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Effect of dietary lipid levels on growth, nutrient digestibility and digestive enzyme activity of the peninsular carp *Hypselobarbus pulchellus* (Day, 1870)

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Abstract

Dietary lipid requirement of *Hypselobarbus pulchellus* was investigated through a feeding trial conducted for 90 days with five isonitrogenous diets formulated to contain lipid levels ranging from 2.5 to 12.5%. Fingerlings (initial weight 1.33±0.06 g and length 5.14±0.32 cm) were fed to apparent satiation twice daily. The final weight and length gain (%) decreased at 12.5% lipid incorporation level with no difference among other treatments. Food conversion and protein efficiency ratios of the experimental diets were the best with 2.5% diet and decreased significantly with 12.5% diet. Fish carcass crude protein values reduced and fat values increased at 10% lipid feeding. Crude protein digestibility was higher with diets 2.5, 5.0 and 7.5% compared to 10 and 12.5% lipid levels. Fat digestibility value was lowest under 2.5% lipid level and comparable among other treatments. Highest activity of gut protease, amylase and lipase was observed at 7.5% lipid level with the lowest activity of protease and amylase at 12.5% level and that of lipase at 2.5% level. The study indicated that the species has relatively low capacity to endure high-energy intakes. Nevertheless, considering the higher activity of digestive enzymes and digestibility of dry matter and crude protein, a dietary fat level ranging between 5 to 7.5% is recommended for the species. A second- order polynomial regression analysis indicated an optimum dietary lipid level of 6.6% in the feed for the species.

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Introduction

Dietary lipid is essential for fish as a source of energy, essential fatty acids, phospholipids, sterols and fat-soluble vitamins and to maintain biological structure as well as normal function of cell membranes (Sargent *et al*., 1999; Glencross, 2009). Moreover, incorporation of proper amount of lipid in the feed is important as lipid level determines the palatability of the diet (Boonyaratpalin, 1991). Dietary lipid was also reported to bring protein sparing effect, so that protein be utilised for the synthesis of muscle tissue and not for metabolic energy (Williams *et al*., 2003; Ozorio *et al*., 2006), reducing organic matter and nitrogenous waste input into culture systems (Miller *et al*., 2005). Supplementation of energy yielding nutrients, mainly lipid, has been suggested as a strategy to improve protein utilisation in fish (Sankian *et al*., 2017). However, a few authors have observed no protein sparing effect of lipid but an increased fat deposition in some fish species (Andersen and Alsted, 1993; Regost *et al*., 2001). Excessive lipid in the diet not only suppresses fatty acid synthesis, but also reduces the ability of fish to digest and assimilate lipids (Sargent *et al*., 1999). Therefore, proper level of lipid in the diet is necessary to achieve high growth rate and feed conversion efficiency. It is essential that the effect of varying dietary lipid levels is evaluated carefully before recommending a dietary lipid level as optimal for a new species.

The effects of dietary lipid levels on growth have been studied in many fish species, such as Indian butter catfish, *Ompok bimaculatus* (Paul *et al*., 2021),

common carp *Cyprinus carpio* (Aminikhoei *et al*., 2015), grass carp (*Ctenopharyngodon idella*) (Du *et al*., 2005; Gao *et al*., 2011), minor carp, *Barbonymus gonionotus* (Paul *et al*., 2010), silver barb *Puntius gonionotus* (Mohanta *et al*., 2009), Crucian carp (*Carassius auratus*) (Wang *et al*., 2008; 2014), dark barbel catfish (*Pelteobagrus vachelli*) (Zheng *et al*., 2010), white sea bass (*Atractoscion nobilis*) (Lopez *et al*., 2009) and cobia (*Rachycentron canadum*) (Wang *et al*., 2005). The gross lipid requirement of Indian major carps is estimated to be around 7 to 8% of the diet (Murthy, 2002).

Hypselobarbus pulchellus (Day, 1870) earlier called *Puntius pulchellus*, endemic to the peninsular rivers of India, has presently declined to the status of a 'Critically Endangered' species (Devi and Ali, 2011). This fish, which is capable of attaining 8 kg could become a welcome addition to pond culture practices, especially for composite fish culture (David and Rahman, 1975). Breeding technology of the species has been standardised (Sridhar *et al*., 2014) and the dietary protein requirement optimised (Barlaya *et al*., 2022). No information is available on the effect of varied levels of dietary lipid on the growth performance of *H. pulchellus* and this information is essential for developing practical diets for this species under culture. Hence the present study was conducted. Additionally, effect of dietary lipid levels on carcass proximate composition, digestive enzyme activity in the gut and *in vivo* digestibility of the experimental diets were also evaluated.

