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Influence of Soya Oil Blended with Fish Oil on Growth Performance and Lipid Profile of Red Seabream *Pagrus major*

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ABSTRACT

Continuous increase in fish feed prices due to deterioration of fish oil sources and quality catalyzes intensive research efforts to study alternatives for dietary fish oil such as soya oil. Being rich in linolenic acid (C18:2*n*-6), soya oil has a competitive edge as an arachidonic acid (ARA) precursor. A 50-day feeding trial was conducted to evaluate the effects of dietary soya oil as a substitute for fish oil on growth performance and lipid composition of juvenile red seabream, *Pagrus major*. Four types of iso-nitrogenous experimental diets formulated in this feeding trial consisted of 100% fish oil (10F), 80% fish oil and 20% soya oil (8F2S), 60% fish oil and 40% soya oil (6F4S), and finally, 40% fish oil and 60% soya oil (4F6S). All diets were fed to triplicate groups of 15 red seabream with an initial mean weight of 4.9 ± 0.1 g twice daily to apparent satiation. Our results demonstrated that inclusion of soya oil as a lipid source to partially replace fish oil in red seabream diet led to the highest body weight gain (BWG) in 10F (837.2 ± 2.2%), followed by 8F2S (786.9 ± 38.3%), 6F4S (764.6 ± 5.2%) and 4F6S (682.0 ± 17.2%), respectively, without any significant differences among 10F, 8F2S and 6F4S. Soya oil inclusion also gradually decreased BWG and specific growth rate (SGR); both BWG and SGR in 4F6S were significantly lower than other treatments. Feed conversion ratio (FCR) and feed intake (FI) showed no significant differences among treatments. Survival rate of all treatments exceeded 90% although the hepatosomatic index (HSI) in 4F6S was significantly higher than other treatments and serum glutamic-oxaloacetic transaminase (GOT) increased gradually with a higher inclusion of soya oil. Lipid deposition in the whole body was the highest in fish fed with dietary 10F and decreased in relation to the elevated concentration of soya oil in diets. The ventral muscles had doubled the amount of lipid deposition as compared to dorsal muscles. Dominance of saturates among total fatty acid composition particularly C16:0, was similarly observed in the dorsal and ventral muscles as well as the liver. Saturates, monoenes, *n*-3 and ratio of *n*-3/*n*-6 observed have a similar gradient degradation in the dorsal and ventral muscles and the liver. An inverse relationship of inclusion level of soya oil on eicosapentanoic acid (EPA) and docosahexanoic acid (DHA) in both dorsal and ventral muscles including the liver was also observed. In conclusion, diets not exceeding 40% soya oil are suitable as fish oil replacement in the diet of juvenile red seabream without significantly affecting the overall growth performance of this fish.

Keywords: *Pagrus major*, red seabream, soya oil, growth performances, lipid profile

ABSTRAK

Kenaikan harga makanan ikan yang berterusan akibat kekurangan sumber dan juga kualiti minyak ikan, telah menggalakkan lebih banyak penyelidikan untuk mengkaji minyak soya sebagai sumber alternatif kepada minyak ikan. Kekayaan sumber asid linolenik (C18:2*n*-6) telah menjadikan minyak soya sebagai pelopor kepada asid arakidonik (ARA). Satu kajian pemakanan selama 50 hari telah dijalankan untuk menilai kesan pemakanan dengan menggunakan minyak soya ke atas tumbesaran dan komposisi lemak dalam ikan merah, *Pagrus major* juvenil. Empat jenis diet kajian iso-nitrogenus telah diformulasikan dalam kajian ini; yang terdiri daripada 100% minyak ikan (10F), 80% minyak ikan dan 20% minyak soya (8F2S), 60% minyak ikan dan 40% minyak soya (6F4S), dan 40% minyak ikan dan 60% minyak soya (4F6S). Semua diet telah diberikan dua kali sehari sehingga kenyang kepada sejumlah 15 ekor ikan merah dengan purata berat awal 4.9 $\pm$ 0.1 g secara triplikat. Keputusan menunjukkan bahawa penggunaan minyak soya sebagai sumber lemak untuk menggantikan sebahagian sumber minyak ikan dalam ikan merah telah memberikan BWG dalam perlakuan 10F (837.2 $\pm$ 2.2%), diikuti dengan 8F2S (786.9 $\pm$ 38.3%), 6F4S (764.6 $\pm$ 5.2%) dan 4F6S (682.0 $\pm$ 17.2%), masing-masing, tanpa perbezaan yang bererti di antara perlakuan 10F, 8F2S and 6F4S. Penggunaan minyak soya juga mengurangkan BWG dan SGR secara perlahan-lahan; kedua-dua BWG and SGR dalam perlakuan 4F6S menunjukkan nilai rendah yang bererti berbanding perlakuan yang lain. Nisbah penukaran makanan (FCR) dan kadar pengambilan makanan (FI) telah menunjukkan tiada perbezaan yang bererti di antara semua perlakuan. Kadar kemandirian untuk semua perlakuan adalah melebihi 90% walaupun indeks hepatosomatik (HSI) dalam perlakuan 4F6S adalah lebih tinggi secara bererti berbanding perlakuan yang lain, dan serum glutamik-oksaloasetik transaminase (GOT) pula berkurangan dengan penambahan minyak soya. Pengumpulan lemak di keseluruhan tubuh adalah tertinggi dalam ikan yang diberi makanan 10F dan berkurangan dengan penambahan kepekatan minyak soya dalam diet. Kandungan lemak pada otot ventral juga telah meningkat sebanyak dua kali ganda berbanding dengan pada otot dorsal. Kandungan saturat dari jumlah komposisi asid lemak terutamanya C16:0 adalah lebih dominan pada otot dorsal, otot ventral dan hati. Saturat, monoene, *n*-3 dan nisbah *n*-3/*n*-6 telah menunjukkan kecerunan degradasi yang sama pada otot dorsal, otot ventral dan hati. Peningkatan minyak soya menyebabkan kesan songsang terhadap aras asid eikosapentanoik (EPA) and asid dokosaheksanoik (DHA) di kedua-dua otot dorsal dan ventral dan juga hati. Sebagai kesimpulan, diet yang mengandungi minyak soya tidak melebihi 40% adalah paling sesuai sebagai pengganti minyak ikan tanpa memberi kesan yang bererti kepada tumbesaran ikan merah juvenil.

