Combined administration of low molecular weight sodium alginate boosted immunomodulatory, disease resistance and growth enhancing effects of *Lactobacillus plantarum* in Nile tilapia (*Oreochromis niloticus*)

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**A B S T R A C T**

The present study investigates the effects of combined or singular administration of low molecular weight sodium alginate (LMWSA) and *Lactobacillus plantarum* on innate immune response, disease resistance and growth performance of Nile tilapia (*Oreochromis niloticus*). Three hundred and twenty fish were supplied and randomly stocked in sixteen glass tanks (150 L) assigned to four treatments as follows: 0 g kg\(^{-1}\) LMWSA (Control, Diet 1), 10 g kg\(^{-1}\) LMWSA (Diet 2), 10⁸ CFU g\(^{-1}\) *L. plantarum* (Diet 3), and 10 g kg\(^{-1}\) LMWSA + 10⁸ CFU g\(^{-1}\) *L. plantarum* (Diet 4). Following 30 and 60 days of the feeding trial, serum lysozyme, phagocytosis, respiratory burst and alternative complement activities as well as growth performance parameters (specific growth rate, feed conversion ratio) were measured. Serum lysozyme, phagocytosis, respiratory burst, and alternative complement activities of fish were significantly stimulated by both LMWSA and *L. plantarum* diets, however, the highest innate immune response were observed in fish fed synbiotic diet. At the end of the experiment, eight fish per replication were randomly selected for a challenge test against *Streptococcus agalactiae*. The survival rate of the fish fed supplemented diets was significantly greater than the control treatment and the highest post challenge survival rate was observed in synbiotic diet. Furthermore, SGR and FCR were significantly improved in fish fed supplemented diets after 60 days and the highest growth performance was observed in fish fed symbiotic diet. These results suggest combined LMWSA and *L. plantarum* can be considered as a promising immunostimulant and growth enhancer in Nile tilapia diet.

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**1. Introduction**

The demand for aquaculture productions has rapidly increased due to the increasing world population and the perception that aquaculture products are good for health [1,2]. Therefore, aquaculture has remarkably expanded to maximize productivity in a limited volume of water [1,3]. As a result, intensive aquaculture has faced many problems, such as the degradation of water environment, the outbreak of diseases and the significant economic losses. Infectious diseases are considered as main obstacle for aquaculture development and are significant factor of economic lost [4–6]. To combat diseases, several strategies, such as vaccination and antimicrobial substances have been intensively employed in aquaculture sector [4]. However, the excessive administration of antibiotics posed adverse impacts on the environment and human health [7], which included emergence of antibiotic resistance bacterial strains, accumulation of residual in aquaculture products, and depression of immune system [8,9]. These issues have prompted interest in developing health promoting and growth enhancing feed additives as an alternative to antibiotics in the intensive aquaculture systems [10–14].

Prebiotics and probiotics are increasingly used as effective tools for controlling diseases and elevation of fish resistance against infections [15–17]. Probiotics are alive or dead, or even a component...
of the microorganism that act under different modes of actions in conferring beneficial effects to the host or surrounding environment [18]. The common probiotic employed in animal feeding include *Saccharomyces, Lactobacillus, Enterococcus* and *Bacillus* [15]. *Lactobacillus* are beneficial bacteria, which was found in the humans and animals’ gut microbiota. Among them, *Lactobacillus plantarum* has recently been considered as an important probiotics in aquaculture [19]. It is a well-adapted bacterium that can effectively suppress the growth of pathogens by releasing bacteriocin [20]. The administration of *L. plantarum* in aquaculture has been shown to stimulate immune system, promote the growth, and increase disease resistance [21–23]. Prebiotics are indigestible substances that allow specific changes in the composition and/or activity of gastrointestinal microbiota, which have a positive effect on the nutrition and health status of the host [24,25]. Recently, the effects of prebiotics on fish and shellfish have been intensively studied which include improve growth performance, modulate gut microbiota, stimulate immunity and increase disease resistance [8,9,24,26]. Sodium alginate is the sodium salt of alginic acid which obtained from the brown algae [27,28]. It has been demonstrated to have antibacterial [29], antitumor [30], and antioxidant properties [31,32]. Recently, low molecular weight sodium alginate (LMWSA) from seaweed has been suggested as novel prebiotic [33] with beneficial effects on innate immune response and growth performance [34]. To our best knowledge there is no available information regarding the combined effect of low molecular weight sodium alginate (LMWSA) and *Lactobacillus plantarum* on fish. Therefore, the present study was conducted to evaluate effects of singular or combined administration of LMWSA (as a prebiotic) and *Lactoba- cillus plantarum* (as a probiotic) on innate immune responses, disease resistance and growth performance of Nile tilapia (*Oreochromis niloticus*) fingerlings.

