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# Combination of dietary pre-gelatinized starch and isomaltooligosaccharides improved pellet characteristics, subsequent feeding efficiencies and physiological status in African catfish, *Clarias gariepinus*, juveniles

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ABSTRACT

An 8-week study was conducted on the use of native tapioca starch (TS) or pre-gelatinized tapioca starch (PGTS), with or without the inclusion of isomaltooligosaccharides (IMO) at 0.5% on the growth, feeding efficiencies, muscle and plasma biochemical composition, intestinal short chain fatty acids (SCFA), differential cell counts, phagocytic ability/capacity, and liver glycogen content in African catfish, *Clarias gariepinus*. Each treatment was triplicated with each replicate consisting of 10 fish (initial weight of 6.2 ± 0.3 g). The bulk density (BD), pellet durability index (PDI), water stability (WS), water absorption index (WAI), water solubility index (WSI), and protein solubility (PS) were measured in all experimental diets. The results showed no significant ($p > 0.05$) growth differences among the treatments, but feed intake was significantly lowest ($p < 0.05$) in the PGTS diet, followed by the PGTS + IMO, while the significantly ($p < 0.05$) highest was in the TS treatment. Catfish fed the PG diets had significantly ($p < 0.05$) higher crude lipid but significantly ($p < 0.05$) lower crude protein and ash compared to those fed the TS diets. Both trypsin and chymotrypsin activities were significantly ($p < 0.05$) higher in the PG diets. The TS + IMO diet significantly increased the white blood cells and phagocytic activity compared to the TS diet. Meanwhile, fish fed the PGTS diet had significantly ($p < 0.05$) lower intestinal acetic and butyric acid than all others, but the inclusion of IMO in the PGTS diet mitigated a decrease of these. The PG starch diets had significantly ($p < 0.05$) higher PDI, WS, WAI, and PS than the TS diets. The uncompromised growth may have been due to dietary PG starch and IMO enhancing available energy to the fish. The mitigating effect of IMO on increasing intestinal SCFA when using PG diets may have implications to disease management, but requires further investigation.

## 1. Introduction

Starches are commonly included in aquafeeds as an inexpensive source of energy to spare proteins and lipids from catabolism (Krogdahl et al., 2005; Glencross et al., 2007). Moreover, when aquafeeds are extruded under high heat conditions, the included starch undergoes gelatinization, which involves the degradation of the more insoluble crystalline regions of starch often by the application of heat and water (Tako et al., 2014). This subsequently increases starch solubility in water that generally leads to improved utilization by the fish as well as increased stability and durability of the pellets (Sørensen, 2012). In some cases, however, farmers choose to manufacture their pellets on site using simple technology that employs much less heat, pressure and shear forces (Gonzalez and Allan, 2007). This process would reduce the amount of starch gelatinization leading to a more fragile pellet while the starch would be less digestible by aquatic animals. A potential way to overcome these drawbacks to the pellet quality is by the dietary inclusion of pre-gelatinized (PG) starches.

*Abbreviations:* BD, bulk density; CF, condition factor; FCR, feed conversion ratio; HSI, hepatosomatic index; IMO, isomaltooligosaccharides; PDI, pellet durability index; PG starch, pre-gelatinized starch; PS, protein solubility; SCFA, short chain fatty acids; SGR, specific growth rates; TS, tapioca starch; WAI, water absorption index; WS, Water stability; WSI, water solubility index

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Researchers will sometimes refer to PG starches as pre-cooked or extruded starches, and are when starches are cooked, dried and made into a fine powder. This ensures that all the starch is gelatinized before being included in feeds, which may not otherwise fully occur during pellet manufacture. The use of dietary PG starches generally leads to improved digestibility in aquatic animals, however, their effects to growth is contradictory that is likely species-specific. For example, increased starch gelatinization decreased feed intake and growth, as observed in the European eel *Anguilla anguilla* (Gallego et al., 1994), European sea bass *Dicentrarchus labrax* (Peres and Oliva-Teres, 2002) and yellowfin seabream *Sparus latus* (Wu et al., 2007). Meanwhile, for rainbow trout *Oncorhynchus mykiss* (Hilton et al., 1981) and sea bream *Sparus aurata* (Venou et al., 2009), there was decreased feed intake but without a growth reduction when fed PG starches, which was suggested to be due to a longer retention time in the gut. In contrast, dietary PG starches improved the growth of common carp *Cyprinus carpio* (Takeuchi et al., 1990), silver perch *Bidyanus bidyanus* (Stone et al., 2003), carp *Labeo rohita* (Kumar et al., 2007), Nile tilapia *Oreochromis niloticus* (Amirkolaie et al., 2006) and red hybrid tilapia *Oreochromis* sp. (Kanmani et al., 2018).

Research on the effects of dietary PG starches on the immunological responses in aquatic animals are limited, however, Kumar et al. (2007) found that increasing ratios of dietary PG starch in *L. rohita* decreased some innate immunological responses as well as resistance to bacterial infection. A potential contributor to these findings may include a decrease to intestinal short chain fatty acids (SCFA) because these volatile fatty acids play important roles in the overall health of animals (Hoseinifar et al., 2017a). This consequence is believed to be from a better utilization of PG starches that would reduce the amount of available carbohydrates for their subsequent fermentation by bacteria, as observed in *O niloticus* (Amirkolaie et al., 2006) and *Oreochromis* sp. (Kanmani et al., 2018). One strategy to mitigate this reduction, and potentially improve immunity, is by the combined administration of PG starches with prebiotics.

