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Determination of Optimum Dietary Carbohydrate Level of Long Whiskers Catfish, *Mystus gulio* Fry

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

The objective of the present study is to evaluate the optimum dietary carbohydrate level of *M. gulio* fry, which will form the basis for formulating the nutritionally adequate, cost-effective nursery diet. Five iso-proteinous (400 g kg^{-1} diet) and iso-lipidic (120 g kg^{-1} diet) semi-purified diets with 100, 150, 200, 250 and 300 g carbohydrate kg^{-1} diet were fed *ad libitum* to *Mystus gulio* fry (0.33 ± 0.004 g) for 90 days in triplicates (10 fish/replicate). Fifteen fibre-reinforced plastic tanks (50 L) with flow-through system (water flow rate of 0.5 L min^{-1}) were used for rearing the fish. At the end of the feeding period, the diet containing 200 g kg^{-1} carbohydrate diet had significantly higher (*P* = .05) weight gain (8.39 g), Specific Growth Rate (SGR; 3.62 %/day), Protein Efficiency Ratio (PER; 1.52),

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Protein Productive Value (PPV; 24.08%), Lipid Productive Value (LPV; 27.13%), Energy Productive Value (EPV; 66.89%) and lower Food Conversion Ratio (FCR; 1.65) as compared to other diet fed groups. The fish fed with carbohydrate beyond 200 g kg^{-1} diet had significantly higher (P = .05) Hepatosomatic index (HIS) and Visecerosomatic index (VSI) than the lower carbohydrate fed groups. Second order polynomial regression analysis of weight gain, SGR, FCR, PER and PPV against the dietary carbohydrate had showed that the optimum dietary carbohydrate requirement of *M. gulio* fry is 197.8-207.3 g kg^{-1} diet.

Keywords: Carbohydrate; catfish; diet; growth; Mystus gulio; nutrient.

1. INTRODUCTION

Carbohydrate is progressively attracting the interest of researchers as it is the most economical dietary source of energy as compared to lipid and protein especially for the formulation of diets of herbivorous and omnivorous fish [1,2]. “Inclusion of carbohydrates in aqua-feeds is also very important because they are readily available, low- budget, act as binder, improve feed palatability and reduce the catabolism of dietary proteins and lipids for energy yielding processes” [3,4]. “Generally, carbohydrates are classified into digestible mono-, di- and polysaccharides and indigestible hemicelluloses and cellulose” [5]. “The digestible polysaccharides commonly used in feedstuff are starch and its products, such as dextrin, which is an intermediate complex of glucose and starch” [6]. “The required level of carbohydrate incorporation in the diet enhances growth performance and feed utilization of fish, increases protein and lipid retention by preventing the catabolism of these expensive nutrients for energy needs (sparing effect), reduce nitrogen load in the farm discharge (environmental safeguard), provide metabolites for biological syntheses, improve feed stability” [7,8,5]. “Carp, catfish and tilapia can utilize carbohydrate more efficiently as an energy source than dietary lipid” [9,10]. In general, optimal levels of dietary carbohydrate for carnivorous fish have been reported to range from 7 to 20% [11,12].

The long whiskers catfish, *Mystus gulio (M. gulio)* is a promising species for aquaculture due to its rapid growth rate, high nutritional value, delicious taste and good market potential (price ranging from 300-500/kg). It can survive very well in the oxygen depleted waters and also tolerate to the crowding condition and therefore, considered as an ideal species for high density fish culture [13,14]. It is distributed in India, Bangladesh and other Asian countries [15,14]. Although *M. gulio* is an important catfish species worthy for culture, the information on nutritional requirement of this species is very limited. Khatua et al. [16] reported that by using the casein-gelatin-dextrin based semi-purified diets the optimum dietary protein and lipid requirement of *M. gulio* fry is 400 g and 120 g kg^{-1} diet. However, the optimum carbohydrate requirement level of *M. gulio* fry has not been studied so far. The results of the present study on optimum dietary carbohydrate level coupled with the optimum protein and lipid requirement levels of this species studied earlier by the same research group will form the basis for formulating the nutritionally adequate, cost-effective nursery diets for this species. A good quality nursery diet is the need of hour not only to produce the quality seed of this species but also to avoid its size disparity, prevent cannibalism and ultimately improve the survival rate.

2. MATERIALS AND METHODS

2.1 Experimental Diets

Five iso-nitrogenous (400 g protein kg^{-1} diet) and iso-lipidic (120 g lipid kg^{-1} diet) semi-purified diets were prepared using various levels of carbohydrate and labelled as D-1 (200), D-2 (150), D-3 (200), D-4 (250) and D-5 (300) g carbohydrate kg^{-1} diets (Table 1). Casein and gelatin were used as protein sources; dextrin and corn starch as carbohydrate sources, and 1:1 fish oil and sunflower oil was used as the source for lipid. Carboxymethyl cellulose (CMC) was used as binder and α-cellulose was applied as filler. Vitamin and mineral mixture is added in the diets [17,18]. The 1.0 mm diameter experimental diets were prepared using a hand pelletizer. Prepared feed pellets were dried overnight at 60˚C in an air oven for 24 h and stocked in a refrigerator at 4˚C for further use [16].

2.2 Experimental Set Up

Two thousand *M. gulio* fry were obtained from the ICAR-Central Institute of Brackishwater Aquaculture, Kakdwip, West Bengal. The fish were accustomed to the laboratory condition in five FRP tanks of 200 L capacity each for 2 weeks. During the period of acclimatization, the fish were fed twice daily close to apparent satiation level throughout the experiment. After acclimatization, 150 similar sized fry (average initial weight of 0.33 ± 0.004 g) were arbitrarily distributed in 15 flow-through (flow rate: 0.5 L min^{-1}) FRP tanks of 50 L capacity each with 40 L water volume in triplicates at a stocking density of 10 fish/tank. Before starting of the experiment, initial fish biomass of each tank was recorded. The experimental fish were fed *ad libitum* for 90 days [16]. After completion of the experiment the final biomass of fish with respect to each tank was determined by batch-weighing the fish.

