
The relationship between dietary carbohydrate level and the levels of blood glucose and amylase in lemon fin barb hybrid (*Barbonymus gonionotus* X *Hypsibarbus wetmorei* male)

By

Muhammad Aliyu Sulaiman^{1,2}, Mohd Salleh Kamarudin^{1*} and Mohammad Fadhil Syukri Ismail¹

¹Department of Aquaculture,

Faculty of Agriculture,

Universiti Putra Malaysia, 43400 UPM Serdang

²Fish Nutrition and Health Programme,

National Institute for Freshwater Fisheries Research, P.M.B. 6006, New Bussa, Niger State, Nigeria

*Email: msalleh@upm.edu.my

ABSTRACT

A feeding trial was conducted to determine the effects of dietary carbohydrate level on the blood glucose and amylase of lemon fin barb hybrid fingerlings. Triplicate groups of fish (1.1 ± 0.5 g) were fed twice a day until apparent satiation with five isonitrogenous (30% protein) and isolipidic (4%) test diets containing increasing level of carbohydrate (20, 25, 30, 35 and 40 %). At the end of the experiment, fish survival, growth, feed efficiency, and blood glucose and amylase were measured. No mortality was observed in this study. The highest growth and blood amylase, and best feed efficiency were achieved at 35% dietary carbohydrate level while blood glucose continued to rise with the increase in the dietary carbohydrate level. This study indicated that blood amylase could be used as a tool to determine the optimal dietary carbohydrate level of a tropical carp and lemon fin barb hybrid could optimally utilize a high dietary carbohydrate level of 31.5-33.5%

Keywords: Lemon fin barb hybrid, carbohydrate, growth, amylase, glucose

INTRODUCTION

Seafood products constitute high-quality protein which is an integral part of the human diet. The culture of fish and crustaceans is been affected by high feed cost, which contributes up to 70% of production cost. There is a need for the replacement or minimizing the use of expensive ingredients such as fish meal and fish oil as feed ingredients (Kristofersson & Anderson, 2006). These alternative ingredients may be derived from plants, animals and microbes (Camacho-Rodríguez et al., 2013) and used to formulate affordable aquafeeds (Stone, 2003). Carbohydrates are cheap and good sources of carbon (Zhou et al., 2013) and are widely used as energy sources in practical fish diets. Studies revealed that suitable carbohydrate levels could stimulate efficient use of other nutrients especially protein (Zamora et al., 2013). An optimal

carbohydrate level in a feed reduces the catabolism of protein for energy which will maximize protein retention and decrease the nitrogen release (Wilson 1994). Nevertheless, the ability to digest and metabolize carbohydrate source and the optimal inclusion level vary among fish species (Krogdahl et al., 2005). Compared to herbivorous and omnivorous fish, carnivorous fish tend to have low specific requirements for dietary carbohydrates and low dietary glucose utilization (Tian et al., 2011). Understanding the ability of the fish species in utilizing carbohydrate is the basis for appropriate feed formulation (Wilson, 1994).

Moreover, carbohydrate utilisation depends on carbohydrate complexity, processing, source and the level of addition in the diet. Though carbohydrates can improve the feeding performance of fish and reduce feed costs, excessive inclusion of

carbohydrates may have negative effects on fish growth, nutrient assimilation and physiological functions (Tian et al., 2012; Niu et al., 2012; Kamalam et al., 2016), which may lead to higher blood glucose content and damage the function of the fish liver (Tran-Duy et al., 2008; Waagbo et al, 1994).

Since its introduction by the Department of Fisheries Malaysia, the production of lemon fin barb hybrid in Malaysia has increased from 29.6 tonnes in 2012 to 190.8 tonnes in 2016 (DOF, 2013; DOF., 2017). In practice, farmers supplemented their fish with Napier grass and aquatic plant cuttings in an effort of reducing the use of commercial tilapia or catfish feeds and production cost as no specific diet has been developed for this carp hybrid. Recent research shows that the hybrid optimally requires 30% protein (Suharmili et al., 2015) and 4% lipid (Ismail et al., 2016). The role of carbohydrate in its nutrition is yet to be established. The present study was conducted to determine the optimal dietary carbohydrate requirement for lemon fin barb hybrid through growth, feed efficiency and blood parameters.

