

The Open Access Israeli Journal of Aquaculture – Bamidgeh

As from **January 2010** The Israeli Journal of Aquaculture - Bamidgeh (IJA) will be published exclusively as **an on-line Open Access (OA)** quarterly accessible by all AquacultureHub (<http://www.aquaculturehub.org>) members and registered individuals and institutions. Please visit our website (<http://siamb.org.il>) for free registration form, further information and instructions.

This transformation from a subscription printed version to an on-line OA journal, aims at supporting the concept that scientific peer-reviewed publications should be made available to all, including those with limited resources. The OA IJA does not enforce author or subscription fees and will endeavor to obtain alternative sources of income to support this policy for as long as possible.

Editor-in-Chief

Dan Mires

Editorial Board

Rina Chakrabarti	Aqua Research Lab, Dept. of Zoology, University of Delhi, India
Angelo Colorni	National Center for Mariculture, IOLR Eilat, Israel
Daniel Golani	The Hebrew University of Jerusalem Jerusalem, Israel
Hillel Gordin	Kibbutz Yotveta, Arava, Israel
Sheenan Harpaz	Agricultural Research Organization Beit Dagan,
Gideon Hulata	Agricultural Research Organization Beit Dagan,
George Wm. Kissil	National Center for Mariculture, IOLR, Eilat, Israel
Ingrid Lupatsch	Swansea University, Singleton Park, Swansea, UK
Spencer Malecha	Dept. of Human Nutrition, Food & Animal Sciences, CTAHR, University of Hawaii
Constantinos Mylonas	Hellenic Center for Marine Research, Crete, Greece
Amos Tandler	National Center for Mariculture, IOLR Eilat, Israel
Emilio Tibaldi	Udine University Udine, Italy
Jaap van Rijn	Faculty of Agriculture, The Hebrew University of Jerusalem, Israel
Zvi Yaron	Dept. of Zoology, Tel Aviv University, Tel Aviv, Israel

Published under auspices of
**The Society of Israeli Aquaculture and Marine Biotechnology (SIAMB),
University of Hawai'i at Mānoa Library**

&

**University of Hawai'i at Mānoa
Aquaculture Program**
in association with
AquacultureHub

<http://www.aquaculturehub.org>



UNIVERSITY
of HAWAII[®]
MĀNOA
LIBRARY



AquacultureHub.org

AquacultureHub
educate • learn • share • engage

ISSN 0792 - 156X

© Israeli Journal of Aquaculture - BAMIGDEH.

PUBLISHER:

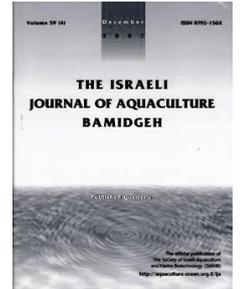
Israeli Journal of Aquaculture - BAMIGDEH -
Kibbutz Ein Hamifratz, Mobile Post 25210,
ISRAEL

Phone: + 972 52 3965809

<http://siamb.org.il>



The IJA appears exclusively as a peer-reviewed on-line open-access journal at <http://www.siamb.org.il>. To read papers free of charge, please register online at [registration form](#).
Sale of IJA papers is strictly forbidden.



The Effects of Fish Meal Replacement by Yeast Based Nucleotides on Growth, Body Composition and Digestive Enzyme Activity in Rainbow Trout Juveniles (*Onchorhynchus mykiss*)

Arzu Özlüer Hunt^{1*}, Ferbal Özkan Yılmaz¹, Kenan Engin¹, Mehmet Berköz², Suna Gül Gündüz¹, Serap Yalın³, Nefise Özlen Şahin⁴

1 Department of Aquaculture, Faculty of Fisheries, Mersin University, 33160, Mersin, Turkey

2 Department of Pharmaceutical Technology, Faculty of Pharmacy, Yuzuncu Yil University, Van, Turkey

3 Department of Biochemistry, Faculty of Pharmacy, Mersin University, Mersin, Turkey

4 Department of Pharmaceutical Technology, Faculty of Pharmacy, Mersin University, Mersin, Turkey

(Received 22.2.13, Accepted 04.5.13)

Key words: growth, nucleotide, protein source, trout, *Onchorhynchus mykiss*, digestive enzymes

Abstract

This 60 day study investigated the effects of organically certified nucleotide yeast-derived protein source (Nu-Pro[®]) on growth, feed efficiency, fillet proximate composition and digestive enzymes in rainbow trout (*Onchorhynchus mykiss*). Diets were isonitrogenous and isocaloric. Three experimental diets wherein 20%, 40% and 60% of the fish meal content was replaced by Nu-Pro[®], were compared to the control diet in which the crude protein content was anchovy fish meal and corn gluten meal. The rainbow trout (initial weight 27 g/fish) were fed twice daily in 200-l Aqaria. Live weight gain increase ranged from 125-195% in fish fed the experimental diets. Results indicated that up to 40% fish meal protein can be replaced by Nu-Pro[®] without compromising growth rates, feed efficiency or the fillet biochemical composition in the rainbow trout. The effect of the dietary Nu-Pro[®] supplementation on digestion was partly observed by assaying the activity of pepsin, intestinal amylase, trypsin and lipase. Apart from amylase activity, results indicated that dietary supplementation of nucleotides is beneficial and may also have differential effects upon digestive enzyme activities.

