The Potential Use of Salted Marine By-Catches and Fish Viscera Meal Mixture as A Replacement for Conventional Fishmeal in the Diet of Indonesian River Catfish (Hemibagrus nemurus)

by Indra Suharman

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The Potential Use of Salted Marine By-Catches and Fish Viscera Meal Mixture as A Replacement for Conventional Fishmeal in the Diet of Indonesian River Catfish (*Hemibagrus nemurus*)

B Hasan[1*], D Iriani[1], T Leksono[1], I Suharman[2] and F Suzanti[3]

[1] Department of Fisheries Product Technology Faculty of Fisheries and Marine Science Universitas Riau, Campus of Bina Widya Km 12.5 Pekanbaru 28293 Indonesia

[2] Department of Aquaculture Faculty of Fisheries and Marine Science Universitas Riau, Campus of Bina Widya Km 12.5 Pekanbaru 28293 Indonesia

[3] Department of Biology Education Faculty of Teacher Training and Education Universitas Riau, Campus of Bina Widya Km 12.5 Pekanbaru 28293 Indonesia

*Email: bustarih@yahoo.com

Abstract. The research was conducted to investigate the substitution effect of dietary salted marine by-catches and fish viscera meal mixtures for conventional fishmeal in physicochemical and sensory quality of Indonesian river catfish. Four experimental diets containing 34% crude protein and 3.20 kcal g^{-1} digestible energy were formulated. A control diet (FM) contains 54% fishmeal and no salted marine bycatch - fish viscera mixture (SMBFVM). In the other diets, the fishmeal was reduced and proportionally replaced with SMBFVM, 50% (SMBFVM-50), 75% (SMBFVM-75) and 100% (SMBFVM-100). A commercial diet (CD) containing 32 % crude protein and 2,94 kcal g^{-1} digestible energy was used as the reference. Feeding trial was performed with 1500 fish (4.79±0.38 g in weight) which was distributed in 2x2x1.5 m triplicate net cages, and the fish was fed the experimental diets at satiation twice daily at 07:00 AM and 05:00 PM for 8 weeks. There was no substitution effect of fishmeal by SMBFVM on the survival rate of the fish ($P>0.05$). Substitution of fishmeal by SMBFVM in the diet up to 75% (SMBFVM-75) increased weight gain, specific growth rate, food efficiency ratio, protein efficiency ratio and protein retention ($p<0,05$) better than control diet; and complete substitution of fishmeal with SMBFVM (SMBFVM-100) gave similar growth performance to control diet ($P>0.05$). Total replacement of fishmeal with SMBFVM in the diets also gave comparable body fish proximate and amino acid profile to control diet. Compared to CD, fish fed SMBFVM diets had similar growth performance, body proximate, and amino acid profile. The SMBFVM, therefore, could completely replace conventional fishmeal in the diet for Indonesian river catfish without negative effects on fish growth, body proximate, and amino acid profile.

1. Introduction

Aquaculture of river catfish species (*Hemibagrus nemurus*) has been developed; artificial breeding and aquaculture techniques for the species are available [1, 2, 3]; however, the production cost is considerably higher as the fish is mostly fed commercially manufactured diets which contain high level of fishmeal as dietary protein [4]. Since fishmeal is expensive, and its production is limited and

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scarcely available in local market [5]. Therefore, the exploring local materials as the alternative protein sources replacing fishmeal in the fish diet are necessary to minimize the production cost.

Marine fish by-catches are a potential alternative for fishmeal in the fish diet due to its high protein content, 14.4-20.8% [6] and abundant supply which is estimated more than 4.392.000 tons a year or equal to 62.6% of total annual marine catch in Indonesia [7, 8]. However, utilization of fresh marine fish by catches for fishmeal is not economical as the their production are scattered at small scales in the remote fishing ground while refrigeration methods are expensive and scarcely available. Salt preservation has been a solution for maintaining good quality marine fish by catches as its production cost is low and easily practiced by fishermen; however high salt concentration of the product may be unacceptable a certain cultured fish.

