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Effects of Dietary Protein Level on Growth and Ammonia Excretion of Leopard Coral Grouper, *Plectropomus leopardus* (Lacepede, 1802)

(Kesan Pemakanan Tahap Protein terhadap Pertumbuhan dan Perkumuhan Amonia pada Kerapu Bara *Plectropomus leopardus* (Lacepede, 1802))

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ABSTRACT

The effects of dietary protein level on the growth performance and ammonia excretion of the leopard coral grouper, Plectropomus leopardus *were investigated for eight weeks. Fish were fed diets with 40, 45, 50, 55 and 60% crude protein levels in separate recirculating systems. Fish fed with the 50% crude protein containing diet showed the best ingestion rate, which was significantly higher than that found in the other groups. As the dietary protein level increased, the specific growth rate increased significantly and it reached the highest level at 50% crude protein containing diet. Based on the results of all measured parameters 50% protein containing diet was the best among all test diets. The regression equation for dietary protein level versus ammonia excretion indicated that the optimal dietary protein level with the least ammonia excretion was 53.14%. More research is still needed to elucidate the effects of 53.14% crude protein containing diet on the specific growth rate, feed conversion ratio, protein efficiency ratio and ingestion rate of leopard coral grouper before recommending this level. Until then, 50% protein containing diet can be recommended for leopard coral grouper culture in the recirculation system.*

Keywords: Ammonia excretion; dietary protein level; Plectropomus leopardus*; water quality*

ABSTRAK

Kesan tahap protein pemakanan terhadap pertumbuhan dan perkumuhan amonia pada kerapu bara, Plectropomus leopardus *telah dikaji selama lapan minggu. Ikan telah diberi makan dengan diet tahap protein mentah 40, 45, 50, 55 dan 60% dalam sistem peredaran air berasingan. Ikan yang diberi makan diet dengan kandungan 50% protein mentah menunjukkan kadar pengambilan terbaik, jauh lebih tinggi daripada yang terdapat di dalam kumpulan-kumpulan lain. Apabila tahap protein dalam diet meningkat, kadar pertumbuhan khusus meningkat dengan ketara dan mencapai tahap tertinggi pada diet yang mengandungi 50% protein mentah. Berdasarkan keputusan semua parameter yang diambil, diet mengandungi 50% protein adalah yang terbaik di antara semua ujian diet. Persamaan regresi untuk tahap protein dalam diet berbanding perkumuhan amonia menunjukkan tahap protein pemakanan yang optimum dengan kurang perkumuhan amonia adalah 53.14%. Lebih banyak kajian perlu dijalankan untuk menjelaskan kesan diet yang mengandungi 53.14% protein mentah terhadap kadar pertumbuhan spesifik, nisbah pertukaran makanan, nisbah kecekapan protein dan kadar pemakanan kerapu bara sebelum ia dapat disyorkan. Sehingga ini, diet yang mengandungi 50% protein dapat disyorkan kepada penternakan kerapu bara dalam sistem peredaran air semula.*

Kata kunci: Amonia perkumuhan; kualiti air; Plectropomus leopardus*; tahap protein pemakanan*

Introduction

Among coral groupers, the leopard coral grouper, *Plectropomus leopardus* (Lacepede 1802) is one of the favorite target fish for all sectors of the fishery because it enjoys high consumer preference and market value locally and overseas (Ayling et al. 2000). Its natural habitat includes open seas and coral reefs. However, it is commonly found in coral-rich areas of lagoon reefs and mid-shelf reefs (Kailola et al. 1993; Kuiter & Tonozuka 2001). The leopard coral grouper population is declining on the Great Barrier Reef and this problem is further aggravated by the increasing harvest in Australia (Ayling et al. 2000). Many other countries have also experienced declining landings, implying that the population of leopard coral grouper may be decreasing (Russell 2007). Depletion of natural stocks together with high commercial value has encouraged stock enhancement and aquaculture for leopard coral grouper. Successful hatching in 2009 enhanced aquaculture of this species in China where land-based intensive culture is one of the main culture methods of this species. To date, studies of leopard coral grouper have mainly focused on its ecology, embryonic development, early life history, molecular classification and behavior (Kenzo et al. 2008; Yang et al. 2012; Zhu & Yue 2008). Only a few studies focused on nutrition of this species. However, study on the optimization of protein in the formulated diet of this species is still lacking.

