

# Evaluation of Different Animal Protein Sources in Formulating the Diets for Blue Gourami, *Trichogaster Trichopterus* Fingerlings

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## Abstract

Based on the nutrient requirement of blue gourami, *Trichogaster trichopterus* fingerlings as reported earlier, nine experimental diets with 350 g protein, 80-100 g lipid and 16-17 MJ digestible energy/kg diet were formulated using snail meal (D-1), freshwater fish processing waste (D-2), surimi by-product (D-3), chicken offal (D-4), earthworm (D-5), squid (D-6), mussel (T-7), chicken liver (T-8) and lean prawn (T-9) as major protein source in addition to fish meal and peanut oil cake and fed *ad libitum* to the fish ( $3.54 \pm 0.02$  g) for a period of 45 days. Twenty seven indoor circular fiber-reinforced plastic tanks with 200 L of water were used for rearing the fish. At the end of the experiment it was found that the fish fed squid meal Diet (D-6) had the best results in terms of weight gain, food conversion ratio (FCR), specific growth rate (SGR) and protein efficiency ratio (PER). However, the freshwater fish processing waste (D-2) and surimi by-product (D-3) diets had almost similar ( $p > 0.05$ ) growth and dietary performance as that of squid, mussel, chicken liver and lean prawn meal diets and therefore, both these fish processing waste and surimi by-product could be used as non-conventional protein sources in formulating the nutritionally balanced cost-effective diets for blue gourami.

**Keywords:** Fish meal; Diets; Animal protein; Blue gourami; Growth; Feed utilization

## Introduction

Nutritive value of fish diet depends on quality of the protein ingredients used in diet formulation [1,2]. Protein is the most expensive component in fish feeds and the fish meal is the major source of protein in fish diet. At present, fish meal is not only expensive but also a scarce commodity due to its large demand in animal and fish feed industries [3]. The global production of fish meal has remained relatively stable over the last decade, and supplies are unlikely to improve [4]. The price of the fish meal will be higher in future because of higher freight cost and the decision made by the Peruvian government to slow down the catch of Peruvian anchovy to replenish its population [5]. Therefore, there is a growing concern to identify other alternate animal protein sources which can minimize/lessen the use of fish meal in fish diet. Several plant and animal protein sources have been used in formulating the practical diets for warm water fish with varying degree of success. However, the main draw back to use the plant protein in fish diets is the presence of variety of endogenous anti-nutritional factors [1]. Generally, the feed stuffs of animal origins are considered better alternative protein sources to fish meal in formulating fish diets because of their higher protein content and the superior indispensable amino acids than that of plant origins [6]. Future fish diets will include a wide range of alternative ingredients, including combinations of ingredients from animal origins [1]. Several animal protein sources were evaluated to formulate the diets for different fish species such as poultry by-product meal [2,7,8], meat and bone meal [2,7,9], blood meal [10-12], feather meal [2,13,14], a mixture of meat meal and blood meal [15], garden snail meal [16], poultry viscera meal [17], turkey meal [18,19], tad pole meal [20], fermented silage made from fish meal [21], shrimp meal, blood meal, maggot meal and tilapia meal [3], fermented fish offal [22,23], fishery by-catch and processing waste [24], fish waste meal [25], tuna by-products [26], a mixture of feather meal, chicken offal and maggot meal [27], a blend of animal protein comprised of meat and bone meal, poultry by-product meal and hydrolysed feather meal [5], chicken concentrate, poultry by-product blend and chicken egg concentrate [28] and surimi by-product meal [29].

