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Nutritional Evaluation of Five Nonconventional Meals as the Substitute of Fishmeal in the Compound Diets of Singhi, *Heteropneustes fossilis*

Das Mousumi

Post Graduate Department of Zoology, Vidyasagar College, Salt Lake Campus, CL Block, Kolkata 700091, West Bengal, India

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Abstract: For the sustainable development of aquaculture practices, the search of cost-effective nonconventional easily available fishmeal alternative animal protein sources is being globally encouraged. Nutritional evaluation of five selected nonconventional animal meals revealed that these were rich in protein content. To evaluate the efficacy of these fishmeal alternatives, a nine-month feeding trial was carried out on *Heteropneustes fossilis* feeding with six iso-proteic compound diets where fishmeal was completely replaced by fish offal meal, poultry by product meal, snail meal, earthworm meal and grasshopper meal separately in the diet (control, D1, D2, D3, D4 and D5, respectively). A total of 540 juveniles (0.61±0.01 g) were randomly distributed in triplicates of six sets each with 30 fishes. At the end of experimental period, significantly higher final weight was noted in D2 diet fed set (52.11±1.77 g) followed by D4 set (43.42±3.30 g), D5 set (37.77±1.83 g), control (27.63±1.78 g) whereas lowest value was recorded in D3. Specific growth rate (SGR%) of control fishes was 2.12±0.03, whereas significantly highest value was obtained from D2 set (2.47±0.03), followed by D4 set, D5 set and lowest in D3 set (1.89±0.04). Similarly, D2 diet fed set showed the best result for feed conversion ratio (FCR). Fishes fed with D4 and D5 diets utilized their diets better than control. Survival percentage ranged from 99.67% to 50%. Compared to control, it was higher in D2, D4 and D5 sets, where values insignificantly varied, and lowest found in D1 set.

Based on the results, poultry by-product meal, earthworm meal and grasshopper meal were found to have the potentiality to replace fishmeal completely in singhi diet with enhanced optimal growth, feed utilization and better survival rate. Hence, these three nonconventional meals are recommended as economic and sustainable protein-rich fishmeal alternatives for the diet of *H. fossilis*.

Keywords: Compound diets, Feed utilization, Fishmeal, Growth, *Heteropneustes fossilis*, Nonconventional animal meals, Survival rate

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Introduction

Worldwide aquaculture industries play a crucial role in combating against malnutrition and in enhancing food and nutritional security through satisfying the excessively increasing demand of fish and fisheries products by contributing a total global fish production of 82.1 million tons (Handbook Fisheries Statistics, 2020). In India, aquaculture is one of the largest and rapidly growing food industries with the national production of 14.18 million tons. Farmed food fish in India has recorded a sustained average annual growth of 8.61% from 2014-15 to 2021-22 (Ministry of Fisheries, Animal Husbandry Dairying, 2023). Inland fisheries and aquaculture production has doubles from 61.36 lakh tons in 2013-14 to 121.12 lakh tons in 2022-23 (Ministry of Fisheries, Animal Husbandry Dairying, 2023). NFP (2020) reported that about 88% of the farmed fish comes from freshwater aquaculture. It is therefore imperative to ensure that the production of nutritionally high-quality fish is sufficiently secured for the sustainable development of nation as aquaculture industry is an important key component of the national key economic areas (NKEA) (Chatla *et al*., 2021). With a goal to enhance fish production, aquaculture practices are gradually shifting from extensive to semi-intensive and intensive culture systems where fish nutrition renders a critical function in fish growth, health, survival, reproductive performance, spawning success and fish-body composition and thereby influencing their production (Kaushik *et al*., 2011). The quality and quantity of protein in fish feed play a vital role in the optimum growth of fish. High-quality protein contains essential or indispensable amino acids (EAAs) which are not synthesized by fish and taken through food for growth and tissue development (Fagbenro *et al*., 2000). Fishmeal is a conventionally used high-quality protein-rich ingredient in fish feed as it contains complete EAAs profile which is able to meet the protein and amino acids requirement of most fish species (Watanabe *et al*., 1997). In recent decades, use of fishmeal in fish feed is becoming a challenging task due to its overexploitation, high-cost, uncertainty and inadequate availability and compromised quality. Feed cost make up a substantial cost of fish farming, accounting for 70% to 90% of the total production cost in which protein is the most expensive dietary ingredient (Da *et al*., 2013).

For the maintenance of sustainable growth and productivity, suitable alternative feeding management is an essential need. In this scenario, search for alternative nonconventional protein sources for fish feed is an international research priority in aquaculture. In recent years, numerous considerable research emphasis has been undertaken towards the utilization of low-cost nonconventional plant as well as animal protein sources in fish feed formulations to replace fishmeal partially or completely with good growth (Ojha *et al*., 2014). Nonconventional feed resources are not the traditional feed ingredients used in aqua-feed industries and their inclusion in fish feed is encouraged for being non-competitive for human consumption, low-cost, by product or waste products from agriculture and processing industries, and a way of waste management (Sogbesan and Ugwumba, 2008).

Plant-based protein sources are cheap, but its incorporation in fish diet at a high level or completely replacing animal protein source mostly results in poor growth and feed efficiency in fish (Lim, 1992) due to inappropriate amino acid balance and the occurrence of high amount of anti-nutritional factors like alkaloids, glycosides, oxalic acids, phytates, protease inhibitors, haemato-glutinin, saponegin, momosine, cyanoglycosides, linamarin etc. (Rahate *et al*., 2021).