Materials and methods

Experimental diets

Five experimental diets were formulated and prepared to contain lipid levels ranging from 2.5 to 12.5% using pure ingredients (Table 1). All the ingredients except gelatin, oils and vitamin and mineral mixture were mixed thoroughly using a mechanical mixer. To this, required quantity of gelatin dissolved in hot water was added, mixed, allowed to cool to room temperature, followed by addition of cod liver oil, sunflower oil and vitamin and mineral mixture. The dough thus obtained was pressed through a hand pelletiser fitted with a 1 mm die and the extruded noodles were sun dried to have moisture level less than 10%. The dried feed was stored in air-tight bags till further use.

Experimental set up

Uniform sized *H. pulchellus* fingerlings (initial weight and length 1.33±0.06 g and 5.14±0.32 cm) were weighed in groups of 10 fish each and distributed into 15 aerated tanks (50 l). Only 10 fish each were stocked in tanks considering the water volume and standing water. Triplicate tanks were maintained for each treatment. Fish were fed to apparent satiation twice daily, at 09.00 and 15.00 hrs. Unconsumed pellets were siphoned out at the end of the feeding period of 1 h. The same were dried and pooled tank-wise and taken into account while calculating the FCR. On the following day, faecal matter collected from each tank by filtering through a 15 µm mesh nylon cloth was dried, pooled and stored (Barlaya *et al*., 2021a, 2022) for proximate analysis (AOAC, 2005). About 50% of water from each tank was replaced with freshwater every day after faecal matter collection. Feeding trial was conducted for a period of 90 days.

Proximate composition, water quality and fish growth analyses

Proximate composition of experimental diets was analysed as per AOAC (2005) (Table 1). Carbohydrate content was calculated as nitrogen free extract (NFE) by the 'Difference method' of Hastings (1976). Energy content of the feed was calculated using the values of 22.6 kJ g^{-1} for protein, 38.9 kJ g^{-1} for lipid and 17.2 kJ g^{-1} for carbohydrate as NFE (Mayes, 1990). Water quality parameters were analysed for pH, temperature, dissolved oxygen and total alkalinity at fortnightly intervals following standard methods (APHA, 2005). At the end of the rearing period of 90 days, length and weight of the fishes were measured. The weight gained by the fingerlings, feed conversion ratio (FCR) and protein efficiency ratio (PER) for each

Table 1. Ingredient proportion (%) and proximate composition (%, mean± SD) of experimental diets

	2.5%L	5.0%L	7.5%L	10.0%L	12.5%L
Ingredients used					
Casein	30	30	30	30	30
Gelatin	6.5	6.5	6.5	6.5	6.5
Dextrin	33	33	33	33	33
Cod liver oil	1.25	2.50	3.75	5.00	6.25
Sunflower oil	1.25	2.50	3.75	5.00	6.25
Carboxymethyl cellulose	2	2	2	2	2
Vitamin mineral mix	2	2	2	2	2
Cellulose	24	21.5	19	16.5	14
Proximate composition (%)					
Moisture	3.74±0.01	3.60±0.33	2.71±0.24	2.52±0.18	2.34±0.16
Crude protein	33.78±1.26	33.41±1.47	33.54±0.36	33.35±1.09	33.46±1.63
Fat	2.47±0.21	4.95±0.13	7.39±0.13	9.87±0.08	12.49±0.42
Ash	4.45±0.01	4.56±0.02	4.62±0.05	4.85±0.06	4.74±0.09
Crude fiber	25.07±1.31	22.49±0.44	20.23±0.22	17.67±0.47	14.83±0.26
Nitrogen-free extract	30.49±1.63	30.99±2.01	31.52±1.17	31.74±1.69	32.14±1.44
Gross energy (kJ g^{-1})	13.84	14.81	15.87	16.83	17.95
P/E ratio (g protein kJ^{-1})	24.24	22.36	21.29	19.57	18.41

feed were calculated. Nine fish from each treatment were dried and analysed for carcass proximate composition (AOAC, 2005).