2.3 Chemical Analysis of Experimental Diet and Fish

Before commencement of the feeding trial, 200 fish were randomly sacrificed with an overdose of MS222 solution and the fish were taken for determining the initial whole-body composition. The proximate composition of experimental diets (Table 1) and fish (oven dried and grounded sample) was analyzed in triplicates as per the standard method [19] in the National Feed Testing and Referral Laboratory, Fish Nutrition and Physiology Division, ICAR-CIFA, Bhubaneswar.

2.4 Water Analyses

Except water temperature which was measured twice daily (06:00 h and 14:30 h), the other water quality parameters were analyzed in every 15 days interval following the methods of APHA [20]. The observed parameters were in the range of: temperature, 24.5-26.3 ˚C; pH, 7.4-7.8; dissolved oxygen, 7.41-8.20 mg L^{-1}; total alkalinity, 111.36-115.39 mg $CaCO_3$ L^{-1}; and total hardness, 103.23-106.76 mg $CaCO_3$ L^{-1}.

Table 1. Formulation and proximate composition of the experimental diets (g kg^{-1} on dry matter basis) with various level of dietary carbohydrate (≠CHO)

	Experimental diets				
Ingredient	**D-1 (100g kg^{-1} diet)**	**D-2 (150g kg^{-1} diet)**	**D-3 (200g kg^{-1} diet)**	**D-4 (250g kg^{-1} diet)**	**D-5 (300g kg^{-1} diet)**
Ingredient composition (g kg^{-1} diet)					
Casein	360.5	360.5	360.5	360.5	360.5
Gelatin	90.1	90.1	90.1	90.1	90.1
Dextrin	50.0	70.5	100.0	120.5	150.0
Corn starch	50.0	70.5	100.0	120.5	150.0
CMC	20.0	20.0	20.0	20.0	20.0
Sunflower oil	60.0	60.0	60.0	60.0	60.0
Fish oil	60.0	60.0	60.0	60.0	60.0
@Vitamin mixture	50.0	50.0	50.0	50.0	50.0
α-cellulose	250.4	200.4	150.4	100.4	50.4
Chemical composition (g kg^{-1} dry matter basis)					
Crude Protein	410.2	390.6	400.1	420.3	390.8
Ether extract	110.9	120.9	120.3	110.7	120.1
Ash	60.5	60.3	60.7	60.4	60.8
Gross energy (MJ/kg)	17.97	18.39	17.97	17.97	17.97

@Vitamin mixture: vitamin A – 3000 IU; vitamin D3 – 15 000 IU; menadione sodium bisulphate – 10 mg; choline chloride – 2000 mg; niacin – 50 mg; riboflavin – 20 mg; pyridoxine – 10 mg; thiamine mononitrate – 10 mg; pantothenic acid – 40 mg; folic acid – 5 mg; vitamin B_{12} – 0.02 mg; biotin – 1 mg; inositol – 400 mg; α-tocopherol acetate – 5 mg and vitamin C – 200 mg (Modified [18])

µMineral mixture: NaCl – 1.0 g; $MgSO_4.7H_2O$ – 15.0 g; NaH_2-$PO_4.2H_2O$ – 25.0 g; KH_2PO_4– 32.0 g; $Ca(H_2PO_4)$. $2H_2O$ – 20.0 g; Fe-citrate – 2.5 g; Ca-lactate – 3.5 g and ωTrace element mixture – 1.0 g [17].

ωTrace element mixture: $ZnSO_4.H_2O$ – 35.3 g; $MnSO_4.4H_2O$ – 16.2 g; $CuSO_4.5H_2O$ – 3.1 g; $CoCl_2.6H_2O$ – 0.1 g; KIO_3 – 0.3 g and cellulose – 45.0 g

≠CHO: Carbohydrate

2.5 Calculation of Nutritional Indices

- Weight gain (g) = Final weight (g) - Initial weight (g)
- Specific growth rate (SGR; %/day) = $\frac{\text{ln final weight- ln initial weight}}{\text{Experimental duration (days)}} \times 100$

- Feed conversion ratio (FCR) = $\frac{\text{Feed consumption (g)}}{\text{Fish weight gain (g)}}$
- Protein efficiency ratio (PER) = $\frac{\text{Fish weight gain (g)}}{\text{Protein intake (g)}} \times 100$
- Nutrient (protein and lipid) and energy productive values:

$$\text{(PPV, LPV and EPV; \%)} = \frac{\text{Nutrients/energy gain in body}}{\text{Nutrients/energy intake}} \times 100$$

- Hepatosomatic Index (HSI) = $\frac{\text{Liver weight}}{\text{Body weight}} \times 100$
- Viscerosomatic Index (VSI) = $\frac{\text{Weight of the whole digestive tract}}{\text{Body weight}} \times 100$

2.6 Statistical Analysis

The statistical significance of different study parameters was analyzed by one-way ANOVA and Duncan's' Multiple Range Test to compare the means (*P* = .05) between different experimental groups. PC-SAS program for Windows, released v 6.12(SAS Institute, Cary, NC, USA [21]) used for data analysis. Second-order polynomial regression analysis was performed by taking weight gain, SGR, PER, PPV and FCR values versus dietary carbohydrate levels and the carbohydrate requirement of *M. gulio* fry was estimated more precisely.

3. RESULTS AND DISCUSSION

At the end of the experiment, there was no fish mortality recorded in any of the experimental tank. The study results indicated that the dietary carbohydrate had a significant effect (*P* = .05) on growth performance and feed utilization in fish (Table 2). The *M. gulio* fry fed with 200 g carbohydrate kg^{-1} (D3) diet had significantly higher (*P* = .05) weight gain, SGR, PER and lower (*P* = .05) FCR, beyond which there was no improvement (*P* = .05) in these parameters. Similar to our results, the poor growth and nutritional indices of fish at lower and higher levels of dietary carbohydrate was reported by many earlier researchers in *Labeo rohita* [22], *Channa striatus* [23], *Mystus monatus* [24], *Nibea japonica* [25], *Apostichopus japonicas* [26], *Epinephelus akaara* [27] and *Puntius gonionotus* [28].

