OPEN ACCESS
International Journal of Aquatic Science
ISSN: 2008-8019
Vol. 9, No. 2, 85-92, 2018

Utilization of different dietary carbohydrate sources in hybrid grouper, Tiger grouper (*Epinephelus fuscoguttatus*, ♀) × Giant grouper (*Epinephelus lanceolatus*, ♂) juveniles

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Received: December-03-2017 **Accepted:** February-07-2018 **Published:** April-02-2018

Abstract: Four isoproteic (50% crude protein) and isolipidic (10% crude lipid) diets containing four different carbohydrate sources (tapioca, corn, sago and dextrin) at the same inclusion level (20%) were fed to triplicate groups of fish for 67 days. The fish were cultured in 150L fiberglass tanks with 20 individuals/tank. At the end of the feeding trial, growth performance and feed intake of fish fed diets tapioca, corn and sago were better than those fed diet dextrin. Body indices, muscle and liver composition were also affected by the dietary treatments. Sago diet produced fish with significantly higher glucose and lower total protein than other diets. Meanwhile, only mean cell volume and platelet count of blood were affected by the carbohydrate sources. In general, the values of digestibility for dry matter, protein and lipid were considered high in all diets, with significant differences detected among them. In conclusion, all tested starches performed significantly better than dextrin as carbohydrate sources, and the findings of the present study had provided the aquafeed industry with more carbohydrate choices in the formulation of diets for grouper species.

Keywords: Sago starch, corn starch, tapioca starch, dextrin, TGGG

Introduction

Carbohydrates are the cheapest non-protein ingredient and most economical source of energy in the diet's formulation for farmed fish (Zamora-Sillero *et al*., 2013). Even though fish and shrimp do not have a specific requirement for carbohydrate; it was commonly incorporated in the diet to prevent the catabolism of expensive nutrient such as protein and lipid at optimal inclusion, which also contributed in good pelleting properties (Wilson, 1994; Stone, 2003). Carbohydrates from polysaccharides group including starch and dextrin are generally utilized more efficiently than mono- and disaccharides in herbivore fish (Ren *et al*., 2015), omnivorous fish (Tan *et al.,* 2006; Sá *et al.,* 2008) and carnivorous fish (Enes *et al.,* 2008; Cui *et al.,* 2010). Based on previous research, the ability of aquatic animals including grouper to utilize different carbohydrate sources varies according to species and research findings have been inconsistent (Shiau and Lin, 2001;2002; Wilson, 1994).

Tiger grouper (*Epinephelus fuscoguttatus,* ♀) × giant grouper (*Epinephelus lanceolatus,* ♂), TGGG was first produced in 2006 at the Borneo Marine Research Institute of Universiti Malaysia Sabah (Ch'ng and Senoo, 2008). Being a strict carnivorous fish, it requires high dietary protein to achieve optimum growth (Rahimnejad *et al*., 2015). Most of the formulated feed focusing on marine fish uses fishmeal and fish oil as the major protein and lipid sources, respectively. This may caused the formulated feed to be very expensive. The alternative way to reduce the cost is by incorporating the non-protein source such as carbohydrate so that the protein can be efficiently used for fish growth (Stone, 2003; Mohanta *et al*., 2007). To date, starch is the most common form of carbohydrate used in the feed formulation due to its competitive price and availability. However, very limited information on the performance of these different starch sources in fish diets. The present study was an endeavour to study the potential carbohydrate sources from polysaccharide group (starches and dextrin) in the diets of TGGG grouper juvenile. Apart from the growth performance, apparent digestibility coefficient, blood profile including serum biochemical and hematological parameters were also investigated.