2. Materials and methods

2.1. Low molecular weight sodium alginate preparation

The sodium alginate was supplied from the local market (Chiang Mai, Thailand) which supplied from brown seaweeds. The low molecular weight sodium alginate (LMWSA) was prepared according to the method described by Ramnani, Chitarrari, Tuohy, Grant, Hotchkiss, Philip, Campbell, Gill and Rowland [33]. The LMWSA dose (10 g kg\(^{-1}\)) used in this experiment was selected based on the results of our previous study [34].

2.2. *Lactobacillus plantarum*

*Lactobacillus plantarum* CR1T5 was kindly provided by Dr. Sowanit Tongpim, (Department of Microbiology, Faculty of Science, Khon Kaen University, Thailand). The administration dose of *L. plantarum* (10\(^8\) CFU g\(^{-1}\)) in this study was selected based on previous studies Son, Chang, Wu, Guu, Chiu and Cheng [35] Giri, Sukumaran and Oviya [36]. *L. plantarum* supplemented diets were daily prepared according to the method described by Irianto and Austin [37].

2.3. Experimental diets

The basal diet used in present study was modified from the work of Tingtam, Khemppaka, Paengkum and Boonanuntanasarn [38] which has been proved its suitable for Nile tilapia (*O. niloticus*). The experimental diets were prepared by inclusion of different additives as follows: 0 (Diet 1-Control), 10 g kg\(^{-1}\) LMWSA (Diet 2), 10\(^8\) CFU g\(^{-1}\) *L. plantarum* (Diet 3) and 10\(^8\) CFU g\(^{-1}\) *L. plantarum* + 10 g kg\(^{-1}\) LMWSA for Diet 4 (Table 1). The ingredients were milled into powder and were thoroughly mixed with soybean oil, and then water was added to produce stiff dough. The dough was then passed through the mincer to form spaghetti like strings as to form pellets. The pellets were finally dried in an oven at 50 °C to achieve a moisture content of approximately 10% then kept at 4 °C until use.

2.4. Experimental design

The tilapia fingerlings were obtained from the Charoen Pokphand Company, Chiang Mai, Thailand and stocked in 1000-L tanks. They were fed with the commercial diet for 2 months. Prior to the experiments, fish were fed a control diet for 2 weeks. Three hundred and twenty individual fish of a similar size (15.56 ± 0.02 g) were randomly allocated into 16 glass tanks (150 L) at the rate of 20 fish tank\(^{-1}\). The experiment was laid out in a Completely Randomized Design with four replications. The diets were hand-fed to the fish *ad libitum* twice a day at 8:00 a.m. and 5:00 p.m. During the experiment, water temperature was maintained at 25–29 °C, and pH in a range of 7.5–8.2. The dissolved oxygen was maintained no less than 5 mg L\(^{-1}\).

2.5. Immunological assays

2.5.1. Samples collection

Blood samples were collected through the caudal vein from 5 fish tank\(^{-1}\) using a 1 ml syringe at the end of the feeding trial. The samples were immediately withdrawn into the Eppendorf tubes without anticoagulant, allowed to clot (1 h at room temperature and 4 h at 4 °C) and centrifuged at 1500 x g, 5 min, and 4 °C. The serum was finally collected and stored at −20 °C until assay.

