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APPLICATION OF SAGO (*METROXYLON SAGU*) STARCH IN THE DIET OF NILE TILAPIA, OREOCHROMIS NILOTICUS (LINNAEUS, 1758) JUVENILES ON NUTRIENT DIGESTIBILITY AND DIGESTIVE ENZYMES

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Abstract: Omnivorous fish utilize dietary carbohydrates better due to the efficiency of nutrient digestibility and enzymes in the digestive tracts. However, the effectiveness of nutrient digestibility and digestive enzymes can be affected by different levels of dietary carbohydrates in the diet. Very limited information is known about the effect on nutrient digestibility and digestive enzymes by different levels of sago starch utilization. A 12 week feeding trial was conducted to identify the effects of sago (*Metroxylon sagu*) starch in the diet of Nile tilapia, *Oreochromis niloticus* juveniles on nutrient digestibility and digestive enzyme activities. Six isoenergetic (20.25±1.35 kJ/g) semi-purified experimental diets were formulated which consisted of dietary protein levels (P22%; P26%; P30%) incorporated with C38% and C44% of sago starch as the carbohydrates source. All the diets were designated as D1 (P:22%, C:38%); D2 (P:26%, C:38%); D3 (P:30%, C:38%); D4 (P:22%, C:44%); D5 (P:26%, C:44%) and D6 (P:30%, C:44%), respectively. A control diet used was formulated from corn starch and labelled as D0 (P30%: C40%). The result showed fish fed on treatment D3 (80.12%) had higher nutrients digestibility followed by D2 (77.54%), D1 (74.72%), D0 (69.83%), D6 (65.67%), D5 (57.40%) and D4 (50.29%). Digestive enzymes (amylase, lipase and protease) activities were significantly affected among all diets. Fish fed on diet, D3 showed high amylase (6.54 Umg^{-1}), lipase (5.68 Umg^{-1}) and protease (0.77 Umg^{-1}) activities compared to fish from other diet treatments. Two-way ANOVA result confirmed that the incorporation of different levels of protein and carbohydrate had significantly influenced nutrient digestibility and digestive enzyme activities of *O. niloticus* juveniles. Overall, fish fed on C38% sago starch-based diets showed positive result and performed better than those fed with C44% diets. The study revealed the ability of *O. niloticus* juveniles to spare protein with sago starch was at optimum level of C38% combined with P26% and P30% protein level.

Keywords: Nile tilapia, sago starch, nutrient digestibility, digestive enzymes, juveniles.

Introduction

Dietary carbohydrates are considered as less expensive energy source components in aquatic animal feeds and are efficiently utilized by omnivorous and/or herbivorous warm water fish (Kong *et al*., 2019). In practical terms for fish, carbohydrates can be broadly categorized as starch and non-starch polysaccharides (NSP). Starch which includes dextrin and glucose are relatively well digested and utilized by fish compared to the non-starch polysaccharides (Song *et al*., 2018). Starch is the predominant carbohydrate in plant such as wheat, corn, field peas, grains and legumes, and has been incorporated into diets for fish (Zhang *et al*., 2016). Although carbohydrate is categorised as non-essential nutrients for fish growth, it is commonly incorporated in aquafeeds, providing an energy source for fish (Wang *et al*., 2016). The application of carbohydrate in fish diets has a great benefit in aquaculture sustainability because it contributes to low-cost feed formulation and increases protein retention, reduces ammonia excretion, and improves stability and floatability of pellets in extruded diets (Corrêia *et al*., 2019).

As the non-protein energy source, carbohydrates also play an important role in maximizing the efficiency of protein digestibility by the protein sparing effect (Chen *et al*., 2020). Kamalam *et al*. (2017) stated that the ability of fish to utilize dietary carbohydrates differs depending on the feeding habits, type of dietary carbohydrate, physical state and complexity of carbohydrates that are consumed. The adaptive responses of an aquatic animal to their natural feeding habits usually determine their capability to utilize feeds from carbohydrate sources. In fact, the optimal level of carbohydrate applied in aquaculture feed production is important aspect in order to formulate a healthy metabolically efficient diets for cultured fish (Feng *et al*., 2019). Generally, fish species (herbivorous/ omnivorous) can utilize high carbohydrate, varied from 350 to 400 g/kg, and their excellent digestive enzymes activity exhibition is the main reason for this high capability of carbohydrate utilization (Azaza *et al*., 2015).

