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Effects of dietary xylan on growth performance, digestive enzyme activity and intestinal morphology of juvenile turbot (*Scophthalmus maximus* L.)

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Abstract

A 12-week feeding trial was conducted to evaluate the effects of dietary xylan on growth performance, digestive enzyme activity, and intestinal morphology of juvenile turbot (*Scophthalmus maximus* L.) with a mean initial body weight of 4.63 ± 0.01 g. Five isonitrogenous and isolipidic diets were formulated to contain 0%, 0.625%, 1.25%, 2.5% and 5% xylan, respectively. The dietary supplementation of 5% xylan significantly decreased ($P<0.05$) fish feed intake, growth performance and feed utilization, but these parameters were significantly improved ($P<0.05$) by 1.25% dietary xylan supplement. Similar trends were observed in whole-body protein and lipid contents of experimental fish. The activity of intestinal caseinolytic, trypsin, and intestinal amylase were inversely related to the supplemented dietary xylan ($P<0.05$). The integrity of the distal intestine was impaired and the length of intestinal epithelium (lIE) significantly declined ($P<0.05$) when 5% xylan was added to the diet. Results of the present study suggest that dietary xylan affected the growth performance and feed utilization of juvenile turbot, with beneficial effects at an intermediate supplemental level of 1.25% but with adverse effects at higher supplemental levels (5%).

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Introduction

With the rapid expansion of aquaculture, the receding supply and high cost of fish meal have forced feed manufacturers to use less expensive plant ingredients as alternatives to fish meal (Ogunji and Wirth, 2001; Sevgili et al., 2011; Olaifa and Bello, 2011). However, high levels of plant ingredients added to fish feed have depressed growth performance and feed utilization in fish (Francis et al., 2001; Ogunji and Wirth, 2001; Olaifa and Bello, 2011; Sevgili et al., 2011). It has been widely demonstrated that the anti-nutritional factors (ANFs) are important factors leading to depressed growth performance, and nutrient utilization in fish (Francis et al., 2001; Slawski et al., 2010; Olaifa and Bello, 2011; Sinha et al., 2011).

Non-starch polysaccharides (NSPs) are generally considered important ANFs in plant ingredients (Ebringerová & Heinze, 2000; Choct et al., 2010; Olaifa and Bello, 2011; Sinha et al., 2011). It has been reported that NSPs in diets could negatively influence growth performance and nutrient utilization in cultured fish such as rainbow trout (*Salmo gairdneri*) (Storebakken, 1985) and Nile tilapia (*Oreochromis niloticus*) (Amirkolaie et al., 2005). The elimination of NSPs from feeds improved growth performance of Japanese seabass (*Lateolabrax japonicus*) (Ai et al., 2007). However, studies have indicated that NSPs do not affect growth performance of African catfish (*Clarias gariepinus*) (Leenhouwers et al., 2006)and the addition of NSP to diets improved growth performance of red sea bream (*Chrysophrys major*) (Morita et al. 1982).

Xylan is one of the many NSPs in plant ingredients and is considered the second most abundant biopolymer in the plant kingdom (Ebringerová & Heinze, 2000; Choct et al., 2010). It is made up of β-(1-4) linked xylopyranose units as the backbone, usually with attached sugar units and O-acetyl groups (Ebringerová & Heinze, 2000). Xylan exists in many plant products and its content in soybean meal can be as high as 5.36%±0.49% (Zhang et al., 2007). Studies on the effects of xylan on animals have mainly focused on terrestrial animals. The addition of xylan in diets has been found to suppress growth performance in chickens. The removal of the endogenous plant xylan, by the addition of specific xylanase in diets, could improve animal growth performance (Choct et al., 1996). It has also been reported that the supplementation of xylan in diets improved the growth performance of pigs (Morel et al., 2001).

Little is known about the influence of xylan on fish. Turbot (*Scophthalmus maximus* L.), a marine flatfish of high economic value, has been extensively farmed in China since the 1990s. The present study was aimed to investigate the effects of dietary xylan on growth performance, feed utilization, digestive enzyme activity, and intestinal morphology of juvenile turbot.

Materials and methods

Experimental diets. The basal diet (control diet) was formulated to contain 50% (dry matter, DM) crude protein and 10.5% (DM) crude lipid (Table 1)

Table 1. Formulation and proximate composition of the experimental diets (g/ kg dry matter).

