REPLACEMENT OF FISH MEAL WITH POULTRY OFFAL MEAL IN DIETS FOR HUMPBACK GROUPER (*Cromileptes altivelis*) GROW-OUT

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ABSTRAK

Fish meal (FM) has traditionally been a major ingredient in fish diets as palatable and high quality protein source. Increasing demand and price as well as uncertain availability of the meal encourage nutritionists to study for alternative sources to replace fish meal in fish diets. Poultry offal meal (POM) is one of the most promising candidates for partial or total replacement of FM in fish diets. Feeding trial was conducted to examine effects of partially replacing FM in diets with POM on growth performance of humpback grouper. Fifteen net cages of 1 x 1 x 1.2 m³, each was stocked with 12 humpback groupers with initial weight of 285 ± 20 g, were set up randomly in seawater. Five isonitrogenous and isocaloric diets were formulated to contain 50% FM (66.40% crude protein) as control diet, 8% POM (65.77% crude protein) + 42% FM, 16% POM + 34% FM, 24% POM + 26% FM, and 32% POM + 18% FM. The fish were fed twice daily to satiation for 20 weeks. The fish fed diet containing 32% POM showed significantly lower ($P<0.05$) specific growth rate than the fish fed control diet or diet containing 8% POM, but not significantly different ($P>0.05$) from fish fed the diet containing 12 to 24% POM. Weight gain, feed efficiency, protein retention, total feed intake and survival rate did not differ significantly ($P>0.05$) among treatments except for fish fed the diet containing 32% POM showed significantly lower ($P<0.05$) weight gain, feed efficiency and protein retention than the fish fed the diet containing 8% POM. These results suggest that up to 24% FM in humpback grouper grow-out diets can be replaced by POM.

KEYWORDS: poultry offal meal, humpback grouper, grow-out

INTRODUCTION

Humpback grouper (*Cromileptes altivelis*) is one of carnivorous marine fish which has recently been successfully developed into one of the promosing species for mariculture. Despite, slow growth rate, its high value, up to $US 90/kg in live fish markets of Asia, make it an economically attractive aquaculture species. However, as in most cultured high-value species, feed constitutes a significant portion of the operating cost. Much of the cost of aquafeed production is due to the extensive use of fish meal in the feed (Tacon & Jackson, 1985; Tacon, 1994). Fish meal has traditionally been a major ingredient in fish feed because of its protein quality and palatability (Lovell, 1984).

In Indonesia, production of large quantities of fish meal is still not feasible because trashfish is also consumed by human, consequently most fish meal use in aquaculture feeds must be imported (Nikijuluw, 1998; Sukadi *et al.*, 1999). Therefore, a pre-requisite for large scale development of aquaculture is identification of a local supply of high quality protein to replace fish meal. Many fish nutritionists have studied alternative protein sources both from plant and animal (Quartararo *et al.*, 1998a; 1998b; Ruchimat *et al.*, 1997; Dabrowski *et al.*, 1989; Fowler, 1991).

Poultry offal meal (POM) or poultry by-product meal (PBM) is one of the promising candidates for partial or total replacement of fish meal in fish diets. However, compared with fish

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meal, animal by products meal may be deficient in one or more essential amino acid (Davies *et al.*, 1991 *in* Abdel-Warith *et al.*, 2005). POM has been previously evaluated in diets for chinook salmon *Oncorhynchus tshawyscha* (Fowler, 1990; 1991), coho salmon *Oncorhynchus kisutch* (Higgs *et al.*, 1979), rainbow trout *Salmon gairdneri* (Alexis *et al.*, 1985), African catfish *Clarias gariepinus* (Abdel-Warith *et al.*, 2001). Unfortunately, no information available on the feasibility of using POM (viscera) as a source of dietary protein in feed formulation for humpback grouper. This study was designed to examine the effect of replacement of fish meal by POM on growth of humpback grouper.

