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Protein sparing capability of carbohydrate in fringed-lipped carp *Labeo fimbriatus* (Bloch, 1795)

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ABSTRACT

The study was undertaken to examine the capability of carbohydrate to spare protein in the diet of fringed lipped carp, *Labeo fimbriatus* (2.93 g±0.15). Three semi-purified experimental diets of iso-caloric nature were formulated having three graded levels of protein (27, 23 and 19% CP) and three levels of carbohydrate (26, 31.5 and 37% CHO). Accordingly, the treatments were designated as T₁ (27% CP and 26% CHO), T₂ (23% CP and 31.5% CHO) and T₃ (19% CP and 37% CHO), where the experimental diet T₁ served as the reference diet. The fish were reared in 200 l fibre reinforced plastic (FRP) tanks under continuous aeration with one third water exchanged daily. After 90 days of feeding trial, it was noticed that the percentage weight gain and specific growth rate (SGR) of 27% protein fed group (209.5±0.74% and 1.26±0.15%) and 23% protein fed group (204.8±0.52% and 1.24±0.13%) were not significantly different (p>0.05). Similarly, the results of feed performance like feed conversion ratio (FCR) among 27% protein fed group (2.24±0.11) and 23% protein fed group (2.29±0.14) did not vary significantly (p>0.05). On the other hand, the protein efficiency ratio (PER) and protein productive value (PPV) of 23% protein fed group (1.87±0.11 and 28.34±0.36) did not vary significantly (p>0.05) with the 19% protein fed group (1.93±0.12 and 27.65±0.32). The carcass protein (CP) and lipid (as ether extract, EE) content of 27% protein fed group and 23% protein fed group were not significantly different (p>0.05). Results of this study indicate that the CP level in the diet of fringed-lipped carp *L. fimbriatus* can be reduced from 27 to 23% by proportionately increasing carbohydrate level from 26 to 31.5% without compromising growth and also without any adverse physiological effect.

Keywords: Fringed-lipped carp, Growth, *Labeo fimbriatus*, Protein sparing effect, Semi-purified diets

Protein is an important component in the diet that affects growth, survival and yield of fish, as it provides the basic amino acids to synthesise body protein and supply energy for maintenance (Shiau and Huang, 1989; Islam and Tanaka, 2004). It is costly and hence influence greatly on the cost of most prepared feeds. It affects immensely on the diet economics as well as on the sustainability of fish farming practices (Hatlen *et al.*, 2005). The optimum protein requirement of any fish depends on its species, stage of growth, ambient temperature, salinity and other stress factors associated with culture. The optimum protein requirement is also influenced by protein energy ratio (P/E), digestibility and amino-acid profile of the protein source present in the diet (NRC, 1993). While, any protein content above optimum level is utilised for energy that results in increased nitrogenous waste as ammonia (Kaushik, 1998, Cho and Bureau, 2001; Kim *et al.*, 2004), protein content below optimum level causes decrease in growth (Kim *et al.*, 2004). Inclusion of ample amount of non-protein energy sources, like carbohydrate and lipid, in the diet could spare protein

to be used as an energy source. Though lipid is known as an important source of non-protein energy for fish (Kaushik *et al.*, 1989), carbohydrate forms the most economical source and is abundantly available throughout the world. Hence, information on optimum protein requirement of fish and the protein sparing effect of cost effective non-protein energy sources like carbohydrate may be useful to reduce the feed cost (Erfanullah and Jafri, 1995; Shiau and Lin, 2001).

Labeo fimbriatus (Bloch, 1795) is a native medium carp of central and peninsular India (Hora and Pillay, 1962) that is known as a potential candidate species for species diversification because of its appreciable growth potential and consumer choice (Jena *et al.*, 2009). The species has now been recognised as a potential endangered species because of its shrinking distribution in most of its parental riverine systems (Gopalkrishnan *et al.*, 1994). The fish is mainly herbivorous bottom feeder that feeds on diatoms, green and blue-green algae, macrophytes, insects and detritus (Talwar and Jhingarn, 1991). Although, demand for this promising species for aquaculture is growing day by day (Power

