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EFFECTS OF SAGO-BASED (*Metroxylon sagu*) CARBOHYDRATE ON GROWTH PERFORMANCE AND BLOOD PLASMA COMPOSITIONS OF NILE TILAPIA, *Oreochromis niloticus* (LINNAEUS, 1758) JUVENILES

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Abstract: Six diets were formulated with three levels of protein, P(22%, 26% and 30%), and two levels of sago starch as source of carbohydrates, C(38% and 44%). The diets were fed to triplicate groups of tilapia, *Oreochromis niloticus*, juveniles (mean weight 4.61 ± 0.04 g), to apparent satiation twice daily for 12 weeks. Fish fed the 38% sago starch diet have a higher ($P < 0.05$) growth than the 44% sago starch and control diets. The whole body proximate compositions were significantly ($P < 0.05$) affected by the 38% and 44% dietary sago starch level among all diets. Fish fed with 38% sago starch diets have a higher ($P < 0.05$) glucose concentration in plasma than those fed with diet containing the 44% group diets. Higher ($p < 0.05$) triglyceride plasma were observed in fish fed with the 44% level diets, compared with the other diets. Two-way ANOVA results confirmed that the interaction between protein and carbohydrate has a significant ($p < 0.05$) influenced on growth performance, whole-body proximate compositions and blood plasma compositions. The study revealed the ability of *O. niloticus* juveniles to spare protein by carbohydrate at the level of 38 % sago starch.

Keywords: Sago starch, tilapia, growth, blood plasma.

Introduction

Tilapia is an omnivorous fish of the family *Cichlidae* (Sklan, *et al*., 2004; Qiang, *et al*., 2014), which includes more than 100 species under three genera; *Oreochromis*, *Sarotherodon* and *Tilapia* (Ridha, 2006; Wang & Lu, 2016). Tilapia cultures have been recognised as economically important and the fish is one of the most cultured fish in the world. Currently, tilapia is the second most farmed fish in the world after carps, with global production estimated to be around 6.3 million tonnes in 2018 (FAO, 2019) and expected to reach 7.3 million tonnes by 2030 (FAO, 2013). The global tilapia market is valued at about US$11.7 billion in 2017 and is estimated to reach US$13.4 billion by the end of 2025, which is mounting at a CAGR of 1.8% during the period. Nile tilapia (*Oreochromis niloticus*) cultures are the most dominant culture farm among the tilapia species (El-Sayed & Kawanna, 2007), and the species has a greater demand for larvae and juveniles (Ribeiro *et al*., 2018). In the aquaculture field, the success and sustainability of culture farms depend on the provision of nutritionally balanced, environmentally friendly and economically viable artificial feeds. Generally, the feed preparation diet is a principal factor that increases the growth and production of the reared fish in an aquaculture (Thankur *et al*., 2004; Liti *et al*., 2005; Abdel-Tawwab *et al*., 2007). However, the high cost and short supply of protein source (fish meal) in feed production is the main problem faced by the aquaculture industry (Sayed *et al*., 2018).

Protein is a macronutrient that is essential to build and repair damaged tissues and for general body maintenance. It is a vital nutrient and is considered the most expensive component in fish diet. The optimum level of protein used in fish diet is crucial to prevent the amino acid

from being catabolised as energy rather than being utilised for growth (Stone *et al*., 2003a). It is reported that the catabolism of protein in the diet can be minimised through adequate levels of non-protein energy sources, such lipid and carbohydrate (Mohanta *et al*., 2007), which also can reduce protein retention and increase the release of nitrogen to the environment, as stated by Lee *et al*. (2003). The use of carbohydrates as a protein-sparing energy source in fish has received less attention compared with lipids, while the utilisation of digestible energy in the form of carbohydrates is considered to be of prime importance under practical aquaculture conditions, as stated by Mohanta *et al*. (2009). Carbohydrates are the lowest expensive energy source among practical diet ingredients and they are efficient as they can be given to omnivorous and/or herbivorous warm water fish (Zhou *et al*., 2011; Fortes-Silva & Sànchez-Vàzquez, 2012). They can be utilised for routine metabolism and daily energy requirements as they yield almost the same energy as protein utilisation. Different carbohydrate levels and sources can affect fish growth as reported by Singh *et al*. (2006). A clear knowledge and better understanding on the optimal level of protein as dietary carbohydrates used is important to study the protein-sparing effect that may be essential in minimising the cost of fish preparation diets (Rahman *et al*., 2017).

In the present study, our principal objective was to evaluate the protein-sparing effects on various levels of sago starch as the carbohydrate source at different protein levels on (i) the growth performance, (ii) body indices, (iii) feed efficiency, (iv) whole-body composition and (iv) blood plasma of *O. niloticus* juveniles.

Material and Methods

Experimental Fish and Husbandry Conditions

O. niloticus juveniles (mean weight of approximately 4.60 g) were purchased from the PM Aquaculture Sdn. Bhd. In Sarawak, Malaysia, and transported to the experimental facilities in aerated polyethylene bags. The fish were acclimated to laboratory conditions for 2 weeks in a 3000-L capacity polyester tank fitted to a flow-through system and were fed a commercially available multi-fish feed (Dindings Soya & Multifeeds Sdn. Bhd., Kuala Lumpur, Malaysia), containing 320 g/kg of crude protein. After this duration, the fish were randomly introduced into 21 rectangular, experimental fish tanks (45×30×30 cm) with a capacity of 40.5 L capacity, and they were supplied with de-chlorinated tap water and aerated continuously in a closed system. Trial conditions included 20 fish per tank, with each diet being experimentally tested in a triplicate. During the experiment, one fourth of the total of volume of water was removed daily to flush out excreta and unfed diet, and then replaced by the same volume of water. The water quality parameters: temperature, pH and dissolved oxygen (DO) were maintained between 26.3–28.7 °C, 5.8–7.3 and 5.4–6.7 mg/L, respectively. The fish were reared under a natural photoperiod of an approximately 12/12 h light/dark schedule and fed experimental diets twice daily at 09:00 and 17:00 h to apparent satiation for 12 weeks.

