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The effects of prebiotics on the digestive enzymes and gut histomorphology of red drum (*Sciaenops ocellatus*) and hybrid striped bass (*Morone chrysops* × *M. saxatilis*)

Maritza Anguiano[1], Camilo Pohlenz[1,2], Alejandro Buentello[1,2]† and Delbert M. Gatlin III[1,2]*

[1]*Department of Wildlife and Fisheries Sciences, Texas A&M University System, College Station, TX 77843-2258, USA*
[2]*Intercollegiate Faculty of Nutrition, Texas A&M University System, College Station, TX 77843, USA*

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Abstract

The effects of four prebiotics (fructo-oligosaccharide, Bio-MOS®, transgalacto-oligosaccharide and GroBiotic®-A) on digestive enzymes and intestinal morphology were studied in juvenile hybrid striped bass (*Morone chrysops* × *M. saxatilis*) and red drum (*Sciaenops ocellatus*) using two separate 8-week feeding trials. Red drum were fed experimental diets with the four prebiotics each individually supplemented at 1 % and hybrid striped bass were fed diets supplemented with GroBiotic®-A at 1 and 2 %. Both trials were conducted with each diet fed to apparent satiation twice per d to three replicate groups of fifteen juvenile fish. For histomorphological analysis, gastrointestinal tract (GIT) samples from three randomly selected fish per tank were taken at 4 and 8 weeks for hybrid striped bass and at 8 weeks for red drum. For both trials, GIT samples from two randomly selected fish per tank were taken at 4 and 8 weeks and analysed for pepsin, trypsin, chymotrypsin, aminopeptidase, α-amylase, lipase, and both acid and alkaline phosphatase activities. The results of the histological evaluation indicated that the inclusion of prebiotics was adequate to elicit structural changes in the GIT of both species. On the other hand, no significant changes in the enzyme activities were detected at week 8 in both species. However, there was a transient effect of Bio-MOS® supplementation on the activities of aminopeptidase, α-amylase and alkaline phosphatase at week 4 in red drum only. Thus, previously observed improvements in nutrient digestibility by these fish in response to prebiotic supplementation appear to be mostly related to changes in GIT structure as opposed to the enhancement of digestive enzyme activity.

Key words: Hybrid striped bass: Red drum: Prebiotics: Intestinal histomorphology: Digestive enzymes

Aquaculture is one of the fastest growing food industries in the world[1] and continues to expand to accommodate the increase in seafood demand from a growing human population. However, this expansion has resulted in large-scale, intensive production facilities that often expose fish to stressful conditions and thus making the fish more susceptible to various diseases[2]. Traditionally, antimicrobial agents have been used to treat disease outbreaks; however, there have been concerns over the misuse of antimicrobials leading to the evolution of antimicrobial resistance in numerous bacterial pathogens and thus prompting research for alternative methods[2,3].

A relatively new alternative to using antibiotics in aquaculture has been to supplement prebiotics in the diet[3–8]. Prebiotics are defined as 'non-digestible food ingredients that beneficially affect the host by selectively stimulating growth and/or activity of one or a limited number of bacteria in the intestinal tract'[9]. Although applications of prebiotics to human subjects and terrestrial livestock have been studied more extensively[4,6,9–12], information on the use of prebiotics in aquatic animals is rapidly accumulating[5,13–19].

Recent reviews[20–22] in prebiotics have documented positive effects in various fish on growth and immune parameters from prebiotic supplementation. In particular, research in two prominent fish species, red drum (*Sciaenops ocellatus*) and hybrid striped bass (*Morone chrysops* × *M. saxatilis*), has demonstrated that dietary inclusion of prebiotics can increase feed efficiency, enhance nutrient and energy digestibility and reduce mortality of these two species[3,8,23]. These benefits may be due to the ability of prebiotics to modify the gastrointestinal microbial community to promote fermentation and immune responses[5,8]. Research also has reported changes in morphological characteristics in the intestine, such as villus height,

Abbreviations: DI, deionised; FOS, fructo-oligosaccharides; GBA, GroBiotic®-A; GIT, gastrointestinal tract; MOS, mannanoligosaccharide; TOS, transgalacto-oligosaccharide.