MATERIALS AND METHODS

Experimental diets

Five isonitrogenous (30% protein) and isolipidic (4%) diets containing increasing levels of carbohydrate (20, 25, 30, 35 and 40%) were formulated and tested (Table 1). Tapioca starch was used as the carbohydrate source. The diets were processed using a single screw extruder (Brabender

KE-19) fitted with a 2 mm Ø die. The three barrel temperatures were set at 80, 100, and 120 °C while the die head temperature was 140 °C. The pellets were dried in an oven (50 °C) for 12 h (Azaza et al., 2015). The test diets were broken into 1 mm crumbles and stored airtight in plastic bags at – 20 °C until used.

Feeding trial

Lemon fin barb hybrid (*Barbonymus gonionotus* × *Hypsibarbus wetmorei*) F₂ fingerlings were supplied by Perlok Aquaculture Extension Centre, Jerantut Pahang, Malaysia and brought to the Department of Aquaculture, Universiti Putra Malaysia, Serdang, Selangor. The fingerlings were then acclimatised in a 1000 L fiberglass tank for fifteen days and fed with a commercial tilapia diet (Cargill Starter 32% crude protein). The source of water was from the city mains which was de-chlorinated and vigorously aerated. The experiment was subjected to indoor natural temperature and photoperiod. After the acclimation period, a total of 300 fish (1.1 ± 0.5 g) were equally and randomly distributed into fifteen 100-L glass aquaria. The aquaria were randomly allocated to one of the five test diets in triplicates. The fish were then fed to apparent satiation twice times daily (0900 and 1600 h) at 4-6% body weight. At every 15 days, the fish were bulk weighed. Fifteen-day intervals were chosen to minimise fish stress (Wu et al., 2016).

Table 1: Feed and proximate composition (% as fed basis) of the experimental diets.

Ingredient	Dietary Carbohydrate Level (%)				
	20%	25%	30%	35%	40%
Fishmeal ¹	52.98	52.98	52.98	52.98	52.98
Crude palm oil ²	3.12	3.12	3.12	3.12	3.12
Vitamin premix ³	1.00	1.00	1.00	1.00	1.00
Mineral premix ⁴	1.00	1.00	1.00	1.00	1.00
Tapioca starch ⁵	20.00	25.00	30.00	35.00	40.00
-cellulose ⁶	21.90	16.90	11.90	6.90	1.90
<i>Proximate composition</i>					
Moisture	8.6 ± 0.30	8.8 ± 0.36	8.5 ± 0.40	8.6 ± 0.26	8.9 ± 0.20
Crude protein	29.99 ± 0.58	30.11 ± 0.77	30.23 ± 0.78	30.34 ± 0.25	30.46 ± 0.46

Ingredient	Dietary Carbohydrate Level (%)				
	20%	25%	30%	35%	40%
Crude lipid	6.32 ± 0.11	6.41 ± 0.10	6.22 ± 0.11	6.31 ± 0.08	6.82 ± 0.09
Crude ash	7.32 ± 0.16	7.51 ± 0.37	7.61 ± 0.08	7.95 ± 0.17	8.07 ± 0.12
Crude fiber	18.31±0.19	13.4±0.34	9.2±0.22	5.3±0.15	1.12±0.36
Nitrogen free extract	29.46±0.16	33.77±0.21	38.24±0.11	41.50±0.09	44.63±0.14
Gross energy (kJ g ⁻¹)	14.98±0.21	15.58 ± 0.13	16.15 ± 0.12	17.06 ± 0.11	16.75 ± 0.14

¹ Fishmeal (local mixed species) with dry matter, crude protein, and crude lipid of 93.4, 55.8, and 5.2 % as fed basis respectively.