Ingredients	Control	X-0.625	X-1.25	X-2.5	X-5
Fish meal [a]	670.0	670.0	670.0	670.0	670.0
α-Starch [a]	160.0	160.0	160.0	160.0	160.0
Menhaden fish oil [a]	35.0	35.0	35.0	35.0	35.0
Soybean lecithin	5.0	5.0	5.0	5.0	5.0
Choline chloride	3.0	3.0	3.0	3.0	3.0
Vitamin premix [b]	10.0	10.0	10.0	10.0	10.0
Mineral premix [c]	5.0	5.0	5.0	5.0	5.0
$Ca(H_2PO_3)_2$	5.0	5.0	5.0	5.0	5.0
Y_2O_3	0.5	0.5	0.5	0.5	0.5
Xylan [d]	0.0	7.4	14.7	29.4	58.8
Microcrystalline	106.5	99.2	91.8	77.1	47.7
Analyzed nutrients compositions (dry matter basis)					
	Control	X-0.625	X-1.25	X-2.5	X-5
Crude protein	484.5	491.4	487.2	487.1	486.9
Crude lipid	94.0	94.1	94.1	99.4	92.6
Ash	84.1	82.6	84.7	81.3	79.8

[a] Fish meal, α-Starch and menhaden fish oil were obtained from Great Seven Bio-tech (Shandong, China). Fish meal: crude protein 74% dry matter, crude lipid 9.7% dry matter.

[b] Vitamin premix (mg kg^{-1} diet): thiamin, 25; riboflavin (80%), 45; pyridoxine HCl, 20; vitamin B_{12}, 10; vitamin K_3, 10; inositol, 800; pantothenic acid, 60; niacin acid, 200; folic acid, 20; biotin (2%), 60; retinyl acetate, 32; cholecalciferol, 5; α-tocopherol, 240; ethoxyquin 3; ascorbic acid 2000; microcrystalline cellulose, 6470.

[c] Mineral premix (mg kg^{-1} diet): $MgSO_4·7H_2O$, 1200; $CuSO_4·5H_2O$, 10; $FeSO_4·H_2O$, 80; $ZnSO_4·H_2O$, 50; $MnSO_4·H_2O$, 45; $CoCl_2·6H_2O$ (1%), 50; $Ca(IO_3)_2$ (1%), 60; Na_2SeO_3 (1%), 20; zeolite, 3485.

[d] 85% xylan and 12% moisture, Shanghai Hualan Chemical Co.

Xylan (85% xylan, 12% moisture, Hualan Chemical Co. Ltd, Shanghai, China) was added to the basal diet to obtain graded levels of dietary xylan, at a levels of 0 (control), 0.625% (X-0.625), 1.25% (X-1.25), 2.5% (X-2.5), 5% (X-5) of dry matter.

The ingredients were ground into fine powder and sieved through 200 μm mesh. All the ingredients were thoroughly mixed with Menhaden fish oil after which water was added to produce stiff dough. The dough was pelleted with a screw extruder [F-26 (II), South China University of Technology, China] through a 4.5 mm die. The moist pellets were dried for 8 h in a ventilated oven at 50 °C and were later broken, sieved and pelleted (4.5mm×4.5mm×4.5mm). All the pellets were stored at -20 °C until use.

Feeding trial. Juvenile turbot (*Scophthalmus maximus*) were obtained from a commercial farm in Laizhou, China. Prior to the start of the 12 week feeding trial, fish were acclimated to a commercial diet (Great Seven Bio-Tech Co. Ltd, Qingdao, China) for two weeks and were then fasted for 24 h and then weighed. A total of 450 fish of similar size (initial weight 4.63±0.01 g) were randomly distributed to 15 cylindrical fiberglass tanks in an indoor flow through, rearing system. Each tank (200 l) was stocked with 30 fish, randomly assigned to triplicate tanks.

Throughout the feeding trial, fish were slowly hand-fed to apparent satiation twice daily (7:30 am and 19:30 pm). The uneaten pellets were siphoned out daily and the feed consumption recorded weekly. The number and weight of dead fish were also recorded weekly.

