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Potential of tilapia (*Oreochromis mossambicus*) meal as a dietary protein source for early growth stages of fringe-lipped carp *Labeo fimbriatus* (Bloch, 1795)

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Abstract

Fishmeal is considered the optimum protein source for aquatic and land animal diets owing to its balanced amino acid profile, phospholipids and favourable fatty acid composition, as well as its outstanding palatability, easy digestion and absorption, The availability of fishmeal (FM) has rapidly decreased and its price has climbed in tandem, due to the constant fall in pelagic wild fish captures and the rising demand for livestock and aquaculture feeds. As a result, there has been a continuous hunt for substitute protein sources that would enable aquaculture to continue being both environmentally and economically viable. This study was conducted to evaluate the effectiveness of the unconventional protein source tilapia meal (TM) in the diet of *Labeo fimbriatus* for seed rearing. Two growth trials, one each with spawn and fry of *L. fibriatus* were conducted. Fish were fed with FM based diet or TM based diet. In Experiment 1, final weight was higher with FM diet. In Experiment 2, no difference was observed between the two diets in terms of growth, survival and condition factor. Similar values of survival and condition factor recorded in two treatments indicate the possibility of incorporation of TM as a protein source in the diet during larval rearing of fringe-lipped carp.

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Introduction

One of the main protein sources in aquaculture feed is fishmeal (FM), which is highly digestible and palatable and has a balanced amino acid content (Tacon *et al.,* 2012). About 70-75% of the global FM production is from small, oily, pelagic fish species and less than 25-30% comes from white fish offal (FAO, 2018). Of the annual world fish catch, about one-third is used as raw material for FM production. Oil sardines (*Sardinella longiceps*) are widely utilised in India to produce FM. Its protein level can range from 40 to 60%, depending on the raw material utilised in production. FM is regarded as a concentrated protein source that is simple to combine with other feed additives to retain the appropriate protein content. However, excessive use of FM in global aquaculture is reported to have negative impacts on wild stock (Naylor *et al.,* 2009). Between 2000 and 2018, there was a 26.50% decline in global FM production (Jannathulla, 2019). Fishmeal production is expected to reduce further in future due to several factors. FM's high price was caused by fewer wild fish captures, which in turn led to less FM being produced. Aquaculture sector is reported to consume >70% of the global FM produced (FAO, 2018). One crucial area that needs immediate attention is the availability of appropriate ingredients to replace FM in order to fulfill the growing demand for feed. High-quality feed ingredients are also necessary for herbivorous and omnivorous animals, particularly during crucial life stages like juveniles and broodstock. Alternative sources, which are locally accessible, economical, and high in protein

(Lim *et al.,* 2011), can be used to optimise the use of FM (Hardy, 2010; FAO, 2014). As a result, there is currently considerable interest in conducting research on assessing less expensive alternatives to FM. Because they are plentiful and less expensive, a variety of plant protein sources are being tested for farmed fish. However, a variety of issues, including their imbalanced amino acid profiles, excessive fiber content, anti-nutritional effects, poor digestibility and competition with use for human consumption, limit the inclusion of vegetable protein sources in fish feed (Sorensen *et al.,* 2011). Furthermore, excessive use of vegetable meal may have negative environmental effects, and so research into new and sustainable protein sources is necessary.

Considering the issues with protein sources of plant origin, research is being focussed on locally available protein sources of animal origin. *Oreochromis mossambicus* (Peters, 1852), the aquatic chicken of the 1960s (Ling, 1977), is widely distributed worldwide (Philippart and Ruwet, 1982; Arthington *et al.,* 1984). However, the fish has lost favour as a preferred aquaculture species due to its propensity to "stunt" and its general poor quality because of small size (Pullin, 1988). The species was introduced in India during 1952. It has spread throughout most of India's warm water bodies, including rivers, lakes, ponds, drainage systems, canals and reservoirs, as a result of its prolific spawning. Since it competes with farmed fish for food, space and dissolved oxygen, it is regarded as a nuisance species and is frequently and inadvertently produced in fish farms. In several other countries also it has established and has been declared an invasive pest (Welcome, 1984; Fortes, 2005; Russel *et al.,* 2012). It is a highly efficient coloniser due to its extensive ecological tolerances, morphological plasticity, serial spawning, extended breeding season, high recruit survival and generalist habitat needs. Once this fish has established a permanent home, it may be very difficult to remove, and the aquatic community may suffer collateral harm (Jhingran, 1988; Natarajan, 1988; Mangum and Madrigal, 1999; Vinson *et al.,* 2010). Maintenance management, in which the invader is kept at a low density by one or more control mechanisms, is a more suitable option than eradication. Use of this fish for meal production could be one such measure. Dried meal produced from tilapia (TM) is a rich source of animal protein (58% crude protein) and hence can be a potential protein source for producing low-cost fish feed. No literature is available on the use of TM as a protein source in fish diets. Studies conducted by Abdelghani (2003) indicated that meal produced from *Gambusia affinis* could replace fish meal in the diet of red tilapia fingerlings up to 50% without affecting growth and survival.