* **Corresponding author:** D. M. Gatlin, fax +1 979 845 4096, email d-gatlin@tamu.edu

† Present address: Schillinger Genetics, 4401 Westown Parkway, Suite 225, West Des Moines, IA 50 266, USA.

and changes to digestive enzyme activities in terrestrial animals[10,11] and fish[7] that seem to correspond with the positive effects seen in growth from prebiotic supplementation. At the present, there are several reports on the effects of prebiotics on the morphological characteristics of the intestine in several fish species[15–18,24,25] but only one study has presented changes in the intestine in red drum[19] and none for hybrid striped bass. Also, to date, there is only one study on prebiotics that reports effects on digestive enzymes in fish[7], and none is available for these two species. As the changes in the morphology of the intestine and digestive enzymes may be complementary to the effects seen in growth, more research is needed to assess the morphological changes in the intestine and digestive enzymes in these two species for better understanding of the effects of prebiotics.

Therefore, the aim of the present study was to further investigate whether enhanced feed efficiency and digestibility associated with prebiotic supplementation may be due to changes in digestive enzymes and/or the morphology of the intestine. To this end, the present comparative study was conducted to evaluate the effects of commercially available fructo-oligosaccharide (FOS) in the form of inulin, Bio-MOS®, containing mannanoligosaccharide (MOS), transgalacto-oligosaccharide (TOS) and GroBiotic®-A (GBA) on the activities of pepsin, trypsin, chymotrypsin, aminopeptidase, α-amylase, lipase, and both acid and alkaline phosphatases in red drum and hybrid striped bass, as well as potential changes in fold length, and both enterocyte and microvillus height of different sections of the gastrointestinal tract (GIT) of these fish.

Experimental methods

Diet formulation

The basal diet for both red drum and hybrid striped bass was formulated and analysed to contain, on a DM basis, 40 % crude protein from a menhaden fishmeal and cooked soyabean meal, 10 % total lipid primarily from menhaden fish oil, and dextrin as the soluble carbohydrate to provide approximately 14·2 kJ digestible energy/g diet. The basal diet was formulated without prebiotic supply (Table 1)[26]. Experimental diets for red drum were prepared by supplementing the basal diet with FOS, MOS, TOS and GBA at 1 % (w/w) of diet in place of cellulose. For hybrid striped bass, GBA was provided at both 1 and 2 % (w/w), in place of cellulose.

Table 1. Composition of the basal diet

Ingredients	g/100 g (dry weight)
Menhaden meal*	29·2
Soyabean meal†	37·5
Dextrin‡	16·5
Menhaden oil*	5·6
Vitamin premix§	3·0
Mineral premix‖	4·0
Carboxymethylcellulose‡	2·0
Fructo-oligosaccharide¶	0·0
Mannanoligosaccharide**	0·0
Transgalacto-oligosaccharide††	0·0
GroBiotic®-A‡‡	0·0
Cellulose‡	2·2
Proximate composition (g/100 g DM)§§	
Crude protein	41·4
Crude lipid	11·4
Crude ash	12·8
Moisture	87·8

* Omega Protein Corporation.
† Rangen, Inc.
‡ USB Corporation.
§ Same as Moon & Gatlin[26].
‖ Same as Moon & Gatlin[26] but prepared by MP Biomedicals LLC.
¶ Inulin; Encore Technologies.
** Bio-MOS®; Alltech, Inc.
†† Vivinal; Friesland Foods Domo.
‡‡ International Ingredient Corporation.
§§ Analysed values are means of two replicate samples.

