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# Effect of different carbohydrate-to-lipid ratios elicits growth, feed utilization, lipid deposition and lipogenic enzyme activity in striped catfish (*Pangasianodon hypophthalmus*, Sauvage, 1878) fingerlings

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**Abstract**

This study evaluated the effects of diets containing various carbohydrate-to-lipid (CHO $L^{-1}$) ratios on growth performance, nutrient utilization body indices and hepatic lipogenic enzyme (malic enzyme, 6-phosphogluconate dehydrogenase and fatty acid synthase) activities. Triplicate–groups of *Pangasianodon hypophthalmus* fingerlings were fed eight isoenergetic and isonitrogenous diets with different carbohydrate-to-lipid ratios (0.51, 0.79, 1.12, 1.79, 2.41, 3.24, 4.43 and 7.62). Higher body fat deposition and lower growth performance were observed in *P. hypophthalmus* fingerlings fed with high-lipid diet than those fed with high-carbohydrate diet. The fish fed the diet with 7.62 CHO $L^{-1}$ ratio exhibited significantly ($p$<0.05) higher hepatosomatic index compared to those fed higher lipid diets (0.51 and 0.79). High dietary carbohydrate level significantly increased ($p$<0.05) the activities of malic, 6-phosphogluconate dehydrogenase and fatty acid synthase enzyme. Based on the second-order polynomial regression analysis of weight gain, the optimal dietary carbohydrate and lipid contents for *P. hypophthalmus* fingerling were 304 and 103 g $kg^{-1}$, respectively, which correspond to a dietary CHO $L^{-1}$ ratio of 2.95.

**Keywords**: Striped catfish, Carbohydrate-to-lipid ratio, Lipogenesis, Fat deposition

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## Introduction

The Striped catfish (*Pangasianodon hypophthalmus*, Sauvage, 1878) is an omnivorous freshwater species (Ali *et al.*, 2005). To achieve the objective of feed cost reduction, preference is given to incorporate more carbohydrates than lipids into diet as the non-protein source of energy because they are relatively cheaper and more available (Zhu *et al.*, 2013). A more carbohydrate-based feed also offers protein-sparing effects. Unfortunately, excess carbohydrates may stimulate lipogenic enzyme activity in some species, which can lead to the formation of undesirable fat deposits in fish tissue (Likimani and Wilson, 1982). Conversely, a high-lipid diet could have adverse effect on fish tissue composition (Nankervis *et al.*, 2000) and consumer acceptance (Gélineau *et al.*, 2001). Any imbalance in carbohydrates to lipids levels could also directly affect fish growth, feed conversion, and body composition (Erfanullah and Jafri, 1998a). An appropriate combination of carbohydrates and lipids in fish diets must therefore be cautiously and meticulously evaluated.

Recent studies have focused on determining the optimal dietary carbohydrate-to-lipid (CHO $L^{-1}$) ratio in different fish species, including yellow croaker (*Larimichthys crocea*, Richardson, 1846) (Xing *et al.*, 2016; Zhou *et al.*, 2016) and Orange-spotted Grouper *Epinephelus coioides* Hamilton, 1822 (Wang *et al.*, 2017). The present study aimed to evaluate the effects of different CHO $L^{-1}$ ratios on growth performance, lipogenesis, and the accumulation of fat in *P. hypophthalmus* body.

## Materials and methods

*Preparation of the experimental diets*

Eight isoenergetic (17.7 MJ $kg^{-1}$) and isonitrogenous (350 g $kg^{-1}$ crude protein) diets were prepared, and their carbohydrate and lipid levels were adjusted to achieve different carbohydrate-to-lipid ratios (0.51, 0.79, 1.12, 1.79, 2.41, 3.24, 4.43 and 7.62) (Table 1). Corn starch and fish meal were used in diets as sources of carbohydrates and protein, respectively, while corn oil and fish oil were used as lipid sources. All dry ingredients were first thoroughly blended into a homogenous mixture using a Hobart mixer. Water and oils were then added to make the dough moist. The dough was then passed through a 3-mm diameter die to form the pellet. The pellets were then oven-dried at 40°C for 24 h and stored at −20°C until used.

**Table 1: Formulation and proximate composition of the experimental diets (g $kg^{-1}$).**

| | CHO : L ratio | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| **Ingredients (g $kg^{-1}$)** | **0.51** | **0.79** | **1.12** | **1.79** | **2.41** | **3.24** | **4.43** | **7.62** |
| [a]Defatted fish meal | 487 | 487 | 487 | 487 | 487 | 487 | 487 | 487 |
| Corn starch | 36.4 | 76.4 | 116.4 | 176.4 | 216.4 | 256.4 | 296.5 | 356.5 |
| Cellulose | 168.5 | 146.2 | 123.6 | 89.8 | 67.2 | 44.6 | 21.9 | 0 |
| Corn oil | 97.4 | 88.5 | 79.8 | 66.7 | 58 | 49.3 | 40.6 | 27.6 |
| Fish oil | 89.7 | 80.9 | 72.2 | 59.1 | 50.4 | 41.7 | 33 | 19.9 |
| [b]Mineral premix | 50 | 50 | 50 | 50 | 50 | 50 | 50 | 50 |
| [c]Vitamin premix | 40 | 40 | 40 | 40 | 40 | 40 | 40 | 40 |
| Vit -C | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 5 |



