Aquaculture Reports 10 (2018) 23–31

Contents lists available at ScienceDirect

Aquaculture Reports

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

Modulation of growth performance, immunological responses and disease resistance of juvenile Nile tilapia (*Oreochromis niloticus*) (Linnaeus, 1758) by supplementing dietary inosine monophosphate

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ARTICLE INFO

Keywords:
Nucleotides
Inosine monophosphate
Growth
Immunity
Disease resistance
Nile tilapia
Streptococcus agalactiae

ABSTRACT

This study was investigated to examine supplemental effects of dietary inosine monophosphate (IMP) on growth performance, feed utilization, biochemical, hematological and immunological parameters of juvenile Nile tilapia *Oreochromis niloticus*. Disease resistance to experimental infection with *Streptococcus agalactiae* was also assessed. A semi-purified basal diet was supplemented with 0 (IMP0, Control), 1 (IMP1), 2 (IMP2), 4 (IMP4) and 8 (IMP8) g purified IMP kg^{-1} diet to formulate five experimental diets. Each diet was randomly allocated to triplicate groups of fish (0.59 g) for 60 days. The results indicated that supplementation of IMP significantly ($P < 0.05$) improved growth performances of fish. The highest final weight was found in IMP2 followed by IMP8, IMP4 and IMP1. Feed utilization parameters were also positively influenced by dietary IMP supplementation and IMP2 supplemented group showed improved values. Feed intake increased with dietary IMP supplementations but not at a significant level ($P > 0.05$). Among whole body proximate composition and somatic parameters, condition factor was significantly influenced by dietary supplementation of IMP. A wide variation in hematological parameters were observed and dietary supplementation increased the hematocrit content ($P < 0.05$) and red blood cells ($P > 0.05$). Total serum protein (TSP), lysozyme activity (LA), superoxide dismutase activity (SOD) and bactericidal activity (BA) tended to increase with the supplementation of dietary IMP. TSP and SOD were significantly improved with ≥ 4 g kg^{-1} supplementation, while LA with 8 g kg^{-1} and BA with ≥ 1 g kg^{-1} supplementations. IMP supplemented groups showed higher ($P > 0.05$) cumulative survival compared to that of supplementation free control group. IMP supplemented diet groups also showed significantly higher BA in the post challenge test. Based on the overall performances, the results of the current study indicated that the inclusion of IMP in Nile tilapia diet can improve growth performance, feed utilization, haematological and immunological parameters; and disease resistance of juvenile Nile tilapia.

1. Introduction

With the increasing expansion of aquaculture activities, the aquaculture industry is presently moving towards more intensive practices that have caused surges in the susceptibility of disease outbreaks (Chen et al., 2014). The application of antibiotics and chemotherapeutics to control these diseases have been widely criticized for their negative impacts such as the spread of drug resistant pathogens, suppression of aquatic animals' immune system and environmental hazards (Brogden et al., 2014; Allameh et al., 2015), thus resulting in reduced consumer preference for food fish treated with antibiotics. As an alternative to the use of therapeutics and vaccines, another approach is to enhance disease resistance, immune responses and other health benefits by the supplementation of functional nutrients which could provide potential health and development benefit beyond satisfying basic nutrition of the cultured species. Recently in aquaculture research, nucleotides and its

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https://doi.org/10.1016/j.aqrep.2018.03.003
Received 25 November 2017; Received in revised form 25 January 2018; Accepted 21 March 2018

related product has been paid attention promisingly as functional nutrients (Hossain et al., 2016a,b,c).

