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Assessment of Soybean Meal as Dietary Fishmeal Replacement in Red Sea Bream (*Pagrus Major*) Juveniles Based on Energy Budget Analysis

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Abstract

Ophirtus Sumule, Agung Sudaryono, Manabu Ishikawa, and Shunsuke Koshio. 2017. Assesment of soybean meal as dietary fishmeal replacement in red sea bream (*Pagrus major)* juveniles based on energy budget analysis. *Aquacultura Indonesiana, 18 (1): 30-40*. The effects of soybean meal (SBM) on the energy budget of red sea bream *Pagrus major* juveniles (3.2 g initial wet weight) were determined by supplementing SBM in the diet at 0% (control diet), 16%, 24%, 32% and 39%, with the fishmeal content correspondingly reduced from 55% to 29%. Diets were made isoenergetic and isonitrogenous by changing the lipid and carbohydrate levels. Fish were fed to apparent satiation for 30 days in duplicates per diet (20 fish per replicate). Ammonia excretion and oxygen consumption were continuously measured during the growth trial, whereas digestibility after the termination of growth trial. Feed intake, body weight gain, and specific growth rate of fish increased to a peak at 24% SBM level, which again decreased as the SBM level was further increased. The apparent digestibility of energy was similar in all dietary treatments, while the digestible of dry matter increased with the SBM level. A lower proportion of energy intake as growth at 39% SBM level was attributed to the higher energy intake channeled to fecal ammonia. This study suggested that the inclusion level of SBM in diets for red sea bream juveniles should be is optimal at the inclusion range 24–32%, thus correspondingly replacing the fishmeal content by 24–32%.

***Keywords**:* Ammonia excretion; Feed intake; Growth; Metabolic rate; *Pagrus major* juvenile Red sea bream; Soybean meal (SBM).

Introduction

Catches of sardines and other species, which are used for fishmeal (FM), have declined in the last decades and, as a consequence, the price of FM has tremendously increased (Aoki, 1996; Watanabe *et al*., 1998; Kikuchi, 1999). Controlling the over-exploitation of the FM species, which are a natural resource, to ensure the reliability of supply is almost impossible. Thus, it is important to look for alternative protein sources to reduce the use of dietary FM in the development of cost-effective aquaculture feeds. Soybean meal (SBM) products are alternative ingredients for replacing FM due to their relatively high protein content, stable supply and generally lower price (Suprayudi *et al.*, 1999; Lovell, 1989; Wilson and Poe, 1985).

In the past, SBM had limited usage as a feed ingredient. Besides limiting in lysine and methionine (Hertrampf and Pascual, 2000), SBM is known to contain certain anti-nutritional factors such as trypsin inhibitors, oligosaccharides, phytoestrogens, allergens, lectins, and antigens that could have a potential negative effect on the performance of fish (NRC, 1993; Wilson and Poe, 1985; Arndt *et al*., 1999). However, further processing treatment of raw SBM by pressing, heating, or treatment with aqueous alcohol, solvent or enzyme have shown to reduce the level of some of the anti-nutritional factors (Kaushik *et al.*, 1995; Van den Ingh, *et al.*,1996; Mustakas *et al*., 1970; Bjork and Asp, 1983). In recent years, improvement of the processing technology, such as twin extrusion, the antigen in defatted SBM could be reduced to 0.4% (40 unit 10 mg-1) of the original activity of 11249 unit 10 mg-1 (Ohisi *et al*., 1994). This twin extruded SBM product has a better digestibility of nutrients than the defatted-only SBM on pig and sheep (Ohisi *et al*., 1995). Saitoh *et al*., (2000) have also demonstrated that twin extruded SBM could reduce about 55% of FM in kuruma shrimp (*Marsupenaeus japonicus*) diet without retardation effects on growth.

It has been reported that about 25% of defatted SBM can be added, while reducing FM, in red sea bream juvenile diet without a growth retardation (Ukawa *et al*., 1994; Aoki *et al*., 1996; Aoki *et al*., 1999). However, the energy budget of red sea bream juveniles fed diets containing SBM remains. Such information is important not only to better understand the energy flow in fish fed SBM diet, but also to provide information for reasonable

aquaculture practices with a minimum waste production by fish.

Energy budget of fish could be constructed by an input-output model equating the energy intake with the energy allocated for metabolism, growth and excretion. The energy intake can be measured by the total energy content of the ingested food, which is partitioned into several components to sustain various physiological and metabolic processes, including growth. Hence, an energy budget can be constructed by equating the energy intake (EI) with the various components of the energy allocation: EI = F + M + U + G, where F is energy loss as feces, M as metabolism, U as non-fecal matters and G is energy for growth (Jobling, 1994). Thus, the decrement in growth due to the presence of SBM in the diet is likely reflected in each or a combination of these variables.

In the present study, various percentages of SBM were incorporated in a formulated red sea bream juvenile diets, while the level of fish meal was reduced from 54% to 29%. The effects of different levels of SBM on red sea bream juveniles were evaluated by an energy budget analysis.

Material and Method

Test diets

SBM used in this study was produced by twin extrusion process, as reported by Saito *et al*. (2001). Five test diets containing 0% SBM (control), 16% (SBM16), 24% (SBM24), 32% (SBM32), and 39% (SBM39) were formulated by replacing the FM. Diets were correspondingly adjusted to become isoenergetic on gross energy basis and isonitrogenous by changing the lipid and carbohydrate contents. Test diets were produced according to the methods of Sakakura *et al*. (1998), with a slight modification. Dry ingredients and lipids with fat-soluble vitamins were well mixed with distilled water using a food mixer (Model KMS, Kitchen Aid Inc., St. Joseph, Michigan, USA) and then extruded using a meat grinder (type 22VR-1500, Royal, Japan) with 1.5 mm diameter diameter. Diets were dried in an oven at 70°C for 1 h, and were stored in a refrigerator at -28°C until use. Diet formulation and proximate composition c are listed in Table 1, whereas amino acid composition is presented in Table 2.

Table 1. Compositions (g per kg dry diet) and proximate analyses (dry basis) of test diets.

