Evaluation of salted trash fish as a protein source replacing fishmeal in the diet for river catfish (*Hemibagrus nemurus*)

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Abstract. The research was conducted to evaluate the effect of substitution of fishmeal by salted trash fish in the diet on growth, feed eficiency, nutrient utilization and body composition of river catfish (*Hemibagrus nemurus*). Five formulated diets were prepared to contain 38% protein and 3.25 kcal g^{-1} digestible energy (DE) as an optimum dietary protein and energy for the fish. One diet was control diet which contained fishmeal without salted trash fish (FM), and other diets were trash fish diets which fishmeal was reduced and proportionally replaced by salted trash fish 25% (STF-25), 50% (STF-50), 75% (STF-75) and 100% (STF-100). Catfish fingerlings, ±5 g in size obtained from local fish hatchery were stocked into triplicate 2 x 2 x 1.5 m floating net cages at a density of 100 fish $cage^{-1}$, and fed experimental diets at satiation, twice a day for 8 weeks. Results indicated that the salted trash fish diets were characterized by higher salt, ash and fiber but similar in amino acid profiles as well as water stability as compared to control diet. The survival rate was reached 100% in all treatments. Inclusion of salted trash fish at a level 25% in the diet increased weight gain as compared to control diet; and inclusion up to 50% did not affect weight gain, specific growth rate, food consumption, food eficiency, protein eficiency and protein retention; but further increase reduced weight gain, specific growth rate, food eficiency, protein eficiency and protein retention. Salted trash fish diets did not affect fish body protein and ash, but increased body moisture and decreased body fat. Essential amino acid profiles of the fish fed salted trash fish diets were similar to that fish fed control diet. It was concluded that the salted trash fish could be included in the river catfish diet up to 50% as replacement for 50% fishmeal without negative effect on weight gain, specific growth rate, food consumption, food eficiency, protein eficiency, protein retention and proximate body composition of river catfish.
Key Words: fishmeal diet, trash fish diet, river catfish, growth performance, fish body composition.

Introduction. River catfish (*Hemibagrus nemurus* Valenciennes, 1840) is a popular and demanded river catfish species for both fresh and smoked fish consumption in Riau Province, Indonesia. Naturally, the fish was cought from the rivers, lakes and resourvoir, however, its population in the wild was decreasing due to overfishing and environmental demage, therefore, the next supply of the fish would depend on aquaculture production. The culture of the river catfish now has been developed; artificial breeding and aquaculture techniques have been available; however, the fish was required relative high dietary protein (approximately 38-42%) for its optimum growth and high flesh quality (Khan et al 1993; Hasan et al 1999; Hasan et al 2013).

Fishmeal (FM) is the most preferable and digestable protein source in the diet for most farmed fish including river catfish due to its amino acid, fatty acid, energy, vitamins and minerals balance (Tacon 1993; Eguia 1998; Khan et al 1993; Hasan et al 1999; 2013; Abdelghany 2003). However, its price is high and its production is limited and scarcely available, import dependent, hence it is not economical for small scale feed manufacturer. Since the demand for FM continues to grow, while its production is expected to remain constant (New & Wijkstrom 2002; Borgeson et al 2006), more attention must be paid to utilizing local material as an alternative protein source replacing FM in the fish diet.

Trash fish from marine by-catch is a potential protein source as a replacement for conventional FM in the fish diet due to its high protein content, averaging 66.1% dry weight (Li et al 2004) and its abundant supply which is estimated more than 4.39 million tons or 62.6% of the total annual marine catches in Indonesia (Davies et al 2009; Anon 2010). However, most trash fish production is scattered at small scale fishermen in remote fishing ground. The fish is highly perishable while refrigeration facilities and ice are expensive and scarcely available, thus an economic and practical preservation method is required to collect and transport it to feed processing industries.

Salting is a cheap and practical method for trash fish preservation as the salt is easily available and the salting process only needs simple technology. In Riau Province, Indonesia, salted trash fish has been used for protein source in the diets for some farm raised fish; and its acceptability by fish was reported to vary with fish species and salt concentration (personal communication). Our personal observation showed that farmers included salted trash fish as protein source up to 30% in the diet for some farm raised fish such as pangasius catfish (*Pangasius hyphopthalmus*) and tilapia (*Oreochromis niloticus*); but there was no scientific information on its effect on growth and fish body composition, especially for river catfish. Hence, this research was to evaluate the effect of substitution of dietary FM by salted trash on growth performance and fish body composition of river catfish.

