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Effects of dietary protein and lipid levels on growth performance, feed utilization and body composition of juvenile hybrid grouper, *Epinephelus fuscoguttatus* × *E. lanceolatus*

Samad Rahimnejad [a], In Chul Bang [b], Jong-Yeon Park [b], Ahmed Sade [c], Jin Choi [a], Sang-Min Lee [a,]*

[a] Department of Marine Bioscience and Technology, Gangneung-Wonju National University, Gangneung 210-702, South Korea
[b] Department of Life Science and Biotechnology, Soonchunhyang University, Asan 336-745, South Korea
[c] Fisheries Department Sabah, Wisma Pertanian, Jalan Tasek, 88628 Kota Kinabalu, Sabah, Malaysia

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ABSTRACT

An 8-week feeding trial was conducted to evaluate the effects of varying dietary protein and lipid levels on growth, feed utilization, and body composition of brown-marbled grouper (*Epinephelus fuscoguttatus*) and giant grouper (*Epinephelus lanceolatus*) hybrid. Eight diets were formulated to contain four protein levels (40, 45, 50 and 55% crude protein) and two lipid levels (7 and 14% crude lipid). Seven hundred and twenty juvenile grouper averaging 2.55 ± 0.10 g were randomly allotted to 24 cages (8 treatments in triplicate groups) and fed the test diets to apparent satiation three times a day. The results showed significant ($P < 0.05$) enhancement of weight gain and specific growth rate with increasing dietary protein from 40 to 50%, and a reduced growth was obtained by further increase of dietary protein to 55%. Increasing dietary lipid level from 7 to 14% did not significantly affect growth performance ($P > 0.05$). Feed efficiency was significantly improved by increasing dietary protein and lipid levels. Also, the results revealed the significant increase of protein efficiency ratio by increasing dietary lipid. Significant reduction in daily feed intake was observed by increment of dietary protein and lipid levels. Whole-body lipid content increased with increasing dietary lipid and inversely correlated with whole-body moisture content. Dietary protein, lipid, and their interaction had a significant effect on liver protein content. Significant changes in dorsal muscle and liver fatty acid compositions were observed by variation of dietary protein and lipid levels. According to these results, a diet containing 50% protein and 14% lipid with protein to energy ratio of 23.9 mg kJ^{-1} is recommended for efficient growth of juvenile hybrid grouper.

Statement of relevance

This research work provides useful information for formulation of cost-effective and environment friendly feeds for hybrid grouper which is considered as a promising candidate for aquaculture.

1. Introduction

Generally feed is the single largest cost item in aquaculture practices and accounts for over 50% of the total production cost. Protein is the most important and expensive nutrient influencing fish growth performance and feed cost (Lee and Kim, 2005), therefore its dietary level should be optimized to cover the fish nutritional requirements and reduce feed cost. Fish, particularly carnivorous species, prefer protein to lipid and carbohydrate as an energy source (Walton and Cowey, 1982), however, the use of protein as dietary energy source is undesirable due to the high cost of protein compared to non-protein energy sources (Watanabe, 2002). Protein requirement of the aquaculture species is determined to provide the minimum amount of essential amino acids required for maximal growth (Chi et al., 2010). Providing dietary protein in higher levels than fish requirement causes diets to be unnecessarily expensive and leads to catabolism of amino acids and subsequently increased ammonia excretion and deterioration of water quality.

One of the most important factors affecting diet efficiency is the protein to energy (P/E) ratio which should be estimated for specific fish species and stages. When the diet has an excess of protein, it will be used as energy source rather than body protein synthesis while a diet with deficient protein and excess energy can lower feed intake and affect growth (NRC, 2011). It has been demonstrated that protein utilization efficiency can be improved by the utilization of lipids and carbohydrates in fish diets (Cho and Kaushik, 1990; Kaushik and Medale, 1994). In this regard, lipids are a more efficient source of energy than carbohydrates because lipids are readily metabolized by fish, especially by carnivorous fish (NRC, 1993). Moreover, lipids play an important role in fish nutrition as a source of essential fatty acids

* Corresponding author at: Department of Marine Bioscience and Technology, Gangneung-Wonju National University, Gangneung 210-702, South Korea.
E-mail address: smlee@gwnu.ac.kr (S.-M. Lee).

