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- RESEARCH ARTICLE-

Investigation of Glutathion S-Transferase, Adenosine deaminase, Paraoxonase Activities in Liver of *Oncorhynchus mykiss* Fed with Nucleotide-Yeast Supplemented Diet

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Abstract

In this study, it was evaluated Glutathione S transferase (GST), Adenosine deaminase (ADA), and paraoxonase (PON) activities in liver tissue of *Oncorhynchus mykiss* fed with nucleotide yeast base protein supplemented diet. Throughout the 60-day period the control group was fed a fish meal based basal diet, and three other groups were fed diets in which 20% (NP 20), 40% (NP 40) and 60% (NP 60) fish meal was substituted with nucleotide (Nu-Pro® (NP) yeast). At the end of experiment, liver tissue GST, PON and ADA activity was increased significantly ($P<0.05$) in nucleotide-yeast groups when compared to control group.

Keywords:

Oncorhynchus mykiss, nucleotide, yeast, oxidative stress

Article history:

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Introduction

Nutritional factors are components of the diet that are essential for normal growth and fish development. The use of micronutrients such as vitamins, trace elements, prebiotics and immunostimulants as dietary supplements can improve fish health by strengthening defense systems (Ringø et al., 2012). They also improve the availability or use of nutrients in a variety of ways. It is known that feeding can affect fish health and immune responses (Ganguly et al. 2010; Özlüer Hunt et al. 2016).

The application of brewer's yeast, mainly the *Saccharomyces cerevisiae* strain, as a source of nutrients and bioactive compounds in aquaculture started as early as the beginning of the 1990s (Ferreira et al., 2010). Nupro® (Alltech Inc.) is a yeast-based product formed from the

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collection of the cytoplasmic contents of the yeast (*Sacharomyces cerevisiae*) cell. The resulting product is of high protein content (>50% crude protein, dry weight basis), and balanced in terms of amino acid profile (Craig & McLean, 2005). Furthermore, NuPro® is a rich source of nucleotides, estimated to be around 5% of the dry weight (Fegan, 2006). Thus, the benefits of using NuPro® in feeds are both nutritional, and as a functional nutrient.

Nucleotides are low molecular weight biological compounds that play important roles in essential physiological and biochemical functions (Carver & Walker, 1995). Nucleotides are synthesized de novo in most tissues, but some immune and intestinal cells are absent from the process and depend on exogenous source (Uauy, 1994). Thus, the application of exogenous nucleotides guarantees greater usability of the body at high demand for a variety of physiological activities (Whitehead et al., 2006). The effects of the use of exogenous nucleotides on digestion, absorption, metabolism and various other physiological events in fish farming have not yet been fully understood. Information is needed in the context of the type of fish, age, and the known doses (Ringø et al., 2012).

Dietary supplementation of nucleotides has been tested in different aquatic species, and various beneficial effects of dietary nucleotides on fish have been reported, including improved growth (Lin et al., 2009), enhanced immune response (Shiau et al. 2015), increased tolerance to stress (Xiang et al., 2011; Welker et al., 2011), enhanced antioxidative capacity (Meng et al. 2017) and improved intestinal morphology (Peng et al., 2013). Research on dietary nucleotides in fishes has shown they may improve growth in early stages of development, enhance larval quality via broodstock fortification, alter intestinal structure, increase stress tolerance as well as modulate innate and adaptive immune responses (Ringø et al., 2012). Fishes fed nucleotide-supplemented diets generally have shown enhanced resistance to viral, bacterial and parasitic infection (Li & Gatlin, 2006).