Several studies have investigated the potential of many animal protein sources such as feather meal, bone meal, poultry by-product meal, freshwater fish processing waste, snail meat, squid, mussel, lean prawn, earthworm, maggot, termite, grub, tadpoles and silkworm pupae meal (Singh *et al*., 2005; Mohanta *et al*., 2016; Parolini *et al*., 2020) as the alternative to fishmeal in fish feed for the different consumable fishes. All these unexplored alternative protein resources could be successfully recycled and utilized by incorporating them in fish feed to replace fishmeal. Hence, further studies are essential to explore locally available nonconventional protein sources to enhance food security.

In aquaculture fish offal, discarded parts of fish, has substantial potentiality to be used in fish feed as animal protein source. Daily huge amount of fish offal which are rich in protein is discarded in bulk from the retail fish markets (Mondal *et al*., 2007). Fish offal have been used previously as partial or total replacement of fishmeal in the diet for common carp, tilapia etc. (El-Sayed, 1999; Farahiyah *et al*., 2015). Limited studies have been conducted using fish offal in fish feed formulation.

Poultry by product meal is one of the potentially rendered animal protein sources which might be an alternative ingredient to fishmeal in the diet of carnivorous fish species due to its palatability, high protein content, digestible protein and most importantly similar energy content as in fishmeal (Zhou *et al*., 2004). It has been tested as partial or complete fishmeal replacer at varying success in tilapia (Felix *et al*., 2020), African catfish *Clarias gariepinus* (Mullabaev *et al*., 2022), minor carp (Emre ***et al*.,** 2003), grass carp (Tabinda and Butt, 2012), spotted rose snapper (Hernandez ***et al*.,** 2014), grouper (Gunben *et al*., 2014), sobaity (Hekmatpour *et al*., 2019), *Lates calcarifer* (Siddik *et al*., 2019), Nile tilapia (Ahmeed *et al*., 2020), yellow croaker (Wang *et al*., 2023) etc.

Bellamya bengalensis, a fresh water snail, distributed throughout Asia and Africa, are sufficiently available in and around rivers or reservoirs banks, ponds and paddy fields, are being used as food mainly by the tribal people from immemorial times. It contains a considerable quantity of protein which is comparable to fish meal (Bar, 2020; Jintasataporn *et al*., 2004). As the protein content in snail vary from 48% (Bar, 2020) to 62% (Serra, 1997), so can play a crucial role in food web by being as food and transferring food energy to higher trophic level. This nonconventional protein source has the potential as an alternative of fishmeal in fish feed. In recent times, several studies have been carried out using snail in fish feed for *Pangasius* sp., *Siganus guttatus* etc. (Miguel and Suzette, 2018; Pertiwi and Saputri, 2020). No such records of studies are available conducted on catfish using *B. bengalensis* as an alternative of fishmeal in feed.

Earthworm could be an alternative protein source to replace fishmeal in fish feed as it contains 50% to 70% crude protein and it has been used in the earlier studies in the diet of *Parachanna obscura* (Vital *et al*., 2016). In this regard, Musyoka *et al*. (2019) and Mohanta *et al*. (2016) reported that *Eisenia fetida* is a highly protein-rich earthworm species which could be used in fish feed as it is suitable for proper growth of fishes (Sogbesan and Ugwumba, 2008).

Many studies have suggested the use of grasshopper meal in livestock and fish feed formulation (Finke, 2013; Rumpold and Schluter, 2013). Earlier studies reported that grasshoppers contain high amount of good quality protein with essential amino acids and other nutrients (Das and Mandal, 2014). These nutrients could be efficiently utilized in fish feed. Several studies have been conducted with grasshopper meal as a partial replacement of fishmeal in fish feed in aquaculture feeding without compromising their growth and feed utilization efficiency (Emehinaiye, 2012; Das, 2023a, b). *Oxya hyla hyla* (Serville) is a shorthorn multivoltine grasshopper species, easily available in agricultural fields and grasslands throughout India, could be farmed for biomass production (Das *et al*., 2012). Furthermore, it contains about 68.77% crude protein, good amount of essential amino acids, fatty acids, minerals, vitamins and negligible amount of anti-nutritional factors (Das and Mandal, 2014).

In fish market, demand of catfishes is very high and their culture in rural areas provide a high profit to the farmers which is a responsible factor of the upliftment of their socio-economic status. In India singhi, *Heteropneustes fossilis* (Bloch) is a commercially and aquaculturally important fish species with high market value and consumer acceptance (Rahman *et al*., 2013) due to its less spine, high protein, low-fat content, delicious taste, nutritional and medicinal values, and high digestibility (Roy *et al*., 2020).

Singhi is an air-breathing catfish which belongs to the family Heteropneustidae and found in India, Bangladesh, Myanmar, Thailand and Pakistan in ponds, ditches, swamps, marshes and muddy rivers of fresh water, and brackish water (pH range: 6.0-8.0)(Chew *et al*., 2020). For small and large-scale rural fish farming, it is an excellent hardy fish species which readily adapts to the local environment, accepts artificial feed and can tolerate poor water quality, low oxygen, resistance to diseases and has ability to maintain high growth at high densities (Chakraborty and Nur, 2012).

Few studies have been conducted on the effect of nonconventional animal protein sources such as fish offal meal, poultry by product meal, snail meat meal, earthworm meal and grasshopper meal, in a formulated compound diet by completely replacing the fishmeal, on the growth performance, feed utilization, survival and body composition of *H. fossilis.* Therefore, the present study aims to evaluate these five selected nonconventional animal proteins for comparative effectiveness as a substitute of fishmeal for *H. fossilis.*

Materials and Methods

Water Quality Analysis

Assessment of water quality from all the experimental aquaria were analyzed twice a week. Physicochemical parameters of water like temperature, dissolved oxygen, pH, free carbon dioxide, hardness and total dissolved solids (TDS) of the water of experimental aquarium sets were estimated and recorded using standard methods (APHA, 2012).

