Animal Feed Science and Technology 207 (2015) 120–129

Contents lists available at ScienceDirect

Animal Feed Science and Technology

journal homepage: www.elsevier.com/locate/anifeedsci

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

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ARTICLE INFO

Article history:
Received 24 July 2014
Received in revised form 10 April 2015
Accepted 16 May 2015

Keywords:
Inulin
Jerusalem artichoke
Helianthus tuberosus
Nile tilapia
Oreochromis niloticus
Prebiotic
Blood profiles
Immune
Intestinal morphology

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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http://dx.doi.org/10.1016/j.anifeedsci.2015.05.008
0377-8401/

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.

Table 2
Ingredients and chemical composition (g kg^{-1}) of the basal diets.

Ingredients	g kg^{-1}
Fish meal (56.2% crude protein)	300
Soybean meal (44.4% crude protein)	270
Rice bran	150
Corn meal	145
Cassava chips	120
Premix[a]	10
Vitamin C	5
Proximate composition (g kg^{-1} dry weight)	
Dry matter	933
Crude protein	343
Crude lipid	79
Ash	104
Crude fiber	42
Nitrogen-free extract[b]	365

[a] Vitamin and trace mineral mix provided the following (IU kg^{-1} or g kg^{-1} diet): biotin, 0.25 g; folic acid, 0.003 g; inositol, 0.25 mg; niacin, 0.0215 g; pantothenic acid, 0.03 g; vitamin A, 5000 IU; vitamin B1, 0.0025 g; vitamin B2, 0.0012 g; vitamin B6, 0.0075 g; vitamin B12, 0.00005 mg; vitamin C, 1 g; vitamin D3, 1000 IU; vitamin E, 100 IU; vitamin K, 0.008 g; copper, 0.02 g; iron, 0.2 g; selenium, 0.3 mg; zinc, 0.32 g.
[b] Nitrogen-free extract = 1000 − (moisture + crude protein + crude lipid + crude fiber + ash).

2.3. Experimental fish and fish culture

The Nile tilapia used in this study were reared at the Suranaree University of Technology Farm (SUT Farm; Nakhon Ratchasima, Thailand). The experimental Nile tilapia were all male fish that were produced by feeding the swim-up fry with a 50 mg kg^{-1} 17α-methyltestosterone-supplemented diet for 4 weeks and then with a diet consisting of 350 g kg^{-1} crude protein until the experiment started.

Twenty cement ponds ($2 \times 2 \times 1$ m^3) (i.e., four replicates of five treatments) were used for the experiment. They were randomly assigned to each treatment diet, and 30 fish (42–47 g) were randomly distributed into each cement pond containing water (depth, 0.7 m) under continuous aeration and with continuous water flow (5 L min^{-1}). In addition, a flow-through water change system was implemented by replacing one-third of the water in each pond with dechlorinated water every week. To acclimatize the Nile tilapia to the experimental conditions, the fish were fed the basal diet for 2 weeks. Throughout the experimental period, the fish were hand-fed ad libitum twice daily, and daily feed consumption by replicate was recorded to determine feed utilization. At the end of week 8, four fish from each pond (replication) were sampled and weighed to asses growth performance. Air and water temperatures were measured daily and were 25–33 °C and 25–28 °C, respectively. Dissolved oxygen (DO) content and pH were measured weekly using a DO meter and pH meter, and values were within acceptable ranges of 5.24–5.98 mg L^{-1} and 7.48–8.16, respectively. Dead fish were removed daily and mortality was recorded.

2.4. Fish sampling and blood collection

At the end of the experimental period (8 weeks), fish were not fed for 18 h before being sampled. Four fish from each diet replicate were removed from the tank and anesthetized with 2-phenoxyethanol (0.2%). Blood samples were collected from the caudal vein using a hypodermic syringe. The collected blood samples were divided into two sets. One set was mixed with K_2EDTA (at 1.5 mg mL^{-1} blood) as an anticoagulant for hematological examination and plasma collection. The other set was left to clot at 4 °C for at least 3 h. Plasma was collected by centrifugation of the K_2EDTA-treated blood at 9000 × *g* for 10 min at 4 °C and stored at −80 °C for further analysis. The serum was collected by centrifuging the clotted blood at 9000 × *g* for 10 min at room temperature.

