

1 **Determination of optimum dietary carbohydrate level of long whiskers catfish, *Mystus***
2 ***gulio* fry**

3 **Abstract**

4 The objective of the present study is to evaluate the optimum dietary carbohydrate level of *M.*
5 *gulio* fry, which will form the basis for formulating the nutritionally adequate, cost-effective
6 nursery diet. Five iso-proteinous (400 g kg⁻¹ diet) and iso-lipidic (120 g kg⁻¹ diet) semi-purified
7 diets with 100, 150, 200, 250 and 300 g carbohydrate kg⁻¹ diet were fed *ad libitum* to *Mystus*
8 *gulio* fry (0.33 ± 0.004 g) for 90 days in triplicates (10 fish/replicate). Fifteen fibre-reinforced
9 plastic tanks (50 L) with flow-through system (water flow rate of 0.5 L min⁻¹) were used for
10 rearing the fish. At the end of the feeding period, the diet containing 200 g kg⁻¹ carbohydrate
11 diet had significantly higher (*P* = .05) weight gain (8.39 g), Specific Growth Rate (SGR; 3.62
12 %/day), Protein Efficiency Ratio (PER; 1.52), Protein Productive Value (PPV; 24.08%), Lipid
13 Productive Value (LPV; 27.13%), Energy Productive Value (EPV; 66.89%) and lower Food
14 Conversion Ratio (FCR; 1.65) as compared to other diet fed groups. The fish fed with
15 carbohydrate beyond 200 g kg⁻¹ diet had significantly higher (*P* = .05) Hepatosomatic index
16 (HIS) and Viscerosomatic index (VSI) than the lower carbohydrate fed groups. Second order
17 polynomial regression analysis of weight gain, SGR, FCR, PER and PPV against the dietary
18 carbohydrate had showed that the optimum dietary carbohydrate requirement of *M. gulio* fry is
19 197.8-207.3 g kg⁻¹ diet.

20 **Keywords:** Carbohydrate, catfish, diet, growth, *Mystus gulio*, nutrient.

21 **1. Introduction**

22 Carbohydrate is progressively attracting the interest of researchers as it is the most economical
23 dietary source of energy as compared to lipid and protein especially for the formulation of diets
24 of herbivorous and omnivorous fish (Erfanullah and Jafri, 1998; Ding et al. 2016). “Inclusion of
25 carbohydrates in aqua-feeds is also very important because they are readily available, low-
26 budget, act as binder, improve feed palatability and reduce the catabolism of dietary proteins and
27 lipids for energy yielding processes” (Gao et al. 2010; NRC, 2011). “Generally, carbohydrates
28 are classified into digestible mono-, di- and polysaccharides and indigestible hemicelluloses and
29 cellulose” (Kamalam et al. 2017). “The digestible polysaccharides commonly used in feedstuff
30 are starch and its products, such as dextrin, which is an intermediate complex of glucose and
31 starch” (Xu et al. 2020). “The required level of carbohydrate incorporation in the diet enhances
32 growth performance and feed utilization of fish, increases protein and lipid retention by
33 preventing the catabolism of these expensive nutrients for energy needs (sparing effect), reduce
34 nitrogen load in the farm discharge (environmental safeguard), provide metabolites for biological
35 syntheses, improve feed stability” (Hardy, 2010; Li et al. 2016; Kamalam et al. 2017). “Carp,
36 catfish and tilapia can utilize carbohydrate more efficiently as an energy source than dietary
37 lipid” (Ogino et al. 1976; Garling and Wilson, 1977). In general, optimal levels of dietary
38 carbohydrate for carnivorous fish have been reported to range from 7 to 20% (Hemre et al. 2002;
39 Amoah et al. 2008).

40 The long whiskers catfish, *Mystus gulio* (*M. gulio*) is a promising species for aquaculture due to
41 its rapid growth rate, high nutritional value, delicious taste and good market potential (price
42 ranging from 300-500/kg). It can survive very well in the oxygen depleted waters and also
43 tolerate to the crowding condition and therefore, considered as an ideal species for high density
44 fish culture (Tripathi, 1996; Kumar et al. 2019). It is distributed in India, Bangladesh and other
45 Asian countries (Day, 1878; Kumar et al. 2019). Although *M. gulio* is an important catfish
46 species worthy for culture, the information on nutritional requirement of this species is very
47 limited. Khatua et al. (2021) reported that by using the casein-gelatin-dextrin based semi-purified
48 diets the optimum dietary protein and lipid requirement of *M. gulio* fry is 400 g and 120 g kg⁻¹
49 diet. However, the optimum carbohydrate requirement level of *M. gulio* fry has not been studied
50 so far. The results of the present study on optimum dietary carbohydrate level coupled with the
51 optimum protein and lipid requirement levels of this species studied earlier by the same research
52 group will form the basis for formulating the nutritionally adequate, cost-effective nursery diets
53 for this species. A good quality nursery diet is the need of hour not only to produce the quality
54 seed of this species but also to avoid its size disparity, prevent cannibalism and ultimately
55 improve the survival rate.