Ornamental fish farming is one of the fastest growing fishery

sectors throughout the world with an annual trade of \$ 15 million dollar and growth rate of over 10%. Freshwater ornamental fish contribute 85% of the total global ornamental fish trade [30]. One of the major problems for the growth of ornamental fish farming is the non-availability of species specific nutritionally balanced diets. So far many of the ornamental fish traders have been using shrimp feeds or other fish feeds meant for rearing the food fishes. Therefore, development of species specific ornamental fish diet as per the nutrient requirement of fish is one of the priority areas in fish nutrition research [31,32]. Among freshwater ornamental fish, blue gourami, *Trichogaster trichopterus* is an important sought after ornamental fish. Using casein-gelatin-dextrin based semi-purified diets, it is reported that the blue gourami fingerlings require 350 g protein and 80 g lipid  $\text{kg}^{-1}$  diet with a digestible energy level of 16.7 MJ  $\text{kg}^{-1}$  for its optimum growth and nutrient utilization [33]. In the present study, based on the nutrient requirement of blue gourami, nine experimental practical diets were formulated using different animal protein sources such as snail meal, freshwater fish processing waste meal, surimi by-product meal, chicken offal meal, earthworm meal, squid meal, mussel meal, chicken liver meal and lean prawn meal in addition to fish meal used at 10% in all diets. Some of the animal protein sources (freshwater fish processing waste meal, surimi by-product meal, chicken offal meal and earthworm meal) used in the present experiment were the agro-industries wastes/by-products. Although these by-products/waste materials have fairly good amount of protein contents, they are not being utilized so far for any productive purposes and are thrown away by the agro-processors.

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Received November 05, 2012; Accepted November 20, 2012; Published November 30, 2012

**Citation:** Mohanta KN, Subramanian S, Korikanthimath VS (2013) Evaluation of Different Animal Protein Sources in Formulating the Diets for Blue Gourami, *Trichogaster Trichopterus* Fingerlings. J Aquac Res Development 4:164 doi:10.4172/2155-9546.1000164

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Even the agro-industries producing these wastes face the problem of safe disposal of these wastes without affecting the soil, water and air environments at the place of their disposal. Therefore, in the present study an attempt was made to utilize these waste materials as protein sources in formulating the diets of blue gourami and compared the growth and dietary performance of fish fed diets containing these protein sources with that of other protein sources (squid meal, mussel meal, chicken liver meal and lean prawn meal).

## Materials and Methods

### Experimental diets

The feed ingredients viz. snail (*Pila globosa*) meat, freshwater fish processing waste (intestines, air bladders, gills and heads of carps), surimi by-product (derived from surimi processing plant where the pink perch, *Nemipterus japonicus* is being used for production of surimi), chicken offal (heads, intestines and skins), earthworm (*Eisenia foetida*), squid (*Loligo duvaucelli*), mussel (*Perna viridis*) meat, chicken liver, lean prawn (*Acetes indicus*), fish meal (sun-dried miscellaneous marine trash fish, mainly the lesser sardines of family Engrolidae and ribbon fish of family Trichuridae), peanut (*Arachis hypogaea*) oil cake (de-oiled, mechanically extracted), wheat bran (the bran obtained from the wheat after milling it), commercial grade vitamin and mineral mixture (M/S Supplevite-M, Sarabhai Zydus, Vadodara, India) and oil (sunflower oil; Marico Industries Limited, Mumbai, India) required for formulating the experimental diets were procured from the local market. Except mineral and vitamin mixture and oil, all the other ingredients were oven-dried for 24 h at 105 °C and then finely powdered using a mixer grinder and sieved through a fine-meshed screen (0.5 mm diameter). The proximate composition of the ingredients was determined [34], the details of which are presented in (Table 1).

As per the nutrient requirement of blue gourami [33], nine experimental diets with 350 g protein, 80-100 g lipid and 16-17 MJ digestible energy kg<sup>-1</sup> diet were formulated using snail meat meal (D-1), freshwater fish processing waste meal (D-2), surimi by-product meal (D-3), chicken offal meal (D-4), earthworm meal (D-5), squid meal (D-6), mussel meal (T-7), chicken liver meal (T-8) and lean

prawn meal (T-9) as major protein source in addition to fish meal and peanut oil cake meal (Table 2). In all experimental diets, fish meal was used at the level of 100 g kg<sup>-1</sup> diet. Wheat bran was used as the major carbohydrate source along with the carbohydrate that present in peanut cake. Sunflower oil was used as lipid source to adjust the required lipid content (80-100 g kg<sup>-1</sup> diet) in the diets. The proximate compositions of the experimental diets were analyzed [34] and presented in Table 3.

