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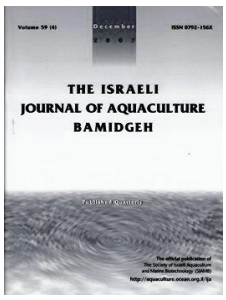

Lipid Requirements in Growing Japanese Seabass (*Lateolabrax japonicus*) of Two Different Sizes

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Key words: Japanese seabass, lipid requirement, fish size, growth performance, body composition, lipid deposition

Abstract

The optimal lipid requirements in Japanese seabass of two different sizes (initial body weight, 34.26±0.37 g, and 343.3±10.0 g) were tested using the same experimental diets in two feeding trials (trial I and trial II). Five experimental diets were formulated to obtain graded dietary lipid levels: 0.6%, 3.9%, 7.3%, 11.7%, and 15.8%. Each diet was randomly assigned to triplicate groups of fish. Both feed trials lasted 10 weeks. The results in both feeding trials showed that specific growth rate significantly increased by increasing dietary lipid levels from 0.6% to 7.3%, and declined thereafter. Based on the specific growth rate, the optimum dietary lipid requirement of the small and large Japanese seabass was 7.4% and 9.9% respectively. The increasing dietary lipid levels significantly increased the fish body protein concentration, but only in feeding trial II. In both feeding trials, the lipid contents in whole fish body and liver significantly increased with increasing dietary lipid levels, but the small fish showed much lower lipid content than the large fish. The serum triglyceride concentrations in the small fish were higher than those in the large fish. High levels of dietary lipid (3.9% and 7.3%) reduced the serum cholesterol concentrations in the small fish, which was much lower than those in the large fish. These results suggested that the lipid requirements, as well as the body composition and lipid deposition differed according to the size of growing Japanese seabass. The results are important for feed formulation for aquaculture of different sized Japanese seabass throughout their production cycle.

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Introduction

Japanese seabass, *Lateolabrax japonicas*, is a euryhaline carnivorous species cultured in both sea and freshwater. It is one of the most commercially valuable aquaculture species in Asia, with an annual production of more than 132,000 tons in China. Japanese seabass is recognized as potentially important for worldwide aquaculture because of its good taste, tolerance to high stocking density, and adaption to wide ranges of environmental factors such as salinity and temperature.

Lipids are the main source of energy and essential fatty acids. Inadequate dietary lipid levels decrease feed efficiency and lead to abnormal lipid deposition in fish tissue. Thus, determining the optimal dietary lipid level is imperative for the nutrition of aquaculture species. Furthermore, previous studies have suggested that the lipid requirements in fish could be relevant to ontogenetic stage and fish size (Lee, 2001; Storebakken, 2002), as observed for other dietary nutrients such as protein (Winfree and Stickney, 1984; Grisdale-Helland and Helland, 1998; Hamre et al., 2003). Thus, the evaluation of dietary lipid requirements in different sized fish seems important, especially for species such as Japanese seabass which is a rapidly-growing fish

The present study was aimed at evaluating the dietary lipid requirements of Japanese seabass with different initial body weights, 34.26±0.37 g and 343.3±10.0 g, and two typical growth phases of growing farmed Japanese seabass. Considering the important role that tissue lipid depositions play in fish health and fillet quality, the effects of dietary lipids on body proximate composition, especially tissue lipid concentrations, were also evaluated. This study provides useful data for real-time feed formulation in the farming of Japanese seabass.

Materials and Methods

Experimental diets. Five isonitrogenous (appr. 48% crude protein) and isoenergetic (appr. 22 kJ/g) experimental diets, were used in the two feeding trials (Table 1).

Table 1. Formulation (%) and proximate composition (%) of the experiment diets (of dry matter).