Ingredients	Dietary treatment				
	Control	SBM 16	SBM 24	SBM 32	SBM 39
Fish meal[b]	550	450	390	340	290
Soybean meal [c]	-	160	240	320	390
Squid liver oil	50	59	64	68	72
Dextrin	57	33	22	10	-
α Cellulose	103	58	44	22	8
Squid powder	60	60	60	60	60
Krill meal	40	40	40	40	40
Soybean lecithin	30	30	30	30	30
Vitamin mix[d]	30	30	30	30	30
Mineral mix[e]	30	30	30	30	30
APM[f]	0.5	0.5	0.5	0.5	0.5
Gluten	50	50	50	50	50
Proximate composition					
Crude protein (%)	52.8	52.9	53.9	53.9	53.5
Crude lipid (%)	15.5	15.1	15.3	15.4	15.7
Crude ash (%)	10.6	10.7	10.7	10.5	10.2
Moisture (%)	7.2	6.7	7.9	6.2	7.6
Gross energy (cal/g)	5281	5282	5263	5294	5257

[a] Control, SBM16, SBM24, SBM32, SBM39 were diet containing no soyben meal 16% soybean meal, 24% soybean meal, 32% esoybean meal and 39 % soy bean meal.
[b] Mackerel meal (Nippon Suisan Ltd.)
[c] Twin-screw extruded soybean meal (Honen Corporation Ltd.)
[d] (mg/100g dry diet) □-carotene, 9.5; vitamin D_3, 1.0; inositol, 379; menadione-$NaHSO^3$, 4.5; □-tocopherol, 38; thiamin-HNO_3, 5.7; riboflavin, 19; pyridoxine-HCl, 4.5; cyanocobalamine, 0.01 ; cellulose, 189; biotin, 0.6; nicotinic acid, 76; D-pantothenate-Ca, 27; folic acid, 1.4; choline chloride, 774; ρ-amino benzoic acid, 38
[e] (mg/100 mg dry diet) : $MgSO_4$, 380; Na_2HSO_4, 242; K_2HSO_4, 665; Fe citrate, 14; Ca lactate, 907; $Al(OH)_3$, 0.2; $ZnSO_4$, 10; $CuSO_4$, 0.3; $MnSO_4$, 2.2; $Ca(IO_3)_2$, 0.4; $CoSO_4$, 2.77
[f] Ascorbyl-2-phosphate-Mg

Table 2. Compositions (g per kg dry diet) and proximate analyses (dry basis) of test diets.

Amino acid	SBM 0	SBM 16	SBM 24	SBM 32	SBM 39
EAA[2]					
Threonine	4.3 ±0.0	4.2 ±0.0	4.2±0.0	4.2 ±0.0	4.2 ±0.0
Valine	4.3±0.1	5.0±0.4	5.2 ±0.1	4.6 ±0.1	4.7 ±0.5
Methionine	2.0±0.5	2.0±0.0	1.8 ±0.1	1.7 ±0.1	1.8±0.1
Isoleucine	4.4 ±0.2	4.8 ±0.2	5.7 ±1.2	4.8 ±0.1	4.5 ±0.0
Leucine	7.7 ±0.1	8.0±0.2	7.5 ±0.7	7.2 ±1.2	7.8 ±0.1
Phenylalanine	4.1 ±0.1	4.5 ±0.1	4.4 ±0.2	4.7 ±0.0	4.5 ±0.2
Histidine	4.4 ±0.3	3.7 ±0.0	3.7 ±0.1	3.6 ±0.0	3.7 ±0.1
Lysine	9.6 ±0.5	9.1 ±0.3	8.8 ±0.2	8.9 ±0.3	8.7 ±0.2
Tryptophan	Tr[3]	tr	tr	tr	tr
Arginine	7.4 ±0.9	6.5 ±0.0	6.5 ±0.1	6.8 ±0.1	6.9 ±0.2
∑ EAA	48.2	47.8	47.8	46.5	46.8
NEAA[4]					
Taurine	1.3 ±0.0	1.1 ±0.0	1.0 ±0.0	1.0±0.0	0.9 ±0.0
Hydroxide Proline	0.9 ±0.1	0.6 ±0.0	0.5 ±0.0	0.5 ±0.0	0.5 ±0.0
Aspartic acid	8.3 ±0.2	8.7 ±0.1	8.9 ±0.1	9.1 ±0.0	9.2 ±0.1
Serine	3.8 ±0.2	3.7 ±0.1	3.9 ±0.0	4.0±0.0	4.3 ±0.0
Glutamic acid	16.6 ±0.1	17.8 ±0.0	18.2 ±0.1	18.9 ±0.0	19.0 ±0.1
Proline	5.4 ±0.2	5.3 ±0.2	5.5 ±0.1	5.7 ±0.1	5.7 ±0.1
Glicine	6.2 ±0.7	5.4 ±0.0	5.2 ±0.0	5.0±0.1	5.00 ±0.0
Alanine	6.0±0.2	5.7 ±0.4	5.3 ±0.0	5.5 ±0.5	5.0±0.0
Tyrosine	3.5 ±0.3	4.0 ±0.3	3.8 ±0.2	4.1 ±0.5	3.9 ±0.4
∑ NEAA	52	52.3	52.3	53.8	53.5
TOTAL	100	100	100	100	100

1mean s.d (n : 2); 2Essential amino acid; 3tr = trace; 4Non essential amino acid

Experiment I : Feeding trial

Red sea bream juveniles (3.2 g mean wet weight, 92 days after hatching) were randomly selected from the stock tank and weighed five fish at a time. Twenty fish were stocked in a 30-L polycarbonate tank (duplicate per treatment), each tank containing 20-L filtered seawater (33 psu, 29+2 °C) with aeration for 30 days. Filtered seawater was supplied in a flow-through system at a rate of 1.5 L min-1. Experimental fish were fed test diets to apparent satiation twice per day. Feed intake (FI) per fish was calculated as food given / number of fish in tank . At the end of the feeding trial, all fish were individually weighed 12 hours after the last feeding, and 6–10 fish from each tank were randomly sampled for the proximate composition analysis.