High cost of feed production is one of the main issues faced by the aquaculture industry. This is due to dietary protein source which is considered as the main and most expensive ingredients in fish diet (Boonanuntanasarn *et al*., 2018). In this study, the alternative approach to spare the dietary protein with sago starch was conducted. Sago starch is extracted from sago palm, *Metroxylon sagu* and regarded as important nutritive plant carbohydrate which is also a potential candidate for protein sparing in aquaculture feed production (Othman *et al*., 2015). Misman *et al*. (2015) stated that the carbohydrate content of sago starch is approximately 89.79% with proximate composition comprises the moisture content (10.6 - 20.0%), ash (0.06 - 0.43%), crude fat (0.10 - 0.13%), fiber (0.26 - 0.32%) and crude protein (0.19 - 0.25%). In Malaysia, the sago production is mainly situated in Mukah, Sarawak, which is well-known as a popular foodstuff especially among Melanau ethnic group in the area (Jong, 2018). The total yield of sago production in Malaysia is estimated around 42,000 to 50,000 metric tonnes per month procuring incomes between US$3.4 million to US$10.8 million per year (DOA, 2020).

Nile tilapia (*Oreochromis niloticus*) is recognized as the second most cultured fish in the world after carp. It is a warm-water omnivorous fish that can use up to 40% of digestible carbohydrate from their diet (Wang *et al*., 2017). It was reported that the application of sago starch as protein sparing in formulated fish feed at optimal level (38% - 40%) showed a positive result which promoted better growth performance and feed utilization in *O. niloticus* juveniles (Senawi *et al*., 2020). Meanwhile, the utilization of sago starch by *O. niloticus* juveniles at 38% level showed no sign of hyperglycaemia and this may be due to the regular absorption of glucose released by glycolysis which helps to maintain the glucose level in blood plasma (Ren *et al*., 2015). Singh and Muthukumarappan (2015) stated that the use of sago starch in aquaculture feed is practically excellent due to its good moisture content in feed mixture to produce high quality of floating pellets. However, the application of sago starch as a protein sparing source of energy still receives less attention in formulated feed for cultured fish (Kamarudin *et al*., 2016). Moreover, there is little information available on the nutrient digestibility and digestive enzymes response in fish that utilized on feeds formulated from sago starch (Jalil *et al*., 2015).

In the present study, our principal objective is to evaluate the application of sago starch as carbohydrate source to spare dietary protein in diets of *O. niloticus juveniles* on the nutrient digestibility and digestive enzyme activities.

Material and Methods

Experimental Fish and Husbandry Conditions

Juveniles *O. niloticus* (mean weight of approximately 4.60 g) were purchased from the PM Aquaculture Sdn. Bhd. Sarawak, Malaysia, and transported to the experimental facilities in aerated polyethylene bags. Fish were acclimated to laboratory conditions for two weeks in a 3000L capacity polyester tank fitted to a flow-

through system and fed with commercial multi fish feed (Dindings Soya & Multifeeds Sdn. Bhd., Kuala Lumpur, Malaysia), containing 320 g/kg of crude protein. After this duration, fish were randomly introduced into 21 rectangular, experimental fish tanks (45 × 30 × 30 cm) of 40.5 L capacity in a closed system supplied with de-chlorinated tap water and aerated continuously (Fujimura & Okada, 2007). About 20 fish per tank were included as trial conditions in which each experimental diet being tested in triplicate. During the experiment, about one fourth of water was removed from the total volume daily. Accordingly, the excreta and unfed diet were flushed out and then, the tank was refilled with the same water volume. 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. Fish were reared at an approximately 12/12 h light/dark schedule under a natural photoperiod during the whole experiment durations. Fish were fed with experimental diets twice daily at 09:00 and 17:00 hour to apparent satiation for 12 weeks. At the end of the feeding trial after 12 weeks, fish were sampled which were randomly grouped within each treatment and used to determine nutrient digestibility and for digestive enzyme assays.