During the feeding period, water temperature was maintained at 15-19 °C, pH 7.5-8.0, and salinity 30-33 g/l. Ammonia nitrogen was lower than 0.4 mg/l, nitrite lower than 0.1 mg/l, and dissolved oxygen higher than 7.0 mg/l. All tanks were continuously aerated.

Sample collection. A sample of twenty fish at the onset of feeding trial and four fish per tank at the end were collected and stored frozen (-20°C) for determination of proximate carcass composition. At the end of the feeding trial, fish were fasted for 24h, anesthetized with eugenol (1:10,000, purity 99%, Shanghai Reagent Corp, China), counted and weighed. Intestine, stomach and hepatopancreas samples for digestive enzyme analysis were taken from another five fish per tank and frozen immediately in liquid nitrogen before being stored at -80 °C. Three more fish from each tank were dissected to extract liver and intestine samples. The body weight, body length, liver weight and visceral weight, of these fish were recorded, and the condition factor, hepatosomatic index, and viscerosomatic index calculated. Rings of tissue from the middle of the distal intestines were sampled (0.5 cm) for histological analysis. The rings were cut-open and rinsed in saline (9 g/l NaCl) to remove remaining gut contents. Bonn's stationary liquid (mixture of saturated water solution of picric acid, 40% formol, and acetic acid at a ratio of 15:5:1) was used to fix the samples for later use.

Composition analysis. Feed ingredients, experimental diets and fish samples were analyzed in duplicates for moisture, crude protein, crude lipid and ash content using standard methods of AOAC (1995). The samples were dried to a stabilized weight at 105 °C to determine moisture levels. Crude protein was determined by measuring nitrogen (N×6.25) using the Kjeldahl method (2300-Auto-analyzer, FOSS, Denmark), crude lipid by ether extraction using Soxhlet method (36680-analyer, BUCHI, Switzerland) and ash by combustion at 550 °C.

Digestive enzyme activity analysis. Pepsin activity was determined colorimetrically according to Anson (1938) with slight modifications. Bovine hemoglobin (Sigma Chemical Co., St. Louis, MO, USA) was used as the substrate. The soluble fraction was determined by Folin-phenol reagent (AppliChem, Darmstadt Germany). The final pH of the reaction system for the analysis of pepsin was 2.0. One unit of protease activity was defined as 1 μg tyrosine released by hydrolyzing bovine hemoglobin for 1 min at 37°C. Enzyme activity was expressed as units per mg tissue protein.

Intestinal caseinolytic activity was determined colorimetrically according to Lowry et al. (1951) with slight modifications. Casein (Sigma Chemical Co., St. Louis, MO, USA) was used as the substrate. The soluble fraction was determined by Folin-phenol reagent. The final pH of the reaction system for the analysis of intestinal caseinolytic activity was 7.5. One unit of protease activity was defined as 1 μg tyrosine released by hydrolyzing casein for 1 min at 37°C. Enzyme activity was expressed as units per mg tissue protein.

Trypsin activity was determined colorimetrically as described by Tseng et al. (1982) with slight modifications. This method used *Na*-benzoyl-arginine-*p*-nitroanilide (BAPNA) (Sigma Chemical Co., St. Louis, MO, USA) as substrate. The final pH of the reaction system for the analysis of trypsin was 8.0. One unit was defined as 1μmol *p*-nitroanilide (PNA) liberated by catalyzing BAPNA for 1 minute at 37 °C. Enzyme activity was expressed as units per g tissue protein.

Intestinal and stomach amylase activities were determined according to the Somogy-Nelson colorimetric method as described by Hidalgo et al. (1999) with slight modifications. This method used starch (Sigma Chemical Co., St. Louis, MO, USA) as substrate. The final pH of the reaction system for the analysis of intestinal and stomach amylase were 7.5 and 2.0 respectively. One unit was defined as the amount of enzyme catalyzing 10mg starch hydrolyzed in 30 minutes at 37 °C. Enzyme activity was expressed as units per mg tissue protein.

