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DIFFERENT STARCH SOURCES IN EXTRUDED DIETS FOR THE MALAYSIAN MAHSEER (*Tortambroides*): EFFECTS ON GROWTH, FEED UTILISATION AND TISSUE HISTOLOGY

SAIRATUL DAHLIANIS ISHAK[1,3]*, YUS ANIZA YUSOF[2], AMBOK BOLONG ABOL-MUNAFI[1] AND MOHD SALLEH KAMARUDIN[3]

[1]Institute of Tropical Aquaculture and Fisheries, Universiti Malaysia Terengganu, 21030 Kuala Nerus, Terengganu, Malaysia. [2]Department of Process and Food Engineering, Faculty of Engineering, Universiti Putra Malaysia, 43400 Serdang, Selangor, Malaysia. [3]Department of Aquaculture, Faculty of Agriculture, Universiti Putra Malaysia, 43400 Serdang, Selangor, Malaysia.

**Corresponding author: sairatul.ishak@umt.edu.my*
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Abstract: Sago (*Metroxylon sagu*), cassava (*Manihot esculenta*) and taro (*Colocasia esculenta*) are potential sources of carbohydrate in lieu of corn starch because of they can easily be grown as crops in Malaysia. We investigated the effects of these starch sources on the growth, feed utilisation efficiency, body composition, and liver and intestine histology in Malaysian mahseer (*Tor tambroides*) (Bleeker, 1854) fingerlings for 10 weeks. Results showed that different starch sources had significant effects ($P<0.05$) on growth, feed utilisation efficiency and biometric indices. Moisture, lipid and gross energy composition of the fish carcass were also significantly different ($P<0.05$). Generally, fish fed with corn and taro starch produced better weight gain, length gain, specific growth rate and lower feed conversion rate values. However, fish fed with corn starch showed high conversion of carbohydrate to lipid compared with taro starch. In tissue histology, *T. tambroides* liver hepatocytes and intestine mucosal epithelial cells were susceptible to lipid vacuolisation in different degrees when starch was included in the diet. In conclusion, our study suggests that *T. tambroides* had limited capability in utilising starch from different sources. However, taro starch might be the best candidate for full/partial replacement of corn starch in *T. tambroides* extruded feed.

Keywords: Fish feed, carbohydrate, starch, *Tor tambroides*.

Abbreviations: weight gain (WG), length gain (LG), feed intake (FI), specific growth rate (SGR), feeding conversion ratio (FCR), protein efficiency ratio (PER), hepato-somatic index (HSI), viscero-somatic index (VSI), intra-peritoneal fat ratio (IPF)

Introduction

Starch inclusion assists the extrusion process for better particle binding and expansion (Rosentrater *et al.*, 2009; Hilton *et al.*, 1981). It is also reported that carbohydrate in starch form for dietary inclusion is better than other raw forms of pulses, oilseed cakes, leaf extracts and plant meals, which have high anti-nutrient compounds and non-starch polysaccharides that must first be broken down (Alonso *et al.*, 2000; Ghavidel & Prakash, 2007; Rathod & Annapure, 2016; Gatlin *et al.*, 2007). In addition, starch gelatinisation will also improve pellet durability, increase expansion ratio and buoyancy, reduce fines, sterilises feed from microbes and toxic compounds, and improve digestibility (Bureau *et al.*, 2002; De Silva & Anderson, 1994; Kannadhason *et al.*, 2011; Sørensen, 2012). Furthermore, an inclusion of digestible starch as exogenous glucose source will spare the use of essential amino acids in gluconeogenesis, thus promoting the protein-sparing effect (NRC, 2011; De Silva & Anderson, 1994; Kamalam *et al.*, 2017).

Fish species of different feeding habits have various anatomical and functional differences, where omnivorous and herbivorous fish can efficiently utilise dietary starch, unlike carnivorous fish (De Silva & Anderson, 1995; Enes *et al.*, 2011; Hardy, 2002; Krogdahl *et al.*,

2005; Wilson, 1994). Specific α-amylase activity has been shown to be influenced by feeding habits and its presence in the digestive tract of carnivorous fishes enables them to digest dietary starch components by weaning (Al-Tameemi *et al.*, 2010; Drewe *et al.*, 2004). Inclusion of starch in diet also directly affects faeces quality, where it will increase the particle size of faeces, therefore allowing aquaculture waste to be easily removed (Keramat-Amirkolaie, 2013). The digestibility of starch in fish varies among species and it is reported that omnivorous fish can derive a high amount of energy from modified starch (Hardy & Barrows, 2003). Various studies on cultured fish have shown that it improves digestibility, which leads to better results in growth (Sørensen *et al*., 2002; Cho *et al*., 2006; Adamidou *et al*., 2009). Work has been done in affirming the feeding behaviour of the *Tor* species of omnivorous fish (Tan, 1980; Islam & Tanaka, 2007). In *T. tambroides*, the optimal dietary carbohydrate requirement is reported to be 23.44 % of corn starch inclusion (Ishak *et al*., 2016).