Materials and Methods

Experimental diets

Four experimental diets were formulated to be

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isonitrogenous (50% crude protein) and isolipidic (10% crude lipid) to contain either 20% corn, sago, tapioca or dextrin as shown in Table 1. The diets were prepared by mixing all dry ingredients and subsequently adding the wet ingredients to form moist dough. The dough was screw-pressed through a 3 mm die and the strands of moist feeds formed were oven dried at 40°C for 3 to 4 hours. The dried diets were stored in sealed plastic bags and kept at 4°C until feeding time. The formulation and proximate composition of each experimental diet are shown in Table 1.

Tab. 1: Formulation and proximate composition of ingredients and diets (% dry weight).

Ingredients	Corn	Sago	Tapioca	Dextrin
Fish meal[1]	70.54	70.54	70.54	70.54
Fish oil[2]	2.76	2.76	2.76	2.76
CMC[3]	1.20	1.20	1.20	1.20
Vitamin premix[4]	3.00	3.00	3.00	3.00
Mineral premix[5]	2.00	2.00	2.00	2.00
Corn starch[6]	20.0	-	-	-
Tapioca starch[7]	-	20.0	-	-
Sago starch[8]	-	-	20.0	-
Dextrin[9]	-	-	-	20.0
Chromic Oxide[10]	0.5	0.5	0.5	0.5
Proximate composition (% of dry matter basis)				
Diets	**Corn**	**Sago**	**Tapioca**	**Dextrin**
Moisture	10.09	11.44	10.27	10.02
Crude protein	51.71	52.15	52.86	51.94
Crude lipid	10.09	10.0	10.07	9.90
Crude ash	15.89	15.77	15.77	15.76
Crude fiber	0.37	0.38	0.82	0.74
Gross energy (Kcal)	379.56	379.56	379.6	379.6

[1] TripleNine Fishmeal, Denmark
[2] Tuna oil, commercial oil
[3] Carboxylmethyl cellulose (Calbiochem, USA)
[4] Vitamin premix. (mg or IU/kg): vitamin A, 5000000 IU, vitamin D3, 1000000 IU; vitamin E, 50000 mg; vitamin B1, 15000 mg; vitamin B2, 15000 IU; vitamin B6, 12000 IU; vitamin B12, 25 mg; biotin, 500 mg; vitamin K3, 5000mg; vitamin c, 300000 mg; calpan, 25000 mg; folic acid, 2500 mg; niacin, 50000 mg; inositol, 125000 mg. Dexchem Industries Sdn. Bhd, Malaysia
[5] Mineral premix (mg/kg): Iron, 25000 mg; copper, 785 mg; zinc, 1894 mg; iodine, 150 mg; manganese, 800 mg; cobalt, 100 mg; sodium, 20 mg; dcp, 723251 mg; sodium chloride, 60000 Mg; anti caking, 1000 Mg; potassium chloride, 50000 mg; magnesium sulphate, 137000 mg. Dexchem Industries Sdn. Bhd, Malaysia
[6,7] AAA brand, Bake with Me Sdn. Bhd., Malaysia
[8] Local made
[9, 10] Sigma, USA

Fish rearing and management

The feeding trial was conducted at the Fish Hatchery of the Borneo Marine Research Institute, Universiti Malaysia Sabah (UMS) for 67 days. Tiger grouper (*Epinephelus fuscoguttatus,* ♀) × Giant grouper (*Epinephelus lanceolatus,* ♂) juveniles were purchased from a local fish farmer and acclimatized for 1 week prior to the start of the experiment. The fish were fed with commercial marine grouper feed (Leong Hup brand, Malaysia: crude protein, 44%; crude lipid, 8%) throughout the acclimatization period. Fish of mean initial wet body weight 13.78 ± 0.08 g (mean ± S.E.M) was randomly distributed into groups of 20 fish in 12 fiberglass tanks (150L) with flow-through system. The system was supplied with continuous aeration (flow rate: 3.5 L/min). Throughout the feeding trial, fish was fed to apparent satiation by hand twice a day (0800 and 1600). Water quality parameters during experimental period were dissolved oxygen monitored with values of 5.05 – 6.83 mg/L, pH 5.1-7.7, temperature 29-32°C, and salinity 30-33. The amount of feed consumption and mortality were recorded daily for calculation of feed intake, feed conversion ratio and survival. Fish were individually weighed at the start and end of feeding trial, and bulk-weighed fortnightly to determine their growth rates.