² Vitamin premix (g kg⁻¹ premix): ascorbic acid, 45; choline chloride, 75; thiamin mononitrate, 0.9; niacin, 4.5; riboflavin, 1; pyridoxine, 1; retinyl acetate, 0.6; cholecalciferol, 0.08; Ca-pantothenate, 3; myo-inositol, 5; vitamin K menadione, 1.7; -tocopheryl acetate (500 IU g⁻¹), 8; biotin, 0.02; folic acid, 0.1; vitamin B₁₂, 0.001

³ Mineral premix (g kg⁻¹ premix): NaCl, 40; CuSO₄.5H₂O, 3; KI, 0.04; MnSO₄.H₂O, 3; ZnSO₄.7H₂O, 4; CaCO₃, 215; KCl, 90; CoSO₄, 0.02; FeSO₄.7H₂O, 20; Ca(H₂PO₄).H₂O, 500; MgOH, 124; Na₂SeO₃, 0.03; NaF, 1.

⁴ -cellulose (Sigma Chemicals C8002)

⁵ nitrogen-free-extract; NFE = 100 – (moisture + protein + lipid + ash + fibre)

Water quality parameters including water temperature, dissolved oxygen, pH and total ammonia were measured at 2-day intervals on non-sampling days, and approximately 50% of the water was exchanged according to Kamarudin et al. (2011). Throughout the experiment, water temperature, dissolved oxygen, pH and ammonia-nitrogen ranged 28.2-29.6 °C, 7.5-7.8 mg L⁻¹, 7.1-7.5 and of 0.01-1.14 mg L⁻¹, respectively.

Blood Analysis

At the end of the feeding period, five fish in each tank were randomly sampled and anaesthetised with 50 mg L⁻¹ tricaine methane sulfonate (MS 222). Each fish was individually weighed and the blood was sampled by puncturing the caudal vein with a heparinised 1 mL syringe. The blood was centrifuged and stored in a freezer (-80 °C) for blood analysis. The blood glucose and amylase were analysed according to Tian et al. (2012).

Statistical analysis

All data were subjected to one-way analysis of variance (ANOVA) after prior confirmation of data

homogeneity using Statistical Analysis System 9.4 for Windows (SAS Inc., USA). Percentage data were arcsine transformed. Differences among dietary treatments were identified using Tukey's test.

RESULTS

No mortality was observed in this 60-day study. The fish fed 35% carbohydrate exhibited the highest specific growth rate and protein efficiency ratio (PER) but these values were not significantly different from fish fed 30% carbohydrate (Fig. 1 and 3). The best feed conversion ratio (FCR) was also observed among fish fed 35% dietary carbohydrate although no significant differences were among the treatment (Fig. 2).

Blood glucose in lemon fin barb hybrid significantly increased when dietary carbohydrate level was increased from 20 to 25% and then continued to slowly increase (Fig. 4). Blood amylase level increased when the dietary carbohydrate level increased up to 35% and then dropped (Fig. 5). The optimal dietary carbohydrate requirement of lemon fin barb hybrid based on SGR and blood amylase were 33.5 and 31.5 %, respectively (Fig. 1 and 5).