In light of increasing global aqua feed cost, this study will explore the usage of domestic plant crops in fish feed in support of the local industry and lessen the dependency on imported ingredients. The aqua feed industry has been actively involved in exploring the use of plant-based ingredients to replace expensive animal-based components, such as fishmeal and fish oil, in order to maximise its carbohydrate contents as an energy source to spare protein, reduce nutrient-loading waste into the environment, and also reduce feed production cost (Gatlin *et al.*, 2007; Trushenski *et al.*, 2006). Starch-rich agricultural products or by-products (barley, corn, canola, oat, pea, soybean, sunflower and wheat) are excellent potential carbohydrate sources for aqua feeds they are cheap and available in abundance (Arnesen & Krogdahl, 1993; FAO, 2017). As such, three locally available starch sources in Malaysia — cassava (*Manihot esculenta*), sago (*Metroxylon sagu*), and taro (*Colocasia esculenta*) — may be potential substitutes for corn starch in the production of extruded-floating and slow-sinking aqua feeds based on their agricultural feasibility in Malaysia (Abd-Aziz, 2002; DOA, 2012; FAO, 2017; Ferraro *et al.*, 2016). Several studies have been reported on the use of these starch sources in producing aqua feeds.

Cassava has been incorporated in the feed for *Cyprinus carpio* (Ufodike & Matty, 1983), *Oreochromis niloticus* (Cavalheiro *et al.*, 2007; Gabriel *et al.*, 2007; Kannadhason *et al.*, 2009), *Pangasius* sp. (Hung *et al.*, 2003), *Salmo salar* (Ah-Hen *et al.*, 2014), *Lates calcarifer* (Glencross *et al.*, 2012) and *T. tambroides* (Ismail *et al.*, 2012; Umar *et al.*, 2013). Sago starch has been successfully used as a carrageenan replacement in the feed for penaeid shrimps (Pascual & Sumalangcay, 1981; Murai *et al.*, 1981; Piedad-Pascual *et al.*, 1978). It has also been incorporated as an ingredient in the feed for *Mystus nemurus* (Hamid *et al.*, 2011), tilapia (Kamarudin *et al.*, 2016) and *T. tambroides* (Umar *et al.*, 2013). Meanwhile, taro is easily cultivated as a staple in the Asia-Pacific region. It is, however, uncommon to find commercial-scale industrial starch processing from taro (Onwueme, 1999; Deo *et al.*, 2009). Nonetheless, usage of lab prepared taro starch has been shown to have good potential in the preparation of *T. tambroides* feed (De Cruz *et al.*, 2015).

This study assessed the nutritional value of selected starch sources incorporated in the diet of *T. tambroides*. For this purpose, their effects on growth, feed utilisation efficiency, body composition, nutrient retention, and liver and intestinal histomorphology are evaluated. It is our hope that the results of this study will provide new opportunities for commercial production of local starch sources in aqua feed, and also to produce aqua feed tailored to the dietary requirements of the mahseer.

Materials and methods

Experimental diets

The nutrient composition of four starch sources (corn, cassava, sago, taro) was predetermined and shown in Table 1. Commercially available

food-grade corn starch, cassava starch and sago starch were purchased from a local supplier (Yummie Bakery Sdn Bhd, Malaysia). Fresh taro corms were purchased from a local market in Selangor, Malaysia, and its starch was prepared following the method described by De Cruz *et al*. (2015).

Experimental diets used for this feeding trial were formulated based on the dietary requirements of the Malaysian mahseer at 40.00 % protein and 23.44 % carbohydrate according to Ishak *et al.* (2016). The dietary lipid (per cent as fed basis) was kept below 11 % as the growth of *T. tambroides* had been reportedly affected above that level (Ng & Andin, 2011; Ishak *et al.,* 2016). The feed and nutrient composition of the experimental diets are shown in Table 2.

