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Effects of inulin and Jerusalem artichoke (*Helianthus tuberosus*) as prebiotic ingredients in the diet of juvenile Nile tilapia (*Oreochromis niloticus*)

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ABSTRACT

This study evaluated the prebiotic effects of dietary inulin and Jerusalem artichoke tuber (JA) on juvenile Nile tilapia (*Oreochromis niloticus*). Five dietary treatments (each diet in four replicates) were formulated to incorporate inulin at 0 (control), 2.5 and 5 g kg^{-1} and JA at 5 and 10 g kg^{-1}. Fish were reared in concrete ponds for 8 weeks. Fish fed the inulin diets exhibited better growth performance than fish fed the control diet, and fish fed the JA diets had the best growth performances among all diets tested. Dietary inulin and JA increased red blood cell number. Among the fourteen blood chemicals examined, dietary inulin or JA led to increased glucose, albumin, protein, magnesium, calcium, and iron content ($P < 0.05$). Inulin supplementation at 5 g kg^{-1} improved lysozyme activity and alternative complement haemolytic 50 (ACH50) activity. Dietary JA increased total immunoglobulin content, lysozyme activity, and ACH50 activity. Dietary inulin or JA increased the height of intestinal villi and goblet cell number. These findings indicate that inulin at 5 g kg^{-1} had beneficial prebiotic effects on juvenile Nile tilapia and that direct supplementation with JA at 10 g kg^{-1} had positive effects on growth and health. Thus, both inulin and JA have great potential for use as prebiotics in fish feed.

1. Introduction

Tilapia production has increased intensely to meet the growing global demand for fishery products (FAO, 2013). In particular, production of Nile tilapia (*Oreochromis niloticus*) has commercially dominated the farm-raised tilapia industry. Although Nile tilapia are easy to culture and fast growing in tropical areas, mass death in tilapia farms due to outbreaks of disease occasionally occurs, particularly when the water temperature is high during summer. Chemotherapeutic agents such as antibiotics have been used to control the risk of disease in tilapia farms. However, the overuse of antibiotics in fish farms may pose a threat to public health and also adversely impact the ecosystem. Application of biotherapeutics such as prebiotics as an alternative to chemotherapy may prove to be an environmentally friendly tool for use in fish farming.

Prebiotics are defined as non-digestible food ingredients that beneficially affect host health by selectively stimulating the growth and/or activity of healthful bacteria and by combating undesired bacteria in the intestinal tract (Gibson and Roberfroid, 1995). Inulin, which belongs to a class of carbohydrates known as fructans, is one of the most common prebiotics

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Table 1
Chemical composition and oligosaccharide contents of JA tuber.

Components	$g\,kg^{-1}$ (dry matter basis)
Dry matter	934.4
Crude protein	57.8
Crude lipid	1.7
Crude fiber	126.0
Ash	80.8
Fructans	502.0

used in feed for livestock and aquatic animals. Inulin is composed of fructosyl residues, which are linked by β-2,1-linkages (Goodwin and Mercer, 1983; Burr et al., 2005; Yousefian and Amiri, 2009; Ringø et al., 2010). In humans and monogastric animals, fructans generally cannot be hydrolysed by digestive enzymes in the proximal intestinal tract (Pool-Zobel et al., 2002). Instead, they are fermented in the large intestine or colon by beneficial bifidobacteria and other lactic acid producing bacteria, thereby enhancing their relative populations (Pool-Zobel et al., 2002; Roberfroid, 2002; Flickinger et al., 2003). Several dietary grades of inulin are available commercially, and their use as a dietary supplement in animal feed has been shown to enhance growth performance, modulate intestinal microbiota, and improve hematological and immune parameters in fish, poultry, and swine (He et al., 2002; Mahious et al., 2006a; Reza et al., 2009; Ibrahem et al., 2010; Mourino et al., 2012; Nabizadeh, 2012; Ortiz et al., 2013). Nevertheless, the use of inulin as a functional feed additive in the animal feed industry is limited by the cost of the inulin extraction process. Therefore, finding eco-friendly sources of fructan-type functional feed ingredients would contribute greatly to aquaculture productivity.

Jerusalem artichoke (*Helianthus tuberosus*; JA), which is a root vegetable native to central-eastern North America (Rogers et al., 1982; Kays and Nottingham, 2007), is widely grown year-round in tropical areas. In Thailand, JA can be harvested after 100–140 days, and crop yields of JA are typically 13–19 $ton\,ha^{-1}$. The JA tuber contains 160–200 $g\,kg^{-1}$ inulin and 120–150 $g\,kg^{-1}$ fructooligosaccharide (FOS) (Moshfegh et al., 1999); therefore, it would be a good source of oligofructose-enriched inulin. Although Nile tilapia production and JA cultivation co-occur in the tropical zone, studies of the potential benefit of the direct use of JA as a prebiotic functional ingredient in aquafeed are limited.

In this study, the prebiotic effects of dietary inulin and JA on juvenile Nile tilapia were evaluated and compared. Prebiotic effects on growth performance, body composition, and intestinal morphology were measured. In addition, hematological, blood chemistry, and immune parameters were examined to better interpret the effects of prebiotic supplementation on the health status of the fish.

2. Materials and methods

2.1. Jerusalem artichoke

JA samples were obtained from Phetchabun Research Station, Agro-Ecological System Research and Development Institute, Kasetsart University, Thailand. Proximate analyses of JA powder were performed according to the standard methods of AOAC (1990) for dry matter, protein, total lipid, fiber, and ash (Table 1). In addition, the content of oligofructose in JA powder was measured according to Joye and Hoebregs (2000). Oxymation and silylation of extracted sugar was carried out and analyzed using high-temperature capillary gas chromatography method.

2.2. Experimental design, feed formulation, and pellet preparation

The experimental design was completely randomized with five treatment diets, each of which was replicated four times. The five treatment diets were as follows: basal diet (control, C), 2.5 $g\,kg^{-1}$ inulin-supplemented diet (2.5 inulin), 5.0 $g\,kg^{-1}$ inulin-supplemented diet (5.0 inulin), 5.0 $g\,kg^{-1}$ JA-supplemented diet (5.0 JA), and 10.0 $g\,kg^{-1}$ JA-supplemented diet (10.0 JA). The 2.5 inulin and 5.0 inulin diets were prepared to incorporate inulin (PREBIOFEED 88; Warcoing, Belgium) to ensure supplementation levels of 2.5 $g\,kg^{-1}$ and 5.0 $g\,kg^{-1}$, respectively. The 5.0 JA and 10.0 JA diets were prepared to incorporate JA at 5.0 $g\,kg^{-1}$ and 10.0 $g\,kg^{-1}$, respectively, which were equal to inulin levels of 2.5 $g\,kg^{-1}$ and 5.0 $g\,kg^{-1}$, respectively.

Table 2 shows the basal dietary ingredients and the proximate composition (moisture, crude protein, crude fat, and ash content) of the experimental diets as determined following standard AOAC methods (1990). All test ingredients were obtained from animal feedstuff companies. Before formulating the feed, all feed ingredients were analyzed to determine gross composition (moisture, crude protein, crude lipid, crude fiber, and ash) according to AOAC methods (1990). All experimental diets were produced using a hammer grinder, mixer, and extruder (Paktongchai Pasusat, Nakhon Ratchasima, Thailand). The dry ingredients were ground using a grinder and mixed using a ribbon screw mixer (22 rpm). The floating pellet was produced using a single screw extruder at an extruding temperature of 120–160 °C. All experimental diets were stored at room temperature until use.