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 a carbohydrate source. Six isoenergetic (20.25 ± 1.35 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 (P:22%, C:38%); D2 (P:26%, C:38%); D3 (P:30%, C:38%); D4 (P:22%, C:44%); D5 (P:26%, C:44%) and D6 (P:30%, C:44%), respectively. Fish meal was used as the source of protein and lipid from fish oil were maintained at 90 g/kg in all the diets. The control diet (D0) contained fish meal (P:30%) and corn starch as the sole carbohydrate source (C:40%) respectively, as reported earlier for this species (Senawi *et al*., 2020). Cellulose was used as the complementary component to the whole compositions of the formulated diet and the variation in amounts may have no effect on the fish growth (Zhang *et al*., 2009). 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) and kept at 55°C. Dried feed pellets were kept separately in tight capped bottles, labelled and stored until used for the feeding trial in a freezer at -20°C. Table 1 showed the formulation and proximate analysis of the experimental diets.

Fish and Preparation of Crude Enzyme Extract

The modified method by Chisty (2005) was applied to extract the fish crude intestinal enzyme. Approximately, 12 fish were randomly collected from each replicate tank after four hours of feeding which consisted of three groups of fish with equal number. The fish were sacrificed, and the intestine was collected to extract the crude enzyme. The fish were dissected, their intestine removed, weighed and cleaned with the autoclave cold distilled water. At a ratio of 1:10 (w/v) (Wang, 2007), the rinsed intestine was cut into smaller pieces and mixed with cold phosphate buffer solution, PBS (pH 7.4). The mixtures were homogenized using homogenizer (Model Ultra Turrax T25, IKA-Werke GmbH & Co. KG, Germany) and collected into 2 ml clean autoclaved eppendorf tubes, then centrifuged (Model CF15RX, Hitachi, Japan) at 10,000 x g for 15 minutes at 4ºC. The supernatant formed which was also the extracted crude intestinal enzyme was collected into separate vials and kept at -70ºC prior for further analysis. Triplicate samples per tank were measured. During all the processes, ice blocks were used

for maintaining cold environment to prevent the enzymes from denaturation. Bradford (1976) method was applied to determine the extracted crude intestinal enzyme protein concentration in which Bovine Serum Albumin (BSA) was used as a standard.

Preparation of Protein Suspension Mixture (PSM)

Danish fishmeal with protein percentage of 74.66 % ± 0.09 as determined by the procedure of AOAC (1997) was the only protein source in the prepared diets. Briefly, the six prepared experimental diets including control diet were finely ground and sieved through a 250 µm meshed sieve. The protein suspension was prepared with an equivalent amount of each diet that contained 312.5 mg of crude protein and mixed thoroughly with 50 ml of distilled water to produce 6.25 mg crude protein ml^{-1} (Saterlee *et al*., 1979). Triplicate samples per diet were analysed. The mixture was incubated for 1 hour at room temperature with occasional stirring, then vortex for homogenous mixing. The mixture was centrifuged at 10,000 x g to obtain the supernatant and the protein concentration was measured by using Bradford (1976) with Bovine Serum Albumin (BSA) as a standard.

Nutrient Digestibility

The pH drop method (Lazo *et al*., 1998) by *in vitro* was used to determine the relative protein digestibility (RPD). The extracted crude enzymes of fish intestine and the protein suspension mixtures, PSM (6.25 mg/ml) were