Experimental fish

A total number of 600 juveniles of *H. fossilis*, purchased from Adhikary Fish Farm and Hatchery, Naihati, Kolkata, India, in February, 2022, were acclimatized to laboratory condition in glass aquaria (36"×12"×12") in the laboratory of Department of Zoology, Vidyasagar College, West Bengal, India, for one week. During this period, the fishes were fed with the control diet used in the feeding trial. Each aquarium (18"×12"×12") was stocked with 30 juveniles (0.61±0.01 g). Triplicates of six dietary treatments were fed one of the experimental diets up to apparent satiation, twice daily (09:00 and 18:00) at a fixed feeding rate of 5% of the body weight for nine months. Experimental fishes were bulk weighted every fortnight and required feed quantity for each aquarium was readjusted. Each aquarium had a continuous aeration via air blowers. To collect the extra uneaten feed particles, water of the experimental aquaria was siphoned daily and then reuse two third of that water with one third of well-aerated tap water. Collected uneaten particles were dried and weighted to find out the amount of diet consumed daily. Total water exchange was done twice a week to maintain a clear and healthy water in the experimental aquaria throughout the feeding trial.

Experimental diet

Discarded fish and chicken slaughter wastes were collected from wet market and then were screened for any impurities and were washed with water. The samples were then dried in hot-air oven at 60˚C for 5 to 6 days until the samples were fully dried with 10% moisture.

Snail, *B. bengalensis* was procured from local wet market and then washed, removed from the shell, oven-dried at 60 °C for 5 to 6 days to make the sample dried with 10% moisture.

Earthworm, *E. fetida* was harvested, rinsed in water and then kept in a tray for 30 min to expel the residual undigested materials from their guts (Sogbesan and Ugwumba, 2008). The worms were kept in hot-air oven at 80˚C for 3 h.

The grasshopper, *O. hyla hyla* were harvested from nearby agricultural and grassland fields of Rajarhat (22.58˚N, 88.48˚E), Kolkata, India using insect net of 30 cm diameter. The specimens were freeze killed and then wings and appendages were removed to get rid of excess chitin, and were oven-dried at 40°C for 3 days.

Then the dried fish offal, poultry by product, snail meat, earthworm and grasshopper were ground into powder form and were packed as fish offal meal, poultry by-product meal, snail meal, earthworm meal and grasshopper meal in air-tight plastic zipper bags until use in the preparing experimental diets for feeding trial and for the proximate analysis.

As per *H. fossilis* nutritional requirement, six iso-proteic (35% crude protein) experimental diets were prepared with raw ingredients such as mustard oil cake, maize, rice bran, fishmeal. The control diet was prepared using 42 g fishmeal while other five diets were prepared by completely replacing the fish meal with the fish offal meal, poultry by-product meal, snail meal, earthworm meal and grasshopper meal and named as D1, D2, D3, D4 and D5 diet, respectively. For the preparation of each experimental diets, all the oven-dried dietary ingredients used in feed formulation were finely ground, well mixed according to the proportion of each diet and thoroughly blended with hot water into dough, and finally each diet type was added separately with 1% chromic oxide (Cr_2O_3). All the diets were prepared in pelleted form by using pelletizer with 2 mm die. The obtained wet pellets were then sun-dried for a few days and then crumbled into desirable particle size and stored at room temperature in airtight plastic container until used.

Proximate Composition Analysis

For the estimation of crude protein, crude lipid, crude fibre, moisture, ash contents of the five selected animal meals used in feed formulation of the experimental diets, prepared experimental diets and fish tissue, collected at the end of feeding trial from the different experimental sets, proximate composition was analyzed following the stipulated method of AOAC - Association of Official Analytical Chemists (AOAC, 2006).

Evaluation of Survival and Growth performance

At the end of the nine-month feeding trial, fishes were counted from each of the aquarium to determine their survival percentage. Fishes were starved for 24 h and then harvested to note the final length and weight of fishes in order to estimate their growth efficiency parameters such as weight gain, specific growth rate (SGR%) and condition factor (CF), and food utilization parameters in terms of feed conversion ratio (FCR).

Statistical Analysis

The results are presented as mean ± standard error (SE). All the data were analyzed by using one-way ANOVA to test the effects of dietary treatments. When a significant treatment effect was found, Duncan's multiple range tests (DMRT) were carried out to compare the mean values. Dietary treatment effects were considered at the level of $P<0.05$ of significance.

Results

Water quality

The ranges of water quality parameters estimated throughout the experimental period (Table 1) from the different dietary treatment sets did not vary significantly and was maintained within acceptable range during the entire experimental period except the water hardness and TDS, their value ranges were found to be quite high. In this regard Chew *et al*. (2020) stated that singhi are capable to withstand a wide range water quality of their habitat and are highly adaptable in culture condition.

Nutrient Composition of animal protein sources and experimental meals

The proximate composition of fishmeal and other five nonconventional animal protein sources used for the preparation of the six different experimental diets are summarized in Table 2. Crude protein content of grasshopper meal was significantly higher ($P<0.05$; DMRT), followed by poultry by product meal, fishmeal, earthworm meal, snail meal and then fish offal meal. Crude lipid content was significantly higher in fish offal meal while crude fibre and total ash content were in poultry by-product meal.