Protein is one of the most expensive and important ingredients in fish feeds. Dietary protein can affect growth and health of cultured fish as well as maintenance of a good farming environment (Rahman et al. 2008a, 2008c, 2012; Rahman & Verdegem 2010). Therefore, it should be carefully apportioned to meet the needs of the cultured organisms (Rajkumar et al. 2013; Yang et al. 2002). If excessive protein is supplied in the diet, only part of it will be used to synthesize new tissues and the surplus will be metabolized as an energy source (Guo et al. 2012). The main end product of protein metabolism in aquatic animals is ammonia, thus fish diet also affects water quality via ammonia excretion (Rahman & Meyer 2009; Rahman et al. 2008b, 2008d, 2010). The appropriate amount of protein in the formulated diet can enhance growth and reduce ammonia excretion. Therefore, optimizing protein in the fish diet is very important for the successful aquaculture of any fish species (Rahman & Verdegem 2007).

Considering the above issues, the goals of this study were to elucidate the effects of different levels of dietary protein on the ammonia excretion and growth of leopard coral grouper and water quality in the culture system.

Materials and Methods

Feed Formulation

Five experimental diets containing 39.9, 44.1, 50.7, 56 and 61.4% crude protein (CP) (CP40, CP45, CP50, CP55 and CP60, respectively) were formulated using feed formulation software REFS3000 (Beijing Resource Group, Beijing City, P.R. China). Soybean meal and defatted fish meal were used as the protein sources in the diets and wheat flour was the carbohydrate source. All ingredients (Table 1) were ground in an ultrafine pulverizer, sieved to a particle size <75 μm and mixed. Pellets were generated using a meat mincer machine (TK-12, Shanghai Yingxiao Food Apparatus Co., Ltd., Shanghai, P.R. China). Each of the different protein level diets was mixed with fresh water (2:1), passed through the mincer machine and dried at 60°C until a constant weight was reached. The pellets were then ground and sieved to a particle size between 3 and 4 mm prior to use.

Experimental Animals and Feeding Trial

Healthy leopard coral groupers were collected from the Tianjin Shengyi Aquatic company located in Tianjin City, P.R. China. One hundred eighty fish with initial body weight of 36.4±1.5 g were randomly stocked into 15 glass aquaria (size of aquaria was 80 × 40 × 40 cm^3) in equal numbers (*n*=12) into five recirculating systems to form five triplicate groups. Prior to the experiment, the fish were acclimatized to the laboratory conditions in a recirculating system for one week. During the experiment, aeration was provided continuously and mean water quality parameters were maintained as temperature 26±2°C, salinity 30±2 gL^{-1} and dissolved oxygen >5 mgL^{-1}.

The five groups of fish were fed with the five different types of diet twice a day at 9:00 h and 15:00 h. Uneaten feed and feces were siphoned from the tanks 24 h later and separated carefully from each other. The uneaten feed was dried at 60°C to a constant weight to calculate feed consumption by fish. At the end of the experiment, all fish in each tank were harvested 24 h after the last feeding and weighed. Specific growth rate (SGR), ingestion rate (IR), feed conversion ratio (FCR) and protein efficiency ratio (PER) were calculated using the following equations:

$$\text{SGR} (\% \ d^{-1}) = 100 \times [\ln W_2 - \ln W_1] / T;$$
$$\text{FCR} = C / (W_2 - W_1);$$
$$\text{IR} (mg\ ind^{-1} d^{-1}) = C / (N * T) \text{ and}$$
$$\text{PER} = (W_2 - W_1) / (C * M)$$

TABLE 1. Ingredients and chemical composition of trial diets (air-dry basis g kg^{-1})

Ingredients	CP40	CP45	CP50	CP55	CP60
Peru fish meal	339.4	402.0	464.6	527.2	589.8
Wheat gluten	111.6	132.3	153.0	173.7	194.5
Soybean protein concentrate	37.2	44.1	51.0	57.9	64.8
Wheat powder	461.6	371.4	281.1	190.9	100.6
Fish oil	20.1	20.1	20.1	20.1	20.1
Soybean lecithin	20.1	20.1	20.1	20.1	20.1
Vitamin premix[1]	5.0	5.0	5.0	5.0	5.0
Mineral premix[2]	5.0	5.0	5.0	5.0	5.0
Total	1000	1000	1000	1000	1000
Chemical composition (g kg^{-1})					
Dry matter	916.6	924.6	919.6	920.6	928.6
Crude protein	398.8	441.3	507.2	559.8	614.1
Crude lipid	75.6	82.9	91.8	105.7	111.2
Crude ash	95.1	107.3	122.0	133.5	145.1

