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Effect of xylan oligosaccharides generated from corncobs on food acceptability, growth performance, haematology and immunological parameters of *Dicentrarchus labrax* fingerlings

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Abstract The objective of this study was to determine the effect of two levels of inclusion of xylan oligosaccharides (XOS) extracted from corncob on growth, feed utilization, immune status and disease resistance of Mediterranean sea bass (*Dicentrarchus labrax*) fingerlings. Specimens of 4.75 ± 0.69 g at initial density of 2.7 ± 0.13 kg/m^3 were fed during 12 weeks at 0 g kg^{-1} diet, 5 g kg^{-1} diet and 10 g kg^{-1} diet, dietary XOS level of inclusion in a commercial sea bass diet. Feeding the fish at both XOS dietary inclusion levels significantly increased weight gain, protein efficiency ratio and feed conversion ratio. Feeding of supplemented diets to fish led to reducing mortalities after challenging with *A. hydrophila*. The haematological and immunological parameters were assayed in both pre-challenged and post-challenged groups. There was an increased trend in red blood corpuscles, white blood corpuscles, pack cell volume, haemoglobin (Hb %) and serum protein content in treated groups over the control as time elapsed with the feeding trials. The serum immunoglobulin level and lysozyme activity showed an increased trend in the fed groups. Histological features of the liver showed lower lipid vacuolization and regular-shaped morphology of hepatocytes around the sinusoidal spaces denoting a better utilization of dietary nutrients supported with the morphometric data. In conclusion, XOS added at a designated dose (5 g kg^{-1} diet) in the diet improves growth and stimulates the immunity and makes *D. labrax* fingerlings more resistant to infection by *A. hydrophila*.

Keywords Xylooligosaccharides · *Dicentrarchus labrax* · Growth performance · Immunology · Liver histology

Abbreviations

XOS	Xylooligosaccharide
PER	Protein efficiency ratio
FCR	Feed conversion ratio
RBC	Red blood corpuscles
WBC	White blood corpuscles
Hb	Haemoglobin
PCV	Pack cell volume

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Introduction

The increasing economical and social concern to decrease the use of antibiotics and other therapeutic chemicals used in fish farming has encouraged more environmentally friendly approaches to disease control (Verschuere et al. 2000). In fact, there is a great interest in the use of different products and organisms to control potential pathogens by competitive exclusion (probiotics and prebiotics), what has been successful in preventing disease outbreaks in other areas of animal production (Corrier et al. 1995; Hansen and Olafsen 1999). A promising alternative approach for controlling fish diseases is the use of probiotics or beneficial bacteria, which control pathogens through a variety of mechanisms. The use of probiotics, in human and animal nutrition, is well documented and recently has been applied to aquaculture. The use of probiotics and prebiotics in fish feeds has a great interest particularly for fortification against infectious diseases (Merrifield et al. 2010) by enhancing the non-specific immune system against potentially insidious disease-causing organisms. For instance, probiotic baker's yeast (*Saccharomyces cerevisiae*) and *Lactobacillus acidophilus* have shown beneficial advantages in aquaculture which have been proved to improve the immune response, growth and fish survival (Al-Dohail et al. 2009; Gopalakannan and Arul 2010).

To date, several studies have been done on administration of fructooligosaccharide, inulin, mannanoligosaccharides (Merrifield et al. 2010; Ringø et al. 2014; Daniels and Hoseinifar 2014) and galactooligosaccharide (Grisdale-Helland et al. 2008; Zhou et al. 2010; Burr et al. 2010; Hoseinifar et al. 2013) as prebiotics in aquaculture and positive results reported on growth performance, feed utilization, immune response and disease resistance of various fish species (Hoseinifar et al. 2013). Xylooligosaccharides (XOS) are xylose-based oligomers which are non-digestible with prebiotics properties that can promote the growth of beneficial bacteria in animal gut microbiota (Crittenden and Playne 2008). Recent studies have proved the efficiency of xylan oligosaccharides (XOS) as they exhibit prebiotic effect when consumed as a part of diet (Hoseinifar et al. 2013; Driss et al. 2014). Thus, the potentiality of XOS is receiving attention in global nutraceuticals market. However, very little scientific information is available on the effects of xylooligosaccharides as prebiotic for fish and shellfish (Li et al. 2008; Xu et al. 2009). This is particularly significant for Mediterranean fingerlings (*Dicentrarchus labrax*), which, despite its importance for Mediterranean aquaculture where it reaches up to 20 % of total fish production, is a species very sensitive to stressors and infections may occasionally cause important losses (Izquierdo 2005). In view of this evidence for other fish species, inclusion of XOS on diets for Mediterranean fingerlings *D. labrax* could improve stress and disease resistance together with growth and feed utilization. Hence, the objective of this study was to evaluate the effect of dietary inclusion of XOS on the rearing performance of Mediterranean fingerling *D. labrax* and its implications in certain immune parameters and resistance to infections.