All fish were weighed individually at the beginning and end of the experiment. The fish were weighed in bulk fortnightly to monitor the growth performance, and feed intake was also measured by subtracting uneaten feed from total feed given. At the end of the feeding trial of 12 weeks, the fish were starved for 24 h prior to sampling and randomly grouped within each treatment and used to determine the whole body proximate composition, body indices and for blood plasma analyses.

Experimental Diets

The feeding trial was carried out at the Aquaculture Laboratory of Universiti Malaysia Sarawak (UNIMAS), Kota Samarahan, Malaysia. The experimental feeding diets were prepared using sago starch as the carbohydrate source. Six isoenergetic (20.25 kJ/g) semi-purified experimental diets were prepared with three levels of protein, P(22%, 26% and 30%), and two levels of carbohydrates, C(38 % and 44 %), and the diets were designated as D1, D2, D3, D4, D5 and D6, respectively. Fishmeal was

used as the protein source and lipids from fish oil were maintained at 90 g/kg in all the diets. The control diet (D0) contained corn starch as the sole carbohydrate source (C40%). In the test diets, corn starch was completely substituted with either 38% or 44% of the sago starch. Ingredients were mixed in a feed mixer (Stand mixer, Model MK-GB1, Panasonic Co. Ltd., Taiwan) and passed through a feed extruder (Model TS102, Kimhill Ltd., Taiwan) to make 3 mm diameter pellets. The pellets were dried in a drying oven (Smith, Model A3018, United Kingdom Ltd., USA) maintained at 55 °C. Dried feed pellets were kept in separate, tightly capped bottles, labelled and stored in a deep freezer at -20 °C until used for the feeding trial. The formulation and proximate analysis of the experimental diets were given in Table 1.

Growth Performance

The following formulae were applied for growth performance calculation of various parameters:

Feed intake (FI) = Total feed intake (g)/ Number of fish (g)

Weight gain (WG %) = (Final weight (g) - Initial weight (g) / Initial weight (g)) x 100

Specific growth rate (SGR %) = (lnfinal weight (g) – lninitial weight (g) / No. of days) x 100

Protein efficiency rate (PER) = Wet weight gain (g) / Total protein intake

Feed conversion ratio (FCR) = Total feed intake (g) / Total weight gain (g)

Hepatosomatic Index (HSI %) = (Liver weight / Body weight) x 100

Viscerosomatic Index (VSI %) = (Viscera weight / Body weight) x 100

Intraperitoneal fat (IPF %) = (Intraperitoneal fat weight / Body weight) x100

Survival rate (SR %) = (Number of fish survived / Initial number of fish) x 100

Proximate Composition of Whole Body

The proximate compositions for feed ingredients, experimental diets and fish whole body were performed using standard AOAC methods (AOAC 1997). The crude protein content was determined by using the Kjeldahl procedure by multiplying nitrogen by 6.25, and chloroform/methanol extraction was used for crude fat determination (Gerhardt, United Kingdom Ltd., USA). The crude ash content was tested by determining the residue after heating in a muffle furnace (Thermconcept, Model KC 20/13- KC120/14, Dr Fischer GmbH and Co. Ltd., Germany) at 550°C for 6 hours and moisture content by oven drying maintained at 100 °C to constant weight. The gross energy was estimated using a bomb calorimeter (Model Parr 6400 calorimeter, Parr Instrument Company, Moline, IL, USA).

Blood Plasma Composition Analysis

Blood samples were obtained randomly from six fish from each treatment for the blood plasma composition analysis. The blood was extracted from the caudal fin vessel of each fish using a 1 mL syringe with a 27-gauge hypodermic needle. Upon collection, the blood was transferred immediately into a 2 mL blood tube containing 5 mg/mL of anticoagulant EDTA. The blood sample was centrifuged at 3500 x g for 5 mins, and the plasma was separated and stored at -80°C. The level of plasma glucose, triglyceride and total protein were analysed using the Haematology Analyzer (Abbott, Model CELL-DYN Emerald 18, Abbott Park, Illinois, USA).

Statistical Analysis

Data for growth performance, body composition and indices, feed utilisation and blood plasma were presented as the mean ± SD and analysed using one-way and two-way analysis of variance (ANOVA) to test the effects of the dietary protein and carbohydrate. P values < 0.05 were considered significant when compared with Duncan's multiple range test. All statistical analyses were compared using the Statistical Package for the Social Sciences

Table 1: Ingredients used and proximate composition of the experimental diets (g/Kg *DM*)

Ingredients	Treatments						
	C40 %	C38 %			C44 %		
	P30 %	P22 %	P26 %	P30 %	P22 %	P26 %	P30 %
	D0	D1	D2	D3	D4	D5	D6
Danish fish meal[1]	300	220	260	300	220	260	300
Fish oil	90	90	90	90	90	90	90
Corn starch	400	-	-	-	-	-	-
Sago starch	-	380	380	380	440	440	440
Cellulose	150	250	210	170	190	150	110
CMC[2]	20	20	20	20	20	20	20
Vitamin Mix[3]	20	20	20	20	20	20	20
Mineral Mix[4]	20	20	20	20	20	20	20
Proximate composition, g/kg							
Moisture	73.4	73.3	74.1	75.3	73.9	74.3	73.1
Crude protein	302.1	224.8	262.7	304.5	223.9	261.4	302.6
Crude lipid	122.5	107.9	114.7	124.3	104.8	112.6	121.1
Crude ash	47.6	47.8	48.1	48.7	46.5	46.9	47.4
Crude fibre	51.7	72.3	66.8	65.1	69.4	62.7	51.1
NFE[5]	402.7	383.9	382.3	381.1	443.5	442.1	440.1
GE (MJ/kg)[6]	216.33	184.69	195.58	214.74	188.36	198.65	217.42

