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Potential of Crab Meal as a Protein Substitute for Fish Meal in Diets for Mudfish *Heterobranchus longifilis* Juveniles

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ABSTRACT

A study aimed at evaluating the suitability of crab meal as a potential substitute for dietary fishmeal protein for mudfish (*Heterobranchus longifilis*) juvenile was carried out. Fishmeal (FM) and crab meal (CM) protein sources were used separately to prepare diets containing 30, 35 and 40% crude protein (CP). The diets were fed to fish at 5% body weight for 70 days in plastic aquaria. Crab meal at 30% CP level showed a performance of approximately 56.6% (percent weight gain), 50% (specific growth rate, protein efficiency ratio and apparent net protein utilization) of FM containing diet of 30% CP but was similar in fish condition, 0.62 ± 0.005 and 0.66 ± 0.005 for CM and FM diets. Fish survival was 100% for all experimental diets. The feed conversion ratio was appreciable, 4.50 ±1.38 for CM compared to 2.60 ± 0.65 and 1.94 ± 0.65 for 30% and 35% protein fishmeal diets. Inadequate essential amino acid balance and digestibility could be responsible for the reduced performance of crab meal compared to fishmeal. Crab meal has potentials and future trails on partial replacement of FM could reveal improved performance on *H. longifilis*.

INTRODUCTION

The mudfish *Heterobranchus longifilis* is of great importance for the Nigeria aquaculture industry and its production is gradually increasing. The high market demand and interest in the species has made some workers to study its biology, including the nutritional requirements. Protein is usually the most expensive component in the diet of fish during culture; hence dietary protein levels directly affect production cost (Lazo et al., 1998). One way to reduce feed costs and cost of production without necessarily compromising growth performance of fish is to partially or totally substitute less expensive animal or plant proteins for more expensive animal proteins. Fishmeal is an important ingredient in aquaculture diets because of its high quality protein and remains the most expensive of all diet ingredients (Turker et al., 2005). Hence, the use of less expensive protein sources as replacement for fishmeal is of important research interest.

Crabs were found to contain 31.25-75.0% crude protein in their tissues (Deekae and Idoniboye – Obu, 1995). Compared to fishmeal, crab meal may be deficient in one or more essential amino acids (Maynard et al., 1979). Crab meal had been tested in diets for the African Mudfish, *Clarias gariepinus* (Ayinla et al., 1993) and could be a valuable alternative animal protein source for species such as *Heterobranchus longifilis*. The objective of this study was therefore to evaluate the effects of totally replacing dietary fishmeal with crab meal on growth performance, feed utilization and body composition of *Heterobranchus longifilis* juveniles.

MATERIALS AND METHODS

Experimental diets

Six practical diets were formulated from commercially available ingredients and produced at the Department of Fisheries Laboratory of the Rivers State University of Science and Technology, Port Harcourt, Nigeria (Table 1). Fishmeal (*Tilapia*) and Crab meal (*Uca tangeri* and *Sesarma huzardii*) were used separately as protein sources to produce diets containing 30%, 35% and 40% protein respectively. The dry ingredients and oil source (red palm oil) were weighed, measured out and hand mixed in a clean basin. Water and garri paste (cassava product) were then added to the mixture to attain a consistency appropriate for passing the mixture through a meat grinder with a 2mm die. After production, each diet was dried, cooled and stored until use. The protein, lipid, ash, fibre and moisture contents of the diets were determined by the methods of AOAC (1990), Table 1.

