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Intestinal histomorphology, autochthonous microbiota and growth performance of the oscar (*Astronotus ocellatus* Agassiz, 1831) following dietary administration of xylooligosaccharide

By S. H. Hoseinifar[1], M. Khalili[2] and Y.-Z. Sun[3]

[1]*Department of Fisheries, Faculty of Fisheries and Environmental Sciences, Gorgan University of Agricultural Sciences and Natural Resources, Gorgan, Iran;* [2]*Medical Cellular and Molecular Research Center, Golestan University of Medical Sciences, Gorgan, Iran;* [3]*Xiamen Key Laboratory for Feed Quality Testing and Safety Evaluation, Fisheries College, Jimei University, Xiamen, China*

Summary

The present study investigates the changes in intestinal histomorphology, autochthonous microbiota and growth performance of the oscar, *Astronotus ocellatus*, following dietary administration of different levels of xylooligosaccharide (XOS). One hundred forty-four oscars (8.88 ± 0.23 g; n = 144) were randomly stocked in 12 aquaria (100-L) assigned to four treatments repeated in triplicate. Fish were fed a commercial diet, Biomar, supplemented with different levels (0 [control], 0.5, 1, 2%) of XOS for 8 weeks. Treatments were investigated under static aerated water conditions with a 70% daily water exchange. Evaluation of intestinal histomorphology (villus height, enterocytes height and thickness of the tunica muscularis) revealed no significant differences between XOS-fed groups and the control treatment ($P > 0.05$). However, administration of XOS in the oscar diet increased the total autochthonous intestinal heterotrophic bacteria significantly ($P < 0.05$). Autochthonous lactic acid bacteria levels were also significantly elevated in XOS-fed groups ($P < 0.05$). Furthermore, dietary XOS remarkably increased growth performance (control: 22.76 ± 2.79, 2% XOS: 29.13 ± 2. 8; n = 12) parameters of the oscar ($P < 0.05$). These results demonstrated the beneficial effects of XOS on the growth performance and intestinal microbiota of *A. ocellatus*.

Introduction

Elevating resistance to disease and stress are important aims in ornamental fish aquaculture. Recently, functional dietary supplements such as pre-, pro- and synbiotics have been suggested and applied in aquaculture with several advantages such as minimizing or avoiding use of antibiotics as well as increasing the production rate by enhancing growth performance and improving the immune response (De et al., 2014; Song et al., 2014). Prebiotics, "a selectively fermented ingredient that allows specific changes, both in the composition and/or activity in the gastrointestinal microflora that confers benefits upon host well-being and health" (Gibson, 2004) are emerging dietary supplements that are receiving increased attention as beneficial, safe and environmentally friendly applications in aquaculture (Ringø et al., 2010, 2014; Daniels and Hoseinifar, 2014). Despite several studies regarding determination of the effects of prebiotics and probiotics on various fish species (Carnevali et al., 2014; Hoseinifar et al., 2016; Ringø et al., 2014), limited information is available regarding their use in ornamental fish (Kiron, 2012; Daniels and Hoseinifar, 2014; De et al., 2014; Ringø et al., 2014). The prebiotic xylooligosaccharide has been shown to improve human health through promotion of potentially beneficial bacteria proliferation (e.g. lactic acid bacteria) in the human intestine (Chapla et al., 2012). Moreover, recent studies revealed beneficial effects of XOS on growth performance, immune response, digestive enzyme activity and disease resistance in several fish species (Xu et al., 2009; Hoseinifar et al., 2014, 2015; Guerreiro et al., 2015). Although the exact mode of prebiotic action remains to be clarified, it has been suggested that XOS and other prebiotics can have a beneficial effect on the fish growth performance and health status through modulation of intestinal microbiota toward potentially beneficial communities (e.g. LAB).

