Fish & Shellfish Immunology 30 (2011) 143–147

Contents lists available at ScienceDirect

Fish & Shellfish Immunology

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

Dietary nucleotides influence immune responses and intestinal morphology of red drum *Sciaenops ocellatus*

Zhenyan Cheng [a,b,1], Alejandro Buentello [a,c], Delbert M. Gatlin III [a,c,*]

[a] Department of Wildlife and Fisheries Sciences, Texas A&M University System, College Station, TX 77843-2258, USA
[b] The Key Laboratory of Mariculture (Ministry Education of China), Ocean University of China, Qingdao 266003, PR China
[c] Intercollegiate Faculty of Nutrition, Texas A&M University System, College Station, TX 77843-2258, USA

ARTICLE INFO

Article history:
Received 1 July 2010
Received in revised form
27 September 2010
Accepted 28 September 2010
Available online 2 November 2010

Keywords:
Red drum
Sciaenops ocellatus
Nucleotide
Immune responses
Intestinal morphology

ABSTRACT

Dietary nucleotides have been shown to benefit many physiological and nutritional functions in higher vertebrates and fish. Therefore, a 6-week feeding trial was conducted to evaluate the effects of graded levels of a commercial nucleotide product on growth performance, immune responses and intestinal morphology of juvenile red drum (initial average weight of 7.1 g). The basal diet was formulated to contain 40% protein, 10% lipid and a digestible energy level of 3.5 kcal g^{-1}. Two levels of nucleotide (Ascogen P®, 0.5% and 1% of diet) were added to the basal diet with menhaden fishmeal and menhaden oil adjusted to provide isonitrogenous and isolipidic diets. Nucleotide supplementation tended to improve weight gain and survival of red drum, but not at a significant level. Neutrophil oxidative radical anion production and serum lysozyme activity tended to be higher for fish fed diets supplemented with nucleotide, while extracellular superoxide anion production of head kidney macrophages from fish fed diets with 1% nucleotide was significantly ($P < 0.05$) increased, although no significant differences were observed between fish fed 0.5% nucleotide diet and the basal diet.

Nucleotide supplementation significantly ($P < 0.05$) increased fold height in the proximal intestine, and enterocyte height in the pyloric caeca, proximal and distal enteric sections. A significantly ($P < 0.05$) higher microvilli height was observed in all evaluated enteric sections of fish fed with diets supplemented with nucleotides. It is therefore possible to use dietary nucleotides supplementation to significantly enhance the intestinal structure of red drum. Likewise, nucleotides in the diet may improve some components of the non-specific immune response of this sciaenid fish.

1. Introduction

Nucleotides are ubiquitous intracellular compounds of crucial importance to cellular function and metabolism [1]. The physiological actions of these compounds include encoding and deciphering genetic information, mediating energy metabolism and cell signaling as well as serving as components of coenzymes, allosteric effectors and cellular agonists [1,2]. Numerous studies with several different aquatic species have reported that dietary nucleotides can enhance growth performance [3–5], immune responses [6–12], disease resistance [7,8,10,11,13] and even gastrointestinal physiology and morphology [5,7].

Red drum (*Sciaenops ocellatus*) is a carnivorous fish that has been an important aquacultured species because of its rapid growth and popularity for both food and stock enhancement. Quantitative nutritional requirements for macro- and micronutrients have been defined for this fish [14], and research concerning the effects of immunostimulants on this fish are expanding [12,15–19]. A preliminary study evaluated the effects of dietary supplementation of brewers yeast and a commercial nucleotide product (Optimûn) on red drum [15], and a subsequent study demonstrated the beneficial, although transient effects of a purified nucleotide mixture on weight gain of this fish [12]. The present study was designed to investigate the influence of another commercial nucleotide product (Ascogen P®) on growth performance, immune responses and intestinal morphology of red drum.

* Corresponding author. Department of Wildlife and Fisheries Sciences, Texas A&M University System, College Station, TX 77843-2258, USA. Tel.: +1 979 847 9333; fax: +1 979 845 4096.
E-mail addresses: chengzy2007@yahoo.com.cn (Z. Cheng), abuentello@tamu.edu (A. Buentello), dgatlin@nature.tamu.edu (D.M. Gatlin III).
[1] Present address. Tianjin Key Laboratory of Aqua-ecology and Aquaculture, Department of Fisheries Science of Tianjin Agricultural University, Tianjin 300384, PR China.