Table 1: Water quality parameters of experimental aquarium sets during the experiment

Parameter	Minimum	Maximum
Temperature (^{O}C)	24	38
Dissolved Oxygen (ppm)	8.3	8.4
pH	7.2	7.4
Free Carbon dioxide (ppm)	1.42	2.12
Hardness (ppm)	588	666
TDS (ppm)	900	1080

Table 2: Proximate nutritional composition of fishmeal and five nonconventional protein sources (%, dry weight basis)

Nutritional composition	Grasshopper meal	Earthworm meal	Snail meal	Poultry by product meal	Fish offal meal	Fish meal
Crude Protein	64.67± 0.13^{f}	52.00±0.06^{c}	48.59 ±1.14^{b}	60.62±0.14^{e}	42.48±0.47^{a}	54.00±0.98^{d}
Crude Fat	6.92±0.03^{b}	10.87±0.15^{d}	5.60± 0.03^{a}	12.05±0.70^{e}	26.02±0.01^{f}	9.74±0.09^{c}
Crude Fibre	6.75±0.04^{e}	4.23±0.05^{c}	0.06±0.002^{a}	12.25±.07^{f}	2.01±0.03^{b}	5.21±0.11^{d}
Total Carbohydrate	17.42±0.66^{a}	24.67±0.14^{b}	37.76±0.04^{d}	18.40±0.56^{a}	24.39±0.35^{b}	32.81±0.10^{c}
Total Ash	10.99±0.04^{e}	8.92±0.04^{c}	4.45±0.09^{a}	12.43±0.08^{f}	7.11±0.07^{b}	8.10±0.02^{d}

Values are mean ± SE. Values with different superscript letters in a row denoting significant differences (one way ANOVA; DMRT) ($P<0.05$).

Proximate composition of all the six prepared experimental diets for singhi are shown in Table 3. Crude protein content in all the experimental diets ranged from 34.67% to 35.33% and did not vary significantly. Crude fibre and total ash content was significantly higher in the D2 diet, and crude fat in the D1 diet, whereas the values of the mentioned parameters did not show significant differences among the remaining diets.

Growth performance

The effect of six different dietary treatments on the growth performance and feed utilization of experimental *H. fossilis* are shown in the Table 4. At the start of experiment, initial length and initial weight of the juveniles of all the six dietary sets varied from 2.15 cm to 2.17 cm and 0.58 g to 0.62 g, respectively and did not vary significantly ($P>0.05$; DMRT) among the sets. At the end of nine-month feeding trial period, significant variations were found in the growth efficiency parameters like final body weight, weight gain, specific growth rate in per cent (SGR%) and also in feed utilization parameters such as total food consumption and food conversion ratio (FCR). Significantly highest final body weight ($P<0.05$; DMRT) was noted in the fishes fed with the D2 diet, followed by the D4 and then D5 set and a significantly lower value was recorded from the D3 dietary set. While the final weight of the fishes from the D1 and D3 sets were found significantly lower than the fishes of control diet fed set. Significantly maximum ($P<0.05$; DMRT) value of the final length was documented from the D2 diet fed set, followed by the D4 and minimum in the D3 diet fed set. The final length of fishes from the control and the D5 set did not vary significantly (DMRT).

A similar result was obtained for weight gain of *H. fossilis,* as found in final weight, where significantly higher weight gain was recorded from the D2 set and lower from the D3 set. In case of SGR in the percent study, juveniles fed with the D2 practical diet where poultry by- product meal

Table 3: Ingredients, their proportion and proximate composition of experimental diets

Ingredients	Diets					
	Control	D1	D2	D3	D4	D5
Fish meal	42.00	0.00	0.00	0.00	0.00	0.00
Fish offal meal	0.00	42.00	0.00	0.00	0.00	0.00
Poultry by product meal	0.00	0.00	42.00	0.00	0.00	0.00
Snail meal	0.00	0.00	0.00	42.00	0.00	0.00
Earthworm meal	0.00	0.00	0.00	0.00	42.00	0.00
Grasshopper meal	0.00	0.00	0.00	0.00	0.00	42.00
MOC	13.00	13.00	13.00	13.00	13.00	13.00
Maize	19.35	20.69	6.46	20.17	19.60	30.70
Rice bran	24.35	23.01	37.24	23.53	24.10	13.00
DCP	1.00	1.00	1.00	1.00	1.00	1.00
Salt	0.20	0.20	0.20	0.20	0.20	0.20
Vit-mineral premix	0.10	0.10	0.10	0.10	0.10	0.10
Total	100 g	100 g	100 g	100 g	100 g	100 g
Crude Protein	35.06±0.06[a]	35.13±0.12[a]	35.19±0.24[a]	35.21 ±0.20[a]	35.17±0.30[a]	35.11± 0.34[a]
Crude Fat	10.59±1.32[a]	17.22±1.19[b]	13.27±1.22[a]	8.75± 0.93[a]	11.11±1.24[a]	7.83±0.86[a]
Crude Fibre	6.76±1.24[a]	5.25±1.22[a]	11.24±0.21[b]	4.43±0.56[a]	6.44±0.25[a]	6.13±0.13[a]
Total Carbohydrate	49.18±2.17[b]	31.37±1.43[a]	44.64±1.57[b]	49.31±2.52[b]	45.19±3.31[b]	49.14±1.03[b]
Total Ash	6.72±0.18[a]	6.28±0.36[a]	8.53±0.08[b]	5.16±1.13[a]	6.89±0.56[a]	7.92±0.52[a]