Since the process of growth and survival following the switch from endogenous to external feeding is complex, larval rearing is a significant and vital step in aquaculture. According to Cahu and Infante (2001), the various digestive systems work well before external feeding begins, but a prepared microdiet must satisfy the particular nutritional needs of fish larvae. Therefore, a proper diet that is easily accessible, easily digested, and supplies the nutrients needed to promote the fish's growth and overall health is crucial to the success of fry rearing (Howell *et al.,* 1998; Cahu and Infante, 2001).

This study was conducted owing to the lack of literature on the use of TM as a non-conventional protein source in carp diets, particularly for *L. fimbriatus* seed rearing.

Materials and methods

Two growth trials were carried out for the rearing of spawn to fry and fry to fingerlings, respectively, in indoor, 50 l, circular, aerated plastic tanks. In trial 1, fimbriatus spawn (2.30 mg, 6.53 mm) were raised @ 100 numbers per tank for 30 days. Tanks were maintained in triplicates for every treatment. The diets were fed daily once until they were satiated and were designed to include 40% crude protein (Sen *et al.,* 1978; Mohanty *et al.,* 1990). After three hours, the leftover feed if any, was siphoned out. The faeces in the aquarium were drained off each day before the fish were fed. Every day, freshwater was added to each tank to replace about half of the water.

Small tilapia from the culture ponds were dried and ground to make the TM used in this investigation. During the drying process, 73.88±0.95% of the biomass was lost as moisture, resulting in 26.12±0.95% dry matter. To get rid of components like skin fragments that couldn't be crushed, the ground biomass was sieved. TM contained 18.16±0.17% ash, 13.51±0.67% fat, 1.46±0.01% crude fibre and 58.08±1.11% crude protein. Liquid chromatography-mass spectrometry (LC-MS) was used to analyse the total amino acids of FM and TM (Table 1).

For six hours, 100 mg of the material was hydrolysed in 6 M HCl at 110°C. The residue was injected into a UPLC-MS/MS system (Waters, USA) after being diluted and filtered using a 0.2 µm nylon filter membrane. Gas chromatography-mass spectrometry (GC-MS) was used to profile fatty acids (Table 2). Chloroform/methanol (3:1 v/v) was used to extract the lipid from the test components (Folch *et al.,* 1957). Boron trifluoride (BF3) in methanol was used to saponify and methylate the isolated lipid (Morrison and Smith, 1964). By comparing the relative retention periods of FAME peaks with reference standards (Sigma-Aldrich, USA) and the spectra with those found in the Wiley and NIST-2007 spectral libraries, fatty acids were identified (Liu, 1994).

Table 1. Amino acid profile (mg g^{-1}, on dry matter basis) of fishmeal (FM) and tilapia meal (TM)

Amino acid	FM	TM
Arginine	36.21	12.42
Histidine	17.28	7.18
Isoleucine	20.80	18.26
Leucine	32.35	41.40
Lysine	13.14	22.19
Methionine	17.35	13.00
Phenylalanine	11.87	13.36
Threonine	9.72	4.22
Tryptophan	0.01	0.01
Valine	11.94	4.78
Cysteine	1.04	1.32
Glutamic acid	49.23	11.48
Tyrosine	7.76	8.10
Glycine	0.06	0.05
Alanine	20.92	13.98
Serine	37.30	7.36
Proline	30.29	11.86
Asparagine	2.85	2.08
Aspertic acid	24.64	4.60
Ethionine	0.04	0.04
Citrulline	0.53	0.20
Beta 3-4 dihydroxy phenyl alanine	0.08	0.12
Total	324.62	179.76