[1]Vitamin premix (g kg^{-1} premix) provided the following levels of nutrients: thiamin HCl, 0.35; riboflavin, 4.5; pyridoxine HCl, 1.0; DL-Ca-pantothenate, 5.0; vitamin A acetate (20 000 IUg^{-1}), 4.0; nicotinic acid, 5.0; biotin, 0.05; vitamin B12, 0.002; folic acid, 0.38; Vitamin C, 200; vitamin D3 (400 000 IUg^{-1}), 0.004; menadione, 2.0; inositol, 10.0; DL-a-tocopheryl acetate (250 IUg^{-1}), 8.0; a–cellulose, 759.71

[2]Mineral premix (g 100 g^{-1}) provided the following levels of nutrients: cobalt chloride, 0.004; zinc sulphate heptahydrate, 10.2; cupric sulphate pentahydrate, 0.30; potassium iodide, 0.06; ferrous sulphate, 10.5; sodium selenite, 0.022; manganous sulphate monohydrate, 0.650; magnesium sulphate heptahydrate, 16.5; filler, 61.76

where W_1 and W_2 are initial and final average individual body weights of fish in each tank, respectively; T is the duration of the experiment; C is the feed consumed (dry weight basis); N is the number of fish in each aquarium; and M is the dietary protein content.

AMMONIA EXCRETION

Ammonia excretion was analyzed for 12 h on the last day of week 5. The different diets were placed in each tank at 9:00 h in the morning. Uneaten feed was siphoned from the tanks at 9:30 h to calculate feed consumption by fish. The total ammonia (ammonia/ammonium in water) concentration was determined at 9:30 h and 12:00 h using the phenol-hypochlorite method (Solorzano 1969). Ammonia excretion was calculated according to Russell (2007) using the equation: Ammonia excretion [mg(g diet)$^{-1}$] = [final ammonia content (mgL^{-1}) - initial ammonia content (mgL^{-1})] × water volume (L) / feed intake (g).

WATER QUALITY

The water quality parameters were measured on the last day of week 4 in the morning before supplying feed. To measure water quality, a 250 mL water sample was removed from each tank. The sample was filtered through a cellulose membrane filter before analysis. Ammonia concentration was measured using phenol-hypochlorite method (Solorzano 1969), nitrite concentration was measured using the hydrochloric acid naphthalene ethylenediamine method (Grasshoff 1999) and phosphate concentration was measured using the phosphorus molybdenum blue spectrophotometry method (Taguchi et al. 1985).

STATISTICAL ANALYSIS

All data were checked for normality and homogeneity of variance before analysis. Growth performance, ammonia excretion and water quality data were subjected to one-way analysis of variance (ANOVA). If any effect was significant, difference between the means were analyzed by Duncan's test for unplanned multiple comparison of mean (p<0.05). All statistical analyses were performed using SPSS software (version 15.0; SPSS, Chicago, IL, USA).

RESULTS

GROWTH PERFORMANCE

The growth performance parameters differed significantly (p<0.05) among groupers fed at the different protein level diets. The SGR of grouper in the CP60, CP55 and CP50 groups were significantly (p<0.05) higher than those in the CP45 and CP40 groups (Figure 1). There was no significant difference between CP60, CP55 and CP50 groups on grouper's SGR, which was statistically the same in the CP45 and CP40 groups. FCR of grouper decreased significantly with increasing dietary protein content (Figure 2). The FCR of grouper in the CP55 and CP60 groups were significantly (p<0.05) lower than those in the CP45 and CP40 groups (p<0.05). There was no significant difference (p>0.05) between CP60, CP55 and CP50 groups on grouper's FCR, which was statistically (p>0.05) similar in the CP45 and CP50 groups. The PER increased first and then decreased as the dietary protein level increased and the highest value occurred at CP55 (Figure 3). No significant differences (p>0.05) were found for PER among the CP60, CP55 and CP50 groups. The highest IR was observed in the CP50 group, followed by the CP60, CP50, CP45 and CP40 groups (Figure 4).