*GOC- groundnut oil cake; *Vit-Mineral Premix (mg/kg diet): retinol-18,000 IU, Choleclaciferol-2000 IU, thiamine-15, menadione sodium bisulphate-10, riboflavin-25, pyridoxine-5, α-tocopherol-35, nicotinic acid-200, Ca-pantothenate-50, biotin-1.5, folic acid-10, cyanocobalamin-0.03, ascorbyl monophosphate-50, inositol-400, copper sulphate-20.2, dibasic calcium phosphate-5.9, sodium fluoride-2.21, potassium iodide-0.78, zinc oxide-37.5, ironsulphate-200, magnesium oxide-840, manganese oxide-26, cobalt sulphate-1.85, sodium selenite-0.65, potassium chloride-1.17, sodium chloride-0.45. Values of proximate composition are presented as mean ± SE. Values with different superscript letters in a row denote significant differences as determined by DMRT after one way ANOVA ($P<0.05$).

replacing 100% fishmeal showed higher significant values ($P<0.05$; DMRT) for SGR (%), followed by the D4 with earthworm meal, completely replacing fishmeal and then D5 where fishmeal totally substituted by grasshopper meal. The values of SGR (%) was found significantly lower in the D1 and D3 sets, when compared with the control set, whereas the lowest was noted in the D3 set. In the current study, obtained results of the condition factor (CF) was found significantly highest in the D5 set and lowest in the D3 set, while in the remaining feeding sets the values of CF did not show significant differences in DMRT.

The results of feed utilization depicted that

Table 4: Growth and food utilization parameters of singhi, *H. fossilis* fed with six experimental diets

Growth parameters	Diets					
	Control	D1	D2	D3	D4	D5
Initial weight (gm)	0.61±0.01^{a}	0.61±0.01^{a}	0.61±0.01^{a}	0.59±0.01^{a}	0.59±0.01^{a}	0.60±0.01^{a}
Initial length (cm)	2.17±0.01^{a}	2.16±0.01^{a}	2.16±0.01^{a}	2.16±0.01^{a}	2.16±0.01^{a}	2.17±0.01^{a}
Final weight (gm)	27.63±1.78^{c}	21.85±1.68^{b}	52.11±1.77^{f}	17.96±1.49^{a}	44.02±3.29^{e}	38.36±1.83^{d}
Final length (cm)	16.83±0.60^{c}	15.17±0.17^{b}	22.33±0.67^{e}	14.33±0.60^{a}	19.37±0.58^{d}	17.31±0.32^{c}
Weight gain (gm)	27.03±1.78^{c}	21.24±1.68^{b}	51.50±1.78^{f}	17.36±1.49^{a}	43.42±3.30^{e}	37.77±1.83^{d}
SGR (%)	2.12±0.03^{c}	1.99±0.05^{b}	2.47±0.03^{f}	1.89±0.04^{a}	2.39±0.05^{e}	2.31±0.02^{d}
Condition factor (CF)	0.58±0.03^{b}	0.61±0.08^{b}	0.61±0.03^{b}	0.48±0.06^{a}	0.63±0.07^{b}	0.74±0.5^{c}
Food consumption (gm)	133±5.57^{c}	114.77±6.76^{b}	149.33±4.33^{e}	93.84±2.69^{a}	167.73±5.20^{f}	145.20±5.20^{d}
FCR	4.94±0.13^{c}	5.51±0.73^{c}	2.91±0.18^{a}	5.46±0.31^{c}	3.93±0.42^{b}	3.86±0.18^{b}

Values are mean ± SE. Values with different superscript letters in a row denote significant differences as determined by DMRT after one way ANOVA (P<0.05).

Table 5: Proximate composition (%) of the whole-body of singhi, *H. fossilis* fed with six experimental diets

Diets	**Crude protein (%)**	**Crude lipid (%)**	**Moisture (%)**	**Ash (%)**
Control	16.06±0.10^{ns}	3.00±0.03^{ns}	76.90±1.95^{ns}	3.22±0.13^{ns}
D1	15.81±0.19^{ns}	3.03±0.03^{ns}	76.85±3.61^{ns}	3.18±0.08^{ns}
D2	16.04±0.18^{ns}	2.8±0.10^{ns}	77.36±3.56^{ns}	3.22±0.08^{ns}
D3	15.86±0.13^{ns}	3.02±0.03^{ns}	78.18±1.04^{ns}	3.16±0.06^{ns}
D4	16.02±0.12^{ns}	2.99±0.03^{ns}	76.65±2.47^{ns}	3.25±0.05^{ns}
D5	16.01±0.20^{ns}	3.03±0.06^{ns}	76.74±2.28^{ns}	3.20±0.06^{ns}

Values are mean ± SE. Values with ns letters are not significantly different (P<0.05) one way ANOVA.

there is a positive effect by the diets with poultry by-product meal, earthworm meal and grass-hopper meal on the experimental *H. fossilis*. In case of total food consumption, the maximum amount of food was consumed by the fishes of the D4 set, followed by the D2 set, D5 set and control, while a lower amount was consumed by the fishes fed with the D3 diet.

Among the dietary treatments, the mean value of food conversion ratio (FCR) was found higher in the D1 set, while no significant differences in FCR of *H. fossilis* were observed in the sets fed with the D1, D3 and the control diet. The best value of FCR i.e. significantly lowest value was recorded from the fishes of the D2 set. The values of FCR of the D2, D4 and D5 set were found better when compared with the control set.