It is investigated that the high level of dietary carbohydrate resulted high glucose level in the blood and glycogen deposition in the liver which reduce the digestion, absorption and assimilation of carbohydrate ultimately leading to poor growth and utilization of nutrient in fish [11,5]. Although we have not measured the glucose level in the blood and the glycogen deposition in the liver, this is one of the reasons for the poor growth and nutrient utilization of *M. gulio* fry. Whereas, low levels of dietary carbohydrate in the diet leads to reduction of daily weight gain due to loss of muscle mass (muscle hypotrophy) [29]. We also observed less whole body protein content in *M. gulio* fry at lower level of dietary carbohydrate. The adequate level of dietary carbohydrate served as energy source so that the fish can directly utilize most of the dietary protein to physical growth rather than energy need and improving animal performance [30]. In this study the carbohydrate level of 200 g kg^{-1} diet might have sufficient to meet the energy need of fish, thereby sparing the dietary protein for growth of fish rather than energy purpose.

Both the HSI and VSI were significantly increased (*P* = .05) with increase in dietary carbohydrate levels (Table 2). "HSI is an important indicator of available energy in fish" [28]. "Excess dietary carbohydrate after assimilation get deposited in the liver in the form of glycogen leading to high HSI values or it may get converted to lipid by lipogenic enzyme and then stored in fish body" [31-33]. In this study, high HSI value in D-4 and D-5 groups might be due to deposition of fat or glycogen in the liver, which is in accordance with earlier findings ([25] for *Nibea japonica*; [28] for *Puntius gonionotus*; Yengkokpam et al. [34] for *Catla catla*). Higher glycogen deposition in liver is caused by excessive available energy obtained from digestible carbohydrate. The higher HSI values in the present study attributed to more lipid deposition in liver when *M. gulio* was fed with higher level of dietary carbohydrate (250 and 300 g carbohydrate kg^{-1} diet). Similar results were reported in gilthead sea bream, European sea bass and Catla [34-36]. "The VSI also showed the similar trend as that of HSI which indicates that the excess dietary carbohydrate beyond the requirement level of *M gulio* fry is converted into lipid and gets deposited in its viscera" [28].

A significant variation (*P* = .05) in nutrient (protein and lipid) and energy gain were also observed in *M. gulio* fry with respect to change in dietary carbohydrate levels. The fish fed 200 g carbohydrate kg^{-1} diet (D-3) had significantly higher (*P* = .05) PPV, LPV and EPV values as compared to other carbohydrate fed groups (Table 3). Any further increase in dietary carbohydrate beyond this level had no significantly higher (*P* = .05) nutrient and energy gain. In this study, the higher growth and nutrient utilization in terms of PPV, LPV and EPV was recorded with diet containing 200 g carbohydrate kg^{-1} (D-3) in *M. gulio* fry and thereafter, there was no significant (*P* = .05) improvement in these nutritional parameters. Primary understanding on the optimum level of protein and protein-sparing effects of non-protein substance like carbohydrates can be utilized efficiently in reducing feed costs [37]. In this study, maximum weight gain, PER and PPV were observed in D-3 group, which advocate that in *M. gulio* fry, the protein is most efficiently utilized at the 200 g carbohydrate kg^{-1} of diet. The maximum growth and utilization of nutrient in *M. gulio* is observed at 200 g carbohydrate kg^{-1} diet in the present study is similar to the requirement of 150-200 g kg^{-1} in *Clarius batrachus* [38]; but lower than the 350 g kg^{-1} of *Heteropneustes fossilis* [39]. A significant increase in protein and energy gain of *M. gulio* fry with an increase in the dietary carbohydrate level up to a certain extent (200 g carbohydrate kg^{-1} diet), beyond which there was no further improvement is in agreement with Erfanullah and Jafri [1] for *Catla catla* and Mohanta et al. [28] for *Puntius gonionotus*. It is reported that the higher amounts of dietary carbohydrate retarded the growth in rainbow trout, *Oncorhynchus mykiss* [40] and red drum, *Sciaenops ocellatus* [41] due to poor nutrient gain. We have also observed poor growth performance at higher carbohydrate level in diet (D-4 and D-5), which might be due to fatty liver (higher HSI) and poor physiological function. Similarly, Hastings [42] reported that if consumption of carbohydrate is used in excess for energy requirement, it increases visceral fat deposits and fatty infiltration in organs, which eventually leads to restriction of normal body function of fish. In our study the LPV was increased with increase in dietary carbohydrate up to a certain level and then remained constant. However, in contrast to our results, Sulaiman et al. [43] observed a positive correlation between dietary carbohydrate level and LPV which indicates that increasing carbohydrate level leads to lipogenesis and spared lipid from catabolism and hence, it gets accumulated.

The composition of whole-body fish fed with various digestible carbohydrate diets are presented in (Table 4). In our study, we observed that the dietary carbohydrate had significant (*P* = .05) effect on whole-body contents of the fish. The moisture content of the whole-body of fish is significantly decreased (*P* = .05) with an increase in dietary carbohydrate levels which is found to be differ from the findings of Mohanta et al. [28 for *P. gonionotus*. However, increase of whole-body moisture content with increase of dietary carbohydrate up to certain level and thereafter, it is decreased was reported by many earlier researchers [24,26,33]. This implies that the effect of different levels of dietary carbohydrate on whole body moisture content is species specific and there is no definite relation/trend exists between dietary carbohydrate and whole body moisture content. The whole-body protein content of *M.gulio* fed with 200 g carbohydrate kg^{-1} diet was significantly higher ($P < 0.05$) than the lower (100 and 150 g carbohydrate kg^{-1} diet) or higher (250 and 300 g carbohydrate kg^{-1} diet) fed groups, which in agreement with Arockiaraj et al. [23] for *Channa striatus* and Mohanta et al. [28] for *Puntius gonionotus*. The maximum protein gain in terms PPV is the reason for significantly higher whole body protein content in fish fed 200 g carbohydrate kg^{-1} diet. The whole-body lipid content is directly proportional to dietary carbohydrate levels, which confirms the findings of other researchers Erfanullah and Jafri [43] in *Labeo rohita*; Wang et al. [44] in *Oreochromis niloticus* × *O. aureus* and Mohanta et al. [28] in *Puntius gonionotus*. They observed lipogenic activity in fish when fed high carbohydrate diets. The whole-body ash content was increased linearly with the rise in carbohydrate levels which is same with the results of Arockiaraj et al. [23] for *Mystus monatus* and Mohanta et al. [28] for *P. gonionotus*. Therefore, maximum ash content was found in fish fed with 300 g carbohydrate kg^{-1} diet (D-5) which suggests that a linear relation coexist between whole body ash content and the carbohydrate level in the diet. In this study, with the increase in dietary carbohydrate concentrations, there was a slight but insignificant increase in whole body gross energy content of *M. gulio* fry which is contrast to the findings of [28] who reported that with an increase in dietary carbohydrate level the whole-body gross energy level is increased.