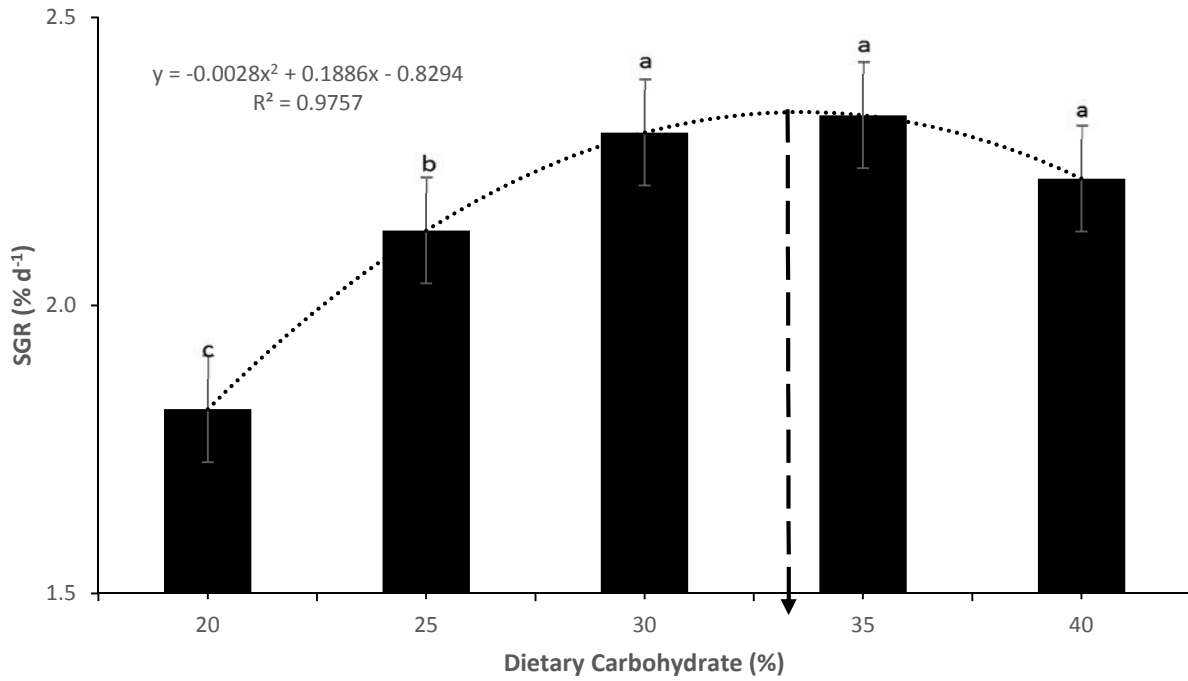


Figure 1: Relationship between dietary carbohydrate level and SGR of lemon fin barb hybrid. Different letters in each treatment indicate significant differences ($P < 0.05$)