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(Sargent et al., 1989) and as carrier of nutrients such as fat-soluble vitamins A, D, and K (Watanabe, 1982). However, fish can utilize dietary lipids up to a certain level beyond which a retarded growth may be achieved due to reduced feed consumption (Daniels and Robinson, 1986; Ellis and Reigh, 1991; Watanabe, 1982). Also, an excess amount of dietary lipid can result in some side effects including feed manufacturing problems, production of fatty liver, body lipid deposition, and lower carcass quality (Chatzifotis et al., 2010; López et al., 2006; Luo et al., 2005; Wang et al., 2005). Therefore, it is very important to optimize dietary protein and lipid levels for formulation of nutritionally balanced cost-effective practical diets of fish.

Groupers are a very diverse family of predatory fish that are widely distributed throughout the tropical and subtropical seas of the world, and are considered as potential candidates for culture due to their favorable taste, fast growth, efficient feed conversion, and high market value. They are high commercial value fish species for Asian aquaculture. The main farmed grouper species are giant grouper (*Epinephelus lanceolatus*) (Yashiro, 2008), brown-marbled grouper (*Epinephelus fuscoguttatus*) (Sugama et al., 2008), Malabar grouper (*Epinephelus malabaricus*) (Yashiro, 2008), orange spotted grouper (*Epinephelus coioides*) (Toledo, 2008), mouse grouper (*Cromileptes altivelis*) (Marte, 2003), and coral trout (*Plectropomus leopardus*) (De Silva, 1998). The giant grouper is considered to be the best candidate for aquaculture due to its faster growth, higher value, and larger size. Brown-marbled grouper is the other widely cultured grouper species because of its relatively faster growth and high adaptability in captivity compared with other grouper species. Interspecific hybridization is a useful technique used in aquaculture to produce fish with desirable traits from two different species (Chevassus, 1983). The brown-marbled grouper and giant grouper hybrid was first produced at Borneo Marine Research Institute (University of Malaysia Sabah, Malaysia) (Chng and Senoo, 2008) and has been globally commercialized especially in Hong Kong market (Senoo, 2010). However, to the best of our knowledge there is no available information on its nutrient requirements. Therefore, the current study was undertaken to evaluate the effects of dietary protein and lipid levels on growth performance, feed utilization, and body composition of the juvenile hybrid grouper.

2. Materials and methods

2.1. Experimental diets

Formulation and proximate composition of the experimental diets are provided in Table 1. Fish meal was used as the primary protein source. Fish oil and a mixture of soybean and linseed oil were used as lipid sources. Eight experimental diets were formulated to contain four different protein levels (40, 45, 50 and 55% crude protein), and each with two lipid levels (7 and 14% crude lipid). P/E ratio of diets ranged from 19.7 to 28.4 mg protein kJ^{-1}. All dry ingredients were thoroughly mixed in a mixer and after addition of oil and ~40% water the pellets were prepared using a laboratory pelleting machine in 3 mm diameter. The pellets were dried overnight at room temperature, crushed into desirable particle size and stored at −30 °C until used.

2.2. Fish and feeding trial

Juvenile hybrid grouper were transported from a private hatchery to Marine Research Institute of University of Malaysia Sabah (Kota Kinabalu, Malaysia). The fish were acclimated to the experimental conditions for one week prior to starting the feeding trial. At the end of the acclimation period, seven hundred and twenty fish (initial mean weight, 2.55 ± 0.10 g) were randomly distributed into 24 plastic cages of 200 L capacity placed in 6000-L circular tanks (8 cages per tank) at a density of 30 fish per cage. A flow-through system was used and the cages were supplied with filtered seawater and aeration to maintain

Table 1
Formulation and proximate composition of the eight experimental diets.