Kata kunci: *Pagrus major*, ikan merah, minyak soya, tumbesaran, profil lipid

INTRODUCTION

The red seabream, *Pagrus major*, is a very important species for aquaculture ventures in East Asia including China, Japan, and Korea, due to its high market value and desirable taste (Ren *et al.*, 2007). Regarded as an alternative protein source besides meat, this species is cultivated to meet the ever increasing demand by consumers on fresh marine fish due to the diminishing landings of red seabream from capture fisheries. However, aquaculture expansion including this species is hampered by the continuous increase in fish feed prices due to a deterioration of fish oil sources (Barlow, 2000) and quality. Thus, the scenario catalyzes more research efforts to study vegetable oils as alternatives for fish oil (Torstensen *et al.*, 2000; Rodríguez *et al.*, 2002; Sargent *et al.,* 2002; Almaida-Pagán *et al.*, 2007). Fish oil, the main source of essential fatty acids (EFA), is rich with omega-3 fatty acid especially docosahexanoic acid (DHA; C22:6*n*-3) and eicosapentanoic acid (EPA; C20:5*n*-3). In addition, arachidonic acid (ARA; C20:4*n*-6) that is sourced from omega-6 fatty acids is also important to meet the growth requirement of fish. As the production pathways of these EFA are still unknown, all EFA must be provided through a proper diet. Therefore, omega-3 can be sourced directly from fish oil while ARA is sourced from vegetable oil. To date,

some documented results demonstrated the possibility of using various vegetable oils such as palm oil, rapeseed oil, linseed oil and sunflower oil to substitute for fish oil. Results have shown the efficacies of these oils in growth performance and feed utilization; in the Atlantic salmon, *Salmon salar* (Bell *et al*., 2002), gilthead seabream, *Sparus aurata* (Izquierdo *et al*., 2005), European seabass, *Dicentrarchus labrax* (Mourente and Bell, 2006) and red seabream, *Pagrus auratus* (Glencross *et al*., 2003).

Soya oil is reputed as a potential substitute for fish oil as it constitutes about half of the worldwide edible vegetable oil. Its richness in linolenic acid (C18:2*n*-6) gives soya oil a competitive edge as an ARA precursor. Currently, little information is available on the suitability of using soya oil as a substitute in feeds for the red seabream. Therefore, this study was conducted to examine the efficacy of partial substitution of fish oil using soya oil on red seabream growth performance, body proximate composition and muscle with liver fatty acid composition profiles. In addition, the health status was also examined to determine whether consumption of dietary incremental soya oil could affect the red seabream's health condition.

MATERIALS AND METHODS

Experimental Diets

Four semi-purified diets were formulated (Table 1). Diets were iso-lipidic and iso-nitrogenous; with 55% protein, 10% lipid and 13% ash, respectively. An experiment with four test diets containing four different levels of fish oil was designed and carried out. Fish diets were formulated for this study, namely 100% fish oil (10F0S), 80% fish oil and 20% soya oil (8F2S), 60% fish oil and 40% soya oil (6F4S), and 40% fish oil and 60% soya oil (4F6S). 10F0S served as the control diet in this experiment. All experimental diets were stored at -80 °C until the time of feeding.

Experimental Fishes and Feeding Protocols

The dietary trial was carried out at the Marine Finfish Hatchery, Kagoshima University, Japan. Red seabream juveniles with a mean weight of 4.9 ± 0.1 g were obtained from a commercial hatchery, Matsumoto Suisan in Miyazaki Prefecture, transported live in a High Density Polyethylene (HDPE) tank aerated with oxygen and acclimatized in indoor rearing tanks a week prior to the experiment. Fish were fed with commercial pellets (Higashimaru Foods, Kagoshima, Japan). Fifteen juveniles were distributed equally in twelve units of 100 L flow-thru tanks in triplicates. Four experimental diets (Table 1), with different ratios of fish oil: soya oil, were fed manually at feeding ration (0800 and 1600 hrs) to the fishes until near satiation. Tanks were cleaned and uneaten feed was collected to determine feed intake.

Fishes were also exposed to a photoperiodic condition of 12 h light/12 h dark, water flow (filtered seawater) maintained at 2.5 L/min in which water temperature was 28.7 ± 1.5 ^{o}C (mean ± S.D.). Fish sampling was done in every ten days, where weight was expressed as bulk weight (g) of all fishes in each respective tank. Survival rate and growth parameters were calculated as follows:

SGR, specific growth rate = 100 × (*ln* final weight - *ln* initial weight)/(duration)
FCR, feed conversion ratio = dry feed intake (kg)/weight gain (kg)
BWG, body weight gain (%) = 100 × (final weight - initial weight)/(initial weight)
HSI, Hepatosomatic index = 100 × (liver weight/body weight)
FI, Feed intake (g/fish/50 days) = (total feed intake (g)/number of fishes) in 50 days feeding period
SR, survival rate (%) = 100 × (initial fish number - dead fish number)/(initial fish number)

Table 1. Basal ratio of experimental diets containing different levels of soya oil.

Ingredients (g/kg DM)	Diets			
	10F0S	8F2S	6F4S	4F6S
Brown fish meal[1]	670.0	670.0	670.0	670.0
Activated gluten	80.0	80.0	80.0	80.0
α –Starch	40.0	40.0	40.0	40.0
Dextrin	40.0	40.0	40.0	40.0
Fish oil	100.0	80.0	60.0	40.0
Soya oil	0.0	20.0	40.0	60.0
Mineral mix[2]	30.0	30.0	30.0	30.0
Vitamin mix[3]	26.5	26.5	26.5	26.5
Stay-C[4]	3.5	3.5	3.5	3.5
α-Cellulose	10.0	10.0	10.0	10.0
Total	1000	1000	1000	1000
Analytical contents (dry matter basis)				
Crude protein (%)	55.8	55.7	55.4	55.7
Total lipid (%)	12.3	11.6	11.6	11.4
Ash (%)	13.9	14.5	13.9	11.1
Gross energy (kcal/g diet)	6.4	6.4	6.4	6.3

Note:

[1] Defatted brown fish meal.