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| **Table 1 continued:** | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| Choline chloride | 6 | 6 | 6 | 6 | 6 | 6 | 6 | 6 |
| [d]CMC | 20 | 20 | 20 | 20 | 20 | 20 | 20 | 8 |
| Proximate composition of diet ([e]DM basis) | | | | | | | | |
| Dry matter | 945.8 | 949.2 | 948.5 | 942.6 | 941.4 | 938.8 | 944.5 | 928.6 |
| Crude protein | 355.6 | 353.2 | 348.9 | 350.7 | 353.3 | 348.5 | 350.8 | 343.9 |
| Crude lipid | 203.0 | 180.0 | 163.0 | 136.5 | 118.6 | 100.5 | 81.4 | 53.1 |
| Ash | 150.2 | 144.2 | 154 | 148.9 | 147.1 | 140.3 | 132.0 | 123.8 |
| Fibre | 187.7 | 180.4 | 151.5 | 119.6 | 95.2 | 85.1 | 75.3 | 74.6 |
| Carbohydrate | 103.5 | 142.2 | 182.6 | 244.3 | 285.8 | 325.6 | 360.5 | 404.6 |
| Gross energy ($MJkg^{-1}$) | 17.6 | 17.5 | 17.6 | 17.6 | 17.6 | 17.7 | 17.6 | 17.5 |
| Protein/Energy (g $^{MJ-1)}$ | 20.1 | 20.1 | 19.9 | 19.9 | 20 | 19.6 | 19.9 | 19.6 |

[a]Danish fish meal defatted with n-Hexane (Grade AR, Esbjerg, Denmark)
[b] Mineral mix: Containing $kg^{-1}$, calcium phosphate (monobasic, 397.5 g; calcium lactate, 327 g; ferrous sulphate, 25 g; magnesium sulphate, 137 g; potassium chloride, 50 g; sodium chloride, 60 g; potassium iodide, 150 mg; copper sulphate, 780 mg; manganese oxide, 800 mg; cobalt carbonate 100 mg; zinc oxide, 1.5 g and sodium selenite, 20 mg.
[c]Vitamin mix (ROVIMIX 6288; Roche Vitamins, Basel, Switzerland): Containing $kg^{-1}$, Vit. A, 50 million IU; Vit. D3, 10 million IU; Vit. E, 130 g; Vit. B1, 10 g; Vit. B2, 25 g; Vit. B6, 16 g; Vit. B12, 100 mg; Biotin, 500 mg; Pantothenic acid, 56 g; Folic acid, 8 g; Niacin, 200 g; Anticake, 20 g; antioxidant, 200 mg; Vit. K3, 10 g and Vit. C, 35 g.
[d] Carboxy methyl cellulose.
[e] dry matter

*Fish and experimental husbandry conditions*

Prior to the trial, *P. hypophthalmus* fingerlings were purchased from a commercial supplier, acclimated to laboratory conditions at the Aquaculture Research Complex of University Sains Malaysia, Penang, Malaysia for three weeks and fed with a commercial diet. During this period, each fish was measured for body weight and sorted into uniform groups. Two hundred and eighty eight fish with a mean initial body weight of 3.04±0.02 g were randomly allocated to 24 glass tanks filled with 30 L of water. Each glass tank was assigned to each replicate of treatments. The fish were hand-fed to apparent satiation twice daily (08:00 and 15:00 h). Uneaten feed, when observed, was siphoned out approximately 45 min after feeding, dried at 60°C for determining the weight of the feed intake. Data on weight gain and feed consumption were collected and recorded. Feed intake was calculated as the difference between the initial dry food weight and the adjusted uneaten dry food weight. The fishes were subjected to 12-h photoperiod of light and dark each day. Each tank was provided with continuous aeration through air-stone to maintain dissolved oxygen levels at or near saturation. The water temperature and dissolved oxygen concentration in the culture tanks were monitored by taking random samples twice weekly.

*Sample collection and chemical analysis*

For sampling, after a 24-h period of food deprivation, the fish were anesthetized with tricaine methanesulfonate (MS-222) and weighed individually. Six fishes from each replicate were sampled randomly and stored at −20°C. Lipid contents were measured for the whole-body, liver, and


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viscera. Each fish's body index parameters were also evaluated. The chemical proximate compositions of the experimental diets, livers, viscera, and carcasses were determined using standard methods (AOAC, 1997).