Table 2. Fatty acid profile (mg g^{-1}, on dry matter basis) of fishmeal (FM) and tilapia meal (TM)

Fatty acid	FM	TM
Lauric acid (C12:0)	0.001	0.001
Myristic acid (C14:0)	0.030	0.062
Palmitic acid (C16:0)	0.857	0.617
Stearic acid (C18:0)	0.226	0.125
Total of saturated fatty acids	1.115	0.806
Palmitoleic acid (C16:1)	0.127	0.149
Oleic acid (C18:1)	0.712	0.451
Eicosaenoic acid (C20:1)	0.024	0.056
Erucic acid (C22:1)	0.002	0.062
Total of mono-unsaturated fatty acids	0.864	0.717
Linolenic acid (C18:3)	0.258	0.125
Total of unsaturated fatty acids	1.122	0.843
Total of fatty acids	2.237	1.648

Table 3 lists the proximate makeup and constituent proportions of the experimental diets. The fishmeal used in the study was procured from a fishmeal manufacturer; the main raw materials of which being oil sardines and other small pelagic fish. A 0.5 mm fine-mesh screen was used to sift all of the ingredients. To make dough, the necessary amounts of each component were combined with hot water. To obtain uniformly sized pellets (2 mm), the dough was cooled, a vitamin-mineral mixture was added, and it was then pushed through a hand pelletiser. After being sun-dried, the pellets were sealed in airtight plastic bags and were powdered while feeding the fish.

In the second growth trial, 30 fimbriatus fry (31.43 mg, 1.61 cm) were raised in each tank for 60 days. Tanks were maintained in triplicate for every treatment. The diets were fed until they were satiated and were designed to include 35% crude protein. Table 4 lists the proximate makeup and constituent proportions of the experimental diets. The diets were made in the manner previously mentioned. The prepared 2 mm pellets were ground into a powder and fed to the fry. After 3 h, any leftover feed was quickly drained away. The faeces in the tub were drained off each day before the fish were fed. Every day, freshwater replenished around half of the water in each tub.

Table 3. Ingredient proportion (%) and proximate composition (n=3, mean±SD) of experimental diets (Growth trial 1)

Ingredient	FM diet	TM diet
Fish meal	32	0
Tilapia meal	0	38
Rice bran	28	22
Groundnut cake	30	30
Finger millet	8	8
Vitamin and mineral mixture	2	2
Proximate composition (%)		
Moisture	9.12±0.20	8.83±0.19
Crude protein	39.90±0.08	39.74±0.95
Fat	8.93±0.05	10.52±0.33
Ash	11.95±0.06	16.27±0.16
Crude fibre	9.50±0.12	7.33±0.04
NFE	20.59±0.36	18.30±0.22
Gross energy (kJ g^{-1})	16.03±0.10	16.00±0.38

Table 4. Ingredient proportion (%) and proximate composition (n=3, mean±SD) of experimental diets (Trial 2)

Ingredient	FM diet	TM diet
Fish meal	22	0
Tilapia meal	0	27
Rice bran	38	33
Groundnut cake	30	30
Finger millet	8	8
Vitamin and mineral mixture	2	2
Proximate composition (%)		
Moisture	9.27±0.01	8.79±0.23
Crude protein	35.19±0.16	35.19±0.15
Fat	10.13±0.06	11.66±0.12
Ash	11.88±0.08	15.52±0.17
Crude fibre	8.54±0.07	7.12±0.20
NFE	24.99±0.21	21.71±0.49
Gross energy (kJ g^{-1})	16.19±0.02	16.22±0.01

The proximate composition of the diets was examined as per AOAC (1995). The finely powdered sample was dried in an oven at 100°C until its weight remained constant in order to measure the moisture content using the thermo-gravimetric method. By burning the sample for six hours at 600°C in a muffle furnace, the amount of ash was estimated. By measuring the nitrogen concentration using the micro-Kjeldahl method and multiplying by a factor of 6.25, the crude protein % was determined. Petroleum ether (boiling point 40-60°C) was used to remove the fat, and acid digestion (1.25%) and alkali digestion (1.25%) were used to extract the crude fibre. Nitrogen free extract (NFE) was used to calculate the amount of carbohydrates using Hastings's difference method (1976). The NFE values of 22.6 kJ g^{-1} for protein, 38.9 kJ g^{-1} for fat and 17.2 kJ g^{-1} for carbohydrates were used to determine the feed's energy content (Mayes, 1990).