[2] Mineral mix (g/kg): NaCl, 0.183; $MgSO_4.7H_2O$, 0.685; $NaH_2PO_4.2H_2O$, 0.436; KH_2PO_4, 1.199; $Ca(H_2PO_4)_2.2H_2O$, 0.679; Fe-Citrate, 0.148; Ca-Lactate, 1.635; $AlCl_3.6H_2O$, 0.00009, $ZnSO_4.7H_2O$, 0.017; $CuCl_2$, 0.00005; $MnSO_4.4H_2O$, 0.004; KCl, 0.008; $CoCl_2$, 0.005.

[3] Vitamin mix (g/kg): p-aminobenzoic acid, 1.45; biotin, 0.02; myo-inositol, 14.5; nicotinic acid, 2.9; folic acid, 0.05; choline chloride, 29.65.

[4] Stay-C: L-Ascorbyl-2-monophosphate-Na/Ca (DSM Nutrition Japan K. K.).

At the end of the experiment, fishes were killed using the hypothermia method *i.e.,* by immersion into slurry-ice cold marine water prepared and maintained at 3 ppt and 0 °C according to a method adapted from Losada *et al.* (2005) and the individual weight of each fish was taken. Livers were removed, pooled and stored at -80 °C until further analysis was performed. Filleting was conducted by separating the dorsal and ventral flesh according to the Japanese standards. Fillets from each treatment were pooled and maintained in ice before being freeze-dried and used in further analysis. Three fishes were also randomly sampled from each respective tank and used for proximate analysis.

Proximate Composition Analysis

All experimental diets were analyzed in duplicates for protein, lipid and ash while homogenized samples of whole body and liver were analyzed in duplicates for protein, lipid, ash, moisture and dry matter. Amount of protein was determined by using the Kjeldahl method (AOAC, 1990), lipid by Bligh and Dyer (1959), whereas ash was analyzed by combustion in a muffle furnace at 600 °C. Moisture was determined on approximately 5 g of minced muscle, by oven-drying at 110 °C to constant weight, according to AOAC (1990); while results of dry matter were expressed as 100 minus moisture (in g of water/100 g of muscle).

Fatty Acid Composition Analyses

Fatty acid composition analysis was performed on lipids isolated from the experimental diets and homogenized samples of dorsal fillet, ventral fillet and liver. Total lipid (TL) was extracted by homogenizing a 2 g sample according to Bligh and Dyer (1959). Fatty acid esters (FAME) were then produced from aliquots of total lipid. Fatty acids were methylated with BF_3 in methanol. Methyl tricosanoate (Nu-Chek Prep. Inc.) was used as the internal standard at 1.000 mg/mL hexane. Fatty acid methyl esters (FAME) was analyzed with a Gas Chromatograph (Shimadzu GC 17A) with flame ionization detector temperature maintained at 260 °C; carrier gas N_2 at 1 mL/min; column temperature at 200 °C; injector temperature at 250 °C and Helium (He) served as the carrier gas. Exactly 0.1 μL sample was manually injected into the injection port and the identified fatty acids were presented as area percentage of total fatty acids.

Blood Collection Protocol

Blood samples were taken from each fish by puncturing the ventral caudal vein using heparinized disposable-syringes with 24-gauge needle. The needle was inserted intramuscularly-perpendicular to the ventral surface of the fish located at the posterior of the anal fin until it reached the spine or blood entered the syringe. The needle needed to be withdrawn slightly once it reached the spine to allow collection of blood. Non-heparinized disposable-syringes were also used to collect blood for serum analysis. Samples were then kept in ice and transported immediately to the laboratory for blood chemistry analysis.

Blood Chemistry Analysis

The micro hematocrit method by Kawadzu (1981) was used for the determination of hematocrit (Ht) levels. Glucose (GLU), total cholesterol (T-CHO), blood urea nitrogen (BUN) in plasma and albumin, total bilirubin (T-Bil), glutamic-oxaloacetic transaminase (GOT) and glutamate-pyruvate transaminase (GPT) in serum were determined by SPOTCHEM EZ SP-4430 system according to Tatsumi *et al.* (2000).

Statistical Analyses

The statistical analyses were performed using ANOVA. Data was expressed as means $\pm$ S.E. Homogeneity of variance between treatments ($P < 0.05$) was analyzed using one-way Analysis of Variance (ANOVA) while significance of differences was determined using the Tukey-Test. Effects of dietary treatments were considered significant at $P < 0.05$.

RESULTS

Growth Performances

Specific growth rate (SGR) and body weight gain (BWG) in fishes fed with 10F, 8F2S and 6F4S formulations noted insignificant differences among the treatments with the exception of 4F6S (Table 2). However, the inclusion of soya oil gradually decreased BWG and in 4F6S, both BWG and SGR were significantly lower than other treatments. Feed conversion ratio (FCR) and feed intake (FI) for all treatments were similar ranging from 1.1 to 1.2 and 45.9 to 54.6, respectively, throughout the 50-day experimental period. Survival rate was relatively high in all treatments ranging from 90 to 96.5% without any significant differences among the treatments. Hepatosomatic index (HSI) in 4F6S was significantly higher than other treatments.

Proximate Composition

Inclusion of soya oil as fish oil replacement in diets indicated significant differences in growth performance of the red seabream. Although the formulated diets contained similar proximate

composition (55.4 - 55.8 g/kg protein, 11.4 - 12.3 g/kg lipid and 11.1 - 14.5 g/kg ash) as shown in Table 1, significant differences in proximate composition in muscles and liver were illustrated in Table 3.