56 2. Materials and Methods

57 2.1 Experimental diets

58 Five iso-nitrogenous (400 g protein kg⁻¹ diet) and iso-lipidic (120 g lipid kg⁻¹ diet) semi-purified
59 diets were prepared using various levels of carbohydrate and labelled as D-1 (200), D-2 (150),
60 D-3 (200), D-4 (250) and D-5 (300) g carbohydrate kg⁻¹ diets (Table 1). Casein and gelatin were
61 used as protein sources; dextrin and corn starch as carbohydrate sources, and 1:1 fish oil and
62 sunflower oil was used as the source for lipid. Carboxymethyl cellulose (CMC) was used as
63 binder and α -cellulose was applied as filler. Vitamin and mineral mixture is added in the diets
64 (Ogino 1977; Modified Lovell et al. 1984). The 1.0 mm diameter experimental diets were
65 prepared using a hand pelletizer. Prepared feed pellets were dried overnight at 60 °C in an air
66 oven for 24 h and stocked in a refrigerator at 4 °C for further use (Khatua et al. 2021).

67 2.2 Experimental set up

68 Two thousand *M. gulio* fry were obtained from the ICAR-Central Institute of Brackishwater
69 Aquaculture, Kakdwip, West Bengal. The fish were accustomed to the laboratory condition in
70 five FRP tanks of 200 L capacity each for 2 weeks. During the period of acclimatization, the fish
71 were fed twice daily close to apparent satiation level throughout the experiment. After
72 acclimatization, 150 similar sized fry (average initial weight of 0.33 ± 0.004 g) were arbitrarily
73 distributed in 15 flow-through (flow rate: 0.5 L min⁻¹) FRP tanks of 50 L capacity each with 40 L
74 water volume in triplicates at a stocking density of 10 fish/tank. Before starting of the
75 experiment, initial fish biomass of each tank was recorded. The experimental fish were fed *ad*
76 *libitum* for 90 days (Khatua et al. 2021). After completion of the experiment the final biomass of
77 fish with respect to each tank was determined by batch-weighing the fish.

78 2.3 Chemical analysis of experimental diet and fish

79 Before commencement of the feeding trial, 200 fish were randomly sacrificed with an overdose
 80 of MS222 solution and the fish were taken for determining the initial whole-body composition.
 81 The proximate composition of experimental diets (Table 1) and fish (oven dried and grounded
 82 sample) was analyzed in triplicates as per the standard method (AOAC, 1990) in the National
 83 Feed Testing and Referral Laboratory, Fish Nutrition and Physiology Division, ICAR-CIFA,
 84 Bhubaneswar.

85 **2.4 Water analyses**

86 Except water temperature which was measured twice daily (06:00 h and 14:30 h), the other water
 87 quality parameters were analyzed in every 15 days interval following the methods of APHA
 88 (1992). The observed parameters were in the range of: temperature, 24.5-26.3 °C; pH, 7.4-7.8;
 89 dissolved oxygen, 7.41-8.20 mg L⁻¹; total alkalinity, 111.36-115.39 mg CaCO₃ L⁻¹; and total
 90 hardness, 103.23-106.76 mg CaCO₃ L⁻¹.

91 **Table 1 Formulation and proximate composition of the experimental diets (g kg⁻¹ on dry**
 92 **matter basis) with various level of dietary carbohydrate ([±]CHO)**

Ingredient	Experimental diets				
	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

93 [@]Vitamin mixture: vitamin A – 3000 IU; vitamin D3 – 15 000 IU; menadione sodium bisulphate
94 – 10 mg; choline chloride – 2000 mg; niacin – 50 mg; riboflavin – 20 mg; pyridoxine – 10 mg;
95 thiamine mononitrate – 10 mg; pantothenic acid – 40 mg; folic acid – 5 mg; vitamin B₁₂ – 0.02
96 mg; biotin – 1 mg; inositol – 400 mg; α -tocopherol acetate – 5 mg and vitamin C – 200 mg
97 (Modified Lovell et al. 1984).

98 ^hMineral mixture: NaCl – 1.0 g; MgSO₄.7H₂O – 15.0 g; NaH₂-PO₄.2H₂O – 25.0 g; KH₂PO₄–
99 32.0 g; Ca(H₂PO₄). 2H₂O – 20.0 g; Fe-citrate – 2.5 g; Ca-lactate – 3.5 g and ^oTrace element
100 mixture – 1.0 g (Ogino, 1977).

101 ^oTrace element mixture: ZnSO₄.H₂O – 35.3 g; MnSO₄.4H₂O – 16.2 g; CuSO₄.5H₂O – 3.1 g;
102 CoCl₂.6H₂O – 0.1 g; KIO₃ – 0.3 g and cellulose – 45.0 g.

103 [#]CHO: Carbohydrate

104

105 **2.5 Calculation of nutritional indices**

- 106 ➤ Weight gain (g) = Final weight (g) - Initial weight (g)
 107
- 108 ➤ Specific growth rate (SGR; %/day) = $\frac{\ln \text{ final weight} - \ln \text{ initial weight}}{\text{Experimental duration (days)}} \times 100$
 109
 110
- 111
- 112 ➤ Feed conversion ratio (FCR) = $\frac{\text{Feed consumption (g)}}{\text{Fish weight gain (g)}}$
 113
 114
- 115 ➤ Protein efficiency ratio (PER) = $\frac{\text{Fish weight gain (g)}}{\text{Protein intake (g)}} \times 100$
 116
- 117 ➤ Nutrient (protein and lipid) and energy productive values:
- 118 (PPV, LPV and EPV; %) = $\frac{\text{Nutrients/energy gain in body}}{\text{Nutrients/energy intake}} \times 100$
 119
 120
- 121 ➤ Hepatosomatic Index (HSI) = $\frac{\text{Liver weight}}{\text{Body weight}} \times 100$
 122
 123
- 124 ➤ Viscerosomatic Index (VSI) = $\frac{\text{Weight of the whole digestive tract}}{\text{Body weight}} \times 100$
 125