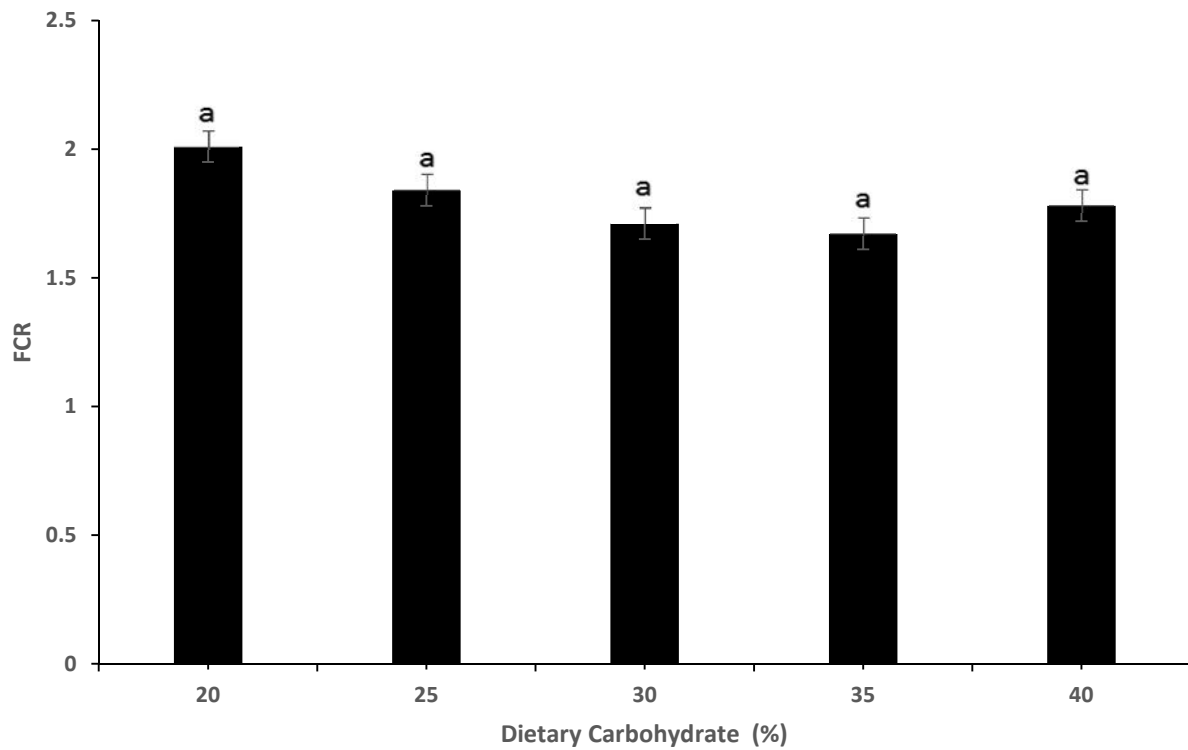


Figure 2: Relationship dietary carbohydrate level and FCR of test diets. Different letters in each treatment indicate significant differences ($P < 0.05$)

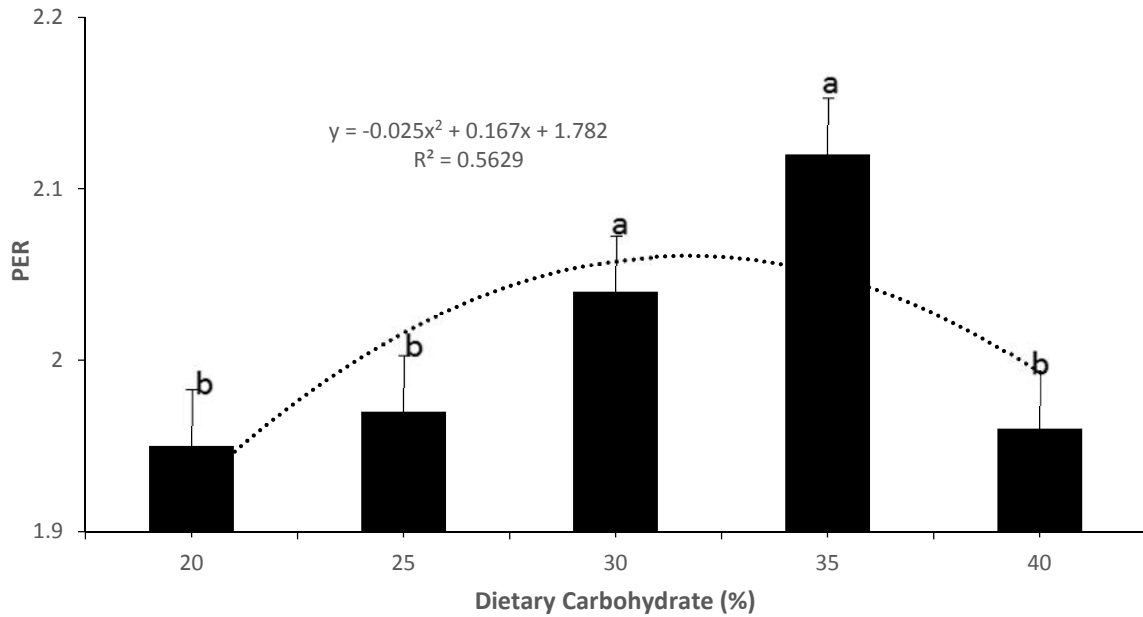


Figure 3: Relationship between dietary carbohydrate level and PER of test diets. Different letters in each treatment indicate significant differences ($P < 0.05$)