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

Ingredients	Treatments						
	C40%	C38%			C44%		
	P30% D0	P22 % D1	P26% D2	P30% D3	P22% D4	P26% D5	P30% 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 bomb calorimeter, Parr 6400 bomb calorimeter

used to determine the RPD (Saterlee *et al*., 1979; Rahmah *et al*., 2016). Accordingly, the PSM (30 ml) was mixed with 3 ml of extracted intestinal crude enzyme into a 50 ml autoclaved test tube and kept at 37ºC water bath. The pH was adjusted to 8.0±0.05 by using 0.01 mol HCl or 0.01 mol NaOH. After that, the pH drop was measured and recorded at one-minute intervals for ten minutes. Triplicate samples per tank were tested. Casein was used as a standard. RPD was calculated as follows:

Relative Protein Digestibility (RPD)
= {(–ΔpH feedstuff) / (–ΔpH casein)} x 100

Digestive Enzymes

The digestive enzyme assays for amylase, protease, and lipase enzyme activities were determined using the same extracted crude enzyme samples as for the determination of relative protein digestibility.

Amylase Assay

Worthington (1993) method as described by Akter *et al*. (2015) was performed to determine the amylase activities of *O. niloticus* juveniles. Briefly, 500 µl of extracted intestinal enzyme was taken into a clean test tube, concurrently another test tube was filled with 500 µl ddH2O as blank. These were incubated at 25ºC for four minutes. About 500 µl of starch solution was then added to each tube and again incubated for three minutes at 25ºC. Next, 1 ml dinitro salicylic acid colour reagent was added to each tube and incubated in the water bath at boiling temperature for five minutes. All tubes were cooled at room temperature, and 10 ml ddH2O was added to each tube followed by thorough mixing. Absorbance was measured by using spectrophotometer (Model SP-880, Metertek Inc., Taiwan) at 540 nm. Triplicate biological samples per tank were assayed. Amylase activity in the extracted intestinal enzyme was determined using maltose as a standard. One unit of amylase activity was defined as the micromoles of maltose released per minute per milligram protein.

Protease Assay

The modified method using casein digestion by Walter (1984) as described by Chong *et al*. (2002) was applied to determine the specific protease activities of *O. niloticus* juveniles. For the enzymatic reaction, the mixture was prepared with 0.25 ml casein (1% w/v in ddH2O), 0.25 ml Tris-HCL buffer (0.1 M, pH 8.5), and 100 µl of extracted intestinal crude enzymes. The mixture was put into a water bath for one hour incubation at 37ºC. After one hour, 0.6 ml of 8% (w/v) trichloroacetic acid (TCL) was added to stop the reaction and incubated at normal freeze (4ºC) for one hour. After the incubation, sample was centrifuged at 1800 x g for ten minutes to collect the supernatant. It was measured at 280 nm wave lengths by using UV-Vis spectrophotometer (Agilent, Model Cary 60 UV, United State Ltd., USA). Blank sample was prepared followed by same procedure conducted earlier except the 100 µl extracted intestinal enzyme was added at the end of the incubation just after stopping the reaction with TCL. Triplicate samples per tanks were tested. The protease activity for *O. niloticus* juveniles was determined using L-Tyrosine as a standard. one unit of specific protease activity was defined as the amount of enzyme needed to release one micromole tyrosine per minute per milligram protein of the enzyme extract.

Lipase Assay

Bier (1955) method modified by Natalia *et al*. (2004) as described by Akter *et al*. (2015) was performed to determine the specific lipase enzyme. Accordingly, 1% solution of 1 ml polyvinyl alcohol (PVA) in ddH2O was used to make the emulsion. Approximately, 5 ml of 0.1 N HCl was added into the PVA solution and heated for one hour at 80ºC. The solution was cooled down at room temperature and filtered. The filtered solution was adjusted to pH 8.0 using 0.1N NaOH. Subsequently, 50 ml of this solution was taken and 500 µl of virgin olive oil was added as substrate and then homogenized for five minutes to make an emulsion that was used for determination of lipase. The mixture

was composed of 1 ml of PVA solution-emulsified substrate, 500 µl McIlvaine buffer (0.1 M citric acid + 0.2 M disodium phosphate, pH 8), and 500 µl extracted intestinal crude enzyme and then incubated with shaking for four hours at 37ºC. After incubation, 3 ml of 1:1 ethanol-acetone solution was added to stop the reaction as well as to disrupt the emulsion. This was titrated with 0.01 M NaOH; before titration, two to three drops of 1% phenolphthalein in ethanol were added. A blank was treated in the same way except the extracted enzymes were added after four hours of incubation. Triplicate samples per tanks were determined. Lipase activity was defined as the volume of 0.01 M NaOH required neutralizing fatty acid release during the four hours incubation period with the substrate and after correction by the appropriate blank.

