DIETARY METHIONINE REQUIREMENT FOR GROWTH OF JUVENILE HUMPBACK GROUPER (*Cromileptes altivelis*)

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ABSTRACT

An experiment to find out amino acid methionine requirement for growth of juvenile humpback grouper has been conducted in 18 polycarbonate 100 L tanks. Each tank was equipped with flow-through water system. Twelve juveniles of humpback grouper (5.6 ± 0.7 g each) were randomly selected and stocked in each tank. Juveniles humpback grouper for the experiment were purchased from back yard hatchery in Gondol. Fish fed test diets twice everyday to satiation level for 49 days. Test diets were prepared as dry pellet with casein and fish meal as the intact protein sources, supplemented with the mixture of crystalline L-amino acids to correspond to the amino acid pattern found in the whole body protein of the juvenile humpback grouper, except methionine. Basal diet (diet-1) containing 0.86% methionine was supplied from casein and fish meal. Graded level (0.3%) of L-methionine was added to the basal diet to get the final methionine level in each test diet of 0.86%, 1.16%, 1.46%, 1.76%, 2.06%, and 2.36 %. The experiment was designed according to completely random design (CRD) with 6 treatments (methionine levels) and three replicates for each treatment. Result of the experiment showed that dietary methionine content influenced final weight, weight gain, specific growth rate, feed efficiency, protein retention, and body protein content of juvenile humpback grouper. Optimum dietary methionine for juvenile humpback grouper was calculated using broken line regression analysis. Optimum dietary methionine requirement for growth of juvenile humpback grouper was 1.18% (2.41% of dietary protein) and 1.16% (2.37% of dietary protein) based on weight gain data and feed efficiency, respectively.

KEYWORDS: **humpback grouper, methionine, growth**

INTRODUCTION

Humpback grouper, *Cromileptes altivelis* is a high economic value fish, particularly in the Asian market. The hatchery technology of this fish has been well operated and has successfully produced seed for grow-out culture (Trijoko *et al.*, 1996; Aslianti, 1996; Sugama *et al.*, 2001). One constraint in the development of grow-out culture of this fish was the appropriate compound feed had not well developed yet.

The development of compound feed applied in grouper culture was hampered due to the inadequate information on dietary nutrient requirement of this fish. Some researchers reported that dietary protein requirements of groupers ranged from 47.8% to 60.0%, and varied by species. For well growth, humpback grouper needs diet contains 54.2% protein and 9%—12% lipid (Giri *et al.*, 1999). This protein requirement is relatively high and therefore it needs to find a way to make it more efficient. The result of an experiment about the protein/lipid ratio showed that the high lipid content of diet was not effective in reducing the total protein requirement of juvenile humpback grouper and the diet that contained 56% protein, 9% lipid, 4.77 kcal/g diet, with the protein/lipid ratio of 118 mg/kcal was the best one (Giri *et al.*, 2002). Dietary protein value for fish was determined by the amino acid composition (Wilson & Poe, 1985). The balance in amino acid composition in the diet strongly determines the effectiveness of the use of the dietary protein

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and reduces feed waste that is detrimental to the environment. Information on essential amino acid requirement of humpback grouper is needed to make a feed formula that is economically feasible.

Methionine is one of essential amino acid for fish (Wilson, 1989). This amino acid that contains sulfur serves as precursor of many body components; the methyl group being a major contributor to the whole body pool of one carbon units required for transmethylations, the biosynthesis of choline, thymidine, etc. A part of methionine is converted to cystine in animals; therefore, the presences of cystine spare the requirement of methionine for growth. Methionine is also one of limiting essential amino acid in fish diet (Lovell, 1989). To support the development of artificial diet that is appropriate for juvenile humpback grouper, it is needed to know methoinine requirement for grouper. The purpose of this study was to determine the amino acid methionine requirement for the growth of juvenile humpback grouper.

MATERIAL AND METHOD

The composition of the test diet is shown on Table 1. Casein and fish meal were the intact protein sources for the test diets. The mixture of crystalline L-amino acid was added to obtain a composition of amino acid that fits amino acid composition of whole body protein of juvenile

Table 1. Composition of experimental diet (g/100 g dry diet) for determining methionin requirement of juvenile humpback grouper