In growth trial 1, key water quality metrics were examined once a week and at fortnightly intervals in trial 2, following standard procedures (APHA, 1998). Fish were counted, weighed and measured for length once the growth trial was terminated. Condition factor (K) was determined using the formula: $K = 100W/L^3$ (Ricker, 1975), where K = Condition factor, W = Weight in g and L = Length in cm. For analysis of digestive enzyme activity, 30 fry from each treatment group (10 from each tank) were sacrificed in the trial 1 (Barlaya *et al.,* 2021). The fry were homogenised in ice cold conditions using distilled water (4 ml g^{-1}) and the homogenate was centrifuged at 10,000 *g* for 20 min at 4°C. Until it was needed again, the supernatant (raw enzyme extract) was kept in 1.5 ml aliquots at -20°C. The Lowry *et al.* (1951) method was used to measure the homogenate's total soluble protein. The method used to measure trypsin activity was that of Erlanger *et al.* (1961). The Kunitz casein digestion method was used to measure total proteolytic activity (1947). The 3-dinitro salicylic acid (DNS) technique (Rick and Stegbauer, 1974) was used to assess amylase activity and p-nitro phenyl acetate (PNPA) was used as the substrate for lipase activity (Licia *et al.,* 2006).

In trial 2, the guts of nine fingerlings, three from each tank were taken out at random from each treatment and placed in icy water. The next steps for enzyme assays were then carried out using the same methods described above after homogenisation. One-way

analysis of variance (ANOVA) was used to compare treatments for different parameters, and Duncan's multiple range test was used for comparison of treatment means (Duncan, 1955; Snedecor and Cochran, 1968).

Results

The amino acid and fatty acid composition of FM and TM are given in Tables 1 and 2, respectively. The total weight of amino acids (mg) per gram of ingredient was 325 for FM as compared to 180 for TM. However, the contribution of essential amino acids was 49% in FM and 69% in TM. Among the essential amino acids, the quantity of Arginine, Histidine, Threonine and Valine were comparatively less, while that of Leucine and Lysine were more in TM as compared to FM. The content of other essential amino acids was comparable between the two ingredients. Among the non-essential amino acids, TM had lower levels of glutamic acid, aspertic acid, alanine, serine and proline than FM.

The total quantity of fatty acids per gram of ingredient was 2.237 mg for FM and 1.648 mg for TM. Saturated fatty acids (SFAs), monounsaturated fatty acids (MUFAs), and polyunsaturated fatty acids (PUFAs) made up 49.84, 38.62 and 11.53% of FM and 48.91, 43.51 and 7.58% of TM 's total fatty acid composition. This indicates that the ingredients are comparable in terms of the quantity of unsaturated fatty acids (UFAs) (50.16% in FM and 51.09% in TM). While the UFAs, Eicosaenoic acid and Erucic acid and the SFA Myristic acid were more in TM, the UFAs, Linolenic acid and Oleic acid and the SFA Stearic acid were more in FM.

In trial 1, fry fed FM diets had a larger mean final weight than those fed TM diets (Table 5). Final length, survival and condition factor did not differ ($p>0.05$). Fish given the TM diet had lower ($p<0.05$) levels of amylase, lipase and proteases than fish fed the FM diet, according to the analysis of the digestive enzyme activity of whole fish (Fig. 1). In trial 2, the mean final weight, length, survival and condition factor did not differ ($p>0.05$) between the two diet regimens (Table 6). Analysis of the gut's digestive enzyme activity showed that the activity of trypsin and lipase was higher ($p<0.05$) in the gut of fish fed TM diet, but there was no difference ($p>0.05$) in the activity of total protease and amylase (Fig. 2).

Table 7 presents the water quality parameters recorded from experimental tanks during the study period.