[1] Danish Fish Meal per kg = Crude protein 765.9 and crude lipid 97.6

[2] Carboxy methylcellulose

[3] Vitamin mix per kg = Vit $_{A}$ 50 million IU, Vit$_{D3}$ 10 million IU, Vit $_{E}$ 130 g, Vit $_{K3}$ 10 g, Vit $_{B1}$ 10 g, Vit $_{B2}$ 25 g, Vit $_{B6}$ 16 g, Vit $_{B12}$ 100 mg, Niacin 200 g, Pantothenic acid 56 g, , Folic acid 8 g, Biotin 500 mg and Anticake 20 g.

[4] Mineral mix per kg = Copper 10 g, Iron 100 g, Manganese 100 g, Zinc 75 g, Cobalt 1 g, Iodine 1 g, Selenium 0.12 g and Anticake 10 g.

[5] NFE = Nitrogen free extract was calculated as 1000 – (Moisture + Protein + Lipid + Ash + Fiber) g/kg.

[6] GE = Gross energy was measured using a bomb calorimeter, the Parr 6400 bomb calorimeter.

(SPSS) programme for Windows (Version 25.0. Armonk, NY: IBM Corp.).

Results

Growth Performance, Nutrient Utilisation and Body Indices

The results on the final body weight (FBW), growth performances (WG% and SGR%), feed utilisation (FCR and PER) and body indices (HSI, VSI and IPF) of the *O. niloticus* observed after 12 weeks were presented in Table 2. The two-way ANOVA results discovered that the interaction between different levels of protein and carbohydrate have significant ($P < 0.05$) effects on the diets fed to the *O. niloticus* juveniles. Different levels of protein and dietary sago starch were found to have a significant ($P < 0.05$) influence on the FBW and growth performance of the *O. niloticus* juveniles. Significant ($P < 0.05$) differences in FBW were recorded. The fish fed with 38% sago starch (D2: 32.32 g and D3: 31.87 g) were heavier than the fish fed with the control diet (D0: 28.16 g) and D6 (27.71 g). There was no numerically significant difference with fish fed with the D1 (29.53 g) , D4 (23.29 g) and D5 (25.65 g) diets. The same trend was observed in the weight gain (WG%) and specific growth rate (SGR%) results. The WG% of fish fed on D3 was the significantly ($P < 0.05$) highest (601.90 %), alongside the SGR (2.18 %), compared with the other test diets. Based on the WG (%), the second-order polynomial regression analysis ($y= -4.6956x2 + 14.23x + 535.73$, $r2= 0.2297$) gave a result of $X= 2.72$, which suggested that the appropriate level of protein and carbohydrates for protein-sparing effects to provide maximum growth of *O. niloticus* juveniles were between diets D2 (P26 %, C38 %) and D3 (P30 %, C38 %) (Figure 1). However, all fish fed with the 38% group diets (D1, D2 and D3) performed better than those fed with diets with 44% sago starch (Figure 2). During the experimental period, all the diets were well accepted by the fish with no observed rejection and all the fish fed on the diets actively. Feed intakes (FI) ranged from 39.47 g to 44.16 g and no significant ($P > 0.05$) differences were observed among all treatments. The feed conversion ratio (FCR) tended to increase with increased sago level in the test diets. The FCR was recorded higher in all fish fed

with the 44% sago starch group diets, and become lower with decreased sago starch at the 38% level. However, it was not significantly ($P > 0.05$) different among all test diets, ranging between 1.55 and 2.12. The protein efficiency ratio (PER) was dependent on the incorporation between the levels of protein and dietary sago starch in the test diets, Fish fed with the optimum level (38 %) performed more effectively ($P > 0.05$). Fish fed with 3 % sago starch group diets exhibited higher PER, compared with those fed with a high sago level (44 %), ranging from 2.12 to 2.75. Based on the body indices (Figure 3) monitored, both HSI (2.04-2.53 %) and VSI (5.60-6.41 %) values were found to have a highly significant ($P < 0.05$) difference in fish fed with the 44% sago starch level (D2 and D3) than those fed with the 38% group diets (D4 and D5). Intraperitoneal fat (IPF %) was showed to be not significantly ($P > 0.05$) different among all dietary treatments, varying from 1.14 - 1.89 %. Overall, no fish died and as such, the fish survival rate was 100 % throughout the feeding trial.

Table 2: The growth performance, feed efficiency, body indices and survival of the *O. niloticus* juveniles fed with diets containing various levels of protein and carbohydrates for 12 weeks (n=20)

