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Optimization of Fish Hydrolysate Preparation and its Effect on Growth and Feed Utilization of Magur (*Clarias batrachus*)

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ABSTRACT
This study provides information on the use of fish hydrolysate as an alternative protein source for magur feeding. Two diets (40% protein) were prepared where fish meal protein was replaced at levels of 0 (control) and 10% with the hydrolysatediets were supplied to magur (10.48 ± 0.11 g initial weight) stocked in 1 × 1 m² cemented tanks in triplicate.Diet containing 10% fish hydrolysate exhibited better water stability and water absorption rate than the diet incorporated fish meal. After 60 days of feeding trial, fish fed the diet containing 10% fish hydrolysate showed significantly (P<0.05) better performance in terms of weight gain (%), specific growth rate, average daily gain, food conversion ratio, protein efficiency ratio, protein productive value, protein growth ratethan those fed the control diet (fish meal). It is concluded that fish hydrolysate is a promising alternative protein source for magur feeding, improving growth rate and it is cost effective as obvious.
Key words: *Fish hydrolysate, fish meal, Growth, Magur.*

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INTRODUCTION

Annual discard of fish industry is 25% of the total production that is estimated to be approximately 21.72 million tons [1]. It is estimated that fish processing waste after filleting accounts for approximately 75% of the total fish weight. About 25% of the total weight remains as waste in the form of skins and bones during preparation of fish fillets. This waste is an excellent raw material for the preparation of high value products including protein foods. Fish waste is a good source of protein[2], but a huge amount of the waste is still being discarded without much effort to recover its protein[3].Fish processing by-products are commonly recognized as low-value resources with negligible market value. Depending on the type of fishery, by-products or waste generated from seafood processing plants usually accounts for about 30–85% of the weight of the landed catch [4]. Additionally, inappropriate disposal is a major cause of environmental pollution. Hydrolysis processes have been developed to convert the fish by-products into the marketable and acceptable forms [5]. Fish protein hydrolysate (FPH) has been used as an ingredient in fish feed [6]. In exchange of FM, fish hydrolysate generally show a beneficial effect on growth performance and feed utilization at low inclusion levels, but decreased performance exceeding a specific dietary level [7] [8]. [9]reported that removing small molecular weight compounds from fish hydrolysate, the growth and feed efficiency were significantly reduced in rainbow trout. Some of these small compounds in fish hydrolysate seem to be essential for biological performance.

Tilapia is a popular freshwater fish with nutritional benefits and wide availability. Due to the growing demand for tilapia fillet in producing fish-based food products, large amounts of waste have been generated. This waste is usually discarded and cause numerous environmental problems [10] and contain considerable amounts of proteins that are known to possess high nutritional value with respect to essential amino acid composition [11] and rich protein content [2] varying from 15-60% [12][13][14]. *Clarias batrachus* is recognized as an important candidate species for aquaculture, as it meets many economic criteria for a candidate species. Its hardy nature to adverse ecological conditions enables its culture with high stocking densities at a production up to 100 tons per hectare [15]. So the aim of the present study was to prepare protein hydrolysates from the tilapia waste and evaluate the effect of fish hydrolysate on growth and feed efficiency on magur.

MATERIALS AND METHODS

Experimental fish

The experiment was conducted at a private fish farm, Poonam Fisheries, Tirga, Durg, Chhattisgarh, India over a period of 60 days. Fingerlings of magur with weight of (10.48 ± 0.11) g were selected for the experiment. Magur fingerlings were produced and reared in the same fish farm. Fishes were selected randomly from the rearing tank, weighed and then transferred to the experimental tanks one week before the start of the experiment for acclimatization to experimental conditions.

Experimental set-up

The experimental set-up consisted of 6 cemented tanks (1 × 1 m^2). 60 fishes were randomly distributed in two distinct experimental groups in triplicate, following a completely randomized design. Each cemented tank was stocked with 10 fish. 25% water exchange was carried out during the trial period.

