EVALUATION OF FERMENTED FISH OFFAL IN FORMULATED DIET OF THE INDIAN MAJOR CARP, ROHU, LABEO ROHITA (HAMILTON)

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Background. Carp fish offal contains high amount of protein and lipid. The quest for protein sources alternative to fish meal has been a priority in aquaculture research because of growing concerns on the future availability of fish meal for incorporation in fish diets. This study determined the extent to which carp fish offal could be fermented and used to replace fish meal in the formulation of diet for fingerlings of *Labeo rohita*.

Materials and Methods. Fish offal, comprising viscera of carp, was fermented along with mustard oil cake and rice bran anaerobically by a microbial suspension (10^8 cells $\cdot$ mL^{-1}) under ambient temperature (27–30ºC). The fermented mixture was mixed with fish meal, mineral- and vitamin mixture to prepare three experimental diets: 1) a reference diet (T1), without any fermentation mixture, containing 40% fish meal, 2) a diet containing 25% fish offal (fermented) and 25% fish meal (T2), and 3) a diet containing 30% fish offal (fermented) and 20% fishmeal (T3). These diets were tested to evaluate growth and biochemical composition of the body of the fingerlings of *Labeo rohita* and apparent protein digestibility (APD) and intake rate of the diets by the fish. Proximate analysis of feed ingredients, experimental diets, faecal samples and the carcass were performed following the AOAC procedures.

Results. Fingerlings of *Labeo rohita*, reared for 60 days with the diets containing fermented fish offal mixture (T2 and T3), showed higher growth and higher values of protein and lipid in the carcass than those reared with the reference diet (T1). Apparent protein digestibility of the diets did not vary significantly, but average feed intake rate of the T2 and T3 diets were higher than the T1 diet.

Conclusions. Fermented fish offal is a viable alternative of fish meal in the formulation of diet for fingerlings of *Labeo rohita*. Fifty percent replacement of fish meal is possible by a mixture containing 30% fish offal. Further studies are required to improve quality of the fermentation mixture and increase the rate of replacement of fish meal.

Keywords: fish offal, recycling, fishmeal, protein, growth, rohu, *Labeo rohita*

INTRODUCTION

There has been a considerable research effort towards utilization of less expensive renewable ingredients in fish diet formulations to alleviate problems related to shortage of quality fish meal (Pongmaneerat et al. 1993, El Sayed 1994, Rangacharyulu et al. 2003, Yang et al. 2004, Mukhopadhyay and Mitra 2007). Fish offal, which is generated in large quantities on a daily basis in the urban and sub-urban markets in India, has immense potential to be used as a predominant nutrient source in fish diet formulations. Very few studies have evaluated its efficacy, so far (Giri et al. 2000). In a preliminary investigation we observed the viscera of carps, which are discarded in bulk

as offal in the retail fish markets contain a substantial amount of crude protein (31.5%–38.9%) and lipid (40.6%–43.8%) on a dry matter basis (Mondal et al. 2006). Collection of these wastes from the urban and suburban markets, treating them suitably to preserve their nutrients and finally recycling them into fish diet production can have a significant benefit in terms of abatement of pollution of environment as well as reduction in the recurring cost of fish production.

Although dried fish, chicken viscera, and related animal by-products have been evaluated previously as protein resource in fish diet formulations (Saha and Ray 1998, Giri et al. 2000), efficacy of fish offal as such has not been

ascertained as nutrient sources probably because of inherent difficulties in handling it either in dried or in raw condition. While drying of the offal results in considerable loss of the nutrients, its direct use leads to rapid spoilage of the whole diet. In the present study an attempt was made to evaluate if fish offal (FO) could be fermented and used to replace fish meal (FM) in the preparation of formulated fish diet. The process of fermentation or composting is widely employed in fish diet formulation to decrease the anti-nutritional factors and crude fibre content of plant diet stuff (Ray and Das 1992, Bairagi et al. 2002). The process of fermentation improves nutritional quality of animal product also. Rangacharyulu et al. (2003) observed that fermented silk worm pupae silage (SWP) was nutritionally superior to untreated SWP or fishmeal. Bertsch and Coello (2005) used a strain of keratinolytic bacteria to ferment poultry feather and obtained poultry feather meal with 71% crude protein and improved bioavailability of some amino acids. The objective of this study was to determine the extent to which fish offal could replace fish meal in carp (*Labeo rohita*) diet while maintaining nutritional quality almost identical to the diet based on fish meal and thus be an ideal cost effective renewable alternative.

