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Suitable Dietary Protein/Lipid Ratio for Hybrid, Female Red Sea Bream *Pagrus major* and Male Black Sea Bream *Acanthopagrus schlegeli* in the Juvenile Stage, Compared with Red Sea Bream

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Abstract

To determine a suitable dietary protein/lipid (CP/CL) ratio in the early juvenile stages of hybrid porgy (F_1), female red sea bream (RSB) × male black sea bream, five diets with various CP/CL ratios—60/7, 55/12, 51/17, 46/23, and 41/28—were prepared and provided to juveniles in triplicate. At the smaller juvenile stage, F_1, weighing 0.32 g, a significantly higher specific growth rate (SGR) and feed efficiency (FE) were seen with 60/7 and 55/12 diets. However, in RSB weighing 0.26 g, SGR and FE were higher with the 60/7 diet than the other diets at 21°C. At the larger juvenile stage, F_1, weighing 3.7 g, there was no significant difference in SGR or FE among the diets, but RSB weighing 4.0 g fed 60/7, 55/12, and 51/17 diets had higher SGR and FE than 46/23 and 41/28 diets at 24°C. Moreover, survival and apparent nutrient retention of F_1 at both stages were significantly higher than those in RSB. These results indicate that both F_1 and RSB weighing ca. 0.3 g require a higher dietary CP/CL than those weighing ca. 4 g. Additionally, F_1 in both trials showed the suitability of a lower dietary CP/CL than RSB, indicating that mass production of F_1 juveniles will be more economical than RSB.

Key words: Hybrid sea bream, Red sea bream, Black sea bream, Dietary protein/lipid, Early juvenile stages

Introduction

Hybridization between fishes is a practical and effective tool for improving the production of fish; the technique has also been used with domestic animals since prehistoric times. Hybrid striped bass *Morone chrysops* × *M. saxatilis*, tilapia *Oreochromis* spp., and channel catfish *Ictalurus punctatus* × *I. furcantus* are economically available, attributable to the rapid growth of industrial aquaculture production worldwide (Swann et al., 1994; Brecka et al., 1995; Riche et al., 2004; Rawles et al., 2006, Phelps et al., 2007; Ponzoni et al., 2007).

Red sea bream *Pagrus major* (RSB) and black sea bream

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Acanthopagrus schlegeli (BSB) are major aquaculture species in East Asia, China, Japan, and Korea. However, these two porgies are classified into different genera, and differ in their ecology and physiology, such as habitats, avoidance of ultraviolet radiation, development of sensory organs, energy allocation patterns, intestinal microflora, and protandrous sex reversal (Muroga et al., 1987; Kinoshita and Tanaka, 1990; Chang et al., 1995; Mana and Kawamura, 2002; Tang et al., 2003; Fukunishi et al., 2006; An et al., 2008). Harada (1991) and Murata (1998) found that the hybrid (F_1) between female RSB and male BSB had higher tolerance against lower water temperature, dissolved oxygen (DO), and salinity than RSB. Murata (1998) investigated the genetic and cytogenetic characteristics of F_1 and found the same number of chromosomes between F_1 and parent species, and that F_1 had hybrid sterility. Additionally, Murata (1998) and Kim et al. (2011) showed faster growth of F_1 juveniles at one year old, compared with RSB and BSB. It seems likely that the F_1 will be an appropriate aquaculture species in Korea and East China where the seawater temperature often falls below 10°C, resulting in mass mortality of RSB. However, there are few reported studies regarding the nutritional requirement of F_1 for establishing an aquaculture industry (Kim et al., 2009a, 2009b).

Dietary protein and lipid levels are essential factors for efficient fish production (National Research Council, 1993). It is known that some hybrids have similar or different dietary protein and lipid requirements compared with their parents (Brecka et al., 1995; Twibell et al., 2003). Moreover, there are some suggestions that the hybrids showed heterosis in growth, survival and disease tolerance, distinguishable from their parent species (Logan, 1968; Blanc and Chevassus, 1982; Tuncer et al., 1990; Brecka et al., 1995; Moreau and Pauly, 1999; Harel and Place, 2003).

Given this background, this investigation was conducted to determine a suitable dietary protein/lipid ratio for early F_1 juveniles, which would be useful information for further promoting and developing the F_1 aquaculture industry in East China and Korea.

Materials and Methods

Fish production

The F_1 were produced artificially by cross-breeding of mature female RSB of the Kinki University strain (4 years old, 4.5 ± 0.4 kg, n = 5) and male BSB (2 years old, 310 ± 89.4 g, n = 4), reared in the Fish Nursery Center of Kinki University, Uragami and Shirahama, in May 2006. The eggs and milt from RSB, of the same group used for F_1, were artificially fertilized to produce RSB. After hatching, F_1 and RSB larvae were fed with rotifer *Brachionus plicatilis* and *Artemia salina* (INVE Aquaculture, Dendermonde, Belgium) from 3 to 29 and from 22 to 40 days after hatching (dAH), respectively. The rotifer and *Artemia* were enriched with *n*-3 highly unsaturated fatty acids (*n*-3 HUFA), using a commercial enrichment product (Marine Glos; Nisshin Marinetech Co., Yokohama, Japan). The juveniles from 28 dAH were fed with commercial pellets (Marubeni Nisshin Feed Inc., Tokyo, Japan), increasing pellet diameter with their growth, until the start of the trials.

Dietary formula and fish

Five diets were prepared with various ratios of crude protein/lipid (CP/CL)—60/7, 55/12, 51/17, 46/23, and 41/28—as shown in Table 1. Brown fish meal (crud protein and lipid contents were ca. 69.2 and 9.4%, respectively) and wheat gluten and fish oil were supplied as the main protein and lipid sources, respectively. All experimental diets were prepared by mixing the ingredients appropriately and adding 30% tap water externally, and were then pelletized using a laboratory pellet machine. The pellets were freeze-dried and sieved to obtain two particle sizes for the two separate trials using F_1 and RSB of different sizes. The pellet particle size was 355-425 μm for smaller F_1 and RSB juveniles, weighing 0.32 ± 0.01 g and 0.26 ± 0.01 g at 43 and 41 dAH, respectively. For larger F_1 and RSB juveniles, weighing 3.67±0.06 and 4.03±0.07 g at 75 and 73 dAH, respectively, pellets of 1.9 mm in diameter were prepared and freeze-dried. The diets for both trials were prepared just before the start of each trial and stored in a freezer at –20°C until used.

