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Original Article

Rearing catfish *Heteropneutes fossilis* on feed supplemented by fermented leaf meal of *Ipomoea aquatica*

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Abstract: Replacement of fish meal by cost effective and sustainable plant resources in the formulation of feed for fish is a viable option to alleviate the current crisis in fish feed industries arising due to high cost and non-availability of fish meal. The present research was aimed to evaluate if fermented leaf meal of the aquatic plant *Ipomoea aquatica* could be used as a fish meal alternative in the formulation of feed for the catfish *Heteropneustes fossilis*. Fresh green leaves of *I. aquatica* were sun dried and finely ground to make *Ipomea* leaf meal (ILM), which was then fermented for 12 days by the phytase producing bacteria *Stenotrophomonas maltophilia* strain KUAKSP1 (GenBank Accession No. KY790423) isolated from rumen of goat. Four iso-proteinous, iso-lipidous and iso-energetic feeds were formulated by replacing 0, 25, 50 and 75% of fish meal by the fermented *Ipomoea* leaf meal (FILM). Protein digestibility of the feeds was evaluated within 12 days in an indoor experiment in glass aquaria and growth performance of the fish was evaluated after 8 weeks rearing in outdoor cement tanks. *H. fossilis* grew better on FILM supplemented feed as compared to fish meal based control feed. Maximum apparent protein digestibility (APD) of the feed, maximum weight gain (WG) and specific growth rate (SGR) and minimum FCR of the fish were found in 50% replacement group. However, crude protein (CP) and crude lipid (CL) deposition in the muscle of the fish and activity of protease in the gut was higher in 25% replacement group. It is concluded that *H. fossilis* accepts and grows well on the plant based FILM supplemented feed. For better growth management of the fish, incorporation of FILM in the feed should be restricted to 25 to 50% of fish meal.

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Introduction

Freshwater aquaculture has been unprecedentedly growing in Indian subcontinent during the last few decades due to application of modern tools and techniques. Fish farmers are coming forward with options of culture of various species of freshwater fish and contributing towards augmentation of aquaculture production. However, the main hurdle behind maximizing aquaculture production still remains the use of nutritionally balanced formulated feed, which constitutes about 70% of the total cost in most successful aquaculture practices. Cost of the commercial fish feed is gradually escalating and becoming out of reach of the poor and marginal fish farmers due to increasing cost and unavailability of fish meal, the main source of protein in the formulated feed. As a result, replacement of fish meal by cost-effective, eco-friendly and environmentally sustainable plant products have become priority research since last two decades (Kaushik et al., 2004; Hardy, 2010; Perez-Velazquez et al., 2018; Daniel, 2018). Although initial researches found soybean and other legumes as nutritionally sound feedstuff to replace fish meal in the formulation of feed for fish, they were proved non-viable due to high cost of the legume by-products and relative non-availability due to large scale human consumption (Gatlin et al., 2007; Dan et al., 2017).

In India, several attempts have been made to utilize easily available and low cost terrestrial (Mondal et al., 2012, 2015; Saha and Ghosh, 2013; Roy et al., 2016) and aquatic plants (Hasan and Chakrabarti, 2009; Saha and Ray, 2011; Debnath et al., 2018) to replace fish meal as a source of protein to develop nutritionally

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Table 1. Formulation of experimental feed by replacing fish meal (FM) with fermented *Ipomea* leaf meal (FILM).

FMRL (%)	T	Ingredients (g/kg)										Proximate Composition		
		FM	FILM	ST	DX	αC	FO	VM	MM	CMC	Cr_2O_3	CP (% dw)	CL (% dw)	Energy (Kj/g)
0	T1	750	0	100	120	15	11	1	1	1	1	29.90	7.92	10.38
25	T2	630	210	70	74	5	6	1	1	1	1	30.18	7.90	9.83
50	T3	478	478	20	14	2	3	1	1	1	1	29.93	7.98	9.92
75	T4	228	682	37	85	8	1	1	1	1	1	30.12	7.88	10.36

FMRL = Fishmeal replacement level: T = Treatment; ST=Starch; DX= Dextrin; αC= α-Cellulose; FO= Fish oil; VM= Vitamin mixture; MM= Mineral mixture; CMC= Carboxymethyl cellulose (Binder); Cr_2O_3 (Marker); CP = Crude protein; CL- Crude lipid.