Our previous feeding study on river catfish revealed that the salted trash fish could be included in the fish diets up to 50 to 75% replacing fishmeal without negative effect on growth, food efficiency, nutrient utilization and flesh quality; however complate replacement of fishmeal by salted trashfish meal reduced the fish performance [6]. In order to increase substitution value of marine fish by catches, they were probably combined with other cheaper protein sour products. Blend of different ingredients has been recommanded to achieve nutritional composition balances, amino acid profile complementation and unpalatable substance masking in feed ingredients [9, 10, 11, 12, 13, 14, 15, 16].

Fish viscera from fish processing by product was abundantly available in the area. The product is high in nutrition but its inclusion value in the fish diet was limited [17]. In this study therefore, the salted marine fish by catches were mixed with fish viscera meal; and the substitution value of the mixtures for fishmeal in the diet of Indonesian river catfish was evaluated.

2. Materials and Methods

2.1. Experimental fish and culture system

Indonesian river catfish averaging 4.79±0.38 g in weight was purchased from local private fish hatchery in Kampar district, Indonesia. The fish was transported to the aquaculture experimental station, Faculty of Fisheries, Universitas Riau, Indonesia. At the station, the fish was acclimatized for one week in 4x4x2 m net cage constructed in an earten pond (25 x 25 m) which was continuously suplied with underground water at a flow rate of 2.5 L min^{-1}. During aclimatization, the fish was fed a commercial diet.

2.2. Experimental diet and analysis

Salted marine by catches consisting of various fish species were purchased from local fish market; and fresh fish viscera of *Pangasius hypopthalmus* was taken from smoked fish processing industry in the area. Salted marine by catches and fish viscera ware boiled separately for 10 minutes, oven-dried at 55-60^{o}C, ground in meat grinder and analized for proximate analysis. Before incorporation in the diet, salted marine by catch meal was mixed with fish viscera meal at 3:1 ratio (dry weight bases). Other ingredients were tofu by product meal, rice bran, vitamin and mineral premix. Four experimental diets were formulated to contain 34% protein and 3.20 kcal g^{-1} digestible energy. The control diet (FM) contains 54% fishmeal and no salted marine by catch - fish viscera mixture (SMB-FVM). The remaining three diets were prepared by replacing fishmeal with SMB-FVM at the level of 50% (SMB-FVM-50), 75% (SMB-FVM-75) and 100% (SMB-FVM-100). A commercial diet (CD) containing 32 % crude protein and 2.94 kcal g^{-1} digestible energy was used as the reference. All formulated diets were thoroughly mixed and pelleted through a meat grinder with an appropriate diameter (2.2–3.2 mm) and the pelleted diet were dried until their moisture content was about 10% and stored at refregerated temperatur (5-7^{0}C). The pelleted diets then were analized for their salt concentration, proximate and amino acid profile. Salt concentration and proximate composition analysis were made according to AOAC method (AOAC, 1990). The salt concentration was determined by titration with $AgNO_3$ 0,1 N after incineration at 500^{o}C. Moisture was determined after the sample was oven-dried at 105^{o}C for 24 hours until constant in weight. Ash was determined after the sample was incinerated at 500^{o}C for 5 hours. Crude protein was analyzed by micro-Kjeldahl procedure and crude protein was estimated as Nx6.25. Crude lipid was determined after the sample was Soxhlet-extracted with petroleum ether. Amino acids analyses were conducted by HPLC using Pico-tag method (Waters, USA) based on [18]. The amino acid profile was determined after hydrolyzed under nitrogen in 6N HCl at 110°C for 24 hours. and the amino acids were calculated in g/100 g protein.

Pellet stability in the water was determined by the method developed by [19, 20]. Triplicate 50 g samples of the pelleted diets were placed on the sieve and slowly immersed in a 40L aquarium containing distilled water at the room temperature for 10 and 30 minutes. The sieves were removed to dry for 1 minute, oven-dried at 105°C for 2 hours. The samples then were cooled in a desiccator and reweighed. Water stability was calculated as the percentage difference in weight after reweighing and the values were expressed as percentage loss of dry matter (% LDM).

