Effect of *Lactobacillus plantarum* and Jerusalem artichoke (*Helianthus tuberosus*) on growth performance, immunity and disease resistance of Pangasius catfish (*Pangasius bocourti*, Sauvage 1880)

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Abstract

This study evaluated the effects of Jerusalem artichoke (JA) and *Lactobacillus plantarum* singly or combined on growth, immunity and disease resistance of *Pangasius bocourti*. In the first experiment, different concentrations of JA (0, 5, 10, 20, 40, 80 and 160 g kg\(^{-1}\)) were administered to determine an optimal concentration on growth of *P. bocourti*. In the second experiment, the optimal concentration of JA (5 g kg\(^{-1}\)) was combined with 10⁸ cfu g\(^{-1}\) *L. plantarum*. In the final experiment, five randomly selected fish from the second experiment were challenged with *Aeromonas hydrophila*. Treatments for second and third experiments were 0 g kg\(^{-1}\) JA (Diet 1), 5 g kg\(^{-1}\) JA (Diet 2), 10⁸ cfu g\(^{-1}\) *L. plantarum* (Diet 3) and 5 g kg\(^{-1}\) JA + 10⁸ cfu g\(^{-1}\) *L. plantarum* (Diet 4). Fish fed 5 g kg\(^{-1}\) JA or 10⁸ cfu g\(^{-1}\) of *L. plantarum* significantly improved specific growth rate (SGR), feed conversion ratio (FCR), serum lysozyme activity and postchallenge survival rate (PCSR). Dietary in the combination of JA and *L. plantarum* showed significantly enhanced SGR, FCR, serum lysozyme, phagocytosis, respiratory burst activities and PCSR compared with control and individual applications. Dietary JA and *L. plantarum* significantly stimulated growth, immunity and disease resistance of *P. bocourti*.

KEY WORDS: *Aeromonas hydrophila*, Jerusalem artichoke, *Lactobacillus plantarum*, *Pangasius bocourti*

Introduction

*Pangasius bocourti* is an important species in the Mekong Delta basin, especially in Vietnam and Thailand (Jiwyam 2010). Pangasius production is approximately 800,000 tons per year with an export value of 1.5 billion USD in 2013 (GLOBEFISH 2013). However, farming intensification and commercialization have resulted in water pollution causing outbreaks of disease with significant economic losses (Anh et al. 2010). Antibiotics and chemotherapeutics have been intensively used in controlling and preventing Pangasius disease (Dung et al. 2009; Rico et al. 2013; Rico & Van den Brink 2014). Nonetheless, abuse of antibiotics can lead to the development of antibiotic-resistant bacterial strains (Le et al. 2005), environmental hazards (Rico et al. 2012), food safety problems (Sapkota et al. 2008; Heuer et al. 2009; Zhang et al. 2014) and decline in human resistance to pathogens (Wu et al. 2013). The problems outlined above and recent restrictions on the use of antibiotics have resulted in natural immunostimulants, probiotics and prebiotics being considered as an alternative strategy to prevent or control pathogens (Hassaan et al. 2014; Dimitroglou et al. 2011; Guzman-Villanueva et al. 2014; Talpur et al. 2014). The use of these additives can minimize risks associated with chemical drugs and constitutes one of the most promising means in controlling disease in aquaculture (Ringø et al. 2010; Zhang et al. 2013, 2014).

Probiotics are microbial cells provided via the diet or in the rearing water that benefit the host fish, fish farmer or fish consumer, at least in part, by improving the microbial balance of the fish (Daniels et al., 2010). Dietary probiotic supplementation in aquaculture has been reported to improve intestinal balance, growth...
performance and disease resistance and enhance immune responses (Lee et al. 2013; Talpur et al. 2014; Zhang et al. 2014). *Lactobacillus plantarum* is known to produce antimicrobial substances (e.g. plantaricin) active against certain pathogens and is used as a probiotic (Cebeci & Gürakkan 2003). Dietary administration of *L. plantarum* has been demonstrated to improve the growth performance, immune response and resistance to disease in numerous aquatic animals (Son et al. 2009; Kongnum & Hongpattarakere 2012; Giri et al. 2013, 2014; Dash et al. 2014; Yeh et al. 2014).

Prebiotics are selectively fermented ingredients that lead to specific changes in the composition and/or activity of the gastrointestinal microbiota, with resulting benefits for the host’s well-being and health (Roberfroid 2007). Inulin is considered as an important prebiotic substrate and has been well-studied due to its effects on intestinal bifidobacteria (Watzl et al. 2005). It is present as a reserve carbohydrate in the roots and tubers of plants such as Jerusalem artichoke, chicory, dahlia and yacon (Chi et al. 2011). Inulin has been reported to stimulate ‘good’ gut bacteria, suppress pathogens and enhance immune response (Mahious et al. 2006). However, information concerning the influence of inulin on fish immune system is relatively limited (Cerezuela et al. 2008).