From the second order polynomial regression analysis of weight gain ($y = -0.000267x^2 + 0.10701x - 3.2607$; $R^2 = 0.7032$) (Fig. 1), SGR ($y = -0.000045x^2 + 0.01824x + 1.6617$; $R^2 = 0.7501$) (Fig. 2), PER ($y = -0.000025x2 + 0.00989x + 0.476$; $R^2 = 0.6628$) (Fig. 3), PPV ($y = -0.000534x^2 + 0.22144x - 0.2073$; $R^2 = 0.7998$) (Fig. 4), and FCR ($y = 0.000041x^2 - 0.01647x + 3.386$; $R^2 = 0.8117$) (Fig. 5) it is observed that the optimum dietary carbohydrate level of *M. gulio* fry is 197.8-207.3 g/kg diet at a 400 g/kg dietary protein and 120 g/kg dietary lipid.

Table 2. Growth performance and nutrient utilization of *Mystus gulio* fry fed with various level of carbohydrate (≠CHO)

	Experimental diet (Carbohydrate level)				
Growth and nutritional Indices	**D-1 (100 g kg^{-1}diet)**	**D-2 (150 g kg^{-1}diet)**	**D-3 (200 g kg^{-1}diet)**	**D-4 (250 g kg^{-1}diet)**	**D-5 (300 g kg^{-1}diet)**
Initial weight (g)	0.33±0.01^{a}	0.33±0.01^{a}	0.33±0.01^{a}	0.32±0.01^{a}	0.33±0.01^{a}
Final weight (g)	4.96±0.07^{a}	7.10±0.09^{d}	8.72±0.14^{e}	5.92±0.09^{c}	5.60±0.07^{b}
Weight gain (g)	4.63±0.07^{a}	6.77±0.08^{d}	8.39±0.14^{e}	5.60±0.09^{c}	5.27±0.07^{b}
FCR	2.17±0.02^{e}	1.80±0.05^{b}	1.65±0.03^{a}	1.92±0.03^{c}	2.05±0.03^{d}
PER	1.21±0.07^{a}	1.39±0.03^{b}	1.52±0.03^{c}	1.30±0.02^{ab}	1.22±0.02^{a}
SGR (%/day)	3.01±0.03^{a}	3.40±0.03^{c}	3.62±0.03^{d}	3.24±0.05^{b}	3.15±0.02^{b}
HSI	0.46±0.03^{a}	0.52±0.05^{a}	0.59±0.03^{a}	0.76±0.06^{b}	0.92±0.07^{c}
VSI	1.52±0.07^{a}	1.61±0.08^{a}	1.70±0.09^{ab}	1.92±0.08^{bc}	2.16±0.07^{c}

FCR: feed conversion ratio; SGR: specific growth rate; PER: protein efficiency ratio; HSI: hepatosomatic index; VSI: visecerosomatic index.
≠CHO: Carbohydrate
Values in the same column with different superscripts are significantly different (P = .05).
Values are means of three replicates of each experimental diet ± standard error (SE).

Table 3. Effect of dietary levels of carbohydrate (≠CHO) on nutrient retention in *Mystus gulio* fry

	Experimental diet (Carbohydrate level)				
Nutrient gain	**D-1 (100 g kg^{-1}diet)**	**D-2 (150 g kg^{-1}diet)**	**D-3 (200 g kg^{-1}diet)**	**D-4 (250 g kg^{-1}diet)**	**D-5 (300 g kg^{-1}diet)**
PPV	16.44±0.13^{a}	20.89±0.57^{c}	24.08±0.55^{d}	20.14±0.25^{c}	18.82±0.36^{b}
LPV	19.36±0.15^{a}	23.83±0.61^{b}	27.13±0.60^{c}	24.76±0.30^{b}	24.56±0.44^{b}
EPV	50.72±0.56^{a}	62.57±2.05^{c}	66.89±1.37^{d}	57.44±0.93^{b}	54.07±1.00^{ab}

PPV: Protein productive value; LPV: Lipid productive values; EPV: Energy productive value
≠CHO: Carbohydrate
Values in the same column with different superscripts are significantly different (P = .05).
Values are means of three samples ± standard error (SE).

Table 4. Whole-body chemical composition (on g kg^{-1} wet weight basis) of *Mystus gulio* fry fed with various level of carbohydrate (≠CHO)

	Experimental diet (Carbohydrate level)				
Nutritional parameters	**D-1 (100 g kg^{-1}diet)**	**D-2 (150 g kg^{-1}diet)**	**D-3 (200 g kg^{-1}diet)**	**D-4 (250 g kg^{-1}diet)**	**D-5 (300 g kg^{-1}diet)**
Moisture	77.13±0.09^{e}	76.73±0.07^{d}	76.28±0.06^{c}	75.97±0.08^{b}	75.65±0.09^{a}
Crude protein	14.22±0.04^{a}	14.95±0.02^{b}	15.79±0.02^{d}	15.34±0.07^{c}	15.31±0.03^{c}
Ether extract	4.89±0.01^{a}	5.05±0.03^{b}	5.26±0.04^{c}	5.52±0.03^{d}	5.77±0.02^{e}
Total Ash	1.71±0.02^{a}	1.88±0.01^{b}	2.08±0.01^{c}	2.26±0.02^{d}	2.41±0.02^{e}
Gross energy (MJ/kg)	4.77±0.00	4.77±0.01	4.77±0.01	4.77±0.01	4.79±0.00

≠CHO: Carbohydrate
Values in the same column with different superscripts are significantly different (P = .05).
Values are means of three samples ± standard error (SE).