Daily dry weight of fish was back estimated using the specific growth rate (SGR, %): SGR = 100 [ln Wt – ln Wo)/t], where Wt is the average dry weight of fish at day 30 of the feeding trial, Wo is the average initial dry weight, t is the duration of experiment in days. The estimated dry weights of fish (W) were related with the feed intake (FI) following the equation: FI = aWb, which was linearized by logarithmic transformation such that a and b were derived as the intercept and slope of the lines. The exponent b expresses the tendency of food consumption of juvenile fish. Additional parameters, such as weight gain, feed conversion efficiency and protein efficiency ratio and survival rate, were used to evaluate the growth performance of fish.

Experiment II: Oxygen consumption and ammonia excretion

Juvenile fish (3.2 g mean wet weight) were randomly selected from the same stock tanks as in the feeding trial and reared in five 100-L polycarbonate tanks with 40 fish tank-1. Fish were acclimatized to the test diets for 4 days. Seawater in tanks was continuously flowing and aerated, as above.

After acclimatization, oxygen consumption of fish was measured using a Strathkelvin instrument (Model 781, Glasgow, Scotland). Prior to measurements, fish were fed for 30 min to apparent satiation as in the growth trial. Thirty minutes after feeding, a satiated fish was transferred into a conical respiratory chamber containing 683 ml of fully saturated and filtered seawater. Temperature of the water in the chamber was maintained at 28+0.1oC by placing the chamber in a water bath equipped with thermostat heaters. Fish were acclimatized in the chamber for 15–20 min, then the water in chamber was carefully changed with new filtered and fully aerated seawater. The reduction of dissolved oxygen in the water was monitored every 10 min for 30 min per fish. At the

end of measurement, 10 ml of water were collected from the respiratory chamber to determine the amount of ammonia content in water following Strickland and Parsons (1972). Ammonia excretion of fish was determined by comparing the ammonia content in water with and without fish. After each trial, fish were rinsed with distilled water and quickly dried with an absorbent paper before determining the individual wet weight. Similar procedure was conducted to determine the oxygen consumption of fish that were starved for 12–16 h from the last feeding. Data were adjusted to weight specific values (ml O2 consumption or mg NH4-N excretion per gram fish) on daily basis. Oxygen consumption of fish after feeding and starved condition was treated as post-prandial and routine metabolism, respectively, following Jobling (1994). Heat increment (HI) by feeding was calculated by the difference of O2 consumption rate between post-prandial and routine metabolisms. Ten fish were used for each dietary treatment on post-prandial and routine O2 consumption, with the measurements conducted once or twice daily for one week. Size of fish ranged from 5.1 to 11.5 g.

Experiment III: Digestibility

After the feeding trial (experiment I), 10 fish (25.1+2.8 g wet weight) from each tank were randomly selected for the apparent digestibility coefficient (ADC) measurement of energy for each diet. Fish were transferred to 100-L polycarbonate tanks and fed to apparent satiation with the same test diets as those in the feeding trial, for 12 days, except □-cellulose, which was replaced by 0.8 g Cr2O3 per 100 g diet. Prior to the collection of feces, test fish were adapted to the test diet for a week. After 30 min of feeding, the water in the tank was slowly drained to make sure there was no diet leftover, and tanks were immediately, but slowly, refilled with new filtered seawater. Feces were then siphoned out from the bottom of the tank hourly for 6 h during daytime. Collected feces in 3 d were pooled and then rinsed carefully with distilled water, resulting in 4 samples per dietary treatment for assay. Samples were stored in a freezer (–30°C) until analyzed. ADC of dry matter and energy were assayed according to the methods of Furukawa and Tsukuhara (1966), with the values obtained multiplied by FI of fish to derive the digestible energy. The difference between energy in the diet consumed and the digestible energy was treated as the energy loss in feces.

Calculations of Energy Budget and Efficiencies

Energy budget was constructed as: EI = F + M + U + G, where EI is the energy intake, F is the energy loss as feces, M is the energy loss for metabolism, U is energy loss as non-fecal matters based on ammonia excretion, and G is energy for growth. M is sub-divided into energy for routine (Mr) and active (Ma) metabolisms, and for heat increment (HI). Mr was obtained from O2 consumption of the starved juveniles. Energy value of diets was used to estimate the EI of fish. Factors 4.825 cal /ml O2 (Bret and Groves, 1979) and 5.94 cal/ mg NH4-N (Elliot and Davison, 1975) were used to calculate the energy for metabolism and non-fecal matters loses, respectively. Finally, Ma was obtained by the difference of EI and the summation of Mr, HI, G, and U, thus completing the energy budget. For comparison, calculations of energy budget in this study was on daily basis for a juvenile size of 10 g wet weight since the measurements of metabolism variables (O2 consumption and ammonia excretion) included that size. Gross conversion efficiency (K1), net conversion efficiency (K2), and assimilation efficiency (AE) were calculated based on Koshio (1985).

Chemical and statistical analysis

The crude protein and lipid contents of the test diets and whole body of fish were determined by Kjeldhal and Bligh and Dyer (1959) methods, respectively, while the gross energy contents were determined using a bomb calorimeter (OSK 150, Ogawa Sampling Co, Ltd., Japan). Ash and moisture were analyzed by methods described by the Association of Official Analytical Method Chemists (AOAC, 1990). Amino acid profiles were obtained by using high-performance liquid chromatography (HPLC) following Teshima *et al.* (1986). Approximately 2 mg of dried samples was weighed and hydrolized with N-methane- sulfonic acid for 22 h at 110°C. The pH of the hydrolysate was adjusted to pH 2.2 and injected into the HPLC unit using an ion exchange resin column. Norleucine was used as an internal standard.

Growth performance data, digestibility of diets, O2 consumption and ammonia excretion of fish were compared by one-way analysis of variance (Package super-ANOVA, Abacus Concepts, Berkeley, California,USA). Significant differences between means were evaluated by Duncan's multiple range test. The relationship between the estimated dry body weight of fish and the amount of food consumed were computed using the Cricket software (version 1.3.2, Malveron, PA, USA). Significant differences between the slope of regression were compared by Student's t-test at 0.05 significance level (Steel and Torrie 1980).