Materials and methods

Xylooligosaccharide preparation

Xylan was obtained from the alkali extraction of corncob. Powders of corncob were treated with 15 % NaOH (1 M) with a solid–liquid ratio of 1:20 (w/v) for 90 min at 90 °C. The purified xylan-rich hemicelluloses were dissolved in 50 mM citrate phosphate buffer (pH = 3) in 50-mL conical flasks to a concentration of 2 % (w/v). Endoxylanase (PoXyn2) was added at a concentration of 50 U g^{-1}. Hydrolysis was carried out at 50 °C with orbital shaking at 150 rpm for 10 h (Driss et al. 2014).

Preparation of diets

In addition to the basal diet (control diet, 0 g kg^{-1} XOS diet), two experimental diets were prepared by supplementation of the basal diet with different levels (5 and 10 g kg^{-1} diets) of XOS. Diets covered nutritional requirements for this species (Izquierdo 2005) and were manufactured by a commercial feed producer (Graneros de Tenerife, Tenerife, Spain) with the composition indicated in Table 1. All ingredients were mixed mechanically for 30 min, and resulting dough was extruded to obtain 3-mm pellets. The pellets were dried in a convection oven at 60 °C for 24 h and stored in airtight plastic bags until use.

Table 1 Ingredients used and proximate composition of the formulated diets (g kg^{-1}, dry matter basis)

Ingredients	Control	XOS 5 g kg^{-1}	XOS 10 g kg^{-1}
Fish meal[a]	436	436	436
Casein	120	120	120
Soybean oil	17	17	17
Corn starch	272	272	272
Cellulose	85	83	83
Vitamins[b]	20	20	20
Minerals[c]	20	20	20
Carboxyl methyl cellulose (CMC)	30	30	30
Supplement	0	5	10
Moisture	48.6	70.8	70.8
Protein	425.2	428.6	428.6
Lipid	63.4	61.4	61.4
Ash	102.9	102.7	101.7
NFC[d]	408.5	407.3	409.3
GE (MJ/kg)[e]	18.3	18.4	18.43

[a] Danish fish meal

[b] Vitamin mix kg^{-1} (ROVIMIX 6288, Roche Vitamins Ltd., Switzerland): Vit. A 50 million i.u., Vit. D3 10 million i.u., Vit. E 130 g, Vit. B1 10 g, Vit. B2 25 g, Vit. B6 16 g, Vit. B12 100 mg, biotin 500 mg, pantothenic acid 56 g, folic acid 8 g, niacin 200 g, anticake 20 g, antioxidant 200 mg, Vit. K3 10 g and Vit. C 35 g

[c] Mineral mix kg^{-1}: calcium phosphate (monobasic) 397.65 g; calcium lactate 327 g; ferrous sulphate 25 g; magnesium sulphate 137 g; potassium chloride 50 g; sodium chloride 60 g; potassium iodide 150 mg; copper sulphate 780 mg; manganese oxide 800 mg; cobalt carbonate 100 mg; zinc oxide 1.5 g and sodium selenite 20 mg

[d] NFE = Nitrogen-free extract (1000 − (protein + lipid + ash)

[e] GE = Gross energy (measured using bomb calorimeter, Parr 1356 bomb calorimeter)

Experimental fish and husbandry conditions

A total of 700 (*D. labrax*) fingerlings (initial weight 4.75 ± 0.69 g) were collected from a brood of south Tunisia (S.A.T: South Tunisian Aquaculture) from a population which had not suffered apparent diseases. Fish were maintained in stocking tanks and fed a commercial extruded diet for 3 weeks (19–22.1 °C) until being well adapted to the environmental conditions (2.7 kg/m^2 stoking density). Afterwards, 692 sea bass fingerlings were randomly distributed in nine indoor, cylindroconical 1000-L fibre glass tanks at an initial stocking density of 2.7 kg/m^3 (67 fish/tank). Tanks were supplied with drilling water (2.14 L min^{-1}), at a temperature of 19.5–22.6 °C, and natural photoperiod (12L/12D). Water dissolved oxygen was kept at 7.9 ± 0.2 ppm. Fish were manually fed until apparent satiation with one of the three experimental diets for 9 weeks (3 times a day, 7 days a week). Each diet was assayed by triplicate. The weight gain, protein efficiency ratio (PER) and feed conversion ratio (FCR) were determined for each group (Nya and Austin 2009).