Ingredients (g 100/ g dry diet)	*Dietary lipid levels*				
	0.6%	*3.9%*	*7.3%*	*11.7%*	*15.8%*
Casein	52.0	52.0	52.0	52.0	52.0
Corn starch	40.0	30.0	20.0	10.0	0.0
Soybean oil	0.0	2.0	4.0	6.0	8.0
Fish oil	0.0	2.0	4.0	6.0	8.0
Vitamin mix[1]	2.0	2.0	2.0	2.0	2.0
Mineral mix[2]	2.0	2.0	2.0	2.0	2.0
Ascorbic acid	0.5	0.5	0.5	0.5	0.5
Choline chloride	0.5	0.5	0.5	0.5	0.5
Monocalcium phosphate	0.5	0.5	0.5	0.5	0.5
Attractant[3]	2.5	2.5	2.5	2.5	2.5
Microcrystalline cellulose	0.0	6.0	12.0	18.0	24.0
Proximate composition					
Crude protein	49.9	49.1	49.1	47.5	47.5
Crude lipid	0.6	3.9	7.3	11.7	15.8
Ash	3.2	3.3	3.5	3.2	3.2
Gross energy (KJ/kg)	21.8	21.7	22.0	22.5	22.5

[1] Vitamin premix (mg or g/kg diet): thiamin 25 mg; riboflavin, 45 mg; pyridoxine HCl, 20 mg; vitamin B_{12} (1%), 10 mg; vitamin K_3, 10 mg; inositol, 800 mg; pantothenic acid, 60 mg; niacin, 200 mg; folic acid, 20 mg; biotin (2%), 60 mg; retinol acetate, 32 mg; cholecalciferol, 5 mg; alpha-tocopherol (50%), 240 mg; wheat middling, 14.47 g.

[2] Mineral premix (mg or g/kg diet): $MgSO_4 \cdot 7H_2O$, 1200 mg; $CuSO_4 \cdot 5H_2O$, 10 mg; $ZnSO_4 \cdot H_2O$, 50 mg; $FeSO_4 \cdot H_2O$, 80 mg; $MnSO_4 \cdot H_2O$, 45 mg; $CoCl_2 \cdot 6H_2O$ (1%), 50 mg; $NaSeSO_3 \cdot 5H_2O$ (1%), 20 mg; $Ca(IO_3)_2 \cdot 6H_2O$ (1%), 60mg; zoelite, 18.485 g.

[3] Attractant: glycine and betaine.

Different levels of soybean oil and fish oil were used in the experimental diets to obtain graded lipid levels of 0% (control), 4%, 8%, 12%, and 16%. The content of corn starch and microcrystalline cellulose were adjusted correspondingly and adjusted to 100%. The analyzed lipid contents of the experimental diets were: 0.6%, 3.9%, 7.3%, 11.7%, and 15.8%, and the corresponding diets were named L0.6%, L3.9%, L7.3%, L11.7%, and L15.8% respectively.

Ingredients were ground into fine powder and sieved through 200 μm mesh. All ingredients were thoroughly mixed with the oils, and water was added to produce stiff dough. The dough was then pelleted with an experimental feed mill and dried for 12 h in a ventilated oven at 45 °C. Once dried, the diets were crumbled to 3.0×5.0 mm for trial I, to 5.0×6.0 mm for trial II, and then stored at -15°C until used.

Experimental procedure. Japanese seabass of two different sizes were used in two feeding trials respectively.

Feeding trial I: Young-of-the-year (YOY) Japanese seabass with initial body weight of 34.26±0.37 g (the small fish) were obtained from Tianyuan Aquaculture Co., Ltd. (Yantai, China). Prior to the start of the experiment, the juveniles were acclimated in indoor concrete ponds (3.0×3.0×0.5 m) with flowing seawater and fed a low-lipid commercial diet for 2 weeks. At the onset of the feeding trial, the fish were fasted for 24 h, anesthetized with eugenol (1:10, 000) (Shanghai Reagent, Shanghai, China) and weighed. The fish were randomly distributed into 15 cylindrical glass fiber tanks (1.2×0.5 m), each stocked with 15 fish. Each diet was randomly assigned to triplicate tanks. Fish were hand-fed to apparent satiation twice daily (07:00 and 17:30). Feces were siphoned 1 h before feeding and feed remnants were collected after feeding. The feeding trial lasted 10 weeks. During the experimental period, the temperature ranged from 20 to 24°C, salinity from 25‰ to 30‰ and dissolved oxygen was approximately 7.5±0.1 mg/l.

