FERMENTED BLOOD MEAL USE FOR TIGER GROUPER, *Epinephelus fuscoguttatus* GROW-OUT DIET

Usman[*], Kamaruddin[*], Neltje N. Palinggi[*], Rachmansyah[*], and Taufik Ahmad[**]

ABSTRACT

The experiment aimed to evaluate the optimal level of fermented blood meal used in grow-out diets for tiger grouper, as an alternative protein source to fish meal. Juvenile tiger grouper, initial weight 31.1 ± 2.1 g, were stocked into 1x1x2 m^3 floating net cages at 20 fish cage^{-1}. The treatment applied was isoprotein and isocaloric diets formulated to contain fermented blood meal (FBM) of 0%, 7.5%, 15.0%, 22.5%, and 30.0% replacement of fish meal protein. The diets were fed to the fish twice a day to satiation for 20 weeks. Based on the Tukey test, the fish fed 0%–15.0% FBM demonstrated similar performance (P>0.05) to those fed the control diet (FBM$_0$) in terms of specific growth rate, weight gain, and feed and protein efficiency. Specific growth rate, weight gain, feed efficiency and protein efficiency of the fish fed 22.5%–30.5% FBM were significantly lower (P<0.05) than those of the fish fed control diet (FBM$_0$). However, there was a significant culvilinier decliner in overall fish performance with increasing inclusion of FPM and most notable for weight gain, feed efficiency and feed consumption. Based on regression analysis, the asymptote where fish growth deteriorates as a function of FBM inclusion was determined to be 8.9%. Tiger grouper diets incorporating up to 9% FBM as fish meal replacement had no adverse affects on fish growth and survival.

KEYWORDS: fermented blood meal, tiger grouper grow-out

INTRODUCTION

Tiger grouper (*Epinephelus fuscoguttatus*) is a piscivorous fish having a short digestive tract, around 0.20—0.25 body length (Smith, 1980a), low enzyme activity for a high carbohydrate diet (Lupatsch *et al.*, 1997) and like other grouper species, is expected to preferentially utilise dietary protein as an energy source (Williams *et al.*, 2004; Tuan & Williams, 2007). Several studies (Smith, 1980b; Laining *et al.*, 2003a; Kabangnga *et al.*, 2004) have demonstrated that tiger grouper grow well when fed 45%—55% protein and <15% lipid. However, to formulate a diet with >45% protein typically requires fish meal to be included at a rate of at least 50%. At a feed conversion ratio of around 2.0:1 and a conversion rate of trash fish into fish meal of 4.0:1, every kilogram of tiger grouper produced requires the equivalence of at least 4 kg of 'trash' fish. The term 'trash' fish is inaccurate in Indonesia because such fish are utilised for human consumption, either directly or for the manufacture of fish sauce. Because of this competition for fish protein, substitution of fish meal with terrestrial sources of protein is a priority for the aquafeed industry.

Blood meal is one of the potential sources of protein that could be used to replace fish meal in fish diets. The protein content of blood meal is high, around 72%—97% (Hertrampf & Piedad-Pascual, 2000; Laining *et al.*, 2003b). However, processing methods affect the quality of blood meal, which in turn, affects the reponse of the fish. With humpback grouper *Cromileptes altivelis*, Laining *et al.* (2003b)

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[*]   Research Institute for Coastal Aquaculture, Maros, Indonesia
[**]   Research Institute for Fresh Water Aquaculture, Bogor, Indonesia

reported that the dry matter and crude protein digestibility of oven-dried blood meal was 48% and 55%, respectively. However, organic acid fermentation of the dried blood meal improved digestibility to 62%–68% and 84%–88%, respectively.

Substitution of fish meal with blood meal products has given conflicting results. With Chinook salmon (*Oncorhynchus tschawynchus*) (Hajen *et al.*, 1993) found that substituting including more than 5% spray dried blood meal as a substitute for fish meal resulted in retarded fish growth. In contrast, 25% replacement of fish meal protein with blood and meat meal had no adverse effect on salmon growth but increasing the replacement to 75% resulted in a 19% reduction in growth and a 25% worsening of feed conversion (Gropp *et al.*, 1976 *in* Hertrampf & Piedad-Pascual, 2000). The objective of this study was to evaluate the optimum rate at which fermented blood meal could substitute for fish meal in the diet of tiger grouper.

