Aquaculture 309 (2010) 253–257

Contents lists available at ScienceDirect

Aquaculture

journal homepage: www.elsevier.com/locate/aqua-online

Effects of dietary prebiotics on growth performance, immune response and intestinal morphology of red drum (*Sciaenops ocellatus*)

Qi-Cun Zhou [a], J. Alejandro Buentello [b], Delbert M. Gatlin III [b,*]

[a] College of Life Science and Biotechnology, Ningbo University, Ningbo 315211, Zhejiang Province, People's Republic of China
[b] Department of Wildlife and Fisheries Sciences and Faculty of Nutrition, Texas A&M University System, College Station, Texas 77843-2258, USA

ARTICLE INFO

Article history:
Received 22 October 2009
Received in revised form 6 April 2010
Accepted 3 September 2010

Keywords:
Sciaenops ocellatus
Prebiotics
Immune responses
Intestinal morphology

ABSTRACT

An 8-week feeding trial was conducted with juvenile red drum to evaluate four different prebiotics: fructooligosaccharides (FOS) in the form of inulin, galactooligosaccharides (GOS), Bio-MOS®, containing mannanoligosaccharides (MOS) derived from yeast, and Previda™ containing galacto-gluco-mannans from hemicellulose extract. Each prebiotic was added at a level of 10 g kg^{-1} to the basal diet which was formulated to contain 41% protein and 10% lipid. Each diet was randomly assigned to three aquaria and fed twice daily to apparent satiation. Fish fed the diet containing Previda™ had significantly higher ($P<0.05$) weight gain than fish fed the basal diet and the one supplemented with Bio-MOS®. Feed efficiency and protein efficiency ratio of fish fed the various diets were not significantly different ($P>0.05$), although fish fed the basal diet had the lowest values. Fish fed the Bio-MOS® diet had lower survival than fish fed the other diets. Hepatosomatic index, muscle ratio and condition factor were not affected by the dietary treatments. Fish fed the FOS diet had a significantly lower neutrophil oxidative radical production than fish fed the other diets. However, serum lysozyme activity was significantly lower ($P<0.05$) in fish fed the basal diet compared with those fed the diets supplemented with prebiotics. Quantitative changes in the ultrastructural characteristics of the gastrointestinal tract of red drum fed the various diets were evaluated using histological methods. Fold height and enterocyte height in pyloric caeca and proximal, mid- and distal intestines were not significantly affected by diet. However, microvilli heights in pyloric caeca, proximal and mid-intestine were significantly increased by the supplementation with prebiotics. In this experiment, Previda™ supplementation resulted in significant improvements in growth performance and immunological responses, even as compared to other commercially available prebiotics. Because prebiotic supplementation may result in enhancement of different performance indicators in various fish species, tailored evaluations are recommended before suggesting specific prebiotic strategies.

1. Introduction

Prebiotics are defined as non-digestible dietary ingredients that beneficially affect the host by selectively stimulating the growth of and/or activating the metabolism of health-promoting bacteria in the gastrointestinal (GI) tract (Manning and Gibson, 2004). Compounds which have been shown to have prebiotic characteristics include mannanoligosaccharides (MOS), lactose, trans-galactooligosaccharide, as well as oligofructose and inulin (Teitelbaum and Walker, 2002). Results from several studies have indicated that prebiotics can improve growth performance and feed utilization of various fish species (Li and Gatlin, 2004; 2005; Mahious et al., 2006; Staykov et al., 2007; Torrecillas et al., 2007; Zhou et al., 2007; Burr et al., 2008; Grisdale-Helland et al., 2008), enhance their non-specific immune responses and resistance to bacterial infections (Li and Gatlin, 2004; 2005; Staykov et al., 2007; Buentello et al., 2010), improve gut function and health by improving the ultrastructure of the intestine mucosa (Salze et al., 2008; Dimitroglou et al., accepted for publication), as well as activate health-promoting bacteria in the intestine (Zhou et al., 2007) and affect whole-body protein concentration (Genc et al., 2007a,b; Torrecillas et al., 2007; Yilmaz et al., 2007).