Table 2. Growth performances of red seabream (*Pagrus major*) fed with different diets[1]

	Diets			
Growth Parameters	10F0S	8F2S	6F4S	4F6S
Initial weight (g/fish)	4.9 ± 0.0	5.0 ± 0.1	5.0 ± 0.0	4.9 ± 0.0
Final weight (g/fish)	49.3 ± 0.6^{b}	43.3 ± 1.2^{a}	42.3 ± 0.6^{a}	40.5 ± 1.7^{a}
SGR[2]	4.5 ± 0.0^{b}	4.5 ± 0.0^{b}	4.4 ± 0.0^{b}	4.3 ± 0.1^{a}
FCR[3]	11.1 ± 0.1	1.1 ± 0.0	1.1 ± 0.0	1.2 ± 0.0
BWG[4]	837.2 ± 2.2^{b}	832.3 ± 3.5^{b}	807.1 ± 8.1^{b}	682.0 ± 1.2^{a}
HIS[5]	1.28 ± 0.1^{a}	1.7 ± 0.0^{a}	1.8 ± 0.0^{a}	2.2 ± 0.0^{b}
FI (g/fish/50 days)[6]	54.6 ± 5.5	49.0 ± 0.1	45.9 ± 0.9	49.2 ± 3.1
SR[7]	96.5 ± 3.5	96.5 ± 3.5	96.5 ± 3.5	90.0 ± 3.1

Notes:
[1] Values were expressed as mean ± S.E. (n = 2). Data with same alphabets were not significantly different (P > 0.05).
[2] SGR, specific growth rate = 100 × (*ln* final weight- *ln* initial weight)/(duration).
[3] FCR, feed conversion ratio = dry feed intake (kg)/weight gain (kg).
[4] BWG, body weight gain (%) = 100 × (final weight - initial weight)/(initial weight).
[5] HSI, Hepatosomatic index = 100 × (liver weight/body weight).
[6] FI, Feed intake (g/fish/50 days) = (total feed intake (g)/number of fishes) in 50 days feeding period.
[7] SR, survival rate (%) = 100 × (initial fish number - dead fish number)/(initial fish number).

Table 3. Lipid, protein, ash and moisture contents of whole body, muscles and liver in red seabream (*Pagrus major*) fed with different diets[1]

Parameters	10F0S	8F2S	6F4S	4F6S
Lipid[2] (%)				
Whole body	31.0 ± 0.1^{b}	30.6 ± 0.2^{b}	28.1 ± 0.5^{a}	26.6 ± 0.2^{a}
Dorsal muscle	10.2 ± 0.1	10.0 ± 0.3	8.0 ± 0.9	10.0 ± 0.1
Ventral muscle	20.4 ± 0.4	22.7 ± 0.7	22.6 ± 0.6	23.1 ± 0.1
Liver	21.8 ± 0.9	23.9 ± 1.9	23.9 ± 1.1	27.9 ± 0.9
Protein[3] (% whole body)	48.2 ± 0.1^{a}	48.9 ± 0.0^{b}	53.0 ± 0.0^{d}	51.8 ± 0.1^{c}
Ash[3] (% whole body)	14.4 ± 0.0^{ab}	14.1 ± 0.2^{a}	15.3 ± 0.1^{c}	14.6 ± 0.1^{b}
Moisture (% whole body)	67.8 ± 0.1^{a}	68.7 ± 0.0^{b}	69.2 ± 0.2^{b}	69.0 ± 0.2^{b}

Notes:
[1] Values were expressed as mean ± S.E. (n = 2). Data with same alphabets were not significantly different (P > 0.05).
[2] Dry weight basis.
[3] Wet weight basis.

Lipid retention in the whole body of the fish demonstrated a gradual decline without any significant difference for fish fed with the 10F, 8F2S and 6F4S formulations (31.0%, 30.6% and 28.1%, respectively); however, a significantly lower lipid content (at 26.6%) was observed for fish fed with 4F6S. Table 3 also indicates plateau deposition patterns of lipid in both dorsal and ventral muscles including the liver. Lipid deposition was lower in the dorsal muscle as compared to the ventral muscle and liver. Dorsal muscles contained lipids ranging from 8.0 to 10.2%, while concentration doubled from 20.4 to 23.1% in the ventral muscle and 21.8 to 27.9% in the liver. Current results also indicated that lipids were elevated with soya oil increment. Ash, protein and moisture contents demonstrated inconsistence pattern among treatments; with ash contents at 14.1 ± 15.3% WM, moisture at 67.8 ± 69.2% and protein at 48.2 ± 53.08% WM, respectively.

Profiles of fatty acid composition

Profiles of fatty acid composition in the dietary feed (Table 4) reflected the expected results of inclusion of soya oil concentration. Saturates dominated total fatty acid composition ranging from 22 to 24.2%. Concentration increment of C16:0 increased from 16.3 to 19.3% while C18:2*n*-6 from 4.2 to 5.0%, affirming positive gain of these fatty acids with higher inclusion of dietary soya oil.

Saturates dominated total fatty acid composition in the dorsal muscle (Table 5), ventral muscle (Table 6) and liver (Table 7) although higher inclusion of soya oil significantly decreased the concentration of saturates. A similar pattern was also observed for monoenes, *n*-6 and *n*-3. Concentration of all fatty acids except C18:2*n*-6 decreased gradually. Therefore, it can be stated that soya oil inclusion intensified linoleic acid concentrations (C18:2*n*-6) in all samples. A significant decline of total polyunsaturated fatty acids (PUFAs) and ratio of *n*-3:*n*-6 accounted in both dorsal and ventral muscles was also observed. Concentrations of essential fatty acids (EFA) like ARA, EPA and DHA also declined to incremental soya oil inclusion, which resulted in an inverse relationship of all respective EFAs as mentioned earlier.

Blood chemistry

The current results as indicated in Table 8 demonstrated degradation of Ht levels in blood serum of fish fed with incremental soya oil inclusion. Other serum biochemical properties; like Hb, Glu, T-Cho, Bun, T-bil and GPT found no significant difference among treatments. However, GOT elevated significantly from 2% inclusion of soya oil in dietary feed at 77.3 IU/L, 79.0 IU/L and 83.8 IU/L, respectively, without any significant difference among treatments. These three treatments were however, significantly higher than GOT in dietary 10F (54.0 IU/L).