Rearing conditions

There were two trials in this study. Both trials were conducted under a photoperiod cycle of 14-h light and 10-h dark.

In Trial 1, 50 smaller juveniles of F_1 and RSB each were introduced into 40-L rectangular tanks. This trial was conducted in triplicate for each diet, and juveniles were fed four times daily until apparent satiation, at 08:00, 11:00, 14:00, and 17:00-h, for 2 weeks. Water temperature and DO were 20.6 ± 0.7°C and 6.5 ± 2.1 mg/L, respectively.

In Trial 2, 20 larger juveniles of F_1 and RSB each were introduced into 40-L rectangular tanks. This trial was also conducted in triplicate for each diet, and juveniles were fed three times daily, at 09:00, 13:00, and 17:00-h, until apparent satiation for 4 weeks. Water temperature and DO were 24.3 ± 2.2°C and 7.6 ± 1.7 mg/L, respectively.

Measurements and assays

At the beginning and end of each trial, juveniles were weighed and sampled after fasting for 24-h. Growth performances of the fishes were evaluated at the end of each trial and compared among the treatments. Growth performance was estimated using daily feeding rate, survival, specific growth rate (SGR), feed efficiency (FE), condition factor (CF), protein efficiency ratio (PER), and apparent protein, lipid, and energy

retention efficiencies (PRE, LRE, and ERE). To evaluate the whole body proximate composition, 10 juveniles from Trial 1 and 5 from Trial 2 were collected from each tank. Also, six juveniles from each tank were sacrificed and dissected for various organ samples to measure hepatosomatic indices (HSI) and viscerosomatic indices (VSI). All samples were immediately transferred to a freezer (–40°C) until analysis.

At the end of Trial 2, blood samples were obtained from the caudal aorta of three fishes per tank with heparinized syringes. Hematocrit levels and hemoglobin content (Hb) were determined using a microhematocrit method (Brown, 1980) and a commercial kit (Wako Pure Chemical Industries, Osaka, Japan), respectively. For assay of hepatopancreatic glutamate-oxalacetate transaminase (GOT) and glutamate-pyruvate transaminase (GPT) activities, the hepatopancreas removed from juveniles was homogenized with nine volumes of deionized water (v/w) using a Potter-Elvehjem glass homogenizer. After centrifugation of the homogenate (10,000 *g*, 15 min), the resulting supernatant was used for assaying GOT and GPT activities using a commercial kit (Wako Pure Chemicals Industries). Enzyme protein was assayed according to Lowry et al. (1951). The enzyme assay was conducted at 30°C for 20 min, and the activity was expressed as international units per mg protein, as specific activity.

The proximate compositions of the diets and whole bodies were assayed using Association of Official Analytical Chemists methods (1995). Sugar contents of diets were measured by the phenol-sulfuric acid method (Hodge and Hofreiter, 1962). The energy concentrations of diets and whole bodies were determined directly using an automatic oxygen bomb calorimeter (IKA-Werke, Staufen, Germany).

Statistical analysis

Data obtained from both trials were subjected to a two-way analysis of variance (ANOVA) to evaluate the effect of experimental diets, fish species, and interactions. Where significant differences were found, the means within and among the treatments were further compared using Duncan's new multiple range test followed by one-way ANOVA at the $P < 0.05$ significance level (Harter, 1960). All statistical analyses were conducted using the SPSS software (ver. 17.0 for Windows; SPSS Inc., Chicago, IL, USA).

Results

Trial 1

The growth performance data from Trial 1 are presented in Table 2. Two-way ANOVA showed that different dietary CP/CL ratios had significant effects on final mean body weight, daily feeding rate, weight gain, SGR, and FE (two-way ANOVA, $P < 0.05$). All growth parameters except SGR showed

Table 1. Feed formula and proximate composition of the experimental diets

	CP/CL				
	60/7	**55/12**	**51/17**	**46/23**	**41/28**
Ingredients (%)					
Brown fish meal*	69	62	54	46	38
Wheat gluten meal	10	10	10	10	10
Fish oil†	-	5	10	15	20
Cellulose	-	2	5	8	11
α-Starch	10	10	10	10	10
Vitamin mixture‡	5	5	5	5	5
Mineral mixture‡	5	5	5	5	5
Choline chloride	1	1	1	1	1
Proximate composition (%, dry matter basis)					
Crude protein	59.5	54.8	50.8	45.8	40.6
Crude lipid	6.5	11.5	16.9	22.6	28.1
Sugar	12.1	13.1	13.6	13.9	14.6
Crude ash	16.6	15.3	14.1	12.9	11.8
Gross energy (MJ/kg)	18.0	19.4	20.4	21.6	22.3
Calculated P/E ratio (g/MJ)§	33.1	28.3	24.9	21.2	18.2

*Itochu Feed Mills Co., Ltd., Japan (crud protein, lipid were ca. 69.2% and 9.4%, respectively).
†Nice feed oil; Marubeni Co., Ltd., Japan.
‡Halver's mixture (1957).
§Protein/energy ratio (P/E ratio), calculation based on crude protein and gross energy values determined for the experimental diets.