A synbiotic is a combination of probiotics and prebiotics; their use may provide the benefits of both pre- and probiotics due to a synergistic effect (Gibson & Roberfroid 1995). However, studies on synbiotics in fish are still in their infancy (Ai et al. 2011; Lin et al. 2012; Mehrabi et al. 2012; Zhang et al. 2013). At present, it seems that the effects of prebiotics, probiotics and synbiotics have not been evaluated in *P. bocourti*. Therefore, the aim of this study was to evaluate the *in vivo* effects of the dietary administration of Jerusalem artichoke (a source of inulin) and *Lactobacillus plantarum* (a probiotic) singly or combined on growth, immune parameters and protection against *Aeromonas hydrophila*.

**Materials and methods**

**Jerusalem artichoke (JA) preparation**

Jerusalem artichoke tubers were obtained from the Department of Plant Science and Agricultural Resources, Khon Kaen University. They were cleaned and sliced longitudinally into thin pieces from the middle of the tubers; samples were oven-dried at 50 °C for 24 h, then ground into powder and kept at 4 °C until use.

**Lactobacillus plantarum**

*Lactobacillus plantarum* CR1T5 was kindly supplied by Dr. Saowanit Tongpim (Department of Microbiology, Faculty of Science, Khon Kaen University, Thailand). It was isolated from rotten cooked rice and multiple cultures in MRS agar. The bacterial suspension content was $4.9 \times 10^9$ cfu g$^{-1}$. The administration of *L. plantarum* $(10^8$ cfu g$^{-1}$) in this study was modified from studies of Son et al. (2009) and Giri et al. (2013). *Lactobacillus plantarum* diets were daily prepared as described by Irianto & Austin (2002). *Lactobacillus plantarum* suspension was adjusted to a concentration of $10^9$ cfu ml$^{-1}$ in 1 ml of 0.09% normal saline solution (NSS). It was then mixed thoroughly with 10 g of Diet 1 feed (Control) to obtain a dose of $10^8$ cfu g$^{-1}$ for Diet 3. Another 1 ml bacterial suspension $(10^9$ cfu ml$^{-1}$) was mixed thoroughly with 10 g of Diet 2 feed (5 g kg$^{-1}$ JA) to form Diet 4. Both Diet 1 and Diet 2 had the same volume of 0.09% NSS.

**Experimental diets**

The basal diet was modified from the work of Phumee et al. (2009). The added vitamins and minerals were similar to the work of Hien & Doolgindachbaporn (2011). The diets contain approximately 379 g kg$^{-1}$ crude protein and 86 g kg$^{-1}$ lipid. Three experimental diets were prepared. The first experiment consisted of seven treatments, that is, 0 (T1, control), 5 (T2), 10 (T3), 20 (T4), 40 (T5), 80 (T6) and 160 g kg$^{-1}$ JA for T7 (Table 1). The second and the third experiments had four treatments, that is, 0 g kg$^{-1}$ JA (Diet 1, control), 5 g kg$^{-1}$ JA (Diet 2), 1 g kg$^{-1}$ *L. plantarum* containing $10^8$ cfu g$^{-1}$ of *L. plantarum* (Diet 3) and 1 g kg$^{-1}$ *L. plantarum* + 5 g kg$^{-1}$ JA for Diet 4 (Tables 2). The ingredients were ground into a fine powder to pass through 320-μm mesh and were thoroughly mixed with soybean oil, and then water was added to produce stiff dough. The dough was then passed through a mincer to form pellets. The pellets were dried in an oven at 50 °C to 10% moisture content and kept in sealed polyethylene bags at 4 °C.

**Experimental design**

The *P. bocourti* fingerlings (an average of 3.57 g fish$^{-1}$) were obtained from the Phayao Fisheries Station, Phayao Province, Thailand. Upon arrival, the fish were kept in 1000-litre fibre tanks and fed a commercial diet for
2 months. Prior to the experiments, the fish were fed the control diet for 2 weeks. Three experiments were carried out. In the first experiment, different concentrations of Jerusalem artichoke (JA) were administrated to determine the optimal concentration for growth of *P. bocourti*. 560 individual fish of a similar size (an average of 35.36 g) were put in 28 glass tanks (capacity: 150 litres), 20 fish tank⁻¹.

In the second experiment, the optimal concentration of JA was combined with *L. plantarum*. 240 individual fish of a similar size (79.05 g) were allocated to 16 glass tanks (capacity: 150 L), 15 fish tank⁻¹. In the final experiment, eight randomly selected fish in each tank were weighed every 3 weeks. Growth performance and survival rate of *P. bocourti* were calculated using the following equations: specific growth rate (SGR) = 100 × (Ln final weight – Ln initial weight)/total duration of experiment; feed conversion ratio (FCR) = feed given (dried weight)/weight gain (wet weight); survival rate (%) = (final fish number/initial fish number) × 100.