Feeding trials

Procedures used in the present study were reviewed and approved by the Texas A&M University System Animal Care and Use Committee. We conducted two separate feeding trials, one for each species, at the Texas A&M University Aquacultural Research and Teaching Facility in 110-litre aquaria maintained indoors in a climate-controlled laboratory. Water flow in the recirculating system was maintained at approximately 1 litre/min and biological/mechanical filtration was used to maintain adequate water quality for red drum and hybrid striped bass culture. Salinity was maintained at 6–8 g/l by mixing synthetic sea salt mixture (Fritz Industries) with well water. Low-pressure electrical blowers provided aeration via air stones to maintain dissolved oxygen levels near air saturation. Water temperature was maintained at 25 (± 2)°C throughout each trial by controlling ambient temperature with dual air-conditioning units. A 12 h light–12 h dark photoperiod was maintained with fluorescent lights controlled by automatic timers.

For each trial, fish with no visual signs of disease were selected, graded by size (10·9 (SD 0·2) and 5·1 (SD 0·3) g for red drum and hybrid striped bass, respectively), and groups of fifteen fish were stocked into each aquarium. Red drum and hybrid striped bass were subjected to a 1-week conditioning period during which the basal diet was fed to satiation based on visual cues of the fish eating. After the conditioning period, triplicate aquaria were randomly assigned to each dietary treatment. The fish were fed close to satiation with pre-weighed rations based on growth and visual cues of the fish eating (initially 5 % of body weight and gradually reduced to 3 % of body weight), twice daily (morning and evening), 7 d/week. Fish in each aquarium were group-weighed every week and feed rations adjusted accordingly. For both fish species, the feeding trials continued for 8 weeks.

Histological sample collection and analysis

Processing for histological analysis was the same in both experiments. At each sample period, three randomly selected fish were taken from each aquarium in both trials. For red drum, samples were taken at 8 weeks and for hybrid striped bass, samples were taken at 4 and 8 weeks. To evaluate

changes in histological structures of the intestinal mucosa, GIT were dissected from the gastro-pyloric region to the anal region, and tied at both ends with cotton twine. Davidson's solution was injected into the intestinal lumen for preventing autolytic changes of the mucosa. Intestinal samples were kept in Davidson's fixative for 24 h and then transferred to 70 % (v/v) ethanol for conservation until processing for slide preparation.

For histological analysis, two cross-sectional rings of approximately 0·5 cm were cut from each of the anterior, mid and posterior regions of the intestine, together with 0·2 cm rings from the most medial region of four pyloric caeca. The anterior region was identified as the region within 1 cm after the pyloric portion of the stomach and the posterior region was identified as the region within 1 cm before the anus. Intestinal regions were embedded in paraffin, and 5 μm sections were made for glass slide mounting and haematoxylin–eosin staining. All slides were evaluated in an Olympus BC-2 series light microscope linked to a digital camera. For each region, three fields at 4 × and five fields at 40 × objectives were captured. Images were then analysed under ImageJ (version 1.4g) Software (National Institutes of Health, freeware). Variables measured were fold length and both total enterocyte and microvillus height for the anterior and posterior intestine, for the red drum trial, whereas for the hybrid striped bass trial, variables measured were fold length for the anterior, mid and posterior intestine, and both enterocyte and microvillus height for the previous regions plus the pyloric caeca; in all cases, ninety measurements per variable in each treatment were made for each sample point. Only appropriately oriented folds were used for measurements.

Enzyme sample collection and analysis

For the red drum and hybrid striped bass trials, two fish were randomly selected from each aquarium and euthanised at weeks 4 and 8. All fish were sampled 4 h after being fed to ensure that the enzymes would be active from digestion. The stomach and the intestine were aseptically dissected and then separated from each other. The entire intestine was then flash-frozen in liquid N_2 and stored at −80°C until analyses.

Frozen intestinal samples were homogenised in cold 50 mM-2-amino-2-hydroxymethyl-propane-1,3-diol (Tris)–HCl, 20 mM-$CaCl_2$ buffer, and supernatants were stored at −20°C before enzyme analyses. The concentration of the soluble protein in the samples was determined by the Bradford method (BioRad Protein Assay), using bovine serum albumin as a standard. The activities of pepsin, trypsin, chymotrypsin, aminopeptidase, α-amylase, lipase, and both acid and alkaline phosphatases were assayed spectrophotometrically in triplicate for each of the two intestinal samples per aquarium per time period.