Diet preparation and feeding

Chemical properties of fish hydrolysate is shown in table 1. Ingredients such as fish meal, soya flour, mustard oil cake, groundnut oil cake, corn flour, rice flour, wheat flour, tapioca, sunflower oil, vitamin and mineral mixture (Valaenza Pharmaceuticals Private Limited) and cod liver oil (Sanofi India Limited, Sanofi Consumer Healthcare Division) were used for feed formulation of control diet. Raw materials were procured from local shops. All the ingredients were grinded first then mixed together and made a dove and cooked ain autoclave for 10 minutes separately for preparation of control diet. Then pelleted those into 1 mm sizes and dried in mechanical drier and then dry feeds were stored in air tight plastic bottles. The preparation method of fish hydrolysate is shown in fig. 1.

Collection of fish processing waste

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Addition of molasses + starter Cultures

↓

Incubation for 28-30 days

↓

Filtration (Whatman No. 4)

↓

Supernatant

↓

Drying in hot air oven at 80 °C

↓

Scrapping

↓

Pulverization

↓

Stored in plastic drums in the air tight condition

Fig. 1. Preparation of fish hydrolysate

Two is nitrogenous (40% crude protein) experimental diets were formulated (Table 2). The diets were designated as T1 (fish meal (FM) as protein source and T2 (10% fish hydrolysate (FH) incorporated diet). Feeding was done at the rate of 10% of body weight initially, and after 10 days fishes were fed ad libitum till the end of each experiment. The daily ration was divided into three equal parts and was given at 09.00, 13.00 and 18.00 hrs.

Chemical analysis

Proximate composition of diets and whole body determined by the standard methods [16]. The crude protein percentage was obtained by multiplying nitrogen percentage by a factor of 6.25. Fat was estimated by Soxhlet apparatus, the moisture content was determined using hot air oven, ash content by muffle furnace, carbohydrate by difference method by summed all the other constituents in the feed (protein, fat, moisture, ash) and subtracted from the total weight of the food (Table 2).

Physical Evaluation of Fish Feed

Water stability:

Feed samples of 5g each in duplicate were placed in wirenet container immersed the 2L beaker containing water. The beaker was kept in a magnetic stirrer to simulate mild water flowing condition for period of 0.5, 1,2,4,6,8,10and 12 hours. After each time interval, the feed samples from containers were collected by draining water and dried at 60 °C till complete drying. Water Stability was calculated from the following formula (Table 3),

$$\text{Water stability } (\%) = (\text{Dry weight of pellet after immersion}/\text{Dry weight of pellets before immersion}) \times 100$$

Water absorption rate:

Feed samples of 5g each in duplicates were placed in wire net container and immersed in 2L beaker. Containing water at room temperature for period of 1, 3, 6 and 10hours. After each specified time period the feed samples were removed and allowed to drain for one minute followed by weighing. The water absorption rate was calculated by water absorption (Table 4).

$$\text{Water absorption } (\%) = (\text{Dry weight of pellet after immersion}/\text{Dry weight of pellets before immersion}) \times 100$$

Calculation

The following calculations were made:

$$\text{Crude protein } (\%) = N_2(\%) \times 6.25$$

$$\text{Ether extract } (\%) = (\text{Weight of the ether extract}/\text{Weight of the sample}) \times 100$$

$$\text{Moisture } (\%) = [(\text{Wet weight of sample} - \text{Dried weight of sample}) / \text{Wet weight of sample}] \times 100$$

$$\text{Ash } (\%) = (\text{Weight of ash}/\text{Weight of sample}) \times 100$$

$$\text{Nitrogen} - \text{free extract} = 10 - [\text{Weight in grams}(\text{protein} + \text{lipid} + \text{moisture} + \text{ash})\text{in 100g food}$$