Parameters	Treatments							Two-Way ANOVA P < 0.05		
	C40 %	C38 %			C44 %					
	P30 % D0	P22% D1	P26% D2	P30% D3	P22% D4	P26% D5	P30% D6	P	C	P x C
IBW[1] (g)	4.62±0.04	4.61±0.05	4.59±0.08	4.60±0.07	4.61±0.09	4.58±0.08	4.62±0.06	-	-	-
FBW[2] (g)	28.16±2.49^{bc}	29.53±2.50^{d}	31.87±2.37^{de}	32.32±2.25^{de}	23.29±2.19^{a}	25.65±2.46^{b}	27.71±2.57^{bc}	0.030	0.001	0.008
WG (%)	504.17±49.17^{bc}	551.60±48.47^{d}	599.78±44.19^{de}	601.90±39.15^{de}	404.13±42.37^{a}	459.25±46.34^{b}	500.81±47.76^{bc}	0.039	0.000	0.014
FI (g)	42.03±1.39	42.45±2.33	43.32±1.17	44.16±1.52	39.47±1.88	40.39±2.80	41.28±2.26	0.022	0.018	0.029
SGR (%)	1.97±0.10^{bc}	2.05±0.09^{d}	2.16±0.08^{de}	2.18±0.06^{de}	1.71±0.10^{a}	1.87±0.10^{b}	1.95±0.09^{bc}	0.008	0.000	0.031
FCR	1.82±0.14	1.76±0.09	1.64±0.10	1.55±0.07	2.12±0.23	1.97±0.12	1.89±0.10	0.035	0.011	0.040
PER	2.20±0.11	2.75±0.14	2.54±0.15	2.32±0.10	2.61±0.17	2.43±0.13	2.12±0.10	0.000	0.021	0.034
HSI (%)	2.25±0.18^{bc}	2.16±0.16^{ab}	2.04±0.13^{a}	2.06±0.19^{a}	2.53±0.25^{d}	2.50±0.21^{d}	2.36±0.15^{bc}	0.019	0.003	0.042
VSI (%)	5.86±0.54^{bc}	5.75±0.52^{b}	5.60±0.49^{a}	5.64±0.57^{a}	6.41±0.73^{d}	6.39±0.68^{d}	5.97±0.55^{bc}	0.028	0.017	0.037
IPF (%)	1.53±0.23	1.42±0.24	1.14±0.20	1.26±0.27	1.89±0.32	1.75±0.29	1.64±0.26	0.013	0.005	0.043
Survival (%)	100±0.00	100±0.00	100±0.00	100±0.00	100±0.00	100±0.00	100±0.00	-	-	-

Mean values in the same row with different superscript letters are significantly different (P < 0.05, one-way ANOVA).

[1] 1IBW, initial mean body weight.

[2] 2FBW, final mean body weight.

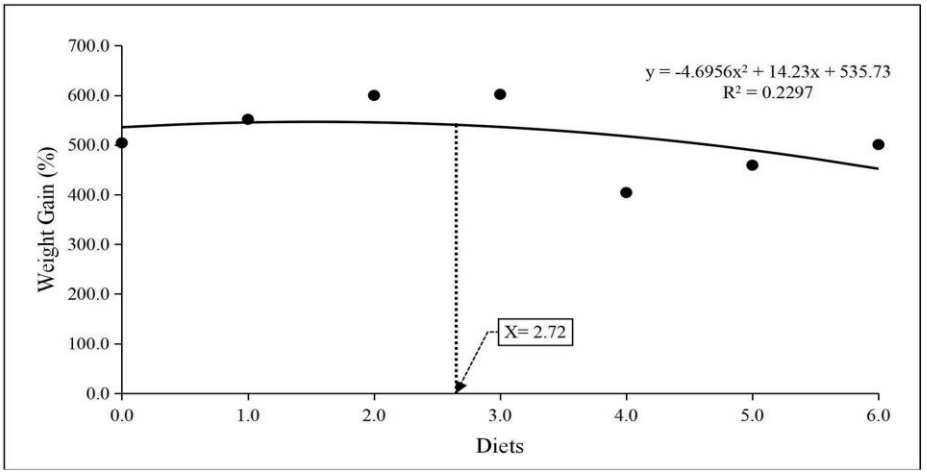


Figure 1: Relationship between diets containing various levels of protein and carbohydrate and weight gain (%) of the *O. niloticus* juveniles

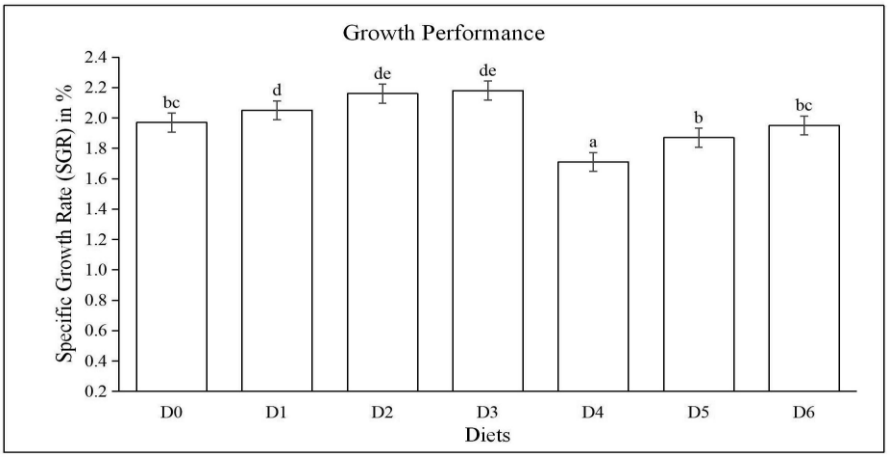
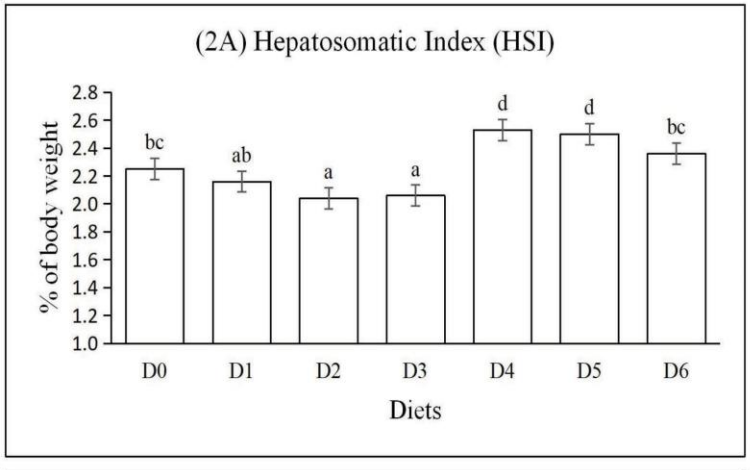
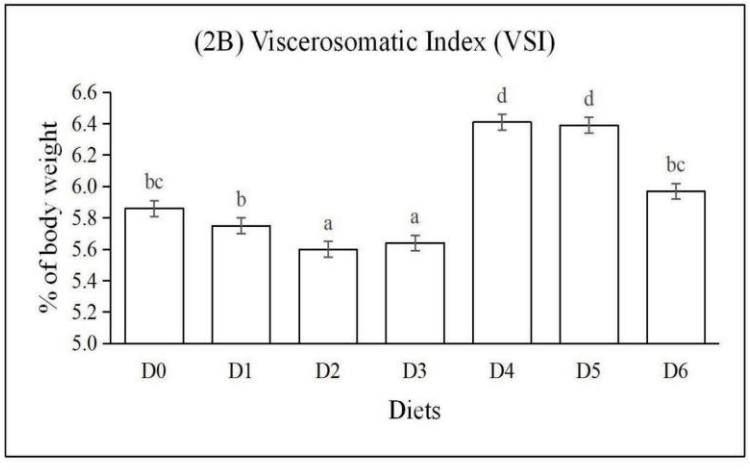