Pepsin activity was assayed based on a method described by Anson[27] using 0·5 % (w/v) Hb in 0·1 M-glycine–HCl buffer (pH 2). The samples were incubated at 37°C for 30 min and then the reaction was stopped with 20 % (w/v) TCA. The samples remained at 4°C for 15–30 min and were then centrifuged. The optical density of the supernatants was measured at 260 nm using deionised (DI) water as a blank.

Trypsin activity was assayed based on the method described by Erlanger *et al.*[28], using *N*-α-benzoyl-DL-arginine 4-nitroanilide hydrochloride in dimethyl sulfoxide and 50 mM-Tris–HCl, 10 mM-$CaCl_2$ buffer (pH 8·2) as the substrate. The samples and the substrate were incubated at 37°C for 30 min, and then the reaction was stopped with 30 % (v/v) acetic acid. Trypsin was measured at 410 nm against a blank containing the substrate, acetic acid and DI water in place of the sample.

Chymotrypsin was assayed based on the method described by Asgeirsson & Bjarnason[29], using 5 mM-*N*-benzoyl-L-tyrosine ethyl ester in dimethyl sulfoxide and 44·4 mM-Tris–HCl, 55·5 mM-$CaCl_2$ buffer (pH 7·8). The samples remained at room temperature for 10 min and then incubated at approximately 100°C for 15 min. The samples were measured at 256 nm against a blank containing *N*-benzoyl-L-tyrosine ethyl ester and buffer.

Aminopeptidase was assayed using L-leucine *p*-nitroanilide in dimethyl sulfoxide and 50 mM-Na_3PO_4 (pH 7·2) as the substrate. The samples and substrate were incubated at 37°C for 10 min, and then the reaction was stopped with 30 % (v/v) acetic acid. Aminopeptidase activity was measured at 410 nm against DI water as the blank.

α-Amylase was assayed based on Vega-Villasante *et al.*[30], using 50 mM-Tris–HCl buffer (pH 7·5) and 1 % soluble starch as the substrate. The samples were incubated for 10 min at room temperature. To initiate the reaction, 2 M-sodium carbonate and reactive dinitrosalicylic acid were added and then the samples were incubated for 15 min at approximately 100°C. DI water was added and then the samples were read at 550 nm against DI water as the blank.

Lipase was measured using the method described by Versaw *et al.*[31]. Sodium cholate hydrate, 50 mM-Tris–HCl buffer (pH 7·2) and β-naphthyl-caprylate were used as the substrate. The samples were incubated for 30 min at room temperature and then 100 mM-Fast Blue BB Salt was added. After 5 min of incubation at room temperature, the reaction was stopped using 0·72 M-TCA and then ethanol–ethyl acetate (1:1, v/v) was added to clarify the solution. The samples were read at 540 nm against DI water as a blank.

Acid and alkaline phosphatases were measured using 2 % (w/v) 4-nitrophenylphosphate as the substrate in either sodium citrate dihydrate (pH 4·8) for acid phosphatase or 30 mM-sodium carbonate (pH 9·8) for alkaline phosphatase. The samples were incubated for 30 min at 37°C and then the reaction was stopped using 0·05 M-NaOH. The samples were read at 405 nm using a blank containing the substrate, NaOH and DI water in place of the samples.

Statistical analysis

Data from the histological and enzyme analyses were subjected to one-way ANOVA, and mean separation was assessed by Duncan's multiple range test using SAS (version 9.2, SAS Institute). Differences in treatment means were considered to be significant at $P \leq 0{\cdot}05$.