Feeding Trial and Sampling

Wild *T. tambroides* fingerlings were obtained in Kelantan, Malaysia, and acclimatised for three weeks in a one-tonne fibreglass tank at the Wet Laboratory in the Department of Aquaculture, Universiti Putra Malaysia. During this period, the fingerlings were graded based on size. Graded fish were then fed *ad libitum* a 32 % crude protein commercial tilapia starter feed (StarFeed (M) Sdn Bhd, Malaysia) and were observed to readily accept the commercial diet. Upon acclimatisation, 12 sets of 60L glass aquaria (38 cm×75 cm×35 cm) were randomly stocked with 20 fish each (initial body weight 1.27±0.01 g; initial total length 5.20±0.02 cm). Each aquarium was supplied with continuous aeration and up to 25 % water change was done weekly using dechlorinated municipal water. Water temperature, pH and dissolved oxygen were maintained at 26.0±1.0ºC, 6.5±0.2 and 5.0±0.5 mg l^{-1}, respectively. Fish were fed experimental diets in triplicates daily at 4 % body weight twice a day (8am and 5pm) for 10 weeks. Body weight and total length were recorded bi-weekly throughout the feeding trial, whereas mortality was recorded daily when applicable. The growth parameters used are weight gain (WG), length gain (LG), and specific growth rate (SGR) were calculated using Equations 1, 2 and 3:

$$WG(\%) = 100 \times (\text{final body weight-initial body weight})/\text{initial body weight} \quad (1)$$

$$LG(\%) = 100 \times (\text{final body length -initial body length})/\text{initial body length} \quad (2)$$

$$SGR(\%) = 100 \times (\ln \text{final body weight} - \ln \text{initial body weight})/\text{days of feeding} \quad (3)$$

The amount of feed throughout the trial was recorded to determine the feeding efficiencies which are feed intake (FI), feeding conversion ratio (FCR) and protein efficiency ratio (PER), using Equations 4, 5 and 6 as below.

$$FI = \text{weight of feed given until treatment ended/total of fish per treatment} \quad (4)$$

$$FCR = \text{weight of feed consumed/body weight gain} \quad (5)$$

Table 1: Proximate composition (% as fed basis) of the four starch sources

	Starch source			
	Corn	**Cassava**	**Sago**	**Taro**
Proximate composition				
Moisture	11.50	15.40	14.80	8.00
Protein	0	0	0	5.80
Lipid	0	0	0	0.79
Carbohydrate	88.50	84.60	85.20	85.41
Gross energy (kJ g^{-1})	14.84	14.59	14.44	15.45

Table 2: Feed ingredient and proximate composition (% as fed basis) of the four experimental diets

	Starch Source			
	Corn	**Cassava**	**Sago**	**Taro**
Feed Ingredient				
Fishmeal[a]	69.00	69.00	69.00	69.00
Vegetable oil[b]	5.56	5.56	5.56	5.56
Vitamin premix[c]	1.00	1.00	1.00	1.00
Mineral premix[d]	1.00	1.00	1.00	1.00
Corn starch	23.44	0	0	0
Cassava starch	0	23.44	0	0
Sago starch	0	0	23.44	0
Taro starch	0	0	0	23.44
Proximate composition				
Moisture	8.48	6.96	7.09	6.42
Protein	40.48	39.89	39.28	41.27
Lipid	8.18	7.31	8.67	7.46
Ash	19.60	17.25	18.63	18.20
Fibre	0.92	0.48	0.87	1.33
Carbohydrate[e]	23.26	28.59	26.33	26.65
Gross energy (kJg^{-1})	17.32	17.91	17.50	17.91

[a] Fishmeal with 59% crude protein.

[b] Mixture of canola and sunflower oil (Naturel™).

[c] Vitamin premix: (g kg $premix^{-1}$); ascorbic acid, 45; myoinositol, 5; choline chloride, 75; niacin, 4.5; riboflavin, 1; pyridoxine, 1; thiaminmononitrate, 0.92; Ca-pantothenate, 3; retinyl acetate, 0.6; cholecalciferol, 0.083; vitamin K menadione, 1.67; α-tocopheryl acetate (500 IU g^{-1}), 8; biotin, 0.02; folic acid, 0.09; vitamin B12, 0.001; cellulose, 845.11.

[d] Mineral premix (g kg $premix^{-1}$): KCL, 90; KI, 0.04; $CaHPO_4.2H_2O$, 500; NaCl, 40; $CuSO_4.5H_2O$, 3; $ZnSO_4.7H_2O$, 4; $CoSO_4$, 0.02; $FeSO_4$ $7H_2O$, 20; $MnSO_4.H_2O$, 3; $CaCo_3$, 215; MgOH, 124; Na_2SeO_3, 0.03; NaF, 1.