et al., 2009), study on its optimum nutritional requirement is limited. Jena *et al.* (2012) have reported the optimum dietary protein (DP) requirement of *L. fimbriatus* as 273.0 g kg⁻¹. As *L. fimbriatus* is a herbivorous fish, carbohydrate utilisation seems to be very important. Adequate knowledge on the influence of dietary carbohydrate on protein sparing effect, growth and physiological performance would be helpful in formulating a low-cost diet for this herbivorous fish. On this backdrop, the present study was carried out to investigate the influence of increasing dietary carbohydrate level by proportionately reducing dietary protein level on feed utilisation, nutrient retention, growth and carcass composition of fringed-lipped carp, *L. fimbriatus* and to determine the protein sparing capability of carbohydrate in this species.

For the experiment, 500 nos. of hatchery reared *L. fimbriatus* advanced fry were procured from Govt. Fish Farm, Kausalyaganga (20° 20' N; 85° 49' E), Bhubaneswar, Odisha. The fish were held in 2 nos. of 500 l fibre reinforced plastic (FRP) tanks under continuous aerations until 15 days for acclimatisation to laboratory rearing condition. The fish were fed twice a day with commercial floating fish feed (ABIS) at a rate of 5% of their total body weight per day before the start of feeding trial. The experiment was initiated with segregation of uniform sized fish fry (average body weight of 2.93±0.35 g) without any physical deformities and randomly assigned at a density of 20 fish per circular FRP tank (72.5 cm dia and 54.5 cm depth) of 200 l capacity containing 160 l bore-well water. The fish were maintained with continuous aeration under 12L:12D photoperiod cycle. Each experimental diet was fed to triplicate groups of fish twice daily at 4% of their body weight per day. Two third of the daily ration was given at 08 00 hrs and remaining one third at 16 00 hrs. The left out feed was collected after 2 h of feeding, dried and pooled fortnightly to find out the feed consumed. Bulk weights of fish were taken fortnightly to evaluate growth, survival and to increase the feeding ration with increase in biomass. One-third water from each tank was exchanged daily by siphoning to remove left over feed and faecal matter. The water quality parameters were analysed at weekly intervals (APHA, 1992). During the experimental period, the range in water quality parameters recorded in the experimental tanks were: pH 8.3-8.5, temperature 24.5-26.8°C, dissolved oxygen 4.8-6.2 mg l⁻¹, total alkalinity 220-250 mg CaCO₃ l⁻¹, total hardness 204-236 mg CaCO₃ l⁻¹, Phosphate 0.3-0.5 ppm, ammonia-N 0.1-0.2 ppm, nitrate-N 0.3-0.5 ppm and a trace of nitrite-N, that were considered to be in the favourable ranges for carp rearing (Ayyappan *et al.*, 2011).

Three semi-purified iso-caloric experimental diets were formulated containing three graded levels of crude protein (*i.e.*, 27, 23 and 19%) and three levels of carbohydrate

(*i.e.*, 26, 31.5 and 37%), such that the reduction of energy due to reduced protein level is compensated by increased carbohydrate level in the diet. The diets were designated as T₁ (27% CP and 26% CHO), T₂ (23% CP and 31.5% CHO) and T₃ (19% CP and 37% CHO), where CP and CHO indicates crude protein and carbohydrate, respectively. The diet T₁ was considered as the reference diet, where the CP level was kept at 27%, being the optimum requirement for this species (Jena *et al.*, 2012) and carbohydrate level was maintained at 26%, being the optimum carbohydrate requirement for carps (Sen *et al.*, 1978; Erafanullah and Jafri, 1995), since no literature is available at present on the carbohydrate requirement for this species. Casein (vitamin free) with 85% CP and gelatin with 90% CP served as the source of protein. They were mixed at casein: gelatin ratio of 4:1, so as to maintain appropriate amino acid balance of the experimental diets. Dextrin served as the source of carbohydrate. Carboxy-methyl cellulose (CMC) was used as binder and α -cellulose as filler and sunflower oil and fish oil (1:1) as the source of lipid. Diets were added with vitamins-minerals premix uniformly at 5%. The levels of incorporation of different ingredients (in g kg⁻¹) in different experimental diets and the proximate composition of such diets have been given in Table 1 and 2, respectively.