Intestine morphology analysis. After fixation, the distal intestines were successively dehydrated in ethanol, equilibrated in xylene, and embedded in paraffin wax according to standard histological procedures (Baeverfjord & Krogdahl, 1996). Then the samples were sliced into 7 μm longitudinal sections following the axis of gut lumen using a Lecia Jung RM 2016 rotary microtome (German) and stained with Hematoxylin-Eosin (H&E). The distal intestine slides were examined under a Nikon eclipse Ti-S microscope (Japan) for lesions as in Baeverfjord & Krogdahl (1996). The intestinal structure (Fig. 1), including the height of the simple fold (hSF), the height of the complex fold (hCF), the total length of the intestinal epithelium over 500 μm distance (lIE), the height of the microvillus (hMV) and the thickness of the muscularis (tM), were chosen as easily assessable markers for the morphological changes of the distal intestine. The macro-morphological parameters were measured by a semi-automatic computer-assisted system as follows: all simple folds and complex folds were measured for hSF and hCF separately and 8, 20, 20 measurements were measured for lIE, hMV, tM respectively per fish. Because of the variable numbers of observations per individual, mean values of simple folds and complex folds per fish were used in the subsequent analyses.
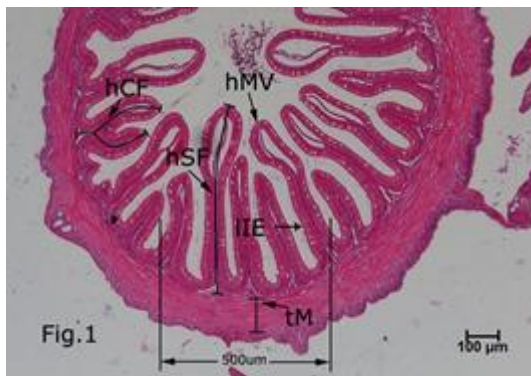

The hSF: the height of the simple fold. The hCF: the height of the complex fold. The lIE: the total length of the intestinal epithelium over 500 μm distance. The hMV: the height of the microvillus estimated (height of the PAS positive stained zone). The tM: the thickness of the muscularis.

Fig. 1 The intestinal structures to be measured.

Calculations. Growth responses were calculated as follow.

Survival rate (SR, %) = $100 \times A_f / A_i$.

Weight gain rate (WGR, %) = $100 \times (W_f - W_i) / W_i$.

Daily growth coefficient (DGC; %/d) = $100 \times (W_f^{1/3} - W_i^{1/3}) /$ days.

Mean metabolic body weight (MBW) = $[(W_i / 1000)^{0.75} + (W_f / 1000)^{0.75}] / 2$.

Feed intake (FI, g/kg MBW per d) = DI / MBW / days.

Feed efficiency rate (FER) = $(W_f - W_i) / DI$.

Protein efficiency ratio (PER) = $(W_f - W_i) / (DI \times FP)$.

Where A_i and A_f are the initial and final number of fish, respectively, W_i and W_f are the initial and final mean body weight of fish (g), separately, DI is the dry diet intake per fish (g, DM/fish), FP is the protein content of feed (%, DM).

Body index was calculated as follows:

Condition factor (CF, %) = $100 \times W_b / L_b^3$.

Hepatosomatic index (HSI, %) = $100 \times W_l / W_b$.

Viscerosomatic index (VSI, %) = $100 \times W_v / W_b$.

Where W_b is the fish body weight (g), W_l and W_v are the liver and viscera weight (g) respectively, L_b is the fish body length (cm).

Statistical analysis. Data from each treatment were subjected to one-way analysis of variance (ANOVA) using SPSS 16.0 for windows. When overall differences are significant ($P < 0.05$), Tukey's test was used to compare the means among individual treatments.

Results

Survival rate, growth performance and body index. No significant difference ($P>0.05$) was observed in the survival rate of fish among all treatments (Table 2).

Table 2. Effects of dietary xylan on survival rate, growth performance, feed utilization and body indexes of juvenile turbot.