Ingredient	Test diet					
	1	2	3	4	5	6
Casein	15.00	15.00	15.00	15.00	15.00	15.00
Fish meal	19.00	19.00	19.00	19.00	19.00	19.00
Fish oil	9.00	9.00	9.00	9.00	9.00	9.00
Vitamin mixture[1]	1.31	1.31	1.31	1.31	1.31	1.31
Mineral mixture[2]	2.50	2.50	2.50	2.50	2.50	2.50
Dextrin	22.14	22.14	22.14	22.14	22.14	22.14
Lecithin	1.50	1.50	1.50	1.50	1.50	1.50
Astaxantin	0.10	0.10	0.10	0.10	0.10	0.10
CMC	3.00	3.00	3.00	3.00	3.00	3.00
L-Methionine	0.00	0.30	0.60	0.90	1.20	1.50
Amino acid mixture[3]	24.95	24.95	24.95	24.95	24.95	24.95
Total Methionine						
% of diet	0.86	1.16	1.46	1.76	2.06	2.36
% of protein	1.76	2.35	2.98	3.58	4.20	4.83
Crude protein (%)	48.9	49.3	49.0	49.1	49.1	48.9
Crude lipid (%)	12.3	11.5	11.6	10.7	10.8	11.7
Ash (%)	6.5	6.6	6.5	6.5	6.5	6.4
Fiber (%)	1.7	1.7	1.9	1.4	1.8	1.8
Energy (kcal/g diet)[4]	4.3	4.2	4.2	4.2	4.2	4.3

[1] Vitamin mixture (mg/100 g diet): thiamin-HCl 5.0; riboflavin 5.0; Ca-panthothenate 10.0; niacin 2.0;pyridoxin-HCl 4.0; biotin 0.6; folic acid 1.5; cyanocobalamine 0.01; inositol 200; p-aminobenzoic acid 5.0; menadione 4.0; b-carotene 15.0; calciferol 1.9; a-tocopherol 2.0; ascorbyl-2-phosphate-Mg 120.0; choline chloride 900.0.

[2] Mineral mixture (mg/100 g diet): KH_2PO_4 412; $CaCO_3$ 282; $Ca(H_2PO_4)$ 618; $FeCl_3.4H_2O$ 166; $MgSO_4$ 240; $ZnSO_4$ 9.99; $MnSO_4$ 6.3; $CuSO_4$ 2; $CoSO_4.7H_2O$ 0.05; KJ 0.15.

[3] See Table 2.

[4] Based on values of 4.0, 9.0, and 4,0 kcal/g for protein, lipid and carbohydrate respectively (Luo *et al.*, 2004).

humpback grouper, except for methionine content. Test diet-1 (basal diet) contained the minimum level of methionine, 0.86% of diet or 1.76% of protein, which obtained from casein and fish meal. Another five test diets were prepared by adding incremental levels (0.3%) of L-methionine to the basal diet at 1.16%, 1.46%, 1.76%, 2.06%, and 2.36% of diet, corresponding to 2.35%, 2.98%, 3.58%, 4.20%, and 4.83% of dietary protein (Table 1). These levels of methionine were ranged from below up to above the methionine content found in juvenile humpback grouper whole body protein. Test diets were prepared in the form of pellet, each with a 3 mm diameter and were dried using a freeze-dryer and then stored in the refrigerator at 4°C during the experiment. The test diets had the same protein content (48.9%—49.3%) and energy (4.2—4.3 kcal/g diet) that obtained by reducing the addition of glutamic acid while increasing of methionine level. Amino acids composition of feed ingredients for the test diet and the amino acid composition of whole body protein of juvenile humpback grouper are shown in Table 2.

Feeding experiment was conducted in 100 L polycarbonate tanks equipped with flow-through water system and aeration. Juvenile humpback groupers for the experiment were purchased from back yard hatchery in Gondol. Twelve fishes (5.6 ± 0.7 g in body weight or 7.4 ± 0.2 cm in total length each) were stocked in each tank. Fish fed test diet twice a day (morning and afternoon) at the satiation level. The experiment was designed using a complete random design (RCD) with 6 treatments of methionine levels and each treatment had 3 replicates. The growth measurement of fish was made every week by weighing all fish individually in the period of 49 days feeding experiment.