Materials and methods

Experimental Diets

Five experimental diets were formulated to contain a variable proportion of POM (viscera) to partially replace fish meal component in the diet. POM was collected from local slaughters, Makassar, South Sulawesi, then cleaned and boiled at a temperature of 100°C for approximately 1 h. prior to be oven-dried (60°C) and extruded at RICA, Maros. A control diet based on fish meal (5-01-985) served as the reference source of dietary protein used to substitute with POM. Besides fish meal, all experimental diets were also included mysid meal, squid liver meal, soybean meal, and wheat gluten as additional protein sources. All ingredients were analyzed for nutrients composition (Table 1) before used in diet formulation.

Five isonitrogenous (47% crude protein content) and isoenergetic (20 MJ/kg) diets were formulated (Rachmansyah *et al.*, 2001; Giri *et al.*, 2001). Table 2 shows composition and proximate analysis of the experimental diets. The diets were made into sinking pellet (10 mm in diameter), oven-dried at 60°C and stored at 5°C.

Experimental Condition, Fish and Feeding

Juveniles humpback grouper originating from a single hatchery cohort were transferred from the Gondol Mariculture Research Centre, Bali to South Sulawesi and reared for several months in floating net seacages at Barru Regency, where the experiment was conducted. From this group, 183 fish were selected free from defects and weight uniformity with initial mean weight of 285 ± 20 g. Afterwards, three fish were randomly taken for determining of body composition of the initial fish and remaining 180 fish were equally (12/seacage) and randomly distributed to 15 floating net cages of 1 ´ 1 m² surface area and 2 m depth. The cages were suspended from a floating platform in seawater of approximately 10 m depth with strong tidal current. During rearing of the fish prior to experimentation, fish were fed pelleted feed (commercial feed), morning and afternoon to satiation. During the experiment, fish were hand-fed twice daily at 0700 and 1700 h to apparent satiation and weighed individually at 4 weeks interval. Feeding was carefully monitored to minimize any food wastage. At conclusion of the experiment, two representative fish from each seacage were taken for determination of the body composition.

Productivity and Nutrient Retention Response Calculations

Growth rate was expressed as % weight gain, determined as the fish's weight increment over the course of the 20 weeks experiment expressed as a percentage of its initial start weight, and as specific growth rate (SGR), calculated using the equation:

$$SGR\,(\%\,day^{-1}) = 100 * \left(\frac{\ln W_e - \ln W_s}{d}\right)$$

where ln is the natural logarithm, W_e and W_s are the weights of the fish at the end and start of the experiment, respectively and d, the number of days on experiment. Feed efficiency (FE) was calculated as the total weight increment (kg) of the fish in each cage divided by the total dry weight (kg) of feed administered to each cage of fish, ignoring any over or under estimation of the value that might result from not accounting for uneaten feed. Protein efficiency ratio (PER) was calculated as for FE but using the weight (kg) of crude protein in the administered feed as the numerator. Protein retentions were calculated as the respective whole body weight increments determined from comparative slaughter analysis procedures, and expressed as a percentage of the total amount of protein contained in the administered feed.

Chemical and Statistical Analyses

For determination of body composition of initial fish, three fish were combined for chemi-

Table 1. Proximate composition of the protein sources used in the experimental diets (% dry matter)

Ingredient	Protein	Lipid	Ash	Moisture
Fish meal (FM)	66.40	5.52	20.86	7.27
Poultry offal meal (POM)	65.77	17.54	5.87	5.80
Mysid meal (MM)	60.93	4.05	15.23	4.66
Squid liver meal (SLM)	47.02	15.36	4.92	6.96
Soybean meal (SBM)	46.23	3.40	6.81	8.04
Wheat gluten (WG)	71.21	3.43	8.44	6.65

Table 2. Composition and proximate analysis of experimental diets (% dry matter)