Items	Control	X-0.625	X-1.25	X-2.5	X-5
SR[1] %	100±0	99.36±0.64	100±0	100±0	100±0
W_i[2] g	4.63±0.02	4.66±0.02	4.64±0.01	4.63±0.01	4.62±0.01
W_f[3] g	22.39±0.60^b	23.02±0.53^{bc}	25.62±1.22^c	23.05±1.08^{bc}	19.55±0.12^a
WGR[4] %	377.33±16.75^{ab}	397.20±11.44^{bc}	445.64±19.11^c	397.86±22.32^{bc}	323.46±1.92^a
DGC[5] %/d	1.37±0.03^b	1.40±0.02^{bc}	1.52±0.05^c	1.40±0.05^{bc}	1.22±0.01^a
FER[6]	1.13±0.02^b	1.13±0.04^b	1.13±0.02^b	1.08±0.03^{ab}	1.04±0.01^a
FI[7] g/kg MBW per d	5.14±0.06^b	5.19±0.06^{bc}	5.47±0.15^c	5.26±0.05^{bc}	4.87±0.01^a
PER[8]	2.58±0.04^b	2.53±0.05^b	2.54±0.03^b	2.40±0.04^{ab}	2.33±0.01^a
CF[9] %	1.97±0.14	1.88±0.06	1.87±0.04	1.90±0.08	1.89±0.08
HSI[10] %	0.97±0.03	0.97±0.17	1.17±0.11	1.19±0.13	1.14±0.19
VSI[11] %	4.60±0.11	4.75±0.29	4.56±0.10	4.83±0.12	4.62±0.17

Values are means±S.E.(n=3) of three replicates. Values within the same row with different letters are significantly different ($P<0.05$).

[1] Survival rate (SR, %) = 100 × (final amount of fish) / (initial amount of fish).

[2] W_i is the initial mean body weight of fish (g).

[3] W_f is the final mean body weight of fish (g).

[4] Weight gain rate (WGR, %) = 100 × (W_f - W_i) / W_i.

[5] Daily growth coefficient (DGC, % d^{-1}) = 100 × ($W_f^{1/3}$-$W_i^{1/3}$) / days.

[6] Feed efficiency rate (FER) = (W_f - W_i) / DI; DI is the dry diet intake per fish (g, DM $fish^{-1}$).

[7] Feed intake (FI, g/kg MBW per d) = DI / MBW / days; Mean metabolic body weight (MBW) = [(W_i / 1000)$^{0.75}$ + (W_f / 1000)$^{0.75}$] / 2.

[8] Protein efficiency ratio (PER) = (W_f - W_i) / (DI × FP).

[9] Condition factor (CF, %) = 100 × W_b / L_b^3; W_b is the fish body weight (g); L_b is the fish body length (cm).

[10] Hepatosomatic index (HSI, %) = 100 × W_l / W_b; W_l is the liver weight (g).

[11] Viscerosomatic index (VSI, %) = 100 × W_v / W_b; W_v is the viscera weight (g).

Compared to the fish in the control group, the fish in X-5 treatment showed a significantly lower final mean body weight (W_f), daily growth coefficient (DGC), feed intake (FI), feed efficiency rate (FER) and protein efficiency ratio (PER) ($P<0.05$) whereas fish in X-1.25 treatment showed significantly higher W_f, weight gain rate (WGR), DGC and FI (P<0.05). No significant difference ($P>0.05$) was observed in condition factor (CF), hepatosomatic index (HSI) and viscerosomatic index (VSI) of fish among all treatments.

Body composition. Compared to the control group, group X-5 showed significantly ($P<0.05$) decreased contents of whole-body protein and lipid whereas group X-1.25 showed significantly increased ($P<0.05$) whole-body lipid content (Table 3).

Table 3. Effects of dietary xylan on whole-body compositions of juvenile turbot.

Items	Control	X-0.625	X-1.25	X-2.5	X-5
Moisture content %	75.91±0.10	76.10±0.25	75.36±0.23	76.87±0.34	77.12±0.09
Protein content %	15.86±0.10^{bc}	16.05±0.23^{bc}	16.11±0.09^c	15.54±0.30^{ab}	15.30±0.06^a
Lipid content %	4.45±0.08^b	4.18±0.04^{ab}	4.82±0.16^c	3.85±0.03^a	3.95±0.10^a
Ash content %	3.80±0.08	3.68±0.03	3.72±0.07	3.74±0.01	3.63±0.04

Values are means ± S. E. (n=3) of three replicates and values within the same row with different letters are significantly different ($P<0.05$).

There was no significant difference in whole-body moisture or ash levels among all treatments (control, X-0.625, X-1.25, X-2.5 and X-5 treatments) ($P>0.05$).

Digestive enzyme activity. The activities of trypsin and intestinal amylase in group X-5 were significantly lower ($P<0.05$) than those of the control treatment, while the activities of trypsin and intestinal caseinolytic in X-1.25 treatment were significantly higher ($P<0.05$) than those of fish fed with the control diet (Table 4). There was no significant difference ($P>0.05$) in the pepsin and stomach amylase activity among all treatments (control, X-0.625, X-1.25, X-2.5 and X-5 treatments).