*Lipogenic enzyme activity*

Upon completing the trial, three fish per tank were randomly picked and used for evaluation of lipogenic enzyme activity. The liver samples were gently washed with ice-cold distilled water and subsequently weighed. The samples were homogenized in a chilled homogenization sucrose buffer (0.2 g of liver in 1 mL buffer; 0.25 M sucrose containing 1 mM DTT, 1 mM EDTA, and protease inhibitors; pH 7.4). The sample homogenates were centrifuged at 30,000 *g* for 59 min at −4°C. Clear supernatant was collected and stored frozen at −40°C to determine lipogenic enzymes activity. The activities of the malic enzyme (ME; EC 1.1.1.40), 6-phosphogluconate dehydrogenase (6PGDH; EC 1.1.1.44), and fatty acid synthase (FAS; EC 2.3.1.85**)** were assayed according to protocols described by Bazin and Ferre (2001). Bradford's (1976) method was employed to determine the protein concentration of the enzyme extract; bovine serum albumin (BSA) was used as standard protein, and fatty acid synthase activity was determined based on measurements of Nicotinamide Adenine Dinucleotide Phosphate (NADPH) oxidation in the spectrophotometer at 340 nm for 10 min at 37°C as described by Halestrap and Denton (1973), while determination of 6PGDH activity was done based on measurements of NADPH formation at 340 nm, according to the procedure described by Glock and Mclean (1953). In the ME assay, the production of NADPH at 340 nm at 37°C for 10 min was measured as described by Ochoa (1955). The NADPH formation was also determined in a spectrophotometer at 340 nm.

The specific activities of the lipogenic enzymes (ME, 6PGDH, and FAS) were expressed as nanomoles of NADPH consumed or generated per minute per milligram of protein at 37°C. All determinations were performed in triplicate, and the results are expressed as mean±standard deviation.

*Calculations and statistical analysis*

The growth performance and body indices of the experimental fish were evaluated based on the following formulas:

Specific growth rate (SGR)=(Ln final weight-Ln initial weight)×100/days of experiment

Weight gain (WG %)=(Final weight (g)– Initial weight (g))/Initial weight (g)×100

Feed conversion ratio (FCR)=[Feed intake(g)/Wet weight gain(g)]

Hepatosoamtic index (HSI)=[Liver weight(g)/ Body weight(g)×100]

Viscerasomatic index (VSI)=[Viscera weight (g)/ Body weight(g)×100]

Interaperitoneal fat (IPF)= [Interaperitoneal fat(g)/Body weight(g)×100]

Protein efficiency ratio (PER)=Fish weight gain(g)×100/protein intake(g)

Conditional factor (CF)=Body weight(g)×100/body length(cm)$^3$

A one-way analysis of variance (ANOVA) was used to compare sample means, and Duncan's multiple range tests were used to determine significant



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differences at the 0.05 probability level. Second-order polynomial quadratic regression analyses were carried out to evaluate the optimal dietary carbohydrate and lipid levels. All statistical analyses were performed using SPSS (version 16.0, for Windows, Chicago, IL, USA).

## Results

The fish fed a 2.41 CHO $L^{-1}$ diet (corresponding to 280 g $kg^{-1}$ carbohydrate and 116 g $kg^{-1}$lipid) exhibited significantly ($p$<0.05) higher final body weight (FBW), weight gain percentages (WG), and specific growth rate (SGR) compared to the fish in the other groups (Table 2). The growth performance (FBW, WG, and SGR) of fish fed 1.79, 3.24, 4.43, and 7.62 CHO $L^{-1}$ diets was significantly better than those that were fed higher levels of dietary lipids (CHO $L^{-1}$ ratios of 0.51–1.12). The feed utilization parameters for the experimental fish are also shown in Table 2. The feed intake of the fish offered diets with high CHO $L^{-1}$ ratios (2.41–7.62) was significantly ($p$<0.05) higher than those that received diets with lower CHO $L^{-1}$ ratios (0.51–1.12).

**Table 2: Growth performance and survival rate of *Pangasianodon hypophthalmus* fingerlings fed experimental diets varied in CHO $L^{-1}$ ratio.**