Nucleotides are low molecular weight intracellular compounds which play key roles in nearly all biochemical processes (Gil, 2002). The modulatory effects of dietary nucleotides on lymphocyte maturation, activation and proliferation, as well as macrophage phagocytosis, immunoglobulin responses and genetic expression of certain cytokines have been reported in humans and animals (Gil, 2002). Nucleotides consists of a nitrogenous base, a pentose sugar, and one or more phosphate groups. Inosine monophosphate (IMP) is the ribonucleotide and is the first compound formed during the synthesis of purine. Also, IMP becomes adenine monophosphate (AMP) and guanine monophosphate (GMP) within several steps. Amino acids, glycinbetaine, oligopeptides, nucleosides and nucleotides have been known to stimulate taste receptors of many fish species (Ishida and Hidaka, 1987). Numerous studies on different aquatic species have reported that dietary inosine or IMP, either alone or in combination with certain free amino acids can enhance growth performance, survival and feed intake of juvenile eel *Anguilla japonica* (Takeda et al., 1984), turbot *Scophthalmus maximus* (Mackie and Adron, 1978; Person-Le Ruyet et al., 1983), dover sole *Solea vulgaris* (Metailler et al., 1983), red sea bream *Pagrus major* (Hossain et al., 2016a,b) while it can also improve immune responses and disease resistance of red sea bream (Hossain et al., 2016b,c) and Japanese flounder *Paralichthys olivaceus* (Song et al., 2012).

In Malaysia, among the three major freshwater species groups (*viz.,* tilapias, catfishes and carps) farmed in freshwater, tilapia constituting the highest percentage of 46% (Ng et al., 2013). Intensive aquaculture of Nile tilapia as well as other tilapia species is often exposed to stressful conditions which have a negative impact on their growth and immunity. Recently, the tilapia aquaculture in Malaysia has been facing many problems related to disease and massive mortality reported in many popular aquaculture areas such as Lake Kenyir, Lake Pergau and Pahang River (Siti-Zahrah et al., 2004; Najiah et al., 2012). It has been investigated that pathogenic bacteria *Streptococcal* spp. are the major causative agent for this devastating disease related losses in tilapia aquaculture (Najiah et al., 2012). Among different genus, *Streptococcus agalactiae* is the main etiological cause of streptococcosis in wild and farmed tilapia fish. This bacterial infection has been thought to be the primary reason for poor growth and declining fish productivity (Ye et al., 2011). Research on supplementation of IMP in tilapia diets is needed to offer insights into interactions between physiological responses and practical solutions in order to prevent infectious diseases (Li and Gatlin, 2006). To our best knowledge, no research was reported on the utilization of IMP or any other nucleotide in tilapia diet, except Barros et al. (2015), who studied the effect of mixed nucleotide on the performance of tilapia. A commercial unknown nucleotide mixture was tested in their research which provided the effects of a group of nucleotides and there might have interaction, antagonism or synergistic effects among the nucleotides. Therefore, effect of single nucleotide should be investigated in order to get insight the mechanism or mode of action on the performance parameters of fish (Hossain et al., 2016a,b). The current study was designed to investigate the effects of dietary supplementation of IMP on growth performance, feed utilization, biochemical, haematological and immunological responses of juvenile Nile tilapia (*Oreochromis niloticus*) and their disease resistance against *S. agalactiae* infection.

2. Materials and methods

2.1. Feed formulation and preparation of experimental diets

The formulation and proximate composition of experimental diets are shown in Table 1. All the dietary ingredients were obtained from commercial sources. Five experimental diets were formulated by supplementing 0 (IMP0, control), 1 (IMP1), 2 (IMP2), 4 (IMP4) and 8 (IMP8) g IMP (Sigma-Aldrich, St. Louis, USA) kg^{-1} semi-purified diet based on casein and fishmeal as major protein sources. All the dietary ingredients were ground through a sieve (500-μm mesh). To prepare diets, at first IMP and L-arginine of the respective test diets were homogenously mixed in a glass beaker by using a stainless laboratory spatula. Then this premix was added to all other dry ingredients in a food mixer and throughly mixed for 10 min. All the lipid sources were premixed with a sonicator (CA-4488Z, Kaijo Corporation, Tokyo, Japan), added to the dry ingredients and mixed for another 10 min. Water was added gradually (35–40% of the dry ingredients) to the premixed ingredients and mixed for another 10 min. The pH of the diets was adjusted to the range of 7.0–7.5 with 4 N sodium hydroxide. The mixture was then passed through a meat grinder at 1.2–2.2 mm diameter. Then the diets were dried at 40 °C for about 4 h in a mechanical convection oven. After drying, all the diets were packed in plastic bags and stored at −20 °C until used.