The red drum is an important fish for United States aquaculture and stock enhancement. Although this carnivorous and euryhaline sciaenid is endemic to the Gulf and Atlantic coasts of the US, its aquaculture production is expanding in several countries. This species has desirable characteristics for aquaculture such as rapid growth and a wide salinity tolerance (Gatlin, 2002). Recently, the beneficial influences of dietary prebiotics on growth performance and immune response of red drum have been investigated (Burr et al, 2009). The purpose of the present study was to further evaluate the effects of different prebiotics including the two commercial mannanoligosaccharides in the form of Bio-MOS® and Previda™ as well as fructooligosaccharide (FOS) in the form of inulin and trans-galactooligosaccharide (GOS).

* Corresponding author. Tel.: +1 979 847 9333; fax: +1 979 845 4096.
E-mail address: dgatlin@nature.tamu.edu (D.M. Gatlin).

0044-8486/$ – see front matter © 2010 Elsevier B.V. All rights reserved.
doi:10.1016/j.aquaculture.2010.09.003

2. Materials and methods

2.1. Diet preparation

The basal diet, which utilized menhaden fish meal and soybean meal as protein sources and menhaden oil as the lipid source, was formulated to contain 41% crude protein and 10% lipid on a dry-matter basis. This diet satisfied all known nutrient requirements of red drum (Gatlin, 2002). The prebiotic products FOS, Bio-MOS®, Previda™ or GOS, were added individually to the basal diet at 1% of dry weight to replace cellulose (Table 1). All ingredients were thoroughly mixed via a Patterson-Kelley V-blender and then placed in the mixing unit of a Hobart meat grinder where lipid and water were added to a homogenous dough consistency. Cold-extruded pellets (3-mm diameter) were produced and air-dried to about 10% moisture, sealed in bags and stored frozen (−20 °C) prior to use in the feeding trial. Proximate composition of the experimental diets is presented in Table 1.

2.2. Fish and experimental conditions

Disease-free juvenile red drum (*Sciaenops ocellatus*) were obtained from the Texas Parks and Wildlife Department Marine Development Center. Prior to the experiment, fish were prophylactically treated with nitrofurazone (broad spectrum antibiotic, 3 h at 30 ppm, SIGMA, Cat no. N9009 St. Louis, MO) and freshwater bath (0 ppt salinity 30 min, against external parasites) and acclimated for 2 weeks to a brackish-water (3‰), recirculating system at the Texas A&M University Aquacultural Research and Teaching Facility. A commercial diet (40% crude protein, 10% crude lipid, Rangen Inc. Idaho, USA) was fed to all fish during the conditioning period. At the beginning of the experiment, fish (initial weight about 7 g) of similar size were weighed and sorted into 15, 38-l aquaria with 12 individuals per aquarium. Each diet was randomly assigned to three replicate groups of fish. Each aquarium was provided with a continuous flow of water (650 ml/min), which was recirculated through a common settling chamber, biofilter and sand filter. Continuous aeration was provided to each aquarium through air stones to maintain dissolved oxygen levels at or near saturation. Water temperature was controlled by ambient air and remained at 26 ± 1 °C throughout the trial. A 12-h light:12-h dark photoperiod was maintained with fluorescent lights controlled by timers. All groups of fish were fed at the same fixed rate, two times daily, initially 6% body weight per day and gradually reduced to 3%. The feeding rate was adjusted every 2 weeks to maintain a level approaching apparent satiation, without overfeeding. During the experimental period, pH was 7.8–8.0, unionized ammonia nitrogen was lower than 0.05 mg/l, and dissolved oxygen was not less than 6.0 mg/l. Each aquarium was cleaned biweekly at the time the fish were removed and weighed as a group. The feeding trial lasted for 8 weeks.

Table 1
Formulation and proximate composition of experimental diets.

Ingredient	Diets				
	Basal	FOS	Bio-MOS®	Previda™	GOS
Menhanden fish meal [a]	35.3	35.3	35.3	35.3	35.3
Soybean meal, dehulled [b]	29.6	29.6	29.6	29.6	29.6
Dextrin [c]	17.0	17.0	17.0	17.0	17.0
Menhanden oil [a]	5.5	5.5	5.5	5.5	5.5
Vitamin premix [d]	3.0	3.0	3.0	3.0	3.0
Mineral premix [e]	4.0	4.0	4.0	4.0	4.0
Carboxymethyl cellulose [c]	2.0	2.0	2.0	2.0	2.0
FOS [f]	0.0	1.0	0.0	0.0	0.0
Bio-MOS [g]	0.0	0.0	1.0	0.0	0.0
Previda [h]	0.0	0.0	0.0	1.0	0.0
GOS [i]	0.0	0.0	0.0	0.0	1.0
Cellulose [c]	3.6	2.6	2.6	2.6	2.6
Proximate composition (%) [j]					
Dry matter	83.8	86.1	89.0	88.4	88.5
Crude protein	41.5	41.2	41.6	41.0	42.2
Crude lipid	10.3	10.8	10.1	11.1	10.4
Ash	12.1	12.2	12.4	12.3	12.3

