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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 10^8 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), 10^8 cfu g^{-1} *L. plantarum* (Diet 3) and 5 g kg^{-1} JA + 10^8 cfu g^{-1} *L. plantarum* (Diet 4). Fish fed 5 g kg^{-1} JA or 10^8 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*

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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 chemo-therapeutics 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 & Gürakan 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 planetarium* (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 4.9×10^9 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

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

	Diets (g kg^{-1})						
Ingredients	T1	T2	T3	T4	T5	T6	T7
Fish meal	417.7	417.7	417.7	417.7	417.7	417.7	417.7
Corn Starch	275.2	275.2	275.2	275.2	275.2	275.2	275.2
Soybean meal	75	75	75	76	77	80	84
Wheat flour	154	149	144	134	114	74	0
Cellulose	40	40	40	39	38	35	25
JA[1]	**0**	**5**	**10**	**20**	**40**	**80**	**160**
Soybean oil	30.1	30.1	30.1	30.1	30.1	30.1	30.1
Vitamin premix[2]	0.179	0.179	0.179	0.179	0.179	0.179	0.179
Minerals[3]	7.821	7.821	7.821	7.821	7.821	7.821	7.821
Proximate composition of the experimental diets (g kg^{-1} dry matter basis)							
Crude protein	379.2	376.5	378.7	379.7	379.9	377.7	378.2
Crude lipid	86.6	84.6	85.4	85.7	86.2	85.8	86.7
Fibre	7.6	7.5	7.0	7.8	7.7	7.9	7.6
Ash	109	107.6	108.5	106.8	107.2	107.7	108.3
Dry matter	981.7	968.3	975.9	971.6	977.3	976.8	969.9
GE (cal g^{-1})[4]	4463	4507	4507	4487	4508	4511	4512

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

[1] JA (g kg^{-1}), Jerusalem artichoke.

[2] 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; cyanocobalamin (10%), 0.5 mg; ascorbic acid (92%), 100 mg; Ca pantothenate, 15 mg.

[3] Mineral mixture (g kg^{-1} mixture): $FeSO_4.7H_2O$, 0.03 g; $CuSO_4.5\ H_2O$ (25.00% copper), 0.006 g; $ZnSO_4.7\ H_2O$ (22.50% zinc), 0.6 g; $MnSO_4.\ H_2O$ (31.80% manganese), 1.183 g; KI (3.8% iodine), 0.001 g; $CaCO_3$, 6 g.

[4] Gross energy was measured in adiabatic bomb calorimeter (Leco AC 500).

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, five randomly selected fish from the second experiment were exposed with *A. hydrophila* for 15 days. All experiments were laid out in a completely randomized design (CRD) with four replications, and a flow-through freshwater system was used. The diets were hand-fed to the fish *ad libitum* twice a day at 9:00 a.m. and 5 p.m., and water temperature was maintained at 25–29 °C and pH in a range of 7.5–8.2. The aerated system was utilized, and the dissolved oxygen level was maintained at no <5 mg L^{-1}.

Growth performance

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

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

Ingredients	Diets (g kg^{-1})			
	Diet 1	Diet 2	Diet 3	Diet 4
Fish meal	417.7	417.7	417.7	417.7
Corn Starch	275.2	275.2	275.2	275.2
Soybean meal	75	75	75	75
Wheat flour	154	149	154	149
Cellulose	40	40	40	40
JA[1]	**0**	**5**	**0**	**5**
Soybean oil	30.1	30.1	30.1	30.1
Vitamin premix[2]	0.179	0.179	0.179	0.179
Minerals[3]	7.821	7.821	7.821	7.821
Proximate composition of the experimental diets (g kg^{-1} dry matter basis)				
Crude protein	379.2	376.5	379.2	376.5
Crude lipid	86.6	84.6	86.6	84.6
Fibre	7.6	7.5	7.6	7.5
Ash	109	107.6	109	107.6
Dry matter	981.7	968.3	981.7	968.3
GE (cal g^{-1})[4]	4463	4507	4463	4507

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

[1] JA (g kg^{-1}), Jerusalem artichoke.

[2] 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; cyanocobalamin (10%), 0.5 mg; ascorbic acid (92%), 100 mg; Ca pantothenate, 15 mg.

[3] Mineral mixture (g kg^{-1} mixture): $FeSO_4.7H_2O$, 0.03 g; $CuSO_4.5H_2O$ (25.00% copper), 0.006 g; $ZnSO_4.7H_2O$ (22.50% zinc), 0.6 g; $MnSO_4.H_2O$ (31.80% manganese), 1.183 g; KI (3.8% iodine), 0.001 g; $CaCO_3$, 6 g.

[4] Gross energy was measured in adiabatic bomb calorimeter (Leco AC 500).

tion, a white buffy coat of leucocytes cells floated on the top of the Histopaque. Opaque interfaces were carefully aspirated with a Pasteur pipette and transferred into a clean 15-ml tube. Phosphate buffer solution (PBS) was then added to attain 6 mL and gently mixed by aspiration. It was centrifuged at 250 g for 10 min. This washing step was repeated three times to remove any residual Histopaque. The isolated leucocytes cells were then re-suspended in the PBS and adjusted to the required cell numbers for phagocytosis and respiratory activities.