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Results

After feeding red drum the experimental diets for 8 weeks, intestinal histological structures were affected by prebiotics (Table 2). All structures in the anterior intestine were consistently affected by TOS, significantly ($P<0·05$) increasing the length of intestinal folds and the height of both enterocyte and microvilli. Similarly, MOS had a significant effect on intestinal fold length and microvillus height, whereas GBA increased only the microvillus height. In contrast, FOS had a significant detrimental effect on anterior intestinal structures, decreasing the fold length and enterocyte height. Conversely, in the posterior intestine, GBA had a significantly greater effect on histological structures, augmenting the length of folds and the height of the microvilli, whereas TOS significantly affected only the microvillus height.

For hybrid striped bass, intestinal histological structures were measured at 4 and 8 weeks after being fed graded levels of GBA. These structures were positively affected by the addition of this prebiotic to the diet (Table 3). At 4 weeks, all structures of all intestinal sections were affected by 2% GBA inclusion, significantly increasing the length of folds and the height of both enterocyte and microvilli. Dietary inclusion of 1% GBA only significantly affected the enterocyte and microvillus height in the anterior intestine. Conversely, after 8 weeks of the feeding trial, the anterior intestine was not affected by the treatments, whereas the inclusion of 1% GBA significantly increased the fold length of both mid and posterior intestine and the enterocyte height of the mid intestine. In addition, 2% GBA significantly increased the fold length of the mid intestine and the microvillus height of pyloric caeca.

Intestinal samples obtained from red drum and hybrid striped bass at weeks 4 and 8 were analysed to measure the specific enzyme activities. For red drum, MOS, in general, had higher activities of the analysed enzymes at week 4. Aminopeptidase activity in fish fed the MOS diet was significantly increased at week 4 compared with the values obtained from fish fed the basal and the GBA-supplemented diets (Table 4). The inclusion of MOS in the diet also increased the activities of alkaline phosphatase and α-amylase in a significant manner when compared with those of fish fed the basal, GBA and FOS diets. Similarly, the TOS diet tended to produce numerically higher values for aminopeptidase, acid and alkaline phosphatases, and α-amylase compared with fish fed the FOS, GBA or basal diet, although these changes were not significantly different. Conversely, GBA, in general, had lower activities of all analysed enzymes except for pepsin and lipase.

The observed changes in enzymatic activities at week 4 for red drum were transient in nature because at the end of week 8, none of the selected enzymes showed any significant difference compared with fish fed the basal diet (Table 4). Interestingly, at week 8, the activities of pepsin, aminopeptidase, trypsin, α-amylase, and both acid and alkaline phosphatases were of higher magnitude in fish fed TOS than in those fed MOS or all other diets, although these values were not significantly different for any dietary treatment.

GBA supplementation at 1% in the diets of hybrid striped bass for 4 weeks tended to increase the activities of chymotrypsin and alkaline phosphatase, while these activities tended to decrease with a supplementation level of 2%. Conversely, GBA supplementation at 1 and 2% elicited a significant decrease in the activity of α-amylase at 8 weeks (Table 5).

Discussion

Prebiotic supplementation in the diets of hybrid striped bass and red drum elicited structural changes in the GIT of the fish. Conversely, prebiotic supplementation did not elicit many significant changes in the activities of digestive enzymes in both fish species. The present study yielded similar results to those reported by a previous study[32], which demonstrated that Atlantic salmon (*Salmo salar*) fed inulin did not have any significant changes in trypsin, amylase, alkaline phosphatase and leucine aminopeptidase. However, these results are different from those seen in allogynogenetic crucian carp (*Carassius auratus gibelio*)[7] which were fed xylo-oligosaccharide. In those studies, treated fish exhibited an increase in several enzyme activities and all also coincided with improvements in condition indices such as weight gain. Observed differences between these studies may be explained in part by the sharp contrast between carnivores (e.g. red

Table 2. Histological parameters (μm) of the gastrointestinal tract of red drum fed different prebiotics for 8 weeks
(Mean values with their pooled standard errors)