* Corresponding author. Tel.: 90-324-3412815-1325, fax: 90-324-3413025, e-mail: huntarzu@hotmail.com

Introduction

Fish meal is the major feed ingredient used in aqua feeds for most finfish and crustacean species cultured worldwide (Tacon and Metian, 2008). Since fish meal and oil are finite resources it is necessary to find cheaper and readily available alternative protein and oil sources to be used in aqua feed for sustainable developments in the aquaculture industry (Craig and Mclean, 2005; Fountoulaki et al., 2009).

The search for alternatives to fish meal as a source of protein in aquaculture diets has been an important area of research. Much of this research has focused on increasing the proportion of plant proteins (Kissil and Lupatch, 2004), in feeds for fish and shrimp. Alternative feed ingredients are required to provide the essential nutrients for the growth and quality of aquaculture production. Several animal or plant derived materials have been tested as alternative protein sources, (Olvera-Novoa et al., 2002; El-Saidy and Gaber, 2003). Many vegetable proteins have disadvantages, including low nutrient densities, anti-nutritional factors, high carbohydrate content, imbalanced amino acid and fatty acid profiles, low palatability, seasonal variability, and potential mycotoxin contamination (Oliva-Teles and Gonçalves, 2001).

Single cell proteins (SCP) such as yeasts are potentially desirable alternate protein sources due to their high nitrogen content (Oliva-Teles, 2006). They have been used as dietary supplements in aquafeeds since it has been found that even in small quantities, immunostimulants, probiotics, and prebiotics usually improve immunity, feed efficiency, growth performance, and enzymatic contribution in the digestion of crustaceans and fish (Ganguly et al., 2010).

One particular product derived from a specific strain of the yeast *Saccharomyces cerevisiae*, and the cell contents, are further processed to produce nucleotides Nu-Pro[®] in which the crude protein content has a 47-50% similarity to fish meal. Yeast extract NuPro[®] is a complex product combining nutritional components, such as protein and vitamins, with functional components such as nucleotides and free amino acids therefore in feeds it is both nutritious and functional (Fegan, 2006).

Several studies have shown that yeasts can successfully replace part of dietary fish meal for different fish species (Oliva-Teles and Goncalves, 2001; Desale et al., 2008). In a series of trials to develop organically certifiable feeds for fish and shrimp, it has been shown that complete replacement of fish and soybean meal with Nu-Pro[®] is possible, although the growth rate of cobia, a marine carnivore, decreased at levels of 50% replacement and higher (Craig and McLean, 2005). Dietary nucleotides positively affect the immune system, hepatic function, lipid metabolism, disease resistance, development of small intestine, and growth (Burrells et al., 2001; Li et al., 2005). Nucleotides have been tested on growth and feed utilization in rainbow trout but information pertaining to their effect on digestive enzyme activity is limited (Staykov et al., 2009 and Güroy et al., 2012).

Since trout is one of the most valuable fresh water fish cultured in the world this study was conducted to evaluate the effect of partial replacement of fish meal with yeast based nucleotides, by measuring growth performance, body chemical composition, and digestive enzyme activity mainly pepsin, amylase, trypsin and lipase in rainbow trout juveniles.

Materials and Methods

Diet formulation and preparation. Three balanced isonitrogenous and isoenergetic diets were formulated to contain commercial organic yeast derived nucleotide protein (Nu-Pro[®] Alltech Inc., Nicholasville, KY, USA) replacing the main protein sources of anchovy meal and corn gluten in the control group fish meal diet with different concentration of Nu-Pro[®], 20%, 40% and 60% (NP20, NP40 and NP60) levels on a dry matter basis (Table 1). Nu-Pro[®] is a functional protein from yeast and contains highly concentrated levels of essential and functional nutrients, which are important in the diets of young animals. It is rich in nucleotides, inositol, glutamic acid, amino acids and peptides. In all diets in the experiment fish oil was the main oil source and dextrin was the carbohydrate source (Table 1).

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

	Diets			
	Control	NP 20 (%)	NP 40 (%)	NP 60 (%)
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:E ratio (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
Phenylalanine	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 standard physiological fuel values: 19 kJ/g for protein, 36 kJ/g for lipid and 15 kJ/g for carbohydrate (Smith, 1989).

The dry ingredients for the diets were mixed in a large bucket for 45 minutes using kitchen hand mixers; with the addition of the dietary oil source and water they were mixed for an additional 30 minutes. Diets were cold pressed using an electric kitchen meat grinder (Arnica, Promeat W1800, Istanbul-Turkey). Feed strands were then dried at room temperature and hand-cut into bite size pellets. The dried pellets for each dietary treatment were individually packaged and stored at -20 °C until use. They were analyzed in duplicate for the proximate compositions using standard methods (AOAC, 1996).