Table 1
Feed formulation and nutritional composition of test diets.

Ingredients (g kg^{-1})	Test diets				
	IMP0	IMP1	IMP2	IMP4	IMP8
Fishmeal	150	150	150	150	150
Casein	300	300	300	300	300
Pollack liver oil[a]	70	70	70	70	70
Soybean lecithin[a]	30	30	30	30	30
Vitamin mixture[b]	30	30	30	30	30
Mineral mixture[c]	30	30	30	30	30
L-arginine[d]	25	25	25	25	25
Vitamin C ester[e]	0.8	0.8	0.8	0.8	0.8
Wheat flour	110	110	110	110	110
Starch	90	90	90	90	90
Activated gluten	40	40	40	40	40
IMP[f]	0	1	2	4	8
ά cellulose	124.2	123.2	122.2	120.2	116.2
Proximate composition (g kg^{-1} dry matter)					
Crude protein	410.2	412.5	415.6	410.1	417.9
Crude lipid	112.8	110.7	116.7	112.3	118.3
Ash	60.2	57.1	61.2	63.1	61.3
Gross energy (KJ/ g DM)[g]	20.37	20.36	20.34	20.25	20.27

[a] Riken Vitamin, Tokyo, Japan.
[b] Vitamin mixture (g kg^{-1} diet): ß-carotene 0.10; vitamin D_3 0.01; menadione $NaHSO_3{\cdot}3H_2O$ (K_3) 0.05; DL-α-tochopherol acetate (E) 0.38; thiamine-nitrate (B_1) 0.06; riboflavin (B_2) 0.19; pyridoxine-HCl (B_6) 0.05; cyanocobalamine (B_{12}) 0.0001; d-biotin 0.01; inositol 3.85; niacine (nicotic acid) 0.77; Ca panthothenate 0.27; folic acid 0.01; choline choloride 7.87; ρ-aminobenzoic acid 0.38; cellulose 1.92
[c] Mineral mixture (g kg^{-1} diet): $MgSO_4$ 5.07; Na_2HPO_4 3.23; K_2HPO_4 8.87; Fe citrate 1.10; Ca lactate12.09; Al $(OH)_3$ 0.01; $ZnSO_4$ 0.13; $CuSO_4$ 0.004; $MnSO_4$ 0.03; Ca $(IO_3)_2$ 0.01; $CoSO_4$ 0.04.
[d] Wako Pure Chemical Industries, Osaka, Japan.
[e] L-Ascorbyl-2-phosphate-Mg.
[f] Inosine mono phosphate, Sigma-Aldrich, St. Louis, USA.
[g] Calculated using combustion values for protein, lipid and carbohydrate of 236, 395 and 172 KJ kg^{-1}, respectively. Carbohydrate calculated by difference: 100- (protein + lipid + ash + moisture).

2.2. Commencement of feeding trial

Juvenile Nile tilapia (*O. niloticus*) was collected from commercial traders at Ampang Selangor, Malaysia and brought to the Freshwater Hatchery, School of Fisheries and Aquaculture Sciences, Universiti Malaysia Terengganu, Malaysia. Prior to the initial stocking, the fish were acclimatized for 10 days in the laboratory condition by feeding with a commercial diet (Cargill Malaysia Sdn Bhd, Port Klang, Malaysia). The feeding trial was conducted in 150 L tanks with a closed water system connected to the biological filtration. All the tanks were covered with fine wire to prevent fishes from jumping out of the tanks. Each tank equipped with an inlet, outlet and continuous aeration. The