	Prebiotic						
	Basal	GBA	FOS	TOS	MOS	$P>F$	PSE
Anterior intestine							
Fold length	$765·4^{b,c}$	$961·8^{a,b}$	$623·1^{c}$	$1009·8^{a}$	$1031·0^{a}$	0·001	39·0
Enterocyte height	$40·3^{b}$	$42·0^{a,b}$	$35·7^{c}$	$43·5^{a}$	$41·4^{a,b}$	<0·0001	0·57
Microvillus height	$3·3^{b}$	$3·8^{a}$	$2·9^{b}$	$4·3^{a}$	$4·0^{a}$	<0·0001	0·09
Posterior intestine							
Fold length	$634·0^{b}$	$875·7^{a}$	$625·2^{b}$	$590·4^{b}$	$689·1^{b}$	0·020	31·8
Enterocyte height	34·6	35·1	33·6	35·1	36·6	0·215	0·53
Microvillus height	$2·2^{b}$	$2·8^{a}$	$2·2^{b}$	$2·7^{a}$	$2·4^{b}$	<0·0001	0·06

GBA, GroBiotic®-A; FOS, fructo-oligosaccharides; TOS, transgalacto-oligosaccharides; MOS, mannan-oligosaccharides; PSE, pooled standard error.
^{a,b,c} Mean values within a row with unlike superscript letters were significantly different ($P<0·05$; Duncan's multiple range test).

Table 3. Histological parameters (μm) of the gastrointestinal tract of hybrid striped bass fed graded levels of GroBiotic®-A (GBA) for 4 and 8 weeks (Mean values with their pooled standard errors)

	Week 4					Week 8				
	Basal	GBA-1 %	GBA-2 %	$P > F$	PSE	Basal	GBA-1 %	GBA-2 %	$P > F$	PSE
Anterior intestine										
Fold length	728·4^{b}	756·6^{a,b}	829·6^{a}	0·005	29·8	931·7	942·0	985·8	0·227	23·6
Enterocyte height	26·6^{b}	28·8^{a}	29·6^{a}	<0·0001	0·49	28·4	29·4	28·5	0·236	0·45
Microvillus height	1·8^{c}	2·03^{b}	2·3^{a}	<0·0001	0·05	1·8	1·9	1·9	0·055	0·04
Mid intestine										
Fold length	390·2^{b}	425·7^{b}	496·3^{a}	<0·0001	14·7	443·5^{b}	541·7^{a}	553·3^{a}	<0·0001	14·8
Enterocyte height	27·4^{a}	25·4^{b}	28·1^{a}	<0·0001	0·43	25·2^{b}	27·2^{a}	25·6^{b}	0·006	0·39
Microvillus height	1·6^{b}	1·7^{b}	2·1^{a}	<0·0001	0·05	1·6	1·6	1·7	0·226	0·03
Posterior intestine										
Fold length	328·6^{b}	340·6^{b}	384·5^{a}	0·001	11·2	330·8^{b}	359·1^{a}	299·7^{c}	<0·0001	7·3
Enterocyte height	25·2^{b}	26·4^{a,b}	27·1^{a}	0·016	0·46	25·7	25·3	26·6	0·082	0·43
Microvillus height	1·9^{b}	1·8^{b}	2·1^{a}	<0·0001	0·05	1·9	1·9	1·9	0·762	0·03
Pyloric caecum										
Enterocyte height	26·2^{b}	24·8^{b}	28·4^{a}	<0·0001	0·51	27·0	26·8	27·1	0·969	0·44
Microvillus height	1·8^{b}	1·9^{b}	2·2^{a}	<0·0001	0·05	1·8^{b}	1·9^{a,b}	2·0^{a}	0·035	0·03

PSE, pooled standard error.
^{a,b,c} Mean values within a row with unlike superscript letters were significantly different at week 4 or 8 ($P<0·05$; Duncan's multiple range test).

drum, hybrid striped bass and Atlantic salmon) and omnivores/herbivores (e.g. crucian carp) in terms of the physiology and the architecture of their GIT. It is possible that the observed prebiotic effects on digestive enzyme activities in the latter correlate with the extended length and passage rate through the GIT of cyprinids, conceivably explaining the improved growth observed in the carp investigations.

