HAYATI Journal of Biosciences 23 (2016) 18–21

HOSTED BY

Contents lists available at ScienceDirect

HAYATI Journal of Biosciences

journal homepage: http://www.journals.elsevier.com/hayati-journal-of-biosciences

Original research article

Evaluation of Smoked Skipjack Processing Byproduct Meal as an Alternative Feed Ingredient for Juvenile Humpback Grouper *Cromileptes altivelis*

Muhamad Agus Suprayudi,[1*] Fatma Hajiali,[1,2] Nur Bambang Priyo Utomo,[1] Julie Ekasari,[1] Ichsan Fauzi[1]

[1] *Department of Aquaculture, Faculty of Fisheries and Marine Science, Bogor Agricultural University, Bogor, Jawa Barat, Indonesia.*
[2] *North Maluku Fisheries and Marine Affair State Agency, Sofifi, Maluku Utara, Indonesia.*

ARTICLE INFO

Article history:
Received 12 February 2014
Accepted 21 August 2015
Available online 9 July 2016

KEYWORDS:
fish meal,
humpback grouper,
smoked skipjack processing byproduct meal

ABSTRACT

This study aimed to evaluate the utilization of smoked skipjack processing byproduct meal (SPBM) to reduce the use of fish meal (FM) for juvenile humpback grouper *Cromileptes altivelis*. This study consisted of digestibility test of SPBM and biological test to observe growth performance. Five isonitrogenous and isocaloric experimental diets were used: Diet A contains 0% SPBM as a control diet, and diets B, C, D, and E contain 25%, 50%, 75%, and 100% of SPBM protein to substitute FM, respectively. Digestibility trial was performed for 14 days by adding Cr_2O_3 into the experimental diets and collecting fecal matter 40–60 minutes after each feeding. For growth trial, juvenile humpback grouper were kept in glass aquariums and fed by the experimental diet until apparent satiation for 60 days. Our result shows that the dry matter and protein SPBM apparent digestibility coefficient is lower compared to FM. The fish that was fed with diet B and C performed a comparable specific growth rate, feed efficiency, and protein retention compared to control ($p > 0.05$). However, lower specific growth rate, feeding efficiency, and protein retention were observed in fish that was fed with diet D (75% SPBM) and diet E (100% SPBM; $p < 0.05$). These results indicate that up to 50% of smoked SPBM can be used for the diet of humpback grouper.

1. Introduction

Humpback grouper *Cromileptes altivelis* is a commercially important marine finfish in Indonesia with a strong market demand in global market. This species is listed as threatened species by the International Union for Conservation of Nature because of overexploitation (Shapawi *et al.* 2008), and thus, expanding culture activity of humpback grouper will fulfill market demand and in the same time protect its natural population. Farming of this species are done around areas such as: Riau Island, Lampung, Bali, East Java, North Sulawesi, and Lombok (Alfero *et al.* 2010) with the selling price of this species reported to reach US$ 50/kg (Harianto 2009).

Aside from the marketing visibility, humpback grouper culture is still facing some issues because this industry still mainly uses trash fish as a feed source. The use of trash fish is strongly discouraged on the grounds of inconsistency of its supply and quality, high risk of infecting pathogen (Kim *et al.* 2007), and increase of nitrogen and phosphorus pollution (Islam 2005; Pomeroy *et al.* 2006). Therefore, there is an urgent need to develop a formulated diet that not only fulfills the species' biological requirement but also produces low waste outputs for a better and more sustainable practice of humpback grouper culture.

The development of practical diets for grouper has been initiated by many researchers (Usman *et al.* 2005; Tuan and William 2007). The research shows that grouper requires around 48%–55% of protein and 12%–18% lipid in the diet. Protein requirement of aquaculture species is determined by the compatibility of amino acids profile of the cultured organism. To date, fish meal (FM) has been considered as the best protein source for aquaculture feed because it has high protein level and suitable amino acid content. With the increasing growth of global aquaculture production, aquaculture industries now consume >80% of FM and fish oil as their feed ingredients (Tacon and Matian 2008). However, most forage fish, which are source of FM and fish oil, are now in the stage

* Corresponding author.
E-mail address: agus.suprayudi@yahoo.com (M.A. Suprayudi).
Peer review under responsibility of Institut Pertanian Bogor.

http://dx.doi.org/10.1016/j.hjb.2015.08.002

of fully exploited, overexploited, or in the process of recovering from overexploitation (Alder *et al.* 2008). Therefore, the use of FM-based diet is not considered as sustainable aquaculture practice. With this aforementioned issue, there is an urgent need to find suitable alternatives protein source to replace or reduce the use of FM. The alternative protein source must fulfill the following requirements, e.g. local based, considered as byproduct materials and not containing hazard materials.