sound feed for carps and catfishes. About 50 species of aquatic plants have been found as potential feedstuffs for fish culture in India (Mandal et al., 2010). *Ipomoea aquatica*, a semi-aquatic tropical plant, that grows profusely in canals, rivers, lakes, ponds and low lands in India and other Asian countries (Austin, 2007), offers a promising and alternative feedstuff in the formulation of fish feed. The plant has hardly been explored as feedstuff for fish, despite being rich in crude protein and crude lipid (Mandal et al., 2010). Recently, we used fermented leaf meal of this plant as fish meal alternative in the formulation of feed for an herbivorous carp, *Labeo rohita* (Ali and Kaviraj, 2018). In this study, the fermented leaf meal of this plant was used as fish meal alternative in the formulation of feed for the catfish *H. fossilis*. Mandal et al. (2011) successfully utilized fermented mulberry leaf meal in the diet of *H. fossilis*, but they used the mulberry leaf meal along with fish offal, an animal-based feedstuff.

The main problem in using plant resources as feedstuff is the presence of anti-nutritional factors (ANFs) which create several adverse effects on the physiology of fish. Phytic acid is a most important ANF, which binds with necessary cations rendering them unavailable to fish, binds to proteins, affects digestive enzyme activities, and reduce digestibility of the feed stuff (Reddy et al., 1989; Afinah et al., 2010; Krogdahlet al., 2010; Khan and Ghosh, 2013). Therefore, several researchers have used fermentation as an effective tool to remove phytic acid from the plant ingredients before using the plant resources as feed stuff (Roy et al., 2009; Mondal et al. 2015; Dey et al., 2016; Ali et al., 2017). Selective use of phytase producing bacteria in the fermentation process serves dual purpose of removing phytic acid and restoring nutrient quality of the plant feedstuff (Roy et al., 2009; Dey et al., 2016; Ali et al., 2017). In this study, we used phytase-producing bacteria isolated from goat rumen to ferment leaf meal of *I. aquatica* and the fermented products were used to partially replace fishmeal in formulation of *H. fossilis* feed and monitored growth performance, digestibility and digestive enzymes' activities of the fish.

Materials and Methods

Experimental feed: We used *Stenotrophomonas maltophilia* strain KUAKSP1 (GenBank Accession No. KY790423), a phytase-producing bacteria isolated from goat rumen (Ali et al., 2017) to ferment leaf meal of *I. aquatica*. Fresh green leaves of the plant were collected from local ponds, sun dried and finely grounded to make the meal before fermentation. The detailed fermentation process has been described by Ali and Kaviraj (2018). After 12 days of fermentation, the wet fermented products were dried in a hot air oven. The proximate composition, phytic acid, tannic acid and some essential mineral contents of the fermented *I. aquatic* leaf meal (FILM) have been reported by Ali and Kaviraj (2018). Four iso-proteinous (30% crude protein), iso-lipidous (8% crude lipid) and iso-energetic (15 kJ/g energy) experimental feed were formulated by replacing 0% (T1), 25% (T2), 50% (T3) and 75% (T4) of fish meal by the FILM (Table 1). To balance the level of nutrients, non-proteinous dietary supplements like fish oil, starch, dextrin and α-cellulose were added to each formulated feed at required amounts. Carboxymethyle

cellulose was used as a binder and Cr_2O_3 was used as a non-absorbent marker. After feed formulation, four types of feed were ground and pelleted separately using a hand pelletizer fitted with a 2 mm diameter to prepare the final experimental feeds. Then, the pelleted feeds were dried before using in the experiments.