Results

A summary of the growth performance of fish is presented in Table 3, while the proximate compositions and amino acid profiles of whole body are listed in table 4. Survival rate was more than 85% in all treatments. Other variables on growth performance showed a general trend that plateau at 24% SBM, then decreased as the SBM in diets increased. However, no statistical difference among dietary treatments for each variable was detected.

Table 3. Growth performance of juvenile red sea bream *Pagrus majo*r fed different level of SBM after 30 days of feeding trial[1]

Parameter	Dietary treatment				
	Control	SBM 16	SBM 24	SBM 32	SBM 39
Survival rate (%)	95.0	97.5	97.5	97.5	85.0
Initial weight (g)	3.1±0.2	3.1±0.0	3.0±0.1	3.3±0.0	3.2±0.2
Final weight (g)	21.9±1.3	21.8±0.1	21.8±0.6	21.0±0.8	19.4±0.2
BWG (%)[2]	603.6±83.9	606.8±3.4	620.3±40.6	544.7±24.7	515.4±41.7
SGR (%)[3]	6.5±0.4	6.5±0.0	6.6±0.2	6.2±0.1	6.1±0.2
FI (g diet/fish/30 days) [4]	17.2±1.1	18.2±0.7	17.2±0.2	15.8±0.6	15.9±0.6
PER[5]	2.1±0.0	1.9±0.0	2.0±0.1	2.1±0.1	1.9±0.1
FE[6]	91.5±1.5	87.5±3.5	92.0±3.0	88.5±0.5	98.0±1.0

[1]Mean ± SE (n=2)
[2]BWG (body weight gain) =[(final weight-initial weight)/initial weight] x 100
[3]SGR (specific growth rate) = [(ln final weight)-ln intial weight]/30] x 100
[4] FI (Feed intake)
[5]PER (protein efficiency ratio) = weight gain/protein intake
[6]FE (feed efficiency) = (feed intake/weight gain) x 100

Table 4. Proximate values and amino acid composition (% of total AA)[a] of whole body of red sea bream *Pagrus major* juvenile after feeding trial of 30 days

Composition	Initial	Dietary treatment				
		Control	SBM 16	SBM 24	SBM 32	SBM 39
Proximate value[b]						
Moisture (%)	68.6	70.2	69.4	69.9	70.8	70.7
Crude Protein (%)	60.9	51.7	50.6	52.0	54.4	54.1
Crude Lipid (%)	28.5	28.7	27.1	28.0	25.3	24.7
Crude ash (%)	17.5	14.2	15.0	15.0	15.3	14.7
Energy (kcal g^{-1})	4.69	5.66	5.77	5.59	5.48	5.48
EAA[c]						
Threonine	4.6±0.2	4.4±0.1	4.4±0.1	4.4±0.0	4.4±0.0	4.2±0.0
Valine	4.7±0.2	4.7±0.1	4.7±0.2	4.5±0.0	4.7±0.0	4.1±0.3
Methionine	2.5±0.0	2.3±0.0	2.4±0.1	2.4±0.0	2.1±0.1	2.8±0.4
Isoleucine	4.4±0.1	4.2±0.1	4.2±0.2	4.0±0.1	4.3±0.1	3.7±0.0
Leucine	8.0±0.5	7.4±0.2	7.5±0.0	7.4±0.2	7.3±0.0	6.6±0.1
Phenylalanine	4.2±0.1	4.1±0.0	4.2±0.1	4.1±0.0	4.1±0.0	3.8±0.0
Histidine	2.7±0.0	2.7±0.1	2.8±0.1	2.8±0.1	2.7±0.0	2.7±0.0
Lysine	9.1±0.1	9.2±0.8	9.6±0.7	9.5±0.8	9.4±0.7	8.7±0.8
Arginine	6.5±0.1	6.7±0.1	6.7±0.2	6.7±0.1	6.9±0.2	6.8±0.2
Tryptophan	1.2±0.0	0.5±0.0	0.6±0.0	0.5±0.0	0.6±0.1	0.6±0.0
Σ EAA	47.8	46.2	47.0	46.2	46.4	43.7
NEAA[d]						
Taurine	1.1±0.0	1.9±0.0	1.7±0.1	1.5±0.1	1.4±0.0	1.2±0.0
Hydroxide proline	1.0±0.0	1.6±0.1	1.4±0.1	1.6±0.1	1.6±0.0	2.5±0.1
Aspartic acid	9.4±0.0	9.0±0.2	9.2±0.2	9.2±0.2	9.5±0.0	8.9±0.0
Serine	3.7±0.1	3.8±0.2	3.8±0.3	4.1±0.2	3.8±0.1	3.8±0.0
Glutamic acid	15.8±0.1	15.4±0.1	15.3±0.3	15.6±0.0	15.5±0.1	15.0±0.0
Proline	4.6±0.1	4.9±0.1	4.5±0.3	4.8±0.2	4.9±0.0	5.9±0.3
Glycine	6.0±0.1	7.2±0.3	7.0±0.1	7.1±0.2	7.2±0.1	8.7±0.0
Alanine	6.8±0.4	6.4±0.1	6.6±0.1	6.4±0.1	6.5±0.0	7.0±0.0
Tyrosine	3.8±0.1	3.5±0.0	3.6±0.1	3.6±0.1	3.4±0.1	3.1±0.0
Σ NEAA	52.2	53.8	53.0	53.8	53.6	56.3
TOTAL	100	100	100	100	100	100

There was a high correlation (R=0.83) between the estimated dry body weight (W) and amount of feeds consumed daily (Y) (Table 5). Although the slope (b) was lowest in fish fed diet 32% SBM, no statistical difference was detected among the dietary treatments.