The amino acid composition of the feed stuff and whole body protein of juvenile

Table 2. Amino acid composition (g/100 g dry diet) of dietary ingredients for determining the methionine requirement of juvenile humpback grouper

Amino acids	Supplied by 15% casein	Supplied by 19% fish meal	Supplied by crystalline amino acid	Total	50% whole body protein of grouper
EAA					
Arginine	0.550	0.735	2.168	3.453	3.453
Histidine	0.335	0.322	0.653	1.310	1.310
Isoleucine	0.720	0.522	0.740	1.983	1.983
Leucine	1.175	0.930	1.491	3.596	3.596
Lysine	0.949	0.819	2.277	4.044	4.044
Methionine	0.580	0.280	0.0--1.50	0.86--2.36	1.665
Phenylalanine	0.611	0.542	0.947	2.099	2.099
Threonine	0.600	0.528	1.801	2.929	2.929
Valine	0.897	0.599	0.613	2.109	2.109
Tryptophan	-	-	0.340	0.340	0.340
NEAA					
Aspartic acid	0.838	1.126	2.903	4.867	4.867
Glutamic acid	3.082	2.337	5.231--3.731	10.650--9.150	9.150
Serine	0.515	0.521	1.190	2.226	2.226
Proline	1.252	0.477	0.568	2.297	2.297
Glycine	0.252	0.693	2.450	3.395	3.395
Alanine	0.431	0.803	2.386	3.621	3.621
Tyrosine	0.817	0.423	0.828	2.068	2.068
Cystine	0.081	0.055	0.145	0.281	0.281

humpback grouper were analyzed by Amino Acid Analyzer. Approximately 2 mg of sample was weighed, put into a cupped tube and hydrolyzed with HCL 6 N for 22 hours at 110°C. After being filtered through 0.2 µm, the sample then was ready to be injected into High Speed Amino Acid Analyzer with ion exchange resin column, 4.6 x 150 mm. To separate an individual amino acid, gradient system with sodium citrate buffer solution (pH 3.3, pH 4.3, and pH 4.9) at the flow-rate of 0.225 mL/minute was used. Post column reaction with ninhydrin solution at the flow rate of 0.3 mL/minute was used to identify each of amino acid at the wavelength of 570 nm and 440 nm. Protein and lipid content of test diet and fish were determined according to Kjeldhal method and the Bligh & Dyer (1959). Moisture and ash content were determined based on AOAC (1990).

The growth data, feed intake, feed efficiency and survival of fish were analyzed using one-way analysis of variance (ANOVA). Significant differences between means were evaluated by Tukey test (Steel & Torrie, 1980). Probabilities of $P<0.05$ were considered significant. The optimum dietary methionine requirement was determined using the broken line regression method (Zeithoun *et al.*, 1976) with the 95% confidence interval.

RESULTS

The final weight, percentage weight gain, specific growth rate, and feed efficiency of juvenile humpback grouper were influenced by dietary methionine content in the diet (Table 3). Fish fed with test diet-1 (without the addition of methionine) had the lowest growth and significantly different from the other treatments ($P<0.05$). This diet had 0.86% methionine content that supplied from casein and fish meal. The addition of crystalline methionine into the diet could improve the growth of fish. However, the increase of methionine content among of test diet from 1.16% to 2.36% resulted in the growth of fish that was not significantly different ($P>0.05$).

Feed intake was not influenced by methionine content of diet (Table 3), but the addition of methionine into the diet increased feed efficiency. Diet without the addition of methionine (methionine content of 0.86%) produced the lowest feed efficiency and its value was significantly different from that of other treatments ($P<0.05$). This indicated that low of methionine in test diet-1 caused the inefficient use of other amino acids in the diet for fish growth. Some amino acids were also deaminated as energy source or to form other compounds.

Survival rate of fish during the experiment was not influenced by the difference of methionine content in diet ($P>0.05$). However, fish fed with test diet-1 (without the addition of methionine) showed weak conditions during the last part of feeding experiment. The decrease of fish conditions followed the decrease of fish growth rate as shown in Figure 1.

Table 3. Final weight (FW), weight gain (WG), feed efficiency (FE), feed intake (FI), survival rate (SR), and specific growth rate (SGR) of grouper fed test diet for 49 days[1]

Dietary methionine level (%)	FW (g)	WG (%)	FE2	FI (g/fish/day)	SR (%)	SGR (%/day)3
0.86	11.4 a	104.3 a	0.61 a	0.19 a	94.5 a	1.46 a
1.16	14.9 b	167.2 b	1.00 b	0.19 a	94.4 a	2.00 b
1.46	15.3 b	174.1 b	1.02 b	0.19 a	94.5 a	2.06 b
1.76	15.3 b	174.8 b	1.03 b	0.19 a	97.2 a	2.06 b
2.06	15.1 b	171.8 b	0.99 b	0.19 a	94.5 a	2.04 b
2.36	15.2 b	172.6 b	0.97 b	0.19 a	94.4 a	2.05 b

[1] Initial weight: 5.6 ± 0.7 g

[2] Feed efficiency = Weight gain (g)/total feed intake (g)

[3] Specific growth rate = [ln (mean final weight) – ln (mean initial weight)] x 100/49