The semi-purified diets were prepared following the methods described by Mohanta *et al.* (2007) with slight

Table 1. Ingredient composition (g kg⁻¹ dry matter basis) of the experimental diets*

Ingredients	T ₁ *	T ₂	T ₃
Casein	254	216	179
Gelatin	60	51	42
Dextrin	260	315	370
Fish oil	50	50	50
Sunflower oil	50	50	50
Vitamin mineral mixture	50	50	50
Carboxy-methyl cellulose (CMC)	20	20	20
Cellulose	256	248	239

*T₁ (CP 27% and CHO 26%); T₂ (CP 23% and CHO 31.5%); T₃ (CP 19% and CHO 37%)

Table 2. Proximate composition of the experimental diets

Particulars	Experimental diets		
	T ₁ *	T ₂	T ₃
Moisture (%)	27.52	25.37	25.14
Crude protein (CP) (%)	27.20	23.33	19.15
Ether extract (EE) (%)	9.06	9.63	9.33
Ash (%)	5.06	5.04	5.37
Total carbohydrate (%)	58.68	62.00	66.15
Gross energy (kcal 100 g ⁻¹)	480.02	477.16	467.78
E/P ratio (kcal g protein ⁻¹)	17.65	20.45	24.43

*T₁ (CP 27% and CHO 26%); T₂ (CP 23% and CHO 31.5%); T₃ (CP 19% and CHO 37%)

modification. In short, all the ingredients for each feed were weighed accurately and kept separately as per requirement (Table 1). Gelatin crystals were dissolved in a small quantity of warm water (80°C) with slow stirring so as to form a jelly. Casein, dextrin, CMC and cellulose weighed and kept separately were then mixed well. Required quantity of sunflower oil and fish oil were poured to the dry mixture and thoroughly mixed. Gelatin jelly was then blended in the mixture and a dough was prepared by adding required quantity of water. The dough was then steam cooked by keeping the dough in a covered pot inside an autoclave for 5 min at 15 psi. The cooking was done to improve gelatinisation of carbohydrate (dextrin) and increase binding strength of CMC. Vitamin mineral mixture was added with the cooked dough after cooling and was mixed well. Then the dough was uniformly spread on a stainless steel plate at a thickness of about 3 mm and was kept overnight in a refrigerator. On the next day, it was taken out of refrigerator and cut into cubes of 3 x 3 x 3 mm size. The cubes were then made into portions equal to one day ration and packed in separate zip lock polythene bags, labelled suitably and were sealed airtight and then stored in the refrigerator at 4°C until further use. One packet each of the designated feeds was taken out everyday from the refrigerator and fed to the test animals. Fresh feeds were prepared once in every seven day using the same lot of ingredients, which had been procured in bulk at the start of the experiment.

Biochemical analysis

Biochemical composition of the experimental diets and the whole body of fish before and after experiment were analysed following standard protocols (AOAC, 1998). Gross energy content of the experimental diets was computed using the average calorific values of 5.65, 9.40 and 4.10 kcal g⁻¹ for protein, lipid and carbohydrate, respectively (Henken *et al.*, 1986).

Computation of growth parameters

The different nutritional indices evaluated and the formula used were as follows :

$$\text{Weight gain (g)} = \text{Final weight (g)} - \text{Initial weight (g)}$$

$$\text{Percentage weight gain} = \frac{\text{Final weight} - \text{Initial weight}}{\text{Initial weight}} \times 100$$

$$\text{Specific growth rate} = \frac{\ln(\text{Final weight}) - \ln(\text{initial weight})}{\text{Experimental periods in days}} \times 100$$

$$\text{Feed conversion ratio} = \frac{\text{Feed given (dry weight)}}{\text{Body weight gain (wet weight)}}$$

$$\text{Protein efficiency ratio} = \frac{\text{Net weight gain (wet weight)}}{\text{Crude protein fed}}$$

$$\text{Protein productive value (PPV)} = \frac{(\text{Final wt. of fish} \times \text{Final carcass protein \% wet wt.} - (\text{Initial wt. of fish} \times \text{Initial carcass protein \% wet wt.}))}{(\text{protein feed}) \times 100}$$

Statistical analysis of data

The data generated were subjected to one-way analysis of variance (ANOVA) followed by Duncans multiple range tests at $p < 0.05$ (Snedecor and Cochran, 1968; Ducan, 1995) to find out the significant difference among the treatments, if any. The PC-SAS programme for Windows, release v6.12 (SAS, 1996) was used for the analysis.