Organisms include a system that permanently deactivates reactive oxygen species (ROS) and other prooxidants with low molecular weight free radical scavengers and antioxidant enzymes. The antioxidant defense system is called the intracellular and extracellular enzyme and non-enzyme defense mechanism against reactive oxygen species (Keleştemur & Özdemir, 2011). Lipids are biomolecules that are most sensitive to the effects of free radicals. Cholesterol and unsaturated bonds of fatty acids in cell membranes easily react with free radicals to form peroxidation products. Lipid peroxidation is the oxidative degradation process of polyunsaturated fatty acids (PUFAs) (Repetto et al., 2012). Antioxidant defenses in fish are dependent on factors such as feeding behavior and nutrition (Martinez-Alvarez et al., 2005).

Paraoxonase (PON, aryldialkyl phosphatase, (EC 3.1.8.1) is an enzyme linked to calcium and is an esterase which hydrolyses aromatic carboxylic acid esters synthesized by the liver (Li et al., 1993). PON is an antioxidant, neutralizing lipid peroxides and showing a protective effect on cell membranes (Aviram & Rosenblot, 2004).

Glutathione S-transferase (GST, EC 2.5.1.18) is a Phase-II detoxification enzyme that protects cellular macromolecules from reactive electrophiles by interacting with electrophilic and hydrophilic compounds with glutathione (Hayes et al., 2005). GST catalyze the nucleophilic attack of glutathione (GSH) tripeptide on electrophilic substrates in catalysis reactions. In addition, it prevents oxidative products or foreign toxic substances from merging with other

macromolecules in the body and allows them to be expelled without harming the cell components. Therefore, GST is one of the most important protective groups of enzymes (Armstrong, 1997).

Adenosine deaminase (ADA, EC 3.5.4.4) is an important enzyme in the purinergic system responsible for irreversible deamination of adenosine levels, a molecule with anti-inflammatory properties (Moreno et al. 2018). This enzyme acts as an endogenous regulator of innate immunity protecting the host tissue from damage and has an important role in immune and inflammatory responses due to the regulation of Adenosine levels (Hasko & Cronstein, 2004).

In this study, it was aimed to understand the importance of Nupro® in terms of fish health in using as feed raw materials. *Oncorhynchus mykiss* was fed with feed containing 20%, 40% and 60% of Nupro® for 60 days. GST, PON and ADA enzyme activities in liver tissue were examined.

Material and Method

Chemicals

Nupro® obtained from Alltech Inc., Nicholasville, KY, USA. All other chemicals were purchased from Sigma Aldrich Chemical Corporation (USA).

Fish diets

Three balanced iso-nitrogenous and iso-energetic diets were formulated with supplemented commercial organic yeast (Nu-Pro® Alltech Inc., Nicholasville, KY, USA) and replaced fish meal at 20 (Nu-Pro®20), 40 (Nu-Pro®40) and 60 (Nu-Pro®60) % levels on a dry matter basis (Table 1), and a control (Nu-Pro® 0). Nu-Pro® is a functional protein from yeast containing highly concentrated levels of essential and functional nutrients. It is rich in nucleotides, inositol, glutamic acid, amino acids and peptides. After pelletizing, each diet was individually bagged and stored at -20 °C until use. The diets were analyzed in duplicate for proximate compositions according to the standard methods of AOAC (1996).

Table 1. Formulation (g/kg diet) and chemical composition of the experimental diets

	Diets			
	Control	**NP20 (%)**	**NP40 (%)**	**NP60 (%)**
Fish meal[1]	605	501	432	355
Corn gluten meal	70	100	93	94
Nu-Pro®[2]	0	121	242	363
Fish Oil[3]	100	108	113	119
Dextrin[3]	140	91	55	10
Binder (CMC)	52	46	32	26
Mineral Mix.[4]	20	20	20	20
Vitamin Mix.[4]	13	13	13	13
Proximate Composition (g/kg)				
Dry Matter	896.5	904.4	909.1	908.6
Crude protein	441.7	436.1	448.6	439.3
Crude lipid	187.3	187.6	187.5	188.9
NFE[5]	159.2	168.2	162.8	179.4