Feed digestibility trial

The feed digestibility trial was conducted in the same experimental tank during the feeding trial period. After 1-2 h post-feeding, fresh and intact strand of feces in each experimental tank were collected by gently siphoning, rinsed with distilled water, dried on filter paper and immediately stored at -20°C until analysis (Shapawi *et al*., 2007). Daily fecal samples from each tank were pooled until sufficient weight for chemical analysis. Chromic oxide of diets and feces were determined following the method by Furukawa and Tsukahara (1966).

Sample collection and analysis

At the beginning of the feeding trial, 10 fish were sampled for whole body proximate analysis. At the end of the feeding trial, fish were starved for 24 h. Commercial anaesthetic transmore (NIKA™) was used to anesthetize the fishes before body weight and total length was recorded at the beginning and end of feeding trial. Samples of viscera, liver, and intraperitoneal fat from fish in each treatment were excised and weighed for viscerasomatic index (VSI), hepatosomatic index (HSI) and intraperitoneal index (IPF). The liver and muscle of fish from each treatment was analysed for glycogen assay following method by Naimo *et al.* (1998). For whole body composition, six fish from each treatment were sampled and stored at -20°C until analysis. The sample of each ingredient, experimental diets, whole body composition, muscle and liver were homogenized for the proximate analysis on dry matter, ash, protein, lipid and fiber following the established methods described in AOAC International (1997). The gross energy content of the diet was calculated using physiological fuel values of carbohydrate (17.2 kJ/g), protein (23.6 kJ/g) and lipid, (39.5 kJ/g) (Halver and Hardy, 2002).

Blood analysis (Serum biochemical and hematology)

Blood samples were collected from caudal vein of anaesthetized fish using a syringe (23 G). The blood samples were centrifuged for 10 mins at 30,000 rpm to separate serum for analysis. Roche reagent kit was used for determination of serum biochemical: triglyceride (cat. 20767107), cholestrol (cat. 03039773), glucose (cat. 03183734) and total protein (cat. 03183734) and the analysis was performed on a Cobas 6000 analyzer series module C501 (Switzerland). While for hematological parameters, the analysis were performed on an XT- 1800i Sysmex.

Statistical analysis

The final body weight (FBW), body weight gain (BWG), specific growth rate (SGR), feed conversion ratio (FCR), protein efficiency ratio (PER), net protein utilization (NPU), survival, feed intake (FI), condition factor (CF), hepatosomatic index (HSI), viscerasomatic index (VSI), intraperitoneal fat index (IPF), apparent coefficient of dry matter and nutrients were calculated at the end of feeding trial (Shapawi *et al.,* 2013). Data obtained were subjected to one-way ANOVA to compare growth performance, feed utilization, body indices, whole body proximate composition, biochemical, hematology parameters and apparent digestibility coefficient. Homogeneity of variance was tested with Levene's test, and multiple comparisons among treatments were performed with a Duncan post-hoc test. Significant level was set at $P<0.05$. Statistical package SPSS v.22.0 was used for all statistical analyses. Results were presented as mean ± standard error of mean (S.E.M).

Results

Growth performance, Feed Utilization and Survival

The growth performance, feed utilization and survival of TGGG are presented in Table 2. TGGG grew from an average initial body weight of 13.78 g to 112.27-124.28 g within 67 days of feeding trial. Final body weight (FBW), body weight gained (BWG) and specific growth rate (SGR) of fish fed Diets Tapioca, Corn or Sago were significantly better than those fed Diet Dextrin, with the best values in these parameters obtained by Diet Sago. Feed intake was significantly influenced by the dietary carbohydrate sources but FCR and PER were not. Net protein utilization (NPU) was the highest in Diet Corn followed by Diets Dextrin, Sago and Tapioca. All experimental fish survived until the end of the feeding trial.