Discussion

The values of water quality parameters estimated during the study period fell within the permissible range for growing carp seed (Chakraborty and Mirza, 2007; Jena *et al.,* 2011). The most important amino acid in fish diet is lysine, which is also typically the most limiting amino acid in plant proteins (Small and Soares, 2000; Ovie and Eze, 2013). It is not only essential for protein deposition, but also aids in calcium absorption, keeps blood arteries healthy, makes collagen, enzymes, antibodies and repairs tissues. Additionally, lysine is crucial for preserving the acid-base balance and osmotic pressure in body fluids (Chiu *et al.,* 1988). Supplementing with dietary lysine may cause weight gain (Khan and

Table 5. Growth and survival (mean ±SD) of *L. fimbriatus* fry at harvest (Trial 1)

Treatment	Final weight (mg)*	Final length (cm)	Survival (%)	Condition factor
FM diet	51.51±3.03[a]	1.71±0.15[a]	90±6[a]	1.03±0.02[a]
TM diet	41.50±2.72[b]	1.51±0.21[a]	87±8[a]	1.20±0.13[a]

*Initial weight and length were 2.30 mg and 6.53±0.86 mm, respectively.
Figures in the same column with same superscript do not differ significantly ($p>0.05$).

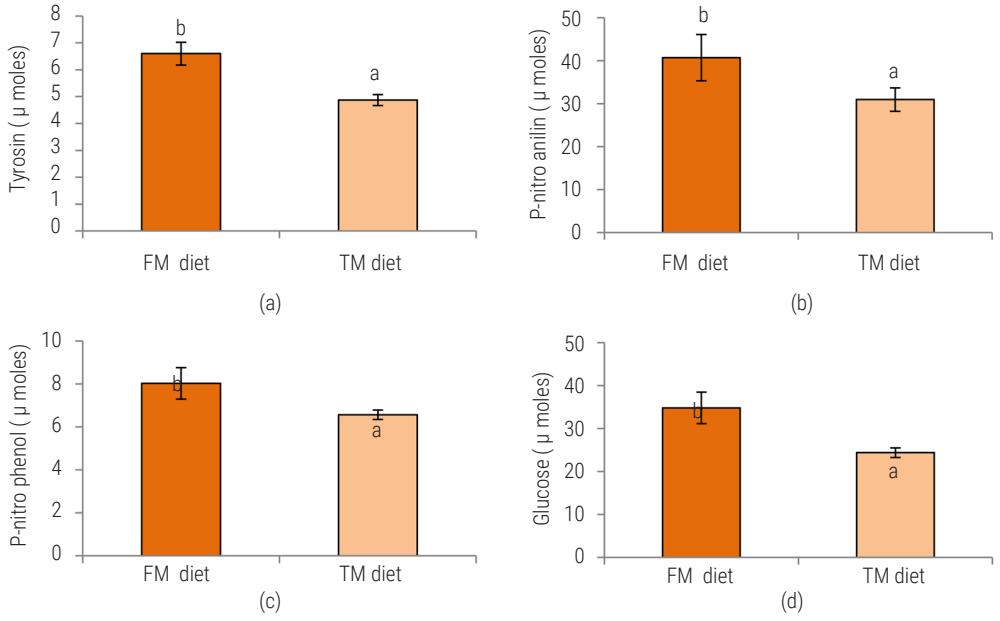

Fig. 1. Activity of digestive enzymes (mean±SD; µ moles of product liberated h^{-1} mg tissue $protein^{-1}$ at 37°C) in *L. fimbriatus* fry fed diets with different protein sources. Different alphabets on bars in the same graph indicate statistical difference ($p<0.05$). (a) Trypsin; (b) Total protease; (c) Lipase and (d) Amylase

Table 6. Growth and survival (mean±SD) of *L. fimbriatus* fingerlings at harvest (Trial 2)

Treatment	Final weight (mg)*	Final length (cm)	Survival (%)	Condition factor
FM diet	882.13±42.85[a]	4.29±0.22[a]	89±5[a]	1.12±0.15[a]
TM diet	1107.17±112.30[a]	4.55±0.25[a]	92±4[a]	1.18±0.08[a]

*Initial weight and length were 31.43±6.94 mg and 1.61±0.20 cm, respectively.
Figures in the same column with same superscript do not differ significantly (p>0.05).

