Fish & Shellfish Immunology 55 (2016) 186–194

Contents lists available at ScienceDirect

Fish & Shellfish Immunology

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

Full length article

Effects of low molecular weight sodium alginate on growth performance, immunity, and disease resistance of tilapia, *Oreochromis niloticus*

Hien Van Doan*, Wanaporn Tapingkae, Tossapol Moonmanee, Apichart Seepai

Department of Animal and Aquatic Sciences, Faculty of Agriculture, Chiang Mai University, Chiang Mai 50200, Thailand

ARTICLE INFO

Article history:
Received 11 March 2016
Received in revised form
24 May 2016
Accepted 25 May 2016
Available online 26 May 2016

Keywords:
Prebiotic
Low molecular weight
Sodium alginate
Seaweed
Streptococcus agalactiae
Tilapia

ABSTRACT

Present study was carried out to evaluate the effects of low molecular weight sodium alginate (LMWSA) as potential prebiotic source on growth performance, innate immunity and disease resistance of tilapia, *Oreochromis niloticus*. Three hundred twenty fish were divided into four treatments and fed following diets 0 (T1- Control), 10 (T2), 20 (T3) and 30 (T4) g kg^{-1} LMWSA for period of 60 days. A Completely Randomized Design with four replications was applied. At the end of experiment, fish in each replication were weighed and specific growth rate (SGR) and feed conversion ratio (FCR) were calculated. Five randomly selected fish were used for innate immune response measurement. Another ten fish were randomly selected for challenge test against *Streptococcus agalactiae* for a period of 18 days. The lysozyme, complement, phagocytosis, and respiratory burst activities were detected after 60 days of feeding trial and after challenge test. The results indicated that fish fed diet 10 g kg^{-1} LMWSA significantly improved SGR and FCR after 60 days of feeding trial. The lysozyme, phagocytosis, respiratory burst, and complement activities were significantly higher in fish fed LMWSA diets compared to control. Fish fed 10 g kg^{-1} LMWSA had greatest values compared to fish fed 20 and 30 g kg^{-1} LMWSA. The survival rate of *O. niloticus* was significantly improved in fish fed LMWSA diets after challenge with *S. agalactiae* for 18 days. However, no significant difference in survival rate was observed among LMWSA supplemented diets. It is indicated that fish fed 10, 20 and 30 g kg^{-1} LMWSA diets can stimulate growth performance, innate immunity and disease resistance in tilapia against *S. agalactiae*.

1. Introduction

Tilapia is an economically important farmed fish world-wide with production has quadrupled over the past decade. This is suitable species for aquaculture because of high market demands and stable market prices [1]. Global production was an approximately of 4.5 million metric tons in 2012 and will be higher in the future [2]. In Thailand, tilapias is the most important cultured fish species with the production of an approximately of 0.9 million tonnes [3,4]. It has been reported that about 30% of production was obtained from intensive cages culture on rivers or irrigation canals [5]. Tilapia raised under these water systems are extremely vulnerable to stress caused by water quality variations and can certainly be infected by naturally pathogens [5]. Infestations with *Streptococcus* spp. and other pathogens such as *Aeromonas* spp., *Pseudomonas* spp., and *Vibro* spp. have been indicated to be the major factors of mortality in caged culture [5]. To deal with outbreak of diseases, chemotherapeutic and antibiotics have been commonly used in tilapia farms [6]. Nonetheless, because side-effects of chemotherapies such as emergence of antibiotic resistance bacterial strains, accumulation of residual in finish products, and depression of immune system has recent extremely affected human health and environment resulted in strict regulation for supplementation of antimicrobial agents in many countries [7–10]. Therefore, alternative strategies to antibiotics that could be applied for better growth performance, disease control and subsequently improve production in intensive fish production systems in a sustainable manner are in needed [11].