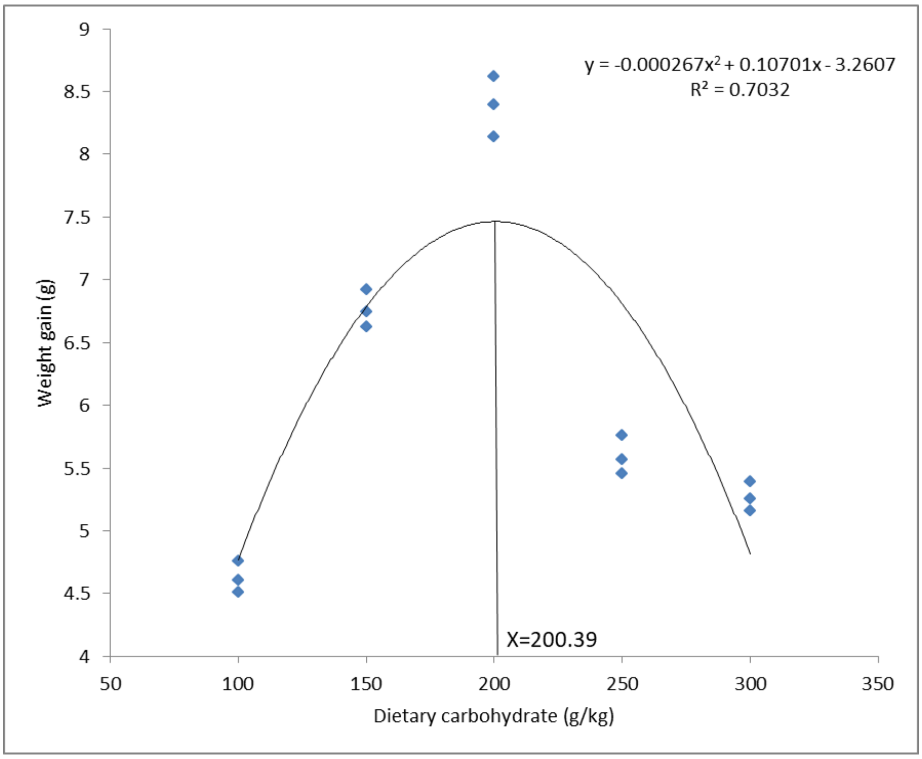

Fig. 1. Second-order polynomial regression analysis of weight gain and dietary carbohydrate levels for *Mystus gulio* fry

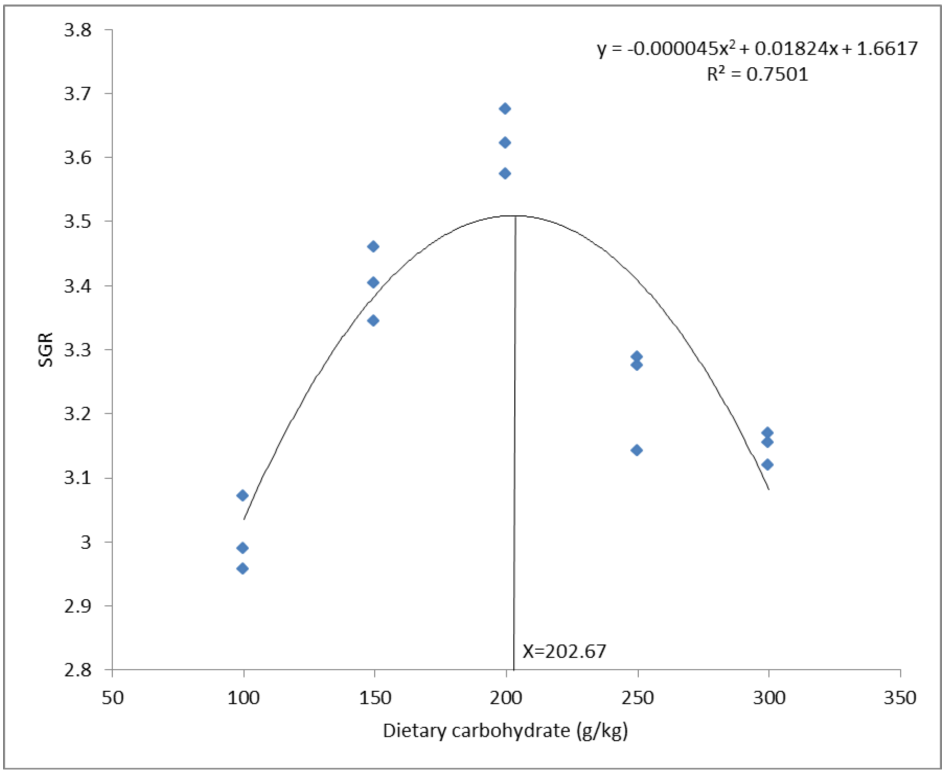

Fig. 2. Second-order polynomial regression analysis of SGR and dietary carbohydrate levels for *Mystus gulio* fry