Experimental Systems and Animals. The experiment was conducted using a semi-closed recirculation system consisting of 12 plastic 200-l aquaria. The system was installed in an environmentally-controlled laboratory at the fish culture unit in the Faculty of Fisheries, Mersin University, where air temperature is maintained at 15-16°C, and the photoperiod at 12L:12D. Water depth in the tanks was kept at 50 cm throughout the experiment by adding fresh water continuously from a reservoir tank following daily siphoning of uneaten feed, and feces. All tanks were thoroughly cleaned every 2 weeks after individual fish weight increments were recorded. Dissolved oxygen was measured every other day using YSI model 58 oxygen meters and kept at, or above, 6 mg/l throughout the experiment (Yellow Springs Instrument Company, Yellow Springs, OH). pH was monitored twice weekly with an electronic pH meter (pH pen. Fisher Scientific, Cincinnati, OH). Total NO₃, NO₂ and NH₄ levels were monitored once a week using a Merck-Spectroquant[®] Nova 60A. Water temperature was recorded daily at 13:00 h using a mercury thermometer suspended at 25-cm depth. Average water quality parameters were temperature 15.5±0.8°C, dissolved oxygen 8.1±0.08 mg/l, pH 8.6±0.25, NO₃ 0.5±0.1 mg/l, NO₂ 0.03±0.05 mg/l, and NH₄ 0.07±0.6 mg/l.

A total of 500 rainbow trout, average initial body weight of 25 g, were obtained from a local trout farm in Gözne-Mersin-Turkey. The trout were acclimated to laboratory

conditions for 2 weeks in two 500-l fiberglass tanks and fed a commercial diet (Crude protein: 48%, crude lipid:22%, Çamli Yem-İzmir/Turkey) at a level of 3% of body weight. Following the acclimatization period, 144 fish were individually weighed and randomly distributed (12 fish/per tank) where they were acclimated to the experimental system for 2 days without feed. Each of the 4 treatments was replicated 3 times, 1 tank per replication. Fish were fed two equal portions of the experimental diets, twice daily (800-900am and 1600-1700pm) at 3% BW/d. The uneaten feed and feces were siphoned out 30 minutes after feeding and half of the water in the system was exchanged daily throughout the experiment to maintain water quality. Fish from each tank were weighed biweekly and daily food rations were adjusted after weighing.

Proximate composition analysis. From each treatment, 24 fish (2 fish per tank, 6 fish per treatment) were randomly chosen from each treatment. Fish fillets were analyzed for: moisture, by heating at 60°C to constant weight; protein, by estimating the Kjeldahl nitrogen ($\times 6.25$) in an automated distillation unit; lipid, by chloroform/methanol extraction; and ash, by incinerating in a muffle furnace at 550°C for 18 h, (AOAC, 1996). All analyses were done in triplicate.

Digestion enzyme assay. In order to study the effect of yeast based nucleotide digestive enzyme activity, six fish were collected from each tank at the end of the study and crude enzymatic extracts were prepared (Ding et al., 2004). A crude mixture of intestine was obtained by dissection at 4°C, and rinsed with cold NaCl (Sigma) solution (0.9%, w/v). Then the total intestinal content was homogenized at 4°C in 50 mm phosphate buffer (pH 7.4) in a homogenizer. The homogenate was centrifuged at 10000 g for 10 min at 4°C. The supernatant was recovered and kept at -80°C; all enzymatic assays were conducted within 24 h after extraction.

Pepsin activity (E.C.3.4.23.1). Pepsin activity was assayed using 2% human hemoglobin in HCl as a substrate (Worthington, 1993). Tissue homogenate (1 ml) in HCl with 5 ml of substrate was incubated at 37°C for 10 min. The reaction was terminated using 5% trichloroacetic acid (TCA) (Sigma) and left to incubate for 5 min. The mixture was then filtered. Absorbance was measured at 280 nm. One unit renders TCA soluble absorption of 0.001 at 280 nm per min. at 37°C from a denatured hemoglobin substrate.

Trypsin activity (E.C.3.4.21.4). Trypsin activity was measured (Erlanger et al., 1961) and the measurements modified using benzoyl-DL-arginin-p-nitroanilide (BAPNA, Sigma) as a substrate (Benjakul et al., 1999). A sample (200 ml) with an appropriate dilution was added to 200 ml of distilled water and 1000 ml of 50 mM Tris-HCl, pH 8.0 containing 10 mM CaCl_2 (Sigma). To initiate the reaction, 200 ml of BAPNA (2 mg/ml) was added and mixed thoroughly. After incubation for 10 min at 25°C, 200 ml of 30% acetic acid (v/v) was added to terminate the reaction. The reaction mixture absorbance was read at 410 nm.

Amylase activity (E.C. 3.2.1.1). The amylase activity was assayed by the dinitrosalicylic acid (DNS, Sigma®) procedure (Bernfeld, 1955) using 1% soluble starch (Sigma) as a substrate. Tissue homogenate (10 μl) was incubated for 30 min at 35°C with 500 μl universal buffer and 40 μl soluble starch. The reaction was terminated by adding 100 μl DNS (D0550, Sigma) and heating in boiling water for 10 min. The reducing groups released from starch by amylase action are measured by the reduction of the color reagent, DNS. The boiling water stops amylase activity, catalyzes the reaction of DNS, and reduces groups of starch. The absorbance of the solution was recorded at 540 nm.

Lipase activity (E.C.3.1.1.3). Tissue lipase activity was measured spectrophotometrically (Winkler and Stuckman, 1979) with slight modifications. The substrate solution containing 10 ml of isopropanol (I9516, Sigma®) and 30 mg of *p*-nitrophenyl palmitate (N2752, Sigma) was mixed with 90 ml of Tris-HCl buffer (50 ml, pH 9.0), containing 0.4% Triton-X-100 (X100, Sigma) and 100 mg of gum arabic (G9752, Sigma®). Freshly prepared substrate solution (2.4 ml) was incubated at 37°C with 25 μl of suitably diluted cell-free supernatant for 15 min. After incubation, absorbance was measured at 410 nm by using a spectrophotometer (Analytikjena-SPECORD 50, Germany) against a control with heat inactivated enzyme.