Because of their color diversity cichlids are common ornamental fish (Maan and Sefc, 2013). The oscar *Astronotus ocellatus* is a popular ornamental fish; therefore improving its growth performance and farming efficiency is important to farmers (Firouzbakhsh et al., 2011). To lessen the research gap on the effects of XOS on ornamental *A. ocellatus*, the present study was designed to assess its effects on the intestinal histomorphology, autochthonous microbiota and growth performance of this species.

Materials and methods

Fish culture and experimental conditions

Oscar fish were purchased from a private ornamental fish centre in Gorgan, Golestan Province, Iran, 144 *Astronotus ocellatus* (8.8 ± 0.2 g; n = 30) were acclimated for 2 weeks to rearing conditions in four 300-L tanks, under static aerated water conditions with a daily exchange rate of 70%. After acclimation, fish were randomly stocked in twelve 100-

L aquaria assigned to four treatments repeated in triplicate. Water quality parameters in each aquarium including water temperature, dissolved oxygen and pH, were monitored daily using a portable instrument (WTW, Munich, Germany) and recorded as 27 ± 0.5°C, 6.9 ± 0.2, 7.38 ± 0.15, respectively.

Prebiotic and experimental diets

Xylooligosaccharide (XOS) (Longlive 95p, China) used as a supplement in this study was kindly provided by the Shandog Longlive Bio-technology Company. The commercial diets (Biomar, France) were supplemented with different levels of XOS (0 [control], 0.5, 1, 2%) according to the method suggested by Merrifield et al. (2011). The diets (Table 1) were stored in plastic bags at 4°C until used. Fish were fed twice daily (3% of body weight) during the 8-week experimental trials. Treatments were investigated under static aerated water conditions with a daily 70% water change.

Intestinal histomorphology

At the end of the 8-week feeding trial intestinal samples were taken from three fish per tank, fixed in 10% buffered formalin for 48 h and then processed following the conventional histological methods for light microscopy, whereby 5 μ sections were obtained with a microtome (Leica™ 820.Nussloch, Germany), rehydrated in a 70% ethanol solution and stained with hematoxylin-eosinon. Distal intestinal villus height, enterocytes height and thickness of the tunica muscularis were measured using a graded ocular lens.

Intestinal microbiota analysis

At the beginning and end of the 8-week feeding trial, the total viable heterotrophic aerobic bacteria (TAC) and lactic acid bacteria (LAB) levels were determined. Briefly, for culture-based analysis of intestinal microbiota, three samples of intestine were pooled and 100 μl of the homogenate intestines in triplicate were spread onto plate count agar (PCA) (Merck, Germany) and deMan, Rogosa and Sharpe (MRS) agar media (Merck, Germany) for determination of total viable heterotrophic aerobic bacteria and lactic acid bacteria, respectively. The plates were then incubated at 25°C (room temperature) for 5 days (Mahious et al., 2006) and colony forming units (CFU) g^{-1} were calculated from statistically viable plates (i.e. plates containing 30-300 colonies) (Rawling et al., 2009).

Table 1
Composition of the basal diet used throughout the experiment

Proximate analysis (%)	
Dry matter	93.6
Crude protein	38.9
Crude lipid	24.0
Ash	5
Gross energy (MJ kg^{-1})	23.1

Growth performance

Growth performance and survival rate of fish were calculated at the end of the 8-week feeding trial using the formula:

$$\text{Weight gain (WG, g)} = \text{Wt (g)} - \text{Wi (g)}$$

$$\text{Specific growth rate (SGR, \% day}^{-1}) = 100 \times (\text{LnWt} - \text{LnWi})/t$$

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

$$\text{Feed conversion ratio (FCR)} = \text{dry feed intake (g)}/\text{weight gain (g)}$$

$$\text{Survival (\%)} = 100 \times (\text{final amount of fish})/(\text{initial amount of fish})$$

where Wt is final body weight (g), W is initial body weight (g); t is experimental duration in days.