MATERIALS AND METHODS

Experimental diet formulation and preparation. Three diets were prepared with mustard oil cake (MOC), rice bran (RB), fish meal, and fish offal. The fish offal, comprising viscera of cultured carps (*Labeo rohita* and *Catla catla*), was obtained from the local retail fish markets. The portion of guts contained in the offal was carefully uncoiled, cut open and the gut contents were removed using clean water. Fresh samples of fish offal were used for diet preparation. Ingredient constituents and proximate composition of the diets have been given in Table 1. The feeds were formulated in such a way that these contained not less than 30% crude protein. The reference diet (T1) was prepared using 40% fish meal, by weight, while two other diets (T2 and T3) were prepared by partially replacing fishmeal with fermented fish offal (Table 1). For fermentation, the fish offal, mustard oil cake, and rice bran were mixed and the mixture was added to a solution of microbial suspension (10^8 cell $\cdot$ mL^{-1}) (the microbial suspension (EM^{TM}) was a gift from M/S, Maple Orgtech Pvt. Ltd. Mumbai.), molasses and water (2.5 mL : 2.5 g : 100 mL) and was fermented anaerobically under ambient temperature (27–30°C) for 12 to 22 days, depending on the pro-

Table 1

Formulation and proximate analyses of the experimental diets

	Contents	Diet		
		T 1 (Reference)	T 2	T 3
Ingredients [%]	Mustard oil cake	29	24	24
	Rice bran	29	24	24
	Fish offal	—	25	30
	Fishmeal	40	25	20
	Vitamin premix[1]	01	01	01
	Mineral premix[2]	01	01	01
Proximate composition [% dry matter basis][3]	Protein	32.5	31.4	30.2
	Lipid	4.5	9.2	11
	Moisture	7	6	8
	Ash	15	13.5	12
	Dry matter	93	94	92
	Gross Energy [kJ·g^{-1}][4]	15.34	16.69	16.93
	P : E ratio[5]	21.19	18.82	17.85

[1] Vitamin mixture [%]: (Ambiplex; Brihans Lab, Pune): vit B1: 7.14, vit B2: 2.55, vit B6: 1.02, vit B12: 0.012, biotin: 0.025, calcium pantothenate: 2.55, niacin: 76.50, cholin chloride (B4): 10.20; vitamin C in the form of ascorbyl polyphosphate was added to vitamin mixture at 100 mg· kg^{-1} mixture.

[2] Mineral mixture [%]: (Agrimin; Glaxo India Ltd, Mumbai) copper: 3.12, cobalt: 0.45, magnesium: 21.14, iron: 9.79, iodine: 1.56, zinc: 21.30, calcium: 30.00, phosphorus: 8.25.

[3] Number of samples per each determination = 3.

[4] Gross energy was calculated on the basis of methodology of Brafield (1985).