Material and Method

Diet preparation and analysis. Dried salted trash fish of marine by catch was taken from a local feed stuff supplayer. The trash fish was desalted in boiling water (1 part of trash fish: 2 parts of water) for 15 minutes and pressed to reduce its salt concentration, dried and ground. Five diets were formulated to contain 38% protein and 3.25 kcal g^{-1} digestable energy (DE), which the levels were within the range of dietary protein and energy requirement for optimum growth of river catfish, 34-42% protein and 2.75-3.25 kcal g^{-1} energy (Khan et al 1993; Hasan et al 1999; Hasan et al 2013; Hasan et al 2016). One diet was control diet containing conventional FM without salted trash fish; and other diets were salted trash fish diets which FM was reduced and proportionally replaced with salted trash fish 25% (STF-25), 50% (STF-50), 75% (STF-75) and 100% (STF-100), respectively.

The diets were pelleted with extruder machine, dried and analized for salt concentration, proximate and amino acid composition as well as stability in the water. Salt concentration and proximate composition analyses were conducted based on AOAC method (AOAC 1990). Salt concentration was analyzed by titration with $AgNO_3$ 0.1 N after incineration at 500°C. Moisture was determined after the sample was oven-dried at 105°C until constant in weight. Ash was determined after the sample was incinerated at 500°C for 5 hours. Protein was analized by micro-Kjeldahl procedure and crude protein was estimated as N x 6.25. Crude fat was determined after the sample was Soxhlet-extracted with petroleum ether. Amino acids analyses were conducted by HPLC using Pico-tag method (Waters USA) according to Cohen et al (1989). 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 percent protein.

Pellet stability in the water was determined based on Fagbenro & Jauncey (1995). Triplicate 50 g samples of pellet diets were placed on sieve and slowly immersed in 40L aquarium containing distilled water at room temperature for 10 and 30 minutes. The sieves were removed to dry for 1 minute, oven-dried at 105°C for 2 hours, cooled in a desicator and reweighed. Water stability was calculated as the percentage difference in weight after reweighing and expressed as percentage loss of dry matter (% LDM).

Feeding trial. River catfish fingerlings, ±5 g in size were taken from a local fish hatchery in Kampar district. The fish was stocked in triplicate 2 x 2 x 1.5 m experimental floating net cages at stocking density of 100 fish per cage; and the experimental fish fed the formulated diets for 8 weeks, from June to August 2015. The fish were fed at satiation twice daily at 9.00 and 16.00. All the fish were weighed at the beginning and two week intervals. Water quality (temperature and dissolved oxygen) and fish mortality were recorded daily. Twenty fish at the beginning of experiment and all fish of each cages at the end of the experiment were taken randomly and analyzed for proximate and amino acid compositions. Growth parameters were calculated by following formula:

Weight gain (g fish^{-1}) = (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 fish^{-1});
Food efficiency ratio = (Weight gain, g)/(Food consumed, g);
Protein efficiency ratio = (Weight gain, g)/(Protein consumed, g);
Protein retention = (Protein gain, g)/Protein consumed, g) x100

***Data analysis**.* Triplicate data were analyzed by the Analysis of Variance using SPSS (SPSS 2000). Least signficant different test was used to determine the differences among individual means.

Results and Discussion

***Feed ingredients, formulation and proximate composition of the diets**.* Proximate and salt composition of salted trash fish and other feed ingredients used in the experimental diets was presented in Table 1. Salted trash fish contained 55.29% protein, 6.35% fat, 9.76% moisture, 14.67% ash, 2.30% crude fiber, 11.93% nitrogen free extract (NFE) and 10.19% salt. Protein composition of salted trash fish was similar to conventional FM, but its fat content was lower; and ash, NFE as well as salt content was higher. Lower fat in salted trash fish in this study may be due to boiling and pressing effect as the salted trash fish before inclusion to the diets was boiled in boiling water and pressed for reducing its salt concentration (desalting). During boiling and pressing process, the trash fish fat may be washed, so its fat content reduced.

Table 1

Proximate composition and salt concentration of the feed ingredients

Feed ingredients	*Proximate composition (%)*						
	Protein	*Fat*	*Moisture*	*Ash*	*Fiber*	*NFE**	*Salt*
Fishmeal	56.68	13.97	8.48	12.24	2.00	6.63	2.84
Salted trash fish	55.29	6.35	9.76	14.67	2.30	11.93	10.19
Soybean meal	40.50	17.69	9.90	8.33	8.50	15.08	-
Rice bran	8.54	6.21	6.83	12.52	15.00	50.90	-

*NFE calculated as 100- (protein, fat, moisture, ash, fiber).