| CHO : L ratio | Initial BW (g) | Final BW (g) | WG (%) | SGR (%/day) | Feed intake (g) | FCR | PER | Survival (%) |
|---|---|---|---|---|---|---|---|---|
| 0.51 | 3.04 ± 0.03 | 10.60 ± 0.55$^{a}$ | 247.86 ± 19.01$^{a}$ | 1.48 ± 0.06$^{a}$ | 13.30 ± 1.51$^{a}$ | 1.75 ± 0.10$^{ab}$ | 1.59 ± 0.10$^{ab}$ | 100 ± 0$^{b}$ |
| 0.79 | 3.05 ± 0.06 | 11.61 ± 0.63$^{a}$ | 279.72 ± 19.58$^{a}$ | 1.58 ± 0.06$^{a}$ | 14.53 ± 0.54$^{a}$ | 1.70 ± 0.08$^{a}$ | 1.66 ± 0.08$^{ab}$ | 100 ± 0$^{b}$ |
| 1.12 | 3.04 ± 0.00 | 11.52 ± 0.35$^{a}$ | 278.46 ± 12.48$^{a}$ | 1.58 ± 0.03$^{a}$ | 14.37 ± 0.28$^{a}$ | 1.70 ± 0.28$^{a}$ | 1.71 ± 0.27$^{ab}$ | 100 ± 0$^{b}$ |
| 1.79 | 3.01 ± 0.08 | 12.82 ± 0.56$^{b}$ | 325.02 ± 22.05$^{c}$ | 1.72 ± 0.06$^{b}$ | 15.74 ± 2.39$^{ab}$ | 1.59 ± 0.16$^{a}$ | 1.79 ± 0.19$^{b}$ | 93.75 ± 4.16$^{a}$ |
| 2.41 | 3.03 ± 0.05 | 14.86 ± 0.89$^{c}$ | 389.88 ± 35.12$^{d}$ | 1.88 ± 0.09$^{c}$ | 19.32 ± 2.17$^{c}$ | 1.63 ± 0.19$^{a}$ | 1.76 ± 0.20$^{b}$ | 97.91 ± 4.16$^{b}$ |
| 3.24 | 3.02 ± 0.05 | 12.72 ± 0.55$^{b}$ | 320.32 ± 21.92$^{bc}$ | 1.70 ± 0.10$^{b}$ | 17.64 ± 1.30$^{bc}$ | 1.82 ± 0.10$^{ab}$ | 1.57 ± 0.09$^{ab}$ | 100 ± 0$^{b}$ |
| 4.43 | 3.06 ± 0.06 | 12.84 ± 0.86$^{b}$ | 318.69 ± 33.57$^{bc}$ | 1.70 ± 0.09$^{b}$ | 18.09 ± 0.44$^{bc}$ | 1.86 ± 0.20$^{ab}$ | 1.56 ± 0.16$^{ab}$ | 95.83 ± 4.81$^{ab}$ |
| 7.62 | 3.03 ± 0.04 | 12.80 ± 1.16$^{b}$ | 321.56 ± 34.52$^{bc}$ | 1.70 ± 0.09$^{b}$ | 19.65 ± 1.35$^{c}$ | 2.02 ± 0.24$^{b}$ | 1.45 ± 0.16$^{a}$ | 100 ± 0$^{b}$ |

Values are expressed as mean±SD of triplicate determinations. SD, standard deviation, CHO $L^{-1}$ ratio, carbohydrate / lipid ratio; BW; body weight (g); WG (%); weight gain percent, SGR (%) specific growth rate. FCR, feed conversion ratio; PER, protein efficiency ratio. Values in the same column with different superscript letters are significantly different ($p$<0.05)

The PER improved as low CHO $L^{-1}$ ratios increased and peaked at ratios of 1.79 and 2.41. This was followed by a decline as dietary carbohydrate levels continued to rise. The diet with the highest CHO $L^{-1}$ ratio (7.62) therefore reflected negatively on the FCR and PER.

The body indices monitored in the experimental fish are reported in Table 3. From the results analyzed, the CF of the fish that were fed 1.79 and 2.41 CHO $L^{-1}$ diets was significantly ($p$<0.05) higher than that of fish offered high-lipid diets (0.51, 0.79, and 1.12 CHO $L^{-1}$). The fish fed a diet with a 7.62 CHO $L^{-1}$ ratio exhibited significantly ($p$<0.05) higher HSI than the fish fed higher lipid diets (0.51 and 0.79 CHO $L^{-1}$; Table 3).

Increased dietary lipid levels, led to significantly ($p$<0.05) higher intraperitoneal fat levels. Whereas, varying dietary CHO $L^{-1}$ ratios did not significantly ($p$<0.05) affect the VSI.

Table 4 shows the whole body composition of the experimental fish as well as their liver and viscera lipid contents. The CHO $L^{-1}$ ratio did not significantly ($p$<0.05) affect the whole



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body' moisture or protein contents. Lipid content in the whole body of the fish that had been fed high- CHO $L^{-1}$ diets (3.24, 4.43, and 7.62 CHO $L^{-1}$) was significantly ($p$<0.05) lower than in the other fish. Significantly ($p$<0.05), lower liver lipid content was observed in the fingerlings that were fed high- CHO $L^{-1}$ diets (4.43 and 7.62) than in fish belonging to the other groups. The relationships between weight gain and both dietary carbohydrate and lipid levels were assessed by a polynomial second-order regression model, which yielded the regression lines $Y=-0.2496x^2+15.162x+110.67$ ($R^2$=0.70) and $Y=-1.0007x^2+20.591x+234.17$ ($R^2$=0.67), respectively. It was determined from the regression analysis that maximum growth performance occurred in the experimental fish when carbohydrate and lipid levels were 303.7 and 102.8 g $kg^{-1}$, respectively (2.95 CHO $L^{-1}$). Data on the activity of the lipogenic enzymes assayed in the livers of *P. hypophthalmus* fingerlings are reported as IU $mg^{-1}$ per mg protein (Table 5). The results showed that ME activity increased with rising CHO $L^{-1}$ ratios. The highest activity was recorded in the fish that were fed the diet with the highest CHO $L^{-1}$ ratio (7.62). Similarly, levels of 6PGDH and FAS activity were also affected by increases to dietary carbohydrate content. Hepatic 6PGDH enzyme activity was highest (5.16±0.08 IU $mg^{-1}$ protein) in fish fed the 7.62 CHO $L^{-1}$ diet, as was FAS activity.