All enzyme activities are expressed as specific activity (U/mg protein). Tissue protein content was determined according to the method developed by Lowry et al., (1951) using bovine serum albumin as standard.

Economic Profit Analysis. Taking into account feed price, the cost of feed required to produce 1 kg of biomass was also calculated. The parameters of economic conversion ratio (ECR) and Economic Profit Index (EPI) were calculated according to Martinez-Llorens et al., (2007); $ECR (US\$/kg) = Feed\ cost (US\$/kg) \times Feed\ conversion\ ratio (kg\ diet/kg\ fish)$, The Economic Profit Index [EPI (US\$ /fish) = final weight (kg/ fish) x fish sale price (US\$ /kg)-ECR (US\$/kg) x weight increase (kg)]. Rainbow trout sale price used in the equation was US\$4.99 per kg.

Statistical analysis. Data were subjected to a one-way analysis of variance (ANOVA). After identification of differences among groups Duncan's new multiple range tests were used to make multiple comparisons among means. Differences were considered significant at $p < 0.05$. Statistical analyses were performed using SPSS 10.0 for Windows (SPSS Inc., Chicago, Illinois).

Results

Optimum growth was reached on NP40% diet, whereas the control diet produced the lowest growth rate. Final fish weight, weight gain (%), daily weight gain increase and specific growth rate increased significantly ($P < 0.05$) with increased dietary nucleotide levels however more than NP40% replacement of fish meal resulted in decreased measured growth parameters.

Table 2. Growth performance, feed utilization data and muscle proximate composition of trout fed with different levels of nucleotide supplementation on feeding trial (n=3).

	Diets			
	Control	NP 20	NP 40	NP 60
Initial weight	27.74±0.18 ^a	27.48±0.27 ^a	27.92±0.30 ^a	27.89±0.28 ^a
Final weight (g)	62.69±0.50 ^a	65.52±0.02 ^b	82.59±0.55 ^c	75.81±0.60 ^d
%LWG (g)	125.80±2.70 ^a	138.46±2.24 ^b	195.83±1.63 ^c	171.91±4.82 ^d
DWG (g)	0.58±0.01 ^a	0.64±0.04 ^b	0.91±0.05 ^c	0.79±0.01 ^d
SGR ¹	1.36±0.02 ^a	1.45±0.01 ^b	1.81±0.01 ^c	1.66±0.03 ^d
FCR ²	1.99±0.02 ^a	1.88±0.02 ^b	1.52±0.02 ^c	1.63±0.04 ^d
PER ³	0.79±0.01 ^a	0.86±0.06 ^b	1.24±0.07 ^c	1.09±0.02 ^d
Proximate Composition (g/kg)				
Moisture	73.46±0.64 ^a	76.34±0.62 ^a	74.68±1.75 ^a	72.84±1.45 ^a
Crude protein	23.05±0.41 ^a	22.10±0.76 ^a	22.75±0.12 ^a	24.77±0.18 ^b
Crude lipid	8.32±0.17 ^a	6.25±0.83 ^b	5.75±0.16 ^b	5.52±0.04 ^b
Crude ash	2.23±0.02 ^a	2.03±0.03 ^a	2.05±0.04 ^a	2.19±0.20 ^a

* Different letters within a same line denote significant differences ($P < 0.05$).

Values are expressed ± SEM of three replicates in each groups

¹SGR= $[(\log_e \text{ average final weight} - \log_e \text{ average initial weight}) / \text{number of days}] \times 100$

²FCR= Feed consumption (g)/wet weight gain (g)

There were no significant changes in survival among the different treatments. The lowest FCR was obtained at 40% NP supplementation. Fish fed the control diet showed a higher FCR. On the other hand, nucleotide supplementation improved nutrient utilization; moreover, fish fed 40% NP supplementation diet showed highest PER. Nucleotide supplementation significantly affected muscle fish composition except for moisture and ash, which did not differ among the dietary treatments. Nucleotide yeast supplementation appeared to increase the protein and decrease the lipid content in the muscle tissue of trout significantly ($P < 0.05$). The level of dietary nucleotide yeast supplementation also significantly ($P < 0.05$) affected the ECR and EPI values calculated for the investigation (Table 3).

Table 3. Results of for bio-economical analysis for the production of 1 kg reared rainbow trout

Parameters	Control	NP 20	NP 40	NP 60	*Different letters within a same line denote sig. diff. ($P < 0.05$). Values are expressed ±SEM of three replicates in each group. Economic Conversion Ratio(ECR) Economic Profit Index(EPI).
FCR	1.99±0.02 ^a	1.88±0.02 ^b	1.52±0.02 ^c	1.63±0.04 ^d	
Feed Cost(US\$/kg)	1.90	1.93	2.03	3.47	
ECR	3.78±0.03 ^a	3.62±0.05 ^{ab}	3.09±0.04 ^c	3.47±0.08 ^b	
EPI	0.18±0.01 ^a	0.19±0.09 ^a	0.24±0.02 ^b	0.21±0.04 ^c	

After the fish were fed different amounts of nucleotide supplementation for 60 days, the mean digestive enzyme activities (with the exception of amylase activity) of all treatment groups were significantly different from that of the control (Figs.1A, B, C, D).

