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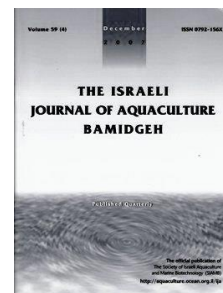
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## **Effect of Dietary Lipids on Growth, Feed Utilization, and Protein Sparing in Black Rockfish, *Sebastes schlegeli***

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### **Abstract**

The effects of dietary lipids on growth and feed utilization of juvenile black rockfish (*Sebastes schlegeli*) were examined. Triplicate groups of 20 juveniles were fed diets containing 42% crude protein, supplemented with 0%, 3%, 6%, 9% and 12% lipid (% dry matter) for 60 days. At the end of the experiment, survival rate of all groups was at least 88%. Fish fed the diet containing 6% lipids had a significantly ( $P < 0.05$ ) higher growth rate, better feed conversion and protein efficiency ratios than the other groups. Muscle lipids increased dramatically as the dietary lipid level increased; the highest lipid content was obtained in fish fed the highest lipid diet. Conversely, muscle protein declined as the dietary lipid level increased, and the lowest protein value was in fish fed the 12% lipid diet. Ash and dry matter content did not significantly differ between groups. Increasing the lipid level to 6% was most effective for improving fish growth and feed efficiency.

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## Introduction

Black rockfish, *Sebastes schlegeli*, is a valuable, tasty, marine fish with few bones. Suitable water temperatures for black rockfish range from 1-27°C, and the optimal temperature range is 4-25°C. Black rockfish efficiently utilizes commercial feed, grows rapidly, and is an increasingly important commercial species in China. Because of its rapid growth, efficient feed conversion, and high market value, aquaculture of this fish plays an important role in the economy of China. With the recent success of artificial propagation and larval production, culture of black rockfish has attracted increasing attention from the scientific community, as well as from the general public (Feng, 2003).

Despite the success of black rockfish culture, research into the nutritional requirements for this important fish has not been extensive. This information is essential to formulate diets that promote optimal growth at minimal cost. Dietary lipids provide a source of concentrated energy and essential fatty acids for carnivorous fish that are limited in their ability to utilize carbohydrates as an energy source (Sargent et al., 2002). The increased digestible energy content in fish diets due to lipid supplementation has a protein sparing effect that reduces nitrogen loss to the environment (Watanabe, 1982; De Silva et al., 1991; Cho and Bureau, 2001; Skalli et al., 2004). However, excessive energy in diets can lead to decreased feed consumption, reducing protein intake and nutrient utilization, resulting in reduced growth (Page and Andrews, 1973; Watanabe, 1982; Daniels and Robinson, 1986; Ellis and Reigh, 1991). Moreover, high dietary lipids can affect carcass composition due to an increase in lipid deposition (Ogunji and Wirth, 2002). Therefore, dietary lipid levels should be carefully evaluated and balanced.

Providing adequate energy from dietary lipids can minimize the use of high-priced proteins as the energy source in fish diets (Ai et al., 2004; Hung et al., 2004). Thus, adequate lipid levels are important for growth performance and product quality in fish (Tibbetts et al., 2005). This study was designed to determine the effects of dietary lipid levels on growth, feed utilization, and muscle composition in black rockfish.

## Materials and Methods

**Diet formulation.** Isonitrogenous diets with 42% protein were formulated to contain five different lipid supplementation: 0%, 3%, 6%, 9%, or 12% (dry matter basis). The major dietary protein sources were freeze-dried white fishmeal and soy meal (Table 1).

Table 1. Formulation and chemical compositions of lipid supplemented diets for juvenile black rockfish (*Sebastes schlegeli*).

	Diet(%)				
	0	3	6	9	12
<b>Ingredient (% supplementary lipid)</b>					
Fishmeal(white)	45	45	45	45	45
Soy meal	25	25	25	25	25
<b>Corn starch</b>			<b>23</b>		
Cod liver oil	0	3	6	9	12
$\alpha$ -starch	2	2	2	2	2
Vitamin*(Norway)		4	4	4	4
Mineral mix*	1	1	1	1	1
<b>Chemical composition (% of)</b>					
Crude protein	42.1	41.8	42.5	42.8	43.1
<b>Crude lipid</b>		<b>3.7</b>	<b>6.8</b>	<b>9.6</b>	
Crude ash	13.2	13.4	13.6	12.9	13.2
NFE + crude	41.0	38.0	34.3	31.2	28.1

\* Lovell (1989, 1998)

for 1991-1998). The percentage of carbohydrates in the diets, including nitrogen free extractives (NFE) and crude fiber, was calculated by subtraction of the percentages of protein, lipid, and ash from 100% (Jobling, 2001).