Smoked skipjack processing byproduct meal (SPBM) is considered as a potential alternative to substitute FM in aquafeeds. According to the Indonesian Ministry of Marine and Fisheries Affair (2009), skipjack production in 2008 was 301.531 ton and only 68.304 ton was processed to smoked skipjack because of the high amount of byproduct. Proximate analyses show that byproduct of smoked skipjack (SPBM) meal contained 55% protein, 3%–4% lipid, and 42% ash content and may be used to substitute FM. Given the abundant availability of the ingredient, the aim of this study was to assess the possibility of SPBM meal as an FM replacement ingredient in the diet of humpback grouper.

2. Materials and Methods

2.1. Experimental fish

Fish that was used for this study is a juvenile humpback grouper with an initial average body weight of 7.8 ± 0.04 g and body length 7.6 ± 0.10 cm. This fish was obtained from Marine Research Center Hatchery of Gondol Bali, Indonesia, and was acclimated with both laboratory condition and experimental diet for 7 days before experimentation.

2.2. Experimental diet

2.2.1. Digestibility trial

Digestibility test for FM and SPBM was performed by indirect methods as described in Watanabe (1998). Chromic oxide at the level of 0.5% was added as an indicator to determine total and protein apparent digestibility coefficients (ADC). Commercial diet with 49% crude protein content was used as reference diet. The formula and proximate composition of experimental diets for digestibility test are presented in Table 1.

2.2.2. Growth trial

Five isonitrogenous and isocaloric diets were formulated to contain 48% protein and 4.5 cal/g diets. As a control diet, 100% protein in the diet was provided by fish meal, whereas in treatment diets B, C, D, and E, SPBM contributed 25%, 50%, 75% ,and 100% of the diet total protein, respectively, in substitution of FM protein. Feed macromaterials were milled to get 70-μm particle sizes. Pelleting was performed to get a pellet size of 2 mm in diameter and 2 mm in length; thereafter, the pellet was dried at 60°C for 24 hours. The formula and proximate composition of the experimental diets are presented in Table 2.

2.2.3. Rearing condition

Juvenile fish was obtained from Marine Research Center Hatchery of Gondol Bali, Indonesia. Fish were acclimated to laboratory condition for 10 days before experimentation.

Fish for digestibility test were reared in glass aquarium (100 × 50 × 40 cm) at a density of 10 fish and fed with experimental diet to satiation level. Feces collection was started after 7 days of feeding acclimation. The feces were collected twice a day, 1 hour after feeding for 14 days. Subsequently, the feces were dried and stored at −20°C until further analysis. The total and protein ADC for the SPBM and fish meal were calculated according to Watanabe (1988).

After adaptation period, the fish for biological test were starved for 24 hours. Fish with an average body weight of 7.80 ± 0.04 g were reared in glass aquaria (50 × 50 × 40 cm) previously filled with 80-L chlorinated sea water and arranged in a recirculating system. The fish were stocked at a density of seven fish/aquarium. Water temperature was maintained at 29 ± 1°C using water heater. Fish were fed three times daily to satiation for 60 days experimental period, and total daily feed consumption was recorded. Water quality was maintained by siphoning the fecal material out two times daily before feeding time. At the end of the rearing period, fish were starved for 24 hours, weighed, and stored at −20°C until further analyses.

For total ammonia nitrogen measurement, the remaining fish of each treatment were collected together and reared on an aquarium at a density of 10 fish and fed for 3 days at the level of 5% of biomass. One hour after feeding, all fish were transferred to new aquarium and all aerations were stopped. Samples of sea water were taken at 0, 2, 4, 8, and 16 hours.