Estimation of apparent digestibility coefficient and digestive enzyme activity

Crude fibre in diet and faecal matter was used as the reference marker (De Silva and Anderson, 1995; Morales, 1999; Krontveit *et al.,* 2014) for calculation of protein and fat apparent digestibility coefficient values of the experimental diets (Maynard and Loosli, 1972).

Nutrient digestibility (%) = 100 - 100 × [% Crude fibre in feed ÷ % Crude fibre in faeces] × [% Nutrient in faeces ÷ % Nutrient in feed]

Gut of six fish from each treatment were dissected out and were individually macerated with ice cold distilled water (gut wt: volume of distilled water; 1:4) and centrifuged in a refrigerated centrifuge at 10,000 *g* for 20 min. The supernatant enzyme extract was collected and the pellets were re-suspended in equal volume of cold distilled water and centrifuged again as before. The washing procedure was repeated and all the extracts collected as supernatant were pooled. The crude enzyme extract thus obtained were divided into 1 ml aliquots and stored at -20ºC. All extraction procedures were carried out at 4ºC. Protein in the crude enzyme extract was estimated according to Lowry *et al*. (1951) using Bovine serum albumin as standard. Total proteolytic activity was determined by the casein digestion method of Kunitz (1947). The assay mixture contained 0.1 ml crude enzyme extract plus 2.0 ml of casein buffer substrate and was incubated at 25ºC for 15 min. The resulting tyrosine was determined using tyrosine as the standard. One unit of protease activity is equivalent to 1 micro mole of tyrosine liberated per hour at 25ºC. Amylase activity was estimated by using 1% starch solution in Tris-HCl buffer (0.1 M, pH 7.0) as the substrate (Rick and Stegbauer, 1974). The assay mixture contained 0.1 ml crude enzyme extract and 1.0 ml of substrate. Incubation was carried out at 25ºC for 10 min. The resulting reducing sugars were determined using maltose as the standard. One unit of amylase activity is equivalent to 1 micro mole of maltose liberated per hour at 25ºC. Lipase activity was determined by the titrimetric method of Bier (1955), which is based on the measurement of fatty acids released by the enzymatic hydrolysis of triglycerides present in a stabilised emulsion of olive oil. One unit of lipase activity is equivalent to 1 nano mole of fatty acids liberated per minute at 25°C. The activities of enzymes were expressed as specific activity.

Statistical analyses

All the data were presented as the mean values ± standard deviation of three replicates. One-way ANOVA followed by Duncan's multiple range test ($p<0.05$) (Duncan, 1955) were applied to compare the differences among the five dietary groups. The data were statistically analysed by SPSS 21.0 for windows. A second order polynomial regression analysis was conducted to analyse the weight gain of the fish in response to dietary fat level.

Results

Water quality parameters analysed during the study period and their range were: Temperature 23.7-24.7ºC, pH 7.95-8.40, Total alkalinity 204.06-223.44 ppm, Hardness 192.75-206.89 ppm and Dissolved oxygen 5.85-7.25 ppm.

The growth parameters recorded are given in Table 2. The final weight and length decreased at 12.5% lipid incorporation level with no significant difference ($p>0.05$) among the other treatments. A second order polynomial regression analysis indicated an optimum dietary lipid level of 6.6% in the feed for the species (Fig. 1). Food conversion and protein efficiency ratio of the experimental diets were the best with 2.5% L diet and decreased significantly with 12.5% L diet. Condition factor did not differ among treatments. Proximate composition analysis of fish carcass revealed no difference in moisture and ash contents (Table 3). However, compared to other treatments, crude protein values reduced and fat values increased at 10% lipid feeding. Activity pattern of digestive enzymes assessed at the end of the study period is given in Fig. 2. Highest activity of all the enzymes was observed at 7.5% lipid level with the lowest activity of protease and amylase at 12.5% level and that of lipase at 2.5% level. Crude protein digestibility was higher with diets 2.5, 5.0 and 7.5% L compared to 10 and 12.5% L (Fig. 3). Fat digestibility value was lowest ($p<0.05$) under 2.5% L and comparable ($p>0.05$) among other treatments.