Formulation and proximate composition of the experimental diets was shown in Table 2. Proporsions of each diet components were made similar, except for convensional FM and salted trash fish. Protein and energy composition of each diet was maintained 38% and 3.25 kcal g^{-1} DE respectively as dietary requirement for optimum growth of fish; and palm oil was used to maintain energy balance in each diet. Proximate compositions of salted trash fish diets were similar to control diet, except salt concentration which was higher in salted trash fish diets than that for control diet. Salt concentration of FM, STF-25, STF-50, STF-75 and STF-100 diet was 0.96%; 1.63%; 2.27%; 2.97% and 3.72% respectively; and their concentrations increased as the increasing amount of salted trash fish in the diets. Essential amino acid profiles of salted trash fish diets were similar to that for control diet.

Essential amino acid profiles of salted trash fish and control diet were similar (Table 3). The A/E ratio (% essential amino acid content/total amino acid) of river catfish which was considered as an indicator for amino acid balance in the fish diets (Arai 1981; Ogata et al 1983; Murai et al 1984; Khan et al 1993; Hasan et al 2001) was also similar between trash fish diets and control diet. As proximate composition and amino acid profile of the salted trash fish diets and control diet was similar, salt concentration therefore was the main factor characterizing salted trash fish diets different from control diet.

Table 2

Proximate and salt concentration of the experimental diets

Ingredients	*Diets (%)*				
	FM	*STF-25*	*STF-50*	*STF-75*	*STF-100*
Fishmeal	35	26.25	17.5	8.75	0
Salted trash fish	0	8.75	17.5	26.25	35
Soybean meal	30	30	30	30	30.3
Rice bran	30.5	30.5	29.5	29.2	28
Palm oil	3.5	3.5	4.5	4.8	5.7
Vitamin and mineral mix[a]	1	1	1	1	1
Proximate composition by analysis					
Dry matter	91.26	91.95	91.62	91.58	91.95
Protein	38.12	37.59	37.43	37.72	37.21
Fat	16.76	16.65	16.49	16.52	16.51
Fiber	8.92	9.77	9.86	9.92	10.38
Ash	12.76	13.17	13.27	13.52	13.62
NFE[b]	23.44	22.82	22.95	22.32	22.28
Energy (Kcal $^{g-1}$ DE)	3.27	3.23	3.21	3.21	3.20
Salt	0.96	1.63	2.27	2.97	3.72

[a]Vitamin 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%;
[b]NFE calculated as 100- (protein, fat, moisture, ash, fiber).

Table 3

A/E ratio (% essential amino acid/total essensial amino acid) of experimental diets and *Hemibagrus nemurus* (% protein)

Essential amino acid	*Diets*					*Hemibagrus nemurus*
	FM	*STF-25*	*STF-50*	*STF-75*	*STF-100*	
Arginine	14.47	14.37	14.69	14.62	14.79	14.37
Histidine	5.45	5.35	5.42	5.37	5.42	5.56
Isoleucine	7.22	6.98	7.12	6.78	6.74	6.94
Leucine	15.20	14.95	15.14	14.90	14.95	13.80
Lysine	14.49	14.19	14.10	14.07	14.03	14.08
Methionine[a]	7.17	6.98	6.97	7.06	7.25	7.13
Phenylalanine[b]	15.24	15.99	15.34	16.24	16.10	15.66
Threonine	8.91	8.69	8.72	8.66	8.21	8.83
Valine	11.85	12.60	12.70	12.46	12.79	12.05
Tryptophan	ND[c]	ND	ND	ND	ND	ND

[a]Methionine + Cystine, **[b]**Phenylalnine + Tyrosine, **[c]**ND: Not determined.

Water stability of pelleted diets. Water stability of experimental pelleted feed diets was presented in Table 4. The water stability of pelleted feed 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 were not different between salted trash fish peleted diets and control peleted diet ($p > 0.05$), indicating that there was no inclusion effect of salted trash fish in the diet on water stability of the pelleted feed. LDM values of control diet and salted trash fish 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 is < 10% for 5 minutes (Wood et al 1985; Fagbenro & Jauncey 1995).