Table 4. Effects of dietary xylan on the digestive enzymes activities of juvenile turbot.

Items	Control	X-0.625	X-1.25	X-2.5	X-5
Pepsin U/mg prot.	41.13±6.43	39.60±11.87	72.43±29.86	33.45±5.96	36.50±11.56
Trypsin U/g prot.	14.04±1.30[b]	15.67±1.09[bc]	19.76±0.51[c]	14.28±1.06[b]	9.25±1.55[a]
Intestinal caseinolytic activity U/mg prot.	12.33±1.64[ab]	16.83±2.97[bc]	22.03±4.24[c]	14.19±0.66[ab]	8.73±2.02[a]
Stomach Amylase U/mg prot.	0.25±0.07	0.22±0.09	0.33±0.05	0.21±0.04	0.22±0.04
Intestinal Amylase U/mg prot.	0.27±0.01[bc]	0.18±0.03[abc]	0.28±0.08[c]	0.17±0.02[ab]	0.13±0.03[a]

Values are means ± S. E. (n=3)of three replicates and values within the same row with different letters are significantly different ($P<0.05$).

Distal intestine morphology and morphometry. No lesion or damage was revealed in the distal intestine of fish fed Control, X-0.625 and X-1.25 diets (Fig. 2, 3, 4 respectively).
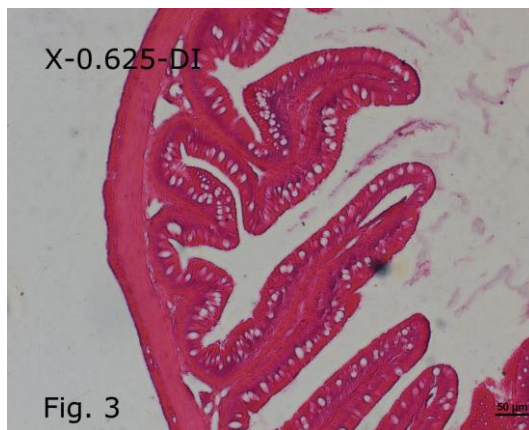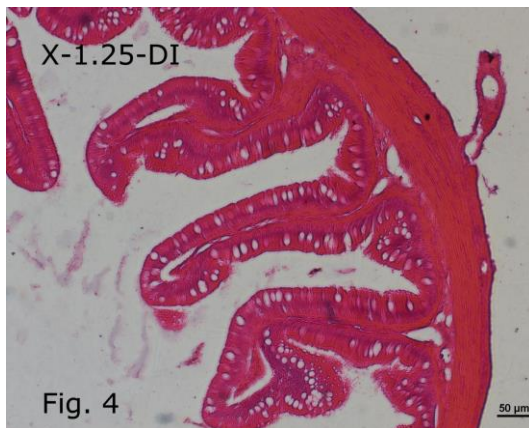

Fig. 2-6 Effects of dietary xylan on the distal intestine morphology of juvenile turbot.
Fig. 2, Control-DI, Distal intestine of fish from Control treatment. Fig. 3, X-0.625-DI, Distal intestine of fish from X-0.625 treatment. Fig. 4, X-1.25-DI, Distal intestine of fish from X-1.25 treatment.

In these groups, the mucosa showed two kinds of folds: simple folds and complex folds characterized by multiple branches, which regularly alternated. The epithelium of the mucosal folds consisted of a single layer of cells which showed various degrees of cytoplasmic supranuclear vacuolation. The nuclei were evenly polarized and basally located. Numerous goblet cells and intraepithelial lymphocytes were scattered among the epithelial cells.. The apical surface of epithelial cells was covered with microvillous border and mucus. The lamina propria and submucosa existed below the mucosa and presented similar width without increased cellularity. However, in group X-5, distal intestine of fish showed obviously impaired integrity of the distal intestine (Fig. 6), including the abnormal development of mucosal folds (mf) and hyperplasia of loose connective tissue (lct) and adipose cells (ac) in the submucosa. Slight variations of these responses were also observed in group X-2.5 (Fig. 5).

Fig. 5, X-2.5-DI, Distal intestine of fish from X-2.5 treatment.