Ingredients	Levels of poultry offal meals in diets (%)				
	0	8	16	24	32
Fish meal	50	42	34	26	18
Poultry offal meal	0	8	16	24	32
Mysid meal	7	7	7	7	7
Squid lever meal	6	6	6	6	6
Soybean meal	7	7	7	7	7
Wheat gluten meal	4	4	4	4	4
Flour meal	13.67	14.27	14.87	15.47	16.07
Fish + soybean oil (2:1)	8.4	7.8	7.2	6.6	6.0
Vitamim premix[1]	2.37	2.37	2.37	2.37	2.37
Mineral premix[2]	1.5	1.5	1.5	1.5	1.5
Carophylpink	0.06	0.06	0.06	0.06	0.06
Proximate composition:					
Moisture	8.88	8.06	8.04	8.52	8.05
Crude protein	46.69	46.31	46.75	46.38	46.88
Total lipid	10.9	10.7	11.1	12.8	12.9
Ash	14.64	12:62	11.22	11.22	9.27
Crude fibre	2.88	2.72	2.64	2.64	2.68
Gross energy (MJ/kg)[c]	19.60	19.91	20.28	20.63	21.02

[a)] Vitamin mix provided (mg/kg diet): Thiamin-HCl, 59.2; riboflavin, 59.2; Ca-panthothenate, 118.5; niacin, 23.7; pyridoxine-HCl, 47.4; biotin, 7.1; folic acid, 17.8; inositol, 2,370; p-aminobenzoic, 59.2; astaxanthin, 177.8; menadione, 47.4; calciferol, 22.5; µ-tocopherol, 237; ascorbic acid, 1,777.5; cyanocobalamin, 1.2; choline-HCL, 10971

[b)] Trace mineral provided (mg/kg diet): KH_2PO_4, 4,000; $CaCO_3$, 2,500; NaH_2PO_4, 6,150; $FeCl_3.2H_2O$, 1,660; $ZnSO_4$, 100; $MnSO_4$, 67.5; $MgSO_4$, 500; $CuSO_4$, 20; KI, 1.5; $CoSO_4.7H_2O$, 1.0

[c)] Calculation from the determined protein, lipid, and NFE of the diet using gross energy conversion coefficients of 23,6; 39,5; and 17,2 MJ/kg respectively (National Research Council, 1993)

cal analysis. For determination of body composition of the end fish, two fish from each seacage were combined for chemical analysis. Preparation of fish for chemical analysis involved whole fish freezing, chopping into small pieces and mincing through a 3 mm die plate fitted to the meat grinder attachment of a Hobart mixer. A uniform sample of fish tissue was obtained by mincing each sample three times through a meat grinder with hand mixing of sample after each passage through the mincer. The ground fish was freeze dried prior to chemical analysis. A representative sample of feed or freeze-dried fish tissue was homogenized using a mortar and pestle and analyzed essentially according to AOAC International (1999) procedures: dry matter (DM) by oven drying at 105°C for 16 h; ash by ignition in a muffle furnace at 550°C for 24 h and crude protein by micro-Kjeldahl analysis with distillation into 4% boric acid and titration with sulphuric acid using methyl red indicator for end point determination. Total lipid was determined gravimetrically following a chloroform: methanol extraction of the sample (Bligh & Dyer, 1959).

Amino acid content of fish meal and poultry offal meal were determined in acid hydrolysates. Amino acids were assayed at Airlangga University, Department of Pharmacy, Surabaya, using high speed amino acid analyzer model 835. Essential amino acid compositions are presented in Table 3.

Response data were analyzed by ANOVA in accordance with completely randomized design of the experiment. Tukey's procedure was used for multiple comparisons. Differences were considered significant at $P<0.05$.

RESULTS

Table 4 shows total feed intake and survival rate of fish were similar for all treatments. However, the specific growth rate of fish fed the diet containing 32% POM was significantly ($P<0.05$) lower compared to fish fed the control diet or the diet containing 8% POM. Percentage of weight gain, feed efficiency, protein efficiency ratio, and protein retention were relatively similar for all treatments, except for fish consuming of the diet containing 8% POM had significantly ($P<0.05$) higher values than fish fed the diet containing 32% POM.