[e] Crude carbohydrate=100-(moisture+crude protein+crude lipid+ash)

$$PER = \text{body weight gain/protein intake} \quad (6)$$

At the end of the feeding trial, 10 fishes from each tank were randomly selected and sacrificed following anesthetisation with NIKA-Transmore® fish stabilizer. Internal organs were removed and weighed, while intraperitoneal fat was obtained by collecting all the fat from the abdominal cavity, as well as those separated from the viscera. Hepatosomatic index (HSI), viscerosomatic index (VSI) and intraperitoneal fat ratio (IPF) were calculated using Equations 7, 8 and 9:

$$HSI(\%) = 100 \times \text{liver weight/total body weight}) \quad (7)$$

$$VSI(\%) = 100 \times (\text{viscera weight/total body weight}) \quad (8)$$

$$IPF(\%) = 100 \times (\text{collected fat weight/total body weight}) \quad (9)$$

Subsequently, extracted livers and intestines (n=9 each treatment, for each respective tissue) were fixed for histological study. The remaining fish bodies without internal organs (n=10 each replicate), which from now on would be referred

to as fish carcass, was freeze-dried for proximate analysis.

Proximate Analysis

All samples (ingredients, diets, fish carcass for initial analysis and fish carcass from treatments) were analysed for protein, lipid, fibre, ash, moisture and gross energy content following their respective methods (AOAC, 1995). Protein content was estimated using the 2400 Kjeltec Analyzer Unit (FOSS, Denmark) following digestion in sulphuric acid at 380 °C for 60 min. Lipid content was analysed using the Foss Tecator Lipid Analyzer (FOSS, Denmark). Defatted samples were then analysed using the Fibertec Cold Extractor unit, followed by acid and alkaline digestion using the Fibertec Hot Extractor Foss Tecator (FOSS, Denmark) to determine crude fibre content. Moisture content was determined by the AD-4715 infrared moisture determination balance (A&D Company, Japan). Ash content was estimated according to the protocols stated by AOAC (1995). Finally, gross energy was determined using an AC-350 bomb calorimeter (LECO, USA). From these data, the following nutrient retention values were calculated according to Equations 9, 10, 11 and 12.

Protein retention value (%) = 100 × [(final body weight×final fish carcass protein)-(initial body weight×initial fish carcass protein)]/ (total dry protein intake) (9)

Lipid retention value (%) = 100 × [(final body weight×final fish carcass lipid)-(initial body weight×initial fish carcass lipid)]/(total dry lipid intake) (10)

Carbohydrate retention value(%) = 100 × [(final body weight×final fish carcass carbohydrate)-(initial body weight × initial fish carcass carbohydrate)]/(total dry carbohydrate intake) (11)

Energy retention value(%) = 100 × [(final body weight×final fish carcass energy) – (initial body weight×initial fish carcass energy)]/(total dry energy intake) (12)

Histology

Procedures for haematoxylin and eosin staining were carried out according to Luna (1968). Excess fat was trimmed from the liver and intestines before the samples were immediately fixed in Bouin's solution for 24 h. Subsequently, fixed samples were stored in 70 % ethanol. Tissues were next dehydrated in a series of graded ethanol as in standard histological protocol and embedded in paraffin wax. Embedded tissues were sectioned at a thickness of 5 µm before being stained with haematoxylin and eosin, and mounted in DPX. Slides were examined under a light microscope (Carl Zeiss GmbH, Germany).

Statistical Analysis

All data values were reported as mean ± standard error (n=3) and were subjected to one-way analysis of variance (one-way ANOVA). Differences between means were tested using Duncan's New Multiple Range Test at $P<0.05$. Percentage data were arcsine transformed before statistical analysis.

Results

Dietary starch source had significant effects ($P<0.05$) on growth performance (WG, LG, SGR), feed efficiency (FCR, PER), HSI and IPF of mahseer fingerlings (Table 3). Fish fed corn starch and taro starch recorded significantly higher ($P<0.05$) WG and also had significantly lower ($P<0.05$) FCR compared to those fed with other starches. Fish fed with corn starch had a higher LG ($P<0.05$) than fish fed with sago starch. The FI for fish fed with cassava starch was the lowest ($P<0.05$) among treatments. Subsequently, PER for fish fed with corn starch was the highest, while fish fed cassava starch had the lowest PER. No significant differences ($P>0.05$) were found in survival among treatments. HSI and IPF were significantly affected ($P<0.05$) by dietary starch sources. Fish fed with corn starch had the highest IPF and HSI. No significant differences ($P>0.05$) were observed for VSI in all treatments.