**Stocking and feeding.** Healthy juvenile black rockfish were provided by Fish Hatchery of Yantai Tai Hua Marine Product Co., Ltd, Shandong, China. Prior to the onset of the experiment, fish were acclimated to laboratory conditions for 14 days during which they were fed a diet with no lipid supplementation. They were individually weighed and selected according to their health, and body size ( $7.13 \pm 0.14$  g;  $6.35 \pm 0.22$  cm). Chosen fish were not fed for 24 h before the growth study, and were then randomly allocated to

Ingredients were weighed, and then blended to produce a homogeneous mixture, pelleted in a commercial meat grinder, air-dried at room temperature, placed in sealed plastic bags, and stored at -20°C until use. All ingredients and chemicals were purchased from 4 Shandong Tian Shen Feed Shandong, China.

Mean total nitrogen was determined by the micro-Kjeldhal method (AOAC, 1995), and crude protein % was calculated as (N x 6.25). Crude lipid was determined after organic extraction by chloroform-methanol (2:1 v/v). Ash was produced with a muffle furnace at 550°C

net cages (60cm×60cm×120cm) held in a round cement tank at a stocking density of 20 fish per cage. Parameters for water quality were monitored during the experimental period. Water temperature was maintained at  $22 \pm 1^{\circ}\text{C}$ , and dissolved oxygen was greater than 5 mg/l. One air stone in each cage provided aeration. All cages were kept at the same natural photoperiod conditions. The cages were cleaned fortnightly. Diets were tested in triplicate groups. Fish were hand-fed the experimental diets to apparent satiation three times a day (08:30, 13:30 and 17:30) for 60 days. Food pellets were distributed slowly, allowing all fish to eat without competing.

Fish were bulk weighed and counted at the beginning and the end of the experiment to the nearest 0.01 g and specific growth rate (SGR), feed conversion ratio (FCR), and protein efficiency ratio (PER) were calculated. Fish were not fed for one day before weighing.

**Chemical composition of fish muscle.** At the end of the experiment, white muscle from 10 fish in each cage was excised and frozen at  $-20^{\circ}\text{C}$  for analysis. After freeze-drying for 48 h, muscle dry weight was obtained from triplicate samples of each dietary treatment. Crude protein, lipid, and ash contents of the muscles were assessed according to the diets, and the calculations were converted to percentages of the total muscle by dry weight.

**Data analysis.** Data from replicates are presented as mean  $\pm$  SE unless otherwise specified. Data were analyzed by one-way analysis of variance (ANOVA) for statistical significance. When differences were significant ( $P < 0.05$ ), Duncan's Multiple Range Test was used to rank the groups. All statistical analyses were performed using the SPSS 17.0 for Windows.

## Results

All fish adapted well to the experimental conditions, and no disease or water quality problems were observed. Survival ranged from 88.33-100.00% and did not significantly differ between groups (Table 2).

Table 2. Weight gain, survival, feed utilization, and muscle composition (Mean  $\pm$  SE; n=3) of black rockfish fed diets containing different percentages of lipid.

	Diet(% supplementary lipid)				
	0	3	6	9	12
Initial body wt (g)	7.04 $\pm$ 0.12	7.12 $\pm$ 0.12	7.20 $\pm$ 0.07	7.20 $\pm$ 0.21	7.11 $\pm$ 0.06
Final body wt (g)	10.46 $\pm$ 0.37 <sup>bc</sup>	11.49 $\pm$ 0.14 <sup>b</sup>	12.61 $\pm$ 0.45 <sup>a</sup>	11.45 $\pm$ 0.17 <sup>bc</sup>	10.42 $\pm$ 0.42 <sup>c</sup>
Wt gain (g)	3.42 $\pm$ 0.37 <sup>bc</sup>	4.37 $\pm$ 0.14 <sup>ab</sup>	5.41 $\pm$ 0.45 <sup>a</sup>	4.25 $\pm$ 0.17 <sup>bc</sup>	3.31 $\pm$ 0.41 <sup>c</sup>
Survival (%)	88.33 $\pm$ 9.28	96.67 $\pm$ 3.33	100.00 $\pm$ 0.00	95.00 $\pm$ 2.89	90.00 $\pm$ 7.64
SGR <sup>1</sup>	0.66 $\pm$ 0.06 <sup>bc</sup>	0.80 $\pm$ 0.02 <sup>ab</sup>	0.93 $\pm$ 0.06 <sup>a</sup>	0.77 $\pm$ 0.02 <sup>abc</sup>	0.63 $\pm$ 0.07 <sup>c</sup>
FCR <sup>2</sup>	1.87 $\pm$ 0.07 <sup>b</sup>	1.74 $\pm$ 0.09 <sup>b</sup>	1.28 $\pm$ 0.02 <sup>c</sup>	2.49 $\pm$ 0.04 <sup>a</sup>	2.55 $\pm$ 0.05 <sup>a</sup>
PER <sup>3</sup>	1.28 $\pm$ 0.05 <sup>b</sup>	1.38 $\pm$ 0.07 <sup>b</sup>	1.86 $\pm$ 0.02 <sup>a</sup>	0.96 $\pm$ 0.02 <sup>c</sup>	0.93 $\pm$ 0.02 <sup>c</sup>
<b>Muscle composition</b>					
Dry matter (%)	21.93 $\pm$ 0.01	21.81 $\pm$ 0.24	22.32 $\pm$ 0.43	21.11 $\pm$ 0.25	21.76 $\pm$ 0.17
Crude lipid (%DM)	5.51 $\pm$ 0.37 <sup>c</sup>	5.54 $\pm$ 0.52 <sup>c</sup>	6.43 $\pm$ 0.33 <sup>c</sup>	7.74 $\pm$ 0.69 <sup>ab</sup>	8.85 $\pm$ 0.43 <sup>a</sup>
Crude protein (%DM)	78.13 $\pm$ 0.99 <sup>a</sup>	77.35 $\pm$ 1.30 <sup>ab</sup>	76.00 $\pm$ 0.59 <sup>ab</sup>	74.90 $\pm$ 0.64 <sup>bc</sup>	73.84 $\pm$ 0.96 <sup>c</sup>
Crude ash (%DM)	8.39 $\pm$ 0.39	8.72 $\pm$ 0.18	8.35 $\pm$ 0.12	8.77 $\pm$ 0.41	8.89 $\pm$ 0.19