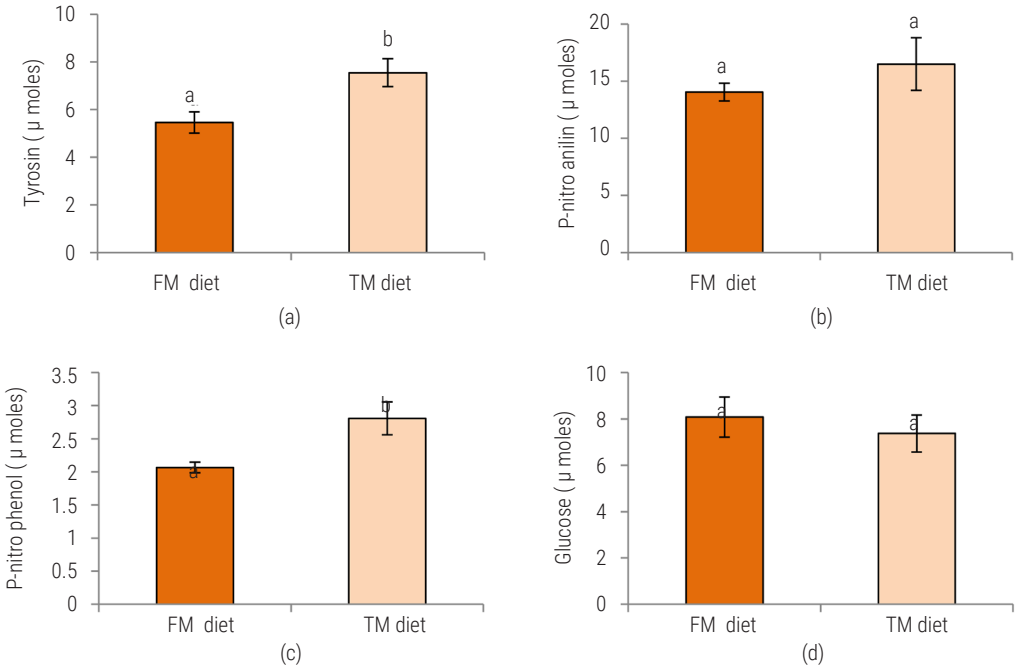

Fig. 2. Activity of digestive enzymes (mean±SD; µ moles of product liberated h^{-1} mg tissue $protein^{-1}$ at 37°C) in the gut of *L. fimbriatus* fingerlings fed different diets. Different alphabets on bars in the same graph indicate statistical difference (p<0.05). (a) Trypsin; (b) Total protease; (c) Lipase and (d) Amylase

Table 7. Water quality parameters recorded during the experimental duration

Trial	Temperature (°C)	pH	Total alkalinity (mg l^{-1})	Hardness (mg l^{-1})	Dissolved oxygen (mg l^{-1})	Ammonia (µg l^{-1})	Nitrate (µg l^{-1})	Phosphate (µg l^{-1})
Trial 1	24.8-25.0	8.09-8.34	127.84-176.72	132-184	5.68-9.6	1.46-52.03	868.18-2748.52	74.91-119.98
Trial 2	25.1-25.9	8.47-8.68	124.08-135.36	112-152	6.8-8.8	1.56-12.36	128.97-2654.21	11.11-95.71

Abidi, 2011; Yang *et al.,* 2011). Wang *et al.* (2020) has shown that in largemouth bass (*Micropterus salmoides*), dietary lysine controls body growth performance through nutrient-sensing signaling pathways. Additionally, it has been noted that the highest quantities of lysine are found in the skeleton of several fish species (Ahmed and Khan, 2004). The bone material of tilapia was crushed in the process of meal preparation in the present study, which could be atributed to the higher levels of Lysine compared to that in FM.

The essential amino acids, Leucine and Lysine were more in TM compared to FM. The branched chain amino acid Leucine increases pathways involved in muscle protein synthesis, inhibits protein degradation and hence is essential for protein synthesis in Indian major carps (Ahmed and Khan, 2006). A lack of Leucine in the diet caused growth retardation in a number of fish species (Ahmed and Khan, 2006; Abidi and Khan, 2007; Deng *et al.,* 2014; Farhat and Khan, 2014). Ren *et al.* (2015) reported that dietary Leucine level affects growth performance in juvenile blunt snout bream, *Megalobrama amblycephala*. Additionally, after bacterial infections, tench, *Tinca tinca*, showed improved growth, immunological response and survival when beta-hydroxyl-beta-methyl-butyrate, a product of leucine catabolism, was administered (Siwicki *et al.,* 2006). Since branched chain amino acids have a major impact on development, health and metabolism in human models, it stands to reason that they could also benefit fish (Andersen *et al.,* 2016).