During experimental period, dissolved oxygen was found to be stable at a concentration of 5 mg/L (Oxygen meter, YSI Model 57; YSI Industries, Yellow Spring, OH, USA) and salinity was 30–31 g/L (Hand Atago refractometer).

Table 1. The formula and proximate composition of experimental diets for digestibility test

Material	Composition (%)		
	Reference	FM	SPBM
Diet A	94.5	64.5	64.5
Binder	5.0	5.0	5.0
Cr_2O_3	0.5	0.5	0.5
FM	0.0	30.0	0.0
SPBM	0.0	0.0	30.0
Total	100	100	100
Proximate composition			
Crude protein	46.3	50.4	47.5
Crude lipid	15.8	14.8	12
NFE	11.02	8.3	7.7
Fiber	6.7	5.9	6.1
Ash	19.9	20.5	27.1

FM = fish meal; NFE = nitrogen-free extract; SPBM = skipjack processing byproduct meal.

Table 2. Feed composition, proximate analysis, energy, Ca, P, and Fe of experimental diets (%)

Feed ingredients	Substitution of FM by SPBM protein (%)				
	(A) 0	(B) 25	(B) 50	(D)75	(E) 100
FM*	35.0	26.3	17.5	8.8	0,0
SPBM†	0.0	10.4	20.7	31.1	41.5
SBM, SHM, PBM, Pollard	50.0	48.3	46.8	45.1	43.5
Fish oil	8.0	8.0	8.0	8.0	8.0
Vitamin and mineral mix	7.0	7.0	7.0	7.0	7.0
Proximate composition (% dry weight)					
Crude protein	48.9	48.6	48.1	48.1	48.1
Crude lipid	16.7	15.8	15.1	14.4	14.6
NFE	11.7	10.4	9.1	7.1	6.3
Fiber	2,0	2.0	2.1	2.2	2.2
Ash	21.1	23.4	25.8	28.3	29,0
Energy (kcal GE‡/100 g)	481.0	465.6	449.4	435.5	433.7
C/P (kcal/g protein)	9.8	9.6	9.4	9.1	9.1

FM = fish meal; NFE = nitrogen-free extract; PBM = poultry byproduct meal; SBM = soybean meal; SHM = shrimp head meal; SPBM = skipjack processing byproduct meal.

* Fish meal: 62.8% crude protein, 13.3% lipid content.;

† SPBM: 53% crude protein, 3.86% lipid content.;

‡ GE (gross energy), protein: 5.6 kcal/g; lipid: 9.4 kcal/g; carbohydrate: 5.1 kcal/g (Halver 2002).

Table 3. Total and protein apparent digestibility coefficient (%) of fish meal and smoked skipjack processing byproduct meal (SPBM) in juvenile humpback grouper

Ingredient	Apparent digestibility coefficient (%)	
	Dry matter	Protein
Fish meal	89.41 ± 0.84^{a}	83.77 ± 2.59^{a}
SPBM	76.45 ± 2.50^{b}	64.84 ± 1.04^{b}

*Superscript letter after mean value (± standard deviation) in the same rows indicates significant difference ($p < 0.05$).

2.2.4. Chemical analysis

Chemical analysis performed in the experiment included amino acid (high-performance liquid chromatography) analyses for both experimental diet and fish body on the beginning of the experiment, proximate analysis at the initial and final day of the experimental period. The proximate and chromium oxide analyses were performed based on Watanabe (1988).

2.2.5. Parameters of observation

The parameters of observation in this experiment were dry matter and protein ADC, specific growth rate, feed efficiency (FE), food consumption rate, survival, protein retention (PR), and total ammonia nitrogen (TAN) to determine total amount of ammonia in the tanks.

2.3. Experimental design

A completely randomized experimental design consisting of five treatments in triplicates was applied in this experiment. Analyses of variance and post hoc Duncan test were performed using SPSS version 16 for windows, whereas TAN will be descriptively analyzed.

3. Results

The digestibility of FM and SPBM is presented in Table 3. It can be seen from the data that dry matter apparent digestibility value of this SPBM ($76.45 \pm 2.50\%$) was statistically lower than FM ($89.41 \pm 0.84\%$). Furthermore, in protein ADC value, SPBM ($64.84 \pm 1.04\%$) was also statistically lower compared to FM ($83.77 \pm 2.59\%$).