2.5. Hematological assays

Immediately after blood sampling, the K_2EDTA-treated blood was used to examine hematological parameters. The red blood cell count (RBC) was analyzed in duplicate for each sample using a Neubauer haemocytometer after dilution with Grower's solution (Voigt, 2000). Haematocrit values (Ht) were measured in duplicate by placing K_2EDTA-treated blood into glass capillary tubes and centrifuging them for 5 min by microhaematocrit centrifugation. The hemoglobin (Hb) content was measured using the photometrical cyanohaemoglobin method.

2.6. Blood chemistry analysis

To determine the effects of dietary supplementation with inulin and JA on fish health and nutritional status, content of the following compounds in the blood was measured: glucose, triglyceride, cholesterol, total protein, albumin, blood urea nitrogen (BUN), total bilirubin (T-bilirubin), direct bilirubin (D-bilirubin), serum glutamic oxaloacetic transaminase (SGOT), serum glutamic pyruvic transaminase (SGPT), calcium, chloride, magnesium, and iron. Immediately after blood

sampling, the K_2EDTA-treated blood was used to measure the blood glucose levels using a hand-held glucometer (AccuTrend; Roche, Mannheim, Germany). Serum triglyceride content was measured using the glycerol-3-phosphate oxidase-sodium N-ethyl-N-(3-sulfopropyl) m-anisidine (GPO-ESPAS) method described by Bucolo and David (1973). Serum cholesterol was quantitatively analyzed using the cholesterol oxidase-phenol+aminophenazone (CHOD-PAP) technique described by Flegg (1973). Plasma protein contents were determined using the Biuret method (Gornall et al., 1949). Serum albumin content was quantitatively estimated using the bromocresol green method (Doumas et al., 1971). BUN content was measured using a modified indophenol colorimetric method (Weatherburn, 1967). T- and D-bilirubin contents were measured using the new diazo-DMSO method (Winsten and Cehelyk, 1969). SGOT and SGPT were analyzed using Reitman and Frankel's colorimetric method (Reitman and Frankel, 1957). The calcium content in the serum was estimated using the o-cresolphthalein direct method (Moorehead and Biggs, 1974). Blood chloride content was measured using the thyocyanate method (Hamilton, 1966). Serum magnesium was estimated by the colorimetric method (Smith, 1955), and iron ferene content was measured using iron quantitative determination in the serum (IDS, Liege, Belgium).

2.7. Immune assay

Immune parameters, including total immunoglobulin, lysozyme activity, and alternative complement haemolytic 50 (ACH50) activity, were measured. Total immunoglobulin was measured according to Siwicki et al. (1994). Using fish serum, lysozyme activity was estimated as described by Pitaksong et al. (2013), and ACH50 activity was measured according to Sunyer and Tort (1995).

2.8. Histological analysis

Two fish from each replicate of each treatment were sampled and prepared for histological analysis as described previously (Phumyu et al., 2012) to investigate the effect of dietary supplementation with inulin or JA on intestinal morphology. Portions of the anterior, middle, and posterior parts of the intestine were dissected and preserved in 10% phosphate-buffered formalin (pH 7.2). After dehydration, the tissue was embedded in paraffin wax, cut into slices 5 μm thick, and mounted on glass slides. After deparaffinization, the slides were dehydrated and stained with hematoxylin and eosin. Villus height was measured on stained sections under a microscope using an ocular micrometer at 100× magnification. The five longest intact villi in each intestinal position were measured in two cross-sections from each sample. In addition, the number of goblet cells along the selected intact villi were counted.

2.9. Data analysis

All data were analyzed by one-way analysis of variance (ANOVA) using SPSS for Windows (Release 10) (SPSS Inc., Chicago, IL, USA). When significant differences were found among the groups, Duncan's multiple range tests were used to rank the groups. The statistical model utilized was $y_{ij} = \mu + \tau_I + \varepsilon_{ij}$, where y_{ij} was the response; μ, the general means; τ_I, the dietary inulin or JA effect; and ε_{ij}, the random error. Throughout the experiment, effects and differences were declared to be significant at $P < 0.05$.