Leukocytes isolates from peripheral blood were taken using a method modified of Chung & Secomes [39]. One ml of the collected bloods from each fish was diluted with 2 ml of RPMI 1640 (Gibbthal). It was then carefully laid onto 3 ml of Histopaque (Sigma) in a 15 ml tube. The tube was centrifuged at 400 g for 30 min at room temperature. Thereafter, a white buffy coat of leukocytes cells

<table>
<thead>
<tr>
<th>Ingredients</th>
<th>Diets (g kg(^{-1}))</th>
<th>Diet 1</th>
<th>Diet 2</th>
<th>Diet 3</th>
<th>Diet 4</th>
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<td>Proximate composition of the experimental diets (g kg(^{-1}) dry matter basis)</td>
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<tr>
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<td>931.0</td>
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<tr>
<td>GE (Cal g(^{-1}))</td>
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<td>4419</td>
<td>4387</td>
<td>4419</td>
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\(^a\) LMWSA – low molecular weight sodium alginete.  
\(^b\) Vitamin and trace mineral mix supplemented as follows (IU kg\(^{-1}\) or g kg\(^{-1}\) diet): retinol acetate 1,085,000 IU; cholecalciferol 217,000 IU; D, L-α-tocopherol acetate 0.5 g; thiamin nitrate 0.5 g; pyridoxine hydrochloride 0.5 g; niacin 3 g; folic 0.05 g; cyanocobalamin 10 g; Ca pantothenate 1 g; inositol 0.5 g; zinc 0.5 g; copper 0.25 g; manganese 1.32 g; iodine 0.05 g; sodium 7.85 g.

\(^c\) GE – Gross energy.
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, pH 7.4) 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 3 times to remove any residual Histopaque. The isolated leukocytes were then re-suspended in the PBS and adjusted to the required cell numbers for phagocytosis and respiratory activities.

2.5.2. Serum lysozyme activity

Serum lysozyme activity was determined according to the method suggested by Parry et al. [40]. The equivalent unit of the activity of the sample (compared with the standard) was determined and expressed in μg ml⁻¹ serum.

2.5.3. Phagocytosis activity

The phagocytic activity assay was modified from the work of Yoshida and Kitao [41]. 200 μl leukocyte suspensions 2 × 10⁶ cells ml⁻¹ were 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-phagocytized 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.

2.5.4. Respiratory burst activity

The respiratory burst activity of Nile tilapia peripheral blood leukocytes was determined using a modification of the method described by Secombes [42]. 175 μl samples of 6 × 10⁶ cells ml⁻¹ in PBS were 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. Thereafter, all supernatant was discarded and each well was washed again twice using 125 μl of 70% methanol. The supernatant in each well was then carefully discarded and the plate was dried at room temperature for 30 min. Then, 125 μl of 0.1 N KOH followed by 150 μl of DMSO were added to each well. The plate was then measured at 655 nm by a microplate reader (Sunrise, TECAN; Germany). Spontaneous O₂ production = (Absorbance NBT reduction of sample) – (Absorbance of blank).

2.5.5. Alternative complement pathway activity

Alternative complement pathway activity (ACH50) was determined as the method described by Yanno [43]. Briefly, rabbit red blood cells (R-RBC) were washed three times in 0.01 M ethylene glycol tetra-acetic acid–magnesium–gelatin veronal buffer (0.01 M – EGTA-Mg-GVB) and the concentration was adjusted to 2 × 10⁸ cells ml⁻¹ in the same buffer. Exactly 100 μl of the suspension was lysed with 3.4 ml of distilled water. The absorbance of hemolysate was measured at 414 nm against distilled water blank and was brought to be close to 0.740. For the ACH50 test, 100 μl of test serum was diluted with 400 μl of 0.01M-EGTA-Mg-GVB and serial two-fold dilution was made. The tubes were carried out at 0 °C to retard the reaction of complement until all tubes of tested serum were prepared. Consequently, 100 μl of R-BSC suspension was added to each tube and incubated at 20 °C for 90 min with occasional shaking. After incubation, 3.15 ml of cold saline was added to each tube and centrifuged at 1600 g for 5 min. One hundred μl of supernatant of each dilution was then transferred to 96-well plate and read at 414 nm. The degree of hemolysis was calculated by dividing the corrected absorbance 414 value by the corrected absorbance 414 of the 100% hemolysis control. The degree of hemolysis and the serum volume were plotted on a log-log paper. The volume of serum that gave 50% hemolysis was used for calculating the ACH50 using the formula:

\[
\text{ACH50 (units/ml)} = \frac{1}{K \times r \times 1/2}.
\]

where K is the amount of serum giving 50% hemolysis, r is the reciprocal of the serum dilution, and 1/2 is the correction factor.