It was also observed that body moisture, lipid and gross energy content of the fish were significantly affected ($P<0.05$) by dietary starch sources (Table 4). Fish fed with corn starch had the lowest body moisture and highest lipid content. In contrast, fish fed with sago starch had the highest gross energy content, although it was not significantly different ($P>0.05$) than that of fish fed with corn starch. Consequently, there was no significant effect ($P>0.05$) of starch source observed for fish body protein, ash, fibre and carbohydrate. Results also showed that starch source significantly affected ($P<0.05$) all nutrient retention values (Table 5). Fish fed with corn starch had the highest ($P<0.05$) protein, lipid and energy retention. In general, the carbohydrate retention was low in all fish groups. However, fish fed with corn and sago starch had significantly higher ($P<0.05$) carbohydrate retention compared to other treatments. Fish fed with cassava starch achieved the lowest values in the retention of all nutrients.

Histological examination of *T. tambroides* liver hepatocytes found a condition of steatosis — accumulation of lipid vacuoles in liver — in all diet treatments (Figure 1). Minor steatosis with small lipid vacuoles was observed in fish fed with corn starch compared to others (Figure 1A). Both fish fed with sago and taro starches developed major steatosis and larger sizes of lipid vacuoles in their liver (Figure 1C, 1D).

Table 3: Growth, feed utilisation and body indices of mahseer fingerlings fed with experimental starch diets for 10 weeks

	Starch Source			
Parameter	**Corn**	**Cassava**	**Sago**	**Taro**
Survival (%)	91.67±4.41	91.67±1.67	91.67±6.00	93.33±4.41
Body Weight				
Initial (g)	1.25±0.01	1.24±0.03	1.29±0.02	1.28±0.01
Final (g)	2.69±0.02^{a}	1.99±0.10^{c}	2.28±0.09^{b}	2.51±0.08^{ab}
WG (%)	114.12±0.56^{a}	62.17±9.40^{b}	77.45±4.90^{b}	96.36±4.14^{a}
Total Length				
Initial (cm)	5.17±0.02	5.14±0.04	5.25±0.01	5.22± 0.03
Final (cm)	6.18±0.07	5.89±0.15	5.90±0.06	6.12±0.09
LG (%)	19.73±1.87^{a}	14.71±2.85^{ab}	12.49±1.20^{b}	17.20±1.14^{ab}
FI	3.91±0.07^{a}	3.03±0.26^{b}	3.83±0.32^{a}	3.88±0.05^{a}
SGR	1.09±0.00^{a}	0.69±0.08^{c}	0.82±0.04^{bc}	0.96±0.03^{ab}
FCR	2.73±0.04^{b}	4.10±0.26^{a}	3.84±0.07^{a}	3.17±0.14^{b}
PER	0.90±0.01^{a}	0.62±0.04^{b}	0.79±0.11^{ab}	0.77±0.03^{ab}
HSI	2.05±0.06^{a}	1.48±0.01^{b}	1.51±0.09^{b}	1.59±0.18^{b}
VSI	2.33±0.05	2.56±0.11	2.62±0.19	2.52±0.14
IPF (%)	1.55±0.31^{a}	0.78±0.07^{b}	1.17±0.10^{ab}	0.92±0.07^{b}

Means ± standard error (SE) within a row and followed by different superscripts are significantly different at $P<0.05$.

Table 4: Body proximate composition (% wet weight) of mahseer fingerlings fed experimental starch diets after 10 weeks

	Starch Source				
	Initial	**Corn**	**Cassava**	**Sago**	**Taro**
Proximate composition					
Moisture	85.23	72.78±0.94^{b}	77.81±0.88^{a}	75.50±0.97^{ab}	76.49±0.40^{a}
Protein	8.29	12.07±0.19	10.47±1.38	11.16±0.58	11.08±0.02
Lipid	1.53	10.60±0.90^{a}	7.50±0.67^{b}	8.44±0.70^{ab}	7.62±0.36^{b}
Ash	2.09	2.47±0.12	2.25±0.04	2.29±0.13	2.44±0.12
Fibre	0.13	0.04±0.03	0.10±0.02	0.05±0.00	0.04±0.00
Carbohydrate	2.86	2.07±0.06	1.98±0.24	2.61±0.63	2.36±0.07
Gross energy (kJ g^{-1})	22.48	25.77±0.13^{a}	24.84±0.03^{b}	25.79±0.26^{a}	24.75±0.10^{b}

Means ± standard error (SE) within a row and followed by different superscripts are significantly different at $P<0.05$