MATERIAL AND METHODS

Test Diets

The reference protein source used in the study was Peruvian fish meal (PFM). Except for the control diet (FBM_0) which contained only PFM as the source of protein, other test diets contain fermented blood meal (FBM) at inclusion rates of 7.5% ($FBM_{7.5}$), 15.0% (FBM_{15}), 22.5% ($FBM_{22.5}$), and 30.0% (FBM_{30}) and substituting for an equivalent amount of PFM protein. Fresh cattle blood from an abattoir in Maros Regency, South Sulawesi was collected in a plastic bucket and homogeneously mixed with 1:1 propionic and formic acid mixture at 3% of blood total weight (Laining *et al.*, 2003b). Fermentation was carried out for 5 days with daily agitation. At the conclusion of fermentation, the acidity of the mixture was pH 3—4 and this was neutralised with 1.6% $Ca(OH)_2$ (Stone *et al.*, 1989) to obtain a product with a pH of 6.0—6.5. The fermented blood was dried and ground to a fine powder. The blood meal product contained 82.7% crude protein (CP), 0.01% total lipid (TL), 2.7% crude fiber (CF), 4.8% ash, and 9.7% nitrogen free extract (NFE). All raw ingredients were stored in a refrigerator prior to their use for feed formulation. The formulation and proximate analysis of the test diets are presented in Table 1. Chromium oxide at 1% was added to the diet at the expense of wheat flour when used for determining diet digestibility.

Dry ingredients for daily-made moist pellet were homogenously mixed in a mixer and than combined with oil. Trash fish was finely grinded and homogenously mixed with flour and oil. The mixture of dry ingredients and trash fish then was blended into dough. Pellets were made by cold extruding the dought through a meat mincer with a die opening of 7—12 mm. The moisture content of the pellets averaged 40%. Prior to be fed, the pellet was kept in a cool box filled with ice.

Experimental Procedures

Hatchery-reared juvenile tiger grouper from a single cohort were stocked into floating net cages for several weeks' acclimatisation during which time they were fed the control formulation moist pellet (FBM_0). Three hundred and ten healthy fish of an uniform size were selected for the experiment. Average initial weight (mean ± SD)of the fish was 31.1 ± 2.1 g. Three hundred fish were stocked into 15 units of 1x1x2 m floating net cages at a density of 20 fish $cage^{-1}$. The remaining 10 fish were sacrificed to determine initial whole body chemical composition. The experimental units were arranged in a completely randomized design with 3 replicates of each diet treatment. The fish were carefully fed twice each day to satiation for 140 days and were sampled every 4 weeks for weight measurement.

Digestitibility of the test diet was determined indirectly by reference to the digestibility marker chromium oxide (Cr_2O_3) as described by Takeuchi (1988). Prior to faeces collection, experimental fish were fed test diets containing chromium oxide to satiation for 5 days. Several hours after feeding, experimental fish were carefully moved into conical bottom fibreglass tanks fitted with a faecal collection trap similar to that as described by Allan *et al.* (1999). While in the digestibility tanks, fish were provided with aeration and circulating sea water. Faeces were collected by procedures similar to those of Allan *et al.* (1999).

Growth and Feed Consumption

The calculated growth variables were relative weight gain (%) in 140 days and specific growth rate (SGR). The calculation of SGR was based on Schulz *et al.* (2005) equation as follows:

Table 1.   Formulation (%), proximate analysis (% dry matter) and energy content
(MJ/kg dry matter) of test diets containing fermented blood meal (FBM)