**Table 3: Body indices of *Pangasianodon hypophthalmus* fingerlings fed diets varied in CHO $L^{-1}$ ratio.**

| CHO $L^{-1}$ ratio | HSI (%) | IPF (%) | VSI (%) | CF (%) |
|---|---|---|---|---|
| 0.51 | $1.28 \pm 0.21^{a}$ | $1.02 \pm 0.57^{b}$ | $11.45 \pm 0.21$ | $1.05 \pm 0.07^{a}$ |
| 0.79 | $1.32 \pm 0.20^{ab}$ | $0.66 \pm 0.18^{ab}$ | $11.90 \pm 0.87$ | $1.04 \pm 0.15^{a}$ |
| 1.12 | $1.37 \pm 0.10^{abc}$ | $0.63 \pm 0.11^{ab}$ | $11.34 \pm 1.87$ | $1.07 \pm 0.07^{a}$ |
| 1.79 | $1.38 \pm 0.12^{abc}$ | $0.63 \pm 0.18^{ab}$ | $10.88 \pm 1.15$ | $1.15 \pm 0.06^{b}$ |
| 2.41 | $1.36 \pm 0.11^{abc}$ | $0.64 \pm 0.24^{ab}$ | $11.83 \pm 1.36$ | $1.17 \pm 0.06^{b}$ |
| 3.24 | $1.38 \pm 0.07^{abc}$ | $0.53 \pm 0.07^{a}$ | $11.33 \pm 0.55$ | $1.12 \pm 0.03^{ab}$ |
| 4.43 | $1.42 \pm 0.11^{bc}$ | $0.54 \pm 0.28^{a}$ | $10.02 \pm 1.20$ | $1.12 \pm 0.05^{ab}$ |
| 7.62 | $1.48 \pm 0.10^{c}$ | $0.56 \pm 0.10^{a}$ | $10.73 \pm 1.64$ | $1.12 \pm 0.07^{ab}$ |

HSI, hepatosomatic index; IPF, intraperitoneal fat; VSI, viscerosomatic index.
Values are expressed as mean±SD of triplicates
Values in the same column with different superscripts are significantly different ($p$<0.05).

**Table 4: Fish whole body proximate composition, liver and viscera lipid of *Pangasianodon hypophthalmus* fingerlings fed on experimental diets.**

| Carcass Composition | Dietary CHO $L^{-1}$ ratios | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | 0.51 | 0.79 | 1.12 | 1.79 | 2.41 | 3.24 | 4.43 | 7.62 |
| Moisture (%) | 75.42 ± 0.55 | 75.36 ± 1.29 | 75.71 ± 1.50 | 76.10 ± 0.69 | 76.35 ± 1.14 | 76.89 ± 0.70 | 77.20 ± 1.25 | 76.93 ± 1.92 |
| Protein (%) | 62.63 ± 1.51 | 63.20 ± 0.92 | 63.57 ± 1.84 | 63.84 ± 1.42 | 64.60 ± 1.38 | 64.21 ± 0.67 | 64.18 ± 1.37 | 63.86 ± 0.84 |
| Lipid (%) | $10.73 \pm 0.73^{b}$ | $10.38 \pm 0.38^{b}$ | $10.35 \pm 0.31^{b}$ | $10.45 \pm 0.38^{b}$ | $10.89 \pm 0.56^{b}$ | $9.56 \pm 0.45^{a}$ | $9.36 \pm 0.65^{a}$ | $9.41 \pm 0.46^{a}$ |
| Ash (%) | $17.58 \pm 0.40^{a}$ | $18.42 \pm 0.32^{b}$ | $18.18 \pm 0.32^{b}$ | $18.45 \pm 0.16^{b}$ | $18.10 \pm 0.30^{b}$ | $19.61 \pm 0.44^{c}$ | $20.95 \pm 0.22^{d}$ | $20.98 \pm 0.40^{d}$ |
| Liver lipid (%) | $22.88 \pm 1.10^{c}$ | $26.54 \pm 0.78^{d}$ | $25.23 \pm 1.07^{d}$ | $24.97 \pm 1.13^{d}$ | $20.85 \pm 0.93^{b}$ | $20.58 \pm 0.48^{b}$ | $18.60 \pm 0.43^{a}$ | $18.17 \pm 1.18^{a}$ |
| Viscera lipid(%) | $22.90 \pm 1.24^{c}$ | $23.18 \pm 1.04^{c}$ | $23.36 \pm 1.42^{c}$ | $22.57 \pm 1.43^{c}$ | $23.79 \pm 1.58^{c}$ | $19.25 \pm 0.45^{b}$ | $19.81 \pm 1.25^{b}$ | $16.21 \pm .0.33^{a}$ |

Values are expressed as mean ± SD of triplicate; Values in the same row with different letters are significantly different ($p$<0.05).