Table 4. Fatty acid composition (% of total fatty acid) in experimental diets[1]

Types of fatty acid	Diet			
	10F0S	8F2S	6F4S	4F6S
14:0	3.2	3.3	2.1	2.4
16:0	16.3	17.6	18.2	19.3
18:0	2.5	2.8	2.7	2.5
∑ saturated fatty acids	22.0	23.7	23.0	24.2
16:1*n*-9	5.0	4.1	3.3	3.5
18:1*n*-7	0.4	0.4	0.3	0.2
18:1*n*-9	9.0	14.4	15.9	17.2
20:1*n*-9	4.0	0.2	2.9	2.0
22:1*n*-9	0.2	0.2	0.5	0.5
20:1*n*-11	0.5	0.4	0.5	0.8
∑ monoenes	19.1	23.4	23.1	24.2
18:2*n*-6	4.2	4.7	5.3	5.0
20:4*n*-6	0.9	0.9	0.9	0.8
22:4*n*-6	8.3	6.9	5.8	4.4
∑ *n*-6 fatty acids	13.4	12.5	12.0	10.2
18:3*n*-3	nd	0.9	1.3	1.8
18:4*n*-3	1.2	0.9	0.7	0.5
20:4*n*-3	0.4	0.3	0.3	0.3
20:5*n*-3	9.4	3.8	2.9	1.8
22:5*n*-3	1.0	0.9	0.9	1.0
22:6*n*-3	12.4	10.7	10.5	11.0
∑ *n*-3 fatty acids	24.4	17.5	16.6	16.4
∑PUFA[3]	37.8	30.3	29.0	26.8
∑*n*-3HUFA[4]	23.2	15.7	14.6	14.1
∑*n*-3/*n*-6 ratio[5]	1.8	1.4	1.4	1.5
∑EPA+DHA[6]	21.8	14.5	13.4	12.8

Notes:

[1] Values were expressed as means ± S.E. (n = 2). Same superscripts were not significantly different (P > 0.05).

[2] nd = Not detected.

[3] Total PUFA was expressed as sum of total *n*-3 fatty acids and total *n*-6 fatty acids.

[4] Total *n*-3 HUFA was expressed as sum of *n*-3 fatty acids in carbons (C) more than 20.

[5] *n*-3/*n*-6 ratio was expressed as total *n*-3 PUFA divided by total *n*-6 PUFA.

[6] Sum of eicosapentanoic acid (C20:5*n*-3) and docosahexanoic acid (C22:6*n*-3) as essential fatty acids.

Table 5. Fatty acid composition (% of total fatty acid) in the dorsal muscle of the red seabream (*Pagrus major*) fed with different diets[1]

	Dorsal			
Types of fatty acid	10F0S	8F2S	6F4S	4F6S
14:0	2.9 ± 0.2^{b}	2.6 ± 0.2^{b}	1.7 ± 0.1^{a}	2.7 ± 0.0^{a}
16:0	25.0 ± 0.2^{c}	23.2 ± 0.3^{b}	18.6 ± 0.2^{a}	17.6 ± 0.3^{a}
18:0	6.4 ± 0.3	7.0 ± 0.1	6.6 ± 0.1	6.4 ± 0.2
∑ Saturated	34.2 ± 0.1^{c}	32.7 ± 0.2^{c}	26.8 ± 0.6^{b}	25.3 ± 0.2^{a}
16:1*n*-9	5.5 ± 0.2^{c}	5.0 ± 0.2^{bc}	3.5 ± 0.4^{ab}	3.2 ± 0.2^{a}
18:1*n*-9	26.1 ± 3.0	25.5 ± 0.1	25.4 ± 0.7	25.9 ± 0.6
20:1*n*-9	6.6 ± 0.5^{c}	5.0 ± 0.9^{bc}	3.6 ± 0.2^{ab}	2.0 ± 0.5^{a}
22:1*n*-9	0.5 ± 0.1	0.5 ± 0.0	0.4 ± 0.0	0.2 ± 0.1
∑ Monoenes	38.6 ± 3.3	35.9 ± 0.5	33.0 ± 0.8	30.7 ± 0.1
18:2*n*-6	3.1 ± 0.1^{a}	3.3 ± 0.2^{a}	5.2 ± 0.2^{b}	6.4 ± 0.1^{c}
18:3*n*-6	0.3 ± 0.0^{c}	0.2 ± 0.0^{b}	0.2 ± 0.0^{b}	nd
20:4*n*-6	1.5 ± 0.1	1.3 ± 0.0	1.2 ± 0.3	1.3 ± 0.0
22:3*n*-6	0.2 ± 0.0^{b}	0.2 ± 0.0^{b}	0.1 ± 0.0^{a}	nd
22:4*n*-6	6.4 ± 0.4	6.7 ± 0.1	5.8 ± 0.1	5.5 ± 0.4
22:5*n*-6	0.5 ± 0.0	0.1 ± 0.0	0.3 ± 0.2	nd
∑*n*-6 fatty acids	12.0 ± 0.5	11.8 ± 0.7	12.8 ± 0.1	13.2 ± 0.9
18:3*n*-3	0.9 ± 0.0^{a}	1.0 ± 0.1^{a}	1.2 ± 0.3^{a}	2.2 ± 0.3^{b}
18:4*n*-3	0.7 ± 0.0^{c}	0.6 ± 0.4	0.4 ± 0.1^{b}	0.3 ± 0.0^{a}
20:4*n*-3	nd	nd	nd	nd
20:5*n*-3	7.3 ± 0.2^{d}	6.0 ± 0.1^{c}	5.2 ± 0.1^{b}	3.8 ± 0.1^{a}
22:5*n*-3	1.8 ± 0.0	1.8 ± 0.1	1.5 ± 0.2	1.4 ± 0.0
22:6*n*-3	7.9 ± 0.1	7.9 ± 0.2	8.1 ± 0.4	7.5 ± 0.0
∑*n*-3 fatty acids	18.6 ± 0.3^{c}	17.3 ± 0.1^{b}	16.4 ± 0.2^{ab}	15.2 ± 0.2^{a}
∑ PUFA[3]	30.6 ± 0.8	29.1 ± 0.3	29.2 ± 0.1	28.4 ± 0.6
∑*n*-3/*n*-6 ratio[4]	1.6 ± 0.0^{c}	1.5 ± 0.1^{bc}	1.3 ± 0.1^{ab}	1.2 ± 0.1^{a}
∑ EPA+DHA[5]	15.2 ± 0.2	13.9 ± 0.3	13.3 ± 2.8	11.3 ± 0.6

Notes:

[1] Values were expressed as means ± S.E (n = 2). Same superscripts were not significantly different (P > 0.05).

[2] nd = Not detected.

[3] Total PUFA was expressed as sum of total *n*-3 fatty acids and total *n*-6 fatty acids.

[4] *n*-3/*n*-6 ratio was expressed as total *n*-3 PUFA divided by total *n*-6 PUFA.

[5] Sum of eicosapentanoic acid (C20:5*n*-3) and docosahexanoic acid (C22:6*n*-3) as essential fatty acids.