Figure 2: The effects of various levels of protein and carbohydrate on growth performance of the *O. niloticus* juveniles. The results are mean±SD of triplicate samples. Values assigned as different superscript letters are significantly different ($P < 0.05$)





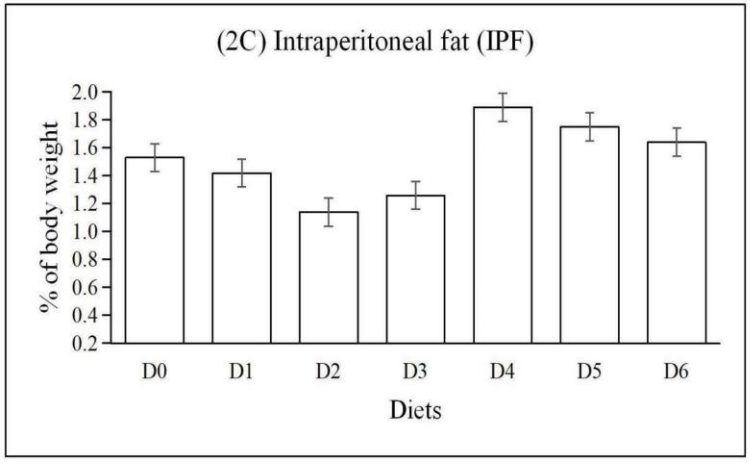


Figure 3: The effects of various levels of protein and carbohydrate on body indices; (2A) Hepatosomatic Index; (2B) Viscerosomatic Index; (2C) Intraperitoneal fat of the *O. niloticus* juveniles. The results are mean±SD of triplicate samples. Values assigned as different superscript letters are significantly different ($P < 0.05$)

Proximate Composition of the Final Whole Body

The proximate of the initial and final body composition of the *O. niloticus* juveniles are shown in Table 3. The moisture content level in the whole-body of the initial fish and the final treatment groups varied from 74.88% to 78.13%. The final moisture content was found to have a highly significant ($P < 0.05$) different in fish fed with the 38% sago starch diet group (D2 and D3) than those fed with D0 and the 44% group test diets However, they have a lower value compared with the initial body moisture contents. The whole-body protein was significantly higher ($P < 0.05$) in the final dietary treatment group than those of the initial group. The whole-body protein content was at the maximum level in the 38% group diets (D2 and D3) and decreased at the 44% sago starch level among the final treatment group. The whole-body lipid content was recorded to be lower in the initial fish than those in final treatment group, and increased significantly ($P < 0.05$) as the dietary starch levels increased from the 38% to the 44% group diets. The whole-body ash content was significantly ($P < 0.05$) influenced by the sago starch level. Significant differences ($P < 0.05$) of the body ash content was higher in the final treatment group compared with the initial fish, and it increased with increased sago starch level in diets. The whole-body gross energy level for the final treatment group was significantly higher ($P < 0.05$) than the initial fish and it increased with the sago starch level increase from 38% to 44% in the diet groups. Overall, the two-way ANOVA results showed that different levels of protein and carbohydrate on the feeding diets significantly ($P < 0.05$) influenced whole-body composition of the *O. niloticus* juveniles.

Blood Plasma Compositions

The blood plasma composition of the *O. niloticus* juveniles fed with different levels of protein and sago starch for 12 weeks is presented in Table 4. The results based on two-way ANOVA revealed that different levels of protein and carbohydrate have significant ($P < 0.05$) interaction on the blood plasma compositions of *O. niloticus* juveniles. The effects of various levels of protein and carbohydrates on blood plasma composition for *O. niloticus* juveniles are statistically showed in Figure 4. The blood plasma glucose concentration was significantly affected by the sago starch level, and decreased as the dietary starch levels increased. Statistically significant ($P < 0.05$) differences with a higher value were recorded in fish fed with the 38% sago starch group (D2: 13.64 mmol/Land D3: 13.68 mmol/L) thant fish fed on D0, D1, D4 and D5, D6 among the group diets. High significant differences ($P < 0.05$) of plasma triglyceride were recorded in fish fed with the 44% sago starch group diets, ranging from 2.47-6.52 mmol/L. However, the plasma protein concentration showed no significant difference ($P > 0.05$), varying from 4.18 and 22.11 g/L among the dietary treatments.