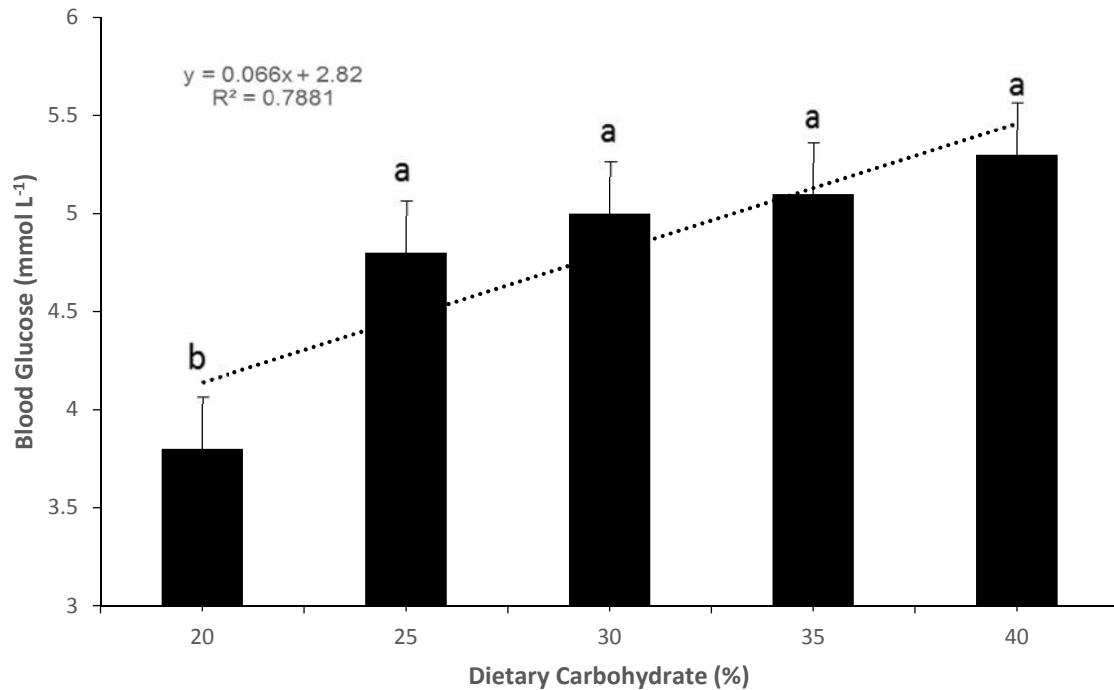


Figure 4: Relationship dietary carbohydrate level and blood glucose in lemon fin barb hybrid. Different letters in each treatment indicate significant differences ($P < 0.05$)

