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Efficacy of nucleotide related products on growth, blood chemistry, oxidative stress and growth factor gene expression of juvenile red sea bream, *Pagrus major*

Md. Sakhawat Hossain [a,b,c,*], Shunsuke Koshio [a,b], Manabu Ishikawa [a,b], Saichiro Yokoyama [b], Nadia Mahjabin Sony [b], Mahmoud A.O. Dawood [a,b], Md. Abdul Kader [b,d], Mahbuba Bulbul [b,e], Takeshi Fujieda [f]

[a] The United Graduate School of Agricultural Sciences, Kagoshima University, Korimoto 1-21-24, Kagoshima 890-0065, Japan
[b] Laboratory of Aquatic Animal Nutrition, Faculty of Fisheries, Kagoshima University, Shimoarata 4-50-20, Kagoshima 890-0056, Japan
[c] Department of Aquaculture, Faculty of Fisheries, Sylhet Agricultural University, Sylhet -3100, Bangladesh
[d] School of Fisheries and Aquaculture Sciences, Universiti Malaysia Terengganu, Kuala Terengganu 21030, Malaysia
[e] Institute of Tropical Aquaculture, Universiti Malaysia Terengganu, 21030, Kuala Terengganu, Malaysia
[f] Research Institute for Bioscience Products & Fine Chemicals, Ajinomoto Co., Inc., Kawasaki, Kanagawa 210-8681, Japan

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ABSTRACT

A feeding trial was conducted to determine the efficacy of nucleotide related products on growth, blood chemistry, oxidative stress and growth factor gene expression of juvenile red sea bream. Five experimental diets were formulated to contain 49% protein, 11% lipid and the diet without nucleotide related product supplementation was the control (D1). Nucleotide related products like; nucleoside by-products (NBPs) and inosine were supplemented at 1, 3 and 0.03, 0.1% consecutively with basal ingredients of D1 and named as D2, D3 & D4 and D5 respectively. Experimental diets were fed to triplicate groups of fish for 60 days. Fish fed diet D5 showed significantly the highest final body weight and % weight gain followed by the diet groups D2 and D4. Fish fed control group showed the lowest growth performance and were not differed significantly with diet group D3. Feed conversion ratio and protein efficiency ratio were also significantly higher in diet group D5, whereas the other supplemented group showed intermediate value. A wide variation in some of the blood parameters was observed. In case of oxidative stress parameters, fish fed inosine supplemented diets showed the best conditions because they performed better under oxidative stress conditions as well as had the highest tolerance against oxidation. Among NBP supplemented groups, diet group D2 also showed acceptable conditions of oxidative stress tolerance. Stress resistance against low salinity exposure (LT_{50}) also increased with dietary supplementation and it was significantly the highest in fish fed diet group D5. Total serum protein, serum lysozyme activity and total peroxidase content tended to be higher ($P > 0.05$) in NBP and inosine supplemented diet groups. In numerically higher hepatic IGF-1, mRNA expression was found in diet groups D2 and D4. However, IGF-1 and IGF-2 mRNA expressions were not significantly altered by dietary supplementations in the present study. Considering overall performance of the present study, we concluded that inosine and low concentration of NBP (1%) could be effectively used as dietary supplements for better growth and health performance of *Pagrus major*.

Statement of relevance: In this study industrial by-products, nucleoside by-products and relatively low cost nucleoside, inosine has been evaluated as potential functional nutrients for marine fish such as red sea bream. Utilization of these functional supplements will help to reduce nucleotide administration cost in fish feed as well as to develop low fishmeal based functional aquafeed in the near future.