Experiments: Fingerlings of *H. fossilis* (average L=6.5 cm; W=2.8 g) were procured from Naihati fish farm and were acclimatized for one week in cement tanks, each containing 250 liters of water. During the acclimatization period, the fish were fed *ad libitum* a feed containing 30% crude protein. Two separate experiments were conducted using *H. fossils* fingerlings: (a) an indoor feeding and digestibility experiment and (b) an outdoor growth experiment. The feeding and digestibility experiments were conducted in the laboratory in glass aquarium of 20-L capacity. Each aquarium contained 10-L of clean deep tube-well water supplied from an overhead tank and five numbers of acclimatized fingerlings of *H. fossilis*. For each experimental feed type three replicates were maintained. Temperature (22-25°C), pH (7.2±0.2), dissolve oxygen (6.3–6.5 mg L^{-1}), free CO_2 (1.99±0.2 mg L^{-1}), total alkalinity (72±0.2 mg L^{-1}) and total hardness (82±0.2 mg L^{-1}) of the aquarium water were maintained regularly. Every day at 8 am, fish of each aquarium were hand fed using the specific experimental feeds based on 5% of the aqurium biomass. Then, the fish were allowed to feed for 6 hours. Left over feed and fecal matter were collected in a particular time interval and processed according to the method of Samaddar et al. (2015) and Roy et al. (2016). The experiment was continued for 12 days. The pooled samples of feed and feces from each aquarium were digested, using a mixture of sulfuric 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 Guhathakurta and Kaviraj (2000). Two parameters were determined from the above experiment: (a) Feed intake rate (FIR) and (b) Apparent protein digestibility (APD). FIR was calculated from the amount of feed given and amount of uneaten feed siphoned out after 6 h and was expressed as gram per 100 g body weight per day (g 100 g^{-1} BW d^{-1}). Apparent protein digestibility (APD) of the feed was calculated from the proportion of Cr and protein in diet and feces based on Ellestad et al. (2002).

The growth experiments were conducted in 400-L outdoor cement circular tanks (diameter 85 cm and average depth 50 cm) under 12 h day-light photoperiod. Each tank contained 4.0 cm thick soil at the bottom, 350-L of the same deep tube-well water, which was used in the feeding and digestibility experiment, and 20 acclimatized fingerlings of *H. fossilis*. The tanks were arranged as complete randomized block design (Gomez and Gomez, 1984); so that, there were three replicates for each of the four fish meal replacement levels (treatments). The experimental fish were hand fed twice daily by the formulated feeds up to satiation. 50% of water of each experimental tank was weekly renewed by fresh tube-well water. Water quality parameters of each experimental tank were determined every week by standard methods (APHA, 1995). The range of values observed during the experiment was: dissolved oxygen (5.55-6.32 mg L^{-1}), free carbon dioxide (0.05-2.08 mg L^{-1}), ammonia-N (0.05-2.05 mg L^{-1}), alkalinity (20.12-82.00 mg L^{-1} as $CaCO_3$), hardness (88-130 mg L^{-1} as $CaCO_3$) and pH (6.76-7.02).

The experiment continued for eight weeks, after which, all fish were sampled from the experimental tanks. Length and weight of the sampled fish were recorded. Then, the sampled fish of each replicate of a treatment were divided into two different sets. One set was used for proximate composition analyses (crude protein, crude lipid and ash). For this purpose, five fish were randomly selected and a small portion of the muscle below the first ray of the dorsal fin from the dorsal side of the fish was first removed, washed with distilled water and preserved at −20°C until proximate composition analyses according to AOAC methods (Helrich, 1990). Daily protein retention (DPR) in the body was determined by [(FBP-IBP) /D], where FBP and IBP are final body protein and initial body protein, respectively, and D is the days of the experiment. The

Table 2. Digestibility of the feed, growth and nutrient deposition in *Heteropneutes fossilis* fed FILM supplemented feeds for 60 days. Data are mean±standard deviation (n = 9); means with dissimilar superscripts in the same row indicates least significant difference (LSD) between the means at $P<0.05$.

	T1	T2	T3	T4
Feed intake and digestibility				
Feed intake rate (g. 100g $^{-1}$BW.d $^{-1}$)	3.22±0.14^{A}	2.39±0.04^{C}	3.09±0.09^{A}	2.83±0.01^{B}
Apparent protein digestibility(APD, %)	78.39±0.43^{B}	66.90±0.86^{C}	82.35±0.20^{A}	65.05±0.10^{D}
Growth				
Total Weight gain (%)	237.15±9.73^{C}	284.17±24.32^{B}	345.41±18.81^{A}	312.15±17.19^{AB}
Specific growth rate (SGR, % d^{-1})	2.02±0.04^{C}	2.24±0.10^{B}	2.48±0.07^{A}	2.35±0.06^{AB}
Feed conversion ratio (FCR)	2.32±0.09^{A}	1.94±0.16^{B}	1.59±0.08^{C}	1.76±0.09^{BC}
Protein efficiency ratio (PER)	1.44±0.05^{D}	1.71±0.14^{CD}	2.09±0.11^{A}	1.88±0.10^{AB}
Apparent net protein utilization (ANPU, %)	103.35±15.80^{B}	246.68±24.6^{A}	247.91±9.84^{A}	211.49±8.42^{A}
Nutrient deposition[1]				
Moisture (%)	75.11±0.77^{A}	67.99±0.73^{B}	68.58±0.24^{B}	69.75±0.05^{B}
Crude protein (%)	20.1±0.56^{B}	25.33±0.89^{A}	25.30±0.35^{A}	24.03±0.30^{A}
Crude lipid (%)	3.06±0.07^{C}	3.95±0.02^{A}	3.62±0.04^{B}	3.64±0.17^{B}
Ash (%)	1.72±0.28^{B}	2.72±0.13^{A}	2.49±0.06^{A}	2.57±0.07^{A}