Fig. 6, X-5-DI, Distal intestine of fish from X-5 treatment.
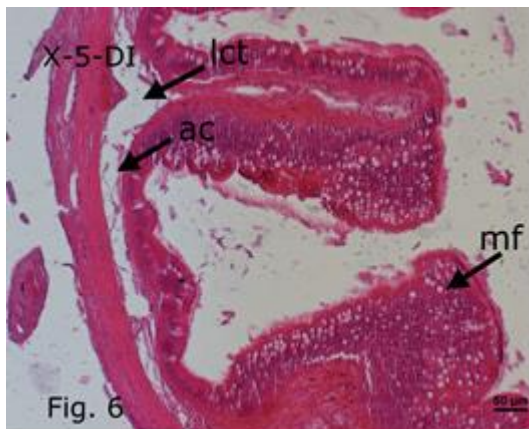
mf: abnormal development of mucosal folds.

Lct: the hyperplasia of loose connective tissue.

ac: the hyperplasia of adipose cells

The total length of intestinal epithelium of 500 μm distance (lIE) in fish fed the X-5 diet was lowest ($P<0.05$) among all treatments (Table 5). The distal intestines of fish fed X-1.25 and X-2.5 showed significantly higher ($P<0.05$) simple folds (hSF) compared to other treatments. Compared to the control treatment, the thickness of muscularis (tM) in the distal intestine of fish fed X-1.25 was significantly higher ($P<0.05$). The height of the complex fold (hCF) or the thickness of muscularis (hMV) in the distal intestine of fish showed no significant ($P>0.05$) differences among all treatments (Control, X-0.625, X-1.25, X-2.5 and X-5 treatments) ($P>0.05$).

Table 5. Effects of dietary xylan on the distal intestine morphometry of juvenile turbot.

Items	Control	X-0.625	X-1.25	X-2.5	X-5
hSF[1] μm	340.27±13.96[a]	336.17±5.71[a]	442.80±15.64[b]	426.83±26.31[b]	315.55±18.88[a]
hCF[2] μm	930.81±120.22	883.89±59.71	943.92±128.55	851.84±65.86	715.05±49.29
lIE[3] μm	2140.33±76.25[b]	2122.94±179.27[b]	2292.94±78.31[b]	2333.29±147.70[b]	1722.88±153.14[a]
hMV[4] μm	3.14±0.18	3.51±0.19	3.55±0.31	3.69±0.16	4.11±0.60
tM[5] μm	63.95±2.66[a]	65.89±5.94[a]	88.36±3.07[b]	76.20±11.90[ab]	67.89±7.41[ab]

Values are means ± S. E. (n=3) of three replicates and values within the same row with different letters are significantly different ($P<0.05$).

[1] The height of the simple fold (hSF, μm).
[2] The height of the complex fold (hCF, μm).
[3] The total length of the intestinal epithelium over 500 μm distance (lIE, μm).
[4] The height of the microvillus (hMV, μm).
[5] The thickness of the muscularis (tM, μm).

Discussion

It has been reported that high levels (≥3%) of dietary xylan could depress growth performance and feed utilization in chickens (Choct et al., 1996). Similar depressions caused by high levels of other NSPs species were also reported in fish species such as rainbow trout (*Salmo gairdneri*) (≥10% guar galactomannan and alginates) (Storebakken, 1985) and Nile tilapia (*Oreochromis niloticus*) (≥8% guar gum) (Amirkolaie et al., 2005). However, there has been no information available on the effects of

purified xylan on fish. Our study has shown that levels of 5% supplemented dietary xylan depressed growth performance and feed utilization of marine flatfish such as juvenile turbot (*Scophthalmus maximus* L.).

Usually, higher NSP levels created viscosity stress in the digesta (Camire et al., 1990), which could damage fish digestive organs and suppress the secretion of digestive enzymes and other digestive components (Sinha et al., 2011). In the present study, a higher level (5%) of dietary xylan depressed the activitiyof fish digestive enzymes. This was consistent with the effects of other
NSPs species on depressing digestive ability of a number of fish species such as rainbow trout (≥2.5% guar gum) (Storebakken, 1985), Nile tilapia (≥8% guar gum) (Amirkolaie et al., 2005) and African catfish (*Clarias gariepinus*) (≥4% guar gum) (Leenhouwers et al., 2006). Higher levels of NSP could also bind a great quantity of digestive enzymes and other digestive components which depressed their interaction with nutrients (Ikegami et al., 1990).