3. Results

Table 3 shows the growth performances and survival rates of juvenile Nile tilapia fed the experimental diets. Fish fed the diets supplemented with inulin had better growth responses and feed utilization efficiency, including final body weight, weight gain (WG), specific growth rate (SGR), and feed conversion ratio (FCR), compared with fish fed the control diet ($P < 0.05$). The increase in growth responses was inulin supplementation level-dependent. Furthermore, among the experimental diets, fish fed diets supplemented with JA exhibited the best growth performances ($P < 0.05$). An increase in JA supplementation level led to improved growth performances, although there were no significant differences ($P > 0.05$). Throughout the experimental period, survival rate of fish in all groups did not differ significantly ($P > 0.05$) (Table 3). To

Table 3
Growth performance of Nile tilapia juveniles fed experimental diets for 8 weeks.

Diet	Initial weight (g)	Final weight (g)	SGR (% day^{-1})	Feed intake (g day^{-1})	FCR	Survival rate (%)
Control	44.6	233.4^{a}	2.76^{a}	4.33^{c}	1.49^{d}	99.2
2.5 Inulin	44.4	257.9^{b}	2.93^{b}	4.35^{c}	1.33^{c}	100.0
5.0 Inulin	44.3	280.3^{c}	3.07^{c}	4.18^{b}	1.16^{b}	99.2
5.0 JA	44.3	308.0^{d}	3.23^{d}	3.93^{a}	0.97^{a}	99.2
10.0 JA	44.3	325.5^{d}	3.32^{d}	4.00^{a}	0.92^{a}	99.2
Pooled SEM	0.1	8.1	0.04	0.04	0.05	0.3

Means with different superscripts in each column differ significantly from each other ($P < 0.05$). Specific growth rate (SGR) = 100 × [(ln final body weight – ln initial body weight)/experimental days]. Feed conversion ratio (FCR) = dry feed fed/wet weight gain. Survival rate = 100 × (initial number of fish/final number of fish).

Table 4
Whole body composition of Nile tilapia juveniles (g kg^{-1}) fed experimental diets for 8 weeks.

Diet	Moisture (g kg^{-1})	Crude protein (g kg^{-1})	Crude lipid (g kg^{-1})	Ash (g kg^{-1})
Control	700.3	120.3	38.5	40.4
2.5 Inulin	700.5	122.1	38.6	40.9
5.0 Inulin	710.6	123.1	42.4	43.7
5.0 JA	700.6	124.5	39.8	42.6
10.0 JA	710.5	125.9	40.4	46.1
Pooled SEM	3.5	0.9	1.0	0.8

Table 5
Hematological parameters of Nile tilapia juveniles fed experimental diets for 8 weeks.

Diet	RBC (cell × 10^{12} L^{-1})	Hemoglobin (g L^{-1})	Hematocrit (L L^{-1})
Control	2.22^{a}	84.80	0.34
2.5 Inulin	2.33^{b}	86.70	0.35
5.0 Inulin	2.34^{b}	88.30	0.35
5.0 JA	2.36^{b}	88.60	0.36
10.0 JA	2.39^{b}	88.80	0.36
Pooled SEM	0.01	0.63	0.00

Means with different superscripts in each column differ significantly from each other ($P < 0.05$). RBC = red blood cell count.

Table 6
Blood chemical parameters of Nile tilapia juveniles fed experimental diets for 8 weeks.