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**Table 5: Activities of hepatic lipogenic enzymes in *Pangasianodon hypophthalmus* fingerlings fed experimental diets varied in CHO $L^{-1}$ ratio.**

| | Malic [1] | 6-Glucose–phosphate dehydrogenase [1] | Fatty acid synthase[1] |
|---|---|---|---|
| CHO $L^{-1}$ ratios | | | |
| 0.51 | 0.303 ± 0.13$^{a}$ | 1.30 ± 0.43$^{a}$ | 23.25±0.59$^{a}$ |
| 0.79 | 0.12 ± 0.04$^{a}$ | 1.65 ± 0.82$^{ab}$ | 23.86±0.15$^{a}$ |
| 1.12 | 1.20 ± 0.58$^{ab}$ | 1.52 ± 0.28$^{ab}$ | 22.58±0.12$^{a}$ |
| 1.79 | 1.14 ± 0.14$^{ab}$ | 2.15 ± 0.48$^{bc}$ | 27.23±0.56$^{b}$ |
| 2.41 | 1.18 ± 0.55$^{ab}$ | 2.14 ± 0.58$^{bc}$ | 27.92±1.11$^{b}$ |
| 3.24 | 2.12 ± 1.26$^{ab}$ | 2.71 ± 0.46$^{cd}$ | 28.15±1.46$^{b}$ |
| 4.43 | 3.57 ± 1.42$^{b}$ | 3.37 ± 0.10$^{d}$ | 30.22±1.23$^{c}$ |
| 7.62 | 10.12 ± 4.68$^{c}$ | 5.16 ± 0.08$^{e}$ | 33.02±1.39$^{d}$ |

Values are expressed as mean ± SD of triplicate; Values in the same row with different letters are significantly different ($p$<0.05).
[1] IU, enzyme activity units, nanomoles of NADPH consumed or generated per minute per milligram of protein at 37°C.

## Discussion

The present study showed that fish fed a 2.41 CHO $L^{-1}$ diet (280 g $kg^{-1}$ carbohydrate and 116 g $kg^{-1}$ lipid) demonstrated the highest growth performance and feed intake. This is comparable to findings reported in other omnivorous fishes. For example, Erfanullah and Jafri (1998a) found that the walking catfish (*Clarias batrachus*) achieved its best growth rate when fed a diet of 272.8 g $kg^{-1}$ carbohydrates and 80.7 g $kg^{-1}$ lipids. Similarly, Vásquez-Torres and Arias-Castellanos (2013) observed that the cachama (*Piaractus brachypomus*) exhibited optimal growth when fed a diet containing 280 g $kg^{-1}$ carbohydrates and 40 g $kg^{-1}$ lipids. According to Erfanullah and Jafri (1998a), carbohydrates and lipids improve fish growth performance and feed conversion when they are provided in optimal amounts. The decreased growth performance of the fish fed high-lipid diets, observed in the present study, could be attributed to lower feed intake, which causes a reduction to the intake of proteins and other nutrient necessary for optimal growth as reported by Pei *et al.* (2004). Additionally, high dietary lipid levels cause an imbalance between protein and digestible energy that may lead to excessive fat deposition in fish carcasses (Company *et al.*, 1999; NRC, 1993). This could adversely affect body composition (Hillestad and Johnsen, 1994) and consequently reduce fish growth (Medale *et al.*, 1991). In this experiment, although the diet with the highest lipid content had fairly high cellulose (168 g $kg^{-1}$) levels, these differences may not have any adverse effect on the growth of fish because studies have previously shown that some fish such as *T. zillii* and channel catfish have consumed diets containing up to 40 g $kg^{-1}$ of cellulose without any adverse effect on growth (El-Sayed and Garling, 1988).

Furthermore, the HSI differences observed in *P. hypophthalmus* became obvious only when the diets contained either high lipid or carbohydrate levels (0.51 and 7.62 CHO $L^{-1}$, respectively), and the highest HSI was observed in fish fed a 7.62 CHO $L^{-1}$ diet. High HSI value was likely attributable to extensive glycogen deposition in the liver, as previously reported by Hu *et al.* (2007). Similar


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observations were reported by Jantrarotai *et al.* (1994) in the omnivorous hybrid *Clarias* catfish (*Clarias macrocephalus*× *Clarias gariepinus*).