**Immunological assays**

**Sample preparation** Blood samples were collected through the caudal vein from 1 fish tank⁻¹ using a 1-mL syringe at weeks 3, 6, 9 and 12 postfeeding. They were immediately withdrawn into Eppendorf tubes without anticoagulant. Blood samples were then allowed to clot (1 h at room temperature and 4 h at 4 °C) and centrifuged at 1500 g, 5 min and 4 °C. The serum was finally collected and stored at minus 20 °C until assayed.

Leucocyte isolates from peripheral blood were taken using a method modified from Chung & Secombes (1988). One mL of the collected bloods from 1 fish tank⁻¹ was diluted with 2 mL of RPMI 1640 (Gibthai). It was then carefully laid onto 3 mL of Histopaque (Sigma, St. Louis, MO, USA) in a 15-mL tube. The tube was centrifuged at 400 g for 30 min at room temperature. After centrifugation, the cell pellet was carefully laid onto 3 mL of Isotine (Sigma, St. Louis, MO, USA) in a 15-mL tube. The tube was centrifuged at 1000 g, 5 min and 4 °C. The cell pellet was finally collected, washed with 2 mL of RPMI 1640 (Gibthai) and used as a cell source for the preparation of leucocyte isolates.

**Growth performance**

For the first experiment, 20 fish in each tank were weighed every 2 weeks. For the second experiment, eight randomly

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**Table 1** Formulation and chemical proximate composition of the first experimental diets (% dry matter)

<table>
<thead>
<tr>
<th>Ingredients</th>
<th>T1</th>
<th>T2</th>
<th>T3</th>
<th>T4</th>
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<th>T6</th>
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<td>76</td>
<td>77</td>
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<td>0.179</td>
<td>0.179</td>
<td>0.179</td>
<td>0.179</td>
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<td>Proximate composition</td>
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<td>379.9</td>
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<td>86.2</td>
<td>85.8</td>
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<td>7.0</td>
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<td>7.9</td>
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<tr>
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<td>108.5</td>
<td>106.8</td>
<td>107.2</td>
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<td>968.3</td>
<td>975.9</td>
<td>971.6</td>
<td>977.3</td>
<td>976.8</td>
<td>969.9</td>
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<tr>
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<td>4507</td>
<td>4507</td>
<td>4487</td>
<td>4508</td>
<td>4508</td>
<td>4511</td>
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</tbody>
</table>

T1: 0 g kg⁻¹ JA; T2: 5 g kg⁻¹ JA; T3: 10 g kg⁻¹ JA; T4: 20 g kg⁻¹ JA; T5: 40 g kg⁻¹ JA; T6: 80 g kg⁻¹ JA; T7: 160 g kg⁻¹ JA

JA (g kg⁻¹), Jerusalem artichoke.

Vitamin mixture (mg kg⁻¹ mixture): retinyl acetate (500 000 IU g⁻¹), 0.6 mg; cholecalciferol (500 000 IU g⁻¹), 0.02 mg; D,L-a-tocopherol acetate, 30 mg; menadione, 5.25 mg; thiamin nitrate, 3.75 mg; riboflavin, 6 mg; pyridoxine hydrochloride, 6 mg; niacin, 10 mg; folic (96%), 2 mg; cyanocobalam (10%), 0.5 mg; ascorbic acid (92%), 100 mg; Ca pantothenate, 15 mg.

Mineral mixture (g kg⁻¹ mixture): FeSO₄·7H₂O, 0.03 g; CuSO₄·5 H₂O (25.00% copper), 0.006 g; ZnSO₄·7 H₂O (22.50% zinc), 0.6 g; MnSO₄·H₂O (31.80% manganese), 1.183 g; KI (3.8% iodine), 0.001 g; CaCO₃, 6 g.

Gross energy was measured in adiabatic bomb calorimeter (Leco AC 500).
Serum lysozyme activity Serum lysozyme activity was measured according to the method of Parry et al. (1965). 25 μL of undiluted serum sample was added to 175 μL of Micrococcus lysodeikticus (Sigma) suspension, 0.3 mg mL⁻¹ in 0.1 M citrate phosphate buffer, pH 5.8. After a rapid mixing, the change in turbidity was measured every 30 s for 10 min at 540 nm and at 25 °C using a micro-plate reader (Sunrise, TECAN; Germany). The equivalent unit of activity of the sample (compared with the standard) was determined and expressed as μg mL⁻¹ serum.

Phagocytosis activity The phagocytosis activity was assayed using a modification of the method of Yoshida & Kitao (1991), 200 μL leucocyte suspensions (2 × 10⁶ cells mL⁻¹) was spread on cover slips and incubated for 2 h. The non-adherent cells were then removed by washing with RPMI 1640. 200 μL of fluorescence latex beads (Sigma) solution 2 × 10⁷ of beads mL⁻¹ was added on each cover slip and incubated for 1.5 h at room temperature. After incubation, the non-phagocytic beads were washed with RPMI 1640. The cover slips were then fixed with methanol and stained with Diff-Quick staining dye (Sigma) for 10 s. Excessive stain was removed by washing with PBS (pH 7.4), and the number of phagocytic cells per 300 adhered cells was counted microscopically. The phagocytic index (PI) was determined as follows: PI = average number of beads per cell divided by the number of phagocytizing cells.