2.6. Challenge study

Streptococcus agalactiae was kindly supported by Dr. Worawit Maneepitaksanti Department of Animal and Aquatic Sciences, Faculty of Agriculture, Chiang Mai University-Thailand. The strain was cultured in Tryptic Soy Broth and incubated overnight in the rotation shaker at 110 rpm and 37 °C. The sub-culture was obtained from the stock. Five ml of the stock solution was transferred into a 50 ml flask containing Tryptic Soy Broth and incubated at 37 °C for 24 h. The sub-cultures were raised in duplicate under the similar conditions for the experiment. Growth was evaluated by optical density of 560 nm and then using plate counting in Tryptic Soy Agar. The LD50 used in present study was obtained from previous work of Wang, Gan, Cai, Wang, Yu, Lin, Lu, Wu and Jian [44]. At the end of the experiment, 32 healthy fish from each treatment were injected intraperitoneally with 0.1 ml of 0.85% normal saline solution (NSS) containing a 10⁵ CFU ml⁻¹ of S. agalactiae. Dead fish were daily removed from the challenge tanks and the bacterium was re-isolated by culturing on TSA plates for 24 h at 27 °C. The isolates were tested morphologically, biochemically; compared with the inoculated strain and confirmed to be S. agalactiae. The numbers of dead fish in each replicate were recorded every day and the mortality (%) of tilapia in different treatment was calculated at the end of challenged test. In addition, the relative percentage of survival (RPS) was calculated after 15 days of challenged test according to the following equation:

\[
\text{RPS} = 100 - (\text{test mortality/control mortality}) \times 100
\]

2.7. Growth performance

At the end of the feeding trial, fish in each replication were weighed. Growth performance and survival rate of Nile tilapia were calculated using following equations: Weight gain (WG) = final weight (g) – initial weight (g); specific growth rate (SGR) = \( \frac{\ln \text{final weight} - \ln \text{initial weight}}{\text{Duration of experiment}} \); feed conversion ratio (FCR) = feed given (dried weight)/weight gain (wet weight); survival rate (%) = (final fish number/initial fish number) × 100.

2.8. Statistical analysis

The obtained data were analyzed using a SAS Computer Program [45] for least significant differences among the treatments where the Duncan’s Multiple Range Test was used. Prior to statistical analysis, the normality of the data was checked and confirmed using Kolmogorov- Smirnov test. Mean values were considered significantly different when \( P < 0.05 \). Data are presented as means ± standard deviation.
3. Results

3.1. Immune response

The effects of dietary supplementation of LMWSA (prebiotic diet) and Lactobacillus plantarum (probiotic diet) and diet in combination of LMWSA and L. plantarum (synbiotic diet) are presented in Figs. 1–4, respectively. Dietary administration of LMWSA and L. plantarum significantly increased serum lysozyme activity compared to the control (P < 0.05). Highest serum lysozyme activity was observed in fish fed symbiotic diet followed by L. plantarum and LMWSA diets (Fig. 1). No significant difference in serum lysozyme activity between diet LMWSA and L. plantarum was observed (Fig. 1). Similarity, fish fed LMWSA and L. plantarum single or combined significantly stimulated the phagocytic index, respiratory burst and alternative complement activities compared to the control diet (P < 0.05) (Figs. 2–4). Fish fed symbiotic diet had significantly higher phagocytic index, respiratory burst and alternative complement activities than those of the control and L. plantarum and LMWSA alone (P < 0.05) (Figs. 2–4). No significant differences in those parameters between L. plantarum and LMWSA diets (Figs. 2–4).