The apparent digestibility coefficient of dry matter increased significantly as the SBM level in the diet increased, with the highest found in diet containing 39% SBM and the lowest in FM-based diet (Figure. 1). However, the digestibility of energy approached a plateau as the SBM in the diet increased. O2 consumption rate per unit wet body weight of fish at post-prandial and routine metabolism are shown in Figure. 2. O2 consumption at both conditions decreased with an increase in SBM inclusion, however statistical difference between treatments was not detected. Similar to oxygen consumption, ammonia excretion rate of fish was not different between dietary treatments. The energy budget of 10 mg wet weight of fish are summarized in Table 6.

Table 5. Relationship between food consumed (Y) and estimation of dry body weight (W) of red sea bream *Pagrus major* juvenile fed different level of SBM ($Y=aW^b$, a is intercept, b is slope)

	Dietary treatment				
Variable	Control	SBM 16	SBM 24	SBM 32	SBM 39
Intercept (a)	-0.597	-0.588	-0.564	-0.582	-0.591
Slope (b)	0.865	0.873	0.786	0.784	0.827
Coeff. Correlation (R^2)	0.87	0.82	0.86	0.92	0.90

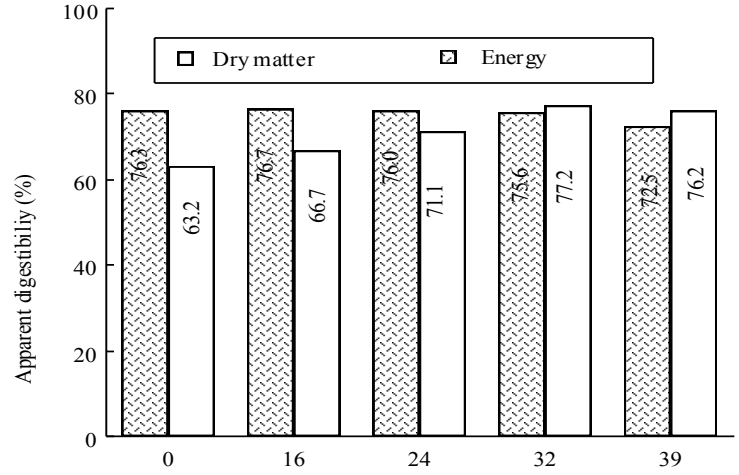

Figure 1. Apparent digestibility of dry matter and energy (%) of red sea bream *Pagrus major* fed artificial diets containing different levels of soybean meal.

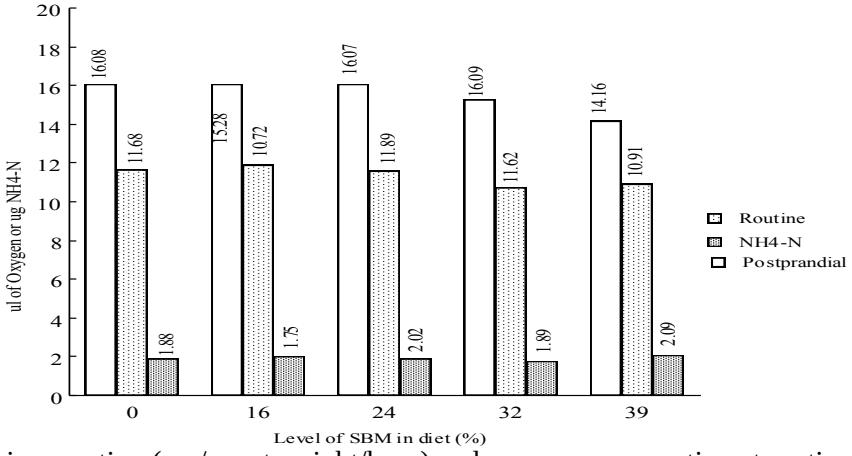

Figure 2. Ammonia excretion (mg/g wet weight/hour) and oxygen consumption at routine and post prandial metabolism (ml/g wet weight/hour) of red sea bream juvenile *Pagrus major* fed artificial diets containing different levels of soybean meal.

Table 6. Distribution of energy intake of 10 g wet weight of Red sea bream *Pagrus major* juvenile (cal $fish^{-1}day^{-1}$) fed diets with different level of soybean meal. Values in parenthesis indicate the percentage relative to energy intake

Variable	Dietary treatment				
	Control	SBM 16	SBM 24	SBM 32	SBM 39
Energy intake (EI)[1]	4348.5 (100)	4577.6 (100)	4189.5 (100)	3717.8 (100)	4028.9 (100)
Feces (F)	1030.6 (23.7)	1066.6 (23.3)	1005.5 (24.0)	907.1 (24.4)	1108.0 (27.5)
Growth (G)[2]	1698.9 (39.1)	1750.1 (38.2)	1657.9 (39.6)	1433.4 (38.6)	1407.9 (34.9)
Metabolism (M)	1506.4 (34.6)	1641.2 (35.9)	1433.5 (34.2)	1301.7 (35.0)	1380.5 (34.3)
	516.2 (11.9)	588.0 (12.8)	538.0 (12.8)	496.3 (13.4)	505.6 (12.5)
Mr					
	230.6 (5.3)	156.0 (3.4)	184.3 (4.4)	124.5 (3.4)	216.7 (5.4)
HI					
Ma[3]	759.6 (17.5)	897.1 (19.6)	711.2 (17.0)	680.8 (18.3)	658.2 (16.3)
Non fecal (U)	112.6 (2.6)	119.8 (2.6)	92.7 (2.2)	75.6 (2.0)	132.6 (3.3)
Efficiency (%)					
A/E	76.3	76.7	76.0	75.6	72.3
K1	39.1	38.2	39.6	38.7	34.9
K2	51.2	49.9	52.1	51.0	48.2

[1]F calculated from the equation of estimated wet weight and feed intake of fish and than converted to energy value of diets

[2]G calculated from SGR value of then converted to gross energy of whole body of each group at size 10 g on dry weight basis

[3]Ma the difference between FI and summation of F, G, HI, Mr and U

The proportion of energy intake and loss as feces increased up to the inclusion level of 39% SBM. Since the portion of energy intake was channeled to metabolism and the small portion was lost as non-fecal matter, the proportion of energy allocated for growth was consequently lower in fish fed 39% SBM. Efficiencies A/E and K1 were similar between fish fed diets with inclusion level up to 32 % SBM, but lower when the inclusion level increased to 39% SBM.