Respiratory burst activity The respiratory burst activity of P. bocourti peripheral blood leucocytes was determined using a modification of the method described by Secombes (1990). 175 μL samples of 6 × 10⁶ cells mL⁻¹ in PBS was placed in the wells of 96-well microtiter plates. 25 μL of nitro blue tetrazolium (NBT) at a concentration of 1 mg mL⁻¹ was added and incubated at 25 °C for 2 h. The supernatant was carefully discarded, and then, 125 μL of 100% methanol was added to each well. Discard all supernatant and wash each well again with 125 μL of 70% methanol well⁻¹ for twice. The supernatant in each well was then carefully discarded, and the plate was dried at room temperature for 30 min. After that, 125 μL of 2N KOH and 150 μL of DMSO were added to each well. The plate was then measured at 655 nm by a microplate reader (Sunrise, TECAN). Spontaneous O₂⁻ production = (absorbance NBT reduction of sample) – (absorbance of blank).

Challenge test Aeromonas hydrophila FW52 was kindly provided by Dr. Saowanit Tongpim. It was isolated from tilapia disease and β-haemolytic and grown in brain heart infusion agar (BHI). After incubation at 35 °C for 18 h, the cells were harvested by centrifugation at 672 g for 15 min at 4 °C. Then bacterial pellets were washed and re-suspended in a normal saline solution, a white buffy coat of leucocytes cells floated on the top of the Histopaque. Opaque interfaces were carefully removed by washing with RPMI 1640. The cover slips were then carefully discarded, and the plate was dried at room temperature for 30 min. After that, 125 μL of 2N KOH and 150 μL of DMSO were added to each well. The plate was then measured at 655 nm by a microplate reader (Sunrise, TECAN). Spontaneous O₂⁻ production = (absorbance NBT reduction of sample) – (absorbance of blank).

Table 2 Formulation and chemical proximate composition of the second and third experimental diets (g kg⁻¹ dry matter)

<table>
<thead>
<tr>
<th>Ingredients</th>
<th>Diet 1</th>
<th>Diet 2</th>
<th>Diet 3</th>
<th>Diet 4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fish meal</td>
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<td>417.7</td>
<td>417.7</td>
<td>417.7</td>
</tr>
<tr>
<td>Corn Starch</td>
<td>275.2</td>
<td>275.2</td>
<td>275.2</td>
<td>275.2</td>
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<tr>
<td>Soybean meal</td>
<td>75</td>
<td>75</td>
<td>75</td>
<td>75</td>
</tr>
<tr>
<td>Wheat flour</td>
<td>154</td>
<td>149</td>
<td>154</td>
<td>149</td>
</tr>
<tr>
<td>Cellulose</td>
<td>40</td>
<td>40</td>
<td>40</td>
<td>40</td>
</tr>
<tr>
<td>JA</td>
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<td>0.001</td>
<td>0.001</td>
<td>0.001</td>
</tr>
<tr>
<td>Soybean oil</td>
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<td>30.1</td>
<td>30.1</td>
<td>30.1</td>
</tr>
<tr>
<td>Vitamin premix²</td>
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<td>0.179</td>
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<tr>
<td>Minerals³</td>
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</tr>
</tbody>
</table>

Proximate composition of the experimental diets (g kg⁻¹ dry matter basis)

<table>
<thead>
<tr>
<th></th>
<th>Diet 1</th>
<th>Diet 2</th>
<th>Diet 3</th>
<th>Diet 4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Crude protein</td>
<td>379.2</td>
<td>376.5</td>
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<tr>
<td>Fibre</td>
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<tr>
<td>Ash</td>
<td>109</td>
<td>107.6</td>
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<tr>
<td>Dry matter</td>
<td>981.7</td>
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<tr>
<td>GE (cal g⁻¹)⁴</td>
<td>4463</td>
<td>4507</td>
<td>4463</td>
<td>4507</td>
</tr>
</tbody>
</table>

Diet 1: 0 g kg⁻¹ JA; Diet 2: 5 g kg⁻¹ JA; Diet 3: 108 cfu g⁻¹ of Lactobacillus plantarum; Diet 4: 5 g kg⁻¹ JA + 108 cfu g⁻¹ of L. plantarum

³ Mineral mixture (g kg⁻¹ mixture): FeSO₄·7H₂O, 0.03 g; CuSO₄·5 H₂O (25.00% copper), 0.006 g; ZnSO₄·7 H₂O (22.50% zinc), 0.6 g; MnSO₄·H₂O (31.80% manganese), 1.183 g; KI (3.8% iodine), 0.001 g; CaCO₃, 6 g.