Statistical Analysis

Data for nutrient digestibility and digestive enzyme assays were presented as the mean ± S.D and analysed using one-way and two-way analysis of variance (ANOVA) to compare the effects between dietary protein and carbohydrate. p values < 0.05 were considered significant when compared by Duncan's multiple range test. All statistical analyses were compared using the Statistical Package for the Social Sciences (SPSS) program for Windows (Version 25.0. Armonk, NY: IBM Corp.).

Results

Nutrient Digestibility

The results on nutrient digestibility of *O. niloticus* juveniles observed after 12 weeks were presented in Table 2. The two-way ANOVA result confirmed that the interaction between different levels of both protein and carbohydrate have significant ($p < 0.05$) effects on nutrients digestibility of *O. niloticus* juveniles. High significant difference ($p < 0.05$) of relative protein digestibility (RPD) was recorded in fish fed on diet, D3 (80.12%) with ratio P30%: C38% among all the diet treatments. Overall, the formulated diets have positive effect on the relative protein digestibility (RPD) of *O. niloticus* juveniles where fish fed with the 38% sago starch diets showed high statistic data compared to those from 44% group diets. This indicates that the formulated diets were well accepted and utilized by the fish at optimum level of 38% sago starch.

Digestive Enzymes

The digestive enzymes (amylase, lipase and protease) activities of *O. niloticus* juveniles were shown in Table 3. Amylase, lipase and protease activities were significantly ($p < 0.05$) affected by 38% and 44% of dietary sago starch among all the diet treatments. The amylase specific activity was found to be significantly high ($p < 0.05$) in fish fed with 38% sago starch diets (D3: 6.54 Umg^{-1}) than those from 44% group diets. Similar trend was observed for specific activities of lipase enzyme ranged between 3.02 Umg^{-1} to 5.68 Umg^{-1} while the protease enzyme was recorded approximately from 0.38 Umg^{-1} to 0.77 Umg^{-1}, respectively. The result based on two-way ANOVA revealed that different levels of protein and carbohydrate had significant ($p < 0.05$) influence on digestive enzymes activities of *O. niloticus* juveniles. Among all the enzyme activities, the amylase specific activity was found to be the highest compared to both lipase and protease enzyme activities.

Table 2: Nutrient digestibility of *O. niloticus* juveniles fed diets containing various levels of protein and carbohydrate for 12 weeks (n=12)

Nutrient digestibility	Treatments							One-Way ANOVA
	C40 %	C38 %			C44 %			
	P30 % D0	P22 % D1	P26 % D2	P30 % D3	P22 % D4	P26 % D5	P30 % D6	*p*-value
Relative protein digestibility (%)	69.83±2.52^{c}	74.72±1.71^{d}	77.54±3.56^{de}	80.12±2.69^{e}	50.29±2.38^{a}	57.40±1.47^{b}	65.67±3.63^{c}	<0.001

Two-Way ANOVA *p*-value

Interaction	Relative protein digestibility (%)
Protein	<0.000
Carbohydrate	<0.000
P x C	<0.020

Mean values in same row with different superscript letters are significantly different ($p < 0.05$). Result is mean ± SD of triplicate sample.