Statistical analysis

For data analysis One-way analysis of Variance (ANOVA) was used followed by the Duncan test after checking the normality and homogeneity of variance. All statistical analyses were conducted using SPSS software 16.0 (SPSS Inc., Chicago, IL).

Results

A study of the intestinal histomorphology (Table 2; Fig. 1) showed normalcy in all experimental groups. Evaluation of some intestinal histomorphological parameters such as villus and enterocyte height as well as tunica muscularis thickness showed no significant differences among treatments ($P > 0.05$). Also, no alterations as a consequence of administering XOS in the diet were observed in the *A. ocellatus* intestine (Table 2).

The results of total intestinal autochthonous bacteria (TAC) and autochthonous lactic acid bacteria (LAB)

Table 2
Morphology of distal intestine of the oscar (*Astronotus ocellatus*) (n = 9, three fish per replicate) fed diets containing different levels (0, 0.5, 1 and 2%) of dietary xylooligosacchride (XOS) for 8 weeks (cultured at 27 ± 0.5°C). Values presented as mean ± SE

	Distal intestine morphology[a]		
XOS level (%)	Villus height (μm)	Enterocytes height (μm)	Tunica muscularis thickness (μm)
Control (0%)	64.86 ± 3.55	4.33 ± 0.23	3.44 ± 0.30
0.5	66.01 ± 2.71	4.38 ± 0.12	3.40 ± 0.39
1	65.33 ± 1.25	4.47 ± 0.36	3.39 ± 0.21
2	68.77 ± 2.07	4.71 ± 0.24	3.60 ± 0.37

[a] 5μ sections obtained with a microtome (Leica™ 820.Nussloch, Germany), prepared and stained with hematoxylin-eosinon.

Fig. 1. Cross section (5 μ) photomicrograph of *Astronotus ocellatus* intestine morphology at end of 8-week feeding trial (scale bar = 10 μ). Distal sections of intestine were sampled and prepared based on the protocol of conventional histology and the slides stained with hematoxylin-eosinon

levels (Log CFU g^{-1}) after 8-week feeding with different levels of dietary XOS are presented in Figs 2 and 3. Oscar fed a XOS-supplemented diet showed significantly higher TAC compared to fish fed the control ($P < 0.05$). There were no significant differences between different prebiotic groups in the TAC ($P > 0.05$). The LAB levels in the intestine of the oscar increased significantly with enhanced levels of XOS in the diets ($P < 0.05$); the highest LAB levels were observed in fish fed the diet supplemented with 2% XOS.

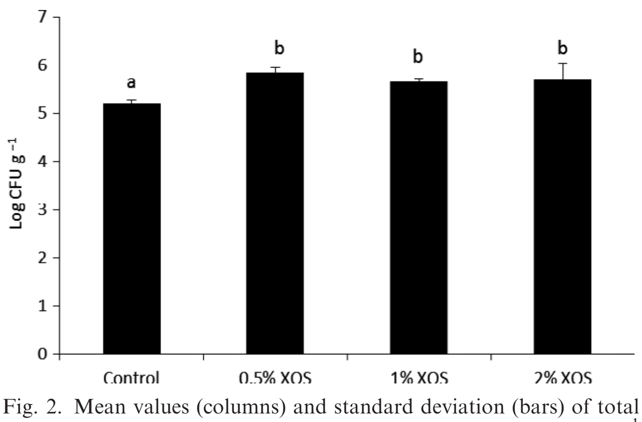

Fig. 2. Mean values (columns) and standard deviation (bars) of total viable heterotrophic aerobic bacteria (TAC) levels (log CFU g^{-1} intestine) (n = 9, three fish per replicate) of the Oscar *Astronotus ocellatus* fed different levels [0% (control), 0.5, 1 and 2%] of dietary xylooligosaccharide (XOS) for 8 weeks (cultured at 27 ± 0.5°C). Samples were spread onto plate count agar (PCA) and incubated at 25°C (room temp) for 5 days. Bars with different superscripts = significantly different ($P < 0.05$); Values are presented as the mean ± SE

