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Enhanced intestinal epithelial barrier health status on European sea bass (*Dicentrarchus labrax*) fed mannan oligosaccharides

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ABSTRACT

The study assesses the effects of dietary mannan oligosaccharides (MOS) in European sea bass (*Dicentrarchus labrax*) posterior intestinal lipid class composition and its possible relation to the potential prostaglandins production and Gut Associated Lymphoid Tissue (GALT) stimulation.

Fish were fed 4 g kg^{-1} MOS (Bio-Mos® Aquagrade, Alltech, Inc., USA) for eight weeks. Fish fed MOS presented higher ($P \leq 0.05$) weight gain, total length, and specific and relative growth rates than fish fed the control diet. Stimulated posterior gut of fish fed MOS showed higher ($P \leq 0.05$) prostaglandins production than fish fed the control diet. Lipid class analyses of posterior gut revealed a reduction ($P \leq 0.05$) in the neutral lipid fraction in fish fed MOS compared to fish fed the control diet, particularly due to a reduction ($P \leq 0.05$) in triacylglycerols content. The polar lipid fraction increased ($P \leq 0.05$) in fish fed MOS compared to fish fed the control diet, mainly due to an increase ($P \leq 0.05$) in phosphatidylethanolamine and phosphatidylcoline contents.

Light microscopy of posterior gut revealed increased number or goblet cells as well as higher level of infiltrated eosinophilic granulocytes for fish fed MOS. Transmission electron microscopy qualitative observations revealed a better preserved cytoarchitecture of the intestinal epithelial barrier in the posterior gut of fish fed MOS. Posterior gut of fish fed MOS presented more densely packed non-damaged enterocytes, better preserved tight junctions structure, healthier and more organized microvilli, and a higher presence of infiltrated lymphocytes and granulocytes compared fish fed the control diet.

The present study indicates that dietary MOS enhances European sea bass posterior gut epithelial defense by increasing membrane polar lipids content in relation to a stimulation of the eicosanoid cascade and GALT, promoting posterior gut health status.

1. Introduction

The mucosal surface of the gastrointestinal tract (GI) is a complex ecosystem composed by epithelial, immune cells and resident microflora [1] and particularly in fish supposes a potential port of entry for some pathogenic bacteria [2,3]. The intestinal epithelial barrier consists of: the supra-epithelial, the epithelial and the sub-epithelial defense mechanisms [4,5]. The supra-epithelial mucus barrier acts as a medium for protection, lubrication and transport between the luminal contents and the epithelial lining [6,7]. Fish mucus is composed by cytokines, peptides, lysozyme, lipoproteins, complement, lectins, proteases, antibodies and mucins [8]. Mucosal mucins are the main mucus component, and play an important epithelium cytoprotective role against: mechanical insults, pathogen colonization and cellular luminal proteases [9].

Intestinal epithelial cells (IECs) provide the second line of the mucosal defense system, and constitute an efficient physical barrier among a broad spectrum of dietary and enteric flora pathogens and the host, allowing also an exchange between nutrients and the systemic circulation [5]. In fish, it consists of a simple epithelial layer composed mainly of absorptive columnar cells (enterocytes)

Abbreviations: ARA, Arachidonic acid (20:4n-6); COX, Cyclooxygenase; EPA, Eicosapentanoic acid (20:5n-3); GALT, Gut associated lymphoid tissue; G, Granulocyte; HMG, High molecular weight glycoprotein; HPTLC, High performance thin-layer chromatography; IEL, Intestinal epithelial lymphocyte; LOX, Lipooxygenase; LPC, Lysophosphatidylcholine; Mo, Macrophage; MOS, Mannan oligosaccharides; NL, Neutral lipids; PAF, Platelet-activating factor; PC, Phosphatidylcholine; PE, Phosphatidylethanolamine; PG, Prostaglandin; PI, Phosphatidylinositol; PL, Polar lipids, phospholipids; PS, Phosphatidylserine; TAG, Tryacylglycerol; TEM, Transmission electron microscopy.

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with the inclusion of mucus-secreting goblet cells and endocrine cells sealed at the apical end of the lateral surface by tight junctions (TJ) [10]. In mammals, IECs are considered immunocompetent (cytokines and chemokines production). Unfortunately, in fish the involvement of IECs in immunity is not well known, however exists some evidence of cytokine production by rainbow trout (*Oncohynchus mykiss*) IECs [8,11].

Besides, intestinal epithelial barrier fluidity, permeability and eicosanoid production are dependent on the fatty acid composition of cellular membranes [12]. Prostanoids (prostaglandins (PG) and prostacyclins), produced from C_{20} long chain fatty acids (LCPUFA) (via cyclooxygenase enzymes), are involved in gastrointestinal cytoprotection [13,14] by influencing the homeostasis regulation and the onset of gastrointestinal inflammation [15]. For example, PG allow plasma exudation and tissue oedema by affecting vascular tone and permeability [14,16], stimulates mucin synthesis and release [17–19], and inhibit the expression of some pro- and anti-inflammatory immune related genes [15,20,21].

Gut associated lymphoid tissue (GALT) represents the intestinal sub-epithelial barrier. For European sea bass, which concentrates the GALT toward the anus [22], the presence of resident intra-epithelial macrophages [23], granulocytes (G; neutrophils and eosinophils), and intestinal epithelial lymphocytes (IEL) [23] has been demonstrated.