Table 3: Digestive enzymes (amylase, lipase and protease) activities of *O. niloticus* juveniles fed diets containing various levels of protein and carbohydrate for 12 weeks (n=12)

Digestive enzymes	Treatments							One-Way ANOVA
	C40 %	C38 %			C44 %			
	P30 % D0	P22 % D1	P26 % D2	P30 % D3	P22 % D4	P26 % D5	P30 % D6	*p*-value
Amylase (Umg^{-1})	5.31±0.28^{c}	6.09±0.37^{d}	6.23±0.24^{de}	6.54±0.43^{e}	4.11±0.19^{a}	4.32±0.21^{b}	5.16±0.32^{c}	<0.000
Lipase (Umg^{-1})	4.25±0.71^{c}	5.33±0.80^{d}	5.46±0.66^{de}	5.68±0.75^{e}	3.02±0.52^{a}	3.16±0.63^{b}	4.10±0.55^{c}	<0.001
Protease (Umg^{-1})	0.58±0.12^{c}	0.61±0.09^{d}	0.69±0.11^{de}	0.77±0.16^{e}	0.38±0.07^{a}	0.43±0.10^{ab}	0.55±0.14^{c}	<0.001

Two-Way ANOVA *p*-value

Interaction	Amylase (Umg^{-1})	Lipase (Umg^{-1})	Protease (Umg^{-1})
Protein	<0.023	<0.009	<0.014
Carbohydrate	<0.000	<0.000	<0.002
P x C	<0.016	<0.004	<0.011

Mean values in same row with different superscript letters are significantly different ($p < 0.05$). Result is ~~are~~ mean ± SD of triplicate sample.

Discussion

The application of *in vitro* protein digestibility is one of the recommended approaches in order to determine the quality of potential feedstuffs which are to be included in the fish diets (Chauranda & Soriab, 2019). The present study revealed high protein digestibility of *O. niloticus* juveniles fed on 38% compared to 44% sago starch group, especially for the diets with 26% and 30% protein. This pattern was comparable with the previous study conducted by Sandrea *et al*. (2017). The incorporation of protein with proper dietary carbohydrate (starch) levels approximately between 35% to 40% in the diet has been reported to show a great impact in the digestibility of fish for better growth rate (Wang *et al*., 2016). This statement proved the current study that 38% sago starch level is at optimal range for the digestibility of *O. niloticus* juveniles which contribute to the better growth performance and feed utilization. However, the result of fish fed on 44% sago starch level was in contrast with those from 38% group diets. Excess dietary starch in diets indeed increased glycogen and lipid deposition (Polakof *et al*., 2012), which is also proven by the low protein digestibility and digestive enzyme activities in the present study. The possible reason might be due to high dietary starch in diets which increased viscosity in intestine which hinder the digestive enzyme activity in the digestive tracts of fish (Haidar *et al*., 2016). Furthermore, the variability of protein digestibility may also be related to the formulation pelleting conditions and differences in feed intake by the fish (Saravanan *et al*., 2013).

Protein digestibility data reflect the percentage of a feed sample which was absorbed from the intestinal tract of an animal (Sales, 2010; Deng *et al*., 2016). Digestibility of protein and carbohydrate in formulated diets was well described by Hoseinifar *et al*. (2017) who stated that appropriate level of both feedstuffs has positive effect on the feed utilization of fish which promotes better growth performance. To take into account, balanced levels of protein incorporated with carbohydrate also had a great influence on digestive enzymes efficiency in the intestinal tracts. This may be due to the positive response on the adaptation of intestinal microbial colonization in the digestive tracts to produce more enzymes to assist in the utilization of feed diets (Maas *et al*., 2019). Fawole *et al*. (2018) mentioned that feed utilization efficiency and growth in fish might also depend on the area of absorption of intestines in which wide area absorbs more nutrients from the diets. Further, one must consider the environmental condition factor as it can also influence the nutrient digestibility and alter intestinal morphology of fish (Tran-Ngoc *et al*., 2018). Additionally, the nutrient digestibility might depend on variability of energy and protein in the diets. Tran-Ngoc *et al*. (2019) reported that changes in the dietary macronutrient (protein) composition and nutrient type (starch) showed an effect on energy and protein utilization in fish.