For individual diet preparation, the powdered ingredients (except vitamin and mineral mixture) were precisely weighed; evenly mixed and lukewarm water (60°C) was added to the ingredient mixture and mixed properly so as to form a dough. The dough (feed mix) with respect to each diet was steam cooked for 15 min in a pressure cooker to increase gelatinization of carbohydrate and to improve the binding of ingredients and also to kill the pathogenic microorganisms present if any in different animal protein sources, particularly in the wastes and by-products. The dough was cooled to room temperature and then the vitamin and mineral mixture was added and thoroughly mixed, and the dough was pressed through a hand pelletizer and 0.5 mm dia feed pellets were prepared. The feed pellets were dried for 24 h at 60°C till it contains 7–8% moisture and stored at -20°C until use [35].

### Fish maintenance

Six hundred uniform sized blue gourami fingerlings (average weight 3.16 g) were procured from local ornamental fish traders. Before conducting the experiment, the fish were acclimatized for 15 days using six cement tanks (500 L of water each) so as to adjust them to the laboratory rearing condition. While acclimatization, the fish were fed with a fish meal protein based diet (300 g kg<sup>-1</sup> diet crude protein and 14.5 MJ kg<sup>-1</sup> diet gross energy) other than the experimental diets. Two hundred seventy healthy fingerlings (3.54 ± 0.02 g; average weight ± SE) were stocked randomly in nine dietary treatments in triplicate groups. Twenty seven circular fiber-reinforced plastic tanks with 200 L of water were used for rearing the fish. The water in the experimental tanks was continuously aerated by using aquarium aerators. The photoperiod was natural cycle of 12 h light/12 h dark (12 Li:12 Dr). The chlorine-free seasoned tap water was used for rearing the fish. The fish were fed *ad libitum* to their respective diets close to apparent satiation level for a period of 45 days. Fish were batch weighed in every 15 days to know growth and survival of fish. The experimental tanks were cleaned in every 15 days during the experiment. In each experimental tank, about 50% of the water was exchanged at every 5 days interval to maintain optimum water quality and hygiene. The ambient water temperature (°C) was recorded twice daily at 06:00 and 14:30 h. The other water quality parameters were analyzed [36] in every 15 days and presented in Table 4. At the end of the experimental period of 45 days, the fish were batch weighed to determine the final weight.

### Chemical analysis

The proximate composition of experimental diets was analyzed in triplicates [34]. Dry matter was estimated by oven drying the samples at 105°C till a constant weight and crude protein percent was calculated by estimating nitrogen content by micro-Kjeldahl method and multiplying with a factor 6.25. Ether extract was determined by solvent extraction with petroleum ether, boiling point 40–60°C, for 10–12 h. Total ash content was determined by incinerating the sample at 650°C for 6 h and crude fiber by acid digestion (1.25%) followed by alkali digestion (1.25%). The total carbohydrate (TC) was calculated using the equation

Ingredient	Parameters					
	Dry matter	Crude protein	Ether extract	Ash	*Total carbohy-drate	§Calculated Digestible energy (MJ kg <sup>-1</sup> )
Snail	302.6	525.0	40.0	160.0	275.0	14.88
Freshwater fish processing waste	322.7	455.0	190.0	180.0	175.0	17.68
Surimi by-product	320.0	560.0	60.0	175.0	205.0	15.05
Chicken offal	297.0	507.5	350.0	135.0	7.5	21.78
Earthworm	172.5	490.0	140.0	132.0	238.0	17.43
Squid	146.7	665.0	80.0	120.0	135.0	16.38
Mussel	172.8	656.2	120.0	96.0	127.8	17.64
Chicken liver	236.6	603.7	236.6	105.0	54.7	19.90
Lean prawn meal	922.0	638.7	45.0	183.6	132.7	14.59
Fish meal	922.0	525.0	81.6	161.2	232.2	15.72
Peanut cake	935.0	420.0	75.0	76.8	428.2	17.01
Wheat bran	913.0	122.5	18.0	73.0	786.5	15.63

\*Total carbohydrate (TC)=100-[%CP+%EE+%Ash], where C=crude protein and EE=ether extract.