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 (2×10^6 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 2×10^7 of beads mL^{-1} was added on each cover slip and incubated for 1.5 h at room temperature. After incubation, the non-phagocyte 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 phagocyte 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 6×10^6 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 O_2^- 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 (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 5×10^7 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 5×10^7 *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 10^8 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 10^8 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 10^8 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

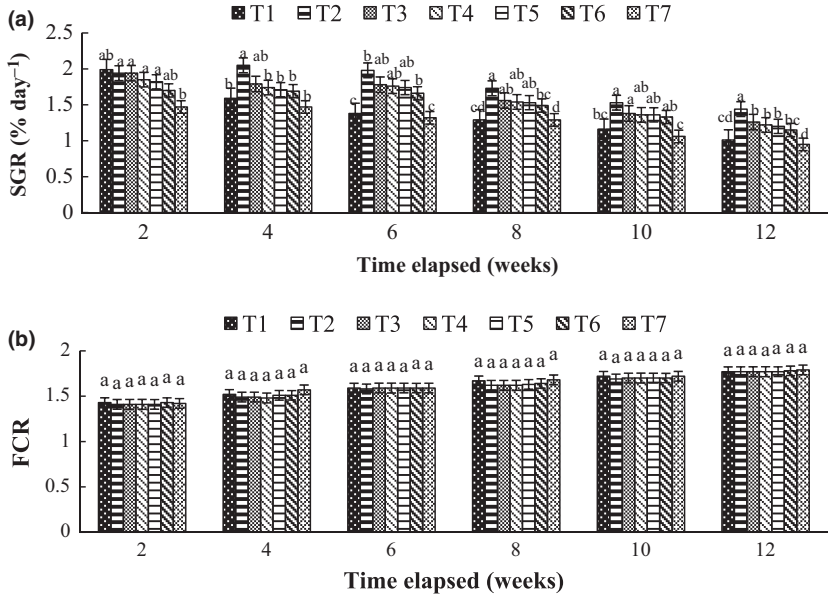

Figure 1 Specific growth rate (a) and feed conversion ratio (b) of *Pangasius bocourti* fed diets with graded levels of dietary Jerusalem artichoke (mean ± SE). Columns sharing the same superscript letter are not significantly different ($P < 0.05$) (by Duncan's multiple range test).

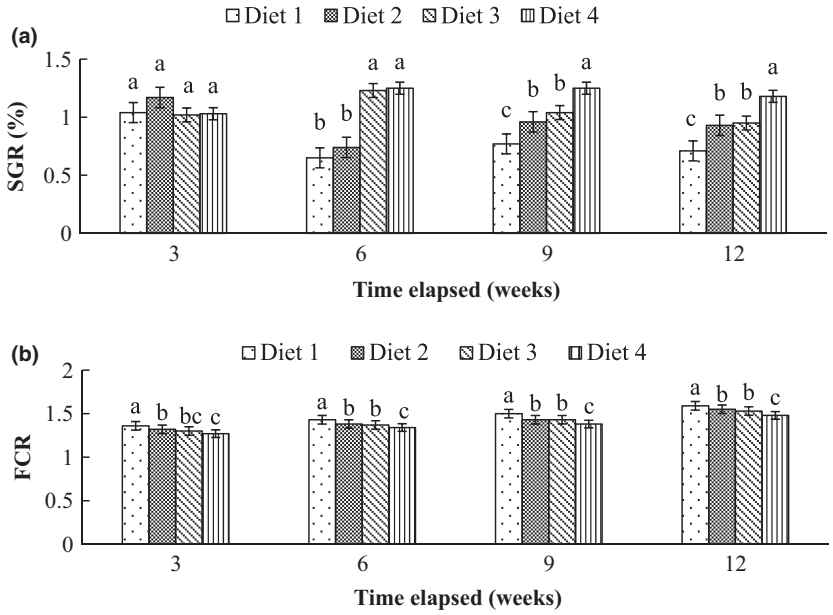

Figure 2 Specific growth rate (a) and feed conversion ratio (b) of *Pangasius bocourti* fed diets with graded levels of dietary Jerusalem artichoke and *Lactobacillus plantarum* (mean ± SE). Columns sharing the same superscript letter are not significantly different ($P < 0.05$) (by Duncan's multiple range test).

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 *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 *et al.* 2010). The effects of inulin on growth performance have been evaluated in several aquaculture species with varied results (Cerezuela *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 *P. bocourti*. This result was in agreement with results from studies on Nile tilapia, *Oreochromis niloticus* (Ibrahem *et al.* 2010); turbot larvae, *Psetta maxima* (Mahious *et al.* 2006); rainbow trout *Oncorhynchus mykiss* (Ortiz *et al.* 2013); sea cucumber, *Apostichopus japonicus* (Zhang *et al.* 2010); juvenile ovate pompano, *Trachinotus ovatus* (Zhang *et al.* 2014); and juvenile white shrimp, *Litopenaeus vannamei* (Zhou *et al.* 2007), but in contrast to studies on large yellow croaker, *Larimichthys crocea* (Ai *et al.* 2011); red drum, *Sciaenops ocellatus* (Burr *et al.* 2009); Atlantic salmon, *Salmo salar* (Grisdale-Helland *et al.* 2008); white shrimp, *Litopenaeus vannamei* (Li *et al.* 2007); white shrimp, *Litopenaeus vannamei* (Luna-González *et al.* 2012); and beluga sturgeon, *Huso huso* (Reza *et al.* 2009). The beneficial effects of prebiotics are associated with an increase in beneficial bacteria (e.g. *bifidobacteria* and *lactobacilli*), an inhibition of pathogens, an enhancement in immunity and an increase in digestibility of feed (Saad *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 *P. bocourti*; this is in agreement with Ringø *et al.* (2010) and Cerezuela *et al.* (2013) who showed adverse effects of high inulin concentration on enterocytes in fish. The