[1], Initial values: moisture 79.12±1.28; crude protein 16.4±0.98 %; crude lipid 2.77±0.17%; Ash 1.30±0.11

data of length and weight, feed consumption rate and crude protein levels were used to determine specific growth rate (SGR), feed conversion ratio (FCR), protein efficiency ratio (PER) and apparent net protein utilization (ANPU) by the experimental fish following the methods of Castell and Tiews (1980).

Another set of five fish sampled from each tank was used for determination of digestive enzyme activities. For this purpose, the sampled fish were kept in clean water containing glass aquaria (20 L) for 24 h to starve the fish. After 24 h of starving, the fish were fed the experimental feed up to satiation. Following 4 h of feeding, they were taken out from each treatment. Then, they were dissected out to collect the digestive tract. Activities for α-amylase, protease and lipase were measured in the homogenized and sonicated samples of the digestive tract by the method of Bernfeld (1955), Marks and Lajtha (1963) and Josep and Kurup (1999), respectively. Protein content of the samples was determined by the method of Lowry et al. (1951). The nature of distribution of the observations for each parameter was evaluated by Kolmogorov–Smirnov (K–S) and Shapiro–Wilks (S–W) tests (Johnson and Wichern, 2001). Since all observations were found normally distributed, all data were subjected to single factor ANOVA, without any transformation, followed by least significant difference (LSD) test to compare mean among the treatments (Gomez and Gomez, 1984).

Results

FIR, APD, WG, SGR, FCR, PER and ANPU of the fish have been presented in Table 2. The results of one-way ANOVA among the treatment means exhibited that FIR and APD decreased significantly in the replacement groups except in 50% replacement level (T3), which showed similar feed intake rate and significantly higher APD as compared to control (T1). WG, SGR and ANPU of the fish significantly ($P<0.05$) increased and FCR significantly decreased in all FILM supplemented feed (T2-T4), compared to T1. PER was similar between T1 and T2 (25% replacement group), but increased significantly in T3 and T4 (75% replacement group) as compared to T1. Best growth was exhibited by 50% replacement of fish meal by FILM (T3). Crude protein (CP), crude lipid (CL) and ash in fish muscle also increased significantly ($P<0.05$) in the fish fed FILM-supplemented feeds (T2-T4). There was no significant difference in CP and ash among the treatments of FILM-supplemented feeds (T2-T4). However, CL deposition was significantly higher in T2 as compared to T3 and T4. DPR by *H. fossilis* has been presented in Figure 1. DPR significantly ($P<0.05$) increased in all supplemented feed (T2-T4) in comparison to control (T1). Maximum retention was found in 25%

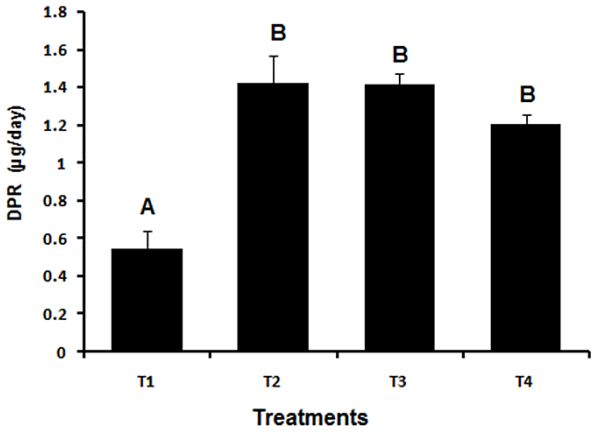

Figure 1. Daily protein retention (DPR) in the body of *Heteropneutes fossilis* fed FIML supplemented feed. Data are mean of three replicates (n=9). The error bars indicate ± standard deviation. Different letters above the error bar indicate least significant difference (LSD) between two treatments at $P<0.05$

replacement level (T2).