Blood chemical	Diet					Pooled SEM
Parameter	Control	2.5 Inulin	5.0 Inulin	5.0 JA	10.0 JA	
Glucose (mmol L^{-1})	2.71^{a}	4.09^{b}	4.81^{b}	4.10^{b}	4.19^{b}	0.24
Cholesterol (mmol L^{-1})	4.10	4.19	4.69	4.37	4.41	0.08
Triglycerides (mmol L^{-1})	1.70	1.66	1.71	1.75	1.89	0.04
Total protein (g L^{-1})	36.40^{a}	39.30^{ab}	40.40^{ab}	41.60^{b}	42.50^{b}	0.70
Albumin (g L^{-1})	16.90^{a}	20.40^{b}	20.90^{b}	21.20^{b}	23.10^{b}	0.60
BUN (mmol L^{-1})	0.85	0.82	0.80	0.77	0.78	0.02
Total bilirubin (μmol L^{-1})	4.62	3.42	2.99	3.17	2.82	0.34
Direct bilirubin (μmol L^{-1})	2.39	1.71	1.50	1.64	1.46	0.17
SGOT (U L^{-1})	34.52	33.18	32.04	29.49	30.29	0.99
SGPT (U L^{-1})	21.00	20.86	19.90	19.58	19.79	0.31
Chloride (mmol L^{-1})	130.70	128.20	132.70	138.20	139.70	2.81
Calcium (mmol L^{-1})	3.48^{a}	3.46^{a}	3.59^{a}	3.71^{a}	4.05^{b}	0.07
Magnesium (mmol L^{-1})	1.00^{a}	0.96^{a}	1.14^{b}	1.15^{b}	1.17^{b}	0.03
Iron (μmol L^{-1})	12.00^{a}	13.73^{ab}	14.04^{ab}	14.52^{ab}	16.05^{b}	0.44

Means with different superscripts in each row differ significantly from each other ($P < 0.05$). BUN = blood urea nitrogen; SGOT = serum glutamic oxaloacetic transaminase; SGPT = serum glutamic pyruvic transaminase.

study dietary inulin or JA effect on the nutrient deposition in experimental fish, the whole-body proximate composition of the fish was analyzed in Table 4. There were no significant differences in the moisture, crude protein, crude lipid, and ash contents among experimental treatments ($P > 0.05$).

The hematological indices of the juvenile Nile tilapia fed the experimental diets are shown in Table 5. While there were no significant differences in Hb and Ht among treatment diets, fish fed the diet supplemented with either inulin or JA had significantly higher RBC compared with that of fish fed the control diet ($P < 0.05$). To study how dietary inulin or JA affects metabolic feed utilization, several blood chemical parameters in experimental fish were examined in Table 6. While dietary supplementation with either inulin or JA did not affect blood triglyceride, cholesterol, BUN, T-bilirubin, D-bilirubin, SGOT, SGPT, and chloride contents, they did modulate several other blood parameters. For instance, both inulin and JA supplementation led to significantly increased blood glucose and albumin levels ($P < 0.05$) and increased total protein in blood, although significant enhancement in the latter was observed only in fish fed diets supplemented with JA ($P < 0.05$). In addition, supplementation with the higher level of inulin and JA at both levels increased serum magnesium content ($P < 0.05$). Moreover, blood calcium and iron levels were markedly increased in fish fed the 10.0 JA diet ($P < 0.05$).

The effects of dietary supplementation with inulin and JA on humoral immune parameters were assessed (Table 7). Dietary supplementation with inulin and JA led to increased total immunoglobulin content and lysozyme activity, although a significant increase in lysozyme activity was observed only in fish fed the 5.0 inulin diet, 5.0 JA diet, and 10.0 JA diet ($P < 0.05$). Compared with fish fed the control diet, higher ACH50 activity was also found in fish fed these three diets, and the highest ACH50 activity was observed in fish fed the 10.0 JA diet.

To evaluate whether prebiotic inulin and JA influence intestinal morphology, these villus height and goblet cell number in intestines of experimental fish in all groups were measured (Table 8). In the anterior and the middle parts of the intestine, fish fed diets supplemented with inulin at 5.0 g kg^{-1} and JA at both levels had higher villus height compared to that of fish

Table 7
Immunological parameters of Nile tilapia juveniles fed experimental diets for 8 weeks.