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doi:10.1016/j.fsi.2010.09.019

2. Materials and methods

The basal diet utilized menhaden fishmeal as the protein source, dextrin and menhaden oil as carbohydrate and lipid sources, respectively, was formulated to contain 40% protein, 10% lipid and an estimated digestible energy level of 3.5 kcal g^{-1}. Two experimental diets were formulated by supplementing the nucleotide product (Ascogen P®, Canadian Bio-Systems Inc.) at the rate of 0.5% and 1% to the basal diet (Table 1). This product contained cytidine-5′-monophosphate, disodium uridine-5′-monophosphate, adenosine-5′-monophosphate, disodium inosine-5′-monophosphate, disodium guanidine-5′-monophosphate and RNA. Procedures for diet preparation and storage were as previously described [20].

Disease-free juvenile red drum (*S. ocellatus*) were obtained from the Texas Parks and Wildlife Department (Lake Jackson, TX) and maintained indoors at the Texas A&M University Aquacultural Research and Teaching Facility. Fish were conditioned on a commercial diet (Ranger, Buhl, ID) and acclimated to the experimental conditions for 1 week prior to the feeding trial. Fish of similar sizes (approximately 7.1 g) were randomly distributed into 15 glass aquaria (38-L), with 12 fish per aquarium. Each diet was randomly assigned to three replicate aquaria. All groups of fish were fed their respective diets at the same fixed rate (initially 6% of body weight per day and gradually reduced to 3%) for 6 weeks. Water temperature remained at 26 ± 2 °C throughout the trial by conditioning ambient air. Salinity was maintained at about 3‰ using well water and synthetic sea salt (Fritz Industries Inc., Dallas, TX). Water flow rate was maintained at approximately 1 L min^{-1} via a recirculating system which maintained adequate water quality through biological and mechanical filtration. Low pressure electrical blowers provided aeration to maintain dissolved oxygen levels near saturation in each aquarium. A 12 h light:12 h dark photoperiod was maintained with fluorescent lights controlled by timers.

At the end of the feeding trial, two fish from each tank were anesthetized with tricaine methane sulfonate (MS-222, 200 mg L^{-1}), and blood was collected from the caudal vasculature using a 1-mL syringe. After a sample of whole blood was taken for assay of neutrophil oxidative radical production, plasma was isolated by centrifugation at $3000 \times g$ for 5 min and kept at −80 °C until analyzed. Serum lysozyme activity was determined by a turbidimetric assay [21]. Previously, the suspension of *Micrococcus lysodeikticus* was adjusted to pH 5.2 to maximize lysozyme activity as determined in preliminary assays with red drum in our laboratory. Whole-blood neutrophil oxidative radical production was determined as described by Siwicki et al. [22]. Absorbance was converted to nitro blue tetrazolium (NBT) units based on a standard curve of NBT diformazan mL^{-1} blood.

An additional two fish per aquarium were collected and the entire gastrointestinal tract was removed and injected with Davidson's fixative solution and then transferred into 70% ethanol after 24 h. Then, segments (~0.5–1 cm length) of proximal, mid-, and distal intestine, as well as the pyloric caeca, were sliced transversely into 4-μm sections and stained with hematoxylin and eosin (H&E). The slides were examined under a light microscope (Olympus, BX60) equipped with a camera (Donpisha, XC-003P) and VGA 460 Osteomeasure software (Osteometrics, Decatur, GA) for image acquisition. Electronic images were further analyzed using Image J software (National Institutes of Health, Bethesda, MD) for

Table 1
Formulation and proximate composition of experimental diets (% dry matter).

Ingredient	Basal	0.5% Nucleotide	1% Nucleotide
Menhaden fishmeal[a]	57.8	57.8	57.8
Menhaden oil[a]	3.7	3.7	3.7
Dextrin[b]	15.0	15.0	15.0
Vitamin premix[c]	3.0	3.0	3.0
Mineral premix[d]	4.0	4.0	4.0
Carboxymethyl cellulose[b]	2.0	2.0	2.0
Celufil[b]	14.5	14.0	13.5
Nucleotide[e]		0.5	1.0
Proximate composition (% dry weight)			
Dry matter	93.8	92.7	92.8
Crude protein	40.2	39.9	41.9
Crude lipid	9.9	10.0	9.8
Ash	13.7	14.3	14.7