Discussion

The equation Y =aWb has been used to describe the relationship between maximum rate of food consumption (Y) and body weight (W) for many fish species. The b values found in this study ranged from 0.85 to 0.87, which are close to the values 0.6–0.8 found in most fish (Jobling, 1994). Although no statistical difference was detected, b and FI (Table 3) decreased as the SBM level in diet increased. Aoki (1996) demonstrated that the palatability and acceptability of diets for juvenile red sea bream were not influenced by the inclusion of SBM up to 30%, which was similar to this finding, whereas FI of fish decreased when SBM in the diet with more than 24% SBM. In addition, an increase in dietary SBM could also reduce the palatability and acceptability of the diets in giant gourami (Suprayudi *et al*., 1999) and yellow tail (Viyakarn *et al.*, 1992; Watanabe *et al.*, 1992). Takagi *et al.* (1999) also reported that diet palatability was influenced by an inclusion of more than 50% soy protein concentrate in juvenile red sea bream.

The lower feed intake of fish fed 32% SBM may lead to growth retardation in fish. The growth performance data (Table 3) showed that final weight, WG and SGR of fish started to decrease as the inclusion level of SBM in the diet reached 32%.

From an energetic point of view, the proportion of EI lost as feces increased as the level of SBM inclusion in diet reached 32% (Table 6). This indicates that the diets with SBM inclusion below 32% can provide similar amounts of energy, as the control diet, for the growth and metabolic processes of fish.

The proportion of energy intake channeled to metabolic rate (M) plays a significant role in energy partitioning of fish. In this study, M ranged from 34.2 to 34.6%, still close to the range 37–51% in many teleost species (Brett and Groves, 1979). It seems that the increment level of dietary SBM up to 39% has no effect on the total metabolism of juvenile red sea bream, probably due to the isonitogenous and the relatively similar amino acid profile of the diets (Table 2). On the other hand, our recent observations in juvenile red sea bream (0.1 g) fed Artemia nauplii with different level of proteins

showed that a portion of EI as M ranged from 30 to 35%, depending on the highly-unsaturated fatty acids (HUFA) composition of the nauplii used (Sumule *et al.*, submitted). Moreover, Takii *et al*. (2000) reported that EI channeled to M is higher (55–58%) in higher salinity (32 psu) compared to that of lower salinity (58–63%, 25 psu). It is likely that the proportion of EI allocated for metabolism was affected by the size of fish, protein content of diet and environmental conditions (De Silva and Anderson, 1995).

Energy as total metabolism consists of energy for routine metabolism (Mr), for activity (Ma) and for heat increment due to feeding (HI). The oxygen consumption of fish as routine metabolism expresses the amount of energy spent for bodily maintenance. It depends on many factors such as size of fish, temperature of water, oxygen availability in the water, stress, etc. (De Silva and Anderson, 1995). The proportion of energy intake, ranging 11.9–13.4%, channeled to routine metabolism in this study is comparable with the values 10–43% found in the same species, as reported by Takii *et al*. (2000). On the basis of digestible energy (gross energy intake minus energy as feces), the proportion of energy in our results, ranging 15.6–17.7%, was also close to that of the rainbow trout (9.9–22.4%) fed commercial diets (Ohta and Watanabe, 1996).

An increase in O2 consumption due to feeding is widely known as heat increment (HI), which reflects the energy requirement for various physiological and biochemical processes related to feeding, such as ingestion, digestion, absorption, and assimilation (Jobling, 1985). Thus, HI reflects the increase in the synthesis of protein and lipids associated with growth as well as the secretion of digestive enzyme and active transport of the product of digestion across the gut mucosa (Jobling, 1994). In comparative analysis, Kiörbe *et al.* (1985) concluded that HI is largely related to the biosynthesis and metabolic transport, whereas feeding, gut activity, amino-acid oxidation, and urea excretion are of lesser importance. Hence, HI represent the cost of growth and high HI likely tends to reflect a high HI (Jobling, 1994). In our previous study, we found that HI of larvae with faster growth is higher (5.9% relative to EI) compared to HI of larvae (2% relative to EI) with lower growth (Sumule *et al.*, submitted).

There was no detectable pattern in HI-SBM level relationship found in this study, probably due to the similar quality of proteins in the diets (Lovell, 1989). The proportion of EI channeled to HI in this study ranged from 3.4% to 5.4%, lower compare to value (10 –15%) of general adults of teleost fish (Lovell,1989). The range was affected by the quality of diet, size of fish, temperature and salinity of water (De Silva and Anderson, 1995), and quality and quantity of protein in diets (Lovell, 1989).

Takii *et al*. (2000) also reported that the portion of EI channeled to HI and voluntary activity (HI+Ma) of 40 g red sea bream at salinity 32 psu is in the range 15–23%, while for bigger size fish (70 g) it may reach 29.8%, which is slightly higher than what in the study (21.7–22.8%). This distinction may be attributed to the composition of diet. Thus, it could be concluded that the total metabolism (M, including Ma, HI, and Mr) of red sea bream is not affected by the increase in dietary SBM levels up to 39%.

Some parts of digestible energy intake is lost as non-fecal excretion of nitrogenous compounds, mainly ammonia and urea in variable proportions (Cho and Kaushik, 1990). This may depend on the dietary quantity and quality (Brafield, 1985; Lovell, 1989), such as amino acid composition. Previous studies have reported that about 3–11% of gross EI is lost as non-fecal matter in red sea bream (Takii *et al.*,1997; Bi *et al.*, 1998; Takii *et al.*, 2000). Ammonia is considered to be dominant, about 60% or more of nitrogenous compound losses (Jobling, 1985; NRC, 1993; Lovel, 1989). Therefore, this parameter could be used to evaluate the quality of diets.