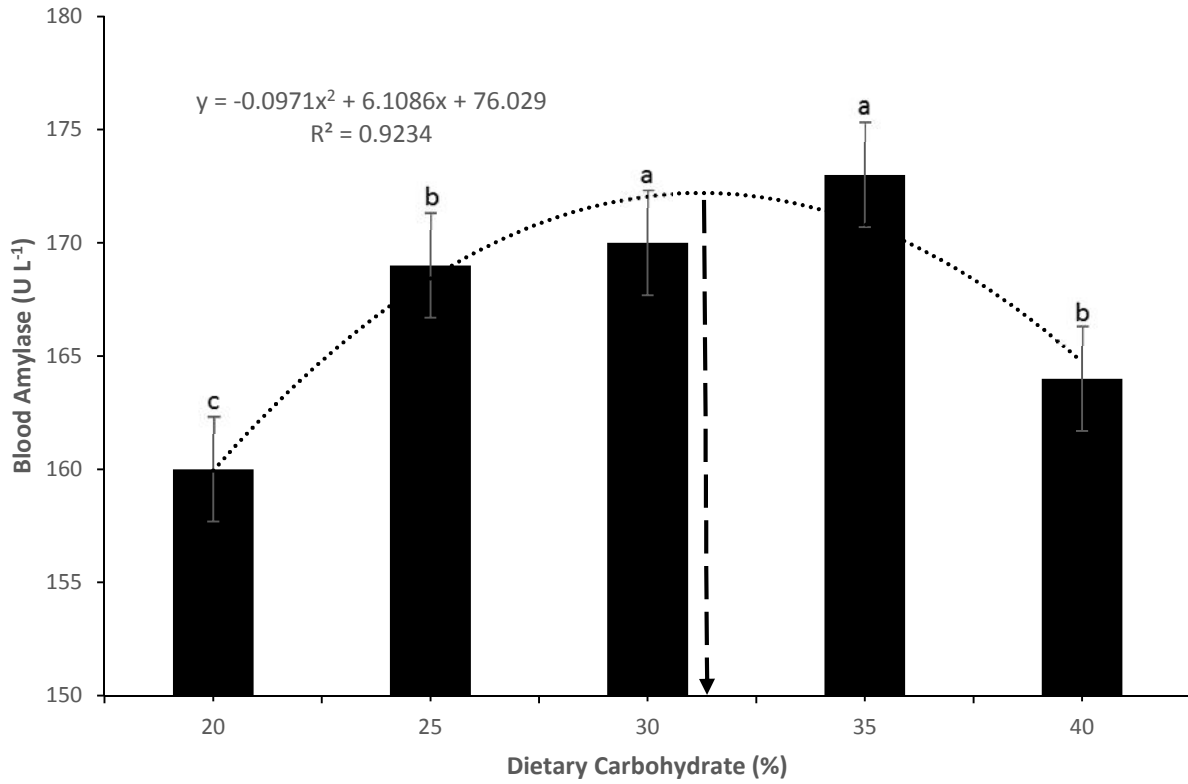


Figure 5: Relationship between dietary carbohydrate level and blood amylase in lemon fin barb hybrid. Different letters in each treatment indicate significant differences ($P < 0.05$)

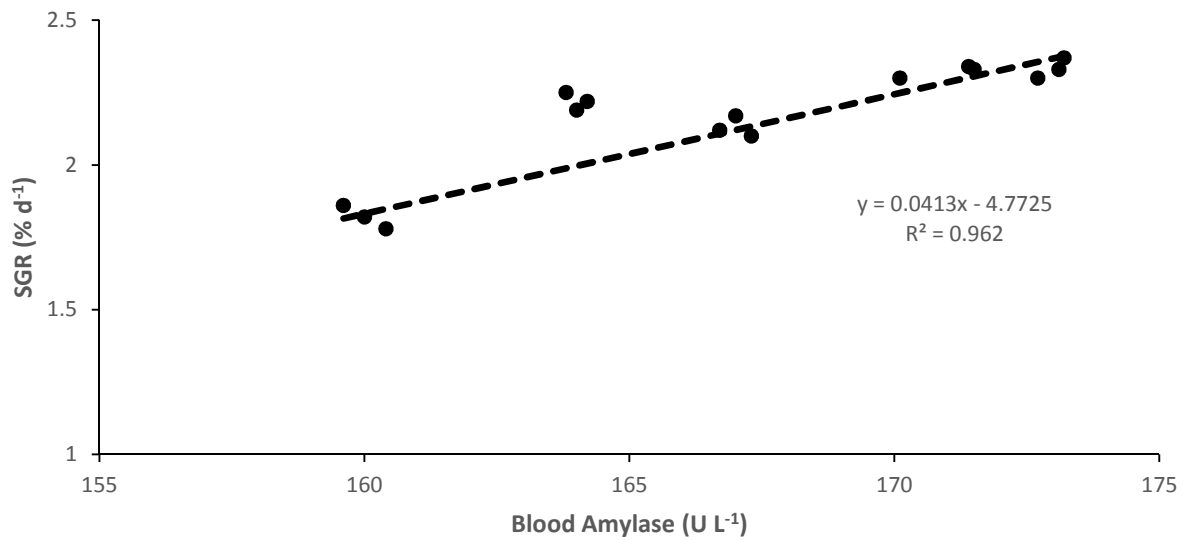


Figure 6: Relationship between blood amylase and SGR of lemon fin barb hybrid.