Table 3: The whole body proximate compositions (%) of the *O. niloticus* juveniles fed with diets containing various levels of protein and carbohydrates for 12 weeks (n=6)

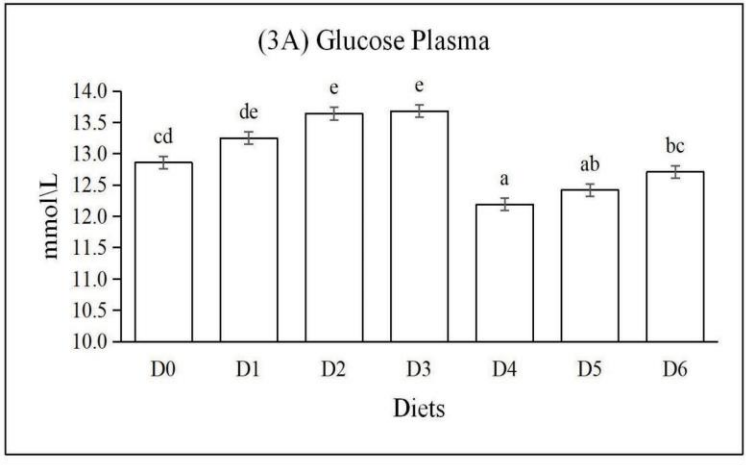
Proximate analysis	Initial	Treatments C40 % P30% D0	C38 % P22% D1	C38 % P26% D2	C38 % P30% D3	C44 % P22% D4	C44 % P26% D5	C44 % P30% D6	Two-Way ANOVA p <0.05 P	C	P x C
Moisture	78.13±0.58^{e}	75.82±0.63^{c}	76.27±0.81^{cd}	76.53±0.96^{d}	76.56±0.87^{d}	74.88±0.77^{a}	75.26±0.65^{ab}	75.69±1.13^{bc}	0.043	0.012	0.038
Crude protein	11.68±0.21^{a}	14.57±0.33^{d}	15.34±0.42^{de}	15.65±0.28^{e}	15.69±0.45^{e}	13.16±0.51^{b}	13.78±0.36^{bc}	14.32±0.19^{cd}	0.012	0.000	0.007
Crude lipid	3.64±0.19^{a}	5.26±0.24^{cd}	4.92±0.33^{bc}	4.75±0.21^{b}	4.63±0.51^{b}	5.89±0.17^{e}	5.81±0.39^{e}	5.42±0.48^{de}	0.024	0.000	0.011
Crude ash	2.49±0.53^{a}	2.83±0.4^{bc}	2.72±0.64^{bc}	2.60±0.72^{b}	2.64±0.48^{b}	3.15±0.36^{e}	2.97±0.69^{de}	2.95±0.29^{de}	0.031	0.026	0.034
GE (kJ/g)[1]	23.51±0.18^{a}	25.86±0.23^{cd}	25.57±0.31^{bc}	25.33±0.38^{b}	25.21±0.26^{b}	26.89±0.45^{e}	26.85±0.32^{e}	26.38±0.40^{de}	0.017	0.000	0.009

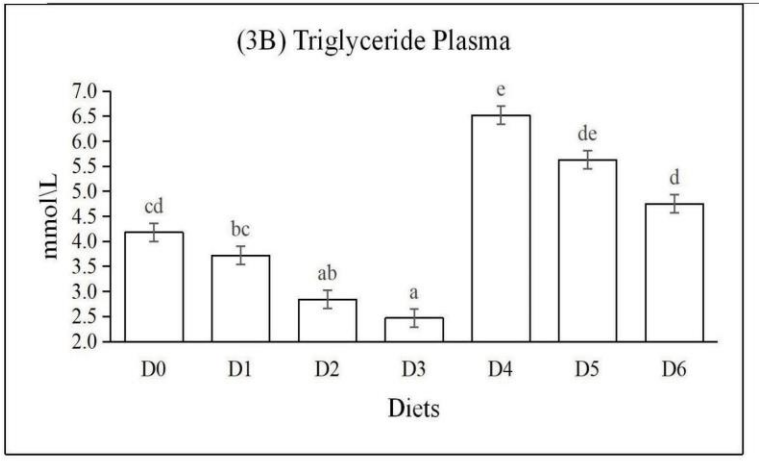
Mean values in same row with different superscript letters are significantly different (P < 0.05, one-way ANOVA).
[1]GE = Gross energy was measured using bomb calorimeter, Parr 6400 bomb calorimeter.

Table 4: The blood plasma composition of the O. niloticus juveniles fed with diets containing various levels of protein and carbohydrate for 12 weeks (n=6)

Blood Plasma	Treatments C40 % P30 % D0	C38 % P22 % D1	C38 % P26 % D2	C38 % P30 % D3	C45 % P22 % D4	C45 % P26 % D5	C45 % P30 % D6	Two-Way ANOVA P<0.05 P	C	P x C
Glucose (mmol/L)	12.86±0.26^{cd}	13.25±0.37^{de}	13.64±0.43^{e}	13.68±0.66^{e}	12.19±0.18^{a}	12.42±0.59^{ab}	12.71±0.71^{bc}	0.022	0.001	0.015
Triglyceride (mmol/L)	4.18±0.86^{cd}	3.72±0.75^{bc}	2.84±0.55^{ab}	2.47±0.38^{a}	6.52±0.61^{e}	5.63±0.29^{de}	4.75±0.42^{d}	0.006	0.012	0.009
Protein (g/L)	17.32±2.12	10.46±2.31	19.25±1.46	22.11±2.19	4.18±2.26	13.27±1.41	15.34±1.38	0.031	0.008	0.014

Mean values in same row with different superscript letters are significantly different (P < 0.05, one-way ANOVA).