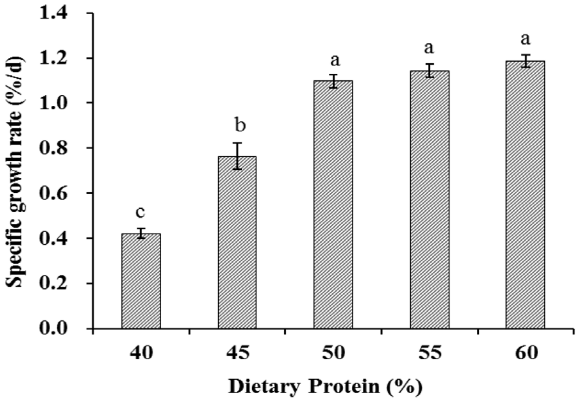

FIGURE 1. Effect of dietary protein levels on specific growth rate of leopard coral grouper based on one-way ANOVA. Bar with no superscript in common differ significantly (p<0.05). Data are mean ± standard error

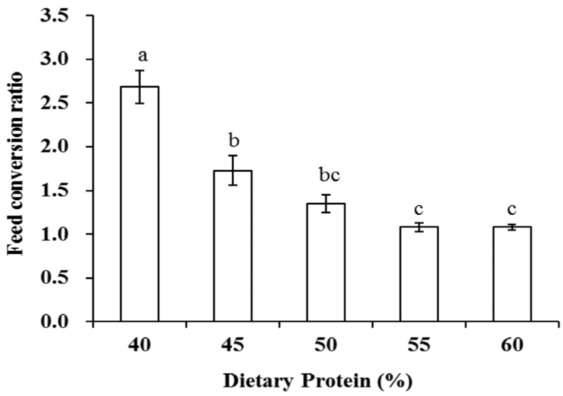

FIGURE 2. Effect of dietary protein levels on feed conversion ratio of leopard coral grouper based on one-way ANOVA. Bar with no superscript in common differ significantly (p<0.05). Data are mean ± standard error

AMMONIA-NITROGEN PRODUCTION

Figure 5 shows the ammonia excretion of leopard coral grouper fed at the different test diets. Dietary protein levels significantly (p<0.05) affected ammonia excretion. As the dietary protein level increased, the ammonia concentration first declined and then increased significantly. The highest

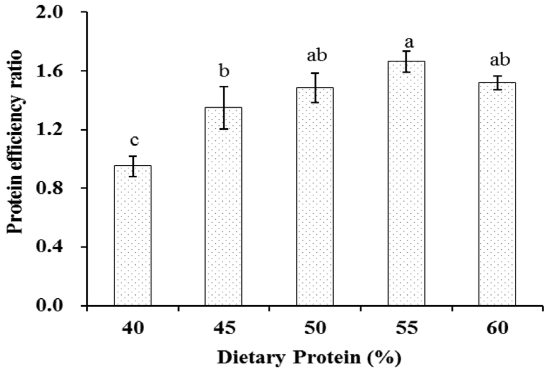

FIGURE 3. Effect of dietary protein levels on protein efficiency ratio of leopard coral grouper based on one-way ANOVA. Bar with no superscript in common differ significantly (p<0.05). Data are mean ± standard error

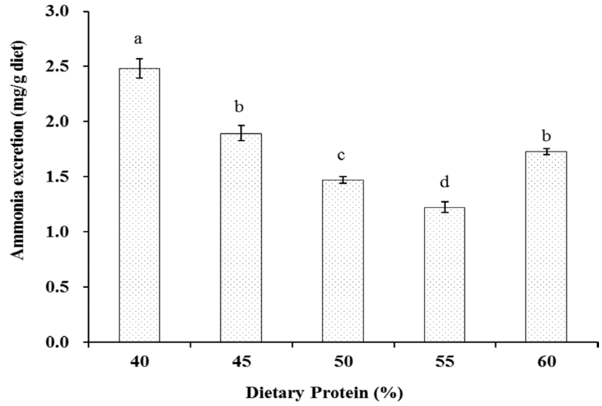

FIGURE 5. Effect of dietary protein levels on ammonia excretion of leopard coral grouper based on one-way ANOVA. Bar with no superscript in common differ significantly (p<0.05). Data are mean ± standard error