1. Introduction

Nucleotides are low molecular weight intracellular compounds which play key roles in diverse essential physiological and biochemical functions including encoding genetic information, mediating energy metabolism and signal transduction (Carver and Walker, 1995). Dietary nucleotides have been reported to be beneficial for humans and animals (Gil, 2002) since they positively influence lipid metabolism, immunity, and tissue growth, development and repair (reviewed in Gil, 2002). In aquatic animals both nucleotides and nucleosides have long been implicated as feed attractants in both vertebrate and invertebrate species (Mackie and Adron, 1978; Carr and Thompson, 1983; Person-Le Ruyet

* Corresponding author at: Laboratory of Aquatic Animal Nutrition, Faculty of Fisheries, Kagoshima University, Shimoarata 4–50–20, Kagoshima City, Kagoshima 890–0056, Japan.
E-mail address: fishsakhawat@yahoo.com (M.S. Hossain).

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et al., 1983; Carr et al., 1984). However, world-wide heightened attention on nucleotide supplementation into potential growth and health benefits in aquaculture species was only instigated after 2000s. To date, research pertaining to nucleotide nutrition in fishes has shown rather consistent and encouraging beneficial results in fish health management.

Inosine, a purine nucleoside containing the base hypoxanthine and the sugar ribose, occurs in transfer RNAs, and is formed during the breakdown of adenosine by adenosine deaminase (Barankiewicz and Cohen, 1985). During the industrial preparation of inosine a liquid form of by-product is produced after the separation of inosine. This liquid nucleoside by-product (NBP) contains considerable portion of inosine nucleoside and small portion of some other nucleotides. Recently, in aquaculture research nucleotides and its related product has been paid attention promisingly as functional nutrients. In aquaculture dietary nucleotide and its related products supplementation has been shown to enhance growth of certain fish species (reviewed by Li and Gatlin, 2006) immune responses and disease resistance of all male hybrid tilapia (*Saratheradon niloticus* ♀ × *Saratheradon aureus* ♂ (Ramadan et al., 1994), Atlantic salmon (*Salmo salar* L.) (Burrells et al., 2001a), common carp (*Cyprinus carpio* L.) (Sakai et al., 2001), hybrid striped bass (*Morone chrysops* × *Morone saxatilis*) (Li et al., 2004), grouper (*Epinephelus malabaricus*) (Lin et al., 2009), red drums (*Sciaenops ocellatus*) (Cheng et al., 2011), rainbow trout (*Oncorhynchus mykiss*) (Tahmasebi-Kohyani et al., 2012) and Japanese flounder (*Paralichthys olivaceus*) (Song et al., 2012). Supplementation of nucleotides was also reported to increase stress tolerance in Atlantic salmon (Burrells et al., 2001b), rainbow trout (Leonardi et al., 2003), red sea bream (Hossain et al., 2016) and even gastrointestinal physiology and morphology of tilapia (Ramadan et al., 1994), Atlantic salmon (Burrells et al., 2001b) and red drum (Cheng et al., 2011).

Red sea bream, *Pagrus major*, is one of the commercially important aquaculture species, whose production reaches the second largest in Japan (Koshio, 2002). Intensive culture of this species often exposed it to stressful conditions which impaired growth and immunity of the fish. Exposure to stress places additional demands on available nucleotides, and an additional exogenous supply of nucleotides provided by dietary supplementation may help to counter the immunosuppressive effects of stress (Low et al., 2003). Although, there are some research into potential growth and health benefits of dietary nucleotides in aquaculture species (Burrells et al., 2001a, 2001b; Li et al., 2004; Cheng et al., 2011; Song et al., 2012, Hossain et al., 2016). However, in most of cases, purified nucleotide mixtures were used and still, some gap exists about current knowledge of nucleotide supplementation in fish diets and its effects on physiology and immunity. Moreover, in aquaculture, nucleotides are more widely studied than relatively cheaper nucleosides. So far, study conducted on nucleosides has mainly focused on its feeding stimulatory properties rather than its functional properties. Until recently, there is also no research on the use of low cost industrial by-product, which can also be used as a source of nucleotide or nucleoside for red sea bream as well as other marine species. In this circumstance, studies on the efficacy of supplementing relative low cost nucleotide related products viz. NBP and inosine on growth and health performance are important for the effective use of these functional supplements. So, the aim of this study was to investigate the efficacy of utilizing nucleotide related products on growth, blood chemistry, oxidative stress and growth factor gene expression of red sea bream.