The present study demonstrated that the increased liver and visceral fat contents were caused by raised dietary lipid intake, which is in agreement with Gao *et al.* (2010) findings. A similar trend was also recorded for IPF values, where values were higher in the fish that were fed high-lipid diets. This matches Jantrarotai *et al.*'s (1994) observations in hybrid *Clarias* catfish. The dietary CHO $L^{-1}$ ratio also influenced the lipid content of *P. hypophthalmus* fingerling carcasses. The lipid content of *P. hypophthalmus* carcasses was significantly higher in those fed low-carbohydrate, high-lipid diets than in those that were fed high-carbohydrate, low-lipid diets. Shimeno *et al.* (1993) documented similar findings in the Nile tilapia (*Oreochromis niloticus*). In general, lipids deposited in adipose tissues originate either from the diet or *de novo* synthesis of glucose (Rossi *et al.*, 2010). Excess carbohydrates in the diet of some fish species cause fat deposition by stimulating lipogenic enzyme activities (Likimani and Wilson, 1982). Consistent with these explanations, the present study found that ME, 6PGDH, and FAS activity increased in *P. hypophthalmus* fingerlings as CHO $L^{-1}$ ratios rose to the highest ratio tested (7.62 CHO $L^{-1}$). Increase in the activities of lipogenic enzymes with increasing dietary carbohydrate levels suggests that in order to metabolize excess glucose, it has to pass through both the tricarboxylic acid cycle (TCA cycle) and pentose phosphate pathway, where the NADPH generated is used for lipogenesis, to be metabolized. Similar observations were noted by Likimani and Wilson (1982), who reported that an increase in dietary carbohydrate levels caused an increase to hepatic lipogenic enzymes (ME, 6PGDH, and FAS) activity in channel catfish (*Ictalurus punctatus*).

A high-lipid diet resulted in higher fat deposition and lower growth performance than a high-carbohydrate diet in *P. hypophthalmus* fingerlings. Overall, the results of the present study indicate that omnivorous *P. hypophthalmus* fingerlings have a higher potential to utilize dietary carbohydrates than dietary lipid. Based on a second-order polynomial regression quadratic model evaluation, maximum fish growth was observed at dietary carbohydrate and lipid levels of 304 and 103 g $kg^{-1}$, respectively, which corresponds to a CHO $L^{-1}$ ratio of 2.95.

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**References**

**Ali, M.Z., Hossain, M.A. and Mazid, M.A., 2005.** Effect of mixed feeding schedules with varying dietary protein levels on the growth of sutchi catfish, *Pangasius hypophthalmus* (Sauvage) with silver carp, *Hypophthalmichthys morlitrix* (Valenciennes) in ponds. *Aquaculture Research*, 36, 627-634.

**AOAC, (Association of official analytical chemist). 1997.** Official methods of analysis of the association of official



[ DOR: 20.1001.1.15622916.2020.19.4.11.9 ]

analytical chemists, Arlington, VA, USA.16th edn, Vol.1, pp. 1-3.

**Bazin, R. and Ferré, P., 2001.** Assays of lipogenic enzymes. *Methods Molecular Biology*, 155, 121-7.

**Bradford, M.M., 1976.** A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Analytical Biochemistry*, 72, 248-254.

**Company, R., Calduch-Giner, J., Kaushik, S. and Pérez-Sánchez, J., 1999.** Growth performance and adiposity in gilthead sea bream (*Sparus aurata*): risks and benefits of high energy diets. *Aquaculture*, 171, 279-292.

**El-Sayed, A.M. and Garling, D.l.Jr., 1988.** Carbohydrate-to-lipid ratios in diets for tilapia zillii fingerlings. *Aquaculture*, 73, 157-163.

**Erfanullah, J.A.K. and Jafri, A.K., 1998a.** Effect of dietary carbohydrate-to-lipid ratio on growth and body composition of walking catfish (*Clarias batrachus*). *Aquaculture*, 161, 159–168.

**Erfanullah, J.A.K. and Jafri, A.K., 1998b.** Growth rate, feed conversion, and body composition of *Catla catla*, *Labeo rohita*, and *Cirrhinus mrigala* fry fed diets of various carbohydrate-to-lipid ratios. *Journal of the World Aquaculture Society*, 29, 84-91.

**Gao, W., Liu, Y.J., Tian, L.X., Mai, K.S., Liang, G.Y., Yang, H.J. and Luo, W.J., 2010.** Effect of dietary carbohydrate-to-lipid ratios on growth performance, body composition, nutrient utilization and hepatic enzymes activities of herbivorous grass carp (*Ctenopharyngodon idella*). *Aquaculture Nutrition*, 16, 327-333.

**Gélineau, A., Corraze, G., Boujard, T., Larroquet, L. and Kaushik, S., 2001.** Relation between dietary lipid level and voluntary feed intake, growth, nutrient gain, lipid deposition and hepatic lipogenesis in rainbow trout. *Reproduction Nutrition Development*, 41, 487-503.