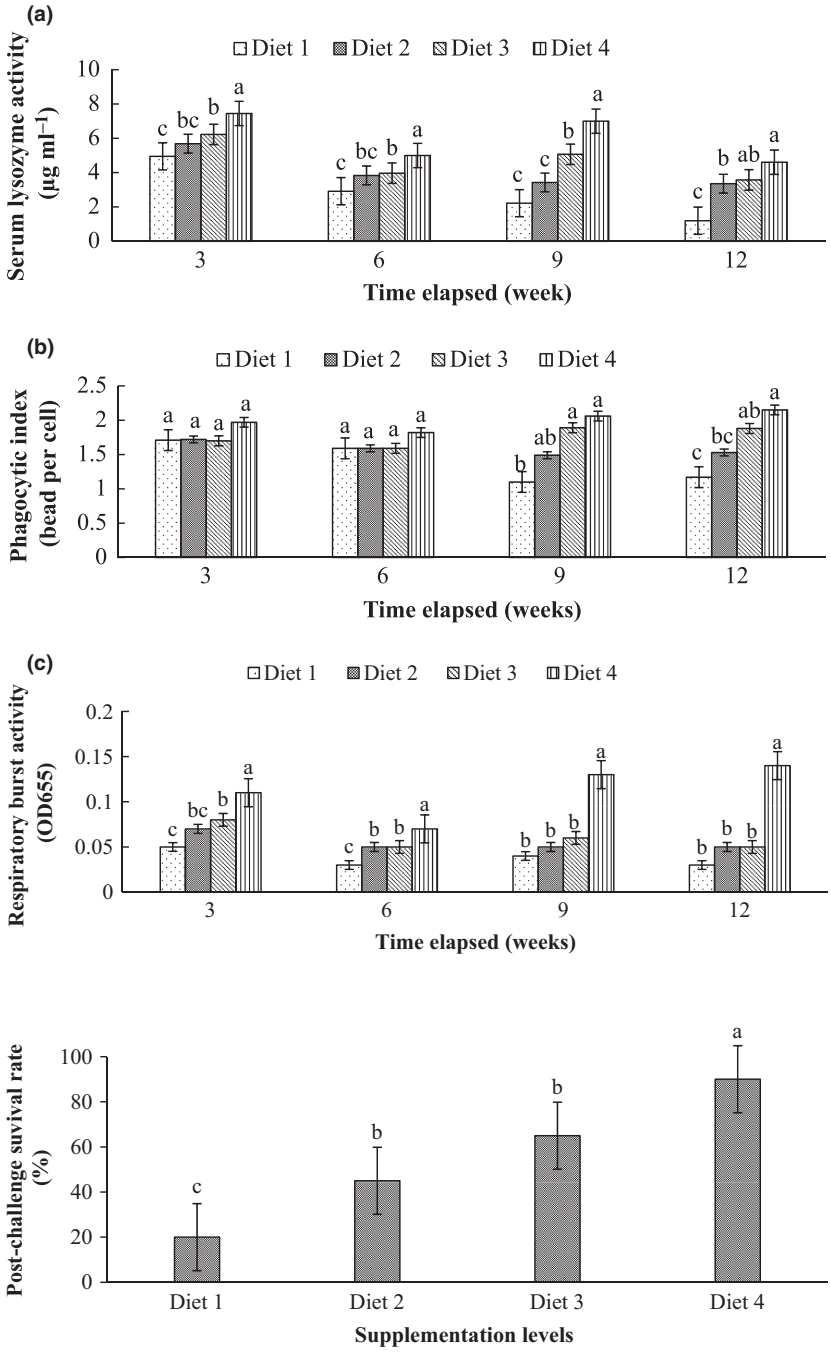

Figure 3 Serum lysozyme activity (a), phagocytic index (b) and respiratory burst activity (c) of *Pangasius bocourti* fed diets with graded levels of dietary Jerusalem artichoke and *Lactobacillus plantarum* (mean ± SE). Columns sharing the same superscript letter are not significantly different ($P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

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). Ibrahem *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. (MouriÑO *et al*. 2012). They reported that diet administrations of inulin had no effects

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 (Kaneko *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, Refstie *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 prebiotics, dietary administration of oligosaccharide 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 (Caspary 1992; Blottiere *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; Balcázar *et al*. 2007; Panigrahi *et al*. 2007; Arijo *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; Díaz-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 & Gürakan 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 prebiotics 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 & Wright 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.

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References

Ai, Q., Xu, H., Mai, K., Xu, W., Wang, J. & Zhang, W. (2011) Effects of dietary supplementation of *Bacillus subtilis* and fructooligosaccharide on growth performance, survival, non-specific immune response and disease resistance of juvenile large yellow croaker, *Larimichthys crocea*. *Aquaculture*, **317**, 155–161.

Al-Dohail, M.A., Hashim, R. & Aliyu-Paiko, M. (2009) Effects of the probiotic, *Lactobacillus acidophilus*, on the growth performance, haematology parameters and immunoglobulin concentration in African Catfish (*Clarias gariepinus*, Burchell 1822) fingerling. *Aquacult. Res*., **40**, 1642–1652.

Andani, H.R.R., Tukmechi, A., Meshkini, S. & Sheikhzadeh, N. (2012) Antagonistic activity of two potential probiotic bacteria from fish intestines and investigation of their effects on growth performance and immune response in rainbow trout (*Oncorhynchus mykiss*). *J. Appl. Ichthyol*., **28**, 728–734.

Anh, P.T., Kroeze, C., Bush, S.R. & Mol, A.P.J. (2010) Water pollution by Pangasius production in the Mekong Delta, Vietnam: causes and options for control. *Aquacult. Res*., **42**, 108–128.

Arijo, S., Brunt, J., Chabrillon, M., Diaz-Rosales, P. & Austin, B. (2008) Subcellular components of *Vibrio harveyi* and probiotics induce immune responses in rainbow trout, *Oncorhynchus mykiss* (Walbaum), against *V. harveyi*. *J. Fish Dis*., **31**, 579–590.

Bakke-McKellep, A.M., Penn, M.H., Salas, P.M., Refstie, S., Sperstad, S., Landsverk, T., Ringo, E. & Krogdahl, A. (2007) Effects of dietary soyabean meal, inulin and oxytetracycline on intestinal microbiota and epithelial cell stress, apoptosis and proliferation in the teleost Atlantic salmon (*Salmo salar* L.). *Br. J. Nutr*., **97**, 699–713.