Table 4

Water stability of feed (% Loss of Dry Matter, LDM) for 10 and 30 minutes

Diets (pellet)	*Water stability (% LDM)*	
	LDM-10 Minutes	*LDM-30 Minutes*
FM	2.04±0.07[a]	6.38±0.07[a]
STF-25	2.02±0.13[a]	6.38±0.05[a]
STF-50	2.03±0.05[a]	6.37±0.05[a]
STF-75	2.03±0.08[a]	6.37±0.06[a]
STF-100	2.04±0.07[a]	6.38±0.04[a]

Note: Means in the same column with the same superscript were not different ($p < 0.05$).

Water quality management. Water quality values along the feeding trial were shown in Table 5. Dissolved oxygen in the morning and afternoon was 3.40-5.50 mg L^{-1} and 3.40-5.50 mg L^{-1} respectively; temperarture was 28-28.9^oC and 31-33.3^oC respectively; and pH was 6.9-7.3 and 6.9-7.3, respectively. The values were within the acceptable ranges for the culture of warm water fishes. Dissolved oxigen which is an important parameter affecting growth responses of fish is acceptable since the recommended value for growth of catfish is greater than 3 mg L^{-1} (Weeks & Ogburn 1973).

Table 5

Water quality during feeding trial

Parameter	*Water quality*	
	Morning	*Afternoon*
Dissolved oxygen (mg L^{-1})	3.40-5.50	3.40-5.50
Temperature (^oC)	28.0-28.9	31.0-33.3
pH	6.9-7.3	6.9-7.3

Growth performance and food utilization. The inclusion effects of salted trash fish as a replacement for convensional FM in the diet on survival rate, weight gain and specific growth rate were shown in Table 6; and food consumption, food efficiency ratio, protein efficiency ratio and protein retention in Table 7. Survival rate of the fish was 100%; and there was no difference in survival rate among the fish fed experimental diets ($p > 0.05$). Inclusion of salted trash fish at a level of 25% in the diet (STF-25) increased weight gain as compared to control diet ($p < 0.05$); and inclusion up to 50% (STF-50) did not affect weight gain and specific growth rate ($p > 0.05$); however, inclusion over 50% decreased weight gain and specific growth rate ($p < 0.05$). Survival rate of the fish was 100%; and there was no difference in survival rate among the fish fed experimental diets ($p > 0.05$). Inclusion of salted trash fish at a level of 25% in the diet (STF-25) increased weight gain as compared to control diet ($p < 0.05$); and inclusion up to 50% (STF-50) did not affect weight gain, specific growth rate, food conversion, protein retention and survival rate ($p < 0.05$). These indicated that the salted trash fish diets were acceptable and utilizable by the fish, and could be included in the fish diet replacing FM up to 50% without adverse effect on growth, food conversion and utilization. The fact that at a low level of salted trash fish in the diet increased food consumption and weight gain was probably due to salt concentration of the diet which at a small amount (1.63%) enhanced palatability of the diets.

However, the fact that the inclusion of salted trash fish > 50% replacing FM in the diet reduced weight gain, specific growth rate, food conversion and protein retention ($p < 0.05$) might be due to higher concentration of salt in the diets (> 2.27%) which reduced palatability of the diet, thus decreased food consumption, weight gain, food eficiency and nutrient utilization. Reduced growth due to the excess amount of salt in the fish diets was also reported in juvenile of flounder (*Paralichthys olivaceus*) by Park et al (2000).

Table 6

The effect of salted trash fish diets on survival rate, weight gain, specific growth rate

Diet	*Initial weight (g)*	*Final weight (g)*	*Survival rate (%)*	*Weight gain (%)*	*Specific growth rate (%)*
FM	5.16	21.52	100	16.36^{c}	2.39^{c}
STF-25	5.25	25.65	100	20.41^{d}	$2.65b^{c}$
STF-50	5.29	20.17	100	14.88^{bc}	2.22^{abc}
STF-75	4.98	16.48	100	11.50^{ab}	1.99^{ab}
STF-100	4.98	15.47	100	10.49^{a}	1.92^{a}

Note: Means in the same column with the same superscript were not different ($p < 0.05$).

Table 7

The effect of salted trash fish diets on food consumption, food eficiency ratio, protein eficiency ratio and protein retention

Diets	*Food consumption (g/fish)*	*Food eficency ratio*	*Protein eficiency ratio*	*Protein retention*
FM	35.88^{bc}	0.45^{a}	1.20^{b}	15.94^{b}
STF-25	45.33^{d}	0.45^{a}	1.18^{b}	15.90^{b}
STF-50	36.03^{bc}	0.41^{a}	1.08^{ab}	13.82^{b}
STF-75	32.56^{a}	0.35^{b}	0.93^{a}	11.71^{a}
STF-100	31.98^{a}	0.33^{b}	0.86^{a}	10.42^{a}

Note: Means in the same column with the same superscript were not different ($p < 0.05$).