126 2.6 Statistical analysis

127 The statistical significance of different study parameters was analyzed by one-way ANOVA and
 128 Duncan's' Multiple Range Test to compare the means ($P = .05$) between different experimental
 129 groups. PC-SAS program for Windows, released v 6.12 [SAS Institute, Cary, NC, USA (SAS,
 130 1996)] used for data analysis. Second-order polynomial regression analysis was performed by
 131 taking weight gain, SGR, PER, PPV and FCR values versus dietary carbohydrate levels and the
 132 carbohydrate requirement of *M. gulio* fry was estimated more precisely.

133 3. Results and Discussion

134 At the end of the experiment, there was no fish mortality recorded in any of the experimental
 135 tank. The study results indicated that the dietary carbohydrate had a significant effect ($P = .05$)
 136 on growth performance and feed utilization in fish (Table 2). The *M. gulio* fry fed with 200 g
 137 carbohydrate kg^{-1} (D3) diet had significantly higher ($P = .05$) weight gain, SGR, PER and lower
 138 ($P = .05$) FCR, beyond which there was no improvement ($P = .05$) in these parameters. Similar
 139 to our results, the poor growth and nutritional indices of fish at lower and higher levels of dietary
 140 carbohydrate was reported by many earlier researchers in *Labeo rohita* (Erfanullah and Jafri,
 141 1993), *Channa striatus* (Arockiaraj et al. 1999), *Mystus monatus* (Arockiaraj et al., 2008), *Nibea*
 142 *japonica* (Li et al. 2015), *Apostichopus japonicas* (Li et al. 2019), *Epinephelus akaara* (Wang et
 143 al. 2016) and *Puntius gonionotus* (Mohanta et al. 2009).

144 It is investigated that the high level of dietary carbohydrate resulted high glucose level in the
 145 blood and glycogen deposition in the liver which reduce the digestion, absorption and
 146 assimilation of carbohydrate ultimately leading to poor growth and utilization of nutrient in fish
 147 (Hemre et al. 2002; Kamalam et al. 2017). Although we have not measured the glucose level in
 148 the blood and the glycogen deposition in the liver, this is one of the reasons for the poor growth
 149 and nutrient utilization of *M. gulio* fry. Whereas, low levels of dietary carbohydrate in the diet
 150 leads to reduction of daily weight gain due to loss of muscle mass (muscle hypotrophy) (Torres
 151 and Castellanos, 2013). We also observed less whole body protein content in *M. gulio* fry at
 152 lower level of dietary carbohydrate. The adequate level of dietary carbohydrate served as energy
 153 source so that the fish can directly utilize most of the dietary protein to physical growth rather
 154 than energy need and improving animal performance (Sulaiman et al. 2020). In this study the
 155 carbohydrate level of 200 g kg⁻¹ diet might have sufficient to meet the energy need of fish,
 156 thereby sparing the dietary protein for growth of fish rather than energy purpose.

157 Both the HSI and VSI were significantly increased ($P = .05$) with increase in dietary
 158 carbohydrate levels (Table 2). “HSI is an important indicator of available energy in fish”
 159 (Mohanta et al. 2009). “Excess dietary carbohydrate after assimilation get deposited in the liver
 160 in the form of glycogen leading to high HSI values or it may get converted to lipid by lipogenic
 161 enzyme and then stored in fish body” (Tian et al. 2012; Azaza et al. 2015; Zhang et al. 2021). In
 162 this study, high HSI value in D-4 and D-5 groups might be due to deposition of fat or glycogen
 163 in the liver, which is in accordance with earlier findings (Li et al. 2015 for *Nibeia japonica*;
 164 Mohanta et al. 2009 for *Puntius gonionotus*; Yengkokpam et al. 2007 for *Catla catla*). Higher
 165 glycogen deposition in liver is caused by excessive available energy obtained from digestible
 166 carbohydrate. The higher HSI values in the present study attributed to more lipid deposition in
 167 liver when *M. gulio* was fed with higher level of dietary carbohydrate (250 and 300 g
 168 carbohydrate kg⁻¹ diet). Similar results were reported in gilthead sea bream, European sea bass
 169 and Catla (Yengkokpam et al. 2007; Enes et al. 2008, Moreira et al. 2008). “The VSI also
 170 showed the similar trend as that of HSI which indicates that the excess dietary carbohydrate
 171 beyond the requirement level of *M. gulio* fry is converted into lipid and gets deposited in its
 172 viscera” (Mohanta et al. 2009).

173

174 **Table 2 Growth performance and nutrient utilization of *Mystus gulio* fry fed with various**
 175 **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	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

weight (g)					
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

176 FCR: feed conversion ratio; SGR: specific growth rate; PER: protein efficiency ratio; HSI:
177 hepatosomatic index; VSI: viscerosomatic index.