DISCUSSION

Both carbohydrate and lipid play important roles as non-protein energy sources for fish species (Gao et al., 2010). The inclusion of optimal carbohydrate level in the diet has been reported to improve growth performance of fish (Anderson et al., 1984). Generally, herbivorous and omnivorous fish such as grass carp (*Ctenopharyngodon idella*) and gibel carp (*Carassius auratus*) can use higher carbohydrate levels (45%) for optimal growth and they have the ability to utilize carbohydrate as an energy source (Tian et al., 2012). The best growth performance and feed efficiency were achieved when lemon fin barb hybrid were fed 35% carbohydrate. However, these were not significantly from those of fish fed 30% carbohydrate. The fish growth and PER dropped when more than 35% carbohydrate was provided in its diet.

Growth performance and feed efficiency have been commonly used to estimate the optimal nutrient requirements of fish (Kamalam et al., 2017). In this study, blood amylase showed a similar trend as the fish growth with the increase of dietary carbohydrate level. The amylase activity dropped when the dietary carbohydrate level were higher than 35%. A strong positive relationship between blood amylase and SGR was observed (Fig. 6). These results indicated that when the dietary carbohydrate went beyond the maximum or optimal level, the amylase activity and growth could be suppressed. Similar findings have been reported in juvenile grass carp, gibel carp (Li et al., 2016) and juvenile spiny lobsters (Simon, 2009) where intestinal amylase activity increases with the increase of dietary carbohydrate but up to a maximum level and later drops due to inhibition. A positive correlation between blood amylase activity and dietary carbohydrate content by several other researchers (Nagase, 1964; Ufodike, 1988; Krogdahl et al., 2005) although some authors suggested that there is no such relationship (Li et al., 2016). High blood amylase level is often associated with damaged pancreas (Pottinger, 1998; Mokhtar, 2015). However, the blood amylase levels found in this study were below the problematic levels

reported for carps and other fish (Das, 1964; Svetina et al., 2002) and should not indicate any damaged pancreas. Histopathology analysis of the livers also did not show any abnormality among the treatments (Sulaiman, unpublished data).

The ability of fish to utilize carbohydrate depends on their ability to oxidize glucose and to store the excess glucose as glycogen or fat (Zhou et al., 2015). In the blood, the excess glucose derived from feeding results in either glycogen synthesis or lipogenesis (Tan et al., 2009). Too high blood glucose level indicates a fish is under stress (Martinez et al., 2009). In this study, the blood glucose continued to increase with the increase of dietary carbohydrate level and seemed to plateau at 40%. However, these levels were lower than the levels reported for stressed carps (Li et al., 2016). Thus, the hybrid was possibly well adapted to the higher carbohydrate level by increasing its glucose utilisation. Similar results were observed in Lebranche mullets (Zamora-Sillero et al., 2013) and black carp (Wu et al., 2016).

Ishak et al. (2016) reported that the optimal carbohydrate requirement of Malaysian mahseer is lower when determined using the molecular tool (22.3%) than that based on fish growth (23.4%). A similar observation was made in this study where the optimal dietary carbohydrate requirement of this hybrid was found at 31.5% and 33.5% based on blood amylase and growth, respectively. The range was comparable to the dietary carbohydrate requirement of silver barb [29.13-29.83%], silver carp [28%] but higher than Malaysian mahseer [23.4%], golden pompano [11.2-16.8%] and cobia [23.14-25.30%] (Mohanta et al., 2009; Radke & Kahl, 2002; Kamarudin et al., 2016; Zhou et al., 2015; Liu et al., 2015). Grass carp, common carp and Nile tilapia have higher requirement ranges of 37-56%, 30-45% and 37-40%, respectively (Iii, 2001; Ufodike et al., 1983; El-Sayed & Garling, 1988).

In conclusion, blood amylase could be used to estimate the optimal dietary carbohydrate requirement of lemon fin barb hybrid. A diet containing 30-35% carbohydrate, 30% protein and 4% lipid was recommended for lemon fin barb hybrid.

Studies on the effects of different carbohydrate types and sources on the growth, blood amylase and intestinal carbohydrases of this hybrid at different stages should be conducted to better understand its carbohydrate requirements.

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