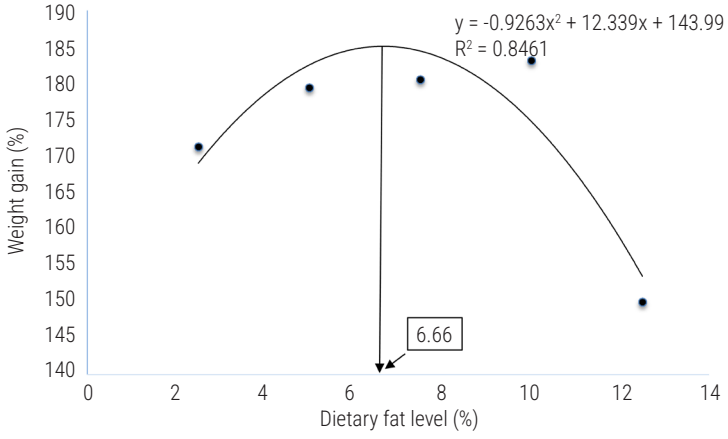

Fig. 1. Relationship between weight gain and dietary fat level for *H. pulchellus* as indicated by second-order polynomial regression

Table 2. Growth parameters (mean ± SD) of *H. pulchellus*

Parameter	2.5%L	5.0%L	7.5%L	10.0%L	12.5%L
Final weight (g)	3.71±0.09^{a}	3.72±0.09^{a}	3.73±0.02^{a}	3.68±0.13^{a}	3.40±0.05^{b}
Weight gain (%)	171.27±8.53^{a}	179.39±17.58^{a}	180.50±13.18^{a}	180.93±10.34^{a}	149.97±16.72^{b}
Length gain (%)	607.33±15.95^{a}	610.33±6.11^{a}	610.33±3.21^{a}	602.00±7.55^{ab}	582.33±4.04^{b}
FCR	2.74±0.28^{a}	2.79±0.20^{a}	2.82±0.10^{a}	3.05±0.18^{ab}	3.38±0.20^{b}
PER	1.09±0.12^{a}	1.06±0.07^{ab}	1.05±0.04^{ab}	0.97±0.06^{ab}	0.88±0.05^{b}
Condition factor	1.05±0.05	1.04±0.01	1.04±0.01	1.13±0.12	1.07±0.01

Initial mean weight and length of fish stocked were 1.33±0.06 g and 5.14±0.32 cm respectively.
Figures in the same row with different superscripts are significantly different (p<0.05).

Table 3. Carcass composition (%, on wet weight basis, mean±SD) of *H. pulchellus*

Diets	Moisture	Crude protein	Fat	Ash
2.5%L	75.39±0.64^{a}	15.84±0.17^{a}	2.77±0.01^{a}	3.86±0.01^{a}
5.0%L	75.32±0.46^{a}	15.76±0.12^{a}	3.12±0.13^{a}	3.84±0.01^{a}
7.5%L	75.99±0.43^{a}	15.58±0.14^{a}	3.15±0.14^{a}	3.69±0.04^{a}
10.0%L	75.65±1.36^{a}	14.86±0.01^{b}	3.78±0.16^{b}	3.65±0.20^{a}
12.5%L	75.58±0.23^{a}	14.88±0.15^{b}	4.22±0.12^{b}	3.82±0.02^{a}

Values with the same superscripts in a column are not statistically different (p>0.05)

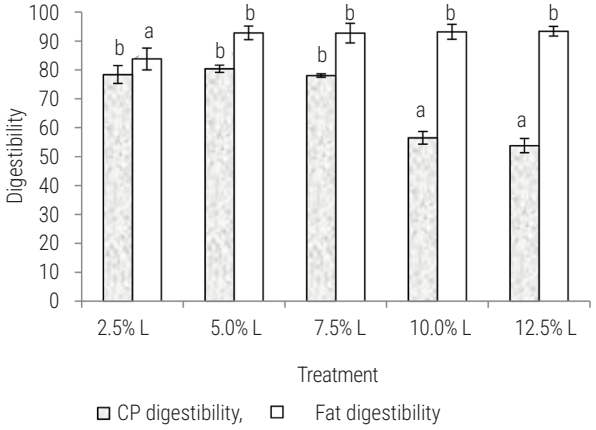

Fig. 3. Apparent digestibility coefficients (mean±SD) of crude protein (CP) and fat in the diets fed to *H. pulchellus*. Different alphabets on bars for the same nutrient indicate significant difference (p<0.05)

Discussion

Growth parameters

The water quality parameters were within the acceptable range for the species studied (Barlaya *et al*., 2021b) and did not show significant (p>0.05) difference between the treatments. The overall weight increment of the fish during the study period was less than 3 times of the initial weight. The fish falls under the category of 'medium carps' owing to its reduced growth in comparison to major carps (Mohanta *et al*., 2008). Barlaya *et al*. (2022) also recorded a similar growth in an indoor experiment conducted with the same species under comparable conditions, justifying the growth of the test species in the present study. In the indoor experiment conducted to determine the effect of dietary lipid levels on growth of juvenile grass carp, Du *et al*. (2005) recorded a weight gain between 81.64 and 148.52% as against the weight gain of 149.97-180.93%, recorded in the present study.