§Calculated digestible energy (DE)=[%CP×4+%EE×9+%TC×4] [37].

**Table 1:** Proximate composition (g kg<sup>-1</sup> dry matter) of ingredients used for formulating the experimental diets for blue gourami, *Trichogaster trichopterus*.

Ingredient	Experimental diets								
	D-1 (Snail meal)	D-2 (Freshwater fish processing waste meal)	D-3 (Surimi by-product meal)	D-4 (Chicken offal meal)	D-5 (Earth-worm meal)	D-6 (Squid meal)	D-7 (Mussel meal)	D-8 (Chicken liver meal)	D-9 (Prawn meal)
Snail meal	400	----	----	----	----	----	----	----	----
Fresh water fish processing waste meal	----	450	----	----	----	----	----	----	----
Surimi by-product meal	----	----	400	----	----	----	----	----	----
Chicken offal meal	----	----	----	350	----	----	----	----	----
Earth-worm meal	----	----	----	----	400	----	----	----	----
Squid meal	----	----	----	----	----	280	----	----	----
Mussel meal	----	----	----	----	----	----	300	----	----
Chicken liver meal	----	----	----	----	----	----	----	330	----
Lean prawn Meal	----	----	----	----	----	----	----	----	320
Fish Meal	100	100	100	100	100	100	100	100	100
Peanut cake meal	160	180	160	220	200	200	200	200	180
Wheat Bran	250	230	260	290	240	350	340	310	310
*Vitamin & Mineral Mixture	20	20	20	20	20	20	20	20	20
≠ Sunflower Oil	50	----	40	----	20	30	20	20	50
§Carboxy methyl cellulose	20	20	20	20	20	20	20	20	20

\*M/S Supplevite-M, Sarabhai Zydus, Vadodara (India) contains (quantities kg-1 mixture): as Vitamin A, 20 00 000 IU; Vitamin D3, 400 000 IU; Vitamin B2, 0.4 g; Vitamin E, 250 IU; Vitamin K, 0.2 g; Calcium pantothenate, 0.5 g; Nicotinamide, 2.0 g; Vitamin B12, 2.4mg; Choline chloride, 30 g; Calcium, 150 g; Manganese, 5.5 g; Iodine, 0.2 g; Iron, 1.5 g; Zinc, 3 g; Copper, 0.4 g; Cobalt, 0.09 g.

≠ Marico Industries Limited, Mumbai, India

§ Himedia, Mumbai, India

**Table 2:** Ingredient composition (g kg<sup>-1</sup> dry matter) the formulated experimental diets used for growth performance of blue gourami, *Trichogaster trichopterus*.

Experimental diet	Dry matter	Crude protein	Ether extract	Crude fibre	Ash	*Total carbohydrate	§Calculated digestible energy (MJ kg <sup>-1</sup> )
D-1 (Snail meal)	923	353.3	82	19.5	124	440.7	16.34
D-2 (Freshwater fish processing waste meal)	928	351.0	98	22.7	135	416.0	16.51
D-3 (Surimi by-product meal)	932	352.5	100	19.4	142	405.5	16.43
D-4 (Chicken offal meal)	924	351.4	89	18.5	95	464.6	16.97
D-5 (Earthworm meal)	935	354.9	80	19.0	123	442.1	16.34
D-6 (Squid meal)	931	352.4	76	18.8	125	446.6	16.22
D-7 (Mussel meal)	936	355.8	104	17.6	121	419.2	16.89
D-8 (Chicken liver meal)	921	354.5	102	16.9	119	424.5	16.84
D-9 (Lean prawn meal)	93.8	353.5	79	23.8	135	432.5	16.13

\*Total carbohydrate (TC)=100-[%CP+%EE+%Ash], where CP=crude protein and EE=ether extract.