Tab. 2: Growth performance, Feed utilization and Survival of TGGG grouper in a 67-days feeding trial.

	Experimental Diets			
	Corn	**Sago**	**Tapioca**	**Dextrin**
FBW (g)	121.69[b] (1.41)	124.28[b] (1.74)	120.84[b] (5.21)	111.27[a] (1.23)
BWG (%)	782.09[b] (11.93)	798.01[b] (13.03)	780.90[b] (36.65)	707.66[a] (7.28)
SGR (%/day)	3.25[b] (0.02)	3.28[b] (0.02)	3.24[b] (0.06)	3.12[a] (0.01)
FI (g/fish)	126.0[b] (1.89)	131.71[b] (1.14)	123.15[ab] (5.59)	114.15[a] (1.32)
FCR	1.17 (0.01)	1.19 (0.03)	1.15 (0.01)	1.17 (0.02)
PER	1.64 (0.01)	1.58 (0.04)	1.63 (0.00)	1.63 (0.02)
NPU	32.92[b] (0.13)	30.11[a] (0.94)	29.38[a] (0.85)	30.28[a] (0.92)
Survival (%)	100.0 (0.0)	100.0 (0.0)	100.0 (0.0)	100.0 (0.0)

Values with different superscripts within row are significantly different (P<0.05).

Body indices, whole body, muscle, and liver proximate composition

Table 3 shows the body indices, whole body, muscle and liver proximate composition of TGGG fed different dietary carbohydrate sources. Viscerasomatic index (VSI), hepatosomatic index (HSI), intraperitoneal fat (IPF) and condition factor (CF) were the highest in Diet Dextrin (VSI 13.76%, HSI 2.77%, IPF 4.37%, CF 1.94% respectively), and the lowest in Diet Sago (VSI 10.26%, HSI 1.39%, VSI 3.02%, 1.78%). In term of the whole body proximate composition, the highest whole body lipid was also found in Diet Dextrin (7.56%) and the value was significantly higher than in other diets (5.84% - 6.83%). TGGG grouper fed Diet Dextrin had resulted significantly lower ash content (3.90%) compared with other diets that ranged from 4.78-5.52%. In muscle composition, the moisture content (78.47 %) of fish fed Diet Dextrin was significantly higher than other diets (76.52-77.52%). The highest muscle lipid deposition (0.79%) was observed in Diet Sago and lowest in Diet Dextrin (0.46%). No significant effect of carbohydrate was observed in muscle glycogen contents among the experimental diets. Liver moisture content was significantly highest in Diet Dextrin followed by Diets Sago, Tapioca and Corn. Liver lipid deposition was significantly higher in Diets Corn (6.07%) and Sago (5.83%), compared to Diet Tapioca (2.88%) and Dextrin (2.21%). Diets Sago and Tapioca had greater liver glycogen contents (0.21 – 0.22%) than other diets.

Tab. 3: Body indices, whole-body, muscle and liver composition (% wet weight) of TGGG grouper in a 67-days feeding trial.