Challenge assay

After 8 and 12 weeks of feeding treated diet and a control diet, 10 fish in duplicate from each group were challenged intraperitoneally with a lethal dose of *A. hydrophila* containing 10^7 cells mL^{-1} (Nya and Austin 2009), and mortalities were observed for 2 weeks. Percentage survival was determined using the following formula:

$$\text{Survival rate } (\%) = (\text{Final number of fish survivor}/\text{initial number of fish stocked}) \times 100$$

Blood collection and analyses

The blood samples from pre-challenged and post-challenged groups were collected through a syringe by caudal vein puncture for haematological, biochemical

and immunological assays as described previously (Al-Dohail et al. 2009; Nya and Austin 2009; Talpur and Ikhwanuddin 2013).

Protein assay, total immunoglobulin concentration and lysozyme activity assay

The protein content and total immunoglobulin concentration of blood serum were determined as described previously by Al-Dohail et al. (2009). Serum lysozyme activity was measured by a turbidimetric assay according to the methods described by Shugar (1952). One unit of lysozyme activity was expressed as the amount of enzyme producing a decrease in absorbance of 0.001 min^{-1} mL^{-1} serum.

Histological studies

Liver samples were taken after 12 weeks of the feeding trial. Samples were fixed in 10 % neutral-buffered formalin, embedded in paraffin and stained with haematoxylin and eosin (H&E) for optical examination (Martoja and Martoja-Pierson 1970).

Morphometric evaluation

Morphometric evaluation liver has been carried out according to Weibel et al. (1969), as the ratio between the fish body and the liver weights, expressing the liver somatic index (LSI) as follows:

$$\text{LSI} = \text{Liver weight } (g) \times 100/\text{body weight } (g)$$

Statistical analysis

One-way analysis of variance (ANOVA) was used to scrutinize the data. Multiple comparisons were performed with Duncan's test to analyse the differences between treatment means to be significant at $P < 0.05$ using SPSS software version 17 for Windows.

Results

Growth performance

Feeding of XOS-supplemented diets to *D. Labrax* led to a significantly increased ($P < 0.05$) growth rate (Table 2). The highest weight gain (40.41 ± 0.9 g) was achieved in those fish fed with 5 g kg^{-1} XOS-added diet. Similarly, FCR was significantly ($P < 0.05$) improved in fish treated with 5 g kg^{-1} XOS-supplemented diets over the control diet (Fig. 1) and over the 10 g kg^{-1} XOS-supplemented diets. In addition, PER was significantly better in groups fed supplemented diets except the control.

Disease resistance

Feeding of XOS after 8 and 12 weeks led to a marked decline in fish mortality after challenge with *A. hydrophila*. Thus, the highest 55 % mortality was recorded for the control compared with XOS fed groups (Fig. 2). Significantly highest ($P < 0.05$) survival of fish was recorded in the groups fed with 10 g kg^{-1} XOS

Table 2 Growth performance and nutrient utilization of Tunisian sea bass fingerlings fed commercial extruded diets with different levels of XOS inclusion

	Days of feeding	Control	XOS 5 g kg^{-1} diet	XOS 10 g kg^{-1} diet
Average weight (g)	0	4.75 ± 0.47	4.72 ± 0.56	4.69 ± 0.70
	8	19.63^{a} ± 0.80	26.30^{b} ± 1.56	25.47^{b} ± 0.95
	12	34.17^{a} ± 0.06	40.41^{b} ± 0.90	39.88^{b} ± 0.10
Length (cm)	0	1.48 ± 1.03	1.55 ± 0.92	1.41 ± 0.95
	8	3.76^{a} ± 0.05	4.26^{b} ± 0.05	4.13^{b} ± 0.29
	12	5.16^{a} ± 0.13	7.73^{b} ± 0.15	7.03^{b} ± 0.25

Different letters within a line denote significant differences ($P < 0.05$). Control = 0 g kg^{-1} diet; XOS = 5 g kg^{-1} diet; XOS = 10 g kg^{-1} diet. Values expressed in mean ± SD

Values in the same line indicated by different superscript letters are significantly different ($P < 0.05$) from the control and each other