In terms of growth and feeding performance of juvenile humpback grouper which is presented in Table 4, there was no significant difference observed in fish survival and feed intake among diet treatments. Specific growth rate, FE and PR of the fish fed with diets B (25%) and C (50%) were comparable to the control. However, higher replacement level of SPBM in diet D (75%) and E (100%) resulted in a significant reduction in growth, FE, and PR and the TAN value seems to increase along with the increase of SPBM inclusion in diet (Figure 1).

In the absence of amino acid requirement data, the amino acid profile of the whole-body tissue of the animal could be used as an index of the EAA requirements. The amino acid profile of whole body of grouper juveniles was set to be 0% (Figure 2) and used as a basis for comparing the effectiveness of the protein sources used in the experimental diets. Arginine content in diet A was 11.7%. This value was lower compared to the requirement index and therefore is referred as deficiency. The replacement of FM by SPBM at the level of 25%, 50%, 75%, and 100% increased arginine deficient level to 12.7%, 14.7%, 15.7%, and 18.7%, respectively.

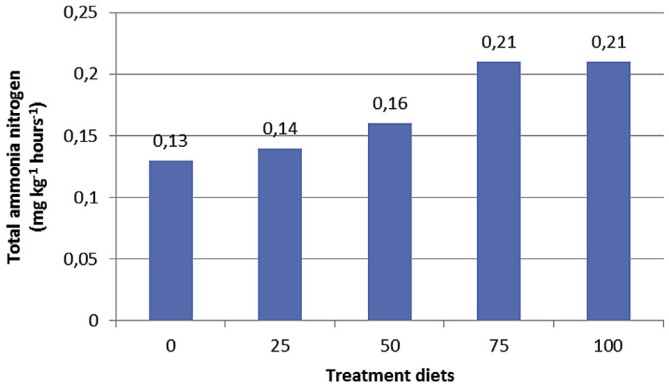

Figure 1. The comparison of total ammonia nitrogen value for every treatment diets.

4. Discussion

ADC of a feed ingredient for aquafeed is influenced by fish age, feed status (kind of substrates), particle size, and enzyme activity (Halver 2002). This study shows that in general, humpback grouper can effectively digest protein, although it can be seen that the ADC value seems to be lower than other animal-based protein source (Wang *et al.* 2012). The differences of ADC values between FM and SPBM might be related to the higher ash content in SPBM than FM. This is supported by previous experiment in gibel carp (Zhang *et al.* 2006) which showed that high ash content reduced the total ADC value.

The total feed consumed by the fish may be used as a parameter to express the feed palatability. Total feed consumption observed in this study generally did not differ significantly among treatments. Previous study by Muzinic *et al.* (2006) on sunshine bass and Thompson *et al.* (2012) on Nile tilapia fry also reported that partial or total replacement of FM with other animal protein source did not affect palatability of the feed.

The use of FM as a protein source has been proven to be a good protein source for many fishes and crustaceans. This study demonstrated that replacement of FM up to 50% by SPBM allowed a similar growth rates comparable to FM-based diet (diet A 100% FM protein). Moreover, feed intake data showed that the humpback grouper juveniles could consume the diets with SPBM at any level tested in this study. The lower growth however was observed in 75% and 100% FM replacement by SPBM which is similar with

Table 4. Survival (SR), feed intake (FI), specific growth rate (SGR), feed efficiency (FE), protein retention (PR), and total ammonia nitrogen (TAN) of humpback grouper juveniles fed experimental diets

Parameter	Substitution of FM by SPBM protein*				
	A (0%)	B (25%)	C (50%)	D (75%)	E (100%)
SR (%)	100 ± 0^{a}	100 ± 0^{a}	100 ± 0^{a}	100 ± 0^{a}	100 ± 0^{a}
FI (g)	200.5 ± 0.62^{a}	199.2 ± 0.61^{a}	198.8 ± 0.15^{a}	198.8 ± 0.64^{a}	198.1 ± 0.87^{a}
SGR (%)	2.24 ± 0.01^{a}	2.21 ± 0.03^{a}	2.12 ± 0.02^{a}	1.79 ± 0.04^{b}	1.62 ± 0.04^{c}
FE (%)	74.2 ± 0.76^{a}	73.2 ± 1.47^{a}	73.1 ± 0.25^{a}	50.4 ± 1.92^{b}	46.1 ± 1.84^{c}
PR (%)	27.7 ± 0.31^{a}	27.2 ± 0.78^{a}	27.0 ± 0.08^{a}	17.4 ± 1.06^{b}	14.5 ± 1.34^{c}
TAN (mg/kg/h)	0.13	0.14	0.16	0.21	0.21

*Superscript letter after mean value (± standard deviation) in the same rows indicates significant difference ($p < 0.05$).