Ingredient	Test diets				
	FBM_0	$FBM_{7.5}$	FBM_{15}	$FBM_{22.5}$	FBM_{30}
Trash fish[1]	18.5	18.5	18.5	18.5	18.5
Fish meal	48.5	38.5	28.5	18.5	8.5
Fermented blood meal	0	7.5	15	22.5	30
Shrimp head meal	9	9	9	9	9
Rice bran	4	4	4	4	4
Wheat flour	13	14.5	16	17.5	19
Fish oil	1	2	3	4	5
Soybean oil	2	2	2	2	2
Vitamin premix[2]	3	3	3	3	3
Mineral premix[3]	1	1	1	1	1
Proximate analysis					
Crude protein	45.8	46.1	46.5	46.6	47.1
Total lipid	10.4	10.5	10.8	10.9	11.1
Fibre	2.4	2.6	3.0	2.5	2.4
Ash	16.9	15.5	14.8	13.7	13.2
Energy[4]	19.1	19.4	19.5	19.7	20.0

[1]   74 g trash fish, equivalent to 18.5 g of dried fish meal was used
[2]   Vitamin premix provided (mg kg^{-1} 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, 2370; p-aminobenzoic, 59.2; astaxanthin, 177.8; menadione, 47.4; calciferol, 22.5;
μ–tocopherol, 237; ascorbic acid, 1777.5; cyanocobalamin, 1.2; choline–HCL, 10971
[3]   Mineral premix provided (mg kg^{-1} diet): KH_2PO_4, 2000; $CaCO_3$, 2500; NaH_2PO_4, 3150;
$FeCl_3.2H_2O$, 1660; $ZnSO_4$, 100; $MnSO_4$, 67.5; $MgSO_4$, 500; $CuSO_4$, 20; KI, 1.5; $CoSO_4.7H_2O$, 1.0
[4]   Calculated from the determined protein, lipid and NFE of the diet using gross energy
convertion coefficients of 23.6, 39.5, and 17.2 MJ kg^{-1}, respectively (National Research
Council, 1993).

$$FE = \frac{\text{fish weight gain (g wet weight)}}{\text{feed consumption (g dry weight)}}$$

$$SGR\ (\%\ day^{-1}) = 100 \times (\ln W_e - \ln W_s)/d$$

Where: We = fish final weight (g), Ws = fish
initial weight (g), and d = rearing period
(days)

Feed efficiency (FE) was calculated using
the equation of Takeuchi (1988) as follows:

Protein efficiency ratio (PER) was calculated
based on the following equations of Takeuchi
(1988) and Hardy (1989):

$$PER = \frac{\text{fish weight gain (g wet weight)}}{\text{total protein consumed (g dry weight)}}$$

The calculation of DM, CP, TL, and energy
(E) apparent digestibility (AD) was based on
Watanabe (1988) and Hardy (1989) equations
as follows:

$$AD\ (\%) = 100 \times \left[1 - \frac{M_D \times A_F}{M_F \times A_D} \right]$$

Where: M_D and M_F are Cr_2O_3 dry weight indicator
concentrations in diet and faeces,
respectively. A_D and A_F are concentrations
of dry weight nutrient in diet and faeces,
respectively.

Chemical and Statistical Analysis

Initial fish whole body analysis was
determined for a pooled sample of 10 fish; a
representative sample of 5 fish from each net

cage was used to determine the final whole body composition of the fish. The fish samples were minced, freeze-dried and pulverized using a mortar and pestle. The dry sample was blended to get a homogenous fine product. Representative samples of feed ingredients, fish, diet, and faeces were analysed based on AOAC International (1999) methods. DM was determined by drying in an oven at 105°C for 16 hours, ash by ignition in a muffle furnace at 550°C for 24 hours and crude protein was analysed using a micro-Kjeldahl procedure with distillation into 4% boric acid and titration with sulphuric acid using methyl red indicator for end point determination. Total lipid was gravimetrically determined with chloroform: methanol extraction (Bligh & Dyer, 1959). Analysis of chromium concentration in diet and faeces samples was analysed based on Takeuchi's (1988) procedures. For amino acid analysis, samples were hydrolysed using 6 molar HCL at 110°C for 22 hours and amino acid content quantified using a Hitachi 835 high speed amino acid analyzer.

Weight gain, daily growth rate, feed efficiency, feed consumption, protein efficiency ratio, fish survival rate, and whole body fish composition data were analysed using ANOVA and regression analytical procedures. Tukey test (Steel & Torrie, 1995) was used to detect significant ANOVA treatment effect. Apparent digestibility was determined for only a single replicate for each treatment and thus was not statistically analysed.