The proportion of energy absorption channeled to non-fecal matter losses as ammonia, i.e. (EI-F)/U x 100, tends to increase as the inclusion level of SBM approaches 39%. Higher energy loss as ammonia may be stimulated by the amino acid composition of that diet, which will meet well with the requirement of fish compared to diet 39% EX-SBM. However, there have been increasing concerns over the quality of water in aquaculture. Efforts have to be made in order to minimize the nutrient added by fish wastes, such as through the excretion of nitrogenous compounds. Thus, in terms of energetic losses as ammonia, inclusion of EX-SBM up to 32% in diets for red sea bream juveniles is more acceptable than the 39% level found in this study.

Assimilation (A/E), gross conversion (K1), and net conversion (K2) efficiencies showed a similar pattern, which similarly started to decrease as the SBM inclusion reached 32%. A/E was in the range 72.3–76.3%, which is still in the range of the reported values (70–90%) in

carnivorous fish (Knight, 1985). Similar to that, Brett and Groves (1979) noted that average K1 of young carnivore fish range 23-35%, still close to values found in this study 34.9-39.6%. Similar to that, our K2 (range 48.5–52.2%) were similar to our recent results on small size (0.1 g) fish i.e 46.7-50.7% but slightly lower compared to 36% as generally found in young fish (Carter *et al*, 2001).

In conclusion, this study shows that SBM can be added in red sea bream juveniles diet at levels 24–32% which implies the reduction of fishmeal from 55% to 34-39%. Since the utilization of SBM was found to improve as fish grows (Murai *et al*., 1989; Shimeno *et al*.. 1992; Gallagher, 1994), EX-SBM could be effectively utilized by larger fish. Further research with extended period studies on the utilization of EX-SBM in aquaculture feeds will still be needed.

Reference

AOAC (Association of the Official Analytical Chemists). 1990. Official Methods of Analysis, 15th edition. Association of the Official Analytical Chemists, Arlington, Virginia, USA.

Aoki, H., T. Watanabe, H. Tsuda, and H. Sakamoto. 1996. Use of defatted soybean meal as substitute protein source for fish meal in a newly developed high energy diet for red sea bream. *Suisanzoshoku*, 44:345-351.

Aoki, H., M. Furuichi, K.Watanabe, S. Satoh, Y. Yamagata, and T. Watanabe. 1999. Use of low or non-fish meal diets for red sea bream. *Suisanzoshoku*, 48:65-72.

Arndt, R.E.R., H. Hardy, S.H. Sugiura, and F.M. Dong. 1999. Effect of heat increment and substitution level on palatability and nutritional value of soy defatted flour in feeds for Coho Salmon, *Oncorhyncus kisutch*. *Aquaculture*, 180:129-145.

Brafield, A.E. 1985. Laboratory studies of energy budget. *In:* Tytler,T. and P. Calow (Eds), *Fish Energetics New Perspectives*. Croom Helm, London and Sydney, pp. 257-282.

Bligh, E.G. and W.J. Dyer. 1959. A rapid method of total lipid extraction and purification. *Com. Bio. Phys.*, 37:911-917.

Bi, N., F. Wang, X. Zhao, and H. Li. 1998. The oxygen consumption and energy metabolism of Crysophyrus major. J. *Shasnghai-Fish.Univ.*, 7:154 – 158.

Bjorck, J. and N.G. Asp. 1983. The effects of extrusion cooking on nutritional value –a literature review. *J.Food.Eng.*, 2:281-308.

Bret, J. R. and T.D.D. Groves. 1979. Physiological energetics. *In:* Hoar, W.S., D.J. Randall, and J.R. Brett (Eds), *Fish fisiology*. Academic Press, New York, pp. 280-352.

Carter, C., D. Houlihan, A. Kieasling, F. Medale, and M. Jobling. Physiological effects of feeding. *In:* Houlihan, D., T. Boujard, M. Jobling (Eds), *Food Intake in Fish*. Blackwell Science, London, pp. 297-319.

Cho, C.Y. and S.J. Kaushik. 1985. Effec of protein intake on metabolizable and net energy values of fish diets. *In:* Cowey, C.B., A.M. Mackie, J.G. Bell (Eds), *Nutrition and Feeding in Fish*. Academic Press, London, pp. 95-117.

De Silva, S.S. and T.A. Anderson. 1995. Fish Nutrition in Aquaculture. Chapman & Hall, London.

Elliot, J. and W. Davison. 1975. Energy equivalent of oxygen consumption in animal energetics. *Oecologia*, 19:195-201.

Furukawa, A. and H. Tsukahara. 1966. On the acid digestion method for determination of chromic oxide as an index substance in the study of digestibility of fish feed. *Bull. Jpn. Soc. Scie. Fish.*, 32:502-506.

Gallagher, M.L. 1984. The use of soybean meal as a replacement for fish meal in diets for hybrid striped bass (*Marone saxatilis* x *M. crypsos*). *Aquaculture*, 126:119-127.

Hertampf, J.W. and Pascual, F.P. 2000. Handbook on Ingredients for Aquaculture Feeds. Kluwer Academic Press, Netherlands

Jobling, M. 1985. Growth. *In:* Tytler, P. and P. Calow (Eds), *Fish Energetics New Perspectives*. Croom Helm, London, pp. 213-230.

Jobling, M. 1994. Fish Bioenergetic. Chapman & Hall, 2-6 Boundary Row, London, pp. 93-205.

Koshio, S. 1985. The effects of eyestalk ablation, diets and environmental factors on growth, survival, and energy utlization of juvenile American Lobster, *Homarus americanus*, as applied to aquaculture. Ph.D Thesis. Department of Biology, Dalhousie University, Halifax, Nova Scotia.

Kikuchi, K. 1999. Use of defatted soybean meal as a substitute for fishmeal in diets of Japanese flounder (*Paralichthys olivaceus*). *Aquaculture*, 199:179:3-11.

Kaushik, S.J., J.P. Cravedi, J.P. Lalles, J. Sumpter, B. Fauconneau, and M. Laroche. 1995. Partial or total replacement of fishmeal by soybean protein on growth, protein utilization, potential esterogenic effects, cholesterolemia and fles quality in rainbow trout Oncorhynchus mykiss. *Aquaculture*, 133: 257-274.