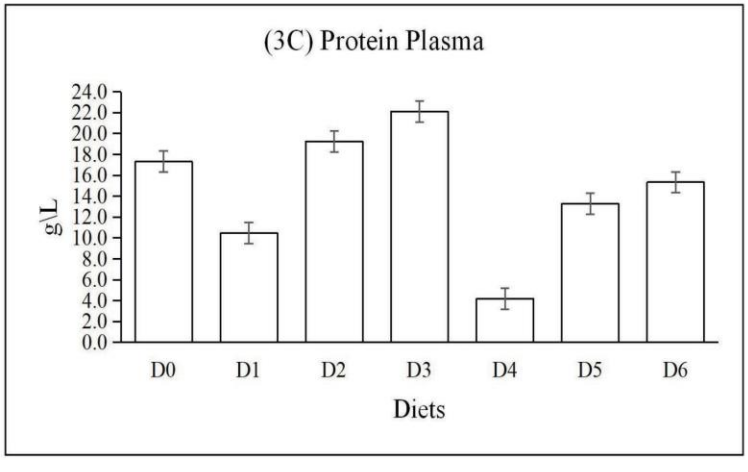


Figure 4: The effects of various levels of protein and carbohydrates on blood plasma; (3A) Glucose plasma; (3B) Triglyceride plasma; (3C) Protein plasma of the *O. niloticus* juveniles. The results are mean±SD of triplicate sample. Values assigned as different superscript letters are significantly different ($P < 0.05$).

Discussion

The incorporation of proper carbohydrate levels in the fish diet has been reported to improve growth performance in different fish species (Stone *et al*., 2003b; Enes *et al*., 2008a; Zhou *et al*., 2016). This study revealed that the optimum sago starch level required for maximum growth performance (WG % and SGR %) of *O. niloticus* juveniles was at the 38% sago starch level (D1, D2 and D3), which was in agreement with the suggested requirement levels of dietary carbohydrate (starch), varying from 350 to 400 g/kg for tilapia (Azaza *et al*., 2015). The decrease in dietary protein level from 30% to 26% incorporated with 38% sago starch was observed to not having much influence on the FBW of the fish, indicating that the level can spare some protein when the dietary protein is low (Mohanta *et al*., 2007). However, the growth performance of fish fed with the 44% sago starch group was in contrast with those fed with the 38% group diets. Azaza *et al*. (2015) stated that excess levels of starch exceed the 401.3 g/kg intake by fish significantly reduce the growth rate and were accompanied by poor feed utilisation. Besides, fish fed with a high carbohydrate (starch) diet can reduce growth as it might depend on the nutrient digestibility efficiency as mentioned by Ren *et al*. (2011).

The survival rate of 100% recorded in all the treatments after 12 weeks shows that tilapia fed with starch-based carbohydrates have high survival rates, as reported by Wang *et al*. (2005). From the results, feed utilisation (FCR and PER) was not significantly affected by the sago starch levels. The FCR was observed to be higher in fish fed on the 44% sago starch group diet (D4) with low protein (2 %), and it showed that a high level of carbohydrate incorporated with a low protein level produces a high FCR value, as recorded by Rahman *et al*. (2017). Our observations also suggested that fish fed on the 44% group diets need to consume more to achieve adequate levels of amino acid consumption for a better growth performance and it was similar with previous findings on juvenile Nile tilapia (Azaza *et al*., 2015). The fish fed with the 38% sago starch group diets have the highest PER and the results were in agreement with Xia *et al*. (2015), which specified that the protein efficiency ratio increased when dietary carbohydrate (starch) levels ranged from 25.61% to 37.55% and decreased in higher carbohydrate levels, approximately at 45.31% starch level. Singh *et al*. (2006) stated that feed utilisation increased when the levels of carbohydrate increases (optimum level at 30-40 %) but protein levels were low, indicating that the protein showed the sparing effect. The data obtained in the present investigation showed that higher growth of *O. niloticus* juveniles could be obtained even at a low dietary protein with optimum levels of carbohydrates in the diet.

In the present study, HSI and VSI with high significant values were observed in fish fed with the 44% group diets of sago starch. The increases in the HSI with dietary starch have been observed by different researchers (Moreira *et al*., 2008; Tian *et al*., 2012; Wang *et al*., 2016). This may occur due to the fact that high dietary starch levels lead to an increase of glycogen and lipid deposition in the liver (Xia *et al*., 2015). The liver functions as the preeminent site of lipogenesis in fish (Segner & Böhm, 1994), meaning that a portion of the absorbed carbohydrate not used for energy are converted and stored in the liver as both lipid and glycogen (Azaza *et al*., 2015). The VSI increasing alongside the starch level intake has been shown in previous studies (Peres & Oliva-Teles, 2002; Moreira *et al*., 2008). In fact, in the juveniles of Nile tilapia, viscera represented about 6-8% of whole-body mass and significantly increased in excess dietary starch. This agreed with other findings that many fish species can deposit significant amounts of lipid and glycogen in visceral depots (Rawles *et al*., 2008; Enes *et al*., 2010) when fed with diets with a high level of starch. Based on the results, intraperitoneal fat (IPF) was not significantly affected by the sago starch level. IPF was found to have high values in fish fed with the 44% group diets (higher) of sago starch, compared with those fed with the 38% group diets (lower). Wang *et al*., (2005), who reported a high IPF ratio appearing in fish

fed with diets with high level of starch, theorised that it is probably caused by the high level of dietary starch being transformed into lipid, and accumulated in the intraperitoneal cavity of the fish. Our results are also in agreement with the findings on other fish species, showing that excess of dietary starch-based carbohydrate intake increased the synthesis of fat in fish (Enes *et al*., 2009).