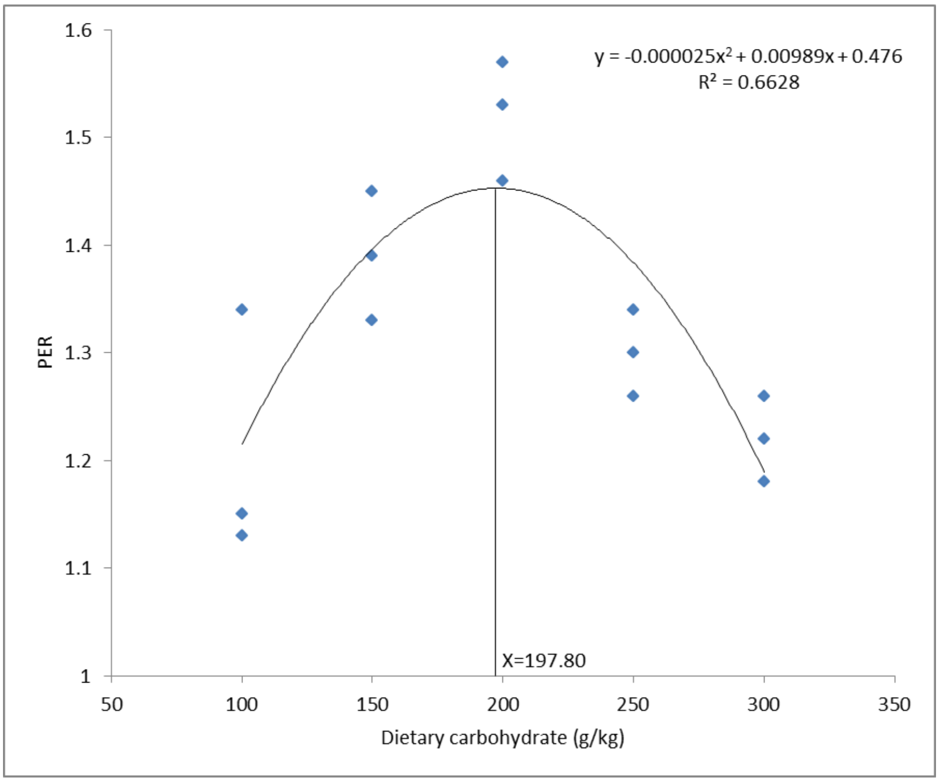

Fig. 3. Second-order polynomial regression analysis of PER and dietary carbohydrate levels for *Mystus gulio* fry

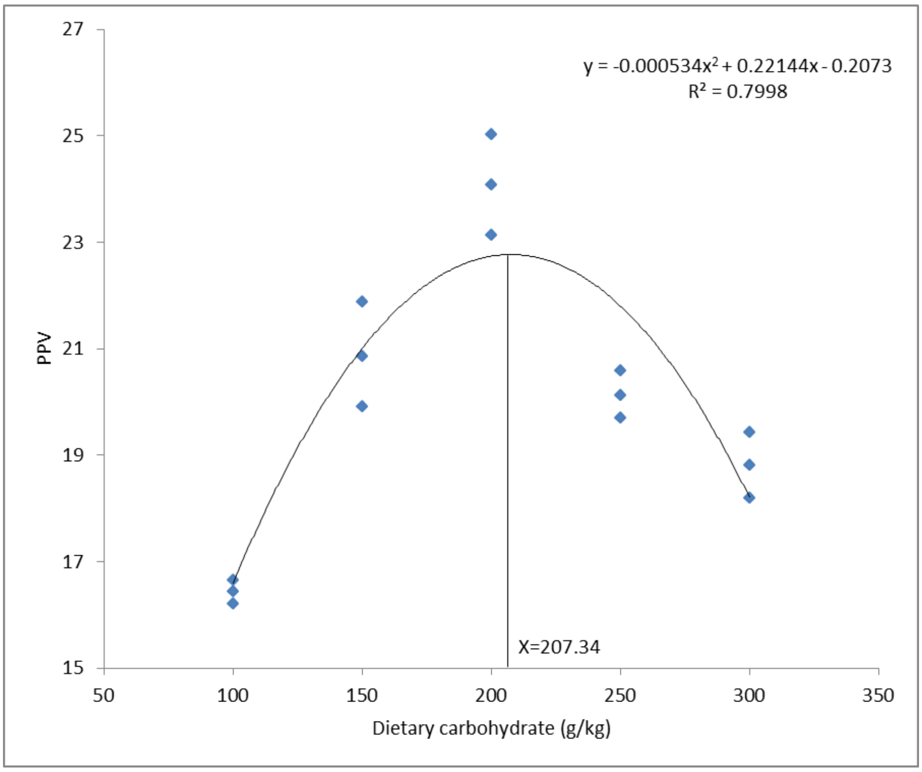

Fig. 4. Second-order polynomial regression analysis of PPV and dietary carbohydrate levels for *Mystus gulio* fry