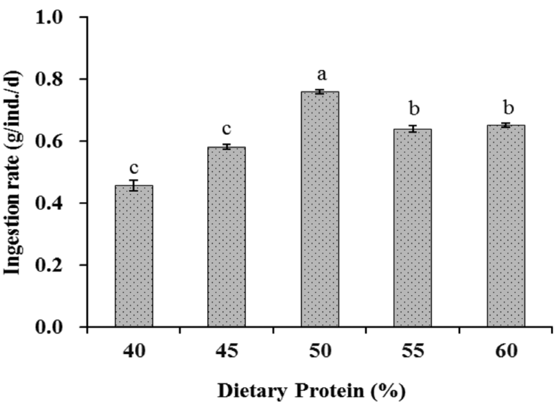

FIGURE 4. Effect of dietary protein levels on ingestion rate of leopard coral grouper based on one-way ANOVA. Bar with no superscript in common differ significantly (p<0.05). Data are mean ± standard error

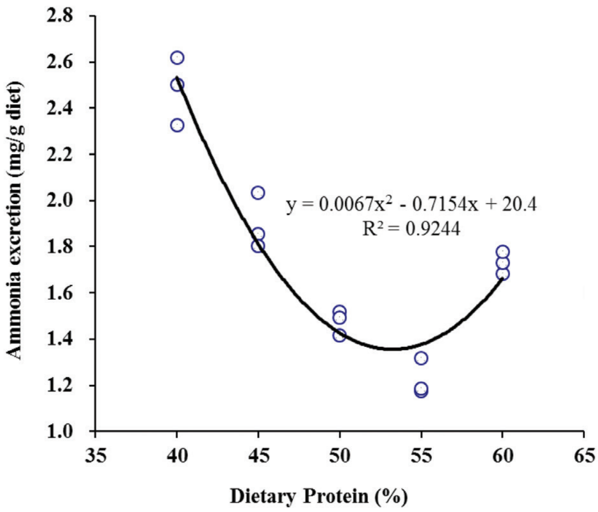

FIGURE 6. Regression analysis illustrating the relationship between dietary protein levels and the ammonia excretion in leopard coral grouper

ammonia excretion by leopard coral grouper was observed in the CP40 group, while the lowest was observed in that CP55 group. There was no significant difference (p>0.05) between CP45 and CP60 groups on the ammonia excretion by leopard coral grouper. The regression equation for the dietary protein level versus ammonia excretion was $y = 0.0067\times^2 - 0.7154\times + 20.4$ ($r^2 = 0.9244, p<0.05$) (Figure 6). Using this equation, the optimal dietary protein level with the least ammonia excretion was calculated to be 53.14%.

WATER QUALITY

The ammonia, nitrite and phosphate concentrations of water varied significantly (p<0.05) with dietary protein level (Table 2). The highest ammonia concentration was observed in the aquaria water supplied with 61.4% crude protein diet (CP60), while lowest was observed in the aquaria water supplied with 56% crude protein diet. Ammonia concentrations were statistically similar in the aquaria water supplied with 39.9, 44.1 and 50.7% crude protein containing diets. The nitrite concentrations were higher in the aquaria water supplied with 61.4% crude protein diet than those tanks that were supplied with 39.9, 44.1, 50.7 and 56% crude protein diets. The nitrite concentration was similar in the tanks with 39.9, 44.1, 50.7 and 56% crude protein diets. Phosphate concentration ranged between 0.273 and 0.420 mg/L and was positively correlated with the dietary protein levels when the dietary protein content was above 44.1%. The phosphate concentration (0.420±0.003) was the highest in the aquaria water supplied with 61.4% crude protein diet (CP60), followed by 55%, 50.7%, 39.9% and 44.1% crude protein diet.

DISCUSSION

Increasing the protein content in the fish feed usually results in increased fish growth rate (Bibiano-Melo et al.