RESULTS AND DISCUSSION

Fermented blood meal contained relatively less methionine, cysteine and isoleucine than PFM or whole body tiger grouper protein (Table 2). However, acid hydrolysis is known to partially destroy sulphur amino acids, resulting in low estimates for methionine and cysteine; tryptophan is also destroyed by acid hydrolysis. While the analysed value for methionine and cysteine detailed in Table 2 are under estimates, it is clear that blood meal is low in sulphur amino acids and isoleucine. Methionine and isoleucine are essential amino acids that are required for fish growth. Asgard & Austreng (1986) reported that blood meal contains less methionine and isoleucine but more leucine than fish meal. Blood meal therefore is not a complete source of dietary protein for fish but could be a useful supplementary source of protein if combined with feed ingredients that contain high amounts of methionine and isoleucine. The fish fed moist pellet containing 22.5% or more of fermented blood meal grew significantly slower ($P < 0.05$) than fish fed the control diet. Fish fed the 30% fermented blood meal diet grew 47% slower than fish fed the control diet.

Table 3 shows that specific growth rate, weight gain, feed efficiency and protein efficiency ratio of fish fed diets containing up to 15% FBM diets were not significantly ($P > 0.5$) different and all were higher than those fed 22.5%–30.0% FBM diets. However, there was a

Table 2. Essential amino acid composition of fish meal, fermented blood meal, and whole body of tiger grouper protein (% dry matter)

Amino acid	PFM	Fermented blood meal	Tiger grouper
Arginine	4.20	4.05	3.63
Histidine	2.40	5.78	1.02
Isoleucine	2.63	0.70	2.42
Leucine	5.43	10.91	3.99
Lysine	5.74	7.36	3.77
Methionine + Cystine	2.13	0.29	0.47
Phenylalanine	3.18	6.34	2.04
Threonine	3.33	4.50	2.47
Valine	3.10	6.40	2.64
Tryptophan	1.68	nd	nd

Note: nd = not detectable

significant culvilinear decline in overall fish performance with increasing inclusion of FPM and most notable for weight gain, feed efficiency and feed consumption (Table 3, Figure 1). Based on regression analysis, the reduction in fish growth is modelled by the equation: FW = 222.4 – 1.319 FBM – 0.07369 FBM^2. From this equation, the asymptote where fish growth deteriorates as a function of FBM inclusion was determined to be 8.9%.

Feed consumption was lower for all diets contained FBM. Unpalatability of the diets containing FBM is the most likely reason for the reduced fish growth associated with increasing dietary inclusion of FBM. Asgard & Austreng (1986) reported a similar result with

salmonids fed a diet containing 33% fermented blood jelly, equivalent to 20% moist pellet protein.

In our experiment, there was no difference in survival rate between the different treatments (P>0.05), indicating that up to 30% fermented blood meal in diet did not affect fish health. Similarly, Asgard & Austreng (1986) found that the survival rate of salmon fed a diet with 33% fermented blood jelly was not different from that of fish fed control diet enriched with fish waste as a source of protein.

According to Hertrampf & Piedad–Pascual (2000), blood meal handling prior to its inclusion in feed formulation affects its digestibility. A similar finding was reported by
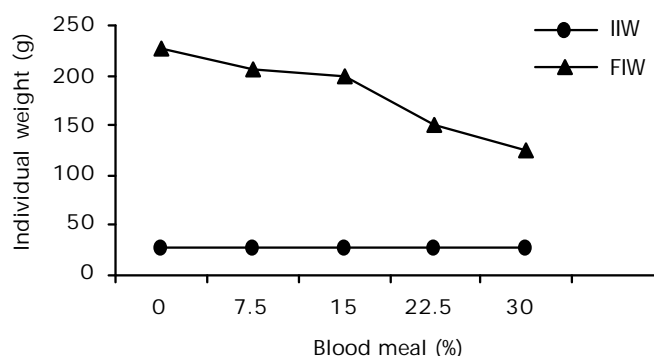