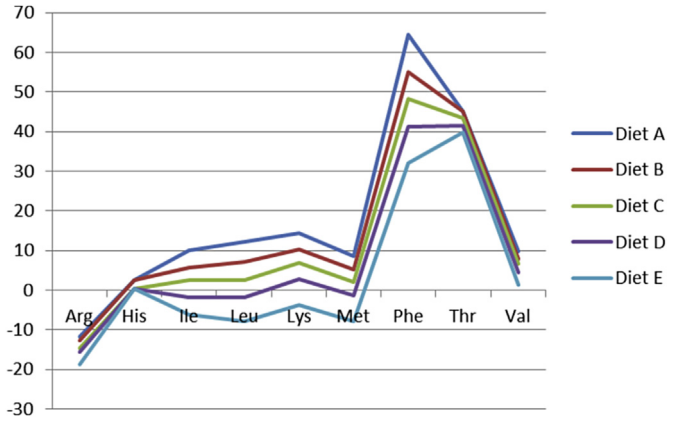

Figure 2. The comparison of essential amino acid profile of experimental diets to the whole body of juvenile humpback grouper.

previous studies related to FM replacement with other animal protein source in *C. altivelis* (Shapawi *et al.* 2007) and gibel carp (Zhang *et al.* 2006) .

PR is greatly affected by protein and total energy in the diet and its protein quality, i.e essential amino acid composition in the diet (Halver 2002). As the experimental diets were formulated to be isonitrogenous and isocaloric, lower growth performance and PR in diet D and E may be related to the imbalance or deficiency in essential amino acids. The requirement of essential amino acids in the diet can be reflected in the essential amino acid profile of whole fish body.

In terms of amino acid balance in this study, inclusion level of SPBM protein in the diet to more than 50% reduced other essential amino acids such as isoleucine, leucine, lysine and methionine. From those amino acids, lysine and methionine are considered as the most limiting amino acid especially when FM was replaced with other ingredients (Mai *et al.* 2006a; Mai *et al.* 2006b). Similar result in amino acid profile was also reported in *Carassius auratus* when fed with high level of animal byproduct in replacement of FM (Zhang *et al.* 2006).

Unbalanced amino acid composition would likely lead to amino acid breakdown or deamination. This would be then followed by reamination or ammonia excretion and lipogenesis or energy production (Wright and Anderson 2007). The nitrogen produced from deamination of amino acid tended to be released. Thus, deamination of amino acids may lead to the release of amino groups that cannot be recycled through the metabolic process. The excretion of amino nitrogen is equal to an energy loss for fish. Therefore, higher level of total ammonia excretion might indicate higher level of amino acid catabolized to produce energy, and contributed to the lower level of amino acid to synthesize protein and growth. This experiment shows that TAN (mg/kg/hour) was increased along with the increase of SPBM level in the diet. This phenomenon could be explained by the imbalance or deficiency in essential amino acid. Survival level of 100% that was observed in all fish fed with the experimental diets may indicate that the experimental diets did not result hazardous effect to the fish.

To summarize this research, the total and protein digestibly of SPBM was significantly lower than those FM. Although, the present study indicated that 50% of FM protein could be substituted by SPBM without showing any negative effects on growth performance, PR, FE and survival.

Conflict of interest

There is no conflict of interest.

References

Alder J, Campbell B, Karpouzi V, Kaschner K, Paul D. 2008. Forage fish: from ecosystem to market. *Annu Rev Environ Resour* 33:153–66.

Alfero F, Miao S, Perez AA. 2010. Economic analysis of tiger grouper *Ephinephelus fuscoguttatus* and humpback grouper *Cromileptes altivelis* commercial cage culture in Indonesia. *Aquac Int* 18:725–9.