Final body composition of fish fed experimental diets is presented in Table 4. Final body composition indicated that replacement fish meal with POM did not affect protein, lipid, and ash content of the fish. Whilst fish fed diet contained 24% POM contains slightly higher fibre in their final body composition.

DISCUSSION

Inclusion of terrestrial alternative protein sources for the partial or total replacement of fish meal has been studied in previous investigation for numerous fish species. These have concluded that increasing animal-derivative protein to replace fish meal has a detrimental effect on growth rate and feed utilization above certain constraints, although partial substitution is quite feasible (Abdel-Warith *et al.*, 2001). Feasibility of poultry offal meal in fish diets was found to depend on fish species and size as well as composition and processing technique. Most result showed that POM

Table 3. Essential amino acid composition of fish meal and poultry offal meal (% dry matter)

Kind of amino acid	Fish meal	Poultry offal meal
Arginine	4.262	4.712
Histidine	1.749	0.829
Isoleucine	3.087	2.263
Leucine	5.247	4.435
Lysine	5.096	3.240
Methionine	0.134	ND[1]
Phenilalanine	2.528	2.036
Threonine	3.061	2.620
Valin	3.338	2.750
Tryptophan	ND[1]	ND[1]

ND: not detected

Table 4. Growth performances and feed utilization of humpback grouper fed different diets

Variables	Levels of poultry offal meals in diets (%) *)				
	0	8	16	24	32
Specific growth rate (%/d)	0.217 [a]	0.220 [a]	0.210 [ab]	0.193 [ab]	0.187 [b]
Weight gain (%)	35.07 [ab]	36.17 [a]	34.32 [ab]	31.26 [ab]	30.14 [b]
Feed efficiency (%)	30.33 [ab]	31.90 [a]	30.41 [ab]	28.38 [ab]	26.71 [b]
Protein eficiency ratio	0.65 [ab]	0.69 [a]	0.66 [a]	0.61 [ab]	0.57 [b]
Protein retention (%)	15.07 [ab]	16.60 [a]	14.78 [ab]	14.13 [ab]	12.27 [b]
Total feed intake (g/fish)	328.6 [a]	323.5 [a]	322.0 [a]	314.4 [a]	322.5 [a]
Survival rate (%)	100 [a]	100 [a]	100 [a]	100 [a]	100 [a]

*) Means in the same row followed bysimilar superscript are not significantly different (P>0.05)

couldn't replace more than 500 g/kg of fish meal protein (Fowler, 1991; Steffens, 1994; Webster *et al.*, 1999).

Some variables of growth and feed utilization (specific growth rate, weight gain, feed efficiency, protein efficiency ratio, and protein retention) were observed in this experiment showed slightly decrease with increase inclusion levels of POM as replacement of fish meal in the diets. The use of POM up to 24% or equivalent 47.5% protein fish meal in diet formulation, could replace fish meal without adverse effects on growth performance of the fish. This is relatively similar to the report by El-Sayed (1994) that silver sea bream (*Rhabdosargus sarba*) fingerlings presented similar growth and feed efficiency when fed chicken offal meal at 25% substitution level with fish meal. Fowler (1991) also reported that 20% PBM inclusion could efficiently substitute 50% fish meal in a practical diet for chinook salmon. Yang *et al.* (2004) reported that PBM could be used as a main protein to replace fish meal up to 500 g kg^{-1} of dietary protein without negative effect on the growth and feed utilization of gibel carp, *Carassius auratus*. The other result reported by Higgs *et al.* (1979) that up to 75% of fish meal could be replaced by defatted PBM in coho salmon diets without adverse effect on growth.