Table 5: Dietary nutrient retention (%) of mahseer fingerlings fed experimental starch diets after 10 weeks

	Starch Source			
	Corn	**Cassava**	**Sago**	**Taro**
Nutrient retention (%)				
Protein	13.91±0.18^{a}	8.66±0.21^{c}	11.26±1.08^{b}	10.72±0.35^{b}
Lipid	83.08±1.09^{a}	68.39±1.78^{c}	77.65±3.73^{b}	72.85±1.63^{bc}
Carbohydrate	3.19±0.05^{a}	1.45±0.18^{c}	3.84±0.45^{a}	2.51±0.12^{b}
Energy	60.59±0.78^{a}	42.80±1.50^{c}	44.68±1.24^{c}	51.61±1.89^{b}

Means ± standard error (SE) within a row and followed by different superscripts are significantly different at $P<0.05$

However, fish fed with sago starch displayed obvious reduction of prominent nucleus, indicating degenerated hepatocytes with signs of hypertrophy (Figure 1C). Observation of intestines showed that fish fed with corn starch had thicker submucosa and slightly decreased villi length compared to other treatments, but did not display enteritis or intestinal inflammation (Figure 2A). In comparison, mucosal epithelial cells in fish fed with corn and taro starches were observed to contain more intracytoplasmic lipid vacuoles (Figure 2A, 2D). Leucocytes appeared to be fewer in fish fed with cassava starch (Figure 2B).

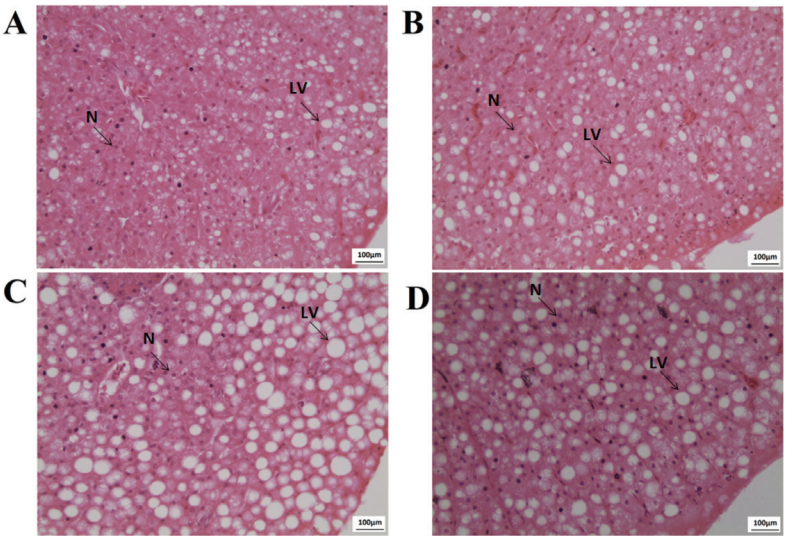

Figure 1: Histology of liver hepatocytes of mahseer fingerlings fed with experimental diets for 10 weeks: (A) fish fed with corn starch showing minor steatosis with small LVs; (B) fish fed with cassava starch showing steatosis with small LVs; (C) fish fed with sago starch showing degenerated hepatocytes with signs of hypertrophy; (D) fish fed with taro starch, showing major steatosis and large LVs. Magnification ×10; scale bar = 100µm; N, nucleus; LV, lipid vacuole