Means in a row with different superscripts significantly differ ( $P < 0.05$ ).

<sup>1</sup> Specific growth rate =  $[(\ln Wt_2 - \ln Wt_1) / (t_2 - t_1)] \times 100\%$ , where  $Wt_2$ =final wt,  $Wt_1$ =initial wt, and  $t_2 - t_1$ =no. days

<sup>2</sup> Feed conversion ratio = dry wt of feed consumed / wet wt gain of fish

<sup>3</sup> Protein efficiency ratio = [final body wt - initial body wt] / protein consumed.

Final body weight, net weight gain, and SGR were significantly highest in fish fed the diet containing 6% lipid. FCR was highest in fish fed the 9% and 12% diets and lowest in fish fed the 6% diet. PER increased with increasing dietary lipid levels to 6%; they then decreased with further increased dietary lipids. The FCR of the fish decreased as the lipid level increased, suggesting a significant effect of dietary lipid on diet utilization.

In muscle of fish fed higher lipid levels, lipid content increased and was highest in fish fed the 12% diet. Protein content decreased as the lipid levels increased and was lowest in fish fed the 12% diet. Ash and dry matter content in all fish groups remained unchanged.

### Discussion

Fish gained weight when fed increased levels (up to 6%) of dietary lipids. The 6% lipid groups had the best feed utilization (lowest FCR and highest PER), suggesting that feed efficiency was generally improved by increasing dietary lipids. However, in fish fed more than 6% lipids in the 42% protein diet there were no increases in weight, SGR, or PER. High dietary lipid levels depress growth in some fish species (Pei et al., 2004; Du et al., 2005). This may be due to the limited ability of fish to digest and absorb high concentrations of lipids, resulting in reduced feed intake, excess lipid accumulation in the liver and other visceral organs, and dietary or metabolic imbalance (Luo et al., 2005). Increased dietary lipid levels were associated with an increased source of energy in the diet. When fish are fed a diet containing excess energy sources, growth rate may decrease due to reduced feed consumption. Other studies also showed that increased dietary lipid levels were associated with a decline in feed intake and growth (Ellis and Reigh, 1991; Lee et al., 2002). In juvenile Asian sea bass which grew from 80 to 210 g when fed a diet containing 60.3% protein and 18% lipid, the FCR was 0.78 (Williams et al., 2003). Due to the protein sparing effects of high lipid diets, dietary protein levels can be reduced by increasing the lipid level without compromising growth (Sargent et al., 2002; Ai et al., 2004). However, high lipid diets can result in decreased body protein (Williams and Robinson, 1988). In our study, increasing dietary lipids resulted in decreased protein content in fish muscle. As protein retention is generally regulated by non-protein energy input of a diet, PER is a better measurement of the protein sparing effect of lipids (Lie et al., 1988).

Despite the overall lower muscle protein content, fish fed higher lipid diets (up to 6%) demonstrated significantly higher PER. Although in some circumstances the protein sparing effect is not demonstrated (Thoman et al., 1999; Vergara et al., 1999), most studies suggest that it does occur (Peres and Oliva-Teles., 2002; Boujard et al., 2004). Our results support the presence of a protein-sparing effect related to a higher level of dietary lipid.

The correlation between dietary lipid and body lipid concentration is well documented and shows that excessive dietary lipid results in excessive fat deposition in the visceral cavity and tissues. Since high body-fat content in fish is unacceptable to consumers (Ottwell and Rickards, 1981), increased body fat caused by high dietary lipid may reduce the commercial value of the fish.

In conclusion, growth rate was greatest in black rockfish juveniles fed a diet containing 6% dietary lipid in and 42% protein. Beyond this level, lipid supplementation resulted in accumulated muscle fat, decreased muscle protein, and less efficient feed and protein utilization. Based on these results, it is recommended to supplement 42% protein fish diets with 6% lipids.

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