Previous studies with prebiotics in red drum and hybrid striped bass have also shown enhancements in several performance parameters including weight gain, feed efficiency and survival[3,13,14,19]. Because the present data indicate that the prebiotic treatments had no persistent effect on the activities of various digestive enzymes, we cannot conclude that the increased weight gain and feed efficiency of red drum and hybrid striped bass observed in the previously mentioned studies are related to changes in endogenous digestive enzymes.

On the other hand, the present set of experiments indicates that intestinal histological structures were affected by prebiotics, which is in line with recent reports for other fish species[15–18,24,25]. From a histological perspective, the most thoroughly evaluated prebiotic is MOS, in fish and other vertebrates. In the present study, MOS (1 %) was evaluated only in red drum, where it had a positive effect on the fold length and microvillus height of the anterior intestine. Consistent with the results reported in the present paper, a previous study observed that 1·5 or 3 % MOS in the diet improved fold length in juvenile rainbow trout (*Oncorhynchus mykiss*)[25]. However, lower inclusion levels (0·2 %) failed to elicit the same response in fish of similar age, but not in subadult trout[16]. Correspondingly, in larval white sea bream (*Diplodus sargus*), MOS supplementation improved intestinal morphology by increasing the villous surface area and the microvillus height[17]. In contrast, histological evaluation of the anterior intestine of gilthead sea

Table 4. Digestive enzyme activities of red drum at 4 and 8 weeks (Mean values with their pooled standard errors)

	Diet	Pepsin (Units)*	Trypsin (μUnits)	Chymotrypsin (mUnits)	Aminopeptidase (Units)	α-Amylase (Units)	Lipase (Units)	Alkaline phosphatase (Units)	Acid phosphatase (Units)
Initial	Basal	7·5	2·0	0·6	26·4	0·8	9·9	9·7	12·4
Week 4	Basal	21·1	4·5	1·4	44·8^{b,c}	0·8^{b}	17·9	13·8^{b}	22·7
	GBA	21·5	3·9	1·2	35·0^{c}	0·8^{b}	20·2	10·6^{b}	19·6
	FOS	17·5	2·6	1·2	47·9^{b,c}	1·3^{b}	13·8	14·9^{b}	24·6
	TOS	19·1	3·9	1·4	58·5^{a,b}	3·0^{a,b}	20·7	17·3^{a,b}	27·7
	MOS	26·9	5·2	1·7	68·4^{a}	4·4^{a}	23·0	23·1^{a}	30·3
$P > F$		0·254	0·507	0·135	0·009	0·015	0·521	0·005	0·167
PSE		1·33	0·48	0·08	2·78	0·36	1·72	0·92	1·41
Week 8	Basal	11·9	2·7	0·7	41·6	0·9	12·2	12·4	18·4
	GBA	12·6	2·7	0·7	42·0	0·5	13·8	9·7	16·3
	FOS	12·7	2·5	0·8	35·4	0·9	13·5	9·8	18·3
	TOS	18·8	4·6	1·3	55·5	1·1	12·7	15·7	28·5
	MOS	12·5	3·0	0·8	43·4	1·0	14·6	13·4	15·8
$P > F$		0·448	0·482	0·193	0·599	0·685	0·974	0·367	0·257
PSE		1·38	0·40	0·09	3·93	0·13	1·20	1·07	1·94

GBA, GroBiotic®-A; FOS, fructo-oligosaccharides; TOS, transgalacto-oligosaccharides; MOS, mannanoligosaccharides; PSE, pooled standard error.
^{a,b,c} Mean values within a column with unlike superscript letters were significantly different at week 4 or 8 ($P<0·05$; Duncan's multiple range test).
* Specific activity/mg soluble protein.