Feeding trial II: One year old Japanese seabass, initial body weight 343.3±10.0g (the large fish) were reared in floating sea cages (1.5×1.5×2.0 m). Fish were acclimated and grouped according to the procedure described for feeding trial I. Each diet was randomly assigned to triplicate cages and each cage was stocked with 12 fish. Fish were hand-fed to apparent satiation twice daily (06:30 and 17:00) for 10 weeks. During the experimental period, the temperature ranged from 19 to 24.5°C, salinity from 29‰ to 33‰ and the dissolved oxygen was approximately 7 mg/l.

Analysis and measurement. At the end of the feeding trials, the fish were fasted for 24 h and weighed. Three fish per tank/cage were then collected to determine their proximate body composition. Samples of liver and serum were also collected from another 5 fish from each tank/cage.

Proximate composition analysis on experimental diets and fish body were performed by the standard methods of AOAC. Samples of diets and fish were dried to a constant weight at 105 °C to determine moisture. Protein was determined by measuring nitrogen (N × 6.25) by the Kjeldahl method; lipid by petroleum ether extraction by the Soxhlet procedure; ash by combustion at 550 °C.

The lipid concentration in the liver was analyzed by extracting 0.1 g liver sample to which 6 ml chloroform-methanol solution (2:1, chloroform:methanol) was added. 1.2 ml of $CaCl_2$ solution (1.6%) was then added to the liver sample solution and left to settle overnight. The supernatant liquid was then discarded, the remaining precipitate was dried with nitrogen and the lipid residue weighed. The concentrations of triglyceride and cholesterol in the serum were determined with commercial kits (Dongou Diagnosis Co. Ltd., Wenzhou, China) following the user manual.

Calculations and statistical methods. The following variables were calculated:

Specific growth rate (SGR) = (Ln W_t - Ln W_0) × 100/t

Survival rate (%) = N_t × 100/N_0

where W_t and W_0 were final and initial fish weight respectively; N_t and N_0 were final and initial number of fish respectively; t is the duration of the feeding trial.

All data were subjected to one-way analysis of variance in SPSS 16.0 for Windows. All percentage data were arcsine transformed before analysis. Differences between the

means were tested by Tukey's multiple range test. The level of significance was chosen at $P<0.05$ and the results are presented as means±SEM.

According to the coefficient of determination (r^2) among the models tested, a second-order polynomial model was used to estimate the optimum dietary lipid requirements of fish in the experiment, based on the special growth rate.

Results

Growth performance and survival. In both feeding trials, at first there was a significant ($P<0.01$) increase in the specific growth rate (SGR) with the increase of dietary lipid level from 0.6% to 7.3% however it subsequently leveled off (Table 2).

Table 2. Growth performance and survival of Japanese seabass fed graded levels of dietary lipid (means±SEM , n=3)*

Growth response	*Dietary lipid levels*					*P value*
	0.6%	*3.9%*	*7.3%*	*11.7%*	*15.8%*	
Feeding trial I						
Initial body weight g			34.26±0.37			
Final body weight g	48.32±0.41^{c}	51.20±0.22^{a}	50.85±0.56^{a}	49.57±0.13^{b}	47.5±0.25^{c}	0.000
Specific growth rate %/d	0.60±0.03^{bc}	0.68±0.05^{ab}	0.71±0.02^{a}	0.65±0.02^{abc}	0.56±0.03^{c}	0.000
Survival %	86.66±6.35	82.22±7.25	75.56±7.70	86.67±5.00	88.89±10.18	0.194
Feeding trial II						
Initial body weight g			343.3±10.0			
Final body weight g	366.33±9.07^{b}	392.67±34.07^{ab}	423.00±21.93^{a}	418.67±4.16^{a}	399.33±12.10^{ab}	0.032
Specific growth rate %/d	0.09±0.04^{b}	0.23±0.07^{ab}	0.30±0.07^{a}	0.28±0.01^{a}	0.22±0.04^{ab}	0.007
Survival %	91.00±8.54	92.33±7.51	94.33±9.81	97.33±4.62	94.67±4.62	0.853

*Values in the same row sharing a same superscript letter are not significantly different determined by Tukey's test ($P>0.05$).