Table 1: Formulations (% of total) and analyzed composition of six practical diets fed to *Heterobranchus longifilis* juveniles at different protein levels

	Diets					
Ingredient	FM-30	FM-35	FM-40	CM-30	CM-35	CM-40
Tilapia fishmeal, 64.25%CP	35.22	44.53	53.84			
Crab meal, 37.20%CP				71.00	60.00	11.00
Soybean meal, 45.24%CP					25.00	77.15
Wheatbran, 18.54% CP	15.00	15.00	15.00	15.00	5.60	5.25
Corn, 10.55% CP	43.18	33.87	24.56	7.40	2.80	
Vitamin and Mineral Premix[a]	0.50	0.50	0.50	0.50	0.50	0.50
Ascorbic acid[b]	0.10	0.10	0.10	0.10	0.10	0.10
Table Salt	0.20	0.20	0.20	0.20	0.20	0.20
Red palm oil 2.80	2.80	2.80	2.80	2.80	2.80	2.80
Binder (Starch)						
Total	100.0	100.0	100.0	100.0	100.0	100.0
Analyzed composition (dry matter basis):						
Moisture (%) 5.57	5.57	5.73	5.53	5.57	5.84	5.67
Crude protein (%)	30.38	35.25	40.50	29.50	35.50	41.00
Crude lipid (%)	11.49	9.42	12.94	8.80	10.84	9.28
Crude fibre (%)	6.25	5.86	5.21	6.45	5.35	4.25
Ash (%)	13.34	14.25	13.07	18.15	17.78	14.14
Nitrogen free extract (%)	32.97	29.49	22.75	31.35	24.69	25.66
Digestible energy (Calculated)[c], kcal/kg	3388.1	3327.1	3640.3	3075.0	3404.1	3559.0

[a]Optimix vitamin – mineral premix: Vit. A,D_3,K,B_1,B_2,B_6,B_{12}, Niacin Pantothenic acid, Folic acid, Biotin, Choline chloride, Antioxidant, Manganese, Zinc, Iron, Copper, Iodine, Selenuim and Cobalt. Produced by Animal care® for Animal Services Konsult (Nig.) Ltd., Agege, Lagos. [b]Supplied by L-ascorbyl-2-phosphate (25% activity). [c]Digestible energy was calculated as 3.0, 4.25, 3.8 and 8.0kcal/g of carbohydrate (non-legume), proteins (animal), proteins (plant) and fats (New, 1987). FM=Fishmeal, CM=Crab meal, CP=Crude protein.

Experimental fish and rearing conditions

Juveniles of mudfish (*Heterobranchus longifilis)* weight range of 61.6-83.05g and 101.58-121.94g were harvested from the Fisheries Department rearing tanks, acclimatized and fed for 7days with a commercial diet containing 30% protein before use in this study. The fish were then randomly distributed among 18 identical plastic aquaria, filled with clean and well aerated tap water to the 40-L mark. Fish were stocked at 1 fish per aquarium in triplicate per treatment.

Continuous aeration was provided by Tacas Air Pumps (AP 1500) fitted with air tubes and air stones. Uneaten feed and faecal matter in each aquarium were siphoned off, water reduced by two-thirds and re-filled to 40-L mark daily. Water temperature was measured using a portable laboratory mercury thermometer (0-100℃) and the pH by a pH meter (Model Jenway 3150). The dissolved oxygen (DO) was determined by Winkler's method, unionized ammonia (NH_3-N) by Nesslerization and nitrite ($N0_2$-N), steam distillation method (Boyd, 1979). Fish were fed twice daily at 1000 and 1600 hours respectively for 70days. The experiment was conducted between December, 2001 and November, 2002.

Fish were weighed individually with a beam balance and body length measured using a metal rule at the start and end of experiment. Before starting the experiment, 5 fish from the stock and 2 fish per treatment at the end of feeding trial were killed, homogenized, stored in clean polyethylene bag and frozen in a refrigerator for subsequent analysis for dry matter after desiccation in an oven (105℃ for 24hours). Ash determination was by incineration at 550℃ for 12 hours, crude protein (micro Kjeldahl, %N x 6.25) and crude fat (ether extraction by Soxlhet method).