	Experimental Diets			
	Corn	Sago	Tapioca	Dextrin
Body Indices				
HSI	1.40^{a} (0.02)	1.39^{a} (0.16)	1.65^{a} (0.24)	2.77^{b} (0.33)
VSI	10.29^{a} (0.50)	10.26^{a} (0.34)	12.51^{b} (0.33)	13.76^{c} (0.11)
IPF	3.02^{a} (0.40)	3.04^{ab} (0.29)	3.01^{a} (0.43)	4.37^{b} (0.65)
CF	1.82^{a} (0.04)	1.78^{a} (0.01)	1.89^{ab} (0.05)	1.94^{b} (0.03)
Whole body composition				
Moisture	70.09^{ab} (0.33)	70.19^{b} (0.22)	69.68^{ab} (0.32)	69.39^{a} (0.34)
Ash	5.52^{b} (0.38)	4.78^{b} (0.20)	5.06^{b} (0.21)	3.90^{a} (0.07)
Protein	18.50 (0.19)	18.75 (0.12)	17.82 (0.50)	18.22 (0.32)
Lipid	6.12^{a} (0.14)	5.84^{a} (0.15)	6.83^{b} (0.11)	7.56^{c} (0.23)
Muscle composition				
Moisture	76.52^{a} (0.23)	77.52^{b} (0.02)	77.51^{b} (0.25)	78.47^{c} (0.34)
Lipid	0.55^{a} (0.10)	0.79^{b} (0.07)	0.49^{a} (0.04)	0.46^{a} (0.07)
Glycogen	0.08 (0.01)	0.14 (0.05)	0.10 (0.04)	0.08 (0.02)
Liver composition				
Moisture	54.50^{a} (2.33	65.30^{b} (0.31	65.21^{b} (0.77)	69.91^{c} (0.23)
Lipid	6.07^{b} (0.41)	5.83^{b} (0.21)	2.88^{a} (0.05)	2.21^{a} (0.42)
Glycogen	0.06^{a} (0.01)	0.22^{c} (0.06)	0.21^{c} (0.07)	0.12^{b} (0.02)

The whole-body composition of initial fish was 72.35±0.22% moisture, 4.12±0.05% ash, 16.38±0.07% protein, and 5.34±0.02% lipid. Values with different superscripts within row are significantly different (P<0.05).

Biochemical parameters

The effects of dietary carbohydrate sources on biochemical parameters are summarized in Table 4**.** The cholesterol and triglyceride did not differ significantly. In contrast, significantly higher total protein was observed in Diet Dextrin (42.97 mmol/L) followed by Diets Tapioca (38.33 mmol/L), Corn (37.33 mmol/L) and Sago (35.0 mmol/L). Diet Sago has significantly higher glucose content (4.27 mmol/L) and no significant difference was observed among Diets Corn (2.53 mmol/L), Tapioca (2.87 mmol/L) and Dextrin (2.90 mmol/L).

Hematological parameters

Only mean corpuscular volume (MCV) and platelet counts were affected by the different carbohydrate sources in the diets (Tab. 5). Diet Corn has significantly lower MCV (168.33 fl) and no difference between Diets Sago (182.67 fl), Tapioca (182.33 fl), and Dextrin (193.0 fl). Platelet count was significantly higher in Diets Corn (15.67) and Sago (16.67) than Diets Tapioca (7.33) and Dextrin (8.67). The highest hemoglobin content and red cell count (RCC) were observed in Diet Dextrin (6.5%, 1.8 × $10^{12}L^{-1}$, respectively). Meanwhile, mean corpuscular hemoglobin (MCH) and mean cell hemoglobin concentration (MCHC) were highest in Diet Tapioca with values of 40.0 pg and 221.0 g/L, respectively. Total white cell count (WCC) was highest in Diet Dextrin (485.10 × $10^{9}L^{-1}$), followed by Diet Tapioca (478.43 ×$10^{9}L^{-1}$), Sago (430.17 × $10^{9}L^{-1}$) and Corn (392.07 × $10^{9}L^{-1}$).

Tab. 4: Serum biochemical of TGGG grouper in a 67-days feeding trial.

	Experimental Diets			
	Corn	Sago	Tapioca	Dextrin
Cholesterol (mmol/L)	2.83 (0.09)	2.77 (0.23)	3.20 (0.31)	3.47 (0.24)
Triglyceride (mmol/L)	0.87 (0.09)	0.87 (0.03)	0.90 (0.15)	0.93 (0.03)
Glucose (mmol/L)	2.53^{a} (0.23)	4.27^{b} (0.12)	2.87^{a} (0.34)	2.90^{a} (0.32)
Total Protein (mmol/L)	37.33^{ab} (1.45)	35.0^{a} (1.15)	38.33^{ab} (2.33)	42.67^{b} (2.73)

Values with different superscripts within row are significantly different (P<0.05).