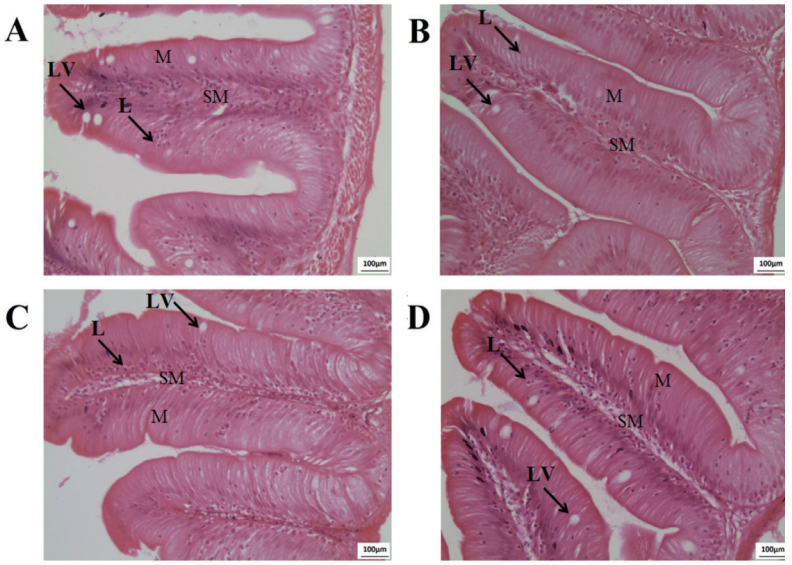

Figure 2: Histology of mahseer fingerling intestines. (A) fish fed with corn starch; (B) fish fed with cassava starch; (C) fish fed with sago starch; (D) fish fed with taro starch. Magnification ×10; scale bar = 100µm; M, mucosa cells; SM, submucosa; LV, lipid vacuole; L, leucocyte

Discussion

Starch comprised two major complex polysaccharides; amylose and amylopectin. Amylose had longer linear α-D-(1,4) glucan chains, whereas amylopectin was made up of a larger branched molecule containing both α-D-(1,4) and α-D-(1,6) chains with higher molecular weight (Ball *et al.*, 1996; FAO, 1998; Keetels *et al.*, 1996; Manners, 1989; Pfister & Zeeman, 2016). Starch ingested in fish would be hydrolysed by α-amylase, breaking down the α-D-(1,4) glycosidic bonds of starch into its constituent sugar and oligosaccharides in the gastrointestinal tract (De Silva & Anderson, 1995; Kamalam *et al.*, 2016; NRC, 2011). As such, omnivorous and herbivorous fish that had this capability could readily accept the dietary inclusion of starch and exhibited a carbohydrate protein-sparing effect compared with their carnivorous counterparts (German *et al.*, 2010; Hemre *et al.*, 2002).

In this study, *T. tambroides* fingerlings were observed to readily accept all experimental diets with fish that had been fed sago and taro starches having similar feed intake to fish fed with corn starch. However, lower intake of cassava starch diet indicated that the mahseer could become selective towards starch sources in its diet. Better FCR values of fish fed with corn and taro diets were observed to be within the range reported for this species (Misieng *et al*., 2011; Ismail *et al*., 2012), compared to the higher FCR values of fish fed with cassava and taro diets. In comparison, the Himalayan mahseer (*Tor pitutora*) and Indian mahseer (*Tor tor*) had been reported to have better FCR range of 2.0 and lower (Islam & Tanaka, 2004; Lone & Lone, 2014). It was reported that starches from different sources had different morphology, and different amylose to amylopectin ratio based on their botanical origins (Singh *et al.*, 2007). Different physiological responses by *T. tambroides* towards dietary treatments were probably due to this dissimilarity in nature, and the complexity of the starches from different plant sources (Gatlin III *et al.*, 2007; Russell *et al.*, 2001).

In this study, fish fed with corn and taro starches showed better growth and feed utilisation than the counterparts fed with cassava and sago starches. Response to different dietary starch sources was reported to be species-dependent. For example, the gilthead seabream (*Sparus aurata*) showed better growth performance when fed diets containing wheat or barley than corn or rye (Couto *et al.*, 2016; Venou *et al.*, 2003), whereas bagrid catfish (*Mystus nemurus*) performed better with diets containing rice or corn, compared with diets with dextrin or sago (Hamid *et al*., 2011). The mirror carp (*Cyprinus carpio*) responded better to the inclusion of rice in its diet compared to cassava (Ufodike & Matty, 1983). In contrast, no significant difference was observed in the growth of European sea bass (*Dicentrarchus labrax*) and Atlantic salmon (*Salmo salar*) fed with different starch sources (Couto *et al.*, 2017; Russell *et al.*, 2001; Storebakken *et al.*, 2000).

Starch gelatinisation and glucose metabolism, however, were not investigated in this study, but other studies had shown that they might also affect growth. Different starch sources were proven to have different gelatinisation rates in formulated *T. tambroides* feed during extrusion and, thus, could influence fish digestibility (De Cruz *et al*., 2015; Umar *et al*., 2013). The jundiá catfish (*Rhamdia quelen*) and Nile tilapia (*Oreochromis niloticus*) displayed differences in starch digestibility when fed different starch sources (Do Carmo Gominho-Rosa, 2015). Wu *et al*. (2007) reported that yellowfin seabream (*Sparus latus*) responded better to raw starch than pregelatinised starch in its diet. In comparison, when fed either gelatinised wheat, corn or oats, *S. salar* was shown to have similar growth although its glucose absorption was affected by parr-smolt transformation (Hemre & Hansen, 1998). The largemouth bass (*Micropterus salmoides*) showed better growth when fed with corn and pea starches, but fish fed with cassava and wheat starches were observed to develop better hepatic glycogen levels (Song *et al*., 2018).