Table 6. Fatty acid composition (% of total fatty acid) in the ventral muscle of the red seabream (*Pagrus major*) fed with different diets[1]

	Ventral			
Types of fatty acid	10F0S	8F2S	6F4S	4F6S
14:0	2.6 ± 0.3^{c}	2.5 ± 0.1^{bc}	1.8 ± 0.1^{ab}	1.3 ± 0.0^{a}
16:0	23.8 ± 0.3^{b}	19.7 ± 0.4^{a}	19.1 ± 0.2^{a}	18.6 ± 0.4^{a}
18:0	6.5 ± 0.2	6.5 ± 0.1	6.7 ± 0.1	7.0 ± 0.1
∑ Saturated	32.9 ± 0.2^{b}	28.7 ± 0.3^{a}	27.6 ±0.4^{a}	26.8 ± 0.5^{a}
16:1*n*-9	5.5 ± 0.2^{b}	4.9 ± 0.3^{b}	3.3 ± 0.1^{a}	2.7 ± 0.0^{a}
18:1*n*-9	30.7 ± 4.8	24.0 ± 0.8	26.1 ± 0.6	21.0 ± 3.9
20:1*n*-9	4.4 ± 0.4^{bc}	4.8 ± 0.4^{c}	3.0 ± 0.2^{b}	0.8 ± 0.4^{a}
22:1*n*-9	0.5 ± 0.1^{b}	0.4 ± 0.1^{ab}	0.3 ± 0.0^{a}	0.3 ± 0.0^{a}
∑ Monoenes	40.1 ± 3.5	34.1 ± 0.0	32.6 ± 0.5	28.7 ± 0.0
18:2*n*-6	3.0 ± 0.0^{a}	4.4 ± 0.5^{a}	4.0 ± 0.3^{a}	7.3 ± 0.4^{b}
18:3*n*-6	0.3 ± 0.0^{b}	0.2 ± 0.0^{a}	nd	nd
20:4*n*-6	1.5 ± 0.1^{c}	1.3 ± 0.1^{bc}	0.7 ± 0.1^{a}	0.5 ± 0.3^{ab}
22:3*n*-6	nd	nd	nd	nd
22:4*n*-6	6.6 ± 0.3^{c}	5.4 ± 0.2^{b}	6.3 ± 0.1^{c}	2.2 ± 0.1^{a}
22:5*n*-6	1.0 ± 0.0^{b}	0.7 ± 0.0^{a}	nd	nd
∑*n*-6 fatty acids	12.4 ± 0.3^{b}	12.0 ± 0.5^{ab}	11.0 ± 0.3^{ab}	9.9 ± 0.5^{a}
18:3*n*-3	0.6 ± 0.1^{a}	1.2 ± 0.1^{b}	1.6 ± 0.1^{c}	2.0 ± 0.1^{d}
18:4*n*-3	0.7 ± 0.1^{b}	0.6 ± 0.1^{b}	0.4 ± 0.0^{a}	0.3 ± 0.0^{a}
20:4*n*-3	0.7 ± 0.1^{c}	0.7 ± 0.0^{c}	0.9 ± 0.0^{b}	0.5 ± 0.0^{a}
20:5*n*-3	8.6 ± 0.2^{d}	7.3 ± 0.4^{c}	5.1 ± 0.1^{b}	3.8 ± 0.0^{a}
22:5*n*-3	1.9 ± 0.1^{b}	2.0 ± 0.2^{b}	1.6 ± 0.1^{ab}	1.4 ± 0.0^{a}
22:6*n*-3	11.6 ± 0.2^{b}	11.0 ± 1.0^{b}	8.7 ± 0.1^{ab}	7.3 ± 0.3^{a}
∑*n*-3 fatty acids	24.0 ± 0.5^{c}	22.7 ± 1.7^{bc}	18.1 ± 0.4^{ab}	15.0 ± 0.3^{a}
∑ PUFA[3]	36.4 ± 0.3^{c}	34.6 ± 2.2^{bc}	28.8 ± 0.1^{ab}	25.2 ± 0.3^{a}
∑*n*-3/*n*-6 ratio[4]	2.0 ± 0.1^{b}	1.9 ± 0.1^{b}	1.7 ± 0.1^{ab}	1.5 ± 0.1^{a}
∑ EPA+DHA[5]	20.2 ± 0.2^{c}	18.3 ± 0.3^{b}	13.8 ± 2.8^{a}	11.1 ± 0.6^{a}

Notes:

[1] Values were expressed as means ± S.E (n = 2). Same superscripts were not significantly different (P > 0.05).

[2] nd = Not detected.

[3] Total PUFA was expressed as sum of total *n*-3 fatty acids and total *n*-6 fatty acids.

[4] *n*-3/*n*-6 ratio was expressed as total *n*-3 PUFA divided by total *n*-6 PUFA.

[5] Sum of eicosapentanoic acid (C20:5*n*-3) and docosahexanoic acid (C22:6*n*-3) as essential fatty acids.

Table 7. Fatty acid composition (% of total fatty acid) in liver of the red seabream (*Pagrus major*) fed with different diets[1]