	Diets							
	P40L7	P40L14	P45L7	P45L14	P50L7	P50L14	P55L7	P55L14
Anchovy meal	46.0	46.0	56.0	56.0	66.0	66.0	76.0	76.0
Wheat flour	38.0	30.0	28.0	20.0	18.0	10.0	8.0	0.0
Corn gluten meal	0.0	1.0	0.0	1.0	0.0	1.0	0.0	1.0
α-potato-starch	6.0	6.0	9.0	9.0	12.0	12.0	15.0	15.0
Fish oil	2.1	2.1	1.4	1.4	0.7	0.7	0.0	0.0
Fish oil + soybean oil + linseed oil (1:1:1)	0.0	7.0	0.0	7.0	0.0	7.0	0.0	7.0
α-Cellulose	6.9	6.9	4.6	4.6	2.3	2.3	0.0	0.0
Vitamin premix[a]	0.4	0.4	0.4	0.4	0.4	0.4	0.4	0.4
Mineral premix[b]	0.4	0.4	0.4	0.4	0.4	0.4	0.4	0.4
Choline salt (50%)	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2
Proximate composition (% dry matter)								
Dry matter	92.9	92.3	90.2	91.6	88.5	90.9	87.7	92.1
Crude protein	38.9	39.8	45.0	45.4	51.8	50.0	57.2	57.4
Crude lipid	7.8	13.9	6.9	14.0	7.3	13.1	7.2	13.7
Ash	7.8	7.8	9.3	9.3	11.2	10.8	12.4	12.3
Crude fiber[c]	7.1	7.1	4.8	4.8	2.5	2.5	0.2	0.3
NFE (%)[d]	38.4	31.4	34.0	26.5	27.2	23.6	23.0	16.4
Gross energy (kJg^{-1})	18.8	20.2	19.1	20.7	19.7	20.9	20.2	21.6
P:E ratio (mg protein kJ^{-1})	20.7	19.7	23.6	22.0	26.3	23.9	28.4	26.5
C18:2n-6	0.76	1.38	0.56	1.14	0.44	1.09	0.42	0.99
C18:3n-3	0.12	0.27	0.07	0.22	0.06	0.15	0.08	0.22
C20:5n-3	0.61	0.95	0.78	1.04	0.92	1.13	0.77	1.34
C22:6n-3	0.53	0.80	0.79	0.97	0.90	1.07	0.74	1.26

[a] Vitamin premix contained the following amount which were diluted in cellulose (g/kg mix): L-ascorbic acid, 121.2; DL-α-tocopheryl acetate, 18.8; thiamin hydrochloride, 2.7; riboflavin, 9.1; pyridoxine hydrochloride, 1.8; niacin, 36.4; Ca-D-pantothenate, 12.7; myo-inositol, 181.8; D-biotin, 0.27; folic acid (98%), 0.68; p-aminobenzoic acid, 18.2; menadione, 1.8; retinyl acetate, 0.73; cholecalciferol, 0.003; cyanocobalamin, 0.003.

[b] Mineral premix contained the following ingredients (g/kg mix): $MgSO_4 \cdot 7H_2O$, 80.0; $NaH_2PO_4 \cdot 2H_2O$, 370.0; KCl, 130.0; Ferriccitrate, 40.0; $ZnSO_4 \cdot 7H_2O$, 20.0; Ca-lactate, 356.5; CuCl, 0.2; $AlCl_3 \cdot 6H_2O$, 0.15; KI, 0.15; $Na_2Se_2O_3$, 0.01; $MnSO_4 \cdot H_2O$, 2.0; $CoCl_2 \cdot 6H_2O$, 1.0.

[c] Calculated based on fiber contents of ingredients.

[d] Nitrogen free extract = 100 − (%crude protein + %crude lipid + %crude fiber + %ash).