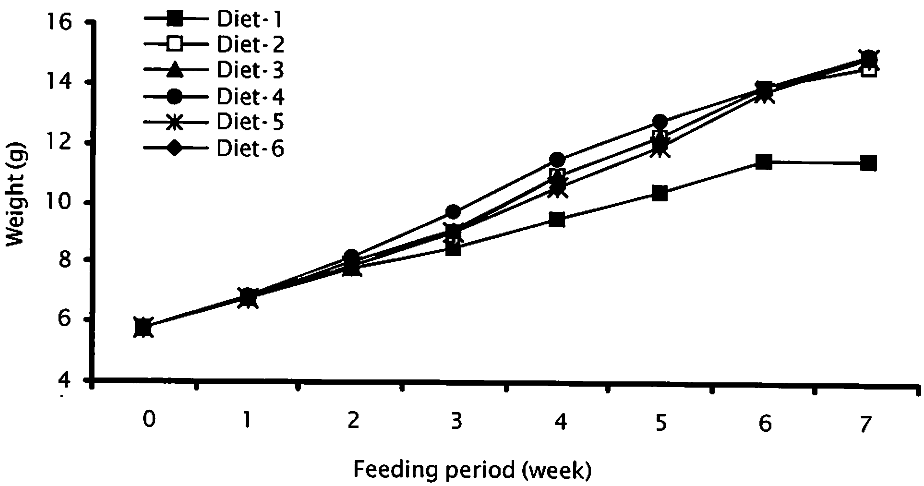

Figure 1. Growth of juvenile humpback grouper fed test diet during 49 day experiment

The effect of dietary methionine content on the proximate composition of whole body of fish is shown in Table 4. The protein content of whole body fish was influenced by methionine content in the test diet. Fish fed with test diet-1 (without addition of methionine) had the lowest body protein content (55.95%) with the highest ash content (17.65%) and significantly different from other treatments ($P<0.05$), except for treatment 2.06% dietary methionine level. However, the increase of dietary methoinine content from 1.16% to 1.46% or above did not increase body protein content. On the other hand, dry matter and crude fiber were not influenced by dietary methionine content ($P> 0.05$). The pattern of the body protein content and fish growth were also followed by the pattern of protein retention in the fish body. The lowest protein retention (18.96%) was observed on fish fed with test diet-1 (without the addition of methionine) and this value significantly different from other treatments ($P<0.05$).

The optimum dietary methionine requirement of humpback grouper was calculated using broken line regression analysis. On the basis of the percentage weight gain, it was obtained that dietary methionine requirement of juvenile humpback grouper was 1.18% of diet (at the 95% confidence interval, the values ranged from 1.15% to 1.21%) or equivalent to 2.41% of protein (Figure 2). When calculation was based on feed efficiency data, it was obtained that dietary methionine requirement was 1.16% of diet (at the 95% confidence interval, the value ranges from 1.13% to 1.19%) or equivalent to 2.37% of protein. This value was slightly lower, but was close to the value obtained on the basis of weight gain data.

DISCUSSION

The optimum dietary methionine requirement of juvenile humpback grouper based on weight gain data and feed efficiency were 1.18% of diet (2.41% of protein) and 1.16% of diet (2.37% of protein), respectively. Dietary methionine requirement has been reported for some economically important fish, such as for yellow tail, *Seriola, quinqueradiata* (2.56%, Ruchimat *et al.*, 1997), red drum, *Sciaenops ocelltus* (2.69%, Moon & Gatlin, 1991) channel catfish, *Ictalurus punctatus* (2.3%, Harding *et al.*, 1977), coho salmon, *Oncorhynchus kisutch* (2.7%, Arai & Ogata, 1993), rainbow trout, *Oncorhynchus mykiss* (2.2%, Walton *et al.*, 1982), read sea bream, *Pagrus major* (2.2%, Forster & Ogata, 1998), milkfish, *Chanos chanos* (3.3%, Borlongan & Coloso, 1993), and Japanese flounder, *Paralichthys olivaceus* (2.98%, Alam *et al.*, 2000). All these values were expressed as percent of protein. Methionine requirement of juvenile humpback grouper obtained in this study closely similar with the values for other economically important fish that have been mentioned previously. No other

Table 4. Whole body composition (% DM) and protein retention (PR) of juvenile humpback grouper fed test diet for 49 days