Balcázar, J.L., de Blas, I., Ruiz-zarzuela, I., Vendrell, D., Calvo, A.C., Márquez, I., Gironés, O. & Muzquiz, J.L. (2007) Changes in intestinal microbiota and humoral immune response following probiotic administration in brown trout (*Salmo trutta*). *Br. J. Nutr*., **97**, 522–527.

Bauer, E., Williams, B.A., Smidt, H., Verstegen, M.W. & Mosenthin, R. (2006) Influence of the gastrointestinal microbiota on development of the immune system in young animals. *Curr. Issues Intest. Microbiol*., **7**, 35–51.

Bielecka, M., Biedrzycka, E. & Majkowska, A. (2002) Selection of probiotics and prebiotics for synbiotics and confirmation of their in vivo effectiveness. *Food Res. Int*., **35**, 125–131.

Blaut, M. (2002) Relationship of prebiotics and food to intestinal microflora. *Eur. J. Nutr*., **41**(Suppl 1), I11–I16.

Blottiere, H.M., Buecher, B., Galmiche, J.P. & Cherbut, C. (2003) Molecular analysis of the effect of short-chain fatty acids on intestinal cell proliferation. *Proc. Nutr. Soc*., **62**, 101–106.

Bornet, F.R. & Brouns, F. (2002) Immune-stimulating and gut health-promoting properties of short-chain fructo-oligosaccharides. *Nutr. Res. Rev*., **60**, 326–334.

Burr, G., Gatlin, D.M. & Hume, M. (2009) Effects of the Prebiotics GroBiotic®-A and Inulin on the Intestinal Microbiota of Red Drum, *Sciaenops ocellatus*. *J. World Aquaculture Soc*., **40**, 440–449.

Burr, G., Hume, M., Ricke, S., Nisbet, D. & Gatlin, D. 3rd (2010) In vitro and in vivo evaluation of the prebiotics GroBiotic-A, inulin, mannanoligosaccharide, and galactooligosaccharide on the digestive microbiota and performance of hybrid striped bass (*Morone chrysops x Morone saxatilis*). *Microb. Ecol*., **59**, 187–198.

Caspary, W.F. (1992) Physiology and pathophysiology of intestinal absorption. *Am. J. Clin. Nutr*., **55**, 299S–308S.

Cebeci, A. & Gürakan, C. (2003) Properties of potential probiotic *Lactobacillus plantarum* strains. *Food Microbiol*., **20**, 511–518.

Cerezuela, R., Cuesta, A., Meseguer, J. & Ángeles Esteban, M. (2008) Effects of inulin on gilthead seabream (*Sparus aurata* L.) innate immune parameters. *Fish Shellfish Immunol*., **24**, 663–668.

Cerezuela, R., Guardiola, F.A., Meseguer, J. & Esteban, M.Á. (2012) Increases in immune parameters by inulin and *Bacillus subtilis* dietary administration to gilthead seabream (*Sparus aura-*

ta L.) did not correlate with disease resistance to *Photobacterium damselae*. *Fish Shellfish Immunol*., **32**, 1032–1040.

Cerezuela, R., Fumanal, M., Tapia-Paniagua, S.T., Meseguer, J., Moriñigo, M.Á. & Esteban, M.Á. (2013) Changes in intestinal morphology and microbiota caused by dietary administration of inulin and *Bacillus subtilis* in gilthead sea bream (*Sparus aurata* L.) specimens. *Fish Shellfish Immunol*., **34**, 1063–1070.

Chi, Z.-M., Zhang, T., Cao, T.-S., Liu, X.-Y., Cui, W. & Zhao, C.-H. (2011) Biotechnological potential of inulin for bioprocesses. *Bioresour. Technol*., **102**, 4295–4303.

Chiu, C.-H., Guu, Y.-K., Liu, C.-H., Pan, T.-M. & Cheng, W. (2007) Immune responses and gene expression in white shrimp, *Litopenaeus vannamei*, induced by *Lactobacillus plantarum*. *Fish Shellfish Immunol*., **23**, 364–377.

Chung, S. & Secombes, C.J. (1988) Analysis of events occurring within teleost macrophages during the respiratory burst. *Comp. Biochem. Physiol. B*, **89**, 539–544.

Clarke, A.J. & Witcomb, D.M. (1980) A study of the histology and morphology of the digestive tract of the common eel (*Anguilla anguilla*). *J. Fish Biol*., **16**, 159–170.

Daniels, C.L., Merrifield, D.L., Boothroyd, D.P., Davies, S.J., Factor, J.R. & Arnold, K.E. (2010) Effect of dietary Bacillus spp. and mannan oligosaccharides (MOS) on European lobster (*Homarus gammarus L.*) larvae growth performance, gut morphology and gut microbiota. *Aquaculture*, **304**, 49–57.

Das, S., Ward, L.R. & Burke, C. (2008) Prospects of using marine actinobacteria as probiotics in aquaculture. *Appl. Microbiol. Biotechnol*., **81**, 419–429.

Dash, G., Raman, R.P., Pani Prasad, K., Makesh, M., Pradeep, M.A. & Sen, S. (2014) Evaluation of *Lactobacillus plantarum* as feed supplement on host associated microflora, growth, feed efficiency, carcass biochemical composition and immune response of giant freshwater prawn, *Macrobrachium rosenbergii* (de Man, 1879). *Aquaculture*, **432**, 225–236.

Deplancke, B. & Gaskins, H.R. (2001) Microbial modulation of innate defense: goblet cells and the intestinal mucus layer. *Am. J. Clin. Nutr*., **73**, 1131S–1141S.