178 [‡]CHO: Carbohydrate

179 Values in the same column with different superscripts are significantly different ($P = .05$).

180 Values are means of three replicates of each experimental diet \pm standard error (SE).

181 A significant variation ($P = .05$) in nutrient (protein and lipid) and energy gain were also
182 observed in *M. gulosus* fry with respect to change in dietary carbohydrate levels. The fish fed 200 g
183 carbohydrate kg⁻¹ diet (D-3) had significantly higher ($P = .05$) PPV, LPV and EPV values as
184 compared to other carbohydrate fed groups (Table 3). Any further increase in dietary
185 carbohydrate beyond this level had no significantly higher ($P = .05$) nutrient and energy gain. In
186 this study, the higher growth and nutrient utilization in terms of PPV, LPV and EPV was
187 recorded with diet containing 200 g carbohydrate kg⁻¹ (D-3) in *M. gulosus* fry and thereafter, there
188 was no significant ($P = .05$) improvement in these nutritional parameters. Primary understanding
189 on the optimum level of protein and protein-sparing effects of non-protein substance like
190 carbohydrates can be utilized efficiently in reducing feed costs (Shiau 1997). In this study,
191 maximum weight gain, PER and PPV were observed in D-3 group, which advocate that in *M.*
192 *gulosus* fry, the protein is most efficiently utilized at the 200 g carbohydrate kg⁻¹ of diet. The

193 maximum growth and utilization of nutrient in *M. gulio* is observed at 200 g carbohydrate kg⁻¹
 194 diet in the present study is similar to the requirement of 150-200 g kg⁻¹ in *Clarius batrachus*
 195 (Mollah and Alam, 1990); but lower than the 350 g kg⁻¹ of *Heteropneustes fossilis* (Akand et al.
 196 1991). A significant increase in protein and energy gain of *M. gulio* fry with an increase in the
 197 dietary carbohydrate level up to a certain extent (200 g carbohydrate kg⁻¹ diet), beyond which
 198 there was no further improvement is in agreement with Erfanullah and Jafri (1998) for *Catla*
 199 *catla* and Mohanta et al. (2009) for *Puntius gonionotus*. It is reported that the higher amounts of
 200 dietary carbohydrate retarded the growth in rainbow trout, *Oncorhynchus mykiss* (Austreng et al.
 201 1977) and red drum, *Sciaenops ocellatus* (Daniels and Robinson 1986) due to poor nutrient gain.
 202 We have also observed poor growth performance at higher carbohydrate level in diet (D-4 and
 203 D-5), which might be due to fatty liver (higher HSI) and poor physiological function. Similarly,
 204 Hastings (1979) reported that if consumption of carbohydrate is used in excess for energy
 205 requirement, it increases visceral fat deposits and fatty infiltration in organs, which eventually
 206 leads to restriction of normal body function of fish. In our study the LPV was increased with
 207 increase in dietary carbohydrate up to a certain level and then remained constant. However, in
 208 contrast to our results, Sulaiman et al. (2020) observed a positive correlation between dietary
 209 carbohydrate level and LPV which indicates that increasing carbohydrate level leads to
 210 lipogenesis and spared lipid from catabolism and hence, it gets accumulated.

211 **Table 3 Effect of dietary levels of carbohydrate ([‡]CHO) on nutrient retention in *Mystus***
 212 ***gulio* fry**

Nutrient gain	Experimental diet (Carbohydrate level)				
	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}

213 PPV: Protein productive value; LPV: Lipid productive values; EPV: Energy productive value

214 [‡]CHO: Carbohydrate

215 Values in the same column with different superscripts are significantly different (*P* = .05).

216 Values are means of three samples \pm standard error (SE).

217 The composition of whole-body fish fed with various digestible carbohydrate diets are presented
218 in (Table 4). In our study, we observed that the dietary carbohydrate had significant ($P = .05$)
219 effect on whole-body contents of the fish. The moisture content of the whole-body of fish is
220 significantly decreased ($P = .05$) with an increase in dietary carbohydrate levels which is found
221 to be differ from the findings of Mohanta et al. (2009) for *P. gonionotus*. However, increase of
222 whole-body moisture content with increase of dietary carbohydrate up to certain level and
223 thereafter, it is decreased was reported by many earlier researchers (Arockiaraj et al. 2008; Li et
224 al. 2019; Zhang et al. 2021). This implies that the effect of different levels of dietary
225 carbohydrate on whole body moisture content is species specific and there is no definite
226 relation/trend exists between dietary carbohydrate and whole body moisture content. The whole-
227 body protein content of *M. gulo* fed with 200 g carbohydrate kg^{-1} diet was significantly higher (P
228 < 0.05) than the lower (100 and 150 g carbohydrate kg^{-1} diet) or higher (250 and 300 g
229 carbohydrate kg^{-1} diet) fed groups, which in agreement with Arockiaraj et al. (1999) for *Channa*
230 *striatus* and Mohanta et al. (2009) for *Puntius gonionotus*. The maximum protein gain in terms
231 PPV is the reason for significantly higher whole body protein content in fish fed 200 g
232 carbohydrate kg^{-1} diet. The whole-body lipid content is directly proportional to dietary
233 carbohydrate levels, which confirms the findings of other researchers Erfanullah and Jafri (1995)
234 in *Labeo rohita*; Wang et al. (2005) in *Oreochromis niloticus* \times *O. aureus* and Mohanta et al.
235 (2009) in *Puntius gonionotus*. They observed lipogenic activity in fish when fed high
236 carbohydrate diets. The whole-body ash content was increased linearly with the rise in
237 carbohydrate levels which is same with the results of Arockiaraj et al. (2008) for *Mystus monatus*
238 and Mohanta et al. (2009) for *P. gonionotus*. Therefore, maximum ash content was found in fish
239 fed with 300 g carbohydrate kg^{-1} diet (D-5) which suggests that a linear relation coexist between
240 whole body ash content and the carbohydrate level in the diet. In this study, with the increase in
241 dietary carbohydrate concentrations, there was a slight but insignificant increase in whole body
242 gross energy content of *M. gulo* fry which is contrast to the findings of (Mohanta et al. 2009)
243 who reported that with an increase in dietary carbohydrate level the whole-body gross energy
244 level is increased.