Prebiotics are indigestible but fermentable carbohydrates that have been shown to improve the growth and health status of various aquatic animals (Ringø et al., 2014; Hoseinifar et al., 2015). This has been attributed to enhanced digestive enzyme activity (Xu et al., 2009; Ye et al., 2011) and altered intestinal microbiota in favor of beneficial bacteria (Mahious et al., 2006; Li et al., 2009; Zhou et al., 2007). The latter case is believed to be responsible for increasing intestinal SCFA in aquatic animals (Geraylou et al., 2013; Chen et al., 2017). Some of the more commonly used prebiotics in aquafeeds include alginate, inulin and various oligosaccharides (Hoseinifar et al., 2015). Among the oligosaccharides, isomaltooligosaccharides (IMO) is a mixture of isomaltose, isomaltotriose, panose, isomaltotetraose and others, which consist of alpha (1, 6) linked glucose homo-oligomers that have sweet-tasting properties but with a low glycemic index (Goffin et al., 2011). There are limited investigations of IMO to fish compared to the other oligosaccharides (e.g. fructoologsaccharides and mannooligosachharides), however IMO was shown to be fermentable in vitro by gut microbes from *C. carpio* (Kihara and Sakata, 2002). Meanwhile, dietary IMO from (0.5 to 3.0%) had no effect on yellowtail *Seriola quinqueradiata* growth, however, those fed 0.5% IMO had more available energy based on significantly higher serum proteins, amino acids, cholesterol, and phospholipids as well as heavier viscera and intestine (Shimeno et al., 1993). Currently, there appears to be no studies on the potential interaction between PG starches and prebiotics in the diets of an aquatic animal. The aim of this study was to investigate the effects of native or PG tapioca starch with or without dietary IMO on various pellet characteristics and subsequent changes to the growth, feeding efficiencies, muscle proximate composition/cholesterol content, plasma biochemistry, trypsin/chymotrypsin activities, liver glycogen and intestinal SCFA of African catfish *Clarias gariepinus*.

## 2. Materials and methods

### 2.1. Experimental diets

A total of four diets were formulated to be isonitrogenous and isolipidic according to the nutritional requirements of *C. gariepinus* by the NRC (2011). In all diets, tapioca starch was added at 25%. Tapioca starch was used since cassava tends to be produced in higher amounts than other starches in countries that farm *C. gariepinus*, such as Nigeria and Indonesia (FAO, 2016).

In two diets, native tapioca starch was used without or with the addition of isomaltooligosaccharides (IMO) at 0.5%, hereafter referred to as the TS and TS + IMO diets, respectively. In the other two diets, the tapioca starch was pre-gelatinized (PG) according to Kanmani et al. (2018) without or with the addition of IMO, hereafter referred to as PGTS and PGTS + IMO diets, respectively. The IMO level of 0.5% was chosen based on this being optimal to *S. quinqueradiata* in a growth trial (Shimeno et al., 1993). The IMO (IMO-900) is a product of Shandong Tianmei Biotechnology Co. Ltd., China, which was in a powder form with a reported isomaltooligosaccharide content of ≥ 90%. Based on high-performance liquid chromatograph by the China Fermentation Industry Association (QBT2491-2000), the IMO product consists of isomaltose (24.5%), isomaltotriose (12.0%), maltose (6.1%), panose (1.6%), maltotriose (1.5%), glucose (0.3%) and others (43.7%).

To make the experimental diets, the main protein sources of fishmeal (35%) and soybean meal (26%) were finely ground and sieved. After thoroughly mixing the dry ingredients, fish oil and palm oil were equally and slowly added at 5% while constantly mixing. After 30 min, distilled water was then added at 20% of the ingredient weight, further mixed for 10 min, and then this mash was pelleted through a meat mincer (TJS22 – Dual Purchase Machine; 410 × 240 × 450 mm; Guangdong Henglian Food Machinery Co., LTD, China) with a 4 mm die. This method of diet preparation was chosen since meat mincers are often used by small-scale farmers that would minimize starch gelatinization compared to extrusion that employs higher heat, pressure and shear forces.

After making each diet, the screw, barrel and die were thoroughly cleaned and left to dry. This was to prevent contamination among the diets as well as allowing the equipment to sufficiently cool to room temperature prior to making the next diet. Once all the diets were made, these were then oven dried at 55 °C for 6 h, placed in air-tight plastic bags and kept at − 4 °C until use.

### 2.2. Pellet characteristics

After making the pellets, triplicate pellet samples from each treatment were measured for bulk density (BD), pellet durability index (PDI), water absorption index (WAI), water solubility index (WSI), water stability (WS), and protein solubility (PS).

The BD was measured according to Aas et al. (2011), by pouring the pellets into a measuring cylinder until the 1 L mark was reached and the weight was recorded. The ratio of pellet weight to volume was then expressed as BD g $l^{-1}$.

The PDI was performed according to Dukić et al. (2007) with slight modifications. Briefly, each sample replicate ($P_{bt}$, 50 g) was placed in a friabilator (DF-3, Distek, North Brunswick, NJ, USA) and the pellets were subjected to falling shocks for 10 min at a speed of 25 rotations per minute. The samples were sieved (2 mm) for 1 min and the remaining pellets on the sieve were weighed ($P_{at}$) to calculate the PDI using the following equation,

$$PDI = \left(\frac{p_{at}}{p_{bt}}\right) \times 100$$

The WAI and WSI of the pellets were determined according to Anderson (1982). Briefly, the pellets were finely ground, sieved