Crude ash	108.4	112.5	110.2	101.0
Gross energy (MJ kg/DM)[6]	18.9	19.0	18.9	18.9
P:Eratio (g /MJ)	23.36	22.95	23.74	23.24
Calculated TAA Profile (g/kg)				
Arginine	16.46	16.01	15.99	15.87
Histidine	7.09	7.15	7.27	7.38
Isoloucine	13.94	14.08	14.35	14.59
Leucine	24.89	26.67	27.26	28.09
Lysine	20.86	20.28	20.62	20.75
Methionine	8.77	8.71	8.73	8.73
Cystine	2.96	3.26	3.41	3.59
Phenyalanine	13.00	13.58	13.88	14.23
Tyrosine	10.53	11.22	11.65	12.12
Thyreonine	12.32	12.89	13.63	14.33
Tryptophane	3.22	3.26	3.36	3.45
Valine	15.52	15.98	16.53	17.06

[1] Anchovy fish meal and oil. SIBAL Black Sea Feed Inc., Sinop, Turkey
[2] Alltech Incorporated, Nicholasville, KY, USA.
[3] SUNAR Inc., Adana, Turkey.
[4] Vitamin and mineral premix added minimum to NRC recommendations, SIBAL Black Sea Feed Inc., Sinop, Turkey (NRC,1993)
[5] Nitrogen-Free Extract: Calculated as the remainder of crude protein + crude lipid + ash.
[6] Calculated based on the standard physiological fuel values: 19 kJ/g for protein, 36 kJ/g for lipid and 15 kJ/g for carbohydrate (Smith, 1989).

Fish and experimental conditions

Rainbow trout fingerlings were obtained from a commercial farm in Gözne-Mersin-Turkey. The fish were transported to the Mersin University Aquaculture Laboratory Unit and acclimatized to laboratory conditions. During the acclimation period, fish were fed a commercial diet (Crude protein: 48 %, crude lipid: 22 %, Çamli Yem-İzmir/Turkey) without dietary nucleotides for 2 weeks at a level of 3 % of their body weight. At the end of the acclimation period, fish (27.76 g ± 0.26, mean weight ± SEM) were randomly distributed into 12 tanks (12 fish/tank, total 144 fish). The tank system was installed in an environmentally-controlled laboratory where temperature was maintained between 15-16 °C, with a photoperiod of 12 h light and 12 h dark. Water depth in the 200 L tanks was kept at 50 cm throughout the experiment by adding fresh water continuously from a reservoir tank after daily siphoning of uneaten feed and feces. Fish were fed two equal portions of the experimental diets, twice daily (8:00-9:00am and 16:00-17:00pm) at 3 % body weight (BW) per day, throughout the 60-day growing period. Average water quality parameters were measured as; temperature 15.5±0.8 °C, dissolved oxygen 8.1±0.08 mg/L, pH 8.6±0.25, NO_3^- 0.5±0.1 mg/L, NO_2^- 0.03±05 mg/L, and NH_4^+ 0.07±0.6 mg/L.

Tissue sampling for biochemical analysis

Biochemical analyzes were performed in the biochemistry laboratory of the Pharmacy Faculty. At the end of the study, 24 fish (6 fish per treatment) were randomly chosen from each dietary group, sacrificed, and used for analysis of liver tissue GST, PON and ADA activities. The tissue samples were obtained from each individual fish, combined and prepared for analysis. Tissue samples were homogenized in 1/5 (w/v) ratio of physiological saline solution (0.8 % NaCl) with

a homogenizer and then centrifuged at 13500 rpm for 15 min in a Sigma 2-16 K centrifuge at -4 ºC. The supernatants were then used for biochemical analyses.

The tissue GST activity was determined spectrophotometrically by following the formation of GSH conjugate with 1-chloro-2,4-dinitrobenzene (CDNB) at 340 nm using extinction coefficient of 9.6 mM^{-1} cm^{-1} (Habig et al. 1974). The reaction mixture contained in 1 mL volume: 0.1 M potassium phosphate buffer (pH 6.5), 1 mM GSH, 1 mM CDNB in ethanol and the tissue supernatant. The GST activity was expressed in U/mg protein.