In feeding trial I, the highest final body weight (FBW) was observed in fish fed L3.9% while in trial II, the highest FBW was observed in the L7.3% group. Based on the SGR, the optimal lipid requirement of Japanese seabass in feeding trials I and II, estimated by second-order polynomial model, was 7.4% and 9.9% respectively (Fig. 1, 2).

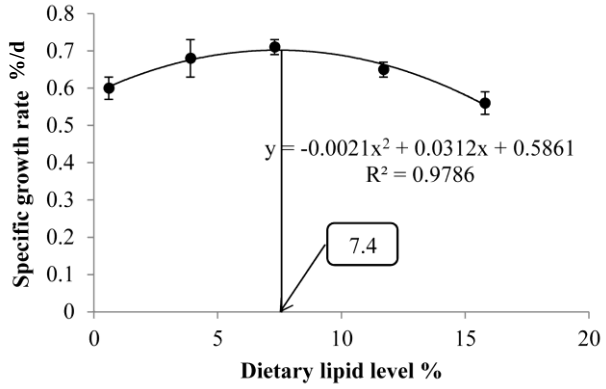

Fig. 1. Requirement of dietary lipid based on specific growth rate of Japanese seabass, *Lateolabrax japonicus* with average initial body weight of 34.26±0.37 g.

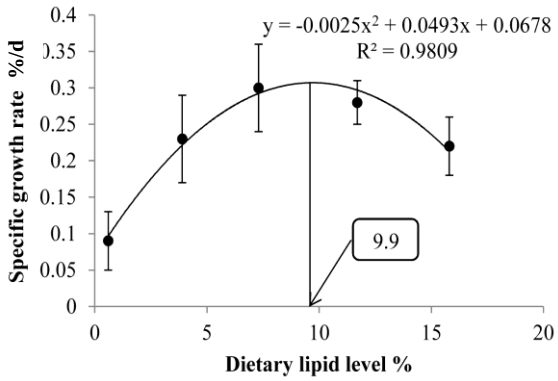

Fig. 2. Requirement of dietary lipid based on specific growth rate of Japanese seabass, *Lateolabrax japonicus* with average initial body weight of 343.3±10.0 g.

No significant difference in survival was observed among dietary treatments in both feeding trials ($P>0.05$). The survival of feeding trial I (75.56~88.89%) was slightly lower than that of feeding trial II (91.00~97.33%).

Body composition. In feeding trial I, no significant differences (P>0.05) were observed in fish body protein concentration among dietary treatments (Table 3).

Table 3. Body composition of Japanese seabass fed experimental diets (means±SEM , n=3; of wet weight)*

Body composition	*Dietary lipid levels*					*P value*
	0.6%	*3.9%*	*7.3%*	*11.7%*	*15.8%*	
Feeding trial I						
Moisture %	72.64±0.82	72.55±1.33	73.28±0.68	72.92±0.18	72.10±0.92	0.570
Crude protein %	17.08±0.14	16.59±0.08	16.64±0.16	16.70±0.10	16.87±0.01	0.053
Crude lipid %	4.14±0.2^{cd}	3.82±0.09^{d}	4.38±0.13^{c}	4.99±0.30^{b}	5.75±0.04^{a}	0.000
Ash %	5.60±0.06^{b}	6.18±0.08^{a}	5.31±0.09^{c}	4.99±0.09^{d}	5.29±0.08^{c}	0.000
Feeding trial II						
Moisture %	69.64±0.65	68.92±0.28	69.28±1.65	69.04±0.68	70.90±0.98	0.160
Crude protein %	15.75±0.22^{c}	16.24±0.28^{bc}	16.61±0.09^{ab}	17.09±0.15^{a}	16.88±0.35^{a}	0.000
Crude lipid %	9.22±0.07^{b}	9.58±0.26^{ab}	10.35±0.23^{a}	10.83±0.38^{a}	10.74±0.11^{a}	0.000
Ash %	3.98±0.26^{c}	4.90±0.37^{ab}	5.46±0.17^{a}	4.37±0.37^{bc}	4.14±0.05^{c}	0.002