Table 2. Effect of feeding diets with different protein/lipid levels on the growth performance of juvenile F_1 and RSB (Trial 1)

		CP/CL*					SEM	*P*-value†		
		60/7	55/12	51/17	46/23	41/28		Diet	Fish	D × F
Initial mean body weight (g)	F_1	0.32	0.32	0.31	0.33	0.32	0.005	-	-	-
	RSB	0.26	0.26	0.25	0.25	0.26	0.007			
Final mean body weight (g)	F_1	1.01^{a}	0.97^{a}	0.90^{b}	0.88^{b}	0.79^{c}	0.021	0.000	0.000	0.000
	RSB	1.02^{a}	0.86^{b}	0.77^{b}	0.65^{c}	0.51^{d}	0.020			
Daily feeding rate (%)‡	F_1	5.2^{b}	5.2^{b}	5.5^{ab}	5.4^{ab}	5.8^{a}	0.140	0.003	0.025	0.462
	RSB	5.2^{a}	5.5^{b}	6.0^{b}	5.5^{c}	6.5^{d}	0.255			
Survival rate (%)	F_1	99.3^{ab}	99.3^{ab}	100.0^{a}	98.7^{ab}	97.3^{b}	0.541	0.463	0.000	0.472
	RSB	67.0	64.3	62.7	56.6	65.0	5.107			
Weight gain (%)§	F_1	219.1^{a}	205.7^{ab}	190.9^{b}	163.2^{c}	144.7^{c}	6.120	0.000	0.000	0.043
	RSB	181.5^{a}	130.5^{b}	111.6^{bc}	71.1^{cd}	51.5^{d}	10.570			
SGR (%/day)¶	F_1	8.3^{a}	8.0^{ab}	7.6^{b}	7.0^{c}	6.6^{c}	0.160	0.000	0.591	0.000
	RSB	9.8^{a}	8.5^{b}	8.0^{b}	6.7^{c}	5.0^{d}	0.186			
FE (%)**	F_1	144.1^{a}	139.1^{ab}	126.3^{bc}	119.8^{c}	103.4^{d}	4.796	0.000	0.000	0.139
	RSB	120.6^{a}	97.5^{ab}	81.7^{b}	66.3^{bc}	45.4^{c}	7.897			
PER††	F_1	2.4	2.5	2.5	2.6	2.5	0.098	0.094	0.000	0.018
	RSB	2.0^{a}	1.8^{ab}	1.6^{ab}	1.4^{ab}	1.1^{b}	0.161			

RSB, red sea bream *Pagrus major*; CP/CL, ratios of crude protein/lipid; SEM, standard error of means.
*Values (mean, $n = 3$) in a row sharing same superscripts (across diets) are not significantly different ($P > 0.05$, ANOVA).
†Results from two-way analysis of variance (ANOVA).
‡Daily feeding rate (%) = total feed intake (g) /{experimental days × [initial mean body weight (g) + final mean body weight (g)/2] × [(initial fish number + final fish number)/2]} × 100.
§Weight gain = 100 × [final fish weight (g) + dead fish weight (g) – initial fish weight (g)]/initial fish weight (g).
¶Specific growth rate (SGR) = 100 × {ln [final body weight (g)] - ln [initial boy weight (g)]}/experimental days.
**Feed efficiency (FE) = 100 × [weight gain (g)/feed intake (g)].
††Protein efficiency ratio (PER) = weight gain (g)/protein intake (g).

Table 3. Carcass compositions and apparent protein, lipid and energy retentions of juvenile F_1 and RSB fed experimental diets with different protein/lipid level (Trial 1)

		Initial	CP/CL*					SEM	*P*-value†		
			60/7	55/12	51/17	46/23	41/28		Diet	Fish	D × F
Proximate composition (%)											
Moisture	F_1	78.8	78.1^{a}	78.1^{a}	75.8^{ab}	77.4^{ab}	75.5^{b}	0.703	0.054	0.013	0.250
	RSB	79.5	79.5	78.4	77.5	77.3	78.6	0.734			
Protein	F_1	13.5	14.6	13.5	13.8	14.0	14.2	0.629	0.225	0.008	0.428
	RSB	13.2	14.1^{a}	13.0^{ab}	13.2^{ab}	12.7^{ab}	12.0^{b}	0.408			
Lipid	F_1	3.7	2.6^{d}	3.6^{c}	4.8^{b}	5.2^{ab}	5.8^{a}	0.276	0.000	0.026	0.027
	RSB	3.0	3.9^{c}	4.8^{bc}	4.6^{ab}	3.6^{a}	2.7^{ab}	0.293			
Ash	F_1	2.9	3.9^{a}	3.4^{ab}	3.6^{ab}	3.4^{b}	3.6^{ab}	0.136	0.334	0.000	0.602
	RSB	3.2	4.1	4.1	4.1	4.0	3.9	0.149			
Energy (kJ/g dry body)	F_1	21.9	20.2^{b}	21.8^{ab}	22.1^{ab}	22.5^{ab}	22.9^{a}	0.650	0.010	0.001	0.430
	RSB	21.1	19.7^{b}	20.6^{ab}	21.3^{a}	21.2^{a}	20.5^{ab}	0.228			
Apparent nutrient retention (%)‡											
PRE	F_1		36.6	34.2	34.7	37.4	37.4	1.390	0.004	0.000	0.001
	RSB		29.6^{a}	22.9^{ab}	21.2^{ab}	17.4^{bc}	10.6^{c}	2.086			
LRE	F_1		45.3^{a}	42.3^{ab}	39.8^{b}	32.1^{c}	26.4^{d}	1.293	0.000	0.000	0.002
	RSB		46.8^{a}	34.6^{b}	29.2^{bc}	21.8^{c}	9.0^{d}	2.054			
ERE	F_1		34.7^{a}	34.8^{a}	35.4^{a}	29.6^{b}	29.2^{b}	1.182	0.000	0.000	0.028
	RSB		26.0^{a}	22.9^{ab}	20.8^{ab}	17.0^{b}	9.1^{c}	1.733			

RSB, red sea bream *Pagrus major*; CP/CL, ratios of crude protein/lipid; SEM, standard error of means.
*Values (mean, $n = 3$) in a row sharing same superscripts are not significantly different ($P > 0.05$).
†Results from two-way analysis of variance.
‡Apparent protein (PRE), lipid (LRE) and energy (ERE) retention efficiency = 100 × [gain (g)/intake (g)].

significant differences between F_1 and RSB, where F_1 showed significantly higher performances in final mean body weight, weight gain, FE, and PER compared with RSB (two-way ANOVA, $P < 0.05$).