Tab. 5: Hematology of TGGG grouper in a 67-days feeding trial.

	Experimental Diets			
	Corn	Sago	Tapioca	Dextrin
Hemoglobin (%)	5.63 (0.64)	5.53 (0.48)	6.47 (0.64)	6.5 (0.42)
RCC ($\times 10^{12}$ L^{-1})	1.5 (0.12)	1.67 (0.12)	1.5 (0.12)	1.8 (0.12)
PCV	0.27 (0.22)	0.31 (0.02)	0.28 (0.03)	0.35 (0.03)
MCH (pg)	33.5 (1.44)	33.0 (0.58)	40.0 (5.2)	35.67 (0.33)
MCV (fl)	168.33^{a} (7.22)	182.67^{b} (1.76)	182.33^{b} (3.18)	193.0^{b} (1.15)
MCHC (g/L)	193.33 (3.76)	179.33 (4.06)	221.0 (1.75)	183.0 (2.31)
Platelet count ($\times 10^{9}$ L^{-1})	15.67^{b} (0.88)	16.67^{b} (0.33)	7.33^{a} (0.88)	8.67^{a} (0.67)
Total WCC ($\times 10^{9}$ L^{-1})	392.07 (30.66)	430.17 (23.33)	478.43 (24.33)	485.10 (29.97)

Values with different superscripts within row are significantly different (P<0.05)

Apparent digestibility coefficient (ADC)

The apparent digestibility of dry matter, crude protein and lipid of experimental diets for TGGG are presented in Table 6. In general, all ADC values were considered high. There were significant differences (*P*<0.05) in the ADC for dry matter, protein and lipid among the experimental diets. ADC of dry matter ranged from 68.75% - 78.48%, where Diet Dextrin had the highest value followed by Diets Tapioca, Corn and Sago. ADC of protein was significantly higher in Diet Tapioca (95.77%) than in other diets (91.91-93.65%). ADC of lipid in Diets Sago and Dextrin (98.74-98.78%) were significantly higher than in Diets Corn and Tapioca (97.06-97.75%)

Tab. 6: Apparent digestibility coefficient (ADC) of dry matter, crude protein and crude lipid of experimental diets in a 67-days feeding trial.

	Experimental Diets			
	Corn	Sago	Tapioca	Dextrin
Dry Matter (%)	72.46[a] (3.28)	68.75[a] (0.65)	78.09[b] (0.58)	78.48[b] (0.60)
ADC Protein (%)	92.38[ab] (0.90)	91.91[a] (0.25)	95.77[c] (0.07)	93.65[b] (0.18)
ADC Lipid (%)	97.06[a] (0.43)	98.74[b] (0.13)	97.75[a] (0.09)	98.78[b] (0.19)

Values with different superscripts within row are significantly different (P<0.05).

Discussion

The influence of dietary carbohydrate source in fish varies with species, carbohydrate complexity, inclusion level, and the environmental factors (Kamalam *et al*., 2017). In carnivorous fish, inconsistent findings were reported on the utilization of different carbohydrate sources and levels. To the best of our knowledge, this is the first report on the utilization of carbohydrate sources in TGGG juvenile.

In the present study, carbohydrate source of starch origins performed equally good with no significant differences observed among them in terms of growth performance and feed conversion ratio (FCR) of TGGG. In malabar grouper (*E. malabaricus*), weight gain, feed efficiency and PER were better when the fish were fed diet with maize starch than glucose (Shiau and Lin, 2002). Meanwhile, the growth and feed efficiency of humpback grouper (*Cromileptes altivelis*) were not influenced by dietary carbohydrate sources and levels (Shapawi *et al.,* 2011). Apart from that, not many similar studies were conducted on grouper species.