*Values in the same row with the same superscript letter are not significantly different determined by Tukey's test (P>0.05).

The body lipid concentration [3.82%-5.75% of dry weight (d.w.)] increased significantly (P<0.01) with increasing dietary lipid level. The ash concentration ranked among dietary groups as follows: L3.9%>L0.6%>L7.3% and L15.8%>L11.7%. These differences were significant (P<0.01).

In feeding trial II, the body protein and lipid concentration (lipid, 9.22%-10.83% d.w.) increased significantly (P<0.01) with increasing dietary lipid level but no significant differences were observed among treatments L7.3%, L11.7%, and L15.8% (P>0.05). The ash content in fish body increased significantly (P<0.01) with increasing dietary lipid levels from 0.6% to 7.3% and thereafter declined significantly (P<0.01).

No significant differences were observed in fish body moisture concentration among dietary groups in both feeding trials (P>0.05).

Tissue lipid depositions. In feeding trial I, the liver lipid concentration significantly increased (P<0.01) with increasing dietary lipid content (Table 4).

Table 4. Tissue lipid concentration of Japanese seabass fed experimental diets (means±SEM,n=3)*

Dietary lipid levels	*Liver lipid concentration % d.w.***	*Serum triglyceride mmol/l*	*Serum cholesterol mmol/l*
Feeding trial I			
0.6%	23.80±0.48^{c}	3.65±0.18^{b}	2.40±0.21^{c}
3.9%	26.94±1.14^{b}	4.97±0.19^{a}	3.39±0.09^{a}
7.3%	28.61±1.02^{ab}	4.94±0.24^{a}	3.15±0.22^{ab}
11.7%	30.80±1.62^{a}	4.21±0.19^{ab}	2.33±0.23^{c}
15.8%	31.32±0.42^{a}	4.32±0.13^{ab}	2.30±0.17^{c}
P value	0.000	0.003	0.001
Feeding trial II			
0.6%	46.30±1.63^{b}	3.35±0.10^{b}	6.25±0.27^{b}
3.9%	42.36±1.08^{c}	3.62±0.16^{b}	6.68±0.06^{ab}
7.3%	43.18±2.08^{bc}	4.21±0.20^{a}	7.54±0.38^{a}
11.7%	53.93±1.45^{a}	4.66±0.05^{a}	6.78±0.69^{ab}
15.8%	50.49±0.19^{a}	4.33±0.36^{a}	7.35±0.37^{a}
P value	0.000	0.000	0.018

*Values in the same column with the same superscript letter are not significantly different determined by Tukey's test (P>0.05).
**d.w.: of dry weight.

Fish fed L0.6% showed significantly lower liver lipid concentration than fish fed other diets, and fish fed L11.7% and L15.8% showed significantly higher values than fish fed

L0.6% and L3.9%. The serum triglyceride concentration was significantly higher (P<0.01) in fish fed L3.9% and L7.3% compared to fish fed L0.6%. The highest serum cholesterol concentration was observed in fish fed L3.9% and the concentration in fish fed L3.9% and L7.3% was significantly higher (P<0.05) than those in fish fed other diets.