[5] Protein to total energy ratio [mg · kJ^{-1}].

portion of fish offal. Based on a preliminary evaluation, 30% fish offal was determined as the maximum level for fermentation by the microbial suspension. Unfermented fish offal putrefied and became unsuitable for use in diet formulation. Samples of fermentation mixture were taken from each level of trial fermentation and were analyzed for aroma, physical condition, total nitrogen, organic carbon, and bacterial population count every 6 days till the end of fermentation. Final fermented product (T2: 19.2% of crude protein, 7.8% of crude lipid, and 10.2% of ash; T3: 20.7% of crude protein, 9.9% of crude lipid, and 9.4% of ash) was mixed with fish meal, vitamin, and mineral mixture in proportion so that one mixture contained 25% fish meal (T2) and the other contained 20% fish meal (T3). The mixtures were ground and pelleted using a hand pelletizer fitted with a 2-mm dia to prepare the final experimental diets (Table 1). To test the protein digestibility of the diet, 1-% chromic oxide (Cr_2O_3) was included in each diet separately as non-absorbed reference substance, blended, and pelleted with 0.5% carboxymethyl cellulose as a binder. This was sun dried before using in the feeding trial.

Feeding trials. Two experimental systems were utilized for the feeding trials: one in outdoor cement tanks (400 L) to evaluate growth and biochemical composition of the body, and the other in indoor glass aquaria (50 L) to evaluate voluntary diet intake and apparent protein digestibility (APD). Deep tube-well water stored in an overhead tank was used in the feeding trial. Fingerlings of rohu (mean initial length 4.65 ± 0.19 cm and mean initial weight 0.98 ± 0.17 g) were obtained from a local fish farm and were acclimatized to the laboratory conditions for a week prior to start of the experiment. The fingerlings were fed to satiation (twice a day six days a week) with the reference diet (T1) during acclimatization. The acclimatized fingerlings were randomly distributed at the rate of 40 pieces per tank and 10 per aquarium. The water temperature in aquarium ranged from 21–24°C and aeration was provided to maintain a dissolved oxygen level of approximately 6–7 mg · L^{-1}. The tanks or aquaria were laid out in a completely randomized block design (Gomez and Gomez 1984) with three replicates for each of the three diet treatments.

For the feeding trial in outdoor tanks all fish were fed twice daily at 1000 h and 1600 h at a fixed feeding rate of 5% body weight per day for the entire experimental period of 60 days. The quantity of the diet given was readjusted every 15 days after weighing the fish. Samples of water were collected every week to determine selected parameters like dissolved oxygen, free carbon dioxide, total ammonia, alkalinity, hardness, and pH—following the APHA procedures (Anonymous 1995). All fish from each outdoor tank were sampled at the end of 60 days trial and their length and weight were recorded. Five fish sampled from each tank were randomly selected and were frozen at –20°C. Biochemical analyses, to determine crude protein, crude lipid, and ash content of the carcass, were done on pooled fish samples from each tank, giving a total of three samples for each diet. Increase in length and weight, spe-

cific growth rate (SGR), food conversion ratio (FCR), protein efficiency ratio (PER), and apparent net protein utilization (ANPU) were calculated using standard methods (Castell and Tiews 1980). For the trial in glass aquaria same ration of diet was given to the fish and the fish were allowed to eat for 6 h. Left-over diet, if any, was collected after 6 h of feeding, oven dried, weighed, and stored frozen at –20°C. Faecal samples were collected from each aquarium in the morning by careful siphoning, dried in an oven at 60°C, and weighed before preserving at –20°C. The feeding trial lasted for 10 days. Pooled samples of diet and faeces from each aquarium were digested, using a mixture of sulphuric acid and perchloric acid, as described by Bolin et al. (1952), and chromium contents in the samples were determined by flame atomic absorption spectrophotometer (Varian Spectra AA240), according to Kaviraj and Ghosal (1998). Apparent protein digestibility (APD) values of the diets were calculated from the proportion of Cr and protein in the diet and faeces (Fontaínhas-Fernandes et al. 1999).

Analytical procedures. Total nitrogen and organic carbon of the fermentation mixture were determined by AOAC procedures (Helrich 1990). The bacterial colony-forming unit (CFU) was isolated by serial dilution, plating, and incubation, as outlined in Atlas et al. (1995). Proximate analyses of the feed ingredients, experimental diets, carcass and faecal samples were performed following the AOAC procedures (Helrich 1990) as follows: moisture was determined by oven drying at 105°C for 24 h; crude protein (nitrogen × 6.25) was determined by Kjeldahl method, after acid hydrolysis; lipid was extracted by petroleum ether (boiling point 40–60°C) for 7–8 h in a Soxhlet apparatus followed by determination of lipid gravimetrically and ash was determined by combustion at 550°C, in a muffle furnace, till a constant weight. Water quality parameters were determined during the outdoor feeding trial by the procedures of APHA (Anonymous 1995).