245 **Table 4 Whole-body chemical composition (on g kg^{-1} wet weight basis) of *Mystus gulo* fry**
246 **fed with various level of carbohydrate ($\% \text{CHO}$)**

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

247 [#]CHO: Carbohydrate

248 Values in the same column with different superscripts are significantly different ($P = .05$).

249 Values are means of three samples ± standard error (SE).

250 From the second order polynomial regression analysis of weight gain ($y = -0.000267x^2 +$
 251 $0.10701x - 3.2607$; $R^2 = 0.7032$) (Figure 1), SGR ($y = -0.000045x^2 + 0.01824x + 1.6617$; $R^2 =$
 252 0.7501) (Figure 2), PER ($y = -0.000025x^2 + 0.00989x + 0.476$; $R^2 = 0.6628$) (Figure 3), PPV ($y = -0.000534x^2 + 0.22144x - 0.2073$; $R^2 = 0.7998$) (Figure 4), and FCR ($y = 0.000041x^2 -$
 253 $-0.000534x^2 + 0.22144x - 0.2073$; $R^2 = 0.7998$) (Figure 4), and FCR ($y = 0.000041x^2 -$
 254 $0.01647x + 3.386$; $R^2 = 0.8117$) (Figure 5) it is observed that the optimum dietary carbohydrate
 255 level of *M. gulo* fry is 197.8-207.3 g/kg diet at a 400 g/kg dietary protein and 120 g/kg dietary
 256 lipid.