Superior performance of the fish receiving the control diet and the diet containing 42% fish meal + 8% POM in this study was possibly a result of the availability of nutrients and amino acid balance. POM 32% in diet formulation had significantly decreased the growth rate of the fish. This reduction in growth rate might have been due to a deficiency of essential amino acids. Based on the amino acid analysis, POM used in this experiment contains lower some essential amino acids such as histidine, isoleucine, leucine, lysine, phenilalanine, threonine valine, and methionene compared to fish meal (Table 3). Abdel-Warith *et al.* (2001) re-

Table 5. Final whole body composition of humpback grouper fed different diets (% dry weight)

Variables	Levels of poultry offal meals in diets (%) *)				
	0	8	16	24	32
Crude protein	59.94 [a]	60.46 [a]	60.54 [a]	59.13 [a]	58.28 [a]
Total lipid	18.70 [a]	18.50 [a]	18.23 [a]	18.36 [a]	18.07 [a]
Ash	15.31 [a]	16.46 [a]	15.47 [a]	15.10 [a]	17.21 [a]
Crude fibre	1.61 [ab]	1.25 [ab]	1.16 [b]	1.77 [a]	1.31 [ab]

*) Means in the same row followed by similar superscript are not significantly different (P>0.05)

ported that the content of some essential amino acids in the Africant catfish tended to decrease with increase inclusion level of PBM expecially above 40% replacement of fish meal. They noted that several other amino acids become progressively reduced at high PBM inclusions, and may have contributed to the inferior protein utilization (protein efficiency ratio) and growth performance of catfish. PBM containing high protein, however it was deficiency one or more essential amino acids (Ogunji, 2004), and it was suitable as a partial or complete replacement of fish meal if lysine and methionine were added (Steffens, 1994). Reduced lysine was listed as a possible contributing factor to reduce growth of snapper on diet containing 10% fish meal, with the reminder of the protein coming from poultry offal meal and soybean meal, compared with growth on a 60% fish meal diet (Quartararo *et al.*, 1998b). Alexis *at al.* (1985) working with rainbow trout, obtained very good result with methionine supplementation. Quartararo *et al.* (1998b) have attempted to solve such a possible deficiency by balancing lysine, methionine and cystein in the test diet of Australian snapper, *Pagrus auratus* with the use of crystalline amino acids. However, crystalline amino acids are not always effective (Lovell, 1989; Murai, 1992).

Besides lower content of some essential amino acids in POM, another cause of reduction in growth rate of the fish fed the diet containing higher of POM might has been due to a deficiency of energy or other nutrients. In spite of similar feed intake by fish for all treatments in this experiment, digestibility of protein, lipid and nitrogen free extract (NFE) tended to decrease with increasing of POM level in the diets (Usman *et al.*, 2006). Nengas *et al.* (1995) recorded lower energy digestibility for poultry by product meal (80.3%) than herring meal (94.1%) for gilthead seabream at 20°C. Apparent digestibility of protein and energy diet was lower in the diet containing PBM than the control diet (containing only fish meal without containing POM) by gibel carp (Yang *et al.*, 2004). Alexis *et al.* (1985) reported that values of digestible protein about 85% for fish meal but only 60% for PBM in the rainbow trout. Hamley (1987) also reported that digestibility of protein and energy for tilapia, *Oreochromis niloticus* was appreciably lower (74% and 59%, respectively) compared with fish meal (86% and 80%, respectively).

Body composition of humpback grouper was not affected by replacement of fish meal with POM with respect to either protein, lipid or ash content. Slightly higher fibre content in fish fed diet containing 24 POM still cannot be clearly explained. Abdel-Warith *et al.* (2001) reported that the African catfish fed with fish meal-based control diet and 20%, 40%, 60% PBM diets did not yield any variation in the protein and lipid content, while fish fed 80% and 100% PBM diets had a slight lower protein level in their final carcass composition.

CONCLUSION

Poultry offal meal is a promising source of protein for fish diet and the use of POM up to 24% or equivalent 47.5% protein fish meal to replace fish meal in diet formulation for humpback grouper grow-out would reduce feed cost without resulting any adverse effect on the fish growth performance.

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