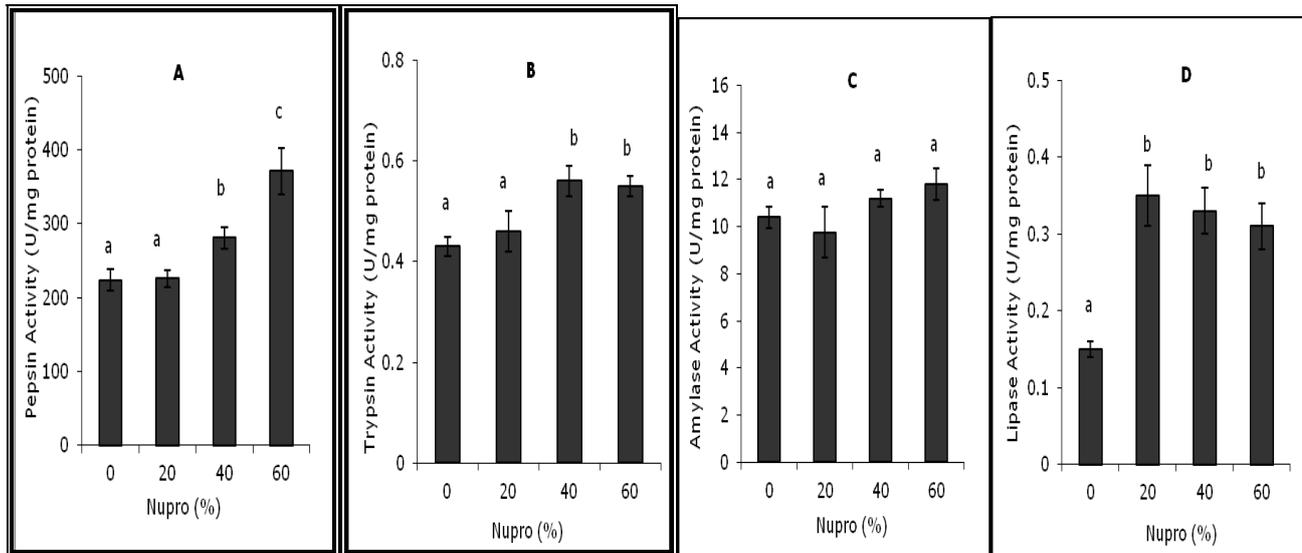


Figure 1. Pepsin (A), Trypsin (B), Amylase (C) and Lipase (D) activity (U/mg protein) in intestinal tissues of rainbow trout fed different levels of nucleotide supplementation. Each value represents the mean±SEM. (n=6). The different letters indicate significant ($P<0.05$) difference between diet groups

Pepsin activity (Fig. 1A) was significantly higher in fish fed 60% NP diet. The lowest activity was observed in fish fed the control diet. Trypsin activity (Fig. 1B) was, however, significantly higher in fish fed 40%NP and 60%NP diets. Amylase activity in fish fed 40%NP and 60%NP increased but differences were not significant (Fig. 2C). Lipase activity was significantly lower in the fish fed control diet. All fish in nucleotide supplemented dietary treatments showed significantly increased lipase activity compared to fish fed control diet ($P<0.05$) (Fig. 2D).

Discussion

The present study demonstrated that growth rates were positively affected when fish meal was replaced with nucleotides of NP20%, NP40% and NP60%. Fish fed the NP40% diet showed significantly higher growth rates ($P<0.05$) and higher weight gain than with other treatments. Weight gain in all nucleotide supplemented groups was higher than the control. Live weight gain (%) ranged from 125%-195% and was significantly affected by the yeast based protein source. The present investigation demonstrated that up to 40% of the fish meal component in trout diets could effectively be replaced by a yeast-based protein source without compromising fish growth performance. No negative effects could be seen when 30-50% yeast-based products were included in fish meal diets fed to lake trout (Rumsey et al., 1990), sea bass (Oliva-Teles and Goncalves, 2001), cobia (Lunger, 2006) and trout (Güroy et al., 2012).

The inclusion of yeast based prebiotics in diets promotes the health of epithelium as well as the microvilli of the gut providing a large surface area for nutrient absorption (Staykov et al., 2009). Improved gut morphology, digestion, absorption and assimilation of the food intake may have occurred due to the inclusion of nucleotides in the diet. Growth rate also increased and FCR was significantly lowered in nucleotide fed groups compared with the control group (Sang and Fotedar, 2010). The findings of the current study may help to explain improved growth performance and feed utilization fish fed with nucleotide supplementation. In addition the PER results indicate that nucleotide supplementation significantly improves protein utilization in trout. Nu-Pro® is a rich source of compounds such as nucleotides and free amino acids known to be potent feeding stimulants and attractants. Nu-Pro® contains significant amounts of glutamic acid, glutamate and active nucleotides such as 5'-IMP (Inosine monophosphate) and 5'-GMP

(Guanosine monophosphate) which are known to enhance flavor (Diehl, 2004). Diet supplementation with amino acids and nucleotides stimulated feed intake in Japanese eel (*Anguilla japonica*) as well as enhanced growth performance (Takeda and Takii, 1992). Although there are no detailed studies to date on the specific effects of NuPro[®] as an attractant or palatability enhancer, trout which are fed diets containing Nu-Pro[®] have better FCR (Tahmasebi-Kohyani et al., 2011) than the control groups. This may be correlated with nucleotide palatability.