Prebiotics are indigestible substances that allow specific changes in the composition and/or activity of gastrointestinal microbiota, which has a positive effect on the nutrition and health status of the host [12,13]. It was well-documented that prebiotics

* Corresponding author.
E-mail address: hienqbuni@gmail.com (H. Van Doan).

http://dx.doi.org/10.1016/j.fsi.2016.05.034
1050-4648/

played a vital role in promoting host health when by-products were fermented by beneficial bacteria [8,14,15]. Recently, effects of prebiotics on fish and shellfish have been intensively studied which includes improve growth performance, modulate gut microbiota, stimulate immunity and increase disease resistance [8,12,13,15–20]. Seaweeds contain many of bioactive compounds with various biological activities such as antibacterial activity [21], antioxidant potential [22,23], anti-inflammatory properties [24], anti-viral activity [25], apoptotic activity [26] and act like a prebiotic [27]. Sodium alginate was extracted from the brown algae [28,29]. It has been demonstrated to have antibacterial [30], antitumor [31], and antioxidant properties [32,33]. The high percentage of polysaccharides in this extracts makes them become a potential choice of investigation as a prebiotic [34]. However, alginates have not been widely used in therapeutics because of their high molecular weight, poor solubility, and absorption [35]. Thus, lower molecular weight forms should be developed. To the best of our knowledge, this is first study about the effects of low molecular weight sodium alginate as a novel prebiotic on tilapia, *Oreochromis niloticus*. Therefore, the study was conducted to determine the effects of dietary low molecular weight sodium alginate on growth performance, innate immune response, and disease resistance against *Streptococcus agalactiae* of tilapia, *O. niloticus*.

2. Materials and methods

2.1. Low molecular weight sodium alginate preparation

The sodium alginate from red seaweeds obtained from the local market was used. Low molecular weight sodium alginate was prepared as the method described by Ramnani, Chitarrari [34].

2.2. Experimental diets

The basal diet used in present study was modified from the work of Tiengtam, Khempaka [6] which has been proved its suitable for tilapia, *Oreochromis niloticus*. The diets contain an approximately of 35.51% crude protein and 9.74% lipid. The experiment was divided into 4 diet treatments as follows: 0 (T1-Control), 10 (T1), 20 (T2) and 30 g kg^{-1} low molecular weight sodium alginate (T4) (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.

2.3. Experimental design

The tilapia fingerlings obtained from the Chiang Mai Patthana Farm, Chiang Mai, Thailand were placed 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 twenty individual fish of a similar size (29.22 ± 0.02 g $fish^{-1}$) were allocated into 16 glass tanks (capacity: 150 L) 20 fish $tank^{-1}$. The experiment was laid 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., 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}.

2.4. Growth performance

At the end of the feeding trial, fish in each replication were weighed. Growth performance and survival rate of *Oreochromis niloticus* were calculated with the use of the 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.

Table 1
The different ingredients and its respective amounts being used in the formula of feed ration for the first and second experiments (g kg^{-1}).

Ingredients	Diets (g kg^{-1})			
	T1	T2	T3	T4
Fish meal	300	300	300	300
Corn meal	125	125	125	125
Soybean meal	200	200	200	200
Wheat flour	60	60	60	60
Rice bran	240	240	240	240
Cellulose	30	20	10	0
LMWSA[a]	0	10	20	30
Soybean oil	30	30	30	30
Premix[b]	10	10	10	10
Vitamin C[c]	5	5	5	5
Proximate composition of the experimental diets (g kg^{-1} dry matter basis)				
Crude protein	353.1	353.6	357.0	356.7
Crude lipid	100.5	99.0	98.9	91.1
Fibre	63.8	60.6	61.8	53.3
Ash	114.4	115.2	117.7	119.8
Dry matter	931.0	931.3	922.4	919.0
GE (cal/g)[4]	4387	4419	4419	4382

[a] LMWSA = low molecular weight sodium alginate.
[b] Vitamin and trace mineral mix supplemented as follows (IU kg^{-1} or g kg^{-1} diet): retinyl acetate 1,085,000 IU; cholecalciferol 217,000 IU; D, L-a-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 kg^{-1}; inositol 0.5 g; zinc 1 g; copper 0.25 g; manganese 1.32 g; iodine 0.05 g; sodium 7.85 g.
[c] Vitamin C 98% 5 g.

2.5. Immunological assays

2.5.1. Samples preparation

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. They were immediately withdrawn into the 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 −20 °C until assay.

Leukocytes isolates from peripheral blood were taken using a method modified from Ref. [36]. One ml of the collected bloods from each fish was diluted with 2 ml of RPMI 1640 (Gibthai). 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. After centrifugation, a white buffy coat of leukocytes 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 3 times to remove any residual Histopaque. The isolated leukocytes cells 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

Lysozyme activity in a serum was determined according to the method of Parry et al. [37]. The equivalent unit of the activity of the sample (compared with the standard) were determined and expressed in μg ml^{-1} serum.