$$\text{Feeding Rate, FR } (\% \text{ Body weight/day}) = [\text{Dry feed intake}/60 \text{ days} \times (\text{Final body weight} + \text{Initial body weight})/2) \times 100$$

$$\text{Food conversion ratio, FCR} = \text{Total amount of the feed consumed (g)}/\text{Wet weight gain (g)}$$

$$\text{Protein efficiency ratio, PER} = \text{Increment in body weight (g)}/\text{Protein intake (g)}$$

$$\text{Protein Productive Value, PPV } (\%) = [\text{Body wet protein gain (g)}/\text{Protein intake (g)}] \times 100$$

$$\text{Protein growth rate, PGR } (\%/\text{day}) = [(\text{Log}\_e \text{ final protein content} - \text{Log}\_e \text{ initial protein content})/\text{Days of feeding}] \times 100$$

$$\text{Survival } (\%) = (\text{Number of fish survived after rearing}/\text{Number of fish stocked}) \times 100$$

Statistical analysis

One way Analysis of Variance (ANOVA) and least significantly difference (LSD) [17] was applied to test the level of significance amongst the treatments.

RESULTS

Growth performance and feed utilization data for magur fingerling fed with control diet (T1) and 10% hydrolysate incorporate diet(T2) are presented in Table 5. Percent weight gain, specific growth rate and average daily gain (ADG) were significantly ($P<0.05$) higher in fish fed diet T2. Same trend was followed for feeding rate, protein efficiency ratio (PER), protein productive value (PPV) and protein growth rate (PGR). Feed conversion ratio (FCR) were better ($P<0.05$) in fish fed diet T2. Water stability and water absorption capacity of feed containing 10% fish hydrolysate were found higher as compared to the control diet. Carcass composition data are given in Table 7. Ether extract of the fish ranged from 9.85% to 9.91% from T1 to T2 diet and whole body moisture of magur decreased from 26.44% to 26.12% from T1 to T2 diet. Production cost for 1 liter of fish hydrolysate was Rs 208.33 which is economically viable (Table 6). However, Protein, ether extract, ash, moisture and nitrogen free extract did not vary significantly ($P>0.05$) among dietary groups (Table 7).

DISCUSSION

60 days feeding trial was conducted to evaluate the growth and feeding efficiency of fish hydrolysate on magur and also observed the water stability and water absorption capacity of fish hydrolysate. Fish hydrolysate contains more than 60 trace minerals which have positive effect son animal cells, plant cell, chlorophyll and plankton health. Again, Fish hydrolysate can be rapidly assimilated when applied as feed

supplement, fertilizer, animal fish and plant feeding as foliar spray. It also produced good quality plankton. [18][19]. Magur feeding experiment has showed that pellets are durable and remain in one pieceuntil eaten by fish and small fractures of feed are not ingested and result in poor feed conversion efficiency [20].In the present experiment, the best growth and feed efficiency were obtained with diet containing fish hydrolysate (Table 5). Fish hydrolysate contains some small molecular weight compounds that are beneficial for growth and feed performance, in accordance with the results obtained by [9] and [21]. The present experiment with magur supports this explanation. Improved palatability is often used to explain observed increases in feed intake and growth performance in studies were fish hydrolysate are exchanged with FM [7] [8]. While, [9] and [21]found that there is no difference in feed intake for either Atlantic cod or Rainbow trout supplemented with different type and level of fish hydrolysate in the diet. Inthe present experiment, magur fed with 10% fish hydrolysate diet showed higher growth performance and feeding rate than fish fed FM.