In the present study muscle proximate composition analysis, specifically crude protein content, appeared to be greatly influenced by the dietary nucleotide supplementation. Muscle protein composition significantly improved after nucleotide supplementation especially in fish fed with NP 60%. Muscle lipid composition significantly decreased in fish fed with all NP supplemented diets. In shrimp fed with nucleotides, whole body protein level increased when compared with the control (Li et al., 2007). Muscle lipid levels decreased in tilapia fed Nu-Pro[®] supplemented diets compared with control group (Craig, and McLean, 2005). Dietary nucleotides may influence the protein biosynthesis by regulating the intracellular nucleotide pool. The addition of yeast supplements plays a role in increasing fish appetite and hence feed intake with a subsequent improvement of fish growth and feed utilization due to improved diet and protein digestibility, and enhancement of body composition or nutrient deposition (Waché et al., 2006). This study also demonstrated that muscle moisture and ash contents were not significantly influenced by the dietary treatments supporting results reported in other studies (Li et al., 2005; Güroy et al., 2012). Nu-Pro[®] significantly increased protein level and reduced muscle lipid levels in the edible component of the fish, thereby providing a leaner and potentially healthier product.

The results of digestive tract enzyme activity revealed that diets supplemented with nucleotides increased gastric pepsin activity, intestinal trypsin, and lipase but did not influence the amylase enzyme activity significantly. The effects of diets supplemented with nucleotides on digestive enzymes in rainbow trout are unknown. Some studies have shown that with the addition of nucleotides in diets some animal gastric pepsin enzymes increased and intestinal mucosa sucrose activity was significantly enhanced (Sato et al., 1999; Lee et al., 2007). Digestive enzymes enhance efficiency of feed utilization in fish and characterization of these enzymes provides information as to the digestive capacity of fish to hydrolyze carbohydrate, protein and lipid of feed ingredients (Lemieux et al., 1999). Information regarding the extracellular enzymes produced by intestinal bacteria and their biochemical significance is limited. Probiotics stimulate the growth and activity of beneficial bacteria in the intestine and can activate the innate immune responses of cultured organisms when used as dietary supplements. Prebiotics have also increased the efficiency of the digestive tract in many organisms by regulating gut viscosity (Ganguly et al., 2010). It is well known that dietary nucleotides have multiple beneficial effects on gastrointestinal (GI) tract function in vertebrates, including positive physiological, morphological and microbiological influences. Some studies on GI tract morphology of humans and other terrestrial animals have reported that dietary nucleotides increase villus height, jejunum wall thickness and villus cell number and gut mucosa (Bueno, 1994). However, limited studies investigating the effects of nucleotide supplementation on GI tract morphology in fish reported an increment in the proximal intestine fold height. Dietary nucleotides have also been shown to enhance lateral branching of the intestinal folds, and may have resulted in increased total gut surface area in salmon and red drum (Burrells et al., 2001, Cheng et al., 2011). In accordance with those findings, data from the present experiment also showed significantly increased ($P < 0.05$) digestive enzyme activity which appears to be correlated to nucleotide supplementation in the diets. Digestive enzyme activity may be enhanced by supplemented nucleotide levels due to the increased gut villi density (Sang and Fotedar, 2010).

The possible effect of nucleotide yeast (Nu-Pro[®]) supplementation on cost production was also calculated. The lowest ECR and highest EPI values were obtained on fish fed 40% dietary nucleotide supplemented rainbow trout. The best FCR was obtained on NP40 supplemented groups thereby significantly lowering final cost of production compared to the control diet. Calculated ECR in trout fed with NP40 supplemented feed (US\$3.09/kg) would represent a saving of US\$0.69/kg (18.3%) compared to the control diet (US\$3.78/kg). Between 40-50% of the variable cost of rainbow trout production is attributed to feed

(Vandenberg and Moccia, 1998). Therefore 18.3% reduction in the feed price per kg would represent a 7.3-9.2% saving in production costs per year.

The replacement of fish meal by nucleotide protein sources increased digestive activity in trout. Our data indicated that the replacement of fish meal with up to 40% nucleotide based protein may be economically viable using the protein sources tested in this study. Future studies should further investigate the distribution of enzymes on various parts of intestinal tract to further understand nutrient digestion in this species. The use of nucleotides in aquaculture diets and the effects of nucleotide supplementation on digestive enzyme activity in rainbow trout is an area for further investigation.

Acknowledgements

This research was funded by the Mersin University Research Fund in Turkey (SÜF-YB/AÖ/2009-6). The authors wish to thank Alltech Company/USA for supplying Nu-Pro® free of charge. The authors also appreciate the help given by Research Assistant Mr. Mustafa Bariş for obtaining fish from a trout farm.