§Calculated digestible energy (DE)=[%CP×4+%EE×9+%TC×4] [37].

**Table 3:** Proximate composition of the formulated experimental diets (g kg-1 dry matter) used for growth performance of blue gourami, *Trichogaster trichopterus*.

=100-[%CP+%EE+%Ash], where CP=crude protein and EE=ether extract. The digestible energy (DE) contents of the experimental diets were determined by using the formula DE=(%CP×4+%EE×9+%TC×4) [37].

### Calculation of nutritional indices

$$\text{Specific growth rate (SGR)} = \frac{\ln \text{Final weight} - \ln \text{Initial weight}}{\text{Experimental duration (days)}} \times 100$$

$$\text{Feed : gain ratio} = \frac{\text{Total feed intake (dry weight)}}{\text{Total live weight gain}}$$

$$\text{Protein efficiency ratio (PER)} = \frac{\text{Total live weight gain}}{\text{Total protein intake}}$$

### Statistical analysis

Data are presented as mean ± standard error (SE; n=3). Statistical significance of data were analyzed following one way analysis of variance

Treatments	Initial weight (g)	Final weight (g)	Weight gain (g)	FCR	SGR	PER
D-1 (Snail meal)	3.57 ± 0.03 <sup>a</sup>	7.40 ± 0.08 <sup>bc</sup>	3.83 ± 0.05 <sup>b</sup>	1.67 ± 0.02 <sup>c</sup>	1.62 ± 0.01 <sup>c</sup>	1.70 ± 0.03 <sup>b</sup>
D-2 (Freshwater fish processing waste meal)	3.52 ± 0.05 <sup>a</sup>	7.55 ± 0.04 <sup>ab</sup>	4.03 ± 0.07 <sup>ab</sup>	1.62 ± 0.03 <sup>cd</sup>	1.70 ± 0.04 <sup>b</sup>	176 ± 0.03 <sup>ab</sup>
D-3 (Surimi by-products meal)	3.51 ± 0.04 <sup>a</sup>	7.60 ± 0.12 <sup>ab</sup>	4.08 ± 0.08 <sup>a</sup>	1.58 ± 0.02 <sup>d</sup>	1.71 ± 0.01 <sup>ab</sup>	1.80 ± 0.03 <sup>a</sup>
D-4 (Chicken offal meal)	3.57 ± 0.05 <sup>a</sup>	6.83 ± 0.08 <sup>d</sup>	3.26 ± 0.03 <sup>d</sup>	2.04 ± 0.04 <sup>a</sup>	1.44 ± 0.01 <sup>e</sup>	1.39 ± 0.03 <sup>d</sup>
D-5 (Earthworm meal)	3.54 ± 0.05 <sup>a</sup>	7.08 ± 0.18 <sup>cd</sup>	3.54 ± 0.12 <sup>c</sup>	1.87 ± 0.03 <sup>b</sup>	1.54 ± 0.02 <sup>d</sup>	1.51 ± 0.02 <sup>c</sup>
D-6 (Squid meal)	3.49 ± 0.04 <sup>a</sup>	7.73 ± <sup>ab</sup>	4.24 ± 0.10 <sup>a</sup>	1.55 ± 0.02 <sup>d</sup>	1.76 ± 0.01 <sup>a</sup>	1.83 ± 0.02 <sup>a</sup>
D-7 (Mussel meal)	3.55 ± 0.05 <sup>a</sup>	7.67 ± 0.13 <sup>ab</sup>	4.12 ± 0.08 <sup>a</sup>	1.57 ± 0.03 <sup>d</sup>	1.71 ± 0.01 <sup>ab</sup>	1.79 ± 0.03 <sup>a</sup>
D-8 (Chicken liver meal)	3.61 ± 0.03 <sup>a</sup>	7.80 ± 0.12 <sup>a</sup>	4.19 ± 0.10 <sup>a</sup>	1.55 ± 0.02 <sup>d</sup>	1.71 ± 0.02 <sup>ab</sup>	1.82 ± 0.02 <sup>a</sup>
D-9 (Lean prawn meal)	3.50 ± 0.03 <sup>a</sup>	7.55 ± 0.06 <sup>ab</sup>	4.05 ± 0.03 <sup>ab</sup>	1.60 ± 0.03 <sup>cd</sup>	1.71 ± 0.01 <sup>ab</sup>	1.78 ± 0.03 <sup>a</sup>