Table 1. Chemical properties of fish hydrolysate as biofertilizers

S. No.	Parameter	Composition
1.	pH	4.25 ± 0.2
2.	Electrical conductivity (dc/m)	5.07
3.	Organic carbon (%)	2.2 ± 0.2
4.	Available nitrogen (mg) in 100 ml	392 ± 0.21
5.	Available nitrogen (mg) in 100 ml	10 ± 0.5
6.	C/N ratio	1.5

Table 2. Proximate composition of control and experimental diet

Parameters	Control	10% FH
Crude protein	40.03	40.21
Ether extract	14.97	15.93
Ash	17.43	16.31
Moisture	8.94	8.76
Nitrogen free extract	18.63	18.79

Table 3. Water Stability (%) of control and fish hydrolysate incorporated pellet

Sl.No.	Time Interval (h)	Water Stability (%)	
		Control	10% FH
1	0.5	89.94	98.37
2	1.0	87.32	97.43
3	2.0	85.89	94.58
4	4.0	80.13	88.76
5	6.0	75.34	84.39
6	8.0	71.06	80.72
7	10.0	68.85	76.41
8	12.0	66.59	72.29

Table.4: Water Absorption (%) of control and fish hydrolysate incorporated pellet

Sl. No.	Time Interval (h)	Water absorption pellets (%) Control	10% FH
1	1	32.19	34.15
2	3	40.83	43.97
3	6	55.68	58.47
4	10	66.42	69.75

Table 5. Growth and feed efficiency parameters and survival of *C. batrachus* fed the test diets for 60 days

Parameters	Control	10% Hydrolysate incorporated diet
Weight gain (%)	96.82±2.86[b]	120.45± 1.76[a]
FR (% bw day^{-1})	1.75±0.03[b]	1.33±0.02[a]
SGR	0.49±0.01[b]	0.57±0.01[a]
ADG (g day^{-1})	0.17±0.01[b]	0.21±0.00[a]
FCR	1.61±0.06[a]	1.43±0.03[b]
PER	1.55±0.06[b]	1.75±0.04[a]
PPV (%)	64.70±2.32[b]	97.42±2.03[a]
PGR (% day^{-1})	0.55±0.01[b]	0.60±0.00[a]
Survival (%)	100±0.00[a]	100±0.00[a]

*Mean±SE within a row followed by with different superscripts are significantly ($p<0.05$) different from each other.

Table 6. Cost of production of hydrolysate Biofertilizer

Category	Cost per Unit	Total cost (Rs)
Molasses 20 liter	Rs 25 /liter	500/-
Culture media 20 ml	Rs 10 /ml	200/-
Plastic drum 5 no.	Rs 250 /piece	1250/-
Labor cost per month	Rs 130 /per day	3900/-
Low cost fish 20 kg	Rs 20 /kg	400/-
Cost of Production	30 liter of fish hydrolysate	6250/-
Cost of Production	1 liter of fish hydrolysate	208.33/-

Table 7. Proximate carcass composition (% dry weight) of magur (*C. batrachus*) fed the experimental diets for 60 days

Parameters	T1 (Control)	10% Hydrolysate incorporated diet
Crude protein	41.12±0.01	41.25±0.02
Ether extract	9.85±0.04	9.91±0.07
Ash	21.21±0.79	21.46±0.63
Moisture	26.44±1.08	26.12±1.03
Nitrogen free extract	1.38±0.13	1.26±0.25

Production of fish protein hydrolysates is one way to add value to proteinaceous fish waste. From the results, it was demonstrated that hydrolysates produced had good functional properties indicating their possible use in different food systems. Production cost of fish hydrolysate is cheaper than the other commercial dietary products (Table 6). However, further research including real food systems is recommended. The use of commercial enzymes for production of highly functional hydrolysates from low commercial value can be a fish feasible technology to make the most of the vast underutilized resources and use it as a food ingredient for direct human consumption.

Generally, nutrients are deposited in fish body at a rate proportional to their levels in diets [22]. The inverse between moisture and lipid in the carcass of treated fish in the present investigation is in agreement with the findings of previous workers [23] [24]. Carcass protein contents were higher than the initial; indicative of the fact that experimental treatments favored body protein deposition as much as the control, and confirmed an adequate protein digestibility in dietary treatments [24](Table 7).

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