Results and discussion

Growth performance

Survival rate of the fish among different experimental groups did not vary significantly ($p > 0.05$), which varied between 92.5 and 97.5% in different experimental tanks. The weight gain (g) and weight gain percentage (%) between the experimental groups T₁ and T₂ did not vary significantly ($p > 0.05$). However, both T₁ and T₂ showed significantly better ($p < 0.05$) weight gain (g) and weight gain percentage (%) than T₃ (Table 3). The specific growth rate (SGR) was found to decrease from 1.26 to 1.07 from T₁ to T₃. This shows that the feed with higher CP resulted in better weight gain and thus had better SGR. However, the reduction in CP from 27.2% (T₁) to 23.33% (T₂) did not decrease SGR significantly ($p > 0.05$). While, further reduction in CP to 19% has significantly affected ($p < 0.05$) SGR in experimental group T₃ (Table 3).

Earlier findings have reported that the optimum protein requirement of *L. fimbriatus* as 27% (Jena *et al.*, 2012) and optimum carbohydrate requirement of carps as 26% (Sen *et al.*, 1978; Erfanullah and Jafri, 1995). However, the optimum dietary protein requirement of silver barb (*Puntius gonionotus*) fry could be reduced from 30 to 25% by increasing carbohydrate from 26 to 34% without sacrificing the growth of the fish (Mohanta *et al.*, 2007). Similarly, Shiau and Peng (1993) found no change in weight gain or feed conversion efficiency in tilapia by decreasing dietary protein from 28 to 24% and increasing carbohydrate (dextrin) from 37 to 41%. Further, Erfanullah and Jafri (1995) and Kumar *et al.* (2006) have reported protein sparing effect of carbohydrate in rohu (*Labeo rohita*) when the fish was fed with dietary protein at its sub-optimum level. Although, the live weight gain in this study was the highest in case of fish fed with 27% CP (T₁), it did not vary significantly ($p > 0.05$) with that of the 23% CP fed group (T₂). This may be attributed to the protein sparing capability of carbohydrate in this species. *L. fimbriatus* being herbivorous in nature (Talwar and Jhingarn, 1991), it might have the capacity to utilise higher levels of carbohydrates than other carps, which needs to be studied. Accordingly, the higher incorporation of carbohydrate might have spared protein at its sub-optimal

Table 3. Growth performance and nutrient utilisation of *L. fimbriatus* fed with varying levels of dietary protein and carbohydrate

Nutritional indices	Experimental diets		
	T ₁ *	T ₂	T ₃
Initial average weight (g)	2.95±0.13	2.91±0.14	2.93±0.16
Final average body weight (g)	9.13±0.14	8.87±0.12	7.70±0.18
Average weight gain (g)	6.18±0.11 ^a	5.96±0.15 ^a	4.77±0.16 ^b
Percentage weight gain (%)	209.5±0.74 ^a	204.8±0.52 ^a	162.8±0.92 ^b
Survival percentage (%)	94.0±0.11	92.5±0.14	97.5±0.10
Specific growth rate (SGR) (%)	1.26±0.15 ^a	1.24±0.13 ^a	1.07±0.12 ^b
Total feed intake (dry wt) (g)	13.84±0.27 ^a	13.64±0.24 ^a	12.92±0.32 ^b
Food conversion ratio (FCR)	2.24±0.11 ^a	2.29±0.14 ^a	2.71±0.13 ^b
Total protein intake (dry wt) (g)	3.76±0.12 ^a	3.18±0.14 ^b	2.47±0.11 ^c
Protein efficiency ratio (PER)	1.64±0.13 ^a	1.87±0.11 ^b	1.93±0.12 ^b
Protein productive value (PPV) (%)	25.76±0.24 ^a	28.34±0.36 ^b	27.65 ±0.32 ^b

*T₁ (CP 27% and CHO 26%); T₂ (CP 23% and CHO 31.5%); T₃ (CP 19% and CHO 37%)

^{a,b,c}: Values having different superscript in a row differ significantly (p<0.05). Values are mean of three samples ± SE.

level for growth of the fish (Mohanta *et al.*, 2007), indicating protein sparing capability of carbohydrate in *L. fimbriatus*.