Diet	Total *Ig* (g L^{-1})	Lysozyme activity (μg mL^{-1})	ACH50 (units mL^{-1})
Control	32.00[a]	8.64[a]	311.97[a]
2.5 Inulin	34.10[a]	8.71[a]	327.50[a]
5.0 Inulin	35.60[ab]	10.01[b]	354.87[b]
5.0 JA	36.10[ab]	10.13[b]	363.55[b]
10.0 JA	38.80[b]	10.42[b]	387.68[c]
Pooled SEM	0.74	0.24	5.49

Means with different superscripts in each row differ significantly from each other ($P < 0.05$). Total *Ig* = total immunoglobulin. ACH50 = alternative complement haemolytic 50.

Table 8
Intestinal villus height and number of goblet cells in different parts of the intestine of Nile tilapia juveniles fed experimental diets for 8 weeks.

Diet	Anterior		Middle		Posterior	
	Villus height (μm)	No. of goblet cells	Villus height (μm)	No. of goblet cells	Villus height (μm)	No. of goblet cells
Control	408.59[a]	31.50[a]	309.61[a]	28.25[a]	206.45	18.42[a]
2.5 Inulin	421.37[ab]	32.25[a]	321.66[ab]	29.00[a]	213.76	18.67[a]
5.0 Inulin	525.58[bc]	38.42[b]	392.37[b]	35.67[b]	225.19	23.59[b]
5.0 JA	530.97[bc]	39.00[b]	394.59[b]	36.83[b]	229.60	24.00[b]
10.0 JA	576.00[c]	40.42[b]	404.11[b]	36.83[b]	243.11	24.25[b]
Pooled SEM	20.94	1.16	13.74	1.20	7.33	0.86

Means with different superscripts in each column differ significantly from each other ($P < 0.05$).

fed the control diet ($P < 0.05$), and the highest villus height was observed in the anterior intestine of fish fed the 10.0 JA diet. There were no significant differences in the villus height in the posterior intestine of fish among treatment diets. In all parts of the intestine, the number of goblet cells in fish fed the diets supplemented with inulin at 5.0 g kg^{-1} and JA at both levels were higher than that of fish fed the control diet ($P < 0.05$).

4. Discussion

Several nutrition management studies have been conducted to quantitatively and qualitatively improve the productivity of commercial tilapia farming (Bhujel, 2001). Functional diet supplementation has recently become a topic of interest for improving not only growth rate and feed utilization but also health status of farmed fish. The recent development of industrial prebiotics requires evaluation of their use as a feed additive and their effects on animal production. Inulin has been shown to have beneficial effects on growth and health status in mammals (Coudray et al., 1997; Trautwein et al., 1998; He et al., 2002; Kaur and Gupta, 2002). However, little is known about its effects on Nile tilapia (Ibrahem et al., 2010). With this study we provide valuable information about the incorporation of inulin in the diet of Nile tilapia at the juvenile stage. The use of commercial inulin as a feed additive inevitably leads to an increase in production cost. Therefore, development of alternative fructan-enriched sources of inulin would contribute to the eco-friendly use of this prebiotic as an animal dietary supplement. This study also demonstrated that JA directly incorporated into the diet of juvenile tilapia had effects comparable to those of inulin.

Dietary supplementation with inulin had a positive effect on growth responses, including final weight and SGR, in juvenile Nile tilapia. The improved growth response observed in the present study was similar to that reported previously in various fish species, including Nile tilapia, Siberian sturgeon (*Acipenser baerii*), and rainbow trout (*Oncorhynchus mykiss*) (Mahious et al., 2006a; Ibrahem et al., 2010; Ortiz et al., 2013). However, dietary supplementation with inulin did not affect the growth response in weaning turbot (*Psetta maxima*), Atlantic salmon (*Salmo salar*), hybrid striped bass (*Morone chrysops* x *Morone saxatilis*) (Mahious et al., 2006b; Bakke-McKellep et al., 2007; Burr et al., 2010), and it had a negative effect on the growth response in beluga (*Huso huso*) (Reza et al., 2009). Thus, the effect of dietary inulin on growth responses in fish appears to vary among fish species, and more parameters need to be examined in order to better understand the metabolism of inulin.