2.3. Feeding experiment and fish analysis

Feeding experiment was carried out with 100 fish in 2x2x1.5 m triplicate net cages which were constructed in an earten pond (25 x 25 m) and continuously supplied with under ground water at a flow rate of 1.5 l min^{-1}. The fish was fed the experimental diets at satiation twice daily at 07:00 AM and 17:00 PM for 8 weeks. All the fish were weighed at the beginning, beweekly feeding intervals and the end of the experiment. A total of 25 fish at the beginning of the experiment and 20 fish of each cage at the end of the experiment were taken randomly for body proximate and amino acid profile analyses. Growth performances were calculated by the following formula:

Weight gain (g) = (Final fish weight – Initial fish weight)

Specific growth rate (% day^{-1}) = [(Ln final weight-Ln initial weight)/days of trial] x 100

Survival rate (%) = (Total final survived fish)/(Total initial fish) x 100.

Food consumption (g fish^{-1}) = (Total food consumed per fish)

Food efficiency ratio = (Weight gain, g)/(Feed consumed, g)

Protein efficiency ratio = (Weight gain, g)/ (Protein consumed, g)

Protein retention (%) = (Protein gain, g)/ Protein consumed, g) x 100

2.4. Data analysis

The data were subjected to a one-way Analysis of Variance (one-way ANOVA) using SPSS software version 17 [21]. The Least Significant Different (LSD) test was used to determine the differences among the treatments.

3. Results and Discussion

3.1. Inggredients and diet formulation

The composition of test ingredients (crude protein, crude lipid, ash, NFE and NaCl concentration) was shown in Table 1. Salted marine by catches composed of 55,51% crude protein, 6,67% crude lipid; 21,61% ash; 11,93% nitrogen free extract (NFE) and 8,30% NaCl. Crude protein and lipid were slightly lower but ash, NFE and NaCl concentrations were higher for salted marine by catches than that conventional fishmeal. Moreover, crude protein and ash were lower but crude lipid and NFE were higher for fish viscera than conventional fishmeal.

The formulation and composition of experimental diets were presented in Table 2. Crude protein, lipid, ash, NFE and energy composition of the formulated diets were similar, except for NaCl concentration which increased as the increasing amount of SMB-FVM in the diets.

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Table 1 Proximate analysis of feed ingredients (triplicate samples)

Ingredients	Proximate composition (% dry basis)				
	Crude Protein	Crude Lipid	Ash	NFE*	NaCl
Fishmeal	57,44	11,81	21,71	9,04	2,11
Salted marine by catches	55,51	6,67	21,61	16,21	9,19
Fish viscera	40,17	15,20	12,71	31,92	-
Tofu by product meal	24,83	4,01	2,39	68,77	-
Rice bran	12,26	6,21	12,52	69,01	-

*NFE=100- (crude protein, crude lipid, moisture, ash)

Table 2 Diet formulation and composition

Ingredients	Diet				
	FM[a]	SMB-FVM50	SMB-FVM75	SMB-FVM100	CD[a]
Fishmeal	54	27	13,50	0	-
SMB-FVM	0	27	40.50	54	-
Tofu by product meal	22	22	22	22	-
Rice bran	23,5	23,5	23,5	23,5	-
Vitamine and mineral mix[b]	0,50	0,50	0,50	0,50	-
Proximate composition by analysis					
Dry matter	89,80	87,90	88,08	87,21	90,39
Crude protein	34,09	34,04	33,57	33,75	31,79
Crude lipid	13,15	13,19	13,11	13,08	6,71
Ash	13,74	13,79	14,17	14,77	8,62
NFE[c]	39,03	38,98	39,15	38,39	52,88
Energy (kcal DE/100g)[d]	3,23	3,24	3,22	3,20	2,94
NaCl (%)	1,15	2,09	3,01	4,29	0,57