Values (means of three replicates in each dietary treatment ± SE, n=3) with different superscripts in the same column signify statistical differences ( $p < 0.05$ ).

**Table 4:** Growth performance of blue gourami, *Trichogaster trichopterus* fed different experimental diets after 45 days of experiment duration.

Parameters	Ranges
Temperature ( °C)	26.2-28.6
pH	6.68-7.12
Dissolved oxygen (ppm)	7.6-7.9
Total alkalinity (mg CaCO <sub>3</sub> L <sup>-1</sup> )	108.2-112.7
Nitrite-Nitrogen (mg CaCO <sub>3</sub> L <sup>-1</sup> )	0.07-0.09
Ammonia-Nitrogen (mg CaCO <sub>3</sub> L <sup>-1</sup> )	0.04-0.07

**Table 5:** Water quality parameters in fish rearing tanks during the experimental period.

(ANOVA) and Duncans' multiple range test at  $p < 0.05$  [38,39]. The statistical package used for the analysis of data was PC-SAS programme for Windows, release v6.12 [40].

## Results

There was no fish mortality found in any of the dietary treatment groups. The water quality parameters were in the optimum range of fish rearing (Table 5). The crude protein and the digestible energy of the various animal protein sources used in the present study varied between 455-665 g kg<sup>-1</sup> diet and 14.59-21.78 MJ kg<sup>-1</sup> diet, respectively (Table 1). The analyzed values of crude protein, ether extract and gross energy of the experimental diets were in the ranges of 351-356 g kg<sup>-1</sup> diet, 80-100 g kg<sup>-1</sup> diet and 16.13-16.97 MJ kg<sup>-1</sup> diet, respectively (Table 3).

Among the different animal protein sources used in the present study, the fish fed diet containing surimi by-product meal (D-3), squid meal (D-6), mussel meal (D-7), chicken liver meal (D-8) and lean prawn meal (D-9) had significantly ( $p < 0.05$ ) higher weight gain, SGR and PER compared to the diet containing snail meal (D-1), chicken offal meal (D-4) and earthworm meal (D-5) as major dietary protein sources (T-4). Significantly lower ( $p < 0.05$ ) FCR was also obtained in fish fed surimi by-product meal, squid meal, mussel meal and chicken liver meal diets than the snail meal, chicken liver meal and earthworm meal diets. The best growth and dietary performance was observed in fish fed diet D-6 where the squid meal was used as major dietary protein source. However, no significant difference ( $p > 0.05$ ) in weight gain, FCR, SGR and PER was observed among the fish fed diets containing freshwater fish processing waste meal, surimi by-product meal, mussel meal, chicken liver meal and lean prawn meal as major dietary protein sources.

## Discussion

The search of alternative protein sources to fish meal is a priority research in aquaculture because of concerns regarding the future availability of fish meal for incorporation into fish diets [22,41]. In the present study, our main objective was to develop nutritionally balanced practical diets for the blue gourami based on the nutrient requirement of this species using different alternate animal protein sources other than fish meal. Therefore, we formulated nine practical diets using nine different animal protein sources (with minimum incorporation of fish meal), out of which some were the by-products from different agro-industries.