**Glock, C.E. and Mclean, P., 1953.** Further studies on the properties and assay of glucose-6-posphate dehydrogenase and 6-posphogluconate dehydrogenase of rat liver. *Biochemical Journal*, 55, 400-408.

**Halestrap, A.P. and Denton, R.M., 1973.** Insulin and the regulation of adipose tissue acetyle coenzyme A carboxylase, *Biochemistry Journal*, 132, 509-513.

**Hillestad, M. and Johnsen, F., 1994.** High-energy/low-protein diets for Atlantic salmon: effects on growth, nutrient retention and slaughter quality. *Aquaculture*, 124, 109-116.

**Hu, Y.H., Liu, Y.J., Tian, L.X., Yang, H.J., Liang, G.Y. and Gao, W., 2007.** Optimal dietary carbohydrate to lipid ratio for juvenile yellowfin seabream (*Sparus latus*). *Aquaculture Nutrition*, 13, 291-297.

**Jantrarotai, W., Sitasit, P. and Rajchapakdee, S., 1994.** The optimum carbohydrate to lipid ratio in hybrid clarias catfish (*Clarias macrocephalus* ×*C. gariepinus*) diets containing raw broken rice. *Aquaculture*, 127, 61-68.

**Likimani, T.A. and Wilson, R.P., 1982.** Effects of diet on lipogenic enzyme activities in channel catfish hepatic and adipose tissue. *The Journal of Nutrition*, 112, 112-117.



[ DOR: 20.1001.1.15622916.2020.19.4.11.9 ]

**Medale, F., Aguirre, P. and Kaushik, S., 1991.** Utilization of dietary carbohydrates by rainbow trout at two water temperatures. Energy metabolism of farm animals. *European Association for Animal Production Publication,* 58, 391-395.

**Nankervis, L., Matthews, S. and Appleford, P., 2000.** Effect of dietary non-protein energy source on growth, nutrient retention and circulating insulin-like growth factor I and triiodothyronine levels in juvenile barramundi, *Lates calcarifer*. *Aquaculture,* 191, 323-335.

**NRC, 1993.** Nutrient requirements of fish. National Academy Press, Washington, DC, 112 P.

**Ochoa, S., 1955.** Malic enzyme. **"***Methods in Enzymology*" (S. P. Colowick and N. O. Kaplan, eds),Vol. 1,pp. 739-753.

**Pei, Z., Xie, S., Lei, W., Zhu, X. and Yang, Y., 2004.** Comparative study on the effect of dietary lipid level on growth and feed utilization for gibel carp (*Carassius auratus gibelio*) and Chinese longsnout catfish (*Leiocassis longirostris Günther*). *Aquaculture Nutrition,* 10, 209-216.

**Rossi, A.S., Lombardo, Y.B. and Chicco, A.G., 2010.** Lipogenic enzyme activities and glucose uptake in fat tissue of dyslipemic, insulin-resistant rats: effects of fish oil. *Nutrition,* 26, 209-217.

**Shimeno, S., Ming, D.C. and Takeda, M., 1993.** Metabolic response to dietary carbohydrate to lipid ratios in *Oreochromis niloticus*. *Bulletin-Japanese Society of Scientific Fisheries,* 59, 827-833.

**Vásquez-Torres, W. and Arias-Castellanos, J.A., 2013.** Effect of dietary carbohydrates and lipids on growth in cachama (*Piaractus brachypomus*). *Aquaculture Research,* 44, 1768-1776.

**Wang, J.T., Jiang, Y.D., Han, T., Li, X.Y., Wang, Y. and Liu, Y.J., 2017.** Effects of dietary carbohydrate-to-lipid ratios on growth and body composition of orange-spotted grouper *Epinephelus coioides*. *North American Journal of Aquaculture*, 79(**1**), 1-7.

**Xing, S., Sun, R., Pan, X., Ma, J., Zhang, W. and Mai, K., 2016.** Effects of dietary carbohydrate-to-lipid ratio on growth performance, body composition, digestive enzyme activities, and hepatic enzyme activities in juvenile large yellow croaker, *Larimichthys crocea*. *Journal of the World Aquaculture Society*,47; 297-307.

**Zhou, P., Wang, M., Xie, F., Deng, D.F. and Zhou, Q., 2016.** Effects of dietary carbohydrate to lipid ratios on growth performance, digestive enzyme and hepatic carbohydrate metabolic enzyme activities of large yellow croaker (*Larmichthys crocea*). *Aquaculture*, 452, 45-51.

**Zhu, H., Jiang, Q., Wang, Q., Yang, J., Dong, S. and Yang, J., 2013.** Effect of dietary carbohydrate-to-lipid ratios on growth performance, body composition, hepatic enzyme activities, and digestive enzyme activities of juvenile Australian redclaw crayfish, *Cherax quadricarinatus* (*von Martens*). *Journal of the World Aquaculture Society,* 44, 173-186.



[ DOR: 20.1001.1.15622916.2020.19.4.11.9 ]