Improvement of growth performance with increasing dietary lipid level was observed in several earlier studies (Gangadhara *et al*., 1997; Harpaz *et al*., 1999; Lee *et al*., 2002; Manjappa *et al*., 2011). Increasing dietary lipid levels improves the diet efficiency (Johnsen *et al*., 1993; Peres and Oli´va-Teles, 1999a) by minimising protein degradation (Beamish and Medland, 1986; Kim *et al*., 2012). Our result was in compliance with the earlier report indicating that higher dietary lipid levels can lead to poor feed conversion and protein efficiency (Du et al., 2005). Positive correlation between growth and feed/protein efficiency has also been recorded in grass carp (Gao *et al*., 2011; Jin *et al*., 2013) and tilapia (*O. noloticus* × *O. aureus*) (Gao *et al*., 2011). Improvement in growth and feed efficiency with increase in dietary lipid content is, however, not universal. Chou and Shiau (1996) reported that there were no significant differences in growth, FCR and PER of hybrid tilapia fed 5, 10 and 15% lipid diets. Hanley (1991)) also reported no protein sparing effect of dietary lipid in Nile tilapia fed diets with varied lipid levels. Peres and Oli´va-Teles (1999b) believed that this lack of protein-sparing effect by dietary lipid may be related to the high/adequate protein levels of the diet. According to Dias *et al*. (1998), the beneficial effects

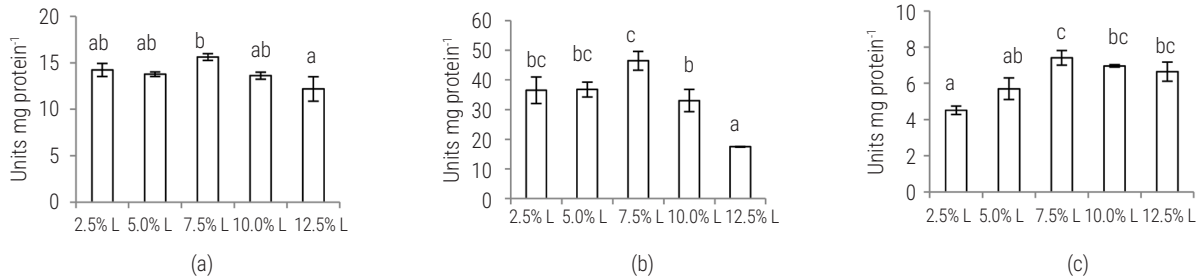

Fig. 2. Activity (mean±SD) of digestive enzymes in the gut of *H. pulchellus* fed experimental diets. (a) Amylase; (b) Protease and (c) Lipase. Different alphabets on bars in the same graph indicate significant difference (p<0.05)

of an increase in lipid level in sea bass diets were significant only with a low-protein diet. In the present study also, the reason for no improvement in growth of pulchellus when the dietary lipid level was increased from 2.5 to 10% may be attributed to the fact that the diet used had uniform crude protein content of 33%, which is optimal for the species (Barlaya *et al*., 2022). These results suggest that 2.5% dietary lipid may meet the minimum lipid requirement of the fish. Du *et al*. (2005) observed best growth performance and feed utilisation in grass carp *Ctenopharyngodon idella* fed low dietary lipid level of 2-4% and attributed it to the natural food of grass carp (water plants), with very low content of usable lipid. *H. pulchellus* is considered to be the only indigenous fish consuming aquatic weeds and submerged grasses both in the juvenile and adult stages (David and Rahman, 1975, 1982). Based on its reported consumption of aquatic weeds like *Chara*, *Hydrilla*, *Vallisneria,* grass, water hyacinth (*Eichhornia* sp.) roots and filamentous algae, this fish is placed next to grass carp (Hora, 1955; Hickling, 1962). Accordingly, it is presumed that under natural conditions this fish feeds mainly on aquatic vegetation having very low lipid content, hence the low lipid requirement.