In feeding trial II, the liver lipid concentration in fish fed L11.7% and L15.8% was significantly higher (P<0.01) than those in fish fed other diets, and fish fed L3.9% showed a significantly lower value (P<0.01) than those in fish fed L0.6%, L11.7%, and L15.8%. The serum triglyceride concentrations in fish fed L7.3%, L11.7%, and L15.8% were significantly higher (P<0.01) than those in fish fed L0.6% and L3.9%. Fish fed L7.3% and L15.8% showed significantly higher (P<0.05) serum cholesterol concentrations than fish fed L0.6%.

Discussion

In the present study, the dietary lipid requirement of the small Japanese seabass juveniles (7.4%, d.w.) was lower than that of the large fish (9.9%, d.w.), based on specific growth rate. This indicates that the lipid requirement of Japanese seabass was influenced by fish size. Similar results were also observed in white sturgeon *Acipenser transmontanus*, which showed that the optimal dietary lipid levels for larvae range from 120 to 200 g/kg (Gawlicka et al., 2002; Guo et al., 2011) but the subyearlings grew well when dietary lipid content ranged from 258 to 357 g/kg (Hung et al., 1997). Studies on channel catfish, Atlantic salmon, and Atlantic halibut have suggested that larger fish appear to have lower protein requirement and higher energy requirement (Winfree and Stickney, 1984; Einen and Roem, 1997; Grisdale-Helland et al., 1998; Storebakken, 2002; Hamre et al., 2003), and thus require more dietary lipid which is a better energy source than carbohydrate for fish. The increasing energy requirement with increasing fish size could be partly attributed to the increase in the percentage of food energy lost to feeding metabolism, i.e., respiration during the specific dynamic action (Brett and Groves, 1979; Keckeis et al., 2001; Azevedo et al., 2004). A study of juvenile barramundi *Lates calcarifer* demonstrated that larger fish (142 g) had higher energy expenditure when compared to small fish (21 g), (Bermudes et al., 2010). Additionally, in the present study, the difference in experimental conditions may have also contributed to the difference in lipid requirement between the small and large Japanese seabass. Compared to the small fish reared in indoor tanks, the large fish reared in sea floating cages swam more actively due to larger area, and waves, and consequently required more energy for swimming.

Higher body lipid content in larger fish was also observed in other studies (Degani et al., 1986; Ortega and Navarro, 1988). A study of Atlantic salmon reported a high correlation (r^2=0.64, P<0.05) between muscle lipid concentrations and fish size (Hemre and Sandnes, 1999). In some salmonid species, lipolytic action of growth hormone was reported (Cameron et al., 2002). It can be speculated that greater growth hormone secretion in fish at earlier ontogenetic stages could contribute to their lower body lipid content. Unlike smaller fish that showed no significant differences among dietary treatments, the whole-body protein content of the larger fish increased in relation to dietary lipid levels. This may be because larger fish require more dietary lipid than smaller ones (Torstensen et al., 2001; Tibbets et al., 2005; Takakuwa et al., 2006). The whole-body ash concentration was influenced by the dietary lipid levels in fish of both sizes but these results are difficult to explain.

Similarly whole-body lipid content and lipid deposition in the liver increased in relation to dietary lipid levels in both feeding trials. Larger fish showed higher liver lipid concentrations than small ones although the serum triglyceride concentrations were lower than those in the smaller fish. This could suggest that the lower tissue lipid deposition in smaller fish is an indication that these differences are due to higher lipid uptake into the tissue instead of their absorption in the digestive tract.

The serum cholesterol concentration in the small fish was much lower than in the larger ones. This may suggest that there is reduced active transport of lipids in smaller fish (Du et al., 2005). On the other hand in the smaller fish, serum cholesterol

concentration was significantly reduced by high levels of dietary lipid. This may be due to high dietary lipid levels that could have caused an impairment of the lipid transport system.

In conclusion, the dietary lipid requirement for the small (initial body weight 34.26±0.37 g) and large (initial body weight 343.3±10.0 g) Japanese seabass was 7.4% and 9.9% respectively. The present results provide useful data for feed formulation in the farming of Japanese seabass.

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