The performance of dextrin-based diet in present study was significantly poorer than those starch-based diets. Growth reduction in fish fed Diets Dextrin and Sago was also observed in bagrid catfish (*Mystus nemurus*) (Hamid *et al.,* 2011). This is contrary with the finding on other carnivorous fish species such as gilthead sea bream (*Sparus aurata*), flounder (*Paralichthys olivaceus)* and Chinese longsnout catfish (*Leiocassis longirotris*) that fed with various carbohydrates and found that dextrin was a better carbohydrate source (Lee *et al.,* 2003; Tan *et al.,* 2006; Enes *et al*., 2010). Dextrin is the smaller metabolic intermediate of starch and highly branched structure which can be quickly hydrolyze by carbohydrate-degrading enzyme to produce glucose compared with starch (Niu *et al.,* 2012). This could be the reason of the poorer growth performance of Diet Dextrin in the present study as a result of lower availability of glucose from dextrin compared to starch (Shiau and Peng, 1992). Through personal observation, a lower FI in Diet Dextrin could probably due to the physical characteristic of the pellet, which appeared to be harder than other diets probably as a result of the stronger binding effect of dextrin compared with other diets. This has reduced the feeding intake of Diet Dextrin. Indirectly, it could reduced the ingestion of other essential nutrient that leads to poor growth (Jafri, 1998; Ali and Jauncey, 2004).

In term of body indices and whole-body analysis, Diet Dextrin had produced fish with significantly higher HSI, VSI, and whole-body lipid content among the tested diets. In blunt snout bream (*Megalobrama amblycephala*) fed with diet wheat starch, corn starch and dextrin also yielded higher whole-body lipid deposition (Ren *et al.,* 2015). The similar effect was also observed in malabar grouper (*Epinephelus malabaricus*) fed diet starch which resulted in higher body lipid content than those fed diet glucose (Shiau and Lin , 2002). However, some studies claimed the HSI and whole-body lipid were not influenced by carbohydrate sources at the same inclusion level (Lee *et al*., 2003; Yengkokpam *et al.,* 2007). Apart from that, the different final fish sizes may also influenced these findings.

Glycogen synthesis and *de novo* synthesis of lipid occured when the plasma glucose reached certain level, and the glycogen will be decomposed normally to supply the glucose if the plasma glucose level was depressed (Kamalam *et al*., 2017). A study by Miao *et al.* (2016) showed that turbot (*Scophthalmus maximus*) fed Diet Dextrin had higher liver lipid and lower liver glycogen and suggested that turbot prefer to convert the absorbed dextrin to lipid and not to glycogen in the liver. Similar pattern was also observed in Diet Corn of the present study where high liver lipid and low glycogen content were observed compared to other tested diets. Additionally, the glycogen content in muscle and liver of fish in the present study were in the range of those reported by Kamalam *et al.* (2017) with values below than 200 mg/gm of fresh tissue and 0.4 to 2 mg/gm in muscle. However, other factors such as fish species and feeding status also has some influence on the glycogen content of the fish (Kamalam *et al.,* 2017).

The liver of fish is an important metabolic organ and responsible for the content of plasma biochemical including blood glucose, cholesterol, triglycerides, and total protein (Andenen *et al.,* 1992; Tan *et al.,* 2013). In addition, blood parameters including serum biochemical and hematology could also provide important information about the general health status of the experimental animal (Li *et al.,* 2016). In the present study, the carbohydrate sources significantly influenced the hematology (MCV and platelet count) and serum biochemical (glucose and total protein) of the TGGG. In orange-spotted grouper *(E. coiodes),* the values reported on serum biochemical including cholesterol, triglyceride, and total protein were 137.3 mg/dl, 68.10 mg/dl and 3.92 mmol/L respectively (Akbary, 2014). The serum protein can be used as an indicator for fish health (Smet and Blust, 2001). The normal range of blood glucose is not yet established for many aquaculture species (Hemre *et al.,* 2002). Plasma glucose of silver perch (*Bidyanus bidyanus*) fed 60% carbohydrate was in the normal range (~ 3.44 mM), meanwhile normal cholesterol level has been reported in the range of 2.28 – 23.63 mmol/L in most marine fish (Larsson and Fange, 1977; Stone *et al*., 2003). In the present study, the value of cholesterol obtained was within this range and close to minimum value. In general, serum biochemical was species-specific and may caused by certain factors including temperature, food, age, season and sex (Kavadias *et al.,* 2003).