Table 5. Digestive enzyme activities of hybrid striped bass at 4 and 8 weeks
(Mean values with their pooled standard errors)

	Diet	Pepsin (Units)*	Trypsin (μUnits)	Chymotrypsin (mUnits)	Aminopeptidase (Units)	α-Amylase (Units)	Lipase (Units)	Alkaline phosphatase (Units)	Acid phosphatase (Units)
Initial	Basal	21·3	0·5	0·5	0·01	0·1	18·2	0·02	29·0
Week 4	Basal	25·6	7·9	1·4	0·05	6·5	31·6	0·13	53·0
	GBA-1 %	22·1	5·9	1·8	0·05	6·9	33·8	0·14	50·6
	GBA-2 %	20·1	6·7	1·3	0·05	5·0	31·4	0·10	47·5
$P > F$		0·192	0·663	0·094	0·460	0·356	0·582	0·079	0·389
PSE		1·86	1·49	0·13	0·004	0·88	0·88	0·01	2·62
Week 8	Basal	16·0	1·1	0·7	0·01	0·9[a]	20·5	0·01	35·9
	GBA-1 %	14·3	0·9	0·5	0·01	0·1[b]	21·3	0·01	32·0
	GBA-2 %	13·8	1·0	0·6	0·02	0·1[b]	22·8	0·01	34·1
$P > F$		0·666	0·939	0·635	0·239	0·044	0·942	0·956	0·822
PSE		1·72	0·25	0·13	0·002	0·21	4·67	0·60	4·30

GBA, GroBiotic®-A; PSE, pooled standard error.
[a,b] Mean values within a column with unlike superscript letters were significantly different at week 4 or 8 ($P<0·05$; Duncan's multiple range test).
* Specific activity/mg soluble protein.

bream (*Sparus aurata*) revealed that dietary MOS had no effect on villous morphology. However, morphological examination of MOS-treated fish indicated improvements in the absorptive area of the posterior intestine[18]. Also, no effects on intestinal structure were observed in either hybrid tilapia (*Oreochromis niloticus* × *O. aureus*)[15] or Gulf of Mexico sturgeon (*Acipenser oxyrinchus*)[24]. The previously listed evidence points to obvious differences in the effects that dietary prebiotic supplementation has on GIT morphological features and these differences appear to be species-specific.

There are very few scientific reports on the intestinal effects available for the additional prebiotic products evaluated in the present experiment[21]. Only one study has evaluated FOS and TOS in addition to MOS and reported increased microvillus height in juvenile red drum[19]. In the present study, similar effects, in addition to longer mucosal folds, were observed for TOS and MOS. However, contrasting results were observed with FOS, where dietary inclusion had a detrimental effect on the red drum intestine in the present experiment.

GBA, a prebiotic proven to increase nutrient digestibility of red drum[23], seemed to have a tendency to affect more the posterior intestine of red rum and hybrid striped bass after 8 weeks of feeding rather than the anterior section. Similar results with MOS have been reported for gilthead sea bream[18] where supplementation for 9 weeks tended to have an effect on the posterior intestinal folds and microvillus height in both anterior and posterior intestine. Interestingly, increasing the inclusion level of GBA to 2% elicited more rapid effects and of greater magnitude on intestinal structures in hybrid striped bass. However, these effects were transient, more accentuated and only significant at week 4 but not at week 8.

Further research is needed to elucidate whether the normal development of the GI tract in fish may have overshadowed possible prebiotic effects at week 8. This could help explain, in part, the observed transitory nature of these effects. It is also possible that prebiotic supplementation may have accelerated gut development, as indicated by the histological assessment at week 4. This finding may coincide with a previous study with MOS in larval cobia that suggests that MOS may drive the gut to develop more rapidly as evidenced by longer microvilli in treated fish[33].

The results from the present experiment, together with the enhanced growth observed in previous studies[3,13,14,19], appear to correlate better with an improved nutrient absorption due to enhanced intestinal features than with possible increases in the activities of the evaluated digestive enzymes.

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