Thus, in parallel to vaccination, a current strategy to reduce the risk of appearance of diseases outbreaks and to promote fish growth is to enhance gut health through the use of dietary prebiotics, among others. Mannan oligosaccharides (MOS) dietary supplementation to European sea bass (*D. labrax*) juveniles has been demonstrated to reduce *in vivo* gut bacterial translocation against *Vibrio alginolyticus* [24] and *Vibrio anguillarum* both *ex vivo* [25] and *in vivo* [26]. These effects have been attributed to an enhancement of both; the intestinal supra-epithelial defense mechanism by enhancing intestinal goblet cells density [25,26] and to a stimulation of the intestinal sub-epithelial defense mechanism in terms of higher number of infiltrated eosinophilic granulocytes in the lamina propria [26]. However, the possible effects of dietary MOS on European sea bass intestinal lipid membrane composition, as well as, on microvilli and GALT structure have not been studied.

Thus, considering the influence of cell membranes fatty acid composition on the production of modulatory prostanoids and on epithelial barrier function [27], the objective of this study is to determine the effect of dietary MOS supplementation on fish posterior GI: lipid class composition, potential PG production and structural morphology.

2. Materials and methods

2.1. Experiment I: intestinal mucus production, lipid class composition and prostaglandins production

2.1.1. Diets

Two experimental dry pelleted diets, based on a commercial formulation, were prepared in order to contain 0 (Control) and 4 g kg^{-1} MOS (MOS; Bio-Mos Aquagrade, Alltech, Inc., USA) replacing standard carbohydrates (corn meal). Diets were pelleted in an industrial mixer, crumbled to the desired size, and air-dried prior to storage at 4 °C until feeding. Diet ingredients, proximate composition and fatty acid profiles are shown in Table 1 and Table 2.

2.1.2. Experimental conditions

Five hundred and fifty commercially reared European sea bass juveniles were maintained in stocking tanks and fed a commercial extruded diet for 3 weeks until being fully adapted to the environmental conditions (4 kg m^{-3} stocking density). Afterward, fish were randomly distributed in 6 indoor cylindroconical 1000 L fiberglass tanks at an initial stocking density of 4.1 kg m^{-3} (90 fish per tank). Fish average initial weight and length were 45.95 ± 0.60 g and 15.05 ± 0.05 cm, respectively (mean ± SD). Tanks were supplied in a flow-through system with filtered sea water, at a temperature of 22.8–23.3 °C, and natural photoperiod (12L:12D). Water dissolved oxygen ranged between 6.5 and 7.2 ppm. Fish were manually fed until apparent satiation with one of the two experimental diets for 8 weeks (3 times a day, 6 days a week). Each diet was assayed in triplicate.

Table 1
Main ingredients and composition of the experimental diets.

Ingredient	Diet (g kg^{-1} dry weight)			
	Experiment I		Experiment II	
	Control	BM4	Control	BM4
Fish meal[a]	515.0	515.0	515.0	515.0
Soybean meal	97.8	97.8	97.8	97.8
Wheat	75.0	75.0	75.0	75.0
Wheat gluten	75.0	75.0	75.0	75.0
Corn meal	65.3	61.3	65.3	61.3
Fish oil[b]	126.9	126.9	126.9	126.9
Fats and oils	20.3	20.3	20.3	20.3
Mineral mix[c]	14.3	14.3	14.3	14.3
Vitamin mix[d]	10.3	10.3	10.3	10.3
Antioxidant (BHT)	0.1	0.1	0.1	0.1
MOS (Bio-Mos Aquagrade)[e]	0	4	0	4
Composition (%, dry weight)				
Crude lipids	20.37	20.32	24.07	24.04
Crude protein	51.62	52.07	48.71	48.33
Ash	9.74	9.82	9.98	9.56

[a] Peruvian fishmeal (65% protein).
[b] Peruvian fish oil.
[c] Mineral mix TROUW Seabream/Seabass (0.8 g), choline chloride (0.17 g) and inositol (0.06 g) (Trouw Nutrition Spain, Madrid, Spain).
[d] Vitamin mix TROUW Seabream/Seabass (1 g), calcium carbonate (0.2 g), potassium monophosphate (0.19 g) and NaCl 97% (0.04 g) (Trouw Nutrition Spain, Madrid, Spain).
[e] Bio-Mos, Alltech, Inc., USA.

After 60 days of supplementation, the whole fish population was sampled for final weight, final length, condition factor (K), relative growth and specific growth rate (SGR) and 5 fish per tank were sampled individually for posterior gut histological studies. Additionally, 5 fish per tank were sampled for prostaglandins levels analyses of posterior gut. Posterior gut fatty acid profiles and lipid class composition were also determined in a pooled sample of 3 fish per tank. The animal experiments described comply with the guidelines of the European Union Council (2010/63/EU) for the use of experimental animals and have been approved by the Bioethical Committee of the University of Las Palmas de Gran Canaria.

2.2. Experiment II: intestinal morphology

2.2.1. Diets

Two diets, based on a commercial formulation, were designed to contain 0 g kg^{-1} (Control), and 4 g kg^{-1} MOS (Bio-Mos®, Alltech Inc., USA), replacing standard carbohydrates (corn meal). Diets covered nutritional requirements for this species [28] and were manufactured by a commercial feed producer (Graneros de Tenerife, Tenerife, Spain) with the composition showed in Table 1.

2.2.2. Experimental conditions

Juvenile European sea bass were transferred from a local farm (ADSA, San Bartolomé de Tirajana, Canary Islands, Spain), to the main facility of the Canarian Institute of Marine Science (ICCM) where they were acclimatized during 8 weeks in indoor 1000 l fiberglass tanks until achieving the initial experimental size (116 g).