We obtained the FCR values of 1.55-2.04 in blue gourami fingerlings using different animal protein sources. As reported by earlier workers, the FCR values varied very widely for various fish when they fed diets containing different animal protein sources (2.37-2.44 for sunshine bass, *Morone chrysops* × *M. saxatilis* with turkey meal [19], 1.78-2.93 for Nile tilapia, *Tilapia niloticus* with fermented feather meal [14], 1.82-1.96 for African catfish, *Clarias gariepinus* with fish waste meal and fish meal [25], 1.27-2.40 for *C. gariepinus* with blood meal [11,12], 1.6-1.9 for rainbow trout with tuna by-products meal [26], 1.20-1.50 for humpback grouper, *Cromileptes altivelis* with poultry by-product meal [42], 1.0-1.08 for Siberian sturgeon, *Acipenser baerii* with rendered animal protein composed of meat and bone meal, poultry by-product meal and hydrolysed feather meal [5], 2.64-2.75 for *C. gariepinus* with rendered animal protein [3], 0.95-1.05 for grouper, *Epinephelus coioides* with meat meal and blood meal [15], 1.36-1.67 for gibel carp, *Carassius auratus gibelio* with rendered animal protein [7], 2.90-6.04 for catfish, *Heterobranchus longifilis* with tadpole meal along with fish meal [20], 2.07-2.27 for sunshine bass, *Morone chrysops* × *M. saxatilis* with poultry

by-product meal [8], 2.01-2.32 for *C. gariepinus* with snail offal meal [43], 1.54-2.67 for catfish, *Heteropneustes fossilis* with fermented fish offal [22], 1.34-1.55 for Nile tilapia fishery by-catch and processing waste [24], 1.29-1.69 for bata, *Labeo bata* with fermented fish offal meal [23]. From the present study and those reported earlier, it was found that the FCR depend on the species of fish and source of dietary protein used in the experiments.

Like FCR, the SGR also varied remarkably for different fish species in relation to dietary protein sources (0.93-1.38 for *H. fossilis* with fermented fish offal [22], 0.6-0.7 for rainbow trout with tuna by-products [26], 0.66-0.69 for *M. chrysops*×*M. saxatilis* with poultry by-product meal [8], 0.79-0.90 for *C. gariepinus* with snail offal meal [43], 0.51-0.71 for *C. gariepinus* with garden snail meal [16], 1.30-1.33 for sunshine bass with turkey meal [19], 1.15-1.21 for *C. gariepinus* with fish waste meal along with fish meal [25], 2.7-3.1 for Nile tilapia with fish by-catch and processing wastes [24], 2.82-3.06 for grouper with meat meal and blood meal [15], 2.32-2.70 for gibel carp with rendered animal protein [7], 1.05-1.34 for bata with fermented fish offal meal [23]. In our study, the SGR varied between 1.44-1.76 which is either higher or lower to the earlier reported values as indicated above. In addition to fish species and the source of dietary protein, the SGR also depend on the initial size of the fish and the duration of fish culture in the study. These are the reasons for the wide variations in SGR in different studies.

In the present study, the blue gourami fed diets with different protein sources had the PER values in the range of 1.39-1.83. The much variation in PER was reported by previous researchers for different fish that fed diets containing various protein sources (0.92-0.99 for sunshine bass with turkey meal [19], 1.3-1.5 for rainbow trout with tuna by-products meal [26], 1.07-1.24 for *C. gariepinus* with snail offal meal [43], 1.03-1.32 for *M. chrysops*×*M. saxatilis* with poultry by-product meal [8], 1.26-1.85 for Nile tilapia with fermented feather meal [14], 1.27-1.37 for *C. gariepinus* with fish waste meal along with fish meal [25], 1.3-1.7 for humpback grouper with poultry by-product meal [42], 1.15-2.15 for *H. fossilis* with fermented fish offal [22], 1.67-1.92 for Nile tilapia with fishery by-catch and processing waste [24], 0.75-1.27 for bata with fermented fish offal meal [23]. The PER depend on the weight gain in relation to protein take and the protein intake depend on the source of protein in the diet. Therefore, the source of protein may be responsible for the wide variation on PER values. The PER values obtained in our study is well within the range of earlier studies as reported by different researchers using different animal protein sources.