The present results showed that dietary supplementation with inulin improved FCR in Nile tilapia, as was also true for Siberian sturgeon (Mahious et al., 2006a). However, several studies reported that dietary supplementation with inulin had no effect on FCR in rainbow trout, hybrid striped bass, and beluga (Reza et al., 2009; Burr et al., 2010; Ortiz et al., 2013). Ibrahem et al. (2010) reported that dietary inulin supplementation led to an increased survival rate in Nile tilapia. Dietary FOS supplementation led to increase survival rate of common carp fry although it did not significantly improve growth performance (Hoseinifar et al., 2014). However, in our study and in reports for other fish species, dietary inulin seemed to have no effects on survival rate (Mahious et al., 2006b; Reza et al., 2009). Thus, the effects of dietary inulin on growth performance and survival rate vary among fish species.

In our study, the growth performances (including final weight, SGR, and FCR) of fish fed the 5.0 JA and 10.0 JA diets were superior to those of fish fed the 2.5 inulin and 5.0 inulin diets, respectively, even though latter two diets contained inulin and FOS at levels equivalent to the 2.5 inulin and 5.0 inulin diets, respectively. Therefore, the superior growth performances

of fish fed the diets containing JA might be due to the differences in degree of polymerization of inulin sources and other substances in addition to inulin and FOS. JA contained high proportion of shorter fructan comparing to inulin from chicory. While high proportion (43–52%) of furctan in JA were short chain fructan (<9 degree of polymerization (dp)), 64–71% of fructan in inulin were medium chain fructan (10–40 dp) (Moshfegh et al., 1999; for review, see Kays and Nottingham, 2007). Although both inulin and FOS exert prebiotic effects, they showed several different prebiotic effects. For example, in fecal cultures, inulin and FOS affected the major fermentation products. Butyric acid was the major product of inulin fermentation whereas FOS fermentation mainly generated acetic acid and lactic acid (Rossi et al., 2005). Comparative study on prebiotic effects in vitro between inulin and FOS revealed that they influenced different microbial community and proteolytic activity (van de Wiele et al., 2006). Dietary supplementation with either inulin or FOS had similar effects on growth performance in rainbow trout (Ortiz et al., 2013), whereas supplementation with FOS had a more positive effect than inulin on the growth rate of turbot larvae (Mahious et al., 2006b). Other substances such as micronutrients in JA also may have had additional positive effects on growth response and feed utilization in juvenile Nile tilapia. In fact, JA contains various minerals and vitamins including iron, calcium, potassium, vitamin B complex, vitamin C, and vitamin A (Van Loo et al., 1995; Kays and Nottingham, 2007). Thus, direct supplementation with JA had positive effects on growth performance in Nile tilapia that were comparable to those of inulin.

Generally, dietary effect on weight gain of fish results from body nutrient contents including water, protein, lipid and ash. The proximate composition of the fish body has been used as a parameter to determine and optimize the supplementation of functional feed ingredients in animal feed (Dumas et al., 2010). This study determined whether the differences in growth response among experimental fish would resulted from the variation in body chemical composition. Our results showed that whole-body composition, including moisture, crude lipid, crude protein, and ash, was not affected by either prebiotic inulin or JA supplementation. Dietary supplementation with inulin also did not significantly change the whole-body composition in hybrid striped bass and beluga (Reza et al., 2009; Burr et al., 2010).

Hematological parameters have been used to assess the health status of fish. In our study, no significant differences in the Hb and Ht values were detected among the experimental groups. Similarly, Ibrahem et al. (2010) and Mourino et al. (2012) reported that the Ht of Nile tilapia and hybrid surubim (*Pseudoplatystoma* sp.) were not affected by dietary inulin. However, the Hb content and Ht were observed to decrease in juvenile beluga that were fed an inulin-incorporated diet at 2 g kg^{-1} and 3 g kg^{-1} (Reza et al., 2009). In our study, the RBC in fish fed the two inulin-supplemented diets and the two JA-supplemented diets increased significantly compared with that of fish in the control group. In contrast, RBC modulation was not observed in hybrid surubim that were fed an inulin-incorporated diet (5.0 g kg^{-1}) for 15 days or in beluga fed an inulin-supplemented diet (10.0–20.0 g kg^{-1}) for 8 weeks (Mourino et al., 2012; Reza et al., 2009). Taken together, these results suggest that the effect of inulin on hematological indices may vary among fish species, level of inulin supplementation, and duration of feeding. Moreover, direct supplementation with JA in fish feed did not exert additive effects on increases in hematological parameters.