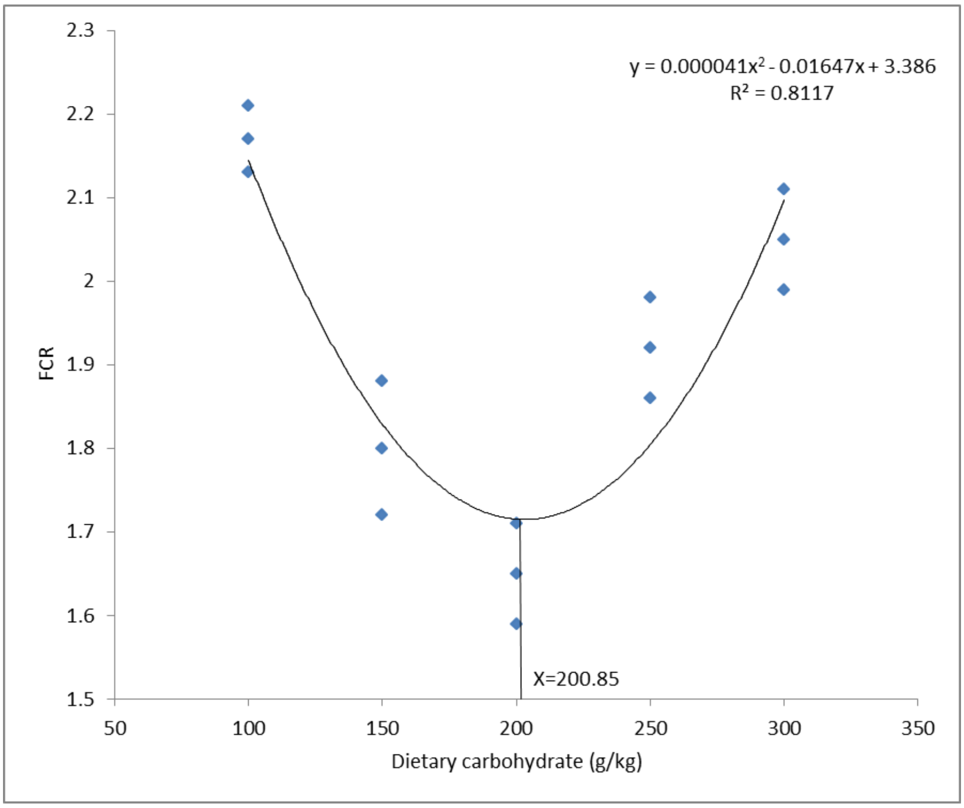

Fig. 5. Second-order polynomial regression analysis of FCR and dietary carbohydrate levels for *Mystus gulio* fry

4. CONCLUSIONS

Results of this study showed that the dietary carbohydrate level of 200 g carbohydrate kg^{-1} diet is optimal for the maximum growth potential of *M. gulio* fry. This primary understanding on optimal dietary carbohydrate requirement level along with the protein and lipid requirement studied earlier by us will be beneficial in formulating the nutritionally adequate and cost-effective nursery diets for the rearing of *M. gulio* fry.

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ANIMAL WELFARE STATEMENT

The authors confirm that they have followed the standards for the protection of animals used for scientific purposes.

AVAILABILITY OF DATA AND MATERIAL (DATA TRANSPARENCY)

It is declared that all data and materials as well as software application or custom code support our claims and comply with field standards.

The data that support the findings of the submitted manuscript are available from the corresponding author upon reasonable request.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Erfanullah, Jafri AK. Growth response, feed utilization and nutrient retention in *Catla catla* (Ham.) fry fed varying levels of dietary carbohydrate. Asian Fish Sci. 1998;11:223-230.
2. Ding Z, Kong YQ, Li JF, Cao F, Zhang YX, Du ZY, Ye JY. Growth and metabolic responses of juvenile *Macrobrachium*

nipponense to different dietary carbohydrate levels. Aquac Nutr. 2016; 23:1136-1144.

3. Gao W, Liu YJ, Tian LX, Mai KS, Liang GY, Yang HJ, Huai MY. Effect of dietary carbohydrate- to- lipid ratios on growth performance, body composition, nutrient utilization and hepatic enzymes activities of herbivorous grass carp (*Ctenopharyngodon idella*). Aquac Nutr. 2010;16:327-333.
4. NRC. Nutrient requirements of fish and shrimp: National Research Council. National Academy Press. 2011.
5. Kamalam BS, Medale F, Panserat S. Utilisation of dietary carbohydrates in farmed fishes: New insights on influencing factors, biological limitations and future strategies. Aquaculture. 2017;467:3-27.
6. Xu W, Zhang Q, Chen Y, Zhang Y, Zhou C. Effects of dietary carbohydrate types on growth performance, innate immunity, antioxidant ability and glucose metabolism of brook trout *Salvelinus fontinalis*. Aquac Res, 2020;1-11.
7. Hardy RW. Utilization of plant proteins in fish diets: effects of global demand and supplies of fishmeal. Aquac Res. 2010;41:770-776.
8. Li X, Zhu X, Han D, Yang Y, Jin J, Xie S. Carbohydrate utilization by herbivorous and omnivorous freshwater fish species: a comparative study on gibel carp (*Carassius auratus gibelio*. var CAS III) and grass carp (*Ctenopharyngodon idellus*). Aquac Res. 2016;47:128-139.
9. Ogino C, Chiu JY, Takeuchi T. Protein nutrition in fish-VI. Effect of dietary energy sources on the utilization of protein by rainbow trout and Carp. Bull Jpn Soc Sci Fish. 1976;42:213-218.
10. Garling DL, Wilson RP. Effects of dietary carbohydrate- to-lipid ratios on growth and body composition of fingerling channel catfish. Progressive Fish Culturist. 1977;39:43-47.
11. Hemre GI, Mommsen TP, Krogdahl A. Carbohydrates in fish nutrition: effects of growth, glucose metabolism and hepatic enzymes. Aquac Res. 2002;8:175-194.
12. Amoah A, Coyle SD, Webster CD, Durborow RM, Bright LA, Tidwell JH. Effects of graded levels of carbohydrate on growth and survival of largemouth bass, *Micropterus salmoides*. J World Aquacult Soc. 2008;39:397-405.
13. Tripathi SD. Present status of breeding and culture of catfishes in south Asia. In: Legendre M, Proteau JP. (Eds.), The Biology and Culture of Catfishes vol. 9. Aquat. Living Resour. 1996;219-228.
14. Kumar P, Biswas G, Ghoshal TK, Kailasam M, Christina L, Vijayan KK. Current knowledge on the biology, captive breeding and aquaculture of the brackishwater catfish *Mystus gulio* (Hamilton, 1822): A review. Aquaculture. 2019;499:243-250.
15. Day F. The fishes of India: Being a natural history of the fishes known to inhabit the seas and fresh waters of India. William Dowson and Sons. 1878;778.
16. Khatua R, Mohanta KN, Chandan NK, Pattanayak R, Mishra CSK, Kumar P. Dietary protein and lipid concentrations affect the growth, nutritional indices, and whole-body composition of long-whisker catfish, *Mystus gulio*, fry. Aquac Int. 2021;29(5):2085-2099.
17. Ogino C. The present situation of studies on fish nutrition: Proceedings of the 7th Japan-Soviet joint symposium aquaculture. 1977;11-18.
18. Lovell RT, Miyazaki T, Rebegnator S. Requirements of α-tocopherol by channel catfish fed diets low in polyunsaturated triglycerides. J Nutr. 1984;114:894-901.
19. AOAC. Official methods of analysis of the association of official analytical chemists, vol. 1 (14th Edn.): Association of official analytical chemists. 1990;1102.
20. APHA. Standard methods for examination of water and wastewater, 17th (Edn.): American public health association. 1992.
21. SAS. PC-SAS Programme for windows, release v6.12. SAS institute. 1996.
22. Erfanullah, Jafri AK. Effects of dietary carbohydrate level on the growth and conversion efficiency of the Indian major carp fingerling, *Labeo rohita* (Ham): A preliminary study. Asian Fish Sci. 1993;6:249-253.
23. Arockiaraj AJ, Muruganandam M, Marimuthu K, Haniffa MA. Utilization of carbohydrates as a dietary energy source by striped murrel *Channa striatus* (Bloch) fingerlings. Acta Zool Taiwanica. 1999;10: 103-111.