[a]FM=Fishmeal; SMB-FVM= Salted marine bycatch and fish viscerae mixture; CD=commercial diet
[b]Vitamine and mineral mix= Vit A, 2750 IU; Vit D, 550,000 IU; Vit E, 25,000 IU; Vit K, 5,000 mg; Choline, 250,000 mg; Niacin, 50,000 mg; Riboflavin, 10,000 mg; Pyridoxine, 10,000 mg; Calcium D-pantothenate, 25,000 mg; Biotin, 50 mg; Folacin, 2,500 mg; Cyanocoblamin, 10 mg; Ascorbic acid, 50,000 mg; K_2HPO_4, 30%; KCL, 8.4%; $MgSO_4$, 14.8%; $CaHPO_4.2H_2O$, 27.4%; $FeCL_3$, 1.4%; $MnSO_4.7H_2O$ 0.2%; $CaCO_3$, 16.8%.
[c]NFE= 100- (crude protein, crude lipid, moisture, ash)

The ratio of individual essential amino acid and total essential amino acid (% individual essential amino acid/total essential amino acid) which was considered as indicator essential amino acid balance in the diet [20, 22, 23, 24, 25] (Table 3) was also comparable between SMB-FVM diets and FM diet.

Table 3 A/E[a] ratio test diet and *Hemibagrus nemurus*

Essential amino acids	*Hemibagrus nemurus*	FM[b]	SMB-FVM50	SMB-FVM75	SMB-FVM100	CD
Arginine	7,72	7,15	6,34	7,03	6,78	5,80
Histidine	7,10	7,81	8,01	8,01	7,49	8,69
Isoleucine	14,02	12,34	13,62	11,72	13,72	13,89
Leucine	17,48	18,54	17,78	17,75	17,39	17,63
Lysine	13,88	17,79	15,17	15,65	14,95	15,93

Methionine	6,54	7,05	6,95	6,89	7,207	5,82
Alanine	7,61	5,05	6,33	6,67	6,63	6,76
Phenylalanine	8,15	7,33	7,73	7,77	7,91	7,92
Threonine	9,68	9,03	9,61	9,52	9,92	8,77
Valine	7,82	7,90	8,43	8,99	7,99	8,78
Tryptophane	ND[c]	ND	ND	ND	ND	ND

[a]A/E= % Essential amino acids/Total essential amino acids
[b]FM=Fishmeal; SMB-FVM= Salted marine bycatch and fish viscerae mixture; CD=commercial diet
[c]ND=Not determined

Since the proximate and amino acid profile of SMB-FVM and FM were similar, NaCl concentration therefore was the component which characterized SMB-FVM diets different from FM diet.

The water stability of the pelleted diets was measured by percentage loss of dry matter (LDM) after 10 and 30 minutes in the water, and the best water stability was the feed with a minimum loss in dry matter. The LDM values in this study (Table 4) were not different between SMB-FVM diets and FM diet (P>0.05), indicating that the inclusion of salted trash fish meal in the diets did not significantly affect water stability of the pelleted feed. LDM values of the FM diet and SMB-FVM diets were 2.02-2.04% for 10 minutes and 6.37-6.38% for 30 minutes. These values were considered very stable as a minimum LDM value for catfish feed was <10% for 5 minutes [26, 27].

3.2. Water quality

Water quality values during feeding experiment was shown in Table 4. Dissolved in the morning and afternoon ranged respectively from 4,34 to 5,85 mg/l and 4,40 to 6,15 mg/l; water temperature from 28° to 29,9°C dan 28° to 33,3°C; and pH from 6,5 to 7.3 and 6,2 to 7,3. These vales were within the conducive values for culture of tropical fish. Dissolved oksigen value, which was the most important factor supporting growth respond of the aquaculture fish was within the kondusive value as the value recommended for catfish was higher than 3 mg/l [28].