3.2. Challenge test

The result of challenge test indicated that fish fed LMWSA and L. plantarum single or combined significantly increased the protection of tilapia, O. niloticus against S. agalactiae infection compared to the control (Fig. 5). Dead fish were observed in control group after 5 days post-challenge, while the dead fish in supplemented groups were observed at day 6 of infection. Dead fish showed the typical symptoms of streptococcosis such as loss of appetite, darkness, exophthalmia, pair-fins basal haemorrhage and pale liver. The highest relative percent of survival (RPS) was observed in tilapia fed symbiotic diet, 10 g kg⁻¹ LMWSA + 10⁸ cfu g⁻¹ L. plantarum (87.50%) following by fish fed diets of 10⁸ cfu g⁻¹ L. plantarum (45.80%) and 10 g kg⁻¹ LMWSA (41.67%). Fish fed symbiotic diet showed highest resistance to S. agalactiae.

3.3. Growth performance

Fish fed 10 g kg⁻¹ LMWSA or 10⁸ cfu g⁻¹ Lactobacillus plantarum showed significantly higher final weight (FW), WG, SGR and lower FCR than the control after 30 and 60 days of the feeding trial (P < 0.05) (Table 2). However, no significant difference in growth performance between diets 10 g kg⁻¹ LMWSA and L. plantarum was observed (Table 2). The highest FW, WG, SGR and FCR were observed in fed diet in combination of LMWSA and L. plantarum (as a synbiotic) compared to those of the control and individual applications (Table 2).

4. Discussion

Dietary administration of immunostimulants, probiotics and prebiotics has been demonstrated as alternative strategies in controlling of disease in aquaculture [4,8]. These feed additives have been proved to improve immune status, feed efficiency, and growth performance of fish and shellfish [9,24]. The present study was conducted to evaluate the effect of singular or combined administration of low molecular weight sodium alginate (LMWSA—a novel prebiotic) and Lactobacillus plantarum (a probiotic) on growth performance and health effects of Nile tilapia. The results indicated that dietary administration of LMWSA and L. plantarum significantly increased the serum lysozyme, respiratory burst, phagocytosis and complements activities of Nile tilapia compared to the control. The highest values were observed in fish fed diet LMWSA + L. plantarum (synbiotic diet). Similarity, significant stimulation of fish immune by dietary prebiotics, probiotics and synbiotics have been reported in previous studies on snakehead, Channa striata [46]; gilthead seabream, Sparus aurata [47]; Pangasius catfish, Pangasius bocourti [48–50]; sea bream, Pagrus major.
Fig. 4. Alternative complement activity of Nile tilapia, O. niloticus fed different diets: Diet 1 (control), Diet 2 (10 g kg\(^{-1}\) LMWSA), Diet 3 (108 CFU g\(^{-1}\) L. plantarum), and Diet 4 (10 g kg\(^{-1}\) LMWSA + 10\(^8\) CFU g\(^{-1}\) L. plantarum) (mean ± S.E., n = 5). Columns sharing the same superscript letter are not significantly different (P < 0.05).

Fig. 5. Survival rate of tilapia, O. niloticus fed different concentrations of dietary LMWSA and L. plantarum: Diet 1 (control), Diet 2 (30 g kg\(^{-1}\) LMWSA), Diet 3 (10 CFU g\(^{-1}\) L. plantarum), and Diet 4 (10 g kg\(^{-1}\) LMWSA + 10\(^8\) CFU g\(^{-1}\) L. plantarum) during 15 days post-challenge with S. agalactiae.

Table 2

<table>
<thead>
<tr>
<th></th>
<th>Diet 1</th>
<th>Diet 2</th>
<th>Diet 3</th>
<th>Diet 4</th>
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<tr>
<td>Final weight (g)</td>
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<tr>
<td>30 days</td>
<td>35.25 ± 0.49(^c)</td>
<td>47.13 ± 0.43(^b)</td>
<td>47.79 ± 0.6(^b)</td>
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<tr>
<td>60 days</td>
<td>82.44 ± 3.71(^c)</td>
<td>95.57 ± 1.49(^b)</td>
<td>102.19 ± 2.73(^b)</td>
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<td>Weight gain (g)</td>
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<td>30 days</td>
<td>19.71 ± 0.44(^c)</td>
<td>31.58 ± 0.43(^b)</td>
<td>32.20 ± 0.60(^b)</td>
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<td>60 days</td>
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<td>84.02 ± 1.49(^b)</td>
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<td>SGR</td>
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<td>30 days</td>
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<td>60 days</td>
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</table>

Data assigned with different letter denote significant difference in a row (P < 0.05).