In contrast, final mean body weight, weight gain, SGR, and FE showed decreasing trends with decreasing dietary CP/CL ratios for both species ($P < 0.05$). For F_1, the final mean body weight, weight gain, SGR, and FE on 60/7 and 55/12 diets were significantly higher than those on 46/23 and 41/28 diets, while all growth parameters on the 60/7 diet were significantly higher than those on 51/17, 46/23, and 41/28 diets for RSB ($P < 0.05$). Overall, the survival rate was significantly higher in F_1 than RSB ($P < 0.05$).

There was no difference in carcass proximate compositions between F_1 and RSB at the start of the rearing trial (Table 3). At the end of Trial 1, two-way ANOVA showed significant differences in all proximate composition parameters, while both diet and species and their interaction had significant effects on apparent nutrients and energy retention ($P < 0.05$). Generally, nutrient retention values were higher in F_1 than RSB, LRE on the 60/7 diet.

Although there was no difference in whole body protein content or PRE among the dietary treatments in F_1 ($P > 0.05$), whole body lipid and energy contents exhibited a significantly increasing trend with decreasing dietary CP/CL ratio, with lower LRE and ERE in both F_1 and RSB ($P < 0.05$). The PRE, LRE, and ERE of RSB decreased markedly with decreasing dietary CP/CL ratio, being significantly higher on the 60/7 diet than the 46/23 or 41/28 diets ($P < 0.05$).

Trial 2

At the end of Trial 2, the experimental diets had significant effects on final mean body weight, weight gain, SGR, FE, and PER for both species (two-way ANOVA, $P < 0.05$) (Table 4). Also, survival, SGR, weight gain, FE, and PER showed significant differences between F_1 and RSB (two-way ANOVA, $P < 0.05$), where F_1 showed higher trends in almost all cases than RSB.

There was no significant difference in final mean body weight, daily feeding rate, survival, weight gain, SGR, or FE among the dietary treatments in F_1. In contrast to Trial 1, the PER of F_1 showed an increasing trend with decreasing dietary CP/CL. The final mean body weight, weight gain, and SGR of RSB were significantly higher on the 60/7 diet than the other diets ($P < 0.05$). However, the PER increased with decreasing dietary CP/CL, as did F_1 (Table 4).

The final whole body proximate compositions, except ash content, were significantly affected by dietary treatments in both species (two-way ANOVA, $P < 0.05$; Table 5). However, the whole body protein, lipid and energy contents were not significant difference between the fish species (two-way ANOVA, $P > 0.05$). Both diet and fish species had significant effects on LER and ERE, with higher values in F_1 than RSB

Table 4. Effect of feeding diets with different protein/lipid levels on the growth performance of juvenile F_1 and RSB (Trial 2)*

		CP/CL					SEM	*P*-value		
		60/7	55/12	51/17	46/23	41/28		Diet	Fish	D × F
Initial mean body weight (g)	F_1	3.6	3.7	3.7	3.6	3.7	0.032	-	-	-
	RSB	4.1	4.1	4.0	4.0	4.0	0.040			
Final mean body weight (g)	F_1	12.8	13.1	12.8	12.5	12.3	0.344	0.000	0.277	0.003
	RSB	14.2^{a}	13.2^{b}	12.8^{b}	11.5^{c}	10.5^{d}	0.235			
Daily feeding rate (%)	F_1	2.9	2.8	3.0	3.1	3.0	0.064	0.545	0.288	0.714
	RSB	3.1	2.9	3.0	3.0	3.0	0.098			
Survival rate (%)	F_1	98.3	96.7	98.3	96.7	98.3	2.000	0.483	0.047	0.542
	RSB	95.0	90.0	88.3	95.0	98.3	4.370			
Weight gain (%)	F_1	249.4	251.8	244.0	238.2	232.3	10.085	0.001	0.000	0.055
	RSB	243.0^{a}	207.1^{b}	202.2^{b}	182.3^{bc}	160.9^{c}	8.046			
SGR (%/day)	F_1	4.5	4.5	4.4	4.4	4.3	0.107	0.000	0.000	0.008
	RSB	4.5^{a}	4.2^{b}	4.1^{b}	3.7^{c}	3.4^{d}	0.066			
FE (%)	F_1	138.6	142.0	134.0	128.5	129.1	5.049	0.009	0.000	0.731
	RSB	129.2^{a}	124.7^{a}	123.1^{a}	114.0^{b}	108.7^{b}	2.109			
PER	F_1	3.2^{c}	3.6^{bc}	3.7^{bc}	3.9^{b}	4.5^{a}	0.132	0.000	0.000	0.039
	RSB	2.2^{c}	2.3^{bc}	2.4^{b}	2.5^{ab}	2.7^{a}	0.044			

RSB, red sea bream *Pagrus major*; CP/CL, ratios of crude protein/lipid; SEM, standard error of means; SGR, specific growth rate; FE, feed efficiency; PER, protein efficiency ratio.

*Refer to table 2 ($n = 3$, except 55/12 group of RSB is $n = 2$).

Table 5. Carcass compositions and apparent protein, lipid and energy retention of juvenile F_1 and RSB fed experimental diets with different protein/lipid level (Trial 2)*