In this study, *T. tambroides* fed with corn starch had the highest HSI and IPF ratios compared with cassava, sago and taro. It could be presumed that the excess energy from the dietary carbohydrate was converted into lipid and deposited as glycogen in the liver, resulting in an increase of HSI as those observed by earlier studies (Hemre *et al.*, 2002; Mohanta *et al.*, 2007; Wu *et al.*, 2015). Subsequently, *D. labrax* fed with fishmeal-based diets including corn starch had higher HSI compared with those fed diets of fishmeal replaced by plant proteins, such as soybean meal, wheat meal and corn gluten (Guerreiro *et al.*, 2015). When fed with a carbohydrate incorporated diet, the hybrid striped bass (*Morone chrysops*×*M. saxatilis*) also attained higher HSI compared to those fed a starch-free and high lipid diet, although fish fed with high lipid diet had higher IPF (Wu *et al.*, 2015). In another study, *T. tambroides* showed an increased IPF with the increase of dietary lipid (Ng & Andin, 2011). This might indicate that a diet containing corn starch was easily digested by *T. tambroides*, although the diet had a slightly lower gross energy content at 17.32 kJ g^{-1} compared to other diets.

Results also showed that body moisture, lipid and energy retention of *T. tambroides* were affected when given different starch sources. Similarly, moisture and lipid compositions in hybrid striped bass and *C. carpio* were affected with different dietary corn starch treatments (Wu *et al.*, 2015; Ufodike & Matty, 1983). In contrast, the body composition of *S.aurata* remained unaffected when fed corn, barley, wheat or rye (Couto *et al.*, 2016). The PER and protein retention of mahseer in the present study indicated a protein-sparing effect by different starch sources. The protein-sparing effect was not observed when different dietary carbohydrate levels of corn starch were tested in an earlier study (Ishak *et al*., 2016). The high IPF ratio, lipid retention and body lipid in *T. tambroides* fed with corn starch indicated that the starch could induce a higher *de novo* conversion of carbohydrate to lipid compared to other starches.

All liver samples of the *T. tambroides* exhibited steatosis of varying degree. However, fish fed with sago starch undertook the most negative effects as indicated by hepatocyte degeneration and major steatosis. Similar results had been reported in the hepatocytes of mahseers fed with diets containing different palm oil to sunflower oil ratios (Ramezani Fard *et al.*, 2014). Consequently, it had been observed that *T. tambroides* was susceptible to hepatic steatosis when given high-energy diets (Ramezani-Fard & Kamarudin, 2012; Ramezani Fard *et al.*, 2014). Lipid vacuolisation in hepatocytes and mucosal epithelial cells were present in all liver and intestine samples. A similar observation had been reported in hepatocytes of Atlantic salmon with the dietary inclusion of plant meals, whereas no lipid vacuoles were found in fish fed with fishmeal only (Gu *et al.*, 2014). Histological observation of the intestines also observed that lipid vacuolisation occurred more in *T. tambroides* fed with corn and taro starches. In contrast, intestinal morphology of *S. salar* and *S. aurata* was unaffected when fed different types of dietary carbohydrates (Couto *et al.*, 2016; Storebakken *et al.*, 2000). Rainbow trout (*Oncorhynchus mykiss*) fed a high inclusion (50%) of yellow lupin kernel meal showed a lower occurrence of lipid vacuoles in hepatocytes compared to those fed a lupin-free diet (Glencross *et al.*, 2004). Slightly shorter mucosal folds and thicker submucosa were observed in fish fed with corn starch compared to other dietary treatments. This indicated that the inclusion of starches derived from plants could influence the formation of lipid vacuoles in *T. tambroides* liver hepatocytes and intestine mucosal epithelial cells.

Conclusion

The inclusion of different starch sources in experimental diets could affect growth, feed utilisation efficiency, body composition and nutrient retention, besides influencing liver and intestinal histomorphology of *T. tambroides* fingerlings. The overall performance of fish fed with taro starch was comparable to the

performance of a corn starch diet. In general, *T. tambroides* was not able to utilise a wide range of starch sources, which indicated that the species had limited capability in using dietary carbohydrates from different botanical origins. In conclusion, taro could be recommended as a potential starch to replace corn starch in the production of extruded feed for *T.tambroides*. A more detailed examination of the enzymes involved in the utilisation of different starches in *T. tambroides* could be carried out in future.

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