The effect of salted trash fish diets on proximate and amino acid composition of fish body was presented in Table 8. There was no effect of salted trash fish diets on protein and ash body composition of fish as compared to control diet ($p > 0.05$); however, moisture increased and fat decreased as the salted trash fish increase in the diet ($p < 0.05$). Amino acid profile of whole body fish was similar between fish fed control and salted fish diets. The inclusion effects of alternative animal and plant protein sources for FM in the diets on proximate body composition of fish has been reported in channel catfish (*Ictalurus punctatus*) (Peterson et al 2012), Nile tilapia (*Oreochromis niloticus*) (Labib et al 2012); olive flounder (*Paralichthys olivaceus*) (Deng et al 2006; Uyan et al 2006; Kikuchi 1999; Lee et al 2012; Kader & Koshio 2012; Kim et al 2014); Atlantic salmon (*Salmo salar*) (Pratoomyot et al 2010; Pratoomyot et al 2011), raibow trout (*Oncorhynchus mykiss*) (Lee et al 2010); black sea bream (*Sparus macrocephalus*) (Zhou et al 2011); cobia (*Rachycentron canadum*) (Watson et al 2014), large yellow croaker (*Larimichthys croceus*) (Yi et al 2015); California yellowtail (*Seriola lalandi*) (Buentello et al 2015). In the present study, inclusion of salted trash fish in the diets did not affect protein and ash body composition of fish; however, fish body fat tended to decrease and body moisture increase as the increasing of the salted trash fish levels in the diets. Moreover, reduced food consumption as the level of dietary FM excessively decreased had also been identified as a factor reducing growth and specific growth rate in Atlantic salmon (Pratoomyot et al 2010; Pratoomyot et al 2011).

Table 8
The effect of salted trash fish diets on proximate composition and amino acid profiles of harvested fish

Proximate composition	*Diets (%)*				
	FM	*STF-25*	*STF-50*	*STF-75*	*STF-100*
Moisture	72.42^{a}	73.29^{b}	74.44^{c}	74.76^{c}	74.80^{c}
Ash	2.91^{a}	3.04^{a}	3.10^{a}	3.25^{a}	3.29^{a}
Protein	13.39^{a}	13.84^{a}	13.43^{a}	13.26^{a}	13.22^{a}
Fat	8.21^{d}	7.59^{c}	6.22^{b}	5.69^{a}	5.51^{a}
Amino acid (% protein)	*FM*	*STF-25*	*STF-50*	*STF-75*	*STF-100*
Aspartic acid	9.44	9.42	8.19	8.08	7.38
Cystine	0.14	0.12	0.19	0.18	0.13
Glutamic acid	13.77	14.04	13.06	13.20	13.13
Serine	2.91	3.07	2.79	2.84	3.02
Glycine	5.31	5.69	5.63	5.58	5.64
Histidine	1.62	1.51	1.38	1.24	1.09
Arginine	4.27	4.25	3.96	3.98	3.89
Threonine	2.63	2.67	2.48	2.35	2.13
Alanine	5.54	5.60	5.12	5.15	5.26
Proline	3.37	3.36	3.12	3.25	3.90
Tyrosine	2.10	2.26	2.09	2.11	2.39
Valine	3.68	3.76	3.46	3.57	3.38
Methionine	2.34	2.42	2.18	2.20	2.05
Isoleucine	3.27	3.36	2.95	2.94	2.74
Leucine	4.50	4.86	4.14	4.24	3.63
Phenylalanine	5.38	5.37	5.00	5.04	4.78
Lysine	4.14	4.21	3.84	3.81	3.65
Total	74.42	75.98	69.59	69.76	68.21

Note: Means in the same row with the same superscript were not different ($p < 0.05$).

Conclusions. Trash fish diets were characterized by higher salt but similar in amino acid profiles and water stability as compared to control diets. Inclusion of salted trash fish 25% in the diet increased weight gain and specific growth rate, and inclusion up to 50% did not affect weight gain, specific growth rate, food conversion, protein retention and survival rate as compared to control diet; but inclusion more than 50% reduced food conversion and protein retention. Salted trash fish diets did not affect fish body protein and ash, but increased body moisture and decreased body fat. There was no marked difference in essential amino acid profiles between the fish fed salted trash fish diets and control diet. The salted trash fish therefore could be included in the river catfish diet up to 50% as replacement for 50% FM without negative effect on growth and body composition.

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