		Initial	CP/CL					SEM	*P*-value		
			60/7	55/12	51/17	46/23	41/28		Diet	Fish	D × F
Proximate composition (%)											
Moisture	F_1	73.3	75.6^{a}	73.4^{b}	72.6^{bc}	71.4^{bc}	70.8^{c}	0.367	0.000	0.043	0.895
	RSB	73.6	74.2	71.3	71.4	71.0	69.9	1.180			
Protein	F_1	16.4	16.1^{a}	16.1^{a}	15.2^{ab}	14.6^{b}	14.2^{b}	0.249	0.000	0.826	0.959
	RSB	15.9	16.2^{a}	16.0^{ab}	15.3^{abc}	14.4^{bc}	14.3^{c}	0.299			
Lipid	F_1	5.5	3.6^{d}	5.6^{c}	7.1^{b}	8.1^{a}	8.7^{a}	0.179	0.000	0.060	0.852
	RSB	5.0	4.6^{c}	5.6^{bc}	7.2^{abc}	8.5^{ab}	9.4^{a}	0.577			
Ash	F_1	4.1	4.2	4.6	4.2	4.1	4.2	0.254	0.857	0.020	0.816
	RSB	4.7	4.8	4.7	4.6	4.7	4.5	0.202			
Energy (kJ/g dry body)	F_1	20.4	19.4^{b}	20.6^{b}	22.7^{a}	23.3^{a}	23.9^{a}	0.378	0.000	0.392	0.306
	RSB	19.9	19.8^{d}	21.1^{c}	21.9^{bc}	22.5^{ab}	23.3^{a}	0.280			
Apparent nutrient retention (%)											
PRE	F_1		37.9	42.6	38.9	39.1	42.0	1.514	0.243	0.000	0.471
	RSB		35.4	36.2	36.3	33.9	35.5	0.640			
LRE	F_1		60.7^{a}	61.7^{a}	61.9^{a}	52.1^{ab}	46.2^{b}	3.339	0.000	0.000	0.132
	RSB		55.9^{a}	46.5^{b}	39.9^{c}	37.4^{d}	35.0^{e}	0.266			
ERE	F_1		34.2^{b}	40.3^{a}	42.8^{a}	42.7^{a}	44.5^{a}	1.535	0.001	0.036	0.069
	RSB		35.9^{c}	41.1^{a}	40.3^{ab}	37.8^{bc}	39.3^{ab}	0.690			

RSB, red sea bream *Pagrus major*; CP/CL, ratios of crude protein/lipid; SEM, standard error of means; PRE, protein retention efficiency; LER, lipid retention efficiency; ERE, energy retention efficiency.
*Refer to Table 3 (n = 3, except that 55/12 group of RSB is n = 2).

Table 6. Hematological characteristics, relative organ weight to somatic weight (%), and hepatopancreatic GOT and GPT activity of juvenile F_1 and RSB fed experimental diets with different protein/lipid level (Trial 2)*

		CP/CL†					SEM	*P*-value‡		
		60/7	55/12	51/17	46/23	41/28		Diet	Fish	D × F
Hematological characteristics										
Ht (%)	F_1	25.1	23.9	24.2	24.6	24.4	0.844	0.294	0.083	0.201
	RSB	23.5^{ab}	25.4^{a}	24.1^{ab}	22.0^{ab}	19.7^{b}	1.317			
Hb (g/dL)	F_1	7.0	6.9	6.6	7.2	6.6	0.339	0.754	0.001	0.963
	RSB	5.9	5.7	5.9	6.0	5.6	0.353			
Relative organ weight to somatic weight										
CF§	F_1	3.0^{b}	3.0^{b}	3.2^{ab}	3.3^{a}	3.1^{ab}	0.058	0.005	0.000	0.006
	RSB	3.6^{ab}	3.5^{b}	3.9^{a}	3.5^{b}	3.6^{ab}	0.059			
HSI¶	F_1	1.3^{b}	1.4^{ab}	1.6^{ab}	1.6^{ab}	1.7^{a}	0.074	0.034	0.000	0.854
	RSB	2.2	2.5	2.5	2.8	2.7	0.132			
VSI¶	F_1	6.0^{c}	7.0^{c}	8.6^{b}	9.9^{ab}	10.5^{a}	0.204	0.000	0.003	0.350
	RSB	7.2^{c}	8.2^{bc}	9.2^{ab}	10.2^{a}	10.6^{a}	0.251			
Hepatic GOT and GPT activity (IU/mg protein)										
GOT	F_1	0.17^{ab}	0.22^{a}	0.19^{ab}	0.18^{ab}	0.11^{b}	0.026	0.410	0.271	0.154
	RSB	0.20	0.17	0.19	0.22	0.19	0.021			
GPT	F_1	0.02^{ab}	0.03^{ab}	0.04^{a}	0.02^{ab}	0.01^{b}	0.006	0.090	0.000	0.237
	RSB	0.11^{ab}	0.16^{a}	0.08^{ab}	0.07^{ab}	0.05^{b}	0.025			

GOT, glutamate-oxalacetate transaminase; GPT, glutamate-pyruvate transaminase; RSB, red sea bream *Pagrus major*; CP/CL, ratios of crude protein/lipid; SEM, standard error of means; Ht, hematocrit; Hb, hemoglobin.
*n = 3, except that 55/12 group of RSB is n = 2.
†Values (mean) in a row sharing same superscripts are not significantly different (P > 0.05).
‡Results from two-way analysis of variance (ANOVA)
§Condition factor (CF) = 100 × [wet body weight (g)/body length (cm)^3].
¶Hepato (HSI) and visceral (VSI) somatic index = 100 × [wet weight of organ (g)/wet body weight (g)].

except ERE on the 60/7 and 55/12 diets (two-way ANOVA, $P < 0.05$).

With decreasing dietary CP/CL, whole body protein showed decreasing trends, but lipid and energy showed increasing trends in F_1 and RSB. LRE also tended to decrease with decreasing CP/CL in both species. While F_1 fed 60/7, 55/12, and 51/17 diets had significantly higher LRE than the 41/28 diet ($P < 0.05$), RSB fed the 60/7 diet showed significantly higher LRE than with the other CP/CL diets ($P < 0.05$). Significantly higher ERE was observed with 55/12, 51/17, 46/23, and 41/28 diets for F_1, and the 55/12 diet for RSB ($P < 0.05$) (Table 5).

Regarding hematological parameters, only Hb contents were affected by fish species, and RSB tended to be lower than F_1 (two-way ANOVA, $P < 0.05$) (Table 6). The CF, HSI, and VSI were affected significantly by diet and by fish species (two-way ANOVA, $P < 0.05$), where the indices tended to lower in F_1 than RSB. Additionally, HSI and VSI showed a tendency to increase with decreasing dietary CP/CL in both species. Although hepatopancreatic GOT activity was not affected by dietary treatment or fish species, hepatopancreatic GPT activity was significantly lower in F_1 (two-way ANOVA, $P < 0.05$).