Statistical analyses. The nature of distribution of the observations of each response variable from both the trials was verified by Kolmogorov–Smirnov (K–S) and Shapiro–Wilks (S–W) tests to ensure a Gaussian distribution. Since all data were found normally distributed they were subjected to Single factor ANOVA, without any further transformation, followed by least significant difference (LSD) test to compare mean between the treatments (Gomez and Gomez 1984, Johnson and Wichern 2005).

RESULTS

Completion of fermentation was indicated by the generation of a characteristic pleasant aroma, which appeared on the 12th day for the 25% FO (fish offal) mixture and on 18th day for the 30% FO mixture (T3, Table 1). Organic carbon content of the T2 mixture gradually reduced from 12.02 mg · g^{-1} at day 1 to 8.4 mg · g^{-1} on day 12 and remained unaltered thereafter, while in T3 mixture it reduced from 13.5 mg · g^{-1} to 11.6 mg · g^{-1} on day 12 and continued to reduce further and became unaltered at 8.5 mg · g^{-1} only after 22 days. A similar trend

was noted for total nitrogen. pH of the fermented mixture gradually decreased from an initial value of 8.0–8.2 to 4.4–4.6. Two distinct bacterial colonies were detected in the bacterial culture of the fermentation mixture. One grew only in the mixture containing the microbial suspension and the other appeared in both the mixtures, irrespec-

of water recorded during the outdoor trial showed values in optimum range required for rearing carp fingerlings (Table 4). There was no significant difference in the apparent protein digestibility among different dietary treatments. But voluntary intake rate of the T2 and T3 diets were significantly higher than the T1 diet (Table 2).

Table 2

Digestibility, diet intake, growth performance, and diet efficiency of *Labeo rohita* fingerlings, fed experimental diets; data are mean ± standard deviation ($n = 15$); means with dissimilar superscripts in the same row indicate significant difference (LSD) between the means at 5% level

Parameter	Diet		
	T 1 (Reference)	T 2	T3
APD[1] [%]	90.1 ± 0.14	86.2 ± 3.96	88.8 ± 1.98
Diet intake [g per 100 g BW per d]	3.75[a] ± 0.08	4.34[b] ± 0.06	4.73[b] ± 0.08
Length increase	22.56[a] ± 1.18	34.00[b] ± 7.02	38.30[b] ± 0.94
Weight increase	76.40[a] ± 6.35	167.27[b] ± 30.78	179.57[b] ± 13.38
FCR[2]	2.90[a] ± 0.97	1.24[b] ± 0.24	1.13[b] ± 0.08
SGR[3] [% per d]	0.86[a] ± 0.25	1.51[b] ± 0.43	1.75[b] ± 0.15
PER[4]	1.00[a] ± 0.07	1.74[b] ± 0.21	1.83[b] ± 0.08
ANPU[5]	14.31[a] ± 1.56	32.20[b] ± 4.81	33.92[b] ± 2.37

[1] APD = 100 – 100 × (% Cr in diet / % Cr in faeces) × (% protein in faeces / % protein in diet).

[2] FCR = dry weight of diet given / increase in weight of the fish.

[3] SGR = ((ln final weight – ln initial weight)/days on trial) × 100.

[4] PER = wet weight gain of fish / protein consumed.

[5] ANPU = (net increase in carcass protein / amount of protein consumed) × 100.

tive of its association with the microbial suspension, indicating that the former was contributed by the microbial suspension, while the later was the native flora of the offal. The former colony started growing from 6 days of fermentation and reached a peak on the 12th day (40×10^3 CFU · g^{-1} FO) in the T2 mixture and on the 22nd day (96×10^3 CFU · g^{-1} FO) in the T3 mixture.