Comparative information on blood metabolic responses would be necessary to investigate variably prebiotic effects in several fish. Blood chemical parameters were measured to help interpret the nutritional and health status of Nile tilapia that were fed dietary inulin and JA. Most of the blood chemical parameters showed the same trends for fish fed diets supplemented with inulin and JA, which suggests that JA could be used directly as a food ingredient, at least in Nile tilapia. However, limited information is available about the effect of dietary inulin on blood chemistry (Reza et al., 2009), and more studies are needed.

In general, prebiotics such as inulin have health benefits because they promote the proliferation of beneficial bacteria (usually bifidobacteria and lactobacilli) in the gut (Kolida et al., 2002; Manning and Gibson, 2004). Dietary FOS supplementation increased intestinal lactic acid bacteria number in common carp fry (Hoseinifar et al., 2014). Similarly, use of FOS as a prebiotic was found to enhance the intestinal digestive enzyme activities (amylase and protease) of blunt snout bream (*Megalobrama amblycephala*) (Wu et al., 2013) and Caspian roach (*Rutilus rutilus*) (Soleimani et al., 2012). Others also have shown that probiotics led to increases in the activities of digestive enzymes such as amylase, protease, and lipase (Ziaei-Nejad et al., 2006; Wang, 2007). Increased intestinal digestive enzyme activities would affect several blood chemical parameters. Our results showed that glucose and albumin contents in fish fed diets supplemented with inulin and JA were significantly higher than those of fish in the control group. In contrast, Reza et al. (2009) reported that supplementation with inulin (10.0–30.0 g kg^{-1}) for 8 weeks had no effect on blood glucose and albumin levels in beluga. BUN seemed to be similar among experimental groups, whereas dietary supplementation with inulin at the highest level and JA at the two levels tested led to increased total protein content. However, dietary inulin caused a decrease in total protein content in beluga (Reza et al., 2009). In some studies of mammals, inulin-incorporated feed was shown to decrease cholesterol and triglyceride levels (Trautwein et al., 1998; Flickinger et al., 2003), but dietary inulin and JA did not modulate triglyceride and cholesterol content in our study of Nile tilapia or in beluga (Reza et al., 2009). Bilirubin (both total and direct), SGOT, and SGPT levels were similar among the experimental fish in our study. Similarly, inulin supplementation did not affect these blood parameters in beluga (Reza et al., 2009). The effects of inulin on several blood parameters were similar between Nile tilapia and beluga, whereas they differed for other blood parameters. The different effects might be due to differences in food habits between the two species, as Nile tilapia are omnivores and beluga are piscivores.

We found that dietary supplementation with inulin or JA significantly increased concentrations of several blood minerals in Nile tilapia, including magnesium, calcium, and iron. Intestinal fermentation of inulin or JA might affect intestinal acidification, and low pH would enhance mineral absorption. A number of mammal studies have shown that prebiotic

oligosaccharides can modulate mineral metabolism, such as by stimulating mineral absorption, particularly of calcium, magnesium, and iron (Chonan et al., 1995; Delzenne et al., 1995; Ohta et al., 1995; Coudray et al., 1997; Scholz-Ahrens et al., 2001). Gill absorption of monovalent salt regulates chloride homeostasis in fish, which may explain the lack of a significant effect of dietary inulin or JA on blood chloride levels in this study.