[a] Special Select™, Omega Protein, Reedville, VA. Menhaden fishmeal composition (dry-matter basis), Crude protein, 69.2%, crude lipid, 11.0%.
[b] US Biochemical Corp., Cleveland, OH.
[c] Contains (as g/kg): $Ca(C_6H_{10}O_6)\cdot 5H_2O$, 348.49; $Ca(H_2PO_4)_2\cdot H_2O$, 136.0; $FeSO_4\cdot 7H_2O$, 5.0; $MgSO_4\cdot 7H_2O$, 132.0; K_2HPO_4, 240.0; $NaH_2PO_4\cdot H_2O$, 88.0; NaCl, 45.0; $AlCl_3\cdot 6H_2O$, 0.15; KI, 0.15; $CuSO_4\cdot 5H_2O$, 0.5; $MnSO_4\cdot H_2O$, 0.7; $CoCl_2\cdot 6H_2O$, 1.0; $ZnSO_4\cdot 7H_2O$, 3.0; Na_2SeO_3, 0.011.
[d] Contains (as g/kg): ascorbic acid, 50.0; DL-calcium pantothenate, 5.0; choline chloride, 36.2; inositol, 5.0; menadione sodium bisulfite, 2.0; niacin, 5.0; pyridoxineHCl, 1.0; riboflavin, 3.0; thiaminemononitrate, 0.5;DL-α-tocopheryl acetate (250 IU/g), 8.0; vitamin A acetate (500,000 IU/g), 0.2; biotin, 0.05; cholecalciferol (1 mg ¼ 40 IU), 0.002; folic acid, 0.18; vitamin B_{12}, 0.002; cellulose, 819.93.
[e] Canadian Bio-systems Inc., Calgary, Alberta, Canada.

Table 2
Weight gain, survival, neutrophil oxidative production (NBT), serum lysozyme, extracellular superoxide anion production of kidney macrophages of red drum fed diets with different concentrations of nucleotides for 6 weeks.[a]

Diet	Weight gain (%)	Survival (%)	NBT (mg mL^{-1})[b]	Lysozyme (10^3 Units L^{-1})[b]	Extracellular superoxide anion (nmol O_2^-)[c]
Basal	295.9	73.1	2.75	117.2	4.1[b]
0.5% nucleotide	318.8	85.9	2.78	121.1	5.6[b]
1% nucleotide	314.0	91.1	2.92	127.8	7.6[a]
Pooled S.E.	9.47	5.07	0.07	7.16	0.59
$Pr > F$[d]	0.646	0.378	0.564	0.167	0.017

[a] Values in a column that do not have the same superscript are significantly different ($P < 0.05$).
[b] Values are means of duplicate determinations on two fish from each of three replicate groups (6 fish/treatment).
[c] Values are means of three composite samples of kidney cells from four fish in each of three replicate groups.
[d] Significance probability associated with the *F* statistic.

Table 3
Micromorphology of the intestine of juvenile red drum fed diets with different concentrations of nucleotides for 6 weeks.[a]

Diets	Basal	0.5% Nucleotide	1% Nucleotide	*F*-Value	$Pr > F$
Distal intestine					
hF(μm)[b]	343.9	351.1	329.2	1.7	0.1794
hE (μm)[c]	33.4[b]	35.8[a]	36.4[a]	6.1	0.0027
hMV (μm)[d]	1.9[c]	2.2[b]	2.3[a]	22.6	<0.0001
Mid-intestine					
hF(μm)	362.2	354.7	351.2	0.2	0.8130
hE (μm)	32.5	31.9	32.2	0.2	0.8013
hMV (μm)	1.8[b]	1.7[b]	2.0[a]	13.4	<0.0001
Proximal intestine					
hF(μm)	551.3[b]	620.1[a]	588.7[a,b]	4.8	0.0091
hE (μm)	40.1[b]	42.6[a]	41.6[a,b]	4.0	0.0199
hMV (μm)	2.6[b]	3.0[a]	3.0[a]	15.7	<0.0001
Pyloric caeca					
hE (μm)	38.0[b]	43.3[a]	42.2[a]	22.8	<0.0001
hMV (μm)	2.5[b]	3.2[a]	3.1[a]	48.5	<0.0001

[a] Values are means of two fish from each of three replicate groups (10 measurements for each fish). Values in a row that do not have the same superscript are significantly different ($P < 0.05$).
[b] hF = fold height.
[c] hE = enterocyte height.
[d] hMV = microvillus height.