The tissue PON enzyme activity was determined using the method specified by Bastos et al. (1998). The formation of para-nitrophenol, which is the result of enzymatic hydrolysis of PON, was determined by measuring the spectrophotometer at 400 nm using Tris-HCI containing paraoxone. For enzyme activity, 1 unit is expressed as 1 μmol of paranitrofenol/ ml/min. The enzyme activity found was expressed as U/mg protein.

The activity of ADA in tissue was determined spectrophotometrically according to the method described by Giusti & Galanti (1984). In brief, the formation of colored indophenol complex from ammonia liberated from adenosine and quantified spectrophotometrically (blue colour occurred because of appearing indophenol blue). This colour was detected by spectrophotometer at 628 nm against to the blank. The values were reported in units per mg protein (U/mg protein).

The tissue protein contents were measured only to determine the specific activity of enzymes according to the method developed by Lowry et al. (1951) using bovine serum albumin as standard. Absorbance of samples were measured at 750 nm wavelength by spectro-photometer.

All data were expressed as mean ± standard error (SE) and analyzed using SPSS statistical package programs. One-way ANOVA was used to compare variables among control and treatments. Duncan's multiple range tests were used to analyze differences between groups. The differences were defined as statistically significant when $P<0.05$.

Results

The GST activity in liver tissues of fish are shown in Figure 1. There was no significant change in NP20 group compared to control. The GST activities were increased significantly ($P<0.05$) in NP40 (43%) and NP60 groups (42%) compared with control (Figure 1).

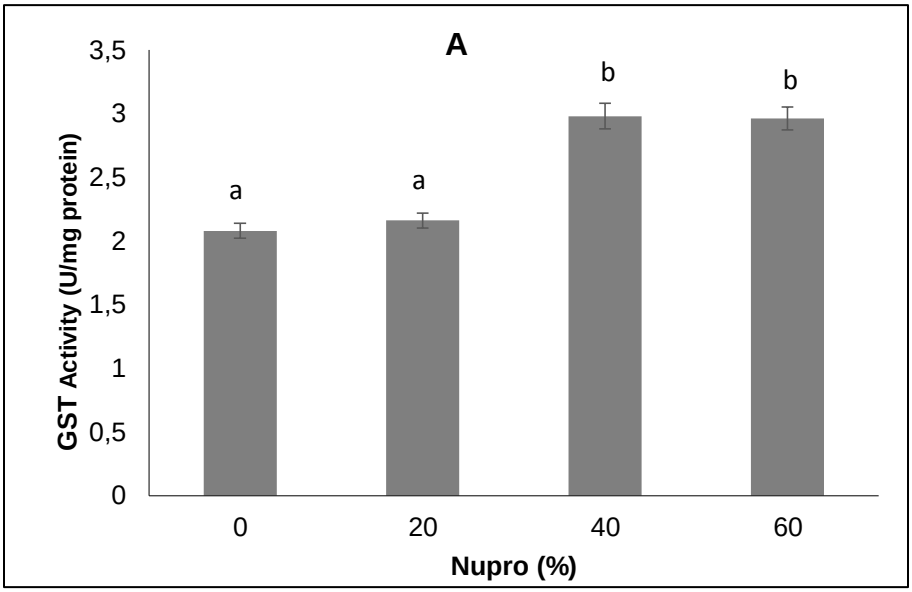

Figure 1. The GST activity in liver tissue of *O. mykiss* fed different levels of nucleotide-rich yeast supplementation for 60 days. Each value represents the mean±SEM. The different letters indicate significant (P<0.05) difference between diet groups.

The PON activity in liver tissues of fish are given in Figure 2. NP20 did not show any significant change in PON activity compared to control. The PON activities were increased significantly (P<0.05) in NP40 (15%) and NP60 (22%) groups compared to control (Figure 2).