Whole-body composition

The whole-body composition of *H. fossilis* from the six different dietary treatments is presented in Table 5. The fishes fed with control diet and the diets where fishmeal was completely replaced by the five nonconventional protein sources such as fish offal meal, poultry by-product meal, snail

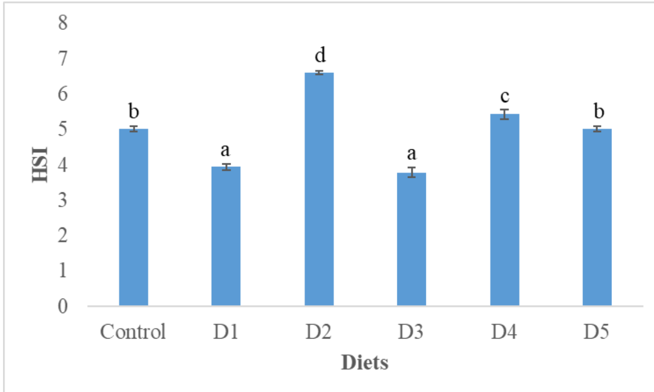

Fig. 1: Hepato-somatic index, HSI of singhi, *H. fossilis* fed with six experimental diets.

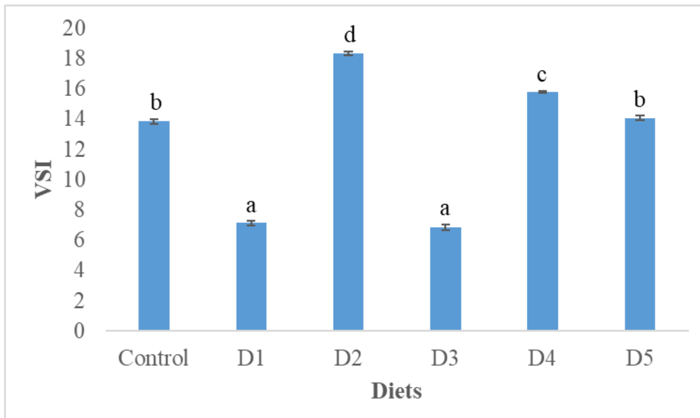

Fig. 2: Viscerosomatic index, VSI of singhi, *H. fossilis* fed with six experimental diets.

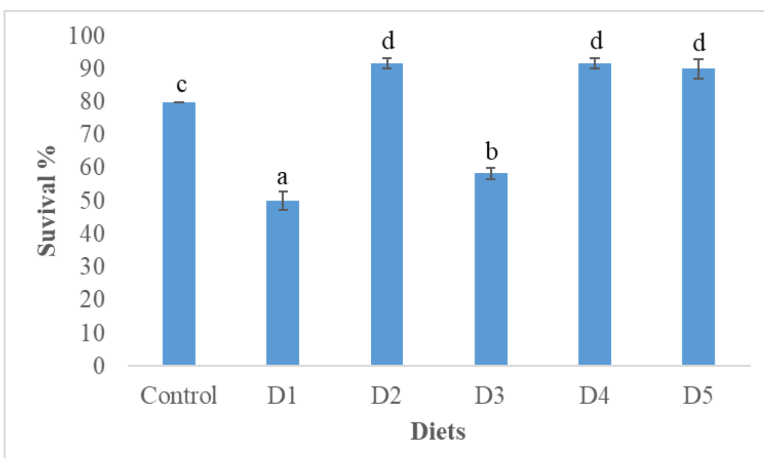

Fig. 3: Survival percentage of singhi, *H. fossilis* fed with six experimental diets. Values are mean ± SE. Bars with different letters are significantly different (P<0.05) using DMRT after one way ANOVA.

meal, earthworm meal and grasshopper meal, did not show any significant differences in whole-body crude protein, crude lipid, ash content and moisture content of the selected fish.

Hepato-somatic index (HIS) and Viscerosomatic index (VSI)

The influence of six different dietary treatments at the termination of feeding trial on the hepato-somatic index (HIS) and viscerosomatic index (VSI) of *H. fossilis* is presented in Figures 1 and 2, respectively. The results of both the mentioned parameters followed a similar trend i.e. significantly higher values were obtained from the D2 set, followed by the D4 set. The value of the D5 set did not vary significantly with the control set. Values recorded from the D1 and D3 set was found lower than the control set.

Survival percentage

The impact of different dietary treatments on the survival percentage of *H. fossilis* after nine months of feeding trial is shown in Figure 3. Survival percentage varied from 50% to 91.67%, where a higher mean value was recorded from the D2 and D4 set. Survival percentages recorded from the D2, D4 and D5 set did not show any significant variations. Significantly lower survival percentage was obtained from the D1 set. Survival percentage was found better in all the dietary sets except the D1 and D3 set when compared with the control.

Discussion

Protein sources and its levels are the most principal criteria in aquaculture fish feed formulation (Cho *et al*., 1985). It is the essential and most expensive component of fish feed. Proper growth, feed utilization, health condition, normal behaviour and external appearance of catfishes mainly depends on the quality of protein present in fish diet (Shukla *et al*., 2019). Fishmeal is the conventional protein source for aquafeed fed to aquaculture fish, especially carnivorous fishes (Zhou *et al*., 2004) due to its amino acid profile, vitamin, mineral content, high digestibility and palatability (Miles and Chapman, 2006). High demand, inadequate production, uncertain availability, deteriorating quality and rising cost have necessitated the exploration and evaluation of cheaper and nonconventional substitutes of fishmeal to replenish the shortage of fishmeal, which could have the potential to provide the protein requirement of fish. In search of complete fishmeal replacers in fish feed of cultivated fish species, several nonconventional animal protein sources are being evaluated throughout the world. Some of the ingredients such as fish offal meal, poultry by-product meal, snail meal, earthworm meal and grasshopper meal were used in the present study to estimate their nutrient contents and to examine their potentiality as alternative protein sources for *H. fossilis*.