Activities of the digestive enzyme, α-amylase and protease, also significantly increased ($P<0.05$) over control (T1) in T2 (Fig. 2). The α-amylase activity significantly decreased in T3 and T4 (50% and 75% replacement level), but there was no significant difference ($P>0.05$) in protease activity between control (T1) and the higher replacement levels (T3 and T4). The lipase activity did not vary significantly ($P>0.05$) between control (T1) and FILM-supplemented feed treatments (T2-T4).

Discussions

The results of the present study indicate that FIR and APD of *H. fossilis* fed the experimental feeds supplemented by FILM decreased as compared to control feed (T1) except in T3 (50% replacement level). *Heteropneutes fossilis*, being an omnivorous fish, can digest high level of protein in feed containing animal-based feedstuffs or mixture of both plant and animal feedstuffs, but digestibility of protein decreases when feed predominantly contains plant based materials (Mondal et al., 2008, 2011). This was also evident from the digestibility experiment of *Mystus vittatus*, a predominantly carnivorous fish, showing high range of APD (91-96%) when fed fishmeal or animal-based feed, but showed a decline

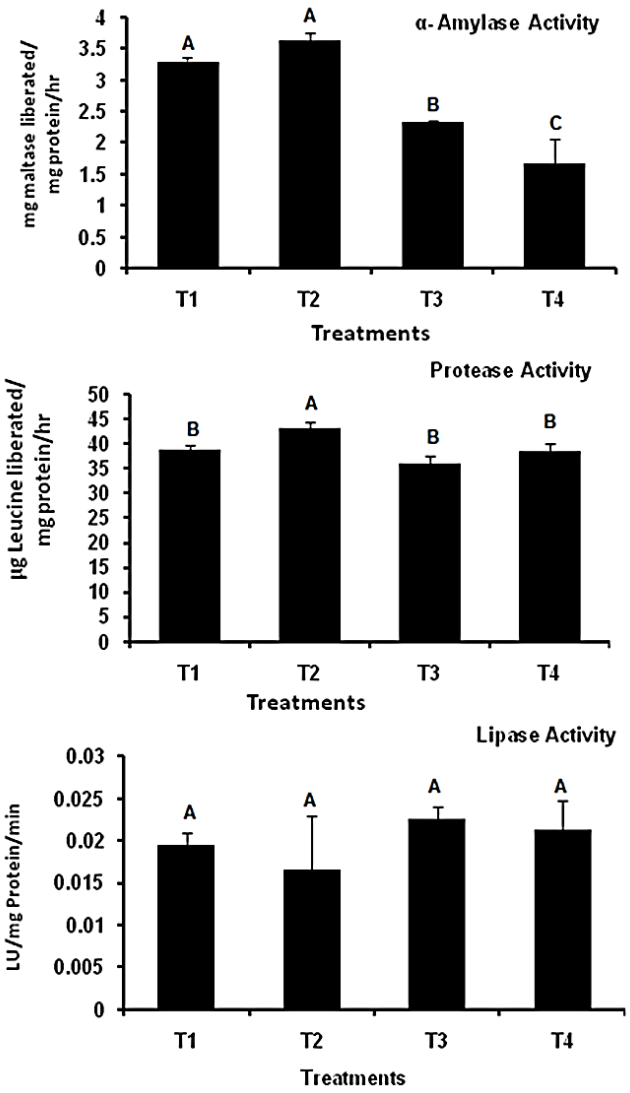

Figure 2. Digestive enzyme activities of *Heteropneutes fossilis* fed FILM supplemented feed. Data are mean of three replicates (n=9). The error bars indicate ± standard deviation. Different letters above the error bar indicate least significant difference (LSD) between two treatments at $P<0.05$

in APD (86-88%) when fed by plant-based feed (Kaviraj et al., 2013). However, *H. fossilis* fed silk worm pupae, an animal protein source, showed only 78.8 to 86.72% APD (Hossein and Jauncy, 1993), indicating that digestibility depended not only on the source of protein, whether animal or plant, but also on other factors like amino acid composition, proportion of lipid etc. Usmani et al. (2003) observed APD of *H. fossilis* to vary widely between practical feed ingredients with rice bran showing the least value (61.1%) and soybean meal the highest value (95.4%).