Calculations

Fish performance was evaluated by calculating the following parameters: weight gain (g) = final weight (g) – initial weight (g), length increase = final length (mm) – initial length (mm) of fish, percent survival = N_i x 100/ N_O (Alatise and Otubusin, 2006), where N_i = number of fish alive at end of experiment and N_O = number of fish at the start, specific growth rate (%day^{-1}) = ln W_2 – ln W_1 x 100/ t_2 – t_1 (Brown, 1957), W_2 and W_1 are final and initial weights (g),t_2 and t_1 = end of growth period and start of growth in days and ln is natural logarithm, condition factor = 100 (Weight, g)/(total length, cm)^3, Bagenal and Tesch (1978), feed conversion ratio (FCR) = feed intake (g) / weight gain (g) (Utne, 1979), protein efficiency ratio (PER) = weight gain (g) / weight of protein fed (g) (Zeitoun et al., 1973) and apparent net protein utilization (ANPU) = P_b – P_a x 100/P_i (Miller and Bender, 1955) where P_b and P_a = body protein at end and start of feeding trial and P_i= protein consumed over feeding trial.

Data Analysis

The data collected were subjected to analysis of variance (ANOVA), using Statistical Analysis System (SAS, 2003) for significant differences among treatment means. Duncan's multiple range test (Duncan, 1955) was used to compare differences among individual means. Treatment effects were considered significant at P<0.05.

RESULTS

The performance of juvenile *Heterobranchus longifilis* fed test diets for 70 days is summarized in Table 2. The weight gain and length increase with diets FM-35 and FM-40 where higher and significantly different (P<0.05) from fish fed crab meal diets. The percent weight gain values for diets CM-35 and CM-30 were comparatively similar and different from other diets. Fish fed diet FM-35 had the highest % weight gain of 29.56 and specific growth rate (0.68 ± 0.02% day^{-1}) than those that fed other diets. The condition factors ranged 0.60 ± 0.009 – 0.66 ± 0.005 for all diets. The feed conversion ratio (1.94 ± 0.65) for fish fed FM – 35 diet was significantly different (P< 0.05) from fish fed other diets. Crab meal containing diets had feed conversion ratio values of 4.50 ± 1.38 – 7.29 ± 3.60. The protein efficiency ratio (PER) and apparent net protein utilization (ANPU) values for fishmeal diets were better and ranged 1.72±0.25-2.37±0.35 and 12.26-15.36 than fish fed crab meal diets, which ranged 0.81 ± 0.18-1.34 ± 0.26 (PER) and 4.52 – 6.24 (ANPU) respectively. The digestible energy in FM diets ranged from 3327.1-3640.3Kcal/kg while CM diets were from 3075.0-3559.0Kcal/kg.

The initial and final body nutrient compositions for juvenile *Heterobranchus longifilis* in this study were expressed as percent wet weight (Table 3). The protein level in fish fed FM-35 diet was highest (21.10% CP) and significantly different from values obtained with other diets (P<0.05). Best performance among crab meal containing diets was CM-30 with body protein of 19.09%. Moisture levels ranged from 70.04 – 71.25% for crabmeal compared to fishmeal diets (70.65-71.66%). Lipid levels also varied between 2.27 – 3.05% for fishmeal diets and 2.05-3.42% for crab meal fed fish. Ash content

Table 2: Effect of Dietary protein sources and levels on growth and feed utilization of *Heterobranchus longifilis* juveniles (± SEM[1])