Table 4 Water stability of pelleted feed (% LDM) for 10 and 30 minutes

Diets (Pellet)	Water Stability (% LDM)[b]	
	LDM-10 Minutes	LDM-30 Minutes
FM[a]	3.04±0.07a	8.48±0.07a
FMB-FVM50	3.02±0.11a	8.38±0.05a
FMB-FVM75	3.03±0.09a	8.47±0.05a
FMB-FVM100	3.03±0.08a	8.37±0.06a

Means in the same column with the same superscript were not different (P<0,05)
[a]FM=Fishmeal; SMB-FVM= Salted marine bycatch and fish viscerae mixture; CD=commercial diet
[b]LDM= Loss of dry matter

Tabel 5 Water quality during feeding trial

Parameter	Water quality	
	Morning	Afternoon
Dissolved oxygen	4,34-5,85 mg/l	4,40 - 6,15 mg/l
Temperature (°C)	28° - 29,9°C	28 - 33,3°C
pH	6,5 - 7.3	6,2 - 7,3.

3.3. Growth performance

Initial weight (IW), final weight (FW), survival rate (SR), feight gain (WG) and specific growth rate (SGR) of the fish fed experimental diets was presented in Table 6; and food eficiency ratio (FER), protein eficiency ratio and protein retention (PR) was shown in Table 7. Complate substitution of

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fishmeal by SMB-FVM in the fish diets (SMB-FVM100) did not affect SR, WG and SGR ($p>0.05$) as compared to FM diet; and substitution by SMB-FVM to the level up to 75% increased in WG and SGR values ($p<0.05$). SMB-FVM therefore could totally replace fishmeal in the fish diet without negative impacts on growth. The replacement value of SMB-FVM for fishmeal in this study was higher than salted marine by catches alone in our previous study, which the value was only 50% for the smaller fish [4], and 75% for the bigger fish [6].

Table 6 Growth and survival rate during feeding trial

Diets	IW (g)	FW (g)	SR (%)	WG (g)	SGR (%)
FM	4.50	16.18	95.33^b	11.68^a	2.29^a
SMB-FVM50	4.32	20.12	94.67^b	15.80^b	2.75^b
SMB-FVM75	4.63	20.85	99.82^b	16.22^b	2.68^b
SMB-FVM100	5.01	18.58	95.33^b	13.58^a	2.36^a
CD	5,26	18,45	$74,00^a$	$13,19^a$	$2,24^a$

Means in the same column with the same superscript were not different (P<0,05)
IW=Initial weight; FW=Final weight; SR=Survival rate; WG= Weight gain; SGR=Specific growth rate; FM=Fishmeal; SMB-FVM= Salted marine bycatch and fish viscerae mixture; CD=commercial diet

Table 7. Food consumption (FC), Food eficiency Ratio (FER), Protein efisiensi ratio (PER) and Protein retention (PR)

Diet	FC[b]	FER	PER	PR
FM[a]	$35,52^a$	$0,33^a$	$0,97^a$	$15,18^a$
SMB-FVM50	$49,65^b$	$0,32^a$	$0,95^a$	$14,97^a$
SMB-FVM75	$48,68^b$	$0,34^a$	$0,99^a$	$15,13^a$
SMB-FVM100	$43,09^a$	$0,32^a$	$0,94^a$	$14,13^a$
CD	$43,89^a$	$0,30^a$	$0,89^a$	$14,06^a$

Means in the same column with the same superscript were not different (P<0,05)
[a]FM=Fishmeal; SMB-FVM= Salted marine bycatch and fish viscerae mixture; CD=commercial diet
[b]FC=Feed consumption; FER=Feed efficiency ratio; PER=Protein efficiency ratio; PR=Protein retention

WG and SGR value in this study showed a consisten trend with FC values, indicating that the higher WG and SGR for the fish fed SMB-FVM50 and SMB-FVM75 diet was caused by increasing FC. NaCl, at low concentration (2.09-3.01%) probably contributed to palatability of the diets. Enhancing palatability for the diets containg a certain amount of NaCl was also reported by [6, 29].