Diaz-Rosales, P., Salinas, I., Rodriguez, A., Cuesta, A., Chabrillon, M., Balebona, M.C., Morinigo, M.A., Esteban, M.A. & Meseguer, J. (2006) Gilthead seabream (*Sparus aurata* L.) innate immune response after dietary administration of heat-inactivated potential probiotics. *Fish Shellfish Immunol*., **20**, 482–492.

Díaz-Rosales, P., Arijo, S., Chabrillón, M., Alarcón, F.J., Tapia-Paniagua, S.T., Martínez-Manzanares, E., Balebona, M.C. & Moriñigo, M.A. (2009) Effects of two closely related probiotics on respiratory burst activity of Senegalese sole (*Solea senegalensis*, Kaup) phagocytes, and protection against *Photobacterium damselae* subsp. piscicida. *Aquaculture*, **293**, 16–21.

Dimitroglou, A., Merrifield, D.L., Carnevali, O., Picchietti, S., Avella, M., Daniels, C., Güroy, D. & Davies, S.J. (2011) Microbial manipulations to improve fish health and production – A Mediterranean perspective. *Fish Shellfish Immunol*., **30**, 1–16.

Dung, T.T., Haesebrouck, F., Sorgeloos, P., Tuan, N.A., Pasmans, F., Smet, A. & Decostere, A. (2009) IncK plasmid-mediated tetracycline resistance in *Edwardsiella ictaluri* isolates from diseased freshwater catfish in Vietnam. *Aquaculture*, **295**, 157–159.

Forchielli, M.L. & Walker, W.A. (2005) The role of gut-associated lymphoid tissues and mucosal defence. *Br. J. Nutr*., **93**, S41–S48.

Gatesoupe, F.J. (1999) The use of probiotics in aquaculture. *Aquaculture*, **180**, 147–165.

Geng, X., Dong, X.-H., Tan, B.-P., Yang, Q.-H., Chi, S.-Y., Liu, H.-Y. & Liu, X.-Q. (2011) Effects of dietary chitosan and *Bacillus subtilis* on the growth performance, non-specific immunity and disease resistance of cobia, *Rachycentron canadum*. *Fish Shellfish Immunol*., **31**, 400–406.

Geng, X., Dong, X.H., Tan, B.P., Yang, Q.H., Chi, S.Y., Liu, H.Y. & Liu, X.Q. (2012) Effects of dietary probiotic on the growth performance, non-specific immunity and disease resistance of cobia, *Rachycentron canadum*. *Aquacult. Nutr*., **18**, 46–55.

Gibson, G.R. (2004) Fibre and effects on probiotics (the prebiotic concept). *Clin. Nutr. Suppl*., **1**, 25–31.

Gibson, G.R. & Roberfroid, M.B. (1995) Dietary modulation of the human colonic microbiota: introducing the concept of prebiotics. *J. Nutr*., **125**, 1401–1412.

Giri, S.S., Sukumaran, V. & Oviya, M. (2013) Potential probiotic *Lactobacillus plantarum* VSG3 improves the growth, immunity, and disease resistance of tropical freshwater fish, *Labeo rohita*. *Fish Shellfish Immunol*., **34**, 660–666.

Giri, S.S., Sukumaran, V., Sen, S.S. & Jena, P.K. (2014) Effects of dietary supplementation of potential probiotic *Bacillus subtilis* VSG1 singularly or in combination with *Lactobacillus plantarum* VSG3 or/and *Pseudomonas aeruginosa* VSG2 on the growth, immunity and disease resistance of *Labeo rohita*. *Aquacult. Nutr*., **20**, 163–171.

GLOBEFISH (2013) Pangasius-June 2013, Vol. 2014. FAO GLOBEFISH, http://www.globefish.org/pangasius-June-2013.html.

Grau, A., Crespo, S., Sarasquete, M.C. & de Canales, M.L.G. (1992) The digestive tract of the amberjack *Seriola dumerili*, Risso: a light and scanning electron microscope study. *J. Fish Biol*., **41**, 287–303.

Grisdale-Helland, B., Helland, S.J. & Gatlin Iii, D.M. (2008) The effects of dietary supplementation with mannanoligosaccharide, fructooligosaccharide or galactooligosaccharide on the growth and feed utilization of Atlantic salmon (*Salmo salar*). *Aquaculture*, **283**, 163–167.

Guzman-Villanueva, L.T., Tovar-Ramirez, D., Gisbert, E., Cordero, H., Guardiola, F.A., Cuesta, A., Meseguer, J., Ascencio-Valle, F. & Esteban, M.A. (2014) Dietary administration of beta-1,3/1,6-glucan and probiotic strain *Shewanella putrefaciens*, single or combined, on gilthead seabream growth, immune responses and gene expression. *Fish Shellfish Immunol*., **39**, 34–41.

Harikrishnan, R., Balasundaram, C. & Heo, M.-S. (2010) *Lactobacillus sakei* BK19 enriched diet enhances the immunity status and disease resistance to streptococcosis infection in kelp grouper, *Epinephelus bruneus*. *Fish Shellfish Immunol*., **29**, 1037–1043.

Hassaan, M.S., Soltan, M.A. & Ghonemy, M.M.R. (2014) Effect of synbiotics between *Bacillus licheniformis* and yeast extract on growth, hematological and biochemical indices of the Nile tilapia (*Oreochromis niloticus*). *Egypt. J. Aquat. Res*., **40**, 199–208.

Heuer, O.E., Kruse, H., Grave, K., Collignon, P., Karunasagar, I. & Angulo, F.J. (2009) Human health consequences of use of antimicrobial agents in aquaculture. *Clin. Infect. Dis*., **49**, 1248–1253.

Hien, D.V. & Doolgindachbaporn, S. (2011) Effect of niacin and folic acid in feed rations on growth and live weights of Green catfish (*Mystus nemurus* Valenciennes 1840). *Pak. J. Biol. Sci*., **14**, 64–68.