Parameter	Fishmeal diets			Crabmeal Diets		
	30%	35%	40%	30%	35%	40%
Initial mean weight of fish (g)	61.60± 3.44^{e}	78.82± 4.30^{d}	83.05± 3.75^{c}	103.43± 3.32^{b}	121.94± 3.44^{a}	101.58± 2.85^{b}
Weight gain (g)	15.66± 3.44^{b}	23.30± 4.30^{a}	20.30± 3.75^{a}	14.87± 3.32^{b}	15.72± 3.44^{b}	11.86± 2.85^{c}
Percent weight gain (%)	25.42	29.56	24.44	14.38	12.89	11.68
Initial mean length (mm)	204.43± 3.07^{d}	223.27± 3.09^{c}	229.53± 3.65^{c}	249.23± 2.42^{b}	264.93± 1.92^{a}	252.23± 2.72^{b}
Specific growth rate (day^{-1})	0.42± 0.04^{a}	0.68± 0.02^{a}	0.46± 0.06^{a}	0.21± 0.02^{b}	0.17± 0.02^{b}	0.20± 0.02^{b}
Condition Factor (K)	0.66± 0.005^{a}	0.65± 0.006^{a}	0.60± 0.009^{a}	0.62± 0.003^{a}	0.62± 0.005^{a}	0.61± 0.003^{a}
Percent Survival (%)	100.0	100.0	100.0	100.0	100.0	100.0
Feed conversion ratio	2.60± 0. 98^{c}	1.94± 0.65^{d}	2.03± 0.05^{cd}	4.50± 1.38^{b}	7.29± 3.60^{a}	5.94± 1.89^{b}
Protein efficiency ratio	2.35± 0.42^{a}	2.37± 0.35^{a}	1.72± 0.25^{b}	1.34± 0.26^{b}	1.03± 0.20^{b}	0.81± 0.18^{c}
Apparent net protein Utilization	12.26	15.36	13.75	6.24	5.39	4.52

[1]SEM: Standard error of the mean. Means with same letter for a given parameter in same horizontal row are not significantly different (P>0.05).

Table 3: Proximate body composition (% wet weight) of *Heterobranchus longifilis* juveniles before and after feeding graded protein levels from different sources

Diet		Nutrient parameter (%)			
		Moisture	Crude Protein	Ether extract	Ash
Fish at start of experiment		72.52^{a}	17.37^{e}	2.96^{ab}	4.48^{b}
Fish fed diets:	FM-30	71.38^{b} 1	9.24^{c}	3.05^{a}	4.15^{b}
	FM-35	70.65^{c}	21.10^{a}	2.27^{b}	4.08^{b}
	FM-40	71.66^{b}	20.44^{b}	2.44^{b}	4.15^{b}
	CM-30	71.25^{b}	19.09^{c}	2.05^{b}	6.32^{a}
	CM-35	71.07^{b}	18.03^{d}	2.98^{ab}	6.46^{a}
	CM-40	70.04^{c}	18.60^{d}	3.42^{a}	6.26^{a}

Values within the same vertical row with same superscripts are not significantly different (P>0.05), FM = Fishmeal, CM = Crab meal at 30, 35, and 40% protein levels.

Table 4: Mean and Range values of water quality parameters for *Heterobranchus longifilis* juveniles fed diets containing different protein sources and levels

Parameter	Diets					
	FM-30	FM-35	FM-40	CM-30	CM-35	CM-40
pH	7.0 (6.9-7.1)	6.9 (6.7-7.4)	6.8 (6.6-7.0)	7.1 (6.6-7.4)	6.9 (6.7-7.4)	7.1 (7.0-7.2)
Temperature (°C)	28.0 (27.0-29.0)	27.5 (26.5-28.0)	27.0 (26.0-28.0)	28.5 (27.5-29.0)	28.0 (27.5-29.0)	27.5 (26.0-28.0)
DO (mg/l)	4.0 (3.5-4.30)	4.9 (4.5-5.1)	4.7 (4.5-5.0)	4.8 (4.5-5.0)	4.10 (3.3-4.5)	4.60 (4.1-5.2)
NH_3-N (mg/l)	0.07 (0.05-0.08)	0.07 (0.06-0.08)	0.05 (0.04-0.07)	0.10 (0.08-0.15)	0.02 (0.01-0.03)	0.15 (0.01-0.25)
NO_2-N (mg/l)	0.007 (0.006-0.009)	0.006 (0.005-0.007)	0.007 (0.005-0.008)	0.005 (0.004-0.007)	0.009 (0.008-0.02)	0.007 (0.005-0.009)

Figures in brackets are range values of parameters, FM = Fishmeal, CM = Crab meal; 30, 35, 40% =dietary protein levels.

The values of water quality parameters of temperature, dissolved oxygen, ammonia and nitrite appeared similar and varied little for the tested diets (Table 4). The pH values indicated slightly acidic water medium (6.8-7.1). Fish used in this study displayed good adaptability in the aquaria, with 100% survival in all diets.