In our study, among all the dietary treatments, the blue gourami fed diet containing squid meal as protein source had resulted the best growth and dietary performance. However, the freshwater fish processing waste meal and surimi by-product meal as dietary protein sources had given almost similar growth and dietary performance as that of squid meal, mussel meal, chicken liver meal and lean prawn meal based diets. This indicates that these two agro-industry wastes which are thrown away by the agro-processing plants could be used as potential dietary protein sources in formulating the cost-effective practical diets for blue gourami. Similar to our results on use of fish processing waste, it is reported that fish offal [22] and the fisheries by-catch and processing waste [24] can be used as dietary protein source in the diet of freshwater catfish and Nile tilapia, respectively. The good growth performance of fish fed surimi by-product in the present study is in agreement with our earlier findings for the same species [29]. Generally, freshwater fish processing waste meal and surimi by-product meal were thrown away by the fish processors. They even face great

difficulty to dispose such a huge quantities of waste materials, which cause environmental pollution, if not disposed properly. Although some authors have reported that snail meat and offal could be used as protein source [16,43,44], we did not find any satisfactory growth and nutrient utilization in blue gourami fed snail meal diet and the reason for poor performance is not known to us. Although earthworm is a good source of protein with favorable amino acids as reported by earlier workers [45,46], in our study, the blue gourami fed earthworm diet did not perform well. Generally, chitin is present in the hard cuticle of earthworm and this chitin in the earthworm meal might have responsible for poor performance of blue gourami. Although we have not measured the chitin contents in the diets, the earthworm used in the present study contains 7.8% fiber (in dry weight basis), which mostly represent its chitin content. Similar to our observations, it is reported that the growth depression and poor feed and protein utilization as well occurred in catfish fed with high levels of meal worm or solely on meal worms due to presence of chitin in the meal worm [47]. Using chicken offal/poultry by-product meal at low inclusion levels (17-24%) in the diet, satisfactory growth and nutrient utilization was observed by different workers in various fish [17,48-50]. The poor performance of blue gourami fed chicken offal diet (D-4) in the present study may be due to the higher inclusion level of chicken offal (35%).

The cost of the nine experimental diets used in the present study is varied between Rs. 30-225/kg while the commercial diets that are sold in the local market are costing between Rs. 1100-3500/kg (as per our market survey). Although we have not used any commercial diets to compare with our diets, but the study results indicated that the growth and dietary performance of blue gourami fed diets containing freshwater fish processing waste, surimi by-product, squid, mussel and lean prawn meal as dietary protein sources is satisfactory.

## Conclusions

Based on results obtained in our study, we concluded that the freshwater fish processing waste, surimi by-product, squid, mussel and lean prawn meal could be used as alternative protein sources in the diet of blue gourami without affecting the growth and dietary performance of blue gourami. However, in order to reduce the cost of feed, we suggest the use of freshwater fish processing waste and the surimi by-product which are available free of cost so far. The cost of transportation and drying are the only costs involved in using these two waste materials as potential protein sources for formulating the diets of blue gourami.

## Acknowledgements

The authors are thankful to Director, ICAR Research Complex for Goa, Old Goa, Goa, India for extending all the infrastructure facilities needed for the experiment and Indian Council of Agricultural Research, Ministry of Agriculture, Government of India for financial support. The technical help extended by Shri Keshav Chodnekar, Technical Officer, ICAR Research Complex for Goa for carrying out the study is duly acknowledged.

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