Kiörbe, T., F. Mohlenberg, and K. Hamburger. 1985. Bioenergetic of planctonic copepod Acartia tonsa : relation between feeding, egg

production and repiration, and composition of specific dynamic action. *Mar.Ecol.Prog.Ser*., 26:85-97.

Knight, B. 1985. Energetics and fish farming. *In:* Tytler, P. and P. Calow, P (Eds.), *Fish energetics New Perspectives*. Croom Helm, London, pp. 309-340.

Lovell, R.T. 1989. Nutrition and Feeding of Fish. Van Nostrand Reinhold, New York, USA.

Mustakas, G.C., W.J. Albrecht, J.E. Bookwalter, and W.F. McGhee. 1970. Extruder-processing to improve nutritional quality, flavor and keeping quality of full-fat soy flour. *Food.Technol.*, 24: 102-108.

Murai, T., H. Ogata, A. Villaneda, and T. Watanabe. 1989. Utilization of soy flour by fingerling rainbow trout having different body size. *Nippon Suisan Gakkaishi*, 55:1067-1073.

NRC (National Research Council). 1993. Nutrient Requirements of Fish. National Academic Press, Washington D.C.,USA.

Ohishi, K., K. Watanabe, M. Urishibata, K. Utsuno, K. Ikuta, K. Sugimoto, and H. Harada. 1994. Detection of soybean antigenicity and reduction by twin-screw extrusion. *J.Am.Oil Chem.Soc*., 71:1391-1396.

Ohishi, K., M. Sakabe, K. Watanabe, M. Urishibata, K. Ikuta, K. Sugimoto, and H. Harada. 1995. Evaluation of nutritive value of twin-screw extruded soybean meal with low antigenicity. *Anim. Scie. Tech*., 66:720-724 (in Japanese).

Ohta, M. and T. Watanabe. 1996. Energy requirements for maintenance of body weight and activity, and for maximum growth in Rainbow Trout. *Fish. Sci.*, 62:737-744.

Saitoh, S., S. Koshio, H. Harada, K. Watanabe, T. Yoshida, S. Teshima, and M. Ishikawa. 2000. The effects of twin-extruded soybean meal on the performance of kuruma Shrimp, *Penaeus japonicus*, juveniles. *Suisanzoshoku*, 48:649-655.

Suprayudi. M.A., M. Bintang, T. Takeuchi, L. Mokoginta, and T. Sutardi. 1999. Defatted soybean meal as an alternative source to substitute fishmeal in the feed of giant gouramy *Osphronaimus gouramy* Lac. *Suisanzoshoku*, 47:551-557.

Sakakura, Y., S. Koshio, Y. Iida, K. Tsukamoto, T. Kida, and J.H. Blom. 1998. Dietary vitamin C improves the quality of yellowtail (*Seriola quinqueradiata*) seedlings. *Aquaculture*, 161:427-436.

Strickland, J.D.H. and T.R. Parsons. 1972. A Practical hand book of sea water analysis. Fisheries Research Board of Canada.

Shimeno, S., H. Hosokawa, M. Kumon, T. Matsumoto, and M. Ukawa. 1992. Inclusion of deffatted soy bean meal in the diet for fingerling yellowtail. *Nippon Suisan Gakkaishi*, 58:1319-1326.

Steel, R.G.D. and J.H. Torrie. 1980 Principles and Procedure of Statistics : A Biometric Approach. McGraw-Hill, New York.

Sumule, O., S. Koshio, S. Teshima and M. Ishikawa. Effects of highly unsaturated fatty acids (HUFA)-enrichment on energy budget of red sea bream (*Pagrus major*) larvae fed Artemia nauplii. *J.Word. Aquacult*.(submited).

Takagi, S., H. Hosokawa, S. Shimeno, M. Maita, M. Ukawa, and S. Ueno. 1999. Utilization of soy protein concentrate in a diet for red sea bream, *Pagrus major*. *Suisanzoshoku*, 47:77-87.

Takii, K., K. Konishi, M. Ukawa, M. Nakamura, and M. Kumai. 1997. Influence of feeding on digestion and energy flow in tiger puffer and red sea bream. *Fisheries sciences*, 63:355-360.

Takii, K., S. Akiyama, H. Maeda, M. Seoka, M. Nakamura, H. Kumai, M. Miki and M. Kurifuji. 2000. Energy budgets of red sea bream under various water salinities and rearing densities. *Suizanshoshoku*. 48:632-636 (in Japanese).

Teshima, S., A. Kanazawa, and M. Yamashita. 1986. Dietary value of several proteins and supplemental amino acids for larvae of the prawn, *Penaeus japonicus*. *Aquaculture*, 51:225-235.

Ukawa, M., K. Takii, M. Nakamura, and H. Kumai. 1994. Utilization of soybean meal for red sea bream diet. *Suisanzoshoku*, 42:335-338.

Van den Ingh, T.S.G.A.M., J.J. Olli, A., and A. Kroghdahl. 1996. Alcohol-soluble components in soybean cause morphological change in the distal intestine of atlantic salmon, *Salmo salar* L. *Journal of fish deseases*, 19:47-530.

Viyakarn, V., T. Watanabe, H. Aoki, H. Tsuda, H. Sakamoto, N. Okamoto, N. Iso, S. Satoh, and T. Takeuchi. 1992. Use of soybean meal as substitute for fishmeal and newly developed soft-dry pellet for yellow tail. *Nippon Suisan Gakkaishi*, 58:1991-2000.

Watanabe, T., V. Viyakarn, H. Kimura, K. Ogawa, N. Okamoto, and N. Iso. 1992. Utilization of soybean meal as a protein source in newly developed soft-dry pellet for yellow tail. *Nippon Suisan Gakkaishi*, 58:1761-1773.

Watanabe,T., H. Aoki, K. Shimamoto, M. Hadzuma, M. Maita, Y. Yamagata, V. Kiron, and S. Satoh. 1998. A trial to culture yellow tail with non-fishmeal diets. *Fish. Sci.* 64:505-512.

Wilson, R.P. and W.E. Poe. 1985. Effects of feeding soybean meal with varying trypsin inhibitor activities on growth of fingerling channel catfish. *Aquaculture*, 46:19-25.