Discussion

The establishment of mass juvenile production for finfish aquaculture industry requires various technical disciplines and basic knowledge related to the biological, chemical, physiological, and environmental aspects of each aquaculture fish. In particular, understanding the relationship between changes in nutritional requirements and biochemical metabolism in the early life stages of fish will produce rapid improvement and success in mass fingerling production. Thus, this study was designed to identify a suitable dietary protein and lipid content and/or ratio for early F_1 juveniles, which are expected to be a promising new aquaculture species in Korea and eastern China where the water temperature becomes very low in the winter.

It is well-known that the survival of fingerling production depends on whether the dietary needs for early juvenile stages are met (National Research Council, 1993; Takeuchi, 2001). In both rearing trials, survival rates were significantly different between species, with markedly higher values in F_1 than RSB ($P < 0.05$) (Tables 2 and 4). These results may be because F_1 had greater adaptable and performance under these experimental conditions (*e.g.*, diet formulation, rearing density) than RSB.

There are few reports dealing with suitable dietary protein and lipid levels in early juvenile stages of F_1 or RSB. We were obligated to end the rearing Trials 1 and 2 at 2 and 4 weeks after the start due to the juveniles' fast growth, and the resulting over-density in the rearing tanks. However, the final mean body weights in each trial increased by 2- to 3-fold relative to the initial mean body weight within this rearing period. Thus, it is reasonable to conclude that the 2- to 4-weeks rearing period for the early juvenile stages of these species are not too short to obtain reliable data.

The growth performance data revealed that suitable CP/CL proportions for F_1 and RSB were 55/12 and above 60/7, respectively, in Trial 1, whereas they were lower, at 41/28 for F_1 and above 51/17 for RSB, in Trial 2. These results for RSB are consistent with the results of other authors (Yone et al., 1974; Takeuchi et al., 1991). Takeuchi et al. (1991) and Yone et al. (1974) reported that RSB weighing 1.6-29.0 g required 52-55% dietary protein and 15% dietary lipid. Watanabe et al. (1984) showed that mature RSB, weighing 689 g, required 45% dietary protein, which is markedly lower than juveniles (Takeuchi et al., 1991). In contrast, Arakawa et al. (1980) indicated a suitable dietary protein level of 40% for BSB weighing 2.9 g, which has been reported to be lower than RSB. A two-way ANOVA revealed significant differences in growth performance and nutrient retention between the two fish species. Generally, F_1 performed better on diets with low CP/CL (46/23, 41/28) than did RSB, providing evidence that F_1 had a lower protein requirement than the female parental fish (RSB). These findings are consistent with those in several other species where hybrid fish ordinarily showed lower protein requirements than the maternal fish (Millikin, 1982, 1983; Berger and Halver, 1987; Shiau and Huang, 1989; Tuncer et al., 1990; Brown et al., 1992; Swann et al., 1994; Brecka et al., 1995; Twibell and Brown, 1998).

Azevedo et al. (2004) found a significant difference in growth between rainbow trout and Atlantic salmon by feeding various CP/CL diets. Regarding the cause of the difference, they considered that it was due to the difference in nitrogen and energy retention efficiency between the two species. Differences in capabilities between fish species were also identified in this experiment, and effects in growth improvement by substituting fish meal for fish oil were observed in both juvenile F_1 and RSB. However, significant differences ($P < 0.05$ by two-way ANOVA) between the two fish species were found in PRE, LRE, and ERE (Tables 3 and 5). In particular, the retention efficiency of F_1 in the low CP/CL diets (46/23, 41/28) showed a markedly higher tendency than RSB. These results suggest that F_1 experiences a protein-sparing effect when fed diets with higher lipid levels, as reported for porgy (Vergara et al., 1996; Schuchardt et al., 2008). However, the juvenile F_1 has a greater capacity to synthesize and accumulate protein using dietary lipid as an energy source than does RSB.

From the hepatic transaminase activity, used as an indicator of the breakdown and consumption of amino acids, the results showed a reduced tendency in GPT activity in both species with a reduced dietary CP/CL ratio. However, there was a significant difference between F_1 and RSB, with lower activity in F_1 than RSB. It has been reported that feeding a high-lipid diet to fish decreases levels of glycogen, blood nitrogenous compounds, gluconeogenesis, and glycolysis enzymes (*e.g.*,

PFK, G6Pase, G6P DH) activities, and GPT activity, along with an increase in hepatic fat content. Thus, gluconeogenesis and glycolysis, fatty acid synthesis, and amino acid decomposition are inhibited by the ingestion of greater quantities of lipid (Shimeno, 1983). This is the so-called "protein-sparing effect" of lipid that decreases nitrogen excretion by inhibiting the breakdown of amino acids and improves the accumulation of dietary protein in the body by using dietary lipid as an energy source (Shimeno et al., 1981; Skalli et al., 2004). Although the amino acid balance in these experimental diets was not considered, the lower GPT activity in F_1 may indicate that this fish has greater protein-sparing capacity than RSB through lipid mobilization. This may be attributable to the rapid growth of F_1, as has been reported previously (Murata, 1998; Kim et al., 2011). However, further studies are necessary to clarify this issue.

In conclusion, these results demonstrate that the hybrid F_1 is a promising aquaculture species because of its superior growth performance and higher survival rate even at a lower dietary CP/CL ratio. The results also indicated that mass juvenile production of F_1 will be more economical than that of RSB.

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References

An KW, Nelson ER, Jo PG, Habibi HR, Shin HS and Choi CY. 2008. Characterization of estrogen receptor β2 and expression of the estrogen receptor subtypes α, β1, and β2 in the protandrous black porgy (*Acanthopagurs schlegeli*) during the sex change process. Comp Biochem Physiol B Biochem Mol Biol 150, 284-291. http://dx.doi.org/10.1016/j.cbpb.2008.03.012.

Association of Official Analytical Chemists. 1995. Official Methods of Analysis. 15th ed. Association of Official Analytical Chemists, Gaithersburg, MD, US.

Arakawa T, Kitajima C and Yone Y. 1980. Effect of dietary protein levels on the growth and feed efficiency of black sea bream, *Mylio macrocephalus*. Bull Nagasaki Pref Inst Fish 6, 49-52.