The body composition results showed considerable responses to the dietary sago starch levels. In the present study, the whole-body moisture content tended to be higher at low dietary starch level (38% group diets). Such effects had been observed for *O. niloticus* X *O. aureus* (Wang *et al*., 2005), *Cirrhinus mrigala* (Singh *et al*., 2006), *Puntius gonionotus* (Mohanta *et al*., 2007), European sea bass (Moreira *et al*., 2008), and Yellow catfish (Ye *et al*., 2009). Crude protein content was recorded to have a highly significant influence on fish fed with the 38% sago starch group diets among all dietary treatments. The current result was agreeable with Mohanta *et al*. (2009), who mentioned that crude protein increased significantly at optimal starch level ranging between 220 g/kg to 380 g/kg. Both whole-body lipid and ash were positively related to the level of starch concentration. A high value in body lipid content with increased dietary starch level could be explained by the *de novo* synthesis of lipid from carbohydrate (Fernàndez *et al*., 2007). In our study, although the body ash content was low, it increased with increased dietary carbohydrate at each level of dietary protein, and it was similar with the results of the study on silver barb, *Puntius gonionotus* (Mohanta *et al*., 2007). The whole-body energy was observed to be higher in fish fed with the 44% sago starch group among all test diets due to the fact that for omnivorous fish, high carbohydrate diets stimulate lipogenic enzyme activities (Rahman *et al*., 2017). The HSI was considered as a sensitive indicator for the available energy in fish because excess digestible energy leads to the deposition of either glycogen or lipid, resulting in higher HSI (Mohanta *et al*., 2009). We presume that the fat deposition in the liver of the Nile tilapia might have arisen from *de novo* synthesis of fat carbohydrates when excess sago starch was fed to the fish, resulting in high energy due to the increased HSI value in the fish fed with the 44% sago starch level.

It was well known that glucose, triglyceride and total protein contents in blood are correlated to animal health, general metabolism and physiological status (NRC, 2011). The amount of total protein in the plasma was found to be not affected by different sago starch levels in this experiment. The plasma protein values varied among all dietary treatments, and it may be due to the protein content levels incorporated in the experimental diets (Boonanuntanasarn *et al*., 2018). Plasma triglyceride and glucose were significantly influenced by the dietary sago starch. The present study showed that plasma triglycerides increased with the excess of dietary sago starch (44 %), compared with fish fed with the 38% group diets. This indicated a more active endogenous lipid transport in response to the higher dietary starch level (Wang *et al*., 2014). The plasma glucose level significantly increased in fish fed with the 38% starch group diets and this may be due to the absorption of the glucose released by glycolysis during efficient utilisation by fish, leading to an increase in the blood glucose level in blood plasma (Ren *et al*., 2011). The intensity of the blood glucose peak increase is also related to the level of digestible carbohydrate (starch) in diets (Stone *et al*., 2003a). The normal range for plasma glucose level has not been not fully defined for many aquaculture fish species (Cui *et al*., 2010). In our study, the plasma glucose level ranged from 12.19 to 13.68 mmol/L. and the trend was similar to the results recorded by Hemre *et al*. (2002), which is approximately above 11 mmol/L. The wide variation observed in the plasma glucose level among fish also depend on the different feeding habit (such as carnivorous, herbivorous and omnivorous) of each species, different stage of life or certain feeding regimes (Hemre *et al*., 2002). Additionally, we presume that the plasma glucose level was significantly influenced by whether fish were fed or not fed the diet when sampling was carried out.

Indeed, the use of starch for carbohydrate-rich diets have the economic advantage as fish would utilise the inexpensive carbohydrate as an energy source instead of proteins, thus the absorbed protein will be spared for growth (Webb *et al*., 2010; Zhao *et al*., 2011). However excessive dietary starch of more than 40% in fish diet may lead to poor growth rate and low feed efficiency (Li *et al*., 2016) and it was in agreement with the present study, in which the 44% sago starch diets show a low growth performance compared with the 38% group diets. Ren *et al*. (2011) also stated that increased carbohydrate starch in the fish diet depressed the growth performance and will impair some physiological conditions. Furthermore, inadequate and excess levels of starch may cause protein and lipid degradation, which results in low physical quality of feed diets and poor protein utilisation (Zhou *et al*., 2016). Hence, the optimum supplementation of the carbohydrate level in fish diet is very important.

Starch-based carbohydrate utilisation by fish varies among species and it also depends on their ability to use the carbohydrates as an energy source, both for digestion and metabolism, respective to their feeding habits (Boonanuntanasarn *et al*., 2018). *Tilapia sp.* are warm water omnivorous fish that can utilise approximately up to 40% of digestible carbohydrate (starch) from their diets (Qiang *et al*., 2014) and the statement supported the current result, in which fish fed with diets with 38% sago starch show a positive impact and performed better. Nevertheless, a dietary starch carbohydrate level that is too high and beyond the optimal levels, as observed in the 44% group diets, may cause problems in fish health through metabolic disturbances and clinical signs, such as decreased growth, prolonged hyperglycaemia, increased glycogen deposition, liver hypertrophy, histopathological and in some cases increased glycosylated haemoglobin, retinopathy, hepatic steatosis and insulin resistance (Prisingkorn *et al*., 2017). Therefore, an appropriate starch level is crucial in fish diet as it also helps to reduce the amount of nitrogen from farms effluents and improve the pellet binding, stability and buoyancy (Guerrero-Zárate *et al*., 2019).

Conclusion

It is suggested that the 38% sago starch level is the optimum level that promotes better growth performance and feed utilisation in *O. niloticus* juveniles. Beyond this level, the fish are unable to obtain good growth performance and feed efficiency utilisation was depressed. In fact, the optimum incorporation level between carbohydrate and protein included in the diet is a very crucial aspect to be considered in formulating healthy and metabolically efficient diets for cultured fish. In this way, diets may also become more economically produced and production will be cost more effectively.

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