24. Arockiaraj AJ, Haniffa MA, Seetharaman S, Appelbaum S. Utilization of various dietary carbohydrate levels by the freshwater catfish *Mystus montanus* (Jerdon). Turk J Fish Aquat Sc. 2008;8:31-35.
25. Li XY, Wang JT, Han T, Hu SX, Jiang YD. Effects of dietary carbohydrate level on growth and body composition of juvenile giant croaker *Nibea japonica*. Aquac Res. 2015;46:2851-2858.
26. Li Z, Xue X, Yang H, Liao M, Han Y, Jiang Z, Ren T. Effect of dietary carbohydrate levels on growth performance, non-specific immune enzymes and acute response to low salinity and high temperature of juvenile sea cucumber *Apostichopus japonicas*. Aquac Nutr. 2019;26:683-692.
27. Wang J, Li X, Han T, Yang Y, Jiang Y, Yang M, Xu Y, Harpaz S. Effects of different dietary carbohydrate levels on growth, feed utilization and body composition of juvenile grouper *Epinephelus akaara*. Aquaculture. 2016; 459:143-147.
28. Mohanta KN, Mohanty SN, Jena JK, Sahu NP, Patro B. Carbohydrate level in the diet of silver barb, *Puntius gonionotus* (Bleeker) fingerlings: effect on growth, nutrient utilization and whole body composition. Aquac Res. 2009;40:927-937.
29. Torres WV, Castellanos JAA. Effect of dietary carbohydrates and lipids on growth in cachama (*Piaractus brachypomus*). Aquac Res. 2013;44: 1768-1776.
30. Sulaiman MA, Kamarudin MS, Romano N, Syukri F. Effects of increasing dietary carbohydrate level on feed utilisation, body composition, liver glycogen, and intestinal short chain fatty acids of hybrid lemon fin barb (*Barbonymus gonionotus* ♀ X *Hypsibarbus wetmorei* male ♂). Aquac Rep. 2020;16:1-7.
31. Tian LX, Liu YJ, Yang HJ, Liang GY, Niu J. Effects of different dietary wheat starch levels on growth, feed efficiency and digestibility in grass carp (*Ctenopharyngodon idella*). Aquac Int. 2012;20:283-293.
32. Azaza MS, Khiari N, Dhraief MN, Aloui N, Kraïem MM, Elfeki A. Growth performance, oxidative stress indices and hepatic carbohydrate metabolic enzymes activities of juvenile Nile tilapia, *Oreochromis niloticus* L., in response to dietary starch to protein ratios. Aquac Res. 2015;46: 14-27.
33. Zhang Q, Chen Y, Xu W, Zhang Y. Effects of dietary carbohydrate level on growth performance, innate immunity, antioxidant ability and hypoxia resistant of brook trout *Salvelinus fontinalis*. Aquac Nutr. 2021;27: 297-311.
34. Yengkokpam S, Sahu NP, Pal AK, Mukherjee SC, Debnath D. Gelatinized carbohydrates in the diets of *Catlacatla* fingerlings: Effects of levels and sources on nutrient utilization, body composition and tissue enzyme activities. Asian-australas J Anim Sci. 2007;20: 89-99.
35. Enes P, Panserat S, Kaushik S, Oliva-Teles A. Growth performance and metabolic utilization of diets with native and waxy maize starch by gilthead sea bream (*Sparus aurata*) juveniles. Aquaculture. 2008;274:101-108.
36. Moreira IS, Peres H, Couto A, Enes P, Oliva-Teles A. Temperature and dietary carbohydrate level effects on performance and metabolic utilization of diets in European sea bass (*Dicentrarchus labrax*) juveniles. Aquaculture. 2008;274: 153-160.
37. Shiau SY. Utilization of carbohydrates in warmwater fish-with particular reference to tilapia, *Oreochromis niloticus* x *O. aureus.* Aquaculture. 1997;151:79-96.
38. Mollah MFA, Alam MS. Effects of different levels of dietary carbohydrate on growth and feed utilization of catfish *Clarius batrachus* L. fry. Indian J Fish. 1990;37 (3):243-249.
39. Akand AM, Hasan MR, Habib MAB. Utilization of carbohydrate and lipid as dietary energy sources by stinging catfish, *H. fossilis* (Bloch). In: De Silva, S. (Ed.), Fish nutrition research in Asia. Asian Fish Soc. 1991;93-100.
40. Austreng E, Risa S, Edwards DJ, Hvidsten H. Carbohydrate in rainbow trout diets II Influence of carbohydrate levels on chemical composition and feed utilization of fish from different families. Aquaculture. 1977;11:39-50.
41. Daniels WH, Robinson EH. Protein and energy requirements of juvenile red drum *(Sciaenops ocellatus)*. Aquaculture. 1986; 53:243-252.

42. Hastings WH. Nutritional score. In: Neuhas OW, Halvar, JE. (Eds.), Fish Research. Academic Press. 1979;263-292.
43. Erfanullah, Jafri AK. Protein sparing effect of dietary carbohydrate in diets for fingerlings *Labeo rohita*. Aquaculture. 1995;136: 331-339.
44. Wang Y, Liu YJ, Tian LX, Du ZY, Wang JT, Wang S, Xiao WP. Effects of dietary carbohydrate level on growth and body composition of juvenile tilapia, *Oreochromis niloticus* X *O. aureus*. Aquac Res. 2005;36:1408-1413.

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