	Liver			
Types of fatty acid	10F0S	8F2S	6F4S	4F6S
14:0	1.5 ± 0.1^{b}	1.5 ± 0.0^{b}	1.4 ± 0.0^{b}	0.9 ± 0.0^{a}
16:0	23.9 ± 0.2$^{\mathbf{d}}$	22.5 ± 0.2$^{\mathbf{c}}$	21.0 ± 0.1$^{\mathbf{b}}$	19.3 ± 0.1$^{\mathbf{a}}$
18:0	14.5 ± 0.9	15.8 ± 0.2	14.4 ± 0.2	14.7 ± 0.4
∑ Saturated	39.8 ± 1.2^{b}	39.9 ± 0.1^{b}	36.8 ± 0.4^{ab}	34.9 ± 0.6^{a}
16:1*n*-9	4.3 ± 0.1$^{\mathbf{c}}$	3.8 ± 0.2$^{\mathbf{bc}}$	3.3 ± 0.3$^{\mathbf{ab}}$	2.4 ± 0.1$^{\mathbf{a}}$
18:1*n*-9	23.5 ± 0.3	25.5 ± 1.0	25.4 ± 1.1	23.0 ± 0.2
20:1*n*-9	3.3 ± 0.2^{b}	3.0 ± 0.1^{ab}	2.4 ± 0.2^{ab}	2.2 ± 0.1^{a}
22:1*n*-9	2.2 ± 0.1^{d}	1.7 ± 0.0^{c}	1.4 ± 0.1$^{\mathbf{b}}$	0.9 ± 0.0$^{\mathbf{a}}$
∑ Monoenes	33.1 ± 0.6^{b}	33.9 ± 0.8^{b}	30.5 ± 1.1^{ab}	28.7 ± 0.3^{a}
18:2*n*-6	1.2 ± 0.0^{a}	4.3 ± 0.1^{b}	7.8 ± 0.1^{c}	12.6 ± 0.0^{d}
18:3*n*-6	nd	nd	nd	nd
20:4*n*-6	nd	nd	nd	nd
22:3*n*-6	nd	nd	nd	nd
22:4*n*-6	1.1± 0.2	1.0 ± 0.0	0.9 ± 0.1	1.1 ± 0.0
22:5*n*-6	nd	nd	nd	nd
∑*n*-6 fatty acids	2.8 ± 0.1^{a}	5.8 ± 0.2^{b}	9.4 ± 0.3^{c}	14.4 ± 0.0^{d}
18:3*n*-3	0.1 ± 0.0$^{\mathbf{a}}$	0.3 ± 0.3$^{\mathbf{ab}}$	0.4 ± 0.1$^{\mathbf{ab}}$	0.6± 0.1$^{\mathbf{b}}$
18:4*n*-3	nd	nd	nd	nd
20:4*n*-3	0.6 ± 0.1	0.5 ± 0.1	0.4 ± 0.0	0.4 ± 0.1
20:5*n*-3	4.7 ± 0.2$^{\mathbf{b}}$	4.5 ± 0.1$^{\mathbf{b}}$	3.8 ± 0.3$^{\mathbf{ab}}$	3.5 ± 0.1$^{\mathbf{a}}$
22:5*n*-3	1.1 ± 0.1	1.2 ± 0.1	1.3 ± 0.1	1.1 ± 0.1
22:6*n*-3	12.5 ± 0.2$^{\mathbf{b}}$	11.2 ± 0.4$^{\mathbf{ab}}$	9.6 ± 0.4$^{\mathbf{a}}$	10.0 ± 0.2$^{\mathbf{a}}$
∑*n*-3 fatty acids	18.9 ± 0.6^{b}	17.6 ± 0.3^{ab}	15.4 ± 0.6^{a}	15.5 ± 0.1^{a}
∑ PUFA[3]	22.3 ± 0.7^{a}	23.7 ± 0.9^{a}	25.1 ± 0.9^{a}	30.4 ± 0.1^{b}
∑*n*-3/*n*-6 ratio[4]	6.8 ± 0.1^{c}	3.0 ± 0.1^{b}	1.7 ± 0.0^{a}	1.1 ± 0.0^{a}
∑ EPA+DHA[5]	17.2 ± 0.2^{c}	15.7 ± 0.3^{b}	13.4 ± 2.8^{a}	13.5 ± 0.6^{a}

Notes:

[1] Values were expressed as means ± S.E. (n = 2). Same superscripts were not significantly different (P > 0.05).

[2] nd = Not detected.

[3] Total PUFA was expressed as sum of total *n*-3 fatty acids and total *n*-6 fatty acids.

[4] *n*-3/*n*-6 ratio was expressed as total *n*-3 PUFA divided by total *n*-6 PUFA.

[5] Sum of eicosapentanoic acid (C20:5*n*-3) and docosahexanoic acid (C22:6*n*-3) as essential fatty acid.

Table 8. Blood serum biochemical analysis of red seabream (*Pagrus major*) fed with four different levels of soya oil inclusion in diets

	10F	8F2S	6F4S	4F6S
Ht[1] (%)	43.9 ± 0.0[b]	37.6 ± 0.6[a]	39.1 ± 0.9[a]	38.7 ± 0.2[a]
Hb[2] (g/dL)	4.9 ± 0.1	4.7 ± 0.0	4.9 ± 0.1	4.7 ± 0.0
GLU[3] (IU/L)	51.0 ± 0.0	53.5 ± 2.5	48.5 ± 3.5	48.5 ± 0.5
T-CHO[4] (IU/L)	262.5 ± 44.5	289.5 ± 21.5	289.5 ± 17.5	329.5 ± 2.5
BUN[5] (mg/dL)	<5	<5	<5	<5
T-Bil[6] (IU/L)	0.4 ± 0.1	0.3 ± 0.0	0.40 ± 0.1	0.4 ± 0.0
GOT[7] (IU/L)	54.0 ± 1.0[a]	77.3 ± 0.3[b]	79.0 ± 2.0[b]	83.8 ± 1.8[b]
GPT[8] (IU/L)	<10	<10	<10	<10

Notes:
Values were means ± pooled from triplicate groups. Means in each column with a different letters were significantly different ($P < 0.05$); [1]Ht = hematocrit; [2]Hb = hemoglobin; [3]GLU = glucose; [4]T-CHO = total cholesterol; [5]BUN = blood urea nitrogen; [6]T-Bil = total bilirubin; [7]GOT = glutamic oxaloacetic transaminase; [8]GPT =glutamate pyruvate transaminase.

DISCUSSION

Results from this study affirmed the potential ability of red seabream to utilize soya oil in a limited amount for optimal growth and health maintenance. Economically accepted, FCR and FI showed no significant difference among treatments. Nevertheless, the 10F, 8F2S and 6F4S feed formulations showed acceptable growth rate but at a degrading gradient whereas the 4F6S formulation demonstrated the slowest SGR (4.2 ± 0.1) and lowest BWG (682.0 ± 17.2%). Degradations may have originated from (i) presence of abnormal fatty acids; (ii) selectivity mechanisms of available fatty acids; (iii) accumulation of lipid droplets and (iv) immunomodulatory response.