Prebiotics have potential for use as alterative biotherapeutics for fish production. Prebiotics are thought to enhance immunity in animals by selectively increasing the number of beneficial intestinal bacteria and/or interacting with carbohydrate receptors on intestinal epithelial cells and immune cells (reviewed in Seifert and Watzl, 2007). Consequently, the cell components (e.g., lipolysaccharides) of some beneficial microbiota can stimulate the immune system in host animals (Sakai, 1999; Bricknell and Dalmo, 2005). We found that fish fed inulin or JA for 8 weeks exhibited increased humoral innate immune responses, as indicated by increased levels of total immunoglobulin and increased lysozyme and ACH50 activities. Similarly, dietary inulin at 10.0 g kg^{-1} for 60 days was reported to increase serum lysozyme activity of Nile tilapia (Ibrahem et al., 2010). However, Cerezuela et al. (2008) reported that dietary inulin (5.0 g kg^{-1} or 10.0 g kg^{-1}) for 2 weeks had no effect on ACH50 activity of gilthead seabream (*Sparus aurata*) compared to the control group (0 g kg^{-1}). Mourino et al. (2012) found that dietary supplementation with 5.0 inulin for 15 days had no effect on total immunoglobulin content and lysozyme activity of hybrid surubim. These contradictory effects of inulin on immune modulation might be explained by the differing periods of prebiotic administration and fish species among different studies. Overall, the existing data indicate that dietary inulin can have beneficial effects on several immune parameters in several fish species. Thus, our finding that direct supplementation with JA (i.e., without extraction of inulin) had beneficial effects on several health parameters demonstrates that JA can be used directly as a functional feed ingredient.

Caspary (1992) reported that increased intestinal villi leads to increased surface area for nutrient absorption, thereby improving growth performance and feed utilization in animals. Several researchers proposed that fermentation of inulin produces several substances that stimulate intestinal cell proliferation, which in turn results in increased villus height (Blottiere et al., 2003; Rehman et al., 2007; Nabizadeh, 2012). The present study demonstrated that dietary supplementation with inulin (5.0 g kg^{-1}) and JA (5.0 g kg^{-1} and 10.0 g kg^{-1}) resulted in greater villus height in all parts of the intestine, although a significant increase in villus height was observed only in the anterior and middle parts. However, the effect of dietary inulin on carnivorous fish appears to be different. For example, Olsen et al. (2001) reported that a high level of dietary inulin (150 g kg^{-1} dietary inclusion) had negative effects on the ultrastructure of the gastrointestinal tract of Arctic char (*Salvelinus alpinus*). In addition, decreased microvillus height was observed in gilthead sea bream fed a diet that included 10.0 g kg^{-1} inulin (Cerezuela et al., 2013). Intestinal goblet cells synthesize mucin, which invades enteric pathogens. Thus, an increase in intestinal goblet cell numbers would help prevent colonization by pathogenic bacteria and promote beneficial bacteria. We found that the fish fed the 5.0 inulin diet and the 5.0 JA and 10.0 JA diets had a higher goblet cell number than the other groups. In another study, however, dietary supplementation with inulin at 10.0 g kg^{-1} had a negative effect on the number of goblet cells in gilthead sea bream (Cerezuela et al., 2013). These findings suggest that the beneficial effect of dietary inulin on intestinal morphology might differ among species, especially those with different feeding habits. The data also show that inulin and JA had comparable effects on villus height in Nile tilapia.

In conclusion, this study demonstrated the beneficial effects of inulin on growth performance and health status in juvenile Nile tilapia. In addition, direct supplementation with JA had superior effects compared to those of inulin at the equivalent inulin levels. The recommended levels of dietary supplementation with inulin and JA are the maximal levels tested (i.e., 5 g kg^{-1} and 10 g kg^{-1}, respectively).

Acknowledgements

This study was supported by grants from the Suranaree University of Technology, the Higher Education Research Promotion and the National Research University Project of Thailand, the Office of the Higher Education Commission, and the National Research Council of Thailand (SUT1-303-55-12-44). In addition, financial support from the Thailand Research Fund through the Royal Golden Jubilee Ph.D. Program (Grant No. PHD/0155/2553) to student's initials and advisor's initials is gratefully acknowledged. We thank Mr. Sunai Plymee (SUT Farm) for maintaining the fish throughout this work.

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