Apparent digestibility coefficient (ADC) of dry matter, protein and lipid were dependent on carbohydrate sources. In a recent paper by Kamalam and Panserat (2016), the ADC decreased with the increase in carbohydrate complexities (glucose > starch). The ADCs of protein and lipid in the present study were high among experimental diets and similar to those reported in other grouper species such as tiger grouper (*Epinephelus fuscoguttatus*) that fed diet with soybean meal to partially replaced fishmeal (Shapawi *et al.,* 2013). In humpback grouper (*C. altiveles*) fed with poultry by-product meal-based diets, the ADC of protein and lipid were 86.2-91.2% and 91.8-96.7%, respectively (Shapawi *et al.,* 2007). The high ADC values obtained in the present study were possibly due to the utilization of readily digestible fishmeal and fish oil as the main source of protein and lipid (Yong *et al.,* 2015). Overall, the differences of the findings on carbohydrate utilization in different fish species might also be influenced by other factors such as environmental factors, culture condition and quality of dietary ingredients (Stone *et al.,* 2003; Kamalam *et al.,* 2017).

Starch consisted of two polymer, amylose and amylopectin. Amylose has a straight-chain structure making α-glycosidic bound less available to enzymatic cleavage whereas amylopectin is highly branched and could be hydrolysed rapidly by enzyme degradation (Copeland *et al.,* 2009). Thus, the amylopectin could release glucose in short time while a linear and longer chain length of amylose could be digested more slowly (Rawles and Lochmann, 2003). In general, more stable glycemic response and better fish growth were observed when carbohydrate are slowly digested (Wilson, 1994). The similar effect observed for starch-origin diets of the present study probably due to their physiochemical properties are quite similar. For example, sago starch exhibit rheological character that seems similar to tapioca but closer to corn starch in term of retrogradation, gelatinization temperature and amylose content (Ahmad, 1999). Hence, the quite similar properties of starch-origin diet could be the reason for the similar growth of TGGG fed these diets. A study using sunshine bass (*Mystus chrysops* × *Morone saxatilis*) also showed a better utilization of diet when starch with greater proportion of amylose was used (Rawles and Lochmann, 2003). However, opposite finding by Hamid *et al.* (2011) where bagrid catfish (*M. nemurus)* performance was related to amylopectin structure of starches used. Other than higher amylose content, sago starch also has bigger average granular size compared to other tested starches (Wattanachant *et al.,* 2002).

The findings of the present study has provided useful information of different types of carbohydrate that can be successfully used in aquafeed industry, especially for carnivorous species. So far, sago, corn and tapioca are all planted in Malaysia, thus open the opportunity for these plants to be diversitified in term of usage. In light of the good performance of sago starch in the present study, it may emerge as a high potential starch source in the near future. Overall, TGGG appeared to be able to utilize starch of different origins without affecting their growth performances, provided at the optimum level.

Conclusion

It can be concluded that starch (sago, corn and tapioca) performed better than dextrin in the diets formulated for TGGG. In particular, sago has high potential to promote growth of TGGG. The findings of the present study had provided the aquafeed industry with more carbohydrate choices in the formulation of diets for grouper species.

Acknowledgement

This research was awarded by Ministry of Higher Education under the Niche Research Grant Scheme (NRGS 0004).

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