The distal intestine is the main site for microbial degradation of NSPs (Choct et al. 2010) and the salient region for ANF induced enteritis in salmon (*Salmo salar* L.) (Baeverfjord & Krogdahl 1996), as well as being important for nutrient digestion and absorption in animals. In this study, the addition of a higher xylan level (5%) to the diet impaired the integrity of fish distal intestine. Moreover, the morphometric analysis of the distal intestine showed that the superficial area of the intestine was reduced by the addition of higher than 5% dietary xylan. Viscosity stress induced by high levels of NSP in digesta could lead to physical erosion of the intestine (Xu et al., 2003; Choct et al., 2010; Sinha et al., 2011). Furthermore, the physical erosion could result in atrophying of the intestinal folds due to increased cell loss from the villous apex via apoptosis and cell sloughing as well as the reduction of crypt-cell proliferation and cell migration along the crypt-fold axis (Montagne et al., 2003; Sinha et al., 2011). Similar results caused by high levels of other NSPs were also reported in pigs (≥4% carboxymethylcellulose) (McDonald, 2001) and chickens (≥2.5% gum xanthan) (Iji et al., 2001). These responses might further contribute to the depressed growth performance and feed utilization of turbot when 5% xylan was added to the diet. In addition, the viscosity stress could also decrease the passage rate and increase the time of digesta in the intestine, which would delay emptying and prolong satiety after meal (Montagne et al., 2003; Choct et al., 2010; Sinha et al., 2011). These effects could contribute to the lowered feed intake of fish fed 5% dietary xylan in this study.

Despite the negative effects of 5% dietary xylan, a lower level (1.25%) of xylan added to the diet improved fish growth performance and feed utilization in this study. This was consistent with the previous study on pigs (7.5% xylan) (Morel et al., 2001). Similar improvement with other NSP was also reported in sea bream (*Chrysophrys major*) (3%-12% carboxymethylcellulose) (Morita et al., 1982). It has been reported that the addition of appropriate levels of dietary NSPs could moderately increase the viscosity stress of the digesta which could increase the secretion of digestive juices, enzymes and components from digestive organs in fish (Poksay & Schneeman, 1983; Ikegami et al., 1990). This study has shown that the activity of digestive enzymes was improved by the addition of a lower level (1.25%) of dietary xylan, and this might contribute to the improved growth performance and feed utilization of fish. In rats, improvement in digestion with appropriate levels of other NSPs was also reported (10% guar gum) (Poksay & Schneeman, 1983). In addition, intestinal amylase and stomach amylase activity in fish fed all treatments was similar; the reason is unclear and needs further study.

In the present study, no lesions or damage were observed on the intestinal structures of fish fed diets with lower levels (0.625%-1.25%) of dietary xylan. Moreover, increased intestine superficial area was observed in fish fed lower levels (1.25%-2.5%) of dietary xylan. Similar improvements with moderate levels of other NSPs were also reported in chicken (2.5% guar gum and 2.5% gum xanthan) (Iji et al., 2001) and rats (2.5% pectin) (Andoh et al., 1999). These improvements may be due to the fact that moderate levels of dietary NSPs could stimulate the crypt-cell proliferation and cell migrations along the crypt-fold axis, which exceed cell loss.(Montagne et al., 2003). Moreover, the trophic substances of NSP degraded by the intestinal microorganisms, including short chain fatty acid (SCFA) and butyrate, could be utilized by the epithelium to stimulate cell proliferation and improve their development (Montagne et al., 2003). The increased thickness of intestine muscularis, which was observed in fish fed lower levels (1.25%) of dietary xylan, may enhance the intestinal activity and finally improve the feed intake of fish.

Conclusion

In this study, the highest level (5%) of dietary xylan produced negative effects on growth performance, digestive enzyme activity and distal intestinal structure of juvenile turbot, however, a lower level (1.25%) of dietary xylan improved these parameters. This was the first time the effects of xylan were evaluated on growth performance, digestive enzyme activity and intestinal morphology of fish. Considering that dietary purified xylan influenced growth performance and feed utilization of fish in a dose related manner, the amount of xylan integrated into commercial diets should be carefully considered.

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