In this study, the proximate analysis of poultry by-product meal revealed that the protein content was 60.62% which was similar (60.52%) as observed by Hekmatpour *et al*. (2019). Tabinda and Butt (2012) reported the protein content of poultry by product meal was 70%, whereas Emre *et al*. (2003) obtained 52.12%.

In the present study estimated value of protein content of fish offal meal was 42.48%, while earlier reports documented about 31.4% (Mandal *et al*., 2008), 54.59% (Farahiyah *et al*., 2015) and 45.50% (Maria-Lizanne *et al*., 2021). These variations in protein percentage of poultry by product meal and fish offal meal might be due to dissimilar proportion of the different discarded parts of chicken and fishes or might be due to their variation in quality.

The result of protein contents of studied earthworm meal and snail meal were noted as 52% and 48.59%, respectively. Previously conducted studies estimated the protein content in earthworm meal and snail meal which varied from 49% to 70% (Sogbesan and Ugwumba, 2008; Maria-Lizanne *et al*., 2021; Kumar *et al*., 2022) and from 48% to 67% (Serra, 1997; Sogbesan and Ugwumba, 2008; Miguel and Suzette, 2018; Bar, 2020; Maria-Lizanne *et al*., 2021), respectively. This variation in protein contents might be due to variation in species composition.

Current result revealed that crude protein content of grasshopper meal (*O. hyla hyla*) was 64.67%and this value is in conformity with the reports of Das and Mandal (2014) and Ghosh and Mandal (2019).

The result of protein content of the nonconventional animal sources showed that the crude protein percentage of grasshopper meal, poultry by-product meal and earthworm meal were higher than the conventional fishmeal, while the crude protein percentage of snail meal and fish offal meal was lower than the traditionally used fishmeal.

The current study offers useful information regarding the complete replacement of fishmeal with fish offal meal, poultry by product meal, snail meal, earthworm meal and grasshopper meal separately in the diets for *H. fossilis* and the potential utilization of these nonconventional animal protein sources as an alternative ingredient. This study revealed that all the chosen alternative animal protein sources could potentially substitute the fishmeal in the formulated compound diet for *H. fossilis*. It was found that diets containing any of the selected fishmeal replacer were well accepted by the chosen fish. Singhi, a carnivorous catfish whuich has a wide range of food habits (Kohli, 1996). To obtain the best growth performance of the selected catfish, there should be an optimum level of protein percentage in their diet as alteration of this level influence the growth pattern of fish. The dietary protein requirement for the singhi has been suggested to be 35% to obtain high growth rate, feed conversion, and nutrient retention (Akand *et al*., 1989). Mondal *et al*. (2007, 2008) also reported similar protein percentage (35%) in formulated diet for singhi to obtain optimum growth. Hence, 35% of dietary protein was considered for all the formulated experimental diets used in the present feeding trial with the singhi.

Nine months feeding experiments clearly revealed that the results of growth performances, food utilization of the fishes from the six dietary sets vary significantly (P<0.05; DMRT). All the growth and feed utilization parameters showed best results for the fishes fed with poultry by-product meal-based diet (D2), followed by earthworm meal- and grasshopper meal- based diet (D4 and D5, respectively). The results recorded that the D2, D4 and D5 diet fed fishes showed better growth performances than the fishes of control set. This depicted that complete replacement of fishmeal with poultry by-product meal, earthworm meal and grasshopper meal did not affect feed utilization and growth performances of *H. fossilis*, rather enhanced growth efficiency and improved feed utilization were observed. On the other hand, lower growth performance of fishes fed the snail meal- and fish offal meal- based diets (D3 and D1, respectively) compared to control set, suggested that the D1 and D3 diets were not efficiently utilized by the fishes.

Condition factor is the indicator of the health of the fishes (Lizama and Ambrosio, 2002). Results of the condition factor followed a similar trend as noted in case of the other mentioned indices, because here also the results of the D2, D4 and D5 sets were the ideal results and the unsatisfactory results were obtained from the D1 and D3 sets.

Partial replacement of fishmeal with poultry by-product meal in fish feed has been reported to result in positive impact on growth performance and feed utilization of *Anabas testudineus*, *Sparidentex hasta*, *Cetenopharyngodon Idella*, *Cyprinus carpio*, *Clarias gariepinus*, *Lates calcarifer*, *Oreochromis* sp. (Emre *et al*., 2003; Mardhati *et al*., 2010; Hekmatpour *et al*., 2019; Tabinda and Butt 2012; Gunben *et al*., 2014; Siddik *et al*., 2018). However, for the optimum growth, the level of partial replacement varies according to the chosen fish species, quality of the poultry by-product meal, tolerance level of fish species, developmental stage of the fish species, protein level of the diets, quality of fishmeal or the other protein sources of the diet (Krogdahl and Bakke-McKellep, 2005).

Many researchers reported that poultry by-product meal could have the potential to replace 100% fishmeal in fish feed without compromising the growth performance and using any additional amino acids as well as processing (Tabinda *et al*., 2013). Improved growth was obtained by complete fishmeal replacement with poultry by-product meal in *Clarias gariepinus* (Mullabaev *et al*., 2022), *Cirrhinus mrigala* (Tabinda *et al*., 2013), tilapia (El-Sayed, 1998), catla (Hasan *et al*., 1993), rohu (Hasan and Das, 1993), carp (Steffens, 1988) etc.

Fish offal has been proved to be a viable source of protein for fish feed. Fishmeal was partially replaced with fish offal in rohu (Mondal *et al*., 2007), common carp (Rao *et al*., 1996), catfish (Mondal *et al*., 2008, 2011) and tilapia (El-Sayed, 1999). Substitution of total fishmeal with fish offal in the diet of tilapia produced enhanced growth performance with no detrimental effect (Farahiyah *et al*., 2015).