Ibrahem, M.D., Fathi, M., Mesalhy, S. & Abd El-Aty, A.M. (2010) Effect of dietary supplementation of inulin and vitamin C on the growth, hematology, innate immunity, and resistance of Nile tilapia (*Oreochromis niloticus*). *Fish Shellfish Immunol*., **29**, 241–246.

Irianto, A. & Austin, B. (2002) Use of probiotics to control furunculosis in rainbow trout, *Oncorhynchus mykiss* (Walbaum). *J. Fish Dis.*, **25**, 333–342.

Jatobá, A., Vieira, F.D., Buglione-Neto, C., Mouriño', J., Silva, B.C., Seiftter, W. & Andreatta, E. (2011) Diet supplemented with probiotic for Nile tilapia in polyculture system with marine shrimp. *Fish Physiol. Biochem.*, **37**, 725–732.

Jiwyam, W. (2010) Growth and compensatory growth of juvenile *Pangasius bocourti* Sauvage, 1880 relative to ration. *Aquaculture*, **306**, 393–397.

Kaneko, T., Yokoyama, A. & Suzuki, M. (1995) Digestibility characteristics of isomaltooligosaccharides in comparison with several saccharides using the rat jejunum loop method. *Biosci. Biotechnol. Biochem.*, **59**, 1190–1194.

Kim, D.-H. & Austin, B. (2006) Innate immune responses in rainbow trout (*Oncorhynchus mykiss*, Walbaum) induced by probiotics. *Fish Shellfish Immunol.*, **21**, 513–524.

Kongnum, K. & Hongpattarakere, T. (2012) Effect of *Lactobacillus plantarum* isolated from digestive tract of wild shrimp on growth and survival of white shrimp (*Litopenaeus vannamei*) challenged with *Vibrio harveyi*. *Fish Shellfish Immunol.*, **32**, 170–177.

Kumar, R., Mukherjee, S.C., Ranjan, R. & Nayak, S.K. (2008) Enhanced innate immune parameters in *Labeo rohita* (Ham.) following oral administration of *Bacillus subtilis*. *Fish Shellfish Immunol.*, **24**, 168–172.

Le, T.X., Munekage, Y. & Kato, S.-I. (2005) Antibiotic resistance in bacteria from shrimp farming in mangrove areas. *Sci. Total Environ.*, **349**, 95–105.

Lee, J.-S., Cheng, H., Damte, D., Lee, S.-J., Kim, J.-C., Rhee, M.-H., Suh, J.-W. & Park, S.-C. (2013) Effects of dietary supplementation of *Lactobacillus pentosus* PL11 on the growth performance, immune and antioxidant systems of Japanese eel *Anguilla japonica* challenged with *Edwardsiella tarda*. *Fish Shellfish Immunol.*, **34**, 756–761.

Li, S.-Z. & Chan-Halbrendt, C. (2009) Ethanol production in (the) People's Republic of China: potential and technologies. *Appl. Energy*, **86**(Supplement 1), S162–S169.

Li, P., Burr, G.S., Gatlin, D.M. 3rd, Hume, M.E., Patnaik, S., Castille, F.L. & Lawrence, A.L. (2007) Dietary supplementation of short-chain fructooligosaccharides influences gastrointestinal microbiota composition and immunity characteristics of Pacific white shrimp, *Litopenaeus vannamei*, cultured in a recirculating system. *J. Nutr.*, **137**, 2763–2768.

Lin, S., Mao, S., Guan, Y., Luo, L., Luo, L. & Pan, Y. (2012) Effects of dietary chitosan oligosaccharides and *Bacillus coagulans* on the growth, innate immunity and resistance of koi (*Cyprinus carpio* koi). *Aquaculture*, **342–343**, 36–41.

Luna-González, A., Almaraz-Salas, J.C., Fierro-Coronado, J.A., Flores-Miranda, M.D.C., González-Ocampo, H.A. & Peraza-Gómez, V. (2012) The prebiotic inulin increases the phenoloxidase activity and reduces the prevalence of WSSV in whiteleg shrimp (*Litopenaeus vannamei*) cultured under laboratory conditions. *Aquaculture*, **362–363**, 28–32.

Mahious, A.S., Gatesoupe, F.J., Hervi, M., Metailler, R. & Ollevier, F. (2006) Effect of dietary inulin and oligosaccharides as prebiotics for weaning turbot, *Psetta maxima* (Linnaeus, C. 1758). *Aquacult. Int.*, **14**, 219–229.

Mehrabi, Z., Firouzbakhsh, F. & Jafarpour, A. (2012) Effects of dietary supplementation of synbiotic on growth performance, serum biochemical parameters and carcass composition in rainbow trout (*Oncorhynchus mykiss*) fingerlings. *J. Anim. Physiol. Anim. Nutr.*, **96**, 474–481.

Merrifield, D.L., Dimitroglou, A., Foey, A., Davies, S.J., Baker, R.T.M., Bøgwald, J., Castex, M. & Ringø, E. (2010) The current status and future focus of probiotic and prebiotic applications for salmonids. *Aquaculture*, **302**, 1–18.

Moriarty, D.J.W. (1998) Control of luminous *Vibrio* species in penaeid aquaculture ponds. *Aquaculture*, **164**, 351–358.

MouriÑO, J.L.P., Do Nascimento Vieira, F., JatobÁ, A.B., Da Silva, B.C., Jesus, G.F.A., Seiffert, W.Q. & Martins, M.L. (2012) Effect of dietary supplementation of inulin and *W. cibaria* on haemato-immunological parameters of hybrid surubim (*Pseudoplatystoma* sp). *Aquacult. Nutr.*, **18**, 73–80.

Nayak, S.K. (2010) Probiotics and immunity: a fish perspective. *Fish Shellfish Immunol.*, **29**, 2–14.