Zhou *et al*. (2016) stated that amylase activity in both herbivores and omnivores are much greater than carnivores and appropriate dietary carbohydrate (starch) levels can improve amylase activity in digestive enzyme. Nile tilapia, *O. niloticus* is an omnivorous fish that can utilize up to 40% starch level in its diet for growth (Azaza *et al*., 2015). These statements support the current study in which *O. niloticus* juveniles fed on 38% sago starch had higher amylase activity in intestines if compared to those from the 44% group diet treatments. Li *et al*. (2014) reported that amylase activity could be stimulated by dietary carbohydrate levels. However, it was not found to increase further but decreased when the dietary carbohydrate level was higher than 44.31%. This indicates that excess carbohydrate level could hinder amylase activity and it is in agreement with the present study. Amylase enzyme utilizes dietary carbohydrates (starch) into glucose and stores as glycogen through glycogenesis which is catalysed by glycogen synthase (NRC, 2011). Xia *et al*. (2015) reported that high starch level in diet may cause insufficient amylase enzyme production in the intestine to breakdown the excess carbohydrate molecules during glycolysis which can lead to low amylase activity in

the digestive tracts. This may result in the accumulation of glycogen and form excess lipid which affect growth improvement in fish (Ren *et al*., 2011). Withal, some researchers thought that the amylase activity is not only affected by different dietary carbohydrate level in diets but also determined by genome and development of the fish species (Li *et al*., 2014).

In this study, *O. niloticus* juveniles fed with 38% starch level appeared to have a positive response on protease enzyme activity in the intestine than the 44% group diets. This is in agreement with Cai *et al*. (2018) who stated that suitable starch level can improve feed utilization and promote protease enzyme secretions in the digestive tracts. Boonanuntanasarn *et al*. (2018) reported that fish fed diets containing 21.8% - 30.4% protein and 16.4% - 39.2% starch are ideal for constant rate of protease enzyme response in the intestine to digest the diets and the statement corresponds with the formulated diets incorporation between protein and carbohydrates in our study. High dietary starch level indeed will reduce the efficiency of protein in formulated diets which resulted in poor activity of protease enzyme to breakdown the amino acid molecules during the diets digestion that is fundamental for growth performance (Wang *et al*., 2017). Evidence has shown that excess starch in diets may increase acidic fluid within the digestive tracts that lead to negative effect on protease enzyme activity (Gominho-Rosa *et al*., 2015).

Similar trends are observed on lipase enzyme activity in the intestine of *O. niloticus* juveniles and our current finding is in agreement with Lu *et al*. (2018) who stated that different levels of starch in diets have a significance influence on digestive lipase enzyme. At average starch level, lipase enzyme helps in the breakdown of the lipid formed from the digestible starch which further converted into energy that may be essential for routine metabolism in fish (Long *et al*., 2015). Excessive absorption of dietary carbohydrate (starch) in diet could result in body and liver lipid depositions due to low capability of digestive lipase enzyme (Wu *et al*., 2007; Qiang *et al*., 2014). This indicates that more of the absorbed starch was used to synthesize lipid and not glycogen which is required for body metabolism energy. As reported in a previous study (Peres & Oliva-Teles, 2002), liver lipid contents increase with the increase in available carbohydrate. Gao *et al*. (2011) reported that lipid accumulation in the liver has potential for serious health issues for cultured fish with the possibility of decreased resistance to disease and might lead to higher levels of oxidative stress.

Furthermore, the intestinal digestive tract enzyme profile is an indicator which corresponds with nutrient digestibility and utilization. The digestibility in fish might depend on the presence of appropriate enzymes in proper locations in the wall and along the lumen of the intestinal tract (Deng *et al*., 2010; Wang *et al*., 2014). Zhang *et al*. (2010) described that digestive enzyme activities were dependable on some factors such as food gene and nutritional habits. Apart from that, the fish feeding strategy and enzymatic ability to digest various kinds of food must also be considered, respectively (Gao *et al*., 2011). Generally, the fish digestive structure reflects their consumption habit which were obtained from their wild natural source. Besides, the homogenized distribution of enzyme in the fish intestine is frequently considered as their adaptation to the diversity of diet compositions from the natural habitat. Therefore, it is more sensible to be concerned about as it is a consequence of the fish history and their position in the evolution course.