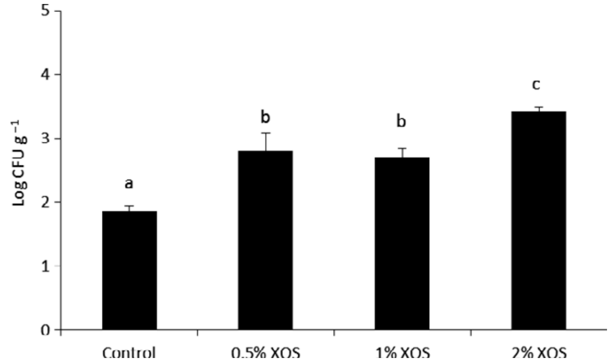

Fig. 3. Mean values (columns) and standard deviation (bars) of the autochthonous lactic acid bacteria (LAB) levels (log CFU g^{-1} intestine) (n = 9, three fish per replicate) of the oscar (*Astronotus ocellatus*) fed different levels [0% (control), 0.5, 1 and 2%] of dietary xylooligosaccharide (XOS) for 8 weeks (cultured at 27 ± 0.5°C). Samples were spread onto deMan, Rogosa and Sharpe (MRS) agar media and incubated at 25°C (room temp) for 5 days. Bars with different superscripts = significantly different ($P < 0.05$); Values presented as mean ± SE

Table 3 represents the effects of different levels of dietary XOS on growth performance parameters, diet utilization and survival rate of the oscar. Administration of XOS in the diet significantly increased weight gain, final length, SGR and remarkably decreased FCR ($P < 0.05$). However, the survival rate was not affected by different dietary treatments and no mortality was observed during the feeding trial.

Discussion

The present study results revealed no remarkable changes in intestinal histomorphology of *A. ocellatus* after 8 weeks feeding on XOS-supplemented diets. The administration of different levels of XOS (1, 2 and 3%) to *Rutilus kutum* fry caused no significant effects on distal intestinal morphology (Hoseinifar et al., 2014). Similar results have been reported following administration of mannanoligosaccharides (MOS) in diets of European sea bass (*Dicentranchus labrax*) (Torrecillas et al., 2007) and Gulf sturgeon (*Acipenser oxyrinchus desotoi*). However, it has been suggested that microbial fermentation of prebiotics and subsequent production of short chain fatty acids beneficially affect gut histomorphology. For instance, prebiotics improved the intestinal morphology of the red drum (*Sciaenops ocellatus*) (Zhou et al., 2010), European sea bass (*Dicentranchus labrax*) (Torrecillas et al., 2011) and rainbow trout (Heidarieh et al., 2013). Reasons for differences in the results of different studies are unclear and merit future research.

Several studies reported that the gut microbiota of fish could be modulated toward potentially beneficial groups (e.g. LAB) through administration of dietary supplements such as probiotics, prebiotics and synbiotics (Ringø et al., 2014). The study of intestinal microbiota in the present study revealed significant elevation of TAC and LAB levels in intestine of XOS-fed *A. ocellatus* (Figs 1 and 2). To the best of our knowledge there is limited information regarding possible effects of XOS as a prebiotic in gut microbiota. In agreement

Table 3
Growth performance and feed utilisation of the oscar (*Astronotus ocellatus*) (n = 12) fed experimental diets at different levels (0, 0.5, 1 and 2%) of dietary xylooligosacchride (XOS) for 8 weeks (cultured at 27 ± 0.5°C). Values presented as mean ± SE