Interestingly, despite low digestibility and feed

intake rate *H. fossilis* grew well with FILM supplemented feed and deposited higher level of crude protein and crude lipid in the muscle of the fish as compared to fishmeal-based control feed (T1). FM based feed is always balanced in amino acids and superior to feed containing fermented products as alternative to FM (Samaddar et al., 2015, 2020). But fermentation results in hydrolyzed nature of the amino acids and most of the catfish can quickly accumulate a high level of amino acids from the feed containing fermented products without any symptom of hyperaminoacedemia due to presence of a proper stomach (Dabrowski et al., 2010; Samaddar et al., 2020). In the present study, higher deposition of CP and increased DPR in fish fed FILM supplemented feed probably resulted from higher assimilation of amino acids in these treatment groups. *Heteropneustes fossilis* fed fermented fish offal supplemented feed also showed a tendency of accumulating lipid at a high rate, which provided a protein sparing effect (Mondal et al., 2008). Moreover, *H. fossilis* is known to consume and digest wide variety of foods (Chondar, 1999). It has been found that the fish grows well on detritus-based food (Kohli, 1996) or feed comprising of composted weed with crude protein level as low as 15-20% (Biswas and Kaviraj, 2003). The experimental tanks in the present study contained 4.0 cm thick bottom sediment, which was the store house of the uneaten feed and its subsequent transformation into nutrient rich detritus. *Heteropneutes fossilis* could well utilize this detritus, in addition to direct consumption of the experimental feed.

The results of the present study also indicate that based on WG, SGR and FCR, *H. fossilis* exhibits maximum growth in T3 (50% replacement level), although there is no significant difference of these parameters between T3 and T4. The fish exhibits maximum deposition of CP, CL and ash in T2 (25% replacement level), although CP and ash did not vary between T2-T4 treatments. There is also no significant difference in DPR between any two FILM supplemented feed (T2-T4). However, protease activity significantly increased in T2 and remaining similar in T3 and T4 as compared to control (T1). On the other hand, amylase activity was similar in T2, but decreased in T3 and T4 as compared to T1. It indicates that *H. fossilis* can utilize maximum amount of CP and carbohydrate of the feed when the feed is supplemented by FILM at low level (25%). Carbohydrate is easily available in feed, but utilization of carbohydrate by fish depends on level, source and complexity of carbohydrates as well as carbohydrate metabolizing enzymes (NRC, 1983; Mollah and Alam, 1990; Tung and Shiau, 1991; Wilson, 1994; Stone et al., 2003; Orire and Sadiku, 2014; Zhou et al., 2015). The results indicate that *H. fossilis* can utilize limited amount of carbohydrate of FILM (T2). Rahman et al. (2017) observed that *H. fossilis* could utilize dextrin better than glucose. Many herbivorous and omnivorous fishes have been found to use oligosaccharides and polysaccharides easier than disaccharides or monosaccharides (Tung and Shiau, 1991; Shiau and Peng, 1993). The implication is that fish meal can be supplemented up to 75% by the FILM in the formulation of feed for *H. fossilis*. But based on APD, growth parameters, nutrient deposition and digestive enzyme activities the best growth of *H. fossilis* can be obtained when fish meal replacement level is restricted to 25 to 50%.

Conclusions: The results of the present study reveal that the FILM is a potential fishmeal alternative in the formulation of feed for catfish *H. fossilis*. Despite low digestibility of this feed and low feed intake rate by *H. fossilis*, the fish grew well with FILM supplemented feed and deposited higher level of crude protein and crude lipid in the muscle of the fish as compared to fishmeal-based control feed, because the fish could better utilize the nutrients contained in the FILM supplemented feed through direct consumption as well as through detritus formed from the uneaten feed at the bottom of the experimental tanks. *Heteropneutes fossilis* could utilize maximum amount of CP and carbohydrate when replacement of fishmeal by the FILM was low. Based on APD, growth parameters and digestive enzyme activities it is concluded that 25 to 50% of fishmeal can be replaced by FILM in the formulation of feed for *H. fossilis.*

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