257

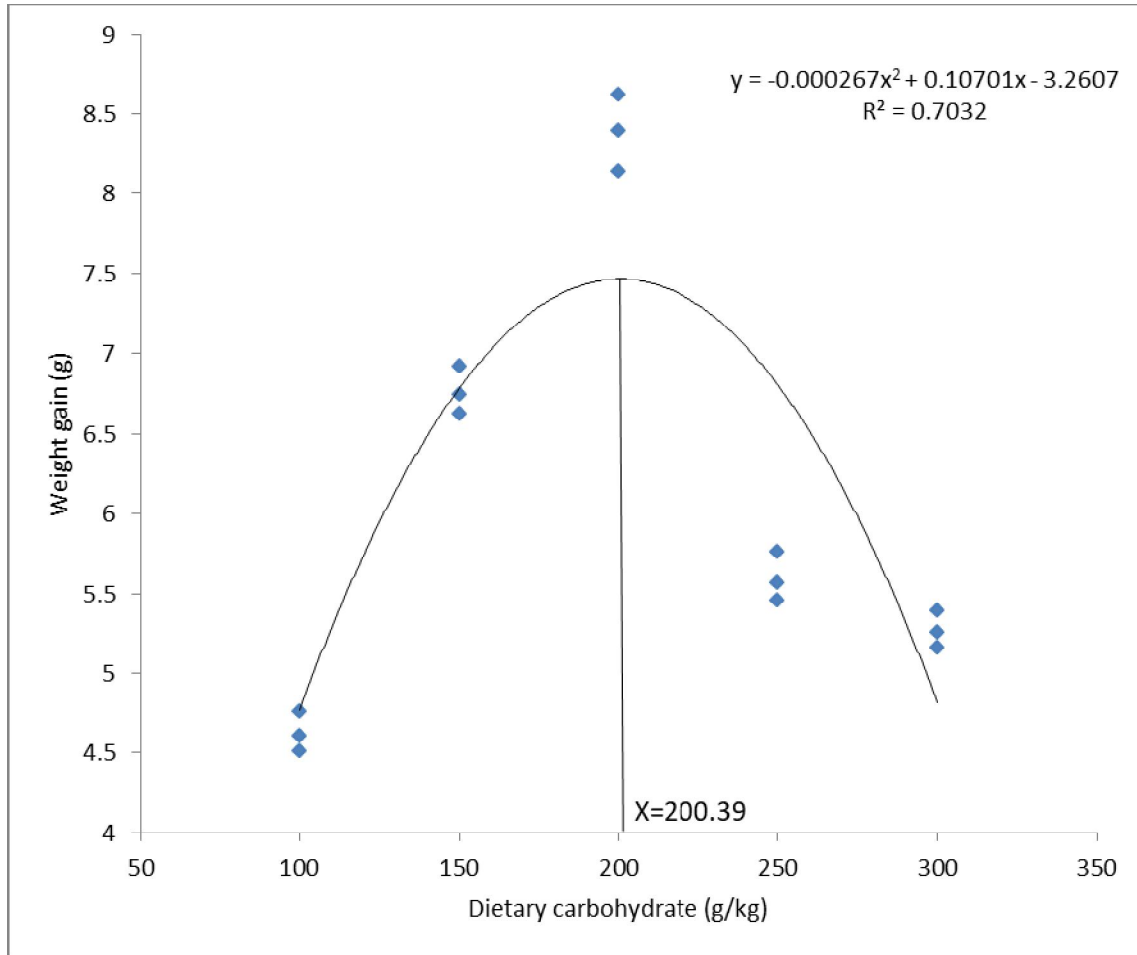
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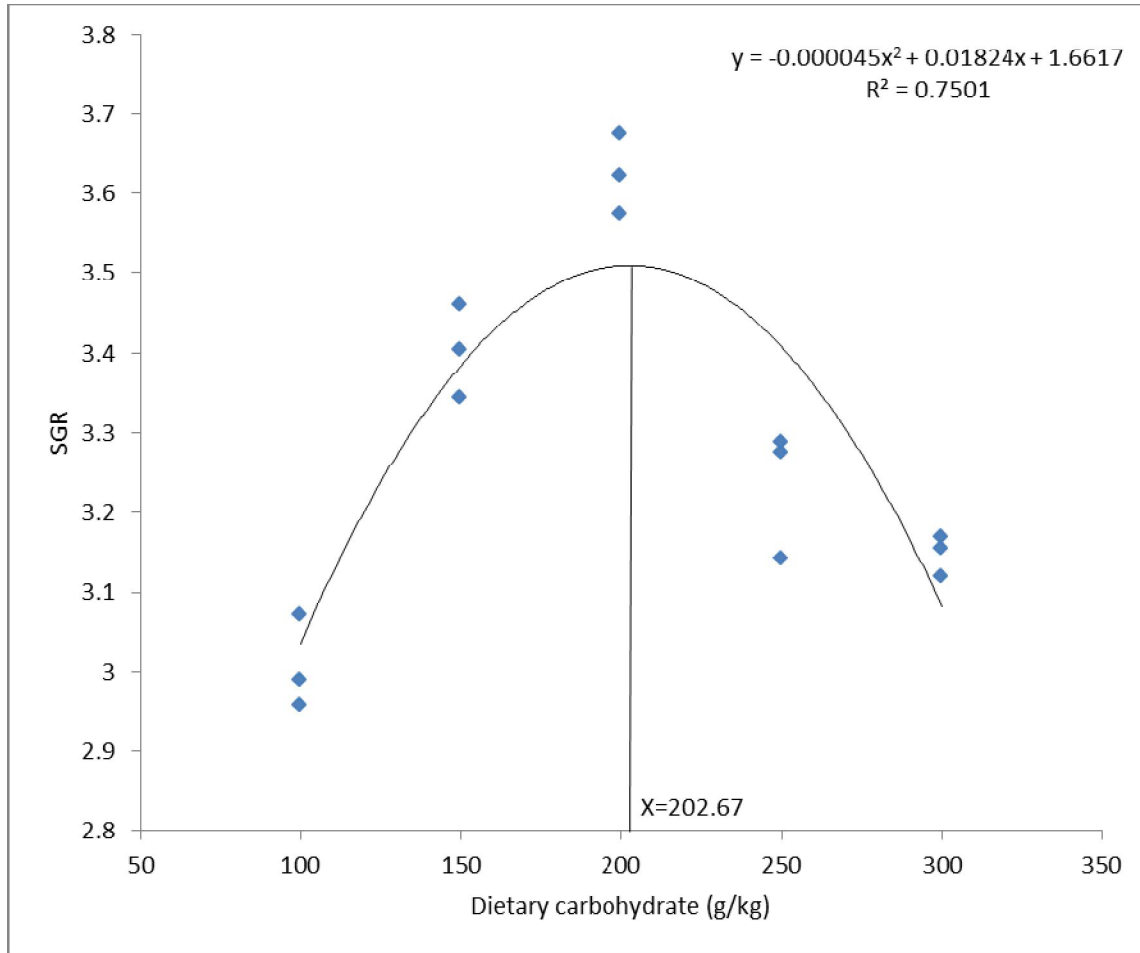
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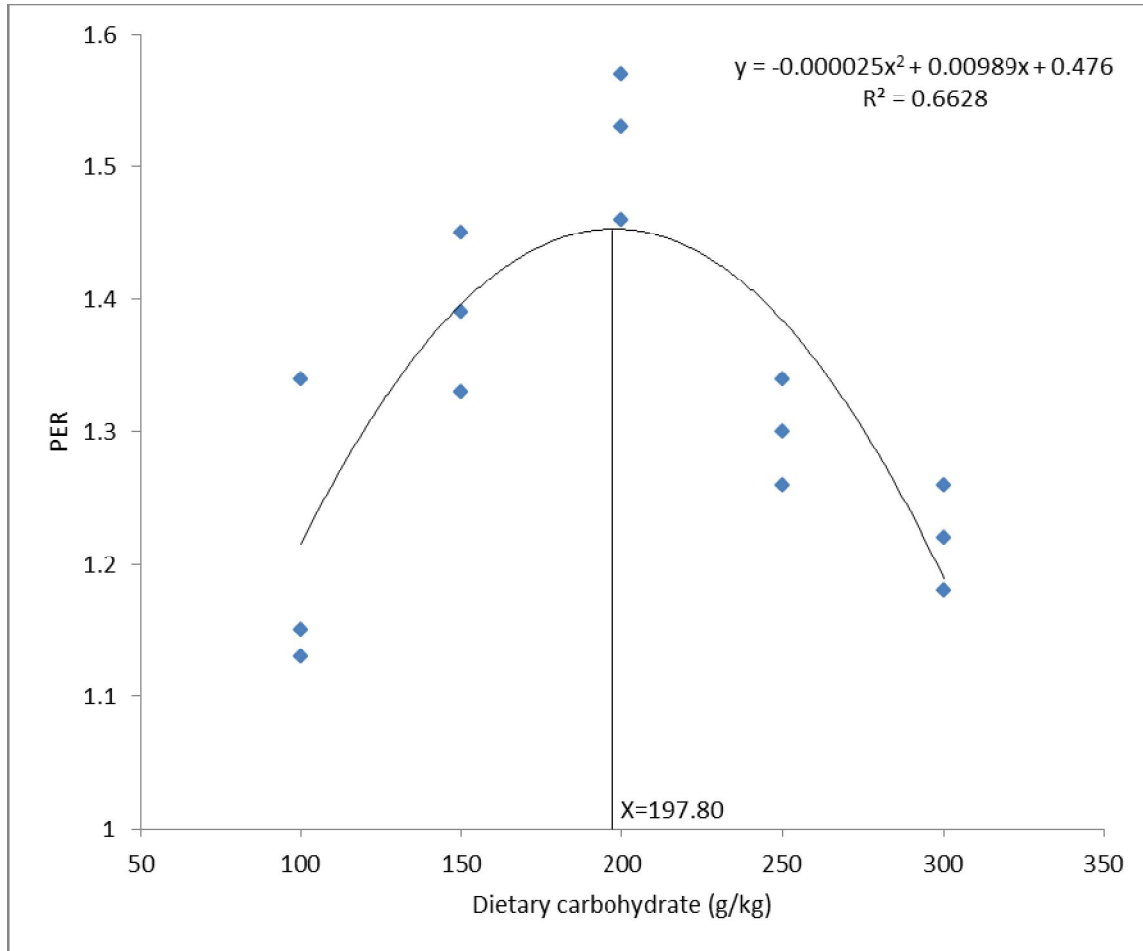
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264 **Figure 1 Second-order polynomial regression analysis of weight gain and dietary**
 265 **carbohydrate levels for *Mystus gulio* fry**