[51]: crayfish, Procambarus clarkia [52]; Pacific white shrimp, Litopenaeus vannamei [53]; angelfish, Pterophyllum scalare [54], and olive flounder, Paralichthys olivaceus [55]. However, administration of dietary synbiotics Bacillus subtilis and chitosan in cobia, Rachycentron canadum [56]; Weissella barba and inulin in hybrid surubim, Pseudoplatystoma sp. [57] and Bacillus subtilis and FOS in juvenile large yellow croaker, Larimichthys crocea [58] had no remarkable effects on immune parameters. Although the mode of action of dietary prebiotics, probiotics and synbiotics on fish immune response has not been clearly demonstrated, recent findings have suggested that the immune stimulatory nature of synbiotics can be attributed to microbial fermentation and short chain fatty acid (SCFA) production [59]. SCFAs alter the immune response through binding to G protein coupled receptor, GPR43, which is expressed mainly on innate immune response and inflammatory cells [60]. When it comes to the mode of action of prebiotics, it has been assumed that immunosaccharides can directly activate the non-specific immune system by contacting with PRRs expressed on microphages receptors, such as β-glucan and dexitin-1 [61]. The interaction between these ligand-receptors will motivate signal transduction molecules, such as NF-kB and finally activate immune cells [62]. Moreover, the pathogen-associated molecular patterns of bacteria, such as teichoic acid, peptidoglycan, glycosylated protein, and the capsular polysaccharide can be detected and thus inspire the immune response [20,63–66]. Therefore, it seems likely that prebiotics activate the non-specific immune system in two different ways. They can directly stimulate the non-specific immune system, or enhance the growth of beneficial bacteria [82,66]. LMWSA has recently demonstrated as novel source of prebiotic, which showed significant increases in total SCFA production and were fermented by gut bacteria [33]. For prebiotics, it has been demonstrated that beneficial bacteria are able to modulate the innate and humoral responses and stimulate host resistance and then facilitate the exclusion of potential pathogens [67,68]. Probiotics can modulate host’s immune response by contacting with epithelial cells and by stimulating the release of anti-inflammatory cytokines, and subsequently result in a reduction in inflammation [69]. The LMWSA and L. plantarum used in present study may have the same mechanisms as above mentioned. It is recently well documented that nutritional manipulation is an effective way for enhancing disease resistance in fish [70,71]. Symbiotic treatment can be taken into account as a promising strategies for disease prevention [72]. The results of the present study clearly indicated that dietary administration of prebiotic, probiotic and synbiotic in O. niloticus significantly increased resistance to Streptococcus agalactiae and the highest resistance was observed in the synbiotic group. The results were in agreement with previous studies on yellow croaker, Larimichthys crocea [58]; orange-spotted grouper, Epinephelus coioides [73]; kelp grouper, Epinephelus bruneus [74]; Apostichopus japonicus [75]; triangular bream, Megalobrama terminalis [35], and olive flounder, Paralichthys olivaceus [21], but in contrast with study on gilthead sea bream, Sparus aurata [76]. The improvement of the survival rate may be due to the activation of innate immune defence of the fish by the LMWSA and L. plantarum as demonstrated in the present study.
study. It has been demonstrated that *L. plantarum* is able to produce antimicrobial substances like plantaricin that are actively manifested against certain pathogens [77,78]. Furthermore, recent studies have indicated that cell wall components of *L. plantarum*, such as surface-bound proteins, peptidoglycans and lipoteichoic acid, play vital roles in the prevention and treatment of intestinal inflammatory diseases [79]. Additionally, lipoteichoic acid isolated from *L. plantarum* has been proved to exert anti-pathogenic effects [80]. They also modified the composition of the intestinal microbiota, thereby providing protection against pathogenic microorganisms [81]. Stimulatory effects of sodium alginate from seaweed on immunity and resistance against pathogens have been well-documented in fish [82]. It has been reported that extracted sodium alginate from brown seaweed significantly improved the resistance of common carp *Cyprinus carpio* against *Edwardsiella tarda* infection [83], and enhanced the innate defence system of *C. carpio* [84]. Seaweeds have been gaining their interest in aquaculture because of rich nutrition [85], and biologically active compounds [86]. The antiviral, antibacterial activities and bioactive substances have been found in seaweeds [87]. Many studies have demonstrated that sulfated polysaccharides from seaweeds can restrain a wide range of bacteria e.g., *Vibrio harveyi* [88] and viruses [89]. Finally, the compounds with cytostatic, antiviral, antimicrobial, antitumour and antibacterial activities have been detected in green, brown and red seaweeds [90].