Fatty acid analysis indicated that the level of SFAs and UFAs in FM was comparable. Alkuraieef *et al.* (2021) reported that Indian mackerel showed similar range of both SFAs and UFAs. We not only recorded differences in the fatty acid profile between FM and TM, but also lower levels of PUFA in TM. According to other studies, freshwater fish have reduced PUFA contents (Vlieg and Body, 1988). According to Suriah *et al.* (1995), finfish from seawater have a slightly different fatty acid composition than fish from freshwater.

TM diet performed poorly in terms of final weight compared to FM diet in Experiment 1, while there was no difference in growth in trial 2, indicating differences in digestion and assimilation of nutrients

from feed ingredients at different life stages. Similar observations were also recorded by Barlaya *et al.* (2021) in *L. fimbriatus* fed FM and silkworm pupae incorporated diets. Mean crude protein and fat content of TM used in the present study were 58.08 and 13.51%, respectively. The values were similar to that reported by Dale *et al.* (2004) for tilapia meal and Farahiyah *et al.* (2015) for fish offal meal. No literature was available comparing the growth of fish fed TM and FM incorporated diets. However, despite the fact that the contribution of essential amino acids was higher in TM compared to FM, it did not have any positive influence on the growth of fish under study. Ahmed (2008) reported reduced growth in red tilapia and Nile tilapia fry fed diets with gambusia meal replacing commercial FM completely. According to Hasan *et al.* (2012), tilapia (*Oreochromis niloticus*) fry feed can only be 50% replaced with meat and bone meal, while 100% substitution stunts growth. El-Sayed and Tacon (1997) found that meat and bone meal could replace up to 75% of the FM in diet of tilapia fry. Farahiyah *et al.* (2015) reported that substitution of 100% FM with fish offal meal, a byproduct from the fish processing industry, consisting of discarded parts like head, bones and internal organs has no negative or detrimental effect on the growth performance of tilapia (*Oreochromis* sp.).

Digestive enzymes are crucial for the breakdown and utilisation of nutrients in aquatic species' diet (Dabrowski and Glogowski, 1977) and the quality of food influences the activity of digestive enzymes and thereby, growth of fish (Fountoulaki *et al.,* 2005) even at larval stages (Suzer *et al.,* 2007a, b; Barlaya *et al.,* 2021). In the present study, the trend of final weight and the pattern of trypsin and total protease activity appeared to be identical, regardless of statistical significance. When kept in comparable settings, other investigators also found a direct correlation between fish weight and protease activity (Mitra *et al.,* 2008; Klahan *et al.,* 2009; Hidalgo *et al.,* 2011; Umalatha *et al.,* 2016). Comparing the quantum of activity, the activity of every enzyme, with the exception of trypsin, was higher in the first experiment than in the second, even though the tissue utilised for the crude enzyme extract was entire fish in the first experiment and gut in the second. According to reports, the fish's optimal digestive capacity happens in the early stages of growth (Poddubny, 1971; Klahan *et al.,* 2009). According to reports, protease, amylase and lipase activity are highest in the early stages of larval development and decrease as carps (Chakrabarti *et al.,* 2006; Chakrabarti and Rathore, 2009; Farhoudi *et al.,* 2013) and marine fish (Cahu and Infante, 2001) develop.

While systematic/published data is not available, it is a well known fact that tilapia is sometimes harvested in bulk in several freshwater aquaculture bodies like tanks and ponds with significant proportion of small-sized fish having no consumer value. They can be sun-dried, powdered and used as a protein source in carp diets. Similar final length, survival, and condition factors observed in the TM and FM treatments suggest that it is feasible to include this protein source in the diet of *L. fimbriatus* during spawn to fry and fry to fingerling rearing, as these factors are the most crucial during nursery rearing, leading to significant cost saving. Further studies on exploitation of this ingredient in the feed for grow-out culture of carps are warranted.

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