References

- AOAC**, 1996. *Official Methods of Analyses of the Association of Official Analytical Chemists*. Assoc. Official Analytical Chemists, Arlington, VA, USA.
- Benjakul, S., Visessanguan, W. and P. Thummaratwasik**, 1999. Isolation and characterization of trypsin inhibitors from some Thai legume seeds. *J. Food Biochem.*, 24: 107-127.
- Bernfeld, P.**, 1955. Amylases, α and β . *Methods in enzymology*, 1:149-158.
- Bueno J., Torres M., Almendros A., Carmona R., Nunez M.C. and A. Rios**, 1994. Effects of dietary nucleotides on small intestinal repair after diarrhea. Histological and ultrastructural changes. *Gut*, 35:926-933.
- Burrells C., Williams P.D. and P.F. Forno**, 2001. Dietary nucleotides: a novel supplement in fish feeds: 1. Effects on resistance to disease in salmonids. *Aquaculture*, 199:159-169.
- Cheng Z., Buentello A, D. and M. Gatlin III**, 2011. Dietary nucleotides influence immune responses and intestinal morphology of red drum *Sciaenops ocellatus*. *Fish & Shellfish Immunology*, 30:143-147.
- Craig S.R. and E. Mclean**, 2005. The organic aquaculture movement: a role for NuPro as an alternative protein source. pp. 285-294. In: *Nutritional Biotechnology in the Feed & Food Industries: Proceedings of Alltech's 21st Annual Symposium* (T.P. Lyons and K.A. Jacques, eds), Nottingham University Press, UK.
- Desale B., Kevin Z., Fitzsimmons M., Collier R. J. and G.J. Duff**, 2008. Evaluation of brewer's waste as partial replacement of fish meal protein in Nile tilapia, *Oreochromis niloticus*, diets. *J. World Aquacult. Society*, 39:4,556-564.
- Diehl, J.** 2004. All in good taste: creating natural savoury flavorings from yeast. In: *Nutritional Biotechnology in the Feed and Food Industries. Proceedings of Alltech's 20th Annual Symposium* (T.P. Lyons and K.A. Jacques, eds). Nottingham University Press, UK, pp. 258-263.
- Ding X., Li Z.J., Chen Y.Q., Lin H.Z., Yang Y.Y. and K. Yang**, 2004. Effects of probiotics on growth and activities of digestive enzymes of *Pennaus vannamei*. *J. Fish. Sci. China*, 11:580-584.
- El-Saidy D. and M. Gaber M.**, 2003. Replacement of fish meal with a mixture of different plant protein sources in juvenile Nile tilapia, *Oreochromis niloticus* (L.) diets. *Aquac. Res.*, 34:1119-1127.
- Erlanger B., Kokowsky N. and W. Cohen**, 1961. The preparation and properties of two new chromogenic substrates of trypsin. *Arch. Biochem. Biophys.*, 95:271-278.
- Fegan D.E.**, 2006. Functional foods for aquaculture: benefits of Nupro and dietary nucleotides in aquaculture feeds. Pp. 419-432. In: *Nutritional Biotechnology in the Feed and food Industries. Proceedings of Alltech's 22nd Annual Symposium* (T.P. Lyons, K.A. Jacques and J.M.Hower, eds). Nottingham University Press, UK.