⁴ Gross energy was measured in adiabatic bomb calorimeter (Leco AC 500).
solution (NSS), 0.09% NaCl. Prior to the experiment, the 96-h LD50 (A. hydrophila dose that killed 50% of the tested fish) was determined. 100 µL of each dilution (10^4, 10^5, 10^6, 10^7 and 10^8 cfu fish⁻¹) was injected intraperitoneally into each fish. For the control, the same volume of the NSS was used instead of the bacterial suspension. The LD50 value was calculated using the method described by Reed & Muench (1938). The results showed that the 4-day LD50 result was 5 x 10^7 cfu fish⁻¹. At the end of the second experiment, 20 fish from each treatment were injected intraperitoneally with 100 µL of 0.09% NSS containing 5 x 10^7 A. hydrophila. The same volume of NSS (100 µL) was intraperitoneally inoculated for each fish in the control treatment. The survival percentage in each treatment was recorded up to the 15th day of challenge.

Statistical Analysis

The data were analysed using SAS computer software (SAS 2003). If significant differences were found among treatments, Duncan’s multiple range test was used to rank the means (P < 0.05).

Results

Growth performance

In the first experiment, no significant difference in specific growth rate (SGR) was observed in fish fed Jerusalem artichoke (JA) supplements after 2 weeks (Fig. 1a). However, significantly lower SGR in fish fed 160 g kg⁻¹ JA was detected (Fig. 1a). Fish fed 5 g kg⁻¹ JA diet had significantly higher SGR than the control and other groups after 12 weeks of the feeding trial (Fig. 1a). No significant differences in feed conversion ratio (FCR) were observed (Fig. 1b).

In the second experiment, fish fed 5 g kg⁻¹ JA or 10⁸ cfu g⁻¹ L. plantarum had significantly higher SGR and FCR compared with the control after 12 weeks of the feeding trial (Fig. 2a,b). A diet combination of JA and L. plantarum showed significantly higher SGR and FCR than the control and individual applications (Fig. 2a,b).

Immune response

Fish fed 5 g kg⁻¹ Jerusalem artichoke (JA) or 10⁸ cfu g⁻¹ L. plantarum singly or combined showed significantly stimulated serum lysozyme activity compared with the control after 12 weeks (Fig. 3a). Fish fed JA combined with L. plantarum showed significantly higher serum lysozyme activity than the control and individual applications. However, no significant difference was observed between diet synbiotic and L. plantarum diet. For the phagocytic index, a significant difference was only observed in fish fed L. plantarum and synbiotic diets after 9 and 12 weeks, respectively (Fig. 3b). Fish fed 5 g kg⁻¹ JA or 10⁸ cfu g⁻¹ L. plantarum significantly increased respiratory burst activity after 6 weeks of the feeding trial (Fig. 3c). Diets with a combination of JA and L. plantarum showed significantly higher respiratory burst activity than both the
control and the individual applications after 9 and 12 weeks (Fig. 3c).

Challenge test

Fish fed 5 g kg\(^{-1}\) Jerusalem artichoke (JA) and 10\(^8\) cfu g\(^{-1}\) \(L.\) \(plantarum\) either singly or combined had a significantly increased postchallenge survival rate compared to the control (Fig. 4). Synbiotic diets resulted in a significantly higher postchallenge survival rate than both the control and individual applications (Fig. 4). No significant difference on postchallenge survival rate was observed between fish fed JA and \(L.\) \(plantarum\) (Fig. 4).

Discussion

Jerusalem artichoke

Jerusalem artichoke (JA) has been found to be one of the most important sources of fructose and inulin; fresh tuber contains 50–70 g kg\(^{-1}\) of inulin-type fructan (Li & Chan-Halbrendt 2009). Inulin is a fructooligosaccharide commonly used as a prebiotic in human and animal feedstuffs (Vulevic \textit{et al.} 2004; Seifert & Watzl 2007). Recently, it has been proposed that inulin may also have useful applications in aquaculture to stimulate beneficial gut bacteria, suppress pathogens and enhance the immune response (Ringø \textit{et al.} 2010). The effects of inulin on growth performance have been evaluated in several aquaculture species with varied results (Cerezuela \textit{et al.} 2013). In the present study, dietary supplementation of JA (the source of inulin) at 5 g kg\(^{-1}\) resulted in beneficial effects on the growth performance of \textit{P. bocourti}. This result was in agreement with results from studies on Nile tilapia, \textit{Oreochromis niloticus} (Ibrahim \textit{et al.} 2010); turbot larvae, \textit{Psetta maxima} (Mahious \textit{et al.} 2006); rainbow trout \textit{Oncorhynchus mykiss} (Ortiz \textit{et al.} 2013); sea cucumber, \textit{Apostichopus japonicus} (Zhang \textit{et al.} 2010); juvenile ovate pompano, \textit{Trachinotus ovatus} (Zhang \textit{et al.} 2014); and juvenile white shrimp, \textit{Litopenaeus vannamei} (Zhou \textit{et al.} 2007), but in contrast to studies on large yellow croaker, \textit{Larimichthys crocea} (Ai \textit{et al.} 2011); red drum, \textit{Sciaenops ocellatus} (Burr \textit{et al.} 2009); Atlantic salmon, \textit{Salmo salar} (Grisdale-Helland \textit{et al.} 2008); white shrimp, \textit{Litopenaeus vannamei} (Li \textit{et al.} 2007); white shrimp, \textit{Litopenaeus vannamei} (Luna-González \textit{et al.} 2012); and beluga sturgeon, \textit{Huso huso} (Reza \textit{et al.} 2009). The beneficial effects of prebiotics are associated with an increase in beneficial bacteria (e.g. \textit{bifidobacteria} and \textit{lactobacilli}), an inhibition of pathogens, an enhancement in immunity and an increase in digestibility of feed (Saad \textit{et al.} 2013). It seems very likely that the reason for the growth promoted by JA in the present study is also due to such beneficial effects. A diet of 160 g kg\(^{-1}\) JA showed adverse effects on growth performance of \textit{P. bocourti}; this is in agreement with Ringø \textit{et al.} (2010) and Cerezuela \textit{et al.} (2013) who showed adverse effects of high inulin concentration on enterocytes in fish.