Oelschlaeger, T.A. (2010) Mechanisms of probiotic actions – A review. *Int. J. Med. Microbiol.*, **300**, 57–62.

Ortiz, L.T., Rebolé, A., Velasco, S., Rodríguez, M.L., Treviño, J., Tejedor, J.L. & Alzueta, C. (2013) Effects of inulin and fructooligosaccharides on growth performance, body chemical composition and intestinal microbiota of farmed rainbow trout (*Oncorhynchus mykiss*). *Aquacult. Nutr.*, **19**, 475–482.

Panigrahi, A., Kiron, V., Puangkaew, J., Kobayashi, T., Satoh, S. & Sugita, H. (2005) The viability of probiotic bacteria as a factor influencing the immune response in rainbow trout *Oncorhynchus mykiss*. *Aquaculture*, **243**, 241–254.

Panigrahi, A., Kiron, V., Satoh, S., Hirono, I., Kobayashi, T., Sugita, H., Puangkaew, J. & Aoki, T. (2007) Immune modulation and expression of cytokine genes in rainbow trout *Oncorhynchus mykiss* upon probiotic feeding. *Dev. Comp. Immunol.*, **31**, 372–382.

Parry, R.M., Chandan, R.C. & Shahani, K.M. (1965) A rapid and sensitive assay of muramidase. *Exp. Biol. Med.*, **119**, 384–386.

Phumee, P., Hashim, R., Aliyu-Paiko, M. & Shu-Chien, A.C. (2009) Effects of dietary protein and lipid content on growth performance and biological indices of iridescent Shark (*Pangasius hypophthalmus*, Sauvage 1878) fry. *Aquacult. Res.*, **40**, 456–463.

Pirarat, N., Pinpimai, K., Endo, M., Katagiri, T., Ponpornpisit, A., Chansue, N. & Maita, M. (2011) Modulation of intestinal morphology and immunity in nile tilapia (*Oreochromis niloticus*) by *Lactobacillus rhamnosus* GG. *Res. Vet. Sci.*, **91**, e92–e97.

Reed, L.J. & Muench, H. (1938) A simple method of estimating fifty percent endpoints. *Am. J. Epidemiol.*, **27**, 493–497.

Refstie, S., Bakke-McKellep, A.M., Penn, M.H., Sundby, A., Shearer, K.D. & Krogdahl, Å. (2006) Capacity for digestive hydrolysis and amino acid absorption in Atlantic salmon (*Salmo salar*) fed diets with soybean meal or inulin with or without addition of antibiotics. *Aquaculture*, **261**, 392–406.

Reiff, C. & Kelly, D. (2010) Inflammatory bowel disease, gut bacteria and probiotic therapy. *Int. J. Med. Microbiol.*, **300**, 25–33.

Reza, A., Abdolmajid, H., Abbas, M. & Abdolmohammad, A.K. (2009) Effect of dietary prebiotic inulin on growth performance, intestinal microflora, body composition and hematological parameters of juvenile beluga, *Huso huso* (Linnaeus, 1758). *J. World Aquaculture Soc.*, **40**, 771–779.

Rico, A. & Van den Brink, P.J. (2014) Probabilistic risk assessment of veterinary medicines applied to four major aquaculture species produced in Asia. *Sci. Total Environ.*, **468–469**, 630–641.

Rico, A., Satapornvanit, K., Haque, M.M., Min, J., Nguyen, P.T., Telfer, T.C. & van den Brink, P.J. (2012) Use of chemicals and biological products in Asian aquaculture and their

potential environmental risks: a critical review. *Rev. Aquacult.*, **4**, 75–93.

Rico, A., Phu, T.M., Satapornvanit, K., Min, J., Shahabuddin, A.M., Henriksson, P.J.G., Murray, F.J., Little, D.C., Dalsgaard, A. & Van den Brink, P.J. (2013) Use of veterinary medicines, feed additives and probiotics in four major internationally traded aquaculture species farmed in Asia. *Aquaculture*, **412–413**, 231–243.

Ringø, E., Olsen, R.E., Gifstad, T.Ø., Dalmo, R.A., Amlund, H., Hemre, G.I. & Bakke, A.M. (2010) Prebiotics in aquaculture: a review. *Aquacult. Nutr.*, **16**, 117–136.

Roberfroid, M. (2007) Prebiotics: the concept revisited. *J. Nutr.*, **137**, 830S–837S.

Robertson, A.M. & Wright, D.P. (1997) Bacterial glycosulphatases and sulphomucin degradation. *Can. J. Gastroenterol.*, **11**, 361–366.

Rodriguez-Estrada, U., Satoh, S., Haga, Y., Fushimi, H. & Sweetman, J. (2009) Effects of single and combined supplementation of *Enterococcus faecalis*, mannanoligosaccharide and polyhydrobutyric acid on growth performance and immune response of rainbow trout *Oncorhynchus mykiss*. *Aquacult. Sci.*, **57**, 609–617.

Saad, N., Delattre, C., Urdaci, M., Schmitter, J.M. & Bressollier, P. (2013) An overview of the last advances in probiotic and prebiotic field. *LWT - Food Sci. Technol.*, **50**, 1–16.

Salinas, I., Cuesta, A., Esteban, M.Á. & Meseguer, J. (2005) Dietary administration of *Lactobacillus delbrüeckii* and *Bacillus subtilis*, single or combined, on gilthead seabream cellular innate immune responses. *Fish Shellfish Immunol.*, **19**, 67–77.

Sang, H.M., Fotedar, R. & Filer, K. (2011) Effects of dietary mannan oligosaccharide on the survival, growth, immunity and digestive enzyme activity of freshwater crayfish, *Cherax destructor* Clark (1936). *Aquacult. Nutr.*, **17**, e629–e635.