- Fountoulaki E., Vasilaki A., Hurtado R., Grigorakis K., Karacostas I., Nengas I., Rigos G., Kotzamanis Y., Venou B. and M.N. Alexis**, 2009. Fish oil substitution by vegetable oils in commercial diets for gilthead sea bream (*Sparus aurata* L.); effects on growth performance, flesh quality and fillet fatty acid profile recovery of fatty acid profiles by a fish oil finishing diet under fluctuating water temperatures, *Aquaculture* 289:317-326.
- Ganguly S., Paul I. and S.K. Mukhopadhyay**, 2010. Application and effectiveness of immunostimulants, probiotics, and prebiotics in aquaculture: a review, *Isr. J. Aquacult. - Bamidgeh*, 62(3):130-138.
- Gibson G.R.**, 1999. Dietary modulation of the human gut microflora using the prebiotics oligofructose and inulin. *J. Nutr.*, 129:1438-1441.
- Güroy D., Tekinay A.A. and S. J. Davies**, 2012. Use of organically certified yeast in the diet of juvenile rainbow trout (*Oncorhynchus mykiss*): growth performance, nutrient utilization, and fatty acid composition, *Isr. J. of Aquacult. - Bamidgeh*, 64:722-730.
- Kissil G.Wm and I. Lupatch**, 2004. Successful replacement of fishmeal by plant proteins in diets for the gilthead seabream, *Sparus aurata* L. *Isr. J. Aquacult. - Bamidgeh*, 56(3), 188-199
- Lemieux H., Blier P. and J.D. Dutil**, 1999. Do digestive enzymes set a physiological limit on growth rate and food conversion efficiency in the Atlantic cod (*Gadus morhua*)? *Fish Physiol. Biochem.*, 20:293-303.
- Li P., Burr G. S., Goff J. B., Whiteman K. W., Davis K. B., Vega R.R., Neill W. H. and D. M. Gatlin III**, 2005. A preliminary study on the effects of dietary supplementation of brewers yeast and nucleotides, singularly or in combination, on juvenile red drum (*Sciaenops ocellatus*). *Aquaculture Research*, 36:1120-1127.
- Li P., Lawrence A. L., Castille F. L. and D. M. Gatlin III**, 2007. Preliminary evaluation of a purified nucleotide mixture as a dietary supplement for Pacific white shrimp *Litopenaeus vannamei* (Boone). *Aquacult. Res.*, 38:887-890.
- Lowry O.H., Rosebrough N.J., Farr A.L. and R.J. Randall**, 1951. Protein measurement with the folin phenol reagent. *J. Biol. Chem.*, 193:265-275.
- Lunger A. N., Craig S.R. and E. McLean**, 2006. Replacement of fish meal in cobia (*Rachycentron canadum*) diets using an organically certified protein, *Aquaculture* 257:393-399.
- Martínez-Llorens, S., Moñino, A.V., Tomás, A., Pla, M. and M. Jover**, 2007. Soybean meal as partial dietary replacement for fish meal in gilthead sea bream (*Sparus aurata*) diets: effects on growth, nutritive efficiency and body composition. *Aquacult. Res.*, 38:82-90.
- Mourente G. and J. G. Bell**, 2006. Partial re-placement of dietary fish oil with blends of vegetable oils (rapeseed, linseed and palm oils) in diets for European sea bass (*Dicentrarchus labrax* L.) over along term growth study: Effects on muscle and liver fatty acid composition and effectiveness of a fish oil finishing diet, *Comparative Biochem. and Physiol. Part B*, 145:389-399.
- National Research Council (NRC)**. 1993. Nutrient requirements of fish. National Academy Press, Washington DC.
- Oliva-Teles A. and P. Goncalves**, 2001. Partial replacement of fishmeal by brewers yeast *Saccharomyces cerevisiae* in diets for sea bass (*Dicentrarchus labrax*) juveniles. *Aquaculture*, 202:269-278.
- Oliva-Teles A., Guedes M.J., Vachot C and S.J. Kaushik**, 2006. The effect of nucleic acids on growth, ureagenesis and nitrogen excretion of gilthead sea bream *Sparus aurata* juveniles. *Aquaculture*, 253: 608-617.
- Olvera-Novoa M.A., Olivera-Castillo L. and C.A. Martínez-Palacios**, 2002. Sunflower seed meal as a protein source in diets for *Tilapia rendalli* (Boulanger, 1896) fingerlings. *Aquac. Res.*, 33:223-229.
- Rumsey G.L., Hughes S.G. and J.E. Kinsella**, 1990. Use of dietary yeast *Saccharomyces cerevisiae* nitrogen by lake trout. *J. World Aquacult. Soc.*, 21, 205-209.
- Sang H. M. and R. Fotedar**, 2010. Prebiotic mannan oligosaccharide diet improves health status of the digestive system of marron, *Cherax tenuimanus* (Smith 1912). *J. Applied Aquacult.*, 22:240-250.
- Sato, N., Nakano, T., Kawakami, H. and T. Idota**, 1999. In vitro and in vivo effects of exogenous nucleotides on the proliferation and maturation of intestinal epithelial cells. *Journal of Nutritional Science and Vitaminology*, 45:107-118.

- Smith, L.S.** 1989. Digestive functions in teleost fishes. Pp.331-421. In: J.E. Halver (ed.). *Fish Nutrition*, Academic Pres., USA.
- Staykov Y., Sweetman J. and P. Spring,** 2009. Effect of NuPro® as a fish meal replacement on the growth rate of rainbow trout (*Oncorhynchus mykiss*) grown in raceways. *Nutritional Biotechnology in the Feed and Food Industries*. Proceedings of Alltech's Twenty Five Annual Symposium.
- Tahmasebi-Kohyani, A., Keyvanshokoh, S., Nematollahi, A., Mahmoudi, N and H. Pasha-Zanoosi,** 2011. Dietary administration of nucleotides to enhance growth, humoral immune responses, and disease resistance of the rainbow trout (*Oncorhynchus mykiss*) fingerlings. *Fish & Shellfish Immunology*, 30:189-193
- Takeda M. and K. Takii,** 1992. Gustation and nutrition in fishes: application to aquaculture. In: *Fish Chemoreception* (T.J. Hara, ed.). Chapman and Hall, London, UK pp. 271-287.
- Tacon, A. G.J. and M. Metian,** 2008. Global overview on the use of fish meal and fish oil in industrially compounded aquafeeds: Trends and future prospects. *Aquaculture*, 285:146-158.
- Vandenberg, G.W. and R.D. Moccia,** 1998. Growth performance and carcass composition of rainbow trout, *Oncorhynchus mykiss* (Walbaum), fed the β -agonist ractopamine, *Aquaculture Research*, 29(7):469-479.
- Verschuere V., Rombaut G., Sorgeloos P. and W. Verstraete,** 2000. Probiotic bacteria as biological control agents in aquaculture. *Microbiology and Molecular Biology Reviews*, 64:4(655-671).
- Waché Y., Auffray F., Gatesoupe F.J., Zambonino J., Gayet V., Labbé L. and C. Quentel,** 2006. Cross effects of the strain of dietary *Saccharomyces cerevisiae* and rearing conditions on the onset of intestinal microbiota and digestive enzymes in rainbow trout, *Oncorhynchus mykiss*, fry. *Aquacult.*, 258:470-478.
- Winkler, U. K. and M. Stuckman,** 1979. Glycogen, hyaluronate, and some other polysaccharides greatly enhance the formation of exo-lipase by *Serratia marescens*. *J. Bacteriol.* 138:663-670.
- Worthington, V.,** 1993. Worthington Enzyme Manual. Enzymes and Related Biochemicals Worthington Chemical, New Jersey, US. 399 pp.