[a] Omega Protein Corporation, Houston, TX, USA.
[b] Rangen Inc. Angleton, TX, USA.
[c] USB Corporation. Cleveland, OH, USA.
[d] Same as Li and Gatlin (2004).
[e] MP Biomedicals LLC, OH, USA.
[f] Inulin, Encore Technologies, Plymouth, MN, USA.
[g] Bio-MOS, Alltech Inc., Nicholasville, KY, USA.
[h] Previda, Temple-Inland, Diboll, TX.
[i] Vivinal GOS, Friesland Foods Domo, Zwolle, The Netherlands.
[j] Means of two replicate analyses per sample expressed in a dry-matter basis.

2.3. Sample collection techniques and analyses

At the termination of the 8-week feeding trial, fish in each aquarium were individually weighed and sampled for tissue analysis 24 h after the last feeding. Hepatosomatic index (HSI) and muscle ratio were determined from two individual fish per aquarium by obtaining tissues (liver and muscle) and expressing ratios as a percent of body weight. Two representative fish from each aquarium also were anesthetized with tricaine methane sulfonate (MS-222), and approximately 0.5 ml of blood was collected from the caudal vasculature using a 1-ml syringe with a 27-gauge needle. Plasma was separated as previously described (Li and Gatlin, 2003) and lysozyme activity was determined by a turbidimetric assay (Jørgensen et al., 1993). A lysozyme activity unit was defined as the amount of enzyme producing a decrease in absorbance of 0.001 min^{-1} at pH 5.2. Whole blood neutrophil oxidative radical production also was determined as described by Siwicki et al. (1994) and Li and Gatlin (2003). Absorbance was converted to Nitro Blue Tetrazolium (NBT) units based on a standard curve of NBT diformazan/ml blood.

2.4. Histological and morphometric analysis

In addition, at the end of the experiment two representative fish from each aquarium were anesthetized with tricaine methane sulfonate (MS-222, 100 mg/l) and their entire intestinal tract was dissected, measured and weighed after removal of the digestive contents. Each intestine was then tied at both ends, injected with Davidson's fixative (water/formalin/ethanol/acetic acid, 3/2/3/1, v/v) and then immersed in Davidson's fixative. After 24 h, the samples were dehydrated and transferred to an ethanol solution (70%). The pyloric caeca, proximal intestine, mid-intestine and distal intestine were embedded in paraffin and sectioned into 4-μm transverse cuts following the axis of the gut lumen. The samples were then mounted on glass slides, and stained with hematoxylin and eosin. Slides were examined on a light microscope (Olympus BX60) equipped with the Adobe Photoshop CS4 software. Digitalized images were analyzed measuring the micrometer length of various enteric structures. Macromorphological (fold length) and micromorphological (total enterocyte height and microvillus height) parameters were measured (10 fields per individual sample) according to the procedures described by Escaffre et al. (2007).

2.5. Calculations and statistical analysis

The parameters were calculated as follows:

$$\text{Percent weight gain (WG, \%)} = [(\text{Final wt.} - \text{initial wt.})/\text{initial wt.}] \times 100$$

$$\text{Feed efficiency ratio (FE)} = \text{weight gain (g)}/\text{feed consumed (g, DW)}$$

$$\text{Protein efficiency ratio (PER)} = \text{weight gain (g)}/\text{protein intake (g)}$$

$$\text{Condition factor (CF)} = 100 \times (\text{body weight, g}) / (\text{body length, cm})^3$$

$$\text{Hepatosomatic index (HSI)} = 100 \times (\text{liver weight} / \text{whole body weight})$$

$$\text{Muscle ratio} = 100 \times (\text{fillet muscle weight, g}) / (\text{whole body weight, g})$$