	Experimental diets			
	Control (0% XOS)	0.5% XOS	1% XOS	2% XOS
Initial weight (g)	8.79 ± 0.39^{a}	8.70 ± 0.38^{a}	9.03 ± 0.41^{a}	9.0 ± 0.45^{a}
Initial length (cm)	7.39 ± 0.28^{a}	7.35 ± 0.34^{a}	7.82 ± 0.39^{a}	7.82 ± 0.48^{a}
Final weight (g)	22.76 ± 2.79^{a}	24.01 ± 0.98^{ab}	26.27 ± 3.56^{bc}	29.13 ± 2.8^{c}
Final length (cm)	10.72 ± 0.11^{a}	10.67 ± 0.52^{a}	10.80 ± 0.19^{ab}	11.35 ± 0.67^{b}
WG (g)	161.98 ± 7.87^{a}	173.58 ± 5.34^{ab}	190.96 ± 11.80^{b}	223.72 ± 11.75^{c}
SGR	2.47 ± 0.09^{a}	2.52 ± 0.12^{b}	2.57 ± 0.10^{b}	2.59 ± 0.11^{b}
FCR	1.75 ± 0.21^{a}	1.53 ± 0.36^{b}	1.44 ± 0.44^{c}	1.23 ± 0.26^{d}
Survival (%)	100	100	100	100

Values in a row with different superscripts = significant differences ($P < 0.05$).

with the findings in this study, dietary administration of 1, 2 and 3% XOS in the Caspian white fish (*Rutilus kutum*) diet caused remarkable elevations of TAC and LAB (Hoseinifar et al., 2014). Furthermore, Geraylou et al. (2013) reported a significant increase of TAC and LAB abundance in the intestine of juvenile Siberian sturgeon (*Acipenser baerii*) following administration of 2% arabinoxylan oligosaccharides (AXOS). An increase in TAC and LAB levels could be due to the use of GOS, because several species of LAB are known to be able to utilize oligosaccharides (Kaplan and Hutkins, 2000; Orrhage et al., 2000; Roller et al., 2004; Ignatova et al., 2009; Ringø et al., 2014).

Evaluation of XOS-fed growth parameters in *A. ocellatus* revealed improved growth performance and diet utilization. However, this observation was not parallel to improvements in intestinal histomorphology. The effects of XOS on growth performance of some fish species have been studied (Li et al., 2008; Xu et al., 2009; Wang et al., 2010; Hoseinifar et al., 2014). The improved growth and feed utilization observed in the present study was similar to that reported for crucian carp (*Carassius auratus gibelio*) fed 50 or100 mg kg^{-1} XOS, as well as for juvenile turbot (*Scophthalmus maximus* L.) fed 0.4 g kg^{-1} XOS. In contrast, administration of 1, 2 and 3% XOS in Caspian white fish (*Rutilus kutum*) fry caused no remarkable effects on growth parameters (Hoseinifar et al., 2014). Such contradictory results are not a case just for XOS, but have been reported in several other prebiotics (Ringø et al., 2014). It is speculated that the administration route, dosage, intestinal microbiota, and fish species are main factors that can affect efficiency of prebiotics on growth performance. However, the observed growth improvement is circumstantial and not necessarily causative. Determination of the exact mode of action of prebiotic on growth performance therefore merits further research.

In summary, 2% dietary XOS can improve growth performance and modulate autochthonous intestinal bacteria toward potentially beneficial communities (i.e. LAB) in *A. ocellatus*. XOS is a cost-effective dietary supplement (US \$5.20–8.50 kg^{-1}) and will not increase feed costs. In the present study we did not determine the XOS health promotion properties that are of high importance in ornamental fish. Determination of other aspects of XOS administration in ornamental fish aquaculture, such as the possible effect on immune responses as well as mechanisms of action, merits further research. Furthermore, evaluation of intestinal microbiota using molecular approaches (i.e. DGGE) should also be considered.

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Author's address: Seyed Hossein Hoseinifar, Department of Fisheries, Faculty of Fisheries and Environmental Sciences, Gorgan University of Agricultural Sciences and Natural Resources, Gorgan, Iran.
E-mail: hoseinifar@gau.ac.ir