Dietary methionine level (%)	Dry matter	Protein	Lipid	Ash	Fiber	PR (%)[1]
0.86	28.57 [a]	55.95 [a]	16.05 [a]	17.65 [a]	2.42 [a]	18.96 [a]
1.16	29.29 [a]	58.94 [b]	15.98 [a]	15.69 [b]	2.58 [a]	36.06 [b]
1.46	29.55 [a]	58.00 [b]	16.52 [a]	15.44 [b]	2.88 [a]	36.41 [b]
1.76	29.22 [a]	58.37 [b]	16.44 [a]	14.86 [b]	2.80 [a]	36.98 [b]
2.06	28.78 [a]	57.43 [ab]	16.21 [a]	15.79 [b]	2.39 [a]	33.79 [b]
2.36	29.57 [a]	58.20 b	16.18 [a]	15.07 [b]	2.41 [a]	36.70 [b]

[1] Protein retention = protein gain x 100/protein intake

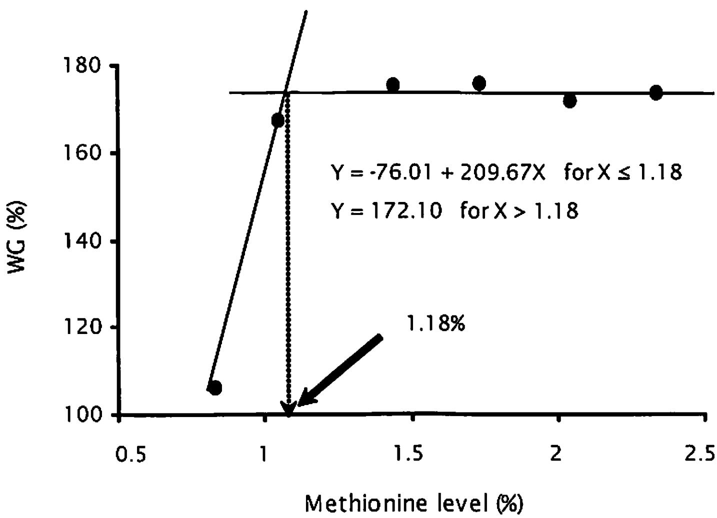

Figure 2. Relationship between dietary menthionine levels and weight gain of juvenile humpback grouper fed test diet for 49 days

information on amino acid requirement was found, including methionine for humpback grouper. Forster & Ogata (1998) calculated methionine requirement of Japanese flounder based on its lysine requirement and obtained the value of 1.9% of protein. This value is much lower than that for other economically important fishes that have been mentioned above, including for Japanese flounder reported by Alam *et al.* (2000), that was 2.98%. Variation in amino acid requirements can be influenced by fish species and age, method used to determine it, protein source, environmental factors and experiment condition (Tacon & Cowey, 1985; Moon & Gatlin, 1991).

The slow growth rate of fish fed diet without addition of methionine might be related to the imbalance of amino acid composition in the test diet. In this case, amino acid methionine becomes limiting amino acid in the diet. Furthermore, other sufficient or excessive amino acid contents could not be used to synthesize body protein (growth) due to the limited available of methionine, and resulted in slow growth rate of fish. The slow growth rate of fish fed with diet that lacked methionine as reported in the literature was also due to the low feed intake, such as the one that occurred to Japanese flounder (Alam *et al.*, 2000) and Atlantic salmon, *Salmo salar* (Rollin *et al.*, 1994). However, in the experiment in which juvenile humpback grouper were used it was obtained that the feed intake was not influenced by dietary methionine levels, but the low fish growth occurrence was thought due to the imbalance of amino acid composition of test diet. Protein retention and whole body protein content tended to increase with the addition of crystalline methionine up to the level required. This might have been caused by increase in nitrogen retention with the increase of dietary methionine content, like the one reported to have occurred on rainbow trout (Kim *et al.*, 1992) and yellow tail (Rochimat *et al.*, 1997). The growth of juvenile humpback grouper fed with diet without addition of methionine in this experiment is the lowest compared to other treatments. This shows that juvenile humpback grouper are capable of using crystalline methionine that was added to the test diet and also shows that amino acid methionine is essential for humpback grouper.

Methionine deficiency symptom in the form of eye cataract was reported to have occurred in rainbow trout (Walton *et al.*, 1982), however, it has not yet been found in other fish, such as Japanese flounder (Alam *et al.*, 2000; Estevez *et al.*, 1997). Visual observation made on juvenile humpback grouper fed with diet lacking in methionine in this experiment did not indicate eye cataract.

CONCLUSIONS

- Dietary methionine content has an effect on the growth and feed efficiency of juvenile humpback grouper.
- Optimum dietary methionine content for the growth of juvenile humpback grouper is 1.18% of diet or equivalent to 2.41% of protein.

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