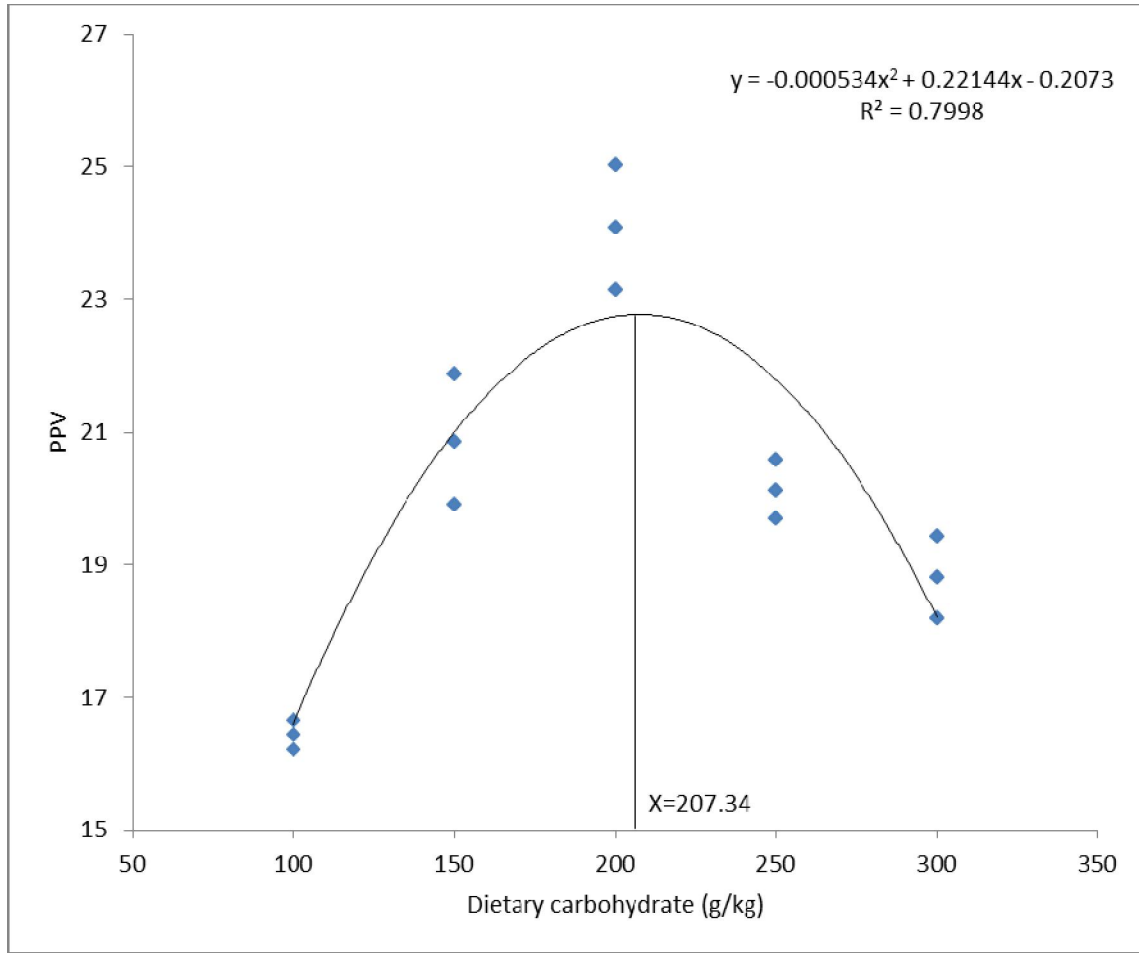
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267 **Figure 2 Second-order polynomial regression analysis of SGR and dietary carbohydrate**
 268 **levels for *Mystus gulio* fry**