Sapkota, A., Sapkota, A.R., Kucharski, M., Burke, J., McKenzie, S., Walker, P. & Lawrence, R. (2008) Aquaculture practices and potential human health risks: current knowledge and future priorities. *Environ. Int.*, **34**, 1215–1226.

SAS (2003). SAS Institute Inc, SAS Campus Drive, Cary, NC USA 27513-2414.

Secombes, C.J. (1990) Isolation of salmonid macrophage and analysis of their killing ability. In: Techniques in Fish Immunology (Stolen, J.S., Fletcher, T.C., Anderson, D.P., Roberson, B.S. & Van Muiswinkel, W.B. eds), pp. 137–152. SOS Publication, Fair Haven, NJ.

Seifert, S. & Watzl, B. (2007) Inulin and oligofructose: review of experimental data on immune modulation. *J. Nutr.*, **137**, 2563S–2567S.

Son, V.M., Chang, C.-C., Wu, M.-C., Guu, Y.-K., Chiu, C.-H. & Cheng, W. (2009) Dietary administration of the probiotic, *Lactobacillus plantarum*, enhanced the growth, innate immune responses, and disease resistance of the grouper *Epinephelus coioides*. *Fish Shellfish Immunol.*, **26**, 691–698.

Suzer, C., Çoban, D., Kamaci, H.O., Saka, Ş., Firat, K., Otgucuoğlu, Ö. & Küçüksari, H. (2008) *Lactobacillus* spp. bacteria as probiotics in gilthead sea bream (*Sparus aurata*, L.) larvae: effects on growth performance and digestive enzyme activities. *Aquaculture*, **280**, 140–145.

Talpur, A.D., Ikhwanuddin, M., Abdullah, M.D.D. & Ambok Bolong, A.-M. (2013) Indigenous *Lactobacillus plantarum* as probiotic for larviculture of blue swimming crab, *Portunus pelagicus* (Linnaeus, 1758): effects on survival, digestive enzyme activities and water quality. *Aquaculture*, **416–417**, 173–178.

Talpur, A.D., Munir, M.B., Mary, A. & Hashim, R. (2014) Dietary probiotics and prebiotics improved food acceptability, growth performance, haematology and immunological parameters and disease resistance against *Aeromonas hydrophila* in snakehead (*Channa striata*) fingerlings. *Aquaculture*, **426–427**, 14–20.

Venter, C.S. (2007) Prebiotics: an update. *J. Family Ecol. Consum. Sci.*, **35**, 17–25.

Vulevic, J., Rastall, R.A. & Gibson, G.R. (2004) Developing a quantitative approach for determining the in vitro prebiotic potential of dietary oligosaccharides. *FEMS Microbiol. Lett.*, **236**, 153–159.

Wang, Y.-B. (2007) Effect of probiotics on growth performance and digestive enzyme activity of the shrimp *Penaeus vannamei*. *Aquaculture*, **269**, 259–264.

Watzl, B., Girrbach, S. & Roller, M. (2005) Inulin, oligofructose and immunomodulation. *Br. J. Nutr.*, **93**, S49–S55.

Wu, Y.-R., Gong, Q.-F., Fang, H., Liang, W.-W., Chen, M. & He, R.-J. (2013) Effect of *Sophora flavescens* on non-specific immune response of tilapia (GIFT *Oreochromis niloticus*) and disease resistance against *Streptococcus agalactiae*. *Fish Shellfish Immunol.*, **34**, 220–227.

Yanbo, W. & Zirong, X. (2006) Effect of probiotics for common carp (*Cyprinus carpio*) based on growth performance and digestive enzyme activities. *Anim. Feed Sci. Technol.*, **127**, 283–292.

Yeh, S.-P., Chiu, C.-H., Shiu, Y.-L., Huang, Z.-L. & Liu, C.-H. (2014) Effects of diets supplemented with either individual or combined probiotics, *Bacillus subtilis* E20 and *Lactobacillus plantarum* 7-40, on the immune response and disease resistance of the mud crab, *Scylla paramamosain* (Estampador). *Aquacult. Res.*, **45**, 1164–1175.

Yoshida, T. & Kitao, T. (1991) The opsonic effect of specific immune serum on the phagocytic and chemiluminescent response in rainbow trout, *Oncorhynchus mykiss* phagocytes. *Fish Pathol.*, **26**, 29–33.

Zhang, Q., Ma, H., Mai, K., Zhang, W., Liufu, Z. & Xu, W. (2010) Interaction of dietary *Bacillus subtilis* and fructooligosaccharide on the growth performance, non-specific immunity of sea cucumber, *Apostichopus japonicus*. *Fish Shellfish Immunol.*, **29**, 204–211.

Zhang, C.-N., Li, X.-F., Xu, W.-N., Jiang, G.-Z., Lu, K.-L., Wang, L.-N. & Liu, W.-B. (2013) Combined effects of dietary fructooligosaccharide and *Bacillus licheniformis* on innate immunity, antioxidant capability and disease resistance of triangular bream (*Megalobrama terminalis*). *Fish Shellfish Immunol.*, **35**, 1380–1386.

Zhang, Q., Yu, H., Tong, T., Tong, W., Dong, L., Xu, M. & Wang, Z. (2014) Dietary supplementation of *Bacillus subtilis* and fructooligosaccharide enhance the growth, non-specific immunity of juvenile ovate pompano, *Trachinotus ovatus* and its disease resistance against *Vibrio vulnificus*. *Fish Shellfish Immunol.*, **38**, 7–14.

Zhou, Z., Ding, Z. & Huiyuan, L.V. (2007) Effects of dietary short-chain fructooligosaccharides on intestinal microflora, survival, and growth performance of juvenile white shrimp, *Litopenaeus vannamei*. *J. World Aquaculture Soc.*, **38**, 296–301.