Azevedo PA, Leeson S, Cho CY and Bureau DP. 2004. Growth and feed utilization of large size rainbow trout (*Oncorhynchus mykiss*) and Atlantic salmon (*Salmo salar*) reared in freshwater: diet and species effects, and responses over time. Aquac Nutr 10, 401-411. http://dx.doi.org/10.1111/j.1365-2095.2004.00322.x.

Berger A and Halver JE. 1987. Effect of dietary protein, lipid and carbohydrate content on growth, feed efficiency and carcass composition of striped bass, *Morone saxatilis* (Walbaum), fingerlings. Aquac Fish Manage 18, 345-356. http://dx.doi.org/10.1111/j.1365-2109.1987.tb00323.x.

Blanc JM and Chevassus B. 1982. Interspecific hybridization of salmonid fish: II. Survival and growth up to the 4th month after hatching in F_1 generation hybrids. Aquaculture 29, 383-387. http://dx.doi.org/10.1016/0044-8486(82)90151-X.

Brecka BJ, Kohler CC and Wahl DH. 1995. Effect of dietary protein concentration on growth, survival and body composition of muskellunge *Esox masquinongy* and tiger muskellunge *Esox masquinongy* × *E. luscius* fingerlings. J World Aquac Soc 26, 416-425. http://dx.doi.org/10.1111/j.1749-7345.1995.tb00837.x.

Brown BA. 1980. Routine hematology procedures. In: Hematology: Principles and Procedures. Brown BA, ed. Lea & Febiger, Philadelphia, PA, US, pp. 71-112.

Brown ML, Nematipour GR and Gatlin DM. 1992. Dietary protein requirement of juvenile sunshine bass at different salinities. Prog Fish-Cult 54, 148-156. http://dx.doi.org/10.1577/1548-8640(1992)054<0148:DPROJS>2.3.CO;2.

Chang CF, Lau EL and Lin BY. 1995. Estradiol-17β suppresses testicular development and stimulates sex reversal in protandrous black progy, *Acanthopagrus schlegeli*. Fish Physiol Biochem 14, 481-488. http://dx.doi.org/10.1007/BF00004348.

Fukunishi Y, Masuda R and Yamashita Y. 2006. Ontogeny of tolerance to and avoidance of ultraviolet radiation in red sea bream *Pagrus major* and black sea bream *Acanthopagrus schlegeli*. Fish Sci 72, 356-363. http://dx.doi.org/10.1111/j.1444-2906.2006.01157.x.

Halver JE. 1957. Nutrition of salmonid fishes. III. Water-soluble vitamin requirements of Chinook salmon. J Nutr 62, 225-243.

Harada T. 1991. Hybridization and select breeding of sea breams. Aquac Sci 39, 110-111.

Harel M and Place AR. 2003 Tissue essential fatty acid composition and competitive response to dietary manipulations in white bass (*Morone chrysops*), striped bass (*M. saxatilis*) and hybrid striped bass (*M. chrysops* × *M. saxatilis*). Comp Biochem Physiol B Biochem Mol Biol 135, 83-94.

Harter HL. 1960. Critical values for Duncan's new multiple range tests. Biometrics 16, 671-685. http://dx.doi.org/10.2307/2527770.

Hodge JE and Hofreiter BT. 1962. Determination of reducing sugars and carbohydrates. In: Methods in Carbohydrate Chemistry. Vol. 1. Analysis and Preparation of Sugars. Whistler RL and Wolfram ML, eds. Academic Press, London, GB, pp. 380-394.

Kim YS, Biswas AK, Ji SC, Yong ASK, Biswas BK, Takaoka O, Murata O and Takii K. 2009a. Dietary soybean meal utilization with phytase supplementation for hybrid F_1, red sea bream (♀) × black sea bream (♂). Aquac Sci 57, 45-52.

Kim YS, Biswas AK, Seoka M, Biswas BK, Yong ASK, Takaoka O, Murata O and Takii K. 2009b. Dietary vitamin C requirement of hybrid, female red sea bream, *Pagurs major* × male black sea bream, *Acanthopagrus schlegeli*. Aquac Sci 57, 127-132.

Kim YS, Ji SC, Biswas BK, Biswas A, Jeong GS, Murata O and Takii K. 2011. Growth and environmental tolerances (water temperature and low salinity) of hybrid female red sea bream *Pagrus major* × male

black sea bream *Acanthopagrus schregeli*. Korean J Fish Aquat Sci 44, 276-283. http://dx.doi.org/10.5657/KFAS.2011.0276.

Kinoshita I and Tanaka M. 1990. Differentiated spatial distribution of larvae and juveniles of the two sparids, red and black sea bream, in Shijiki Bay. Nippon Suisan Gakkaishi 56, 1807-1813. http://dx.doi.org/10.2331/suisan.56.1807.

Logan HJ. 1968. Comparison of growth and survival rates of striped bass and striped bass × white bass hybrids under controlled environments. Proc Annu Conf Southeast Assoc Game Fish Comm 21, 260-263.

Lowry OH, Rosebrough NJ, Farr AL and Randall RL. 1951. Protein measurement with the folin phenol reagent. J Biol Chem 193, 265-275.

Mana RR and Kawamura G. 2002. A comparative study on morphological differences in the olfactory system of red sea bream (*Pagrus major*) and black sea bream (*Acanthopagrus schlegeli*) from wild and cultured stocks. Aquaculture 209, 285-306. http://dx.doi.org/10.1016/S0044-8486(01)00732-3.

Millikin MR. 1982. Effects of dietary protein concentration on growth, feed efficiency and body composition of age-0 striped bass. Trans Am Fish Soc 111, 373-378. http://dx.doi.org/10.1577/1548-8659(1982)111<373:EODPCO>2.0.CO;2.

Millikin MR. 1983. Interactive effects of dietary protein and lipid on growth and protein utilization of age-0 striped bass. Trans Am Fish Soc 112, 185-193. http://dx.doi.org/10.1577/1548-8659(1983)112<185:IEODPA>2.0.CO;2.