The feed conversion ratio (FCR) varied significantly (2.24 to 2.71, p<0.05) with the change in protein and carbohydrate levels in the diets (Table 3). However, no significant difference in FCR (p>0.05) was found between 23.33 and 27.2% protein fed group. On the other hand, the protein productive value (PPV) of 27.2% protein fed group was significantly (p<0.05) lower than other two groups and the PPV of 23.33% protein fed group was the highest. However, the PPV of 23.33 and 19.15% protein fed group did not differ significantly (p>0.05), showing better protein retention in fish body at sub-optimal CP level in the diet. The decrease in PPV with the increase in dietary protein may be due to higher protein consumption when the diet contains higher amount of dietary protein, but at the same time the body protein gain was not in proportionate with protein intake. The better protein retention observed in the study when fed at sub-optimal level has earlier been reported in several other fish species (Erfanullah and Jafari, 1995; Kim *et al.*, 2004; Mohanta *et al.*, 2007).

The protein efficiency ratio (PER) of T₂ and T₃ was significantly better than that of T₁ (p<0.05). However, there

was no significant difference in PER values among T₂ and T₃. The higher PER values with increasing carbohydrate level agrees with the findings of Erfanullah and Jafri (1995) for rohu, *L. rohita*. The lower PER values at the higher dietary protein level substantiates the earlier findings in several other fish species (Daniels and Robison, 1986; Arzel *et al.*, 1995).

Analysis of PER in several other fish species concluded that protein is better retained when fed at its sub-optimum level (Dabrowski, 1977; Garling and Wilson, 1977; Mazid *et al.*, 1979; Santiago and Laron, 1991; Mohanta *et al.*, 2007). Although in the present study the protein synthesis could not be measured, the greater protein retention when fed at lower level of dietary protein may be due the high protein synthesis and hence the growth (Mohanta *et al.*, 2007).

Proximate composition of the carcass of *L. fimbriatus* was significantly (p<0.05) affected by varying dietary CP and carbohydrate level. The carcass CP content was found to increase significantly (p<0.05) from initial value after feeding with CP rich diet, T₁ and T₂. On the other hand, carcass lipid content as ether extract (EE) was found to increase significantly (p<0.05) after feeding with carbohydrate rich diet (Table 4). The increase in carcass CP content with increase in dietary protein content corroborates the findings

Table 4. Initial and final carcass composition (% wet weight basis) of *L. fimbriatus* fed varying levels of dietary protein and carbohydrate

Parameters	Before feeding trial	After feeding trial		
		T ₁ *	T ₂	T ₃
Moisture	71.92±0.21 ^a	70.76±0.09 ^b	70.14±0.11 ^b	68.78±0.18 ^c
Crude protein	13.90±0.27 ^a	15.10±0.16 ^b	14.72±0.19 ^b	14.16±0.20 ^a
Ether extract	6.87±0.26 ^a	7.04±0.17 ^a	7.36±0.14 ^a	8.60±0.16 ^b
Crude Ash	5.68±0.11 ^a	5.90±0.17 ^{ab}	6.13±0.12 ^b	6.50±0.21 ^c

*T₁ (CP 27% and CHO 26%); T₂ (CP 23% and CHO 31.5%); T₃ (CP 19% and CHO 37%)

^{a,b,c}: Values bearing different superscript in a row differ significantly (p<0.05). Values are mean of three samples ± SE.

of Shiau and Lin (2001) and Jena *et al.* (2012). The increase in carcass lipid level in the experimental group T₃ might be due to the conversion of excess carbohydrate into lipid and that is stored in the fish body (Shiau and Lin, 2001).

To summarise, the dietary group T₂ showed almost similar growth and physiological performance and better PER and PPV than T₁ suggesting that replacing protein with carbohydrate at the former's sub-optimum level might have spared more protein for growth. Hence, the study indicates that dietary protein content can be reduced from 27 to 23% by proportionately increasing carbohydrate content in the diet without significant influence on the growth of fringed-lipped carp *L. fimbriatus*.

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