\(Lactobacillus\) \textit{plantarum} Jerusalem artichoke

\textit{Aquat. Nutr.}, 22; 444–456 © 2015 John Wiley & Sons Ltd
higher concentrations of JA used in this study may have a similar effect on *P. bocourti* gut cells, resulting in a decrease of growth performance of *P. bocourti*. However, the gut morphology of *P. bocourti* needs to be examined in further studies to confirm this hypothesis.

Dietary administrations of inulin have been reported to stimulate the immune system of gilthead seabream, *Sparus aurata*, affecting factors such as serum complement, leucocyte phagocytic, leucocyte respiratory burst activities and IgM level (Cerezuela et al. 2012). Ibrahim et al. (2010) reported that Nile tilapia fed 5 g kg\(^{-1}\) inulin showed significantly improved respiratory burst and serum lysozyme activities. Different results were observed in studies on gilthead seabream (Cerezuela et al. 2008) and hybrid surubim, *Pseudoplatystoma* sp. (Mourião et al. 2012). They reported that diet administrations of inulin had no effects

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**Figure 3** Serum lysozyme activity (a), phagocytic index (b) and respiratory burst activity (c) of *Pangasius bocourti* fed diets with graded levels of dietary Jerusalem artichoke and *Lactobacillus plantarum* (mean ± SE). Columns sharing the same superscript letter are not significantly different (\(P < 0.05\)) (by Duncan’s multiple range test).

**Figure 4** Postchallenge survival rate following a 15-day *Aeromonas hydrophila* challenge of *Pangasius bocourti* fed with graded doses of dietary Jerusalem artichoke and *Lactobacillus plantarum* (mean ± SE). Columns sharing the same superscript letter are not significantly different (\(P < 0.05\)) (by Duncan’s multiple range test).
on fish immune system. In the present study, significantly increased serum lysozyme and respiratory burst activities were detected in fish fed 5 g kg$^{-1}$ Jerusalem artichoke (JA) compared with the control treatment. However, no significant effects of dietary JA were observed on phagocytic activity. The effect of inulin on the immune response is contradictory. The reasons may be attributable to the levels and contents, the particular aquatic species and experimental conditions. The beneficial effects of dietary inulin are possibly conferred through gastrointestinal microbiota changes (Bakke-McKellep et al. 2007; Burr et al. 2010). Additionally, oligosaccharides can be selectively fermented by Bifidobacterium to reproduce probiotic bacteria and inhibit the adherence and colonization of pathogens (Kane-ko et al. 1995). Mahious et al. (2006) reported that dietary inulin administration significantly enhanced the growth of Bacillus sp. in the gut of larval turbot. These beneficial bacterial can use inulin as a single source of carbon. The authors inferred that significantly increasing the numbers of Bacillus sp. could play a role in the beneficial effect of inulin on turbot growth, as Bacillus sp. have been demonstrated as effective probiotics in fish. Similarly, Reffie et al. (2006) revealed that inulin could selectively stimulate growth of beneficial bacteria in the gastrointestinal tract of Atlantic salmon, Salmo salar.

Regarding the disease resistance effects of dietary probiotics, dietary administration of oligosaccharides effectively enhanced the immunity of animals and thus enhanced the resistance to pathogen infection (Bornet & Brouns 2002; Zhang et al. 2010; Ai et al. 2011). The improvement in resistance against A. hydrophila with 5 g kg$^{-1}$ JA (the source of inulin) could be due to the stimulation of the growth of Bacillus subtilis, which can enhance the non-specific immune response of fish (Mahious et al. 2006). The functions of prebiotics are related to physiological changes in the host’s gut; metabolic products of bacteria are probably the major effectors of most detected effects. The most important are the short-chain fatty acids (SCFA) such as acetate, propionate and butyrate. It has been reported that the consumption of prebiotics could double the pool of SCFA in the alimentary tract. These SCFA acidify the gastrointestinal environment, which is beneficial for the development of probiotic bacteria and disadvantageous to the growth of pathogens (Blaut 2002; Venter 2007).