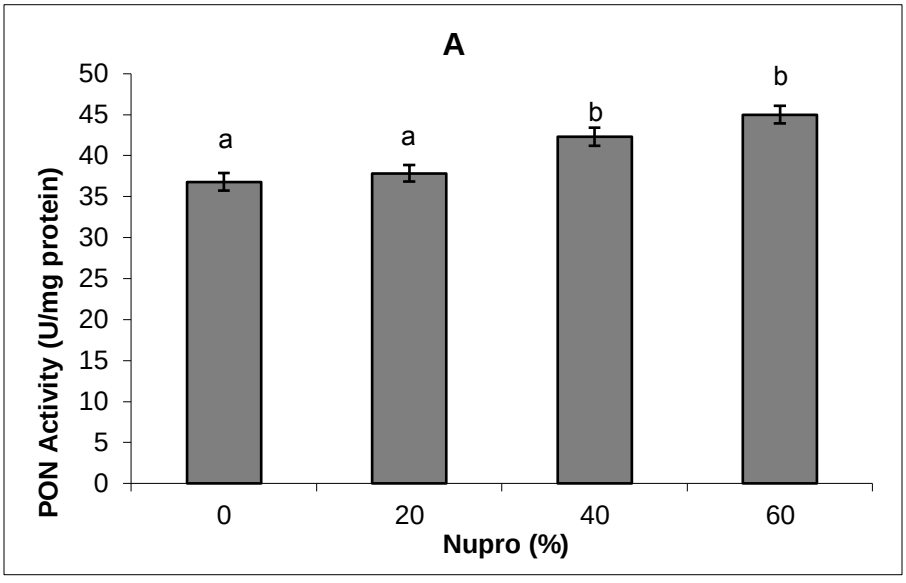

Figure 2. The PON activity in liver tissue of *O. mykiss* fed different levels of nucleotide-rich yeast supplementation for 60 days. Each value represents the mean±SEM. The different letters indicate significant (P<0.05) difference between diet groups.

The ADA activity in liver tissues of fish are given in Figure 3. The ADA activities were increased significantly (P<0.05) in NP40 (35%) and NP60 (46%) groups compared to control Figure 3).

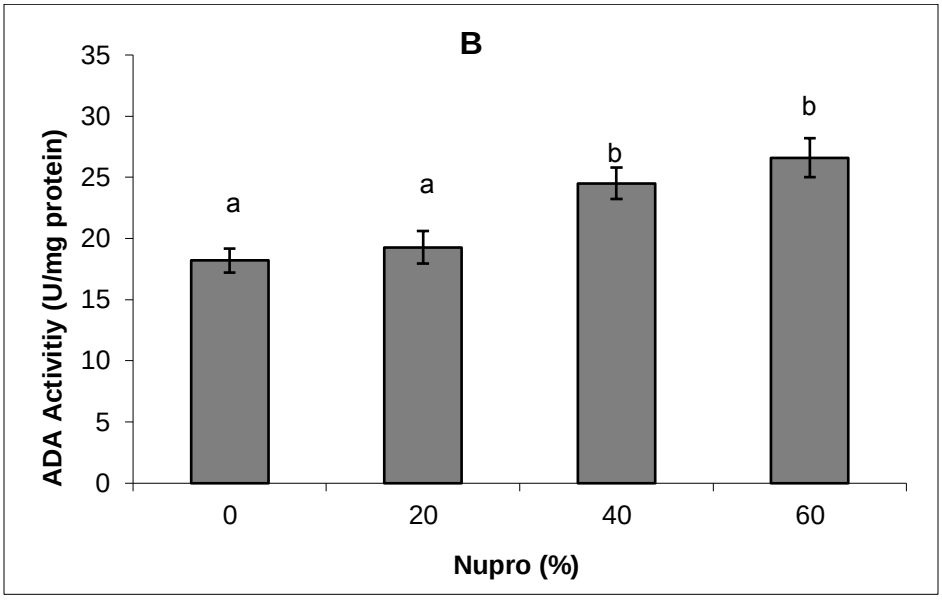

Figure 3. The ADA activity in liver tissue of *O. mykiss* fed different levels of nucleotide-rich yeast supplementation for 60 days. Each value represents the mean±SEM. The different letters indicate significant (P<0.05) difference between diet groups.