DISCUSSION

Growth response as observed with *Heterobranchus longifilis* juveniles in this study showed that all the diets could support growth and good survival of fish. The best growth of fish was obtained with a diet of 35% protein containing fishmeal. Fishmeal containing diet even at 30%CP had superior growth performance than the best performing CM diet. The trend in fish growth across diets with crab meal as total replacement for fishmeal showed decrease and lower biological performance. This could be due to poor feed consumption. Chude (2001) explained that growth was also influenced primarily by food supply and depended on food consumed. Diets that contained crab meal had higher ash levels than fishmeal diets. Such ash levels had their limiting effects on diet consumption and could cause possible pollution of the culture environment.

The variation in the values of FCR obtained in this study was probably reflected in the composition of diets from a protein source (Hepher, 1988), hence the higher FCR values in crab meal diets than those of fishmeal. The juvenile of *Heterobranchus longifilis* had a general decrease of PER with increasing dietary protein for both fishmeal and crab meal protein sources. Turker et al. (2005) suggested that the proportion of dietary protein used for catabolic processes increased with the level of fishmeal replacement. The reduction in PER the authors suggested was partly due to reduced growth as protein required for maintenance consumed a greater share of the protein intake. The decline of ANPU in the experimental fish for both fishmeal and crab meal protein sources agreed with observations on *Oreochromis niloticus* (Faturoti and Akinbote, 1986) and sub – adult of Black Sea Bass, *Centropristis striata* (Copeland et al., 2002). However, the PER and ANPU values were higher for fish fed fishmeal diets than those fed crab meal diets. This indicated that fish fed fishmeal diets utilized the protein more efficiently than their crab meal fed counterparts.

Ufodike et al. (2011) reported that the extent of protein utilization by fish depended on availability, ease of processing and the nutritive value. Fish fed crab meal containing diets might have found it increasingly necessary to metabolize part of the protein consumed when fishmeal was completely replaced by crab meal in the diets. Hence, utilization of protein for incorporation into the body structure was consequently reduced. Other possible reasons for a decline in biological performance such as growth could be inadequate levels of some essential amino acids (Lysine and / or Methionine, Carlos et al., 1988) and higher amount of ash coupled with lower digestibility in crab meal (De Silva and Anderson, 1995). In contrast, fishmeal diets appeared superior in essential amino acid balance, digestibility and acceptability (Lovell 1988; Ufodike et al., 2011).

Carcass composition may be influenced by the quality of feed, feeding frequency, ration size and water quality, hence the flesh quality of the final fish product. Fish fed a diet of 35% protein containing fishmeal had higher tissue protein than those of crab meal while those diets of 30% protein containing crab meal and fishmeal were similar. The elevated body protein level in fish fed fishmeal diets could be attributed to the superior quality and quantity of protein consumed, the protein/calories ratio (Faturoti et al., 1996) and nutrient utilization within each treatment (Omoniyi and Fagade, 2003). The observed range of protein levels, 18.03-21.10 corroborated the results of Effiong and Tafa (2006) for *Heterobranchus longifilis.* Except for the higher ash content in crab meal fed fish, there were no noticeable variations in lipid and moisture levels between fishmeal and crab meal fed fish in the study. The observed increase in growth and high survival rate of fish for all diets could be attributed to the positive response to the diets, culture environment and handling techniques. The values of the observed water quality parameters were within acceptable tolerant ranges recommended for fresh water fish culture including catfish (Boyd, 1982).

CONCLUSION

Crab meal with 37.20% CP and dietary digestible energy of 3075.0Kcal/kg at 100% substitution of fishmeal could not give similar or greater biological performance than the least fish meal diet of 30% protein in this study. However it showed potentials in weight gain, SGR, condition, PER, ANPU and fish survival at 30% dietary protein level. Partial substitution of fishmeal could be investigated to reveal a better use of crab meal in aqua feeds for *Heterobranchus longifilis.*

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