Moreau J and Pauly D. 1999. A comparative analysis of growth performance in aquaculture of tilapia hybrids and their parent species. Asian Fish Sci 12, 91-103.

Murata O. 1998. Studies on the breeding of cultivated marine fishes. Bull Fish Lab Kinki Univ 6, 1-101.

Muroga K, Higashi M and Keitoku H. 1987. The isolation of intestinal microflora of farmed red seabream (*Pagrus major*) and black seabream (*Acanthopagrus schlegeli*) at larval and juvenile stages. Aquaculture 65, 79-88. http://dx.doi.org/10.1016/0044-8486(87)90272-9.

National Research Council. 1993. Nutrient requirements of fish. Committee on Animal Nutrition, Board of Agriculture. National Academy Press, Washington, DC, US.

Phelps RP, Hastey R, Pendetar A, Linley L, Papanikos N and Dunham RA. 2007. Effects of temperature on the induced spawning of channel catfish and production of channel × blue catfish hybrid fry. Aquaculture 273, 80-86. http://dx.doi.org/10.1016/j.aquaculture.2007.09.019.

Ponzoni RW, Nguyen NH and Khaw HL. 2007. Investment appraisal of genetic improvement programs in Nile tilapia (*Oreochromis niloticus*). Aquaculture 269, 187-199. http://dx.doi.org/10.1016/j.aquaculture.2007.04.054.

Rawles SD, Riche M, Gaylord TG, Webb J, Freeman DW and Davis M. 2006. Evaluation of poultry by-product meal in commercial diets for hybrid striped bass (*Morone chrysops* ♀ × *M. saxatilis* ♂) in recirculated tank production. Aquaculture 259, 377-389. http://dx.doi.org/10.1016/j.aquaculture.2006.05.053.

Riche M, Haley DI, Oetker M, Garbrecht S and Garling DL. 2004. Effect of feeding frequency on gastric evacuation and the return of appetite in tilapia *Oreochromis niloticus* (L.). Aquaculture 234, 657-673. http://dx.doi.org/10.1016/j.aquaculture.2003.12.012.

Schuchardt D, Vergara JM, Fernández-Palacios H, Kalinowski CT, Hernández-Cruz CM, Izquierdo MS and Robaina L. 2008. Effects of different dietary protein and lipid levels on growth, feed untilization and body composition of red progy (*Pagrus pagrus*) fingerlings. Aquac Nutr 14, 1-9. http://dx.doi.org/10.1111/j.1365-2095.2007.00484.x.

Shiau SY and Huang SL. 1989. Optimal dietary protein level for hybrid tilapia (*Oreochromis niloticus* × *O. aureus*) reared in seawater. Aquaculture 81, 119-127. http://dx.doi.org/10.1016/0044-8486(89)90237-8.

Shimeno S. 1983. Carbohydrate metabolizing route. In: Metabolism in Fish. Nakayama F, ed. Kouseisha, Tokyo, JP, pp. 9-22.

Shimeno S, Hosokawa H, Takeda M, Takayama S, Fukui A and Sasaki H. 1981. Adaptation of hepatic enzymes to dietary lipid in young yellowtail. Bull Jpn Soc Sci Fish 47, 63-69.

Skalli A. Hidalgo MC. Abellán E, Arizcun M and Cardenete G. 2004. Effect of the dietary protein/lipid ratio on growth and nutrient utilization in common dentex (*Dentex dentex* L.) at different growth stages. Aquaculture 235, 1-11. http://dx.doi.org/10.1016/j.aquaculture.2004.01.014.

Swann DL, Riepe JR, Stanley JD, Griffin ME and Brown PB. 1994. Cage culture of hybrid striped bass in Indiana and evaluation of diets containing three levels of dietary protein. J World Aquac Soc 25, 281-288. http://dx.doi.org/10.1111/j.1749-7345.1994.tb00192.x.

Takeuchi T. 2001. A review of feed development for early life stages of marine finfish in Japan. Aquaculture 200, 203-222. http://dx.doi.org/10.1016/S0044-8486(01)00701-3.

Takeuchi T, Shiina Y and Watanabe T. 1991. Suitable protein and lipid levels in diet for fingerling of red sea bream *Pagrus major*. Nippon Suisan Gakkaishi 57, 293-299. http://dx.doi.org/10.2331/suisan.57.293.

Tang QS, Sun Y and Zhang B. 2003. Bioenergetics models for seven species of marine fish. J Fish China 27, 443-449.

Tuncer H, Harrell RM and Houde ED. 1990. Comparative energetics of striped bass (*Morone saxatilis*) and hybrid (*M. saxatilis* × *M. chrysops*) juveniles. Aquaculture 86, 387-400. http://dx.doi.org/10.1016/0044-8486(90)90327-J.

Twibell RG and Brown PB. 1998. Otimal dietary protein concentration for hybrid tilapia *Oreochromis niloticus* × *O. aureus* fed all-plant diets. J World Aquac Soc 29, 9-16. http://dx.doi.org/10.1111/j.1749-7345.1998.tb00294.x.

Twibell RG, Wilson KA, Sanders S and Brown PB. 2003. Evaluation of experimental and practical diets for bluegill *Lepomis macrochirus* and hybrid bluegill *L. cyanellus* × *L. macrochirus*. J World Aquac Soc 34, 487-495. http://dx.doi.org/10.1111/j.1749-7345.2003.tb00087.x.

Vergara JM, Robaina L, Izquierdo M and de la Higuera M. 1996. Protein sparing effect of lipids in diets for fingerlings of gilthead sea bream. Fish Sci 62, 624-628.

Watanabe T, Itoh A, Kitajima C and Fujita S. 1984. Effect of dietary

protein levels on reproduction of red sea bream. Bull Jpn Soc Sci Fish 50, 1015-1022. http://dx.doi.org/10.2331/suisan.50.1015.

Yone Y, Sakamoto S and Furuichi M. 1974. Studies on nutrition of red sea bream. IX. The basal diet for nutrition studies. Rep Fish Res Lab Kyushu Univ 2, 13-24.