Earthworms could be the best friend for fish farmers if utilized as alternative of fishmeal (Bhuvaneshwaran *et al*., 2019). Partial replacement of fish meal by earthworm meal in fish feed for common carp (*Cyprinu scarpio*), rohu (*Labeo rohita*), *Parachanna obscura* and tetra showed a positive effect on growth performance (Tutan, 2010; Deborah *et al*., 2011; Vital *et al*., 2016) while total replacements in feeds resulted in a growth performance as found with fish meal (Tuan, 2010; Maria-Lizanne *et al*., 2021; Mohanta *et al*., 2016).

In recent times, snail meal has been incorporated as partial fishmeal replacer in fish feed and proved as a good alternate substitute of fishmeal. Snail meal has been used as partially replacer of fishmeal in the diet of Nile tilapia (Cagauan and Doria, 1989), rabbitfish (Miguel and Suzette, 2018), Pasupati catfish (Pertiwi *et al*., 2020) etc. Jintasataporn *et al.* (2004) stated that snail meal can totally substitute fishmeal in the diet of freshwater prawn.

Aquatic insects are the most acceptable basic prey for carnivorous fishes (Severo-Neto *et al*., 2015; Abilhoa *et al*., 2016) and in recent decades, insects play an important role in aqua-feed production as an alternative of fishmeal (Pulcini *et al*., 2020). Grasshopper as an alternative protein supplement in fish diet provided satisfactory results of growth and nutrient utilization. Partial replacement of fishmeal with grasshopper meal supported better growth performance for black molly (Ganguly *et al*., 2014), rohu (Ghosh and Mandal, 2019), *Clarias gariepinus* (Balogun, 2011), Nile tilapia (Emehinaiye, 2012),*Clarias batrachus* (Johri *et al*., 2010) etc.

Das (2023a) carried out a feeding trial on Platy fish fed with three nonconventional insects and commercial meal, and showed that solely used grasshopper meal increased the growth efficiency and feed utilization of Platy fish. In a similar experiment it was also observed that grasshopper meal might be the alternative of fishmeal as grasshopper meal fed swordtail fishes showed higher growth performance and survival rate.

Webster and Lim (2002) completely replaced the fishmeal by terrestrial protein sources in the formulation of diets for several fresh water fishes. Maria-Lizanne *et al.* (2021) conducted feeding experiment with Swordtail fish and reported that growth performance and feed utilization were best in poultry by-product meal-based diet fed fishes, followed by snail meal-based diet fed fishes and earthworm meal-based diet fed fishes.

The results of condition factor indicated that grasshopper-based diet was the most suitable for the singhi for maintaining good health status, while snail meal-based diet might have an adverse effect on health of fish as significantly lowest condition factor was obtained from this set. Condition factor of the remaining experimental sets was found insignificantly varied with the control set, depicted that fishes of all these sets are healthier.

Significantly higher value of HSI obtained from poultry by-product meal-based and earthworm meal-based diet fed fishes than the control set confirmed maximum conversion of excess glucose into glycogen and its deposition in liver, while snail meal-based and fish offal meal-based diet fed fishes had lower value of HSI compared to control indicated depletion of glycogen storage due to nutrient deficiency (Debnath *et al*., 2007). In this feeding experiment, HSI of the fishes fed on control diet and grasshopper meal-based diet neither higher nor lower and that gave a hint of no nutrient deficiency was occurred by feeding of the D5 diet.

Body composition of fish ascertains the flesh quality of fish, that indirectly depends on the quality of feed taken by the fish (Khan *et al*., 2004). So, during quality feed formulation, body composition of the fishes of feeding trial should be taken into consideration. In the present study, body composition of fishes from the different dietary treatment groups showed no significant variations. Hence, it confirmed that all the experimental diets had no adverse effect on the flesh composition of singhi.

In this study, fish survival percentage was noted significantly lower in the snail meal-based and fish offal meal-based diet fed sets compared to the control, whereas the survival of fishes was not varied significantly among the remaining dietary treatment sets and significantly higher than the control set. The results ensured that feeding singhi with conventional and nonconventional meal-based diets such as poultry by-product meal, earthworm meal and grasshopper meal-based diets could able to enhance their survivorship. This might be due to the fact that singhi is a carnivorous fish which efficiently utilized the animal protein sources to meet their energy requirements (NRC, 2011).

This finding clearly alluded that the protein quality of feed formulated with poultry by-product meal, earthworm meal and grasshopper meal were well accepted by the *H. fossilis*. Moreover, the growth efficiency and feed utilization of *H. fossilis* were successfully enhanced by the earthworm meal-, grasshopper meal- and poultry by-product meal-based diets and were better than the fishmeal-based diet fed fishes. In this context, there is a need of further investigations on singhi fed with diets having partial replacement of fishmeal with fish offal meal and snail meal as complete replacement had an adverse impact on growth performance and feed utilization of singhi.

Conclusion

In conclusion, the present study revealed that earthworm meal, grasshopper meal and poultry by-product meal have the potential to replace fishmeal in the diet of *H. fossilis* and all the three nonconventional animal proteins were found highly beneficial for the growth performance, feed utilization and survival of *H. fossilis.* Therefore, earthworm meal, grasshopper meal and poultry by-product meal might be the fishmeal alternative animal sources in aqua-feed formulation. The results of this investigation will reduce the cost of fish farming and lead to better outcome for aquaculture with ecological benefit.

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