2. Materials and methods

2.1. Test fish and experimental system

Juvenile red sea bream were obtained from a local hatchery, in Kagoshima prefecture, Japan, and transported to the Kamoike Marine Production Laboratory, Faculty of Fisheries, Kagoshima University, Japan. The fish were maintained in 500 L tank with continuous aeration and flow through sea water and fed a commercial formulated diet (54% crude protein, Higashimaru Foods, Kagoshima Japan) for one week to acclimatize with the laboratory facilities. The feeding trial was carried out in 100 L polycarbonate tanks (filled with 80 L of water) in a flow through sea water system where each tank was equipped with an inlet, outlet, and continuous aeration. The tanks were maintained with natural light/dark regime. The seawater was pumped from the deep basin of Kagoshima bay, Japan; gravel filtered and supplied to the system. A flow rate of 1.5 L min^{-1} was maintained throughout the experimental period.

2.2. Ingredients and test diets

Tables 1, 2, 3 and 4 summarize the formulation and chemical composition, total and free amino acid composition of the experimental diets, respectively. Five experimental diets were formulated to contain 49% protein, 11% lipid, without nucleotide related products supplementation considered as control (D1). Nucleotide related products like; nucleoside by-products (NBPs) and inosine were supplemented at 1, 3 and 0.03, 0.1% consecutively with basal ingredients of D1 and named as D2, D3 & D4and D5 respectively. The diets were prepared by thoroughly mixing all the dry ingredients in a food mixer for 10 min. Liquid NBP were also simultaneously added with dry ingredients of the respective diets. Pollack liver oil and soybean lecithin 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 (30–40% 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 with an appropriate diameter (1.2–2.2 mm) to prepare pellets, which were then dried in a dry-air mechanical convection oven (DK 400, Yamato Scientific, Tokyo, Japan) at 55 °C for about 150 min. The test diets were stored at −28 °C in a freezer until use.

Table 1
Ingredients and formulation of the experimental diets (% o f the dry ingredients).

Ingredients	Diet groups				
	D1[a]	D2	D3	D4	D5
Fishmeal[b]	46.53	46.15	45	46.53	46.53
Soybean meal[c]	20	20	20	20	20
Pollack liver oil[d]	3.65	3.68	3.73	3.65	3.65
Soybean lecithin[e]	2.5	2.5	2.5	2.5	2.5
Vitamin mixture[f]	3	3	3	3	3
Mineral mixture[g]	3	3	3	3	3
Stay-C[h]	0.3	0.3	0.3	0.3	0.3
Wheat flour	11	11	11	11	11
Activated gluten[i]	5	5	5	5	5
α-Cellulose	5.02	4.37	3.47	4.99	4.92
NBP[j]	0	1	3	0	0
Inosine[j]	0	0	0	0.03	0.1

[a] According to Kader et al. (2012).
[b] Nippon Suisan Co. Ltd., Tokyo, Japan.
[c] J. Oil Mills, Japan.
[d] Riken Vitamin, Tokyo, Japan.
[e] Kanto Chemical Co., Inc. Tokyo, Japan.
[f] Vitamin mixture (g kg^{-1} diet): β-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; Cyanocobalamin (B_{12}), 0.0001; 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.
[g] 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 lactate,12.09; 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.
[h] Stay-C 35.
[i] Glico Nutrition Company Ltd. Osaka, Japan. Commercial name "A-glu SS".
[j] NBP (nucleoside by-products) Ajinomoto Co., Inc., Tokyo, Japan.
[j] Tokyo Chemical Industry Co., Ltd. Tokyo, Japan.