Dietary lipid level of 2.5 - 10% used in the present study was providing sufficient amount of metabolisable energy for growth of pulchellus and a further increase in dietary lipid level had a negative effect on growth. Decline in growth performance and feed utilisation with increasing dietary lipids above 4% have been reported in grass carp (Du *et al*., 2005), common carp (Murai *et al* ., 1985), trout (Regost *et al*., 2001) and salmon (Silverstein *et al* l., 1999). Negative effects of high lipid levels on growth performance have also been reported for the Indian butter catfish, *O. bimaculatus* (Paul *et al*., 2021), Crucian carp (Wang *et al*., 2014), Japanese sea bass (Xu *et al*., 2011), tiger puffer (Kotaro *et al.,* 2009), cobia (Wang *et al.*, 2005), halibut (Nortvedt and Tuene, 1998) and red drum (Ellis and Reich, 1991).

Aminikhoei *et al*. (2015) recorded a positive correlation between P/E ratio (g protein kJ^{-1}) and growth of juvenile Israeli carp *C. carpio* with values ranging from 21.5 to 27.3 resulting in higher growth compared to lower values (11.4 to 18.6). In the present study also, P/E ranging from 21.29 to 24.24 resulted in higher growth compared to 18.41 to 19.57, which is similar to the ratio of 23-27, reported for sunshine bass (Keembiyehetty and Wilson, 1998) and tilapia and grass carp (Gao *et al*., 2011) but higher than 20, reported for silver barb *P. gonionotus* (Mohanta *et al*., 2009) and bagrid catfish *Mystus nemurus* (Ng *et al*., 2001) and lower than 29-31, reported for juvenile Chinese sucker *Myxocyprinus asiaticus* (Yuan *et al*., 2009). The apparent differences in the optimum P/E ratio among fish species indicate that adequate levels of protein and energy in the diets must be carefully considered when optimum P/E ratio is estimated for a given species. The P/E ratios for optimum growth of several fish species ranged from 19 to 27 (NRC, 1993).

Carcass proximate composition

In fish, besides geographic location, age, sex and maturity, the feed consumed is regarded as one of the primary factors that influence its carcass composition (Jobling, 2001; Aryani *et al*., 2017). An inverse relationship between carcass moisture and lipid content on feeding varied levels of dietary lipid was observed in grass carp (Du *et al.,* 2005), European sea bass (Peres and Oliva-Teles, 1999b), turbot (Andersen and Alsted, 1993), salmonids (Arzel *et al*., 1994; Alvarez *et al.,* 1998), gilthead sea bream (Vergara *et al*., 1996, 1999) and Atlantic halibut (Aksnes *et al*., 1996). Increase of dietary lipid levels is usually associated with an increased lipid deposit in the body (Martino *et al*., 2002; Satpathy *et al*., 2003). However, in the present study, the carcass lipid levels increased with dietary lipid levels without any change in the moisture level. Similar to our observation, Gao *et al*. (2011) and Koprucu (2012) in grass carp and Manjappa *et al*. (2011) in common carp, did not observe any change in carcass moisture content but an increase in carcass lipid content, when fed increasing levels of dietary lipid. The positive correlation between carcass lipid content and dietary lipid indicates that when dietary lipid is supplied in excess, higher lipid levels are not utilised by the fish for energy and a proportion of this lipid is deposited on viscera or the carcass (Shiau and Huang, 1990). Sridhar (2017) reported visceral fat deposition and its negative effect on breeding performance in *H. pulchellus* maintained in a confined pond environment and fed the traditional feed containing rice bran and groundnut oil cake. Similar results were observed in studies with common carp *C. carpio* (Manjappa *et al.,* 2002), grass carp and tilapia (Gao *et al*., 2011), grouper *Epinephelus malabaricus* (Williams, 2007), cobia *R. canadum* (Craig *et al*., 2006), grass carp (Du *et al*., 2005), grass carp and hybrid tilapia (Chou and Shiau, 1996; Du *et al*., 2005; Gao *et al*., 2011), Eurasian perch *Perca fluviatilis* (Mathis *et al*., 2003) and rockfish *Sebastes schlegelii* (Lee *et al*., 2002). Raghunath *et al*. (2017) reported that *H. pulchellus* is a medium fat fish. The decrease in carcass crude protein content and increased fat deposition with increased incorporation of dietary lipid as observed in the present study, was also reported by Silver *et al*. (1991) in chinook salmon (*Oncorhynchus tshawytscha*) and Gao *et al*. (2011) in tilapia (*Oreochomis niloticus* x *O. aureus*). Hence it is apparent that the excess energy is stored in the form of fat and not protein in *H. pulchellus*.