Figure 1.   Initial (IIW) and final (FIW) individual weight of tiger grouper fed diets containing different levels of fishmeal substitution with fermented blood meal

Table 3.   Growth performance and feed utilization of tiger grouper fed diets containing different level of fermented blood meal (FBM)

Variable	Test diets*				
	FBM_0	$FBM_{7.5}$	FBM_{15}	$FBM_{22.5}$	FBM_{30}
Initial weight (g)	31.1	31.2	31.3	31.2	31.1
Final weight (g)	224.0	201.4	196.7	148	118.5
Specific growth rate (% day^{-1})	1.41[a]	1.33[a]	1.31[a]	1.11[b]	0.96[b]
Weight regression slope	38.6[a]	33.5[a]	32.7[a]	23.3[b]	17.1[b]
Weight gain (%)	621.6[a]	545.2[a]	529.1[a]	373.6[b]	280.8[b]
Feed efficiency	0.71[a]	0.68[a]	0.68[a]	0.49[b]	0.39[b]
Feed consumption (g fish^{-1})	265.0[a]	247.3[b]	241.4[b]	234.4[bc]	222.4[c]
Protein efficiency ratio	1.54[a]	1.47[a]	1.48[a]	1.04[b]	0.83[c]
Survival rate (%)	91.7[a]	93.3[a]	95.0[a]	88.3[a]	95.0[a]

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

Laining *et al.* (2003) who demonstrated that DM digestibility of blood meal was only 48% while that of the protein was only slightly higher at 55%. However, blood meal fermented with formic and propionic acid had higher digestibilities of 62%–68% for DM and 84%–88% for CP. The digestibility of feed containing fermented blood meal in this experiment was relatively high, 71.4%–76.5% for dry matter, 84.4%–88.1% for protein, and 93.3%–94.7% for lipid (Table 4). However, the digestibility of the feed appears to decrease with increasing blood meal content but the digestibility of lipid remaining high for all diets. Digestibility of salmon feed containing 24.5% fermented blood jelly was not different from the feed containing 54% fish waste; 71% for dry matter, 87% for protein, and 86% for lipid (Asgard & Austreng, 1986).

The whole body composition of fish fed at the end of the experiment was similar for all diet treatments (Table 5). Protein content ranged from 57.5% to 58.1% and lipid from 21.3% to 21.7%. The similarity of the chemical composition of the fish across all treatments indicates that including up to 30% of fermented blood meal in the diet, while depressing fish growth rate, had little effect on the nutrient composition of the fish.

CONCLUSION

Fermented blood meal added to the diet at less than 9 % does not adversely affect tiger grouper growth. Including more than 9% fermented blood meal as a replacement for fish meal protein is likely to depress fish performance, most likely due to the unpalability of the diet and its effect of reducing feed consumption. Depending on relative ingredients costs, it may be economic to include more than 9% fermented blood meal in the diet, with the lower fish productivity being compensated for by the lower feed cost.

ACKNOWLEDGMENT

Tabel 4. Apparent digestibility coefficients of tiger grouper diets containing different levels of fermented blood meal

Test diet	Variable of feed digestibility (%)		
	Dry matter	Protein	Lipid
FBM_0	76.5	88.1	94.5
$FBM_{7.5}$	75.7	87.5	94.7
FBM_{15}	74.5	86.8	93.7
$FBM_{22.5}$	71.7	84.9	93.3
FBM_{30}	71.4	84.4	93.9

Table 5. Whole body composition of tiger grouper fed diet containing different levels of fermented blood meal (% dry weight)

Nutrient	Test diets*				
	FBM_0	$FBM_{7.5}$	FBM_{15}	$FBM_{22.5}$	FBM_{30}
Crude protein	57.5[a]	57.7[a]	58.1[a]	57.6[a]	58.1[a]
Total lipid	21.7[a]	21.4[a]	21.3[a]	21.6[a]	21.5[a]
Ash	16.3[a]	16.0[a]	15.8[a]	17.3[a]	15.7[a]
Crude fibre	2.1[a]	2.1[a]	2.0[a]	2.1[a]	2.1[a]

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

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