TABLE 2. Effect of dietary protein level diets on water quality of leopard coral grouper based on one-way ANOVA. Data are mean ± standard error

Parameter (mg/L)	CP40	CP45	CP50	CP55	CP60
Ammonia	0.166±0.004[b]	0.158±0.004[b]	0.162±0.004[b]	0.123±0.003[c]	0.188±0.003[a]
Nitrite	0.064±0.010[b]	0.066±0.005[b]	0.111±0.003[b]	0.100±0.002[b]	0.222±0.031[a]
Phosphate	0.286±0.001[c]	0.273±0.002[d]	0.291±0.001[c]	0.362±0.001[b]	0.420±0.003[a]

Values in the same row with different superscripts differ significantly ($p<0.05$)

2006). This statement is true in the present study up to a certain point for leopard coral grouper. The SGR of leopard coral grouper increased with increasing dietary protein level up to 50.7% crude protein. This result concurs with Gunasekera et al. (2000) and Mohanty and Samantaray (1996). Gunasekera et al. (2000) used five different diets containing 40, 45, 50, 55 and 60% crude protein to culture murray cod, *Maccullochella peelii peelii* and observed the highest SGR in fish fed with 50% crude protein containing diet. Mohanty and Samantaray (1996) used almost similar diets to culture snakehead, *Channa striata* and observed the highest SGR in fish fed with 55% crude protein containing diet.

Based on the present observation, 50.7% crude protein containing diet may be considered as the optimal dietary protein level for leopard coral grouper culture. This statement is further supported by the feed conversion ratio, dietary protein ingestion rate and ammonia excretion by leopard coral grouper, which ingested the highest quantity of protein but excreted the lowest quantity of ammonia when fed with the 50.7% crude protein containing diet. However, regression analysis of dietary protein level versus ammonia excretion showed that the optimal dietary protein level was 53.14% for leopard coral grouper culture. This dietary protein level is within the range that allows for high growth rate and production of fish. According to NRC (1993), the dietary protein requirements for most fishes vary from 30 to 55% depending on species, size, source of protein and environmental conditions. Millikin (1982) reported that juvenile striped bass, *Morone saxatilis* required a diet containing 55% protein for their best growth. For commercial farming of juvenile Asian sea bass or other marine fish in China, feeds normally contain 45% or higher crude protein. The metabolism of carnivorous fish is generally adapted to high levels of dietary protein (Bibiano-Melo et al. 2006). Leopard coral grouper is piscivorous; juveniles mostly eat crustaceans especially prawns and adults feed on a variety of reef fish particularly damselfish. This piscivorous feeding habit may explain the requirement of relatively high levels of dietary protein for their high growth.

In this study, the protein efficiency ratio increased significantly when the dietary protein level was below 50%. However, an opposite trend was almost obtained in case of ammonia excretion by leopard coral grouper. We observed a significant negative correlation ($r = -0.8541$, $p<0.01$) between protein efficiency ratio and ammonia excretion by leopard coral grouper. This finding is in agreement with Kim et al. (1991) and Rychly (1980), who stated that the increased nitrogenous excretion is a consequence of using amino acids as energetic compounds and this process reduce protein efficiency ratio. When lipids are not available to provide the energy required for growth and activity, proteins are generally metabolized as energy instead and this situation leads to a lower protein efficiency ratio and higher ammonia excretion (Cho & Kaushik 1985; Engin & Carter 2001; Shiau & Huang 1989).

Dietary proteins that are above the optimal levels influence ammonia excretion in fish and shrimp. For example, ammonia excretion is greatly increased by white-leg shrimp when the dietary protein level is more than 43% (Xia et al. 2010). Similarly, Jiang et al. (2005) observed that turbot, *Scophthalmus maximus* excreted much higher quantity of ammonia in the environment when the dietary protein content was 54.3% compared to 52.1%. In the present study, leopard coral grouper excreted more ammonia when fed with 61.4% protein containing diet than the 56% protein containing diet. The reason might be the amino acids surplus from the 61.4% protein containing diet (Ballantyne 2001; Stone et al. 2003). According to Ballantyne (2001) and Stone et al. (2003), the amino acid surplus from protein-rich diets cannot be directly stored in fishes and they are deaminated and converted into energetic compounds. The augmented protein breakdown in fish results in an increased concentration of plasma ammonia, which is promptly excreted through the gills into the environment (van Waarde 1983; Yang et al. 2002).

In the present study, 53.14% protein containing diet produced the least ammonia in the culture system. However, nothing is known about the effects of 53.14% protein containing diet on SGR, FCR, PER and IR of the leopard coral grouper. Therefore, more research is still needed to elucidate the effects of 53.14% protein containing diet on SGR, FCR, PER and IR of the leopard coral grouper before recommending this level. Until then, 50.7% protein containing diet can be recommended for leopard coral grouper culture in a recirculation system.

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