The survival rate of the fingerlings during growth trial in outdoor tanks ranged from 90% to 95% and showed no significant variation between the dietary treatments. Survival rate of the fingerlings during digestibility trial in indoor aquaria was 100%, irrespective of treatments. Fingerlings reared in outdoor tanks with T2 and T3 diets showed significantly higher growth (increase in length, weight, and specific growth rate) than the reference diet (T1) (Table 2). Food conversion ratio (FCR), Protein Efficiency Ratio (PER), and Average Net Protein Utilization (ANPU) also showed similar trends. There was no significant difference of any parameters evaluated between T2 and T3 diets. Proximate composition of the carcass at the end of the trial also showed significantly higher value of crude protein and lipid in T2 and T3 as compared to T1 diet (Table 3). Temperature, pH, dissolved oxygen, free carbon dioxide, total alkalinity, and hardness

DISCUSSION

The present results indicate that the high amount of crude protein and lipid present in the FO can be utilized in carp diet formulation if it is fermented with suitable microorganisms. FO could be used up to 30% and with the microbial suspension used in the present study it required 22 days for complete fermentation. Further research is necessary to increase the efficiency of fermentation with specific strains of bacteria.

It has been reported that optimum protein requirement of Indian major carps ranges between 30% and 45% (Renukardhya and Varghese 1986, Mohanty et al. 1990). However, concentrating on level of crude protein alone may be misleading in formulation of practical diet for fingerlings of *Labeo rohita* since fishes generally regulate their feed intake in relation to the energy content of the diet. The dietary energy level, particularly lipid to carbohydrate ratio, not only affects the growth and body composition of fish but also affects its digestive enzyme activity and plasma concentration (Ali and Jauncey 2005). Garling and Wilson (1977) observed that fish fed higher level of dietary lipid exhibited higher liver protease activity. Ray and Das (1992) observed that fingerlings of *Labeo rohita* fed a diet containing composted weed (20%)

Table 3

Proximate composition of carcass [% dry weight] determined from pooled sampled of fish ($n = 5$) from each tank; data are mean of three replicates ± standard deviation; different superscript letters in a row indicate significant difference between treatments (LSD, $P < 0.05$)

Component	Initial	Final		
		T1	T2	T3
Crude protein	39.2 ± 0.53	42.55[a] ± 0.21	46.6[b] ± 0.2	48.6[c] ± 0.2
Crude lipid	14.6 ± 0.28	16.3[a] ± 0.14	19.7[b] ± 0.14	18.7[c] ± 0.14
Ash	14.3 ± 0.14	18.5[a] ± 0.14	18.7[a] ± 0.14	19.3[b] ± 0.14

and fish meal (80%) with 30.72% crude protein showed higher growth than those reared with a diet containing only fish meal (100%) with 35.7% protein. In the present study the crude protein level in the diet ranged from 30.2% in T3 diet to 32.5% in the T1 diet. Interestingly, best growth was obtained in fish fed the diet containing the lowest level of crude protein (30.2%, T3). This diet contained the highest level of lipid.

In any fish feeding trial, acceptability of the diet (Hasan et al. 1997) and digestibility of the nutrients (Cho and Kaushik 1990) are crucial factors besides source of protein (Kaushik et al. 1995), fat (Guillou et al. 1995) and dietary fat content (Bjerkeng et al. 1997) that can have profound influence on the growth and flesh quality of fish. In the present study, voluntary intake rate of the diet containing FO (25% and 30%) was significantly higher than the reference diet (T1) containing 40% fish meal. The rate of diet intake by fingerlings of *Labeo rohita* observed in the present investigation (3.75 to 4.73 g per 100 g BW per day) was higher than the rate observed by Bairagi et al. (2002) for a reference diet containing 40% fish meal and diets containing different levels of duckweed leaf meal. In addition to ambient water quality condition, quality of the ingredients play crucial role in voluntary intake of a formulated diet. Gomes et al. (1995) observed depression in voluntary diet intake of rainbow trout when fishmeal was totally replaced by vegetable protein. But no such depression was found in tilapia fed 100% vegetable protein. The three diets used in the present investigation did not show any significant difference in the protein digestibility, which varied from 86.2 to 90%. Although protein