Complate substitution of fishmeal by SMB-FVM in the diet (SMB-FVM100) did not impair FER, PER and PR as compared to FM diet ($p>0.05$). The reason might be the NaCl concentration in the diet was still acceptable by fish and did not influece feed utilization. [5] reported that *Hemibagrus nemurus* could tolerate NaCl in the diet up to about 5%, while NaCl concentration of SMB-FVM100 was only 4,29%, within tolerable value. Compared to CD, SMB-FVM75 diet produced higher FER, PER and PR; and SMB-FVM100 give comparable FER, PER and PR with FM diet.

3.4. Proximate composition and amino acid profile of fish

The substitution effects of fishmeal by SMB-FVM on proximate and amino acid profile was summerized in Table 8. There was no substitution impact of fishmeal by SMB-FVM on body protein, lipid, ash and moisture (P>0,05). The body composition of cultured fish usually depends on the nutritional composition of the fed diet [30], specifically dietary protein and fat [31, 32, 4]. As the proximate composition of the SMB-FVM diets was maintained similar to FM diet, the body composition of the fed fish was also similar between that fed SMB-FVM diets and FM diet. The fact

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that the substitution of fishmeal in the diet do not impair body composition of the fish were also reported in tilapia fed shrimp industrial waste by [33].

Body amino acid profiles were also similar between the fish fed SMB-FVM diets and FM diet, indicating that the was no effect of fishmeal substitution by SMB-FVM diet on essential amino acid profiles of the fish. This may be due to essential amino acid profiles of the SMB-FVM and FM diets which was similar; and A/E ratio of both SMB-FVM and FM diets was also comparable with A/E ratio of *Hemibagrus nemurus*, which was considered as indicator of essential amino acid requirement in the fish diet. The fact that there was no effect of fishmeal substitution on body amino acid profile was also reported in the same species which was fed fish silage diets as a replacement for dietary fishmeal [20].

Table 8 The effect of substitution fismeal by SB-FVM on proximate and amino acid composition of fish

Proximate composition	Diet (%)				
	FM[a]	SMB-FVM50	SMB-FVM50	SMB-FVM50	CD
Moisture	72.44[a]	72.42[a]	72.39[a]	72.46[a]	72.30[a]
Ash	2.91[a]	3.04[a]	3.10[a]	3.25[a]	3.29[a]
Protein	13.39[a]	13.34[a]	13.43[a]	13.26[a]	13.22[a]
Lipid	6.62[a]	6.69[a]	6.59[a]	6.61[a]	6.50[a]
Amino acid profiles (% sample)					
Asp	2,13	1,72	1,31	2,14	1,72
Glu	3,62	3,23	2,89	3,79	3,23
Ser	0,59	0,56	0,77	0,59	0,56
Gli	1,52	2,24	2,80	1,79	2,24
His	0,61	0,95	1,13	0,65	0,95
Arg	0,58	0,81	0,61	0,60	1,08
Tre	0,82	0,30	0,40	0,75	0,30
Ala	0,59	0,49	0,59	0,42	0,49
Pro	1,55	1,34	1,94	1,55	2,14
Tir	0,82	0,87	0,43	0,88	0,87
Val	0,65	0,53	0,67	0,66	0,53
Met	0,59	0,67	0,51	0,59	0,67
Sis	0,72	0,69	0,60	0,70	0,69
Iso	1,03	0,86	0,37	1,03	0,86
Leu	1,52	1,62	1,28	1,55	1,62
Phe	0,62	0,88	0,43	0,61	0,88
Lys	1,40	1,16	1,36	1,49	1,16
Total	19,34	18,90	18,09	19,78	19,97

Means in the same column with the same superscript were not different ($P<0,05$)
aFM=Fishmeal; SMB-FVM= Salted marine bycatch and fish viscerae mixture; CD=commercial diet

4. Conclusion

SMB-FVM can complately replaced FM in *Hamibagrus nemurus* diet without negative effects on growth, feed convertion and body composition

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