Discussion

In the present study, GST, ADA and PON activities were increased in the NP40 and NP60 groups. Glutathione-S-transferases are a family of multifunctional enzymes that are involved in the detoxification of both xenobiotics as well as endogenous reactive compounds of cellular metabolism (Mani et al. 2014). They are mainly involved in the free radical scavenging and peroxide reduction through the formation of GSH conjugates (Sugimoto, 1995). PON has many enzymatic activities and is known to have an antioxidant function. PON plays a role in decreasing the oxidative stress (Bodolay et al. 2008). The cysteine of the paraoxonase has been shown to be an antioxidant due to its amino acid and its role in protecting against low-density lipoprotein (LDL) oxidation and also to reduce hydroperoxide accumulation in HDL and LDL by its ability to hydrolyze lipid peroxides (Aviram et al. 1998; Camps et al. 2009). ADA activity has been associated primarily with lymphocytes and ADA activity occurs as an attempt to improve the immune system (Baldissera et al. 2018).

The extract obtained from yeast *S. cerevisiae* is an excellent example of a functional food. *S. cerevisiae* yeast protein has been used successfully as an alternative protein source with a few fish species (Li & Gatlin 2006; Craig & McLean 2005). The main component of the yeast cell wall is *β*-glucan (50-60%), which stimulates the immune function of fish (Manoppo et al. 2015). *S cerevisiae* yeast is a rich source of prebiotics, mainly β-glucan. Some studies have demonstrated that brewer's yeast (*S. cerevisiae*), yeast culture or yeast extract is able to improve

growth and animal health (Deng et al., 2013). The reasons may be due to the nutritional value of their nutrients such as proteins, vitamins and peptides in the yeast (Yuan et al., 2017). It has been demonstrated that nucleotide-rich yeast supplemented to diets can positively affect in aquculture growth, intestinal structure, innate and adaptive immune responses, disease resistance and anti-oxidant status (Xu et al. 2015; Xiong et al., 2018). Due to the limited knowledge about dietary supplemented nucleotides in fish, especially on their metabolism and influence on various physiological processes (Xu et al. 2015), yet detailed explanations cannot be reported.

Exogenous nucleotides can function in cell signaling events and can also serve as nutrients for biosynthesis (Li & Gatlin, 2006). Nucleotide effects on gene transcription rate have been reported in murine intestinal epithelial cells (Walsh et al. 1992) and intestine (Sanchez-Pozo & Gil, 2002). Although nucleotides can be synthesised de novo, several tissues may benefit from an exogenous supply to decrease energy expenditures (Sauer et al. 2011). The beneficial effects of dietary nucleotides on hepatocytes in animal have been more widely investigated, and nucleotides were found to improve hepatic function (Sauer et al. 2011). Nucleotides have been used as functional nutrients to improve the health of animals, including fish (Xu at al. 2015; Özlüer-Hunt et al. 2016).

The liver damage may be associated with oxidative stress and lipid peroxidation; in particular, oxidative stress and lipid peroxidation are key factors in the development and progression of liver damage (Atamer et al. 2008). Increased antioxidant enzyme activities demonstrate improved control of oxidative stress by the use of dietary nucleotide-rich yeast (Xu et al. 2015).

In conclusion, compared with control, dietary nucleotide-rich yeast supplementation increased GST, PON and ADA activities in liver of *O. mykiss*. It can be suggested that dietary nucleotide use may improve anti-oxidant status and immune response for *O. mykiss*. The nucleotide research in fishes are new area, so detail studies are needed to understand the mechanism of nucleotide.

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