digestibility generally increases with the levels of dietary protein (Ali and Jauncey 2004) it varies widely depending upon the sources of protein used in the feed (Fontaínhas-Fernandes et al. 1999). With high quality of protein, teleosts show 95%, or higher, proportion of digestibility (Cho and Kaushik 1990, Anonymous 1993, Regost et al. 2001). Using practical diets, Bairagi et al. (2002) observed 89.46% protein digestibility of the reference diet (40% FM) for the fingerlings of *Labeo rohita*. Maximum value of protein digestibility (94.43%) was obtained by Bairagi et al. (2002) when fish meal was replaced (10%) by fermented duckweed leaf meal. Full-fat toasted soybean meal showed lower digestibility co-efficient than defatted soybean meal (Fontaínhas-Fernandes et al. 1999) indicating that ingredients containing higher level of fat might result in decreased digestibility of protein. In the presently reported study, the diets containing the FO had a higher level of crude lipid and the average protein digestibility of these diets, although not significantly different, was lower than the reference diet containing only fish meal.

Growth rate of fish increases with increase in the level of dietary protein till the optimum level is reached. Interestingly in the present investigation, the T3 diet having lowest level of dietary protein (30.2%) and protein energy ratio (17.8 mg protein per kJ) of the three diets produced best growth of the fish. Although average intake rate of this diet was significantly higher than the reference diet there is a question if increased intake rate could compensate for the low level of dietary protein. Tilapia fed diets with low protein and energy levels were found unable to compensate by increased rate of intake to

Table 4

Selected water quality parameters recorded during the feeding trial in outdoor tanks

Parameter	Treatment (diet)		
	T1	T2	T3
Temperature [ºC]	22.6 ± 0.58	22.53 ± 2.83	21.33 ± 0.057
pH	7.47 ± 0.06	7.58 ± 0.03	7.60 ± 0.01
Dissolved oxygen [mg·L⁻¹]	5.71 ± 0.14	5.51 ± 0.47	6.11 ± 2.14
Free CO_2 [mg·L⁻¹]	4.09 ± 0.13	3.52 ± 0.26	3.37 ± 0.31
Alkalinity [mg·L⁻¹]	197.9 ± 11.34	190.4 ± 11.32	200.41 ± 8.86
Hardness [mg·L⁻¹]	190 ± 3.7	201.87 ± 8.26	197.91 ± 10.63
Total ammonia nitrogen [mg·L⁻¹]	0.036 ± 0.01	0.041 ± 0.01	0.045 ± 0.01

achieve comparable growth to fish fed high protein and energy levels (Ali and Jauncey 2004). On the other hand, there is a possibility that increased level of lipid in the diet containing FO might influence sparing of protein. Lipid as a non-protein energy source allows protein sparing by effectively reducing organic matter and nitrogen losses. Protein sparing effects of dietary lipids has been demonstrated for salmonids, sea bass (Cho and Kaushik 1990, Dias et al. 1998), common carp (Manjappa et al. 2002, Gilbey et al. 2001), and grass carp (Du et al. 2005). High level of dietary lipid may lead to deposition of fat in the body of fish and depression of activities of lipogenic enzymes (Arnesen et al. 1993, Alvarez et al. 1998). Little is know about lipogenesis in rohu.

The outcome of the study is that fish offal (FO) can be effectively used as a feed ingredient to replace fish meal partially in the diet for fingerlings of *Labeo rohita*. Fifty percent replacement of fish meal is possible when 30% FO is fermented along with MOC and RB (24% each) and is used as ingredient in the formulated diet.

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