2	13.2±0.3[c]	13.9±0.2[b]	14.7±0.5[a]	14.0±0.4[b]
Crude lipid (%)	1.2±0.1	1.9±0.1[a]	1.9±0.1[a]	1.9±0.1[a]	1.4±0.1[b]
Crude ash (%)	3.0±0.1	3.3±0.2[a]	3.4±0.2[a]	3.0±0.1[b]	3.5±0.1[a]

Values in a raw with different letters are significantly different ($P<0.05$).

content was significantly higher ($P<0.05$) in PBT fed the SPC diet compared to PBT fed the other dietary treatments. Crude ash content was significantly lower in fish fed the SPC diet compared with other dietary treatments.

Results from this feeding trial demonstrated that the majority of measured growth parameters were significantly lower in PBT fed the FFS diet compared to PBT reared on the fishmeal control (EFM) or diets containing 20% SM or SPC, respectively. The reasons for the poor performance of PBT reared on the diet containing 20% FSS were not identified in this study, but it could be related to the presence of anti-nutritional factors in FFS which might have inhibited the digestion and absorption of nutrients or affected palatability of this diet. A similar observation was reported in Atlantic salmon and rainbow trout.[8-10] On the other hand, the diets containing 20% SM or SPC promoted better growth and feed efficiency. Both SM and SPC are processed in different ways using specialized production techniques.

These processing techniques have likely removed or deactivated anti-nutritional factors and or reduced the level of soluble carbohydrates and fiber in these products. In addition, the protein content of SPC is much higher than that of SM and FFS and it

is reported to have a relatively balanced amino acid profile.[11,12]

Fish fed on the SPC diet had the highest protein content in their carcass. Encouraging results with survival and condition factor indicate potential for further research with protein substitutes such as SPC and SM in diets for juvenile Pacific bluefin tuna.

Acknowledgements

This study was supported by the Global COE program of the Ministry of Education, Culture, Sport, Science and Technology, Japan.

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