Nile tilapia fed single or combined dietary of LMWSA and *L. plantarum* also showed significantly improved specific growth rate (SGR) and feed conversion ratio (FCR) compared with the control. Similarly, significant improvement in SGR and FCR by dietary administration of prebiotics and probiotics have been observed in previous studies on snakehead, *Channa striata* [46,91]; gilthead seabream, *Sparus aurata* [47]; Nile tilapia, *Oreochromis niloticus* [92]; Pangasius catfish, *Pangasius bocourti* [48–50]; sea bream, *P. major* [51]; *Procambarus clarkia* [52]; angelfish, *Pterophyllum scalare* [54], and Olive flounder, *Paralichthys olivaceus* [55]. In contrast, no significant effects on the SGR and FCR were observed in shrimp fed with prebiotic or probiotic diets [93] and in symbiotic diet in carp [94]. This may be attributable to the supplementation of an inappropriate prebiotic as the substrate in symbiotic mixture which could not be fermented by selected probiotic to produce short chain fatty acid (SCFA) [71]. Significant enhancement of growth performance in fish fed symbiotic diet may be due to the enhancement digestibility of the probiotic, or the increase of survival and colonization of the probiotic [58,95] compared to the individual administration. It is well-documented that dietary probiotics and prebiotics intake can produce the bioactive microbial metabolites, such as vitamins, bioactive peptides, organic acids or fatty acids during fermentation [91,98]. These bioactive compounds will stimulate digestion process in the intestine and consequently increase growth rates. Moreover, the presence of beneficial bacteria in the gut could improve microbial balance, which in turn enhances nutrient absorption and utilization [99,100]. The LMWSA used in present study may have similar effects on stimulating growth performance of Nile tilapia. Ramnani, Chittarrari, Tuohy, Grant, Hotchkiss, Philip, Campbell, Gill and Rowland [33] have demonstrated that low molecular weight polysaccharides derive from sodium alginate bearing seaweed were fermented by intestinal microflora and showed their potential to be utilized as novel prebiotics. Our previous work have indicated that tilapia, *O. niloticus* fed dietary administration of low molecular weight sodium alginate significantly improved growth performance and feed conversion ratio compared to the control [34]. For probiotic of *Lactobacilli*, it has been revealed that *Lactobacilli* could produce short chain fatty acids (SCFAs) in the digestive tract of the animal as a by-product of carbohydrate metabolism. These SCFAs are used by intestinal epithelial cells as major sources of energy and hence possibly play a vital role in increasing the villi height of the digestive tract which can improve nutrient absorption by providing more absorptive surface area [101]. Furthermore, the studies with the use of zebra fish, *Danio rerio* as model animal have indicated that live probiotic such as *Lactobacillus rhamnosus* can improve the growth performance by modulating the transcription of genes involved with differentiation and maturation of vital tissues [102,103].

In conclusion, the present study revealed that combined administration of LMWSA with *L. plantarum* in Nile tilapia diet showed elevated beneficial effects in case of innate immunity, protection against infection as well as performance compared singular administration. These findings are of great importance and interest for aquaculture research and the fish farming industry. However, the relationships and mechanisms of action of probiotic *L. plantarum* and LMWSA on immune response and disease resistance of *O. niloticus* need further investigations.

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