Digestive enzyme activity and digestibility

Nutrient utilisation in fish is indicated by the activity of digestive enzymes that ultimately affect the growth and development of fish (Chen and Zhang, 2004; Wei *et al*., 2010; Mandal and Ghosh, 2018). The trend in the activity pattern of protease and amylase was almost similar to the trend in growth of *H. pulchellus*, with the lowest activity and growth at 12.5% lipid level. Earlier reports also indicate a direct relationship between the activity of proteases and fish weight when maintained under similar conditions (Mitra *et al*., 2008; Klahan *et al*., 2009; Hidalgo *et al*., 2011; Umalatha *et al*., 2016; Siddiqua and Khan, 2022;).

Excepting the higher activity at 7.5%, the values for protease at dietary lipid levels 2.5, 5 and 10% and that of amylase at dietary lipid levels 2.5, 5.0, 10 and 12.5% were comparable. This is attributable to similar ($p>0.05$) crude protein and available carbohydrate (NFE) content of experimental diets. Manjappa *et al*. (2011) reported no difference in amylase in *C. carpio* fed increasing lipid levels. Several other studies also revealed that the relative activity of the digestive enzymes can be correlated with the biochemical composition of the food consumed (Kuzmina, 1996; Gangadhara *et al*., 1997; Fountoulaki *et al*., 2005). A direct relationship between lipase activity and dietary lipid levels, as observed in the present study, was also reported in *Tor khudree* (Bazza and Keshavanath, 1993), *L. rohita* (Gangadhar *et al*., 1997), *Dicentrarchus labrax* (Peres and Oliva-Teles, 1999) and *O. niloticus* × *O. aureus* (Gao *et al*., 2011).

The decreased activity of digestive enzymes, protease and amylase and stagnated values of lipase at 10 and 12.5% levels indicate negative effect of higher levels of dietary lipid on digestive enzyme activity in *H. pulchellus*. Fountoulaki *et al*. (2005) and Paul *et al*. (2021) reported that amylase activity in *O. bimaculatus* larvae and *Sparus aurata* was significantly decreased with elevated dietary lipid levels. Manjappa *et al*. (2002 and 2011) reported decrease in protease in the gut of *C. carpio* fed increasing lipid levels. It is apparent from the present study that dietary lipid levels beyond 7.5% is not advisable for *H. pulchellus,* a herbivorous fish, whose natural food is aquatic plants and vegetation, having very low amounts of usable lipids. Adaptations of the digestive system in different species exhibit close association with their diet (Fernandez *et al*., 2001).

Activity pattern of protease and lipase corroborated the apparent digestibility coefficient values for protein and fat. Crude protein digestibility was higher with diets 2.5, 5.0 and 7.5% L compared to 10 and 12.5% L. Digestibility of protein decreased significantly with an increase in dietary lipid level from 7.5%, which probably is one of the reasons for the lower protein accretion as indicated by the carcass protein content and ultimately the poor growth performance of *H. pulchellus* fed 12.5% lipid diet. Koprucu (2012) observed similar phenomenon in grass carp fed diets having lipid ranging from 4 to 8%. The digestibilities of protein and lipid for a diet with high lipid seems to differ among fish species. Hernandez *et al*. (2001) reported no decrease in protein digestibility but an increase of lipid digestibility with an increase in dietary lipid level in sharp snout sea bream (*Diplodus puntazzo*). The second order polynomial regression analysis based on weight gain showed that the optimal dietary lipid level for *H. pulchellus* was 6.66%. Jin *et al*. (2013) recommend a dietary lipid of 6.5% for juvenile grass carp, although Du *et al.* (2005) reported that 4% dietary lipid could meet the requirement of juveniles.

In conclusion, the present study established that dietary lipid levels of 2.5 to 12.5% did not result in protein sparing in *H. pulchellus*. This species has relatively low capacity to endure high-energy intakes. Owing to low energy requirement, excess dietary lipid level should be avoided in the diet for *H. pulchellus*. Nevertheless, considering the higher activity of digestive enzymes and digestibility of dry matter and crude protein, a dietary fat level ranging between 5 to 7.5% is recommended for the species. Based on the findings of regression analysis, it is concluded that *H. pulchellus* fingerlings require a dietary fat level of 6.66%.

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