**Lactobacillus plantarum**

In the present work, *P. bocourti* fed with a diet of $10^8$ cfu g$^{-1}$ *L. plantarum* showed significantly improved SGR and FCR after 12 weeks. Similar results were observed in rainbow trout, Oncorhynchus mykiss (Andani et al. 2012); rohu, Labeo rohita (Giri et al. 2013, 2014); Nile tilapia, Oreochromis niloticus (Jatobá et al. 2011); white shrimp, Litopenaeus vannamei (Kongnum & Hongpattarakere 2012); grouper, Epinephelus coioides (Son et al. 2009), gilthead seabream, Sparus aurata, L. (Suzer et al. 2008); and blue swimming crab, Portunus pelagicus (Talpur et al. 2013). The available evidence suggests that beneficial bacteria involved in the decomposition of nutrients in the gastrointestinal tract provide physiologically active compounds such as enzymes, amino acids and vitamins (Gatesoupe 1999; Yanbo & Zirong 2006; Wang 2007; Ringø et al. 2010) and hence improve feed utilization and digestion. Carbohydrates in the gastrointestinal tract (GI) are consumed by probiotic bacteria for their metabolism and produce short-chain fatty acids (SCFA). These by-products are the main source of energy for intestinal epithelial cells. They may play an important role in increasing the villi height of the GI which improves nutrient absorption by providing more absorptive surface area (Casparry 1992; Blottiire et al. 2003; Pirarat et al. 2011; Cerezuela et al. 2013). This could account for the enhancement of FCR by dietary *L. plantarum* supplementation in this study. The possible mechanisms whereby dietary probiotics affect villi height in gut epithelial cells are not well understood, and more studies are necessary to elucidate this.

The ability to augment non-specific and specific immune responses of probiotics has been well documented. For mammals, probiotics beneficially stimulated host health in several ways. They include activating the mucosal innate immunity and the gut-associated T cells, releasing antimicrobial substances, competition for chemicals, energy and adhesion sites, and inhibition of virulence expression (Forchielli & Walker 2005; Bauer et al. 2006; Oelschlaeger 2010; Reiff & Kelly 2010). For fish, the effects of probiotics on immunomodulation and disease resistance are still poorly understood (Ai et al. 2011). Probiotic bacteria have been shown to enhance non-specific and specific immune responses in the gut-associated lymphoid tissue and the systemic immunity of fish. For example, phagocytic, lysozyme, alternative complement activities, superoxide anion production and expression of certain cytokines and antibodies are well studied (Kim & Austin 2006; Balázar et al. 2007; Panigrahi et al. 2007; Ariojo et al. 2008; Al-Dohail et al. 2009; Nayak 2010). In the present work, dietary supplementation with $10^8$ cfu g$^{-1}$ *L. plantarum* significantly increased serum lysozyme activity after 12 weeks. Similar
results in stimulating lysozyme activity were observed in kelp grouper, *Epinephelus bruneus* (Harikrishnan et al. 2010); grouper, *E. coioides* (Son et al. 2009); and rohu, *Labeo rohita* (Giri et al. 2013). However, lysozyme activity in the rainbow trout did not exhibit any significant differences after 2 weeks and 30 days under probiotic feeding with lactic acid bacteria (Andani et al. 2012). For phagocytosis and respiratory burst activities, our results indicated that fish fed with *L. plantarum* had significantly higher activities than the control. These were similar to previous results in grouper, *E. coioides* (Son et al. 2009); cobia, *Rachycentron canadum* (Geng et al. 2012); kelp grouper, *E. bruneus* (Harikrishnan et al. 2010); and rohu, *Labeo rohita* (Giri et al. 2013) but did not agree with previous studies on gilthead seabream, *Sparus aurata* L. (Salinas et al. 2005). This discrepancy may be attributable to different aquatic species and experimental conditions such as water quality, hardness, dissolved oxygen, temperature, pH, osmotic pressure, mechanical friction and the environmental microbiota (Zhang et al. 2014). These environmental factors affect probiotics in the gastrointestinal tract mainly with respect to establishment, proliferation and function (Das et al. 2008). The discrepancy may also be due to different doses given, reported as one of the factors affecting immunostimulatory activities of probiotics (Panigrahi et al. 2005; Kumar et al. 2008). Discrepancies in stimulating immune parameters of the same probiotics are also dependent on the feeding duration (Diaz-Rosales et al. 2006; Diaz-Rosales et al. 2009).