Halver JE. 2002. Fish Nutrition, 3rd edition. California, USA: Academic Press Inc.

Harianto. 2009. Feasibility study assessment of grouper business for export by applying packaging technique for land and air transportation. *Jurnal Sains dan Teknologi Indonesia* 5:25.

Islam MS. 2005. Nitrogen and phosphorus budget in coastal and marine cage aquaculture and impacts of effluent loading on ecosystem: review and analysis towards model development. *Mar Pollut Bull* 50:48–61.

Kim JH, Gomez DK, Choresca Jr CH, Park SC. 2007. Detection of major bacterial and viral pathogens in trash fish used to feed cultured flounder in Korea. *Aquaculture* 272:105–10.

Mai K, Zhang L, Ai Q, Duan Q, Zhang C, Li H, Wan J, Liufu Z. 2006a. Dietary lysine requirement of juvenile seabass *Lateolabrax japonicas*. *Aquaculture* 258:535–42.

Mai K, Wan J, Ai Q, Xu W, Liufu Z, Zhang L, Zhang C, Li H. 2006b. Dietary methionine requirement of juvenile yellow croaker Pseudosciaena crocea. *Aquaculture* 251: 564–72.

Ministry of Marine and Fisheries Affair. 2009. Perikanan dan Kelautan Dalam Angka 2009. Jakarta: DKP.

Muzinic LA, Thompson KR, Metts LS, Dasgupta S, Webster CD. 2006. Use of turkey meal as partial and total replacement of fish meal in practical diets for sunshine bass *Morone chrysops x Morone saxatilis* grown in tanks. *Aquacult Nutr* 12: 71–81.

Pomeroy RS, Parks JE, Balboa CM. 2006. Farming the reef: is aquaculture a solution for reducing fishing pressure on coral reef? *Mar Policy* 30:111–30.

Shapawi R, Ng WK, Mustafa S. 2007. Replacement of fish meal with poultry by-product meal in diets formulated for the humpback grouper *Cromileptes altivelis*. *Aquaculture* 273:118–26.

Shapawi R, Mustafa S, Ng WK. 2008. Effects of dietary fish oil replacement with vegetable oils on growth and tissue fatty acid composition of humpback grouper, *Cromileptes altivelis*. *Aqua Res* 39:315–23.

Tacon AGJ, Metian M. 2008. Global overview on the use of fish meal and fish oil in industrially compounded aquafeeds: trends and future prospects. *Aquaculture* 285:146–58.

Thompson RT, Velasques A, Patterson JT, Metss LS, Webster CD, Brady YJ, Gannam AL, Twibell LG, Ostrand SL. 2012. Evaluation of plant and animal source as partial or total replacement of fish meal in diets for Nile tilapia fry and juvenile stage. *N Am J Aquacult* 74:365–75.

Tuan LA, Williams KC. 2007. Optimum dietary protein and lipid specifications for juvenile malabar grouper *Epinephelus malabaricus*. *Aquaculture* 267:129–38.

Usman, Rachmansyah, Laining A, Ahmad T, Williams KC. 2005. Optimum dietary protein and lipid specifications for grow-out of humpback grouper *Cromileptes altivelis*. *Int Aqua Res* 36:1285–92.

Wang J, Yun B, Xue M, Wu X, Zheng Y, Li P. 2012. Apparent digestibility coefficients of several protein sources, and replacement of fish meal by porcine meal in diets of Japanese seabass, *Lateolabrax japonicus*, are affected by dietary protein levels. *Aqua Res* 43:117–27.

Watanabe T. 1988. Fish Nutrition and Mariculture, JICA Textbook the General Aquaculture Course. Tokyo: Kanagawa International Fish Training Center.

Wright PA, Anderson PM. 2007. Nitrogen excretion. In: Anderson PM, Hoar WS, Randall DJ, Farrell AP, Wright PA (Eds.). *Fish Physiology*. New York: Academic Press.

Zhang S, Xie S, Zhu A, Lei W, Yang Y, Zhao M. 2006. Meat and bone meal replacement in diets for juvenile gibel carp *Carassius auratus gibelio*: effects on growth performance, phosphorus and nitrogen loading. *Aquacult Nutr* 12:353–62.