Indeed, dietary carbohydrates are the most economical source of energy for protein sparing in the aquaculture feed production (Zhou *et al*., 2016; Tran-Ngoc *et al*., 2019). Although no real carbohydrate requirements have been demonstrated in fish, an adequate level of carbohydrates in the diet is necessary to reduce the catabolism of protein and lipids for energy and to provide metabolic intermediates for the synthesis of other biologically important compounds, such as ribose-5-phosphate for nucleotides and nucleic acid synthesis (Feng *et al*., 2019). Therefore, if the dietary carbohydrate

is well utilized by fish, it would be more economic because of its cheaper cost and better availability (Cheng *et al*., 2017). Likewise, replacing the dietary protein by carbohydrate not only reduces production cost but also important for nitrogen effluent from the culture system (Koch *et al*., 2016). Hence, the provision of appropriate amount of dietary carbohydrates as sources of energy in fish formulated diets is important to spar the use of lipids and protein in the aquaculture feeds production (Haridas *et al*., 2017).

The protein sparing ability of fish on dietary carbohydrate is correlated with the efficiency of digestive enzymes and nutrient digestibility. Generally, fish utilize less carbohydrate source than terrestrial animal, however in terms of feeding habits, herbivorous and omnivorous fish can utilize dietary carbohydrate better than carnivorous fish species (Hamid *et al*., 2011; Marandel *et al*., 2015). The main reason may be due to high digestive enzymes in the digestive tracts of herbivorous and omnivorous fish species and also high affinity of insulin receptors compared to the carnivorous fish (Liu *et al*., 2019). Therewithal, the capability of fish to utilize carbohydrate for nutrient digestibility varies among fish species and this might be due to the attributes of their natural habitat, digestive tract characteristic and rate of metabolic activities in digestive tracts and livers (Li *et al*., 2019). Besides, the complexity and utilization of the carbohydrate in fish depends on the source and the practical ingredients such as corn or a mixture of corn where wheat is common carbohydrate source used in feed formulations (Wang *et al*., 2017). *O. niloticus* is able to digest 57.9 g/kg) of carbohydrates in corn and 71.7 g/kg) in wheat and teleost is generally considered to be glucose-intolerant capable of protein sparing ability (Azaza *et al*., 2015).

In addition, the fish growth is directly affected by incorporation level of carbohydrate in the formulated feed. Further discussion on this section was based on our previous finding which is prequel to the current study (Senawi *et al*., 2020). The earlier study reported that the optimum sago starch level required for maximum growth performance of *O. niloticus* juveniles was at C38% than those fish fed on the C44% diet treatments. The data recorded that the diet treatment, D3 (P:30%, C:38%) had higher final body weight, ranging from 32.32 g to 23.29 g, respectively. Apart from that, our finding also discovered that the decrease in dietary protein level from P30% to P26% incorporated with 38% sago starch was observed and had not influenced much the final weight of fish. This indicates that at this optimal range, *O. niloticus* juveniles could spare some protein when the dietary protein level is low. Meanwhile, fish fed on 44% sago starch level showed statistically high body indices in which hepatosomatic index, HSI (2.04 - 2.53%), viscerosomatic index, VSI (5.60 - 6.41%) and intraperitoneal fat, IPF (1.14 - 1.89%), respectively. This might be due to high level of dietary starch utilization which resulted in the accumulation of fat on those particular part of the body indices. Overall, the fish survival rate recorded at 100% throughout the feeding trial indicates that the experimental diets were well accepted by *O. niloticus* juveniles.

Conclusion

In conclusion, our results indicated that nutrient digestibility and digestive enzymes were positively influenced by 38% sago starch formulated in diets especially with higher protein levels. This suggests that *O. niloticus* juveniles can effectively digest and utilize the 38% sago starch formulated level compared to those fed with 44% group diets. The present study confirmed that different levels of dietary starch formulated in fish diets may have a significant effect on digestive ability and enzymes for body metabolism.

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