Several studies have reported that dietary probiotic administration effectively increased disease resistance of fish (Merrifield et al. 2010; Nayak 2010); dietary administration of *L. plantarum* to white shrimp (*Litopenaeus vannamei*) promoted disease resistance by activating non-specific immune defences (Chiu et al. 2007). Son et al. (2009) indicated that dietary supplementation of *L. plantarum* significantly improved growth and survival rate of grouper and protected against *Streptococcus* sp. and an iridovirus by increasing alternative complement, phagocytic, respiratory burst and lysozyme activities. Andani et al. (2012) showed that *L. plantarum* stimulated both cellular and humoral immune responses by increasing the total immunoglobulin level as well as enhancing alternative complement and lysozyme activity and thus protected rainbow trout against a pathogen, *Yersinia ruckeri*. Giri et al. (2013) reported that dietary administration of *L. plantarum* significantly enhanced serum lysozyme, alternative complement, phagocytosis, respiratory burst and superoxide dismutase of rohu and hence protected fish against *A. hydrophila* infection. In the present study, dietary administration of *L. plantarum* stimulated non-specific immunity and resistance to *A. hydrophila* infection. The increase in resistance against *A. hydrophila* in fish fed with *L. plantarum* may be due to increased non-specific immune response. *L. plantarum* is also able to produce antimicrobial substances such as plantaricin that are active against certain pathogens (Cebeci & Gu¨rakan 2003). However, the precise mechanism of how probiotics stimulate the non-specific immune system of fish is not clarified as yet, and further research on this aspect is needed.

**Interaction**

Prebiotics could be selectively fermented by specific intestinal bacteria and modulate the growth and/or the activity of the bacteria (Gibson 2004). Administration of probiotics in the form of synbiotics to the host yielded significantly better results than that given in individual application (Rodriguez-Estrada et al. 2009). Supplementation of probiotics in the synbiotics can increase the survival of probiotics in the gastrointestinal tract and hence stimulate faster reproducibility of probiotics in vivo to beneficial effect (Bielecka et al. 2002; Bornet & Brouns 2002). Recently, scientists have paid great attention in the interactions, especially synergistic actions, between prebiotics and probiotics (Zhang et al. 2014). Synergistic actions between inulin and *B. subtilis* in gilthead seabream (*Cerezuela et al. 2012*), chitosan and *B. subtilis* in cobia (Geng et al. 2011), yeast extract and *Bacillus licheniformis* in Nile tilapia (*Hassaan et al. 2014*), fructooligosaccharide and *B. licheniformis* in triangular bream (Zhang et al. 2013), and fructooligosaccharide and *B. subtilis* in juvenile ovate pompano (Zhang et al. 2014) have been conducted. Similar significant interactions between *L. plantarum* and Jerusalem artichoke (the source of inulin) on growth, non-specific immunity and disease resistance of *P. bocourti* were observed in the present study. The improvement effect of synbiotics can possibly explain the immunostimulatory nature of prebiotic stimulated growth of beneficial bacteria such as *Lactobacillus* spp. and *Bacillus* spp. (Sang et al. 2011). Prebiotics could be selectively fermented by bifidobacterium to reproduce probiotic bacteria and inhibit the adherence and colonization of pathogens, resulting in an enhanced immune function of the host (Bornet & Brouns 2002). Unlike the prebiotics, the probiotics gave beneficial effects when available in a high numbers by improving intestinal microbial balance and the microbiota bacteria which
secrete many enzymes to compete for nutrients and sites, while inhibiting other bacteria (Moriarty 1998). Cerezuela et al. (2013) indicated that inulin and B. subtilis administration seemed to provoke a great liberation of mucin. It has been suggested that acidic mucin could protect against bacterial translocation (Robertson & Witcomb 1997; Deplancke & Gaskins 2001), whereas neutral mucin has been related to digestion processes (Clarke & Witcomb 1980; Grau et al. 1992). However, the mechanisms responsible for this secretion remain unknown. More studies are necessary to understand the importance of alteration of mucin types in fish intestine.

**Conclusions**

Dietary administration of JA and L. plantarum either singly or combined significantly increased the growth, innate immunity and protection against infection for P. bocourti. These findings are of great importance and interest to aquaculture research and the fish farming industry. However, the relationships and action mechanisms of probiotic L. plantarum and Jerusalem artichoke (the source of inulin) in significantly increasing non-specific immunity and disease resistance in juvenile P. bocourti need to be further investigated. The expression of immune genes and the modulation of microbiota in the gastrointestinal tract of P. bocourti also require further study.

**Acknowledgements**

The authors wish to thank the Khon Kaen University, and Higher Education Research Promotion and National Research University Project of Thailand, Office of the Higher Education Commission, through the Food and Functional Food Research Cluster of Khon Kaen University, for financial assistance. Thanks are also due to Drs. Ong-ard Lawhavinit, Prapansak Srisapoome, Saowanit Tongpim, Nicha Charoensri, Ratchanu Meidong (Ph.D student), Dr. David Higgs and Dr. David JH Blake for their kind assistance in carrying out the experiments or editing.

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