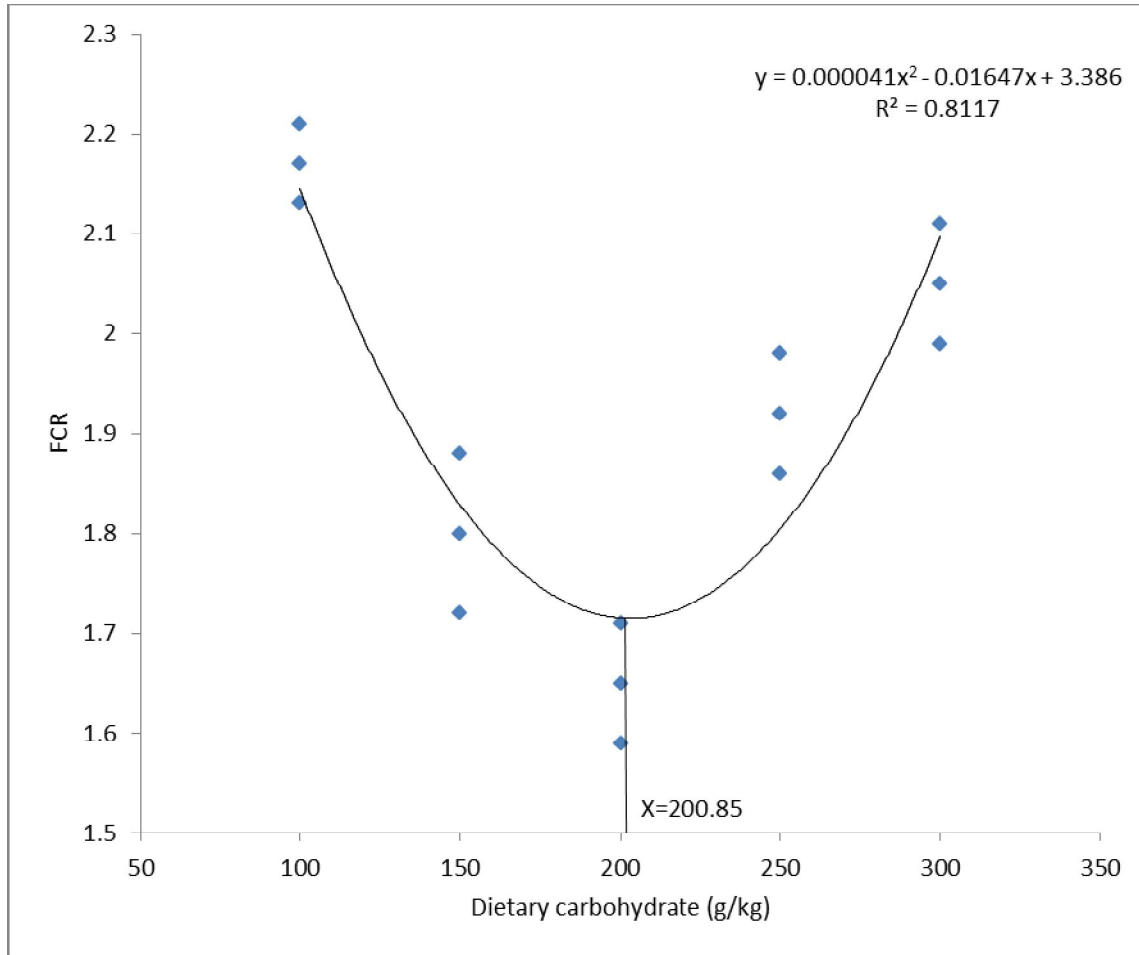
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270 **Figure 3 Second-order polynomial regression analysis of PER and dietary carbohydrate**
 271 **levels for *Mystus gulio* fry**



272

273 **Figure 4 Second-order polynomial regression analysis of PPV and dietary carbohydrate**
 274 **levels for *Mystus gulio* fry**



275

276 **Figure 5 Second-order polynomial regression analysis of FCR and dietary carbohydrate**
 277 **levels for *Mystus gulio* fry**

278 **4. Conclusions**

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

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288

289 **6.1 Conflict of interest:** The authors declare that there is no conflict of interest.

290 **6.2 Animal welfare statement:** The authors confirm that they have followed the standards for
291 the protection of animals used for scientific purposes.

292 **6.3 Availability of data and material (data transparency)**

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

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

297

298 **7. References**

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