Increase in concentration of soya oil in diets amends presence of fatty acids, reduces EFAs and subsequently degrades growth performances including health status in fish. Results demonstrated that saturates dominated the total fatty acid composition of the dorsal and ventral muscles, and the liver (Table 5 – Table 7). However, the concentration level declined at a gradual gradient in all samples. Others like monoenes, *n*-6 except C18:2*n*-6, and *n*-3 also demonstrated a similar declining pattern as saturates. Similar observations using soyabean oil dietary feed were reported by Xue *et al.* (2006) on the Japanese seabass, *Lateolabrax japonicus* and Izquierdo *et al.* (2005) on gilthead seabream, *Sparus aurata.* The declining pattern indicated above, implies that the fish were having difficulties in metabolizing concentrated soya oil-based diets. EFAs mainly consist of *n*-3 (C18:3*n*-3, C20:5*n*-3 and C22:6*n*-3) and *n*-6 (C18:2*n*-6 and C20:4*n*-6). The current study confirms expected degradation of *n*-3 EFAs with the higher inclusion of soya oil in diets although, linolenic acid (C18:3*n*-3) was elevated in the dorsal and ventral muscles and also in the liver. C18:3*n*-3 is important as a source for EPA (C20:5*n*-3) and further elongation transforms this source to DHA (C22:6*n*-3). Nevertheless, results showed that elevated C18:3*n*-3 in diets did not contribute much to the elevation of EPA and DHA; instead EPA and DHA declined further. In addition to that, the decline of essential fatty acids concentration in the dorsal and ventral muscles may be inhibited by receptors responsible in producing certain metabolic enzymes. This state could probably prohibit further elongation of C18:3*n*-3 to C20:5*n*-3 as well as further elongation to C22:6*n*-3. Therefore, low amounts of C22:6*n*-3 in the dorsal and ventral muscles, as well as the liver with higher inclusion of soya oil could be accounted. This is suggestive of the

inability of the liver to function under such abnormality in lipid esterification and instead, the liver has to reposition its function to β-oxidation metabolism. In relation to this inhibitory pattern, significant decline of BWG and SGR in 4F6S as compared to other treatments occurred. This confirmed that the poor growth rate in the fish is due to reduced amount of EFAs like C22:6*n*-3 that is required by the fish. Nevertheless, 60% inclusion of soya oil suggested acceptability in terms of growth performance for fish and this conforms to the study by Montero *et al.* (2003) on the gilthead seabream, *S. aurata* by using 60% soybean oil replacing fish oil. In other words, fatty acid composition in fish muscles and also liver depend on each respective fish metabolism pathway in channeling incorporated dietary fatty acids as pointed out by Robin *et al.* (2003).

Elevated C18:2*n*-6 from dietary feed also suggests reposition of the liver's function to β-oxidation metabolism instead of elongating C18:3*n*-3. The liver, which functions as a regulator in fat metabolism, has to function at a more vigorous manner to either desaturated or elongated fatty acids especially at higher inclusion of soya oil (Table 5 – Table 7). The mechanism derived here conforms to Robin and Skalli (2006), whose relative incorporation theory explained the influences of catabolic mechanisms by diet characteristics and possible interactions between various fatty acids. The function of the liver may be protracted during metabolism including catabolism. Excessive C18:2*n*-6 from determined inclusion of soya oil will further depress desaturation and elongation of fatty acid and this was observed in the decline of desaturated fatty acids such as C20:4*n*-6 in all samples. This observation obviously reflected the inability of fish to desaturate and elongate selected fatty acids within the feeding period of 50 days. Due to selective metabolic rate, these excessive soya oil-based fatty acids are channeled into β-oxidation instead of synthesized into triglycerides (TAG). Stubhaug *et al.* (2005) also showed increased β-oxidation in the Atlantic salmon, *Salmo salar* with incremental rapeseed oil although this species has high resistance to β-oxidation (Bell *et al.,* 2001; Bell *et al.,* 2002).

Therefore, inherent complexities occurred in the liver as a result of excessive concentration of C18:2*n*-6 for β-oxidation metabolism pathways and the low C_{18} resulted in deficiencies in HUFA, like EPA, DHA and ARA, which is similar to what Ghioni *et al.* (1999) have reported on the turbot. The loss in the ability to convert C18:2*n*-6 as shown by the red seabream in this study is in line with other observations made by Mourente and Tocher (1994), Sargent *et al.* (2002) and Mourente *et al.* (2005a, 2005b) on marine species of fish.

The plateau pattern of lipid deposition in the dorsal and ventral muscles and also the liver (Table 3) did not indicate possibilities of fat accumulation with higher inclusion of soya oil. Although inclusion of soya oil at 60% may still be acceptable in terms of fat accumulation, HSI in fish fed with dietary 60% soya oil elevated significantly from other treatments. However, elevation of HSI does not necessarily indicate a correlation of inflammatory response to the degree of fat accumulation (Stubhaug *et al.*, 2005) but may instead be due to an implication of desaturation and elongation mechanisms within the liver itself. Apparently, rigorous β-oxidation metabolism due to excessive C18:2*n*-6 in the liver may have exhausted and eventually inflamed this organ.

Blood chemical properties are important indicators of fish health status. Degradation of hemoglobin (Ht) count in blood plasma obviously demonstrates the impact of DHA decline in fish and its importance in maintenance of red blood cell membrane properties as confirmed by Waagboo *et al.* (1995). Higher inclusion of soya oil also seemed to inhibit RBC in generating new cells most probably due to higher β-oxidation metabolism. Elevated GOT according to higher inclusion of soya oil indicates possible symptom of liver congestion. GOT and GPT have been used in fish for the detection of liver damage in flounder (Jung *et al.*, 2003; Hernandez-Hernandez *et al.*, 2007) but there is little information on the effects of vegetable oil diet on the red seabream. Substitution of soya oil as a lipid source may have created a temporary shocking condition for the fish and therefore, causes impaired enzyme activities to meet the liver's aggressive efforts in carrying out the β-oxidation of lipids. Therefore, prolonged high intake of soya oil may induce these functional organs to deteriorate and subsequently confers poor health to the fish.

CONCLUSION

Substituting fish oil with soya oil at limited ratio as lipid source demonstrated acceptable growth performances and optimum health status. However, prolonged high intake of soya oil may contribute some negative influences to fish hepatic metabolism that could be due to increased β-oxidation metabolism. This study concluded that dietary 60% fish oil mixed with 40% soya oil (6F4S) may contribute to optimal fish growth and acceptable health status in the red seabream.

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