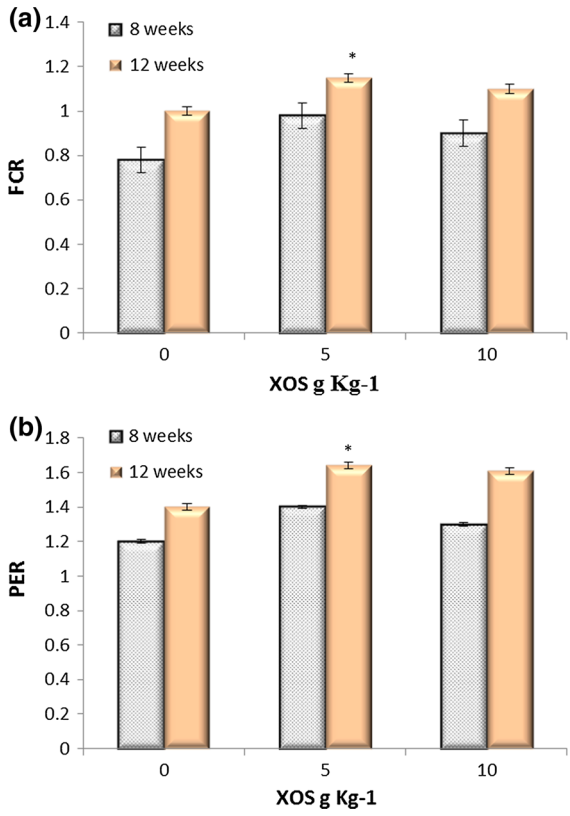

Fig. 1 **a** Feed efficiency (FCR) and **b** protein efficiency ratio (PER) for Tunisian sea bass fed at different levels of XOS inclusion during 8 and 12 weeks. Data represent the mean ± SD. Significant differences ($P < 0.05$) between treatments for a given feeding period are indicated by *asterisk*

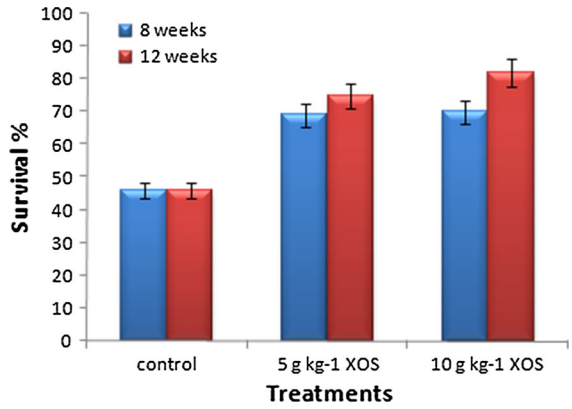

Fig. 2 Effect of XOS on survivability of *D. labrax* after challenge with *Aeromonas hydrophila* (values are mean ± SD)

diet for both 8 and 12 weeks. Survivors did show a localized disease sign in the shape of abrasion at the site of injection. This sign of injury disappeared after 1 week of the injection in fish fed treated diets.

Haematological parameters

The XOS-treated fish after feeding 8 weeks had significantly greater ($P < 0.05$) numbers of erythrocytes (RBC) than those of the control (Table 3). An increased trend in RBC was obvious as time elapsed with the feeding of XOS. Feeding the fish with 5 g kg^{-1} XOS-supplemented diets for 12 weeks led to a significant increase ($P < 0.05$) in RBC. The number of leucocytes (WBC) in pre-challenged groups of fish fed XOS at 5 and 10 g kg^{-1} diets was significantly higher than that of the control fed for 8 weeks (Table 3). Relatively higher proportions of WBC were observed for 12 weeks in pre-challenged groups over the fish fed for 8 weeks and control (Table 3). The result shows a significant increase ($P < 0.05$) in haemoglobin (Hb %) concentration in the groups that were fed XOS-supplemented diets over the control. A decline trend in Hb % concentrations was apparent for all post-challenged groups over the fish fed for 12 weeks (Table 3). A significant increase ($P < 0.05$) in mean PVC concentration in the pre-challenged fish was observed in those groups that received 5 g kg^{-1} XOS and 10 g kg^{-1} XOS diets for 8 and 16 weeks over the control. All groups of fish fed supplemented diets for 16 weeks had shown a non-significant increased level of PVC. Conversely, there was a clear significant reduction in the concentration of PVC in the post-challenged groups (Table 3).

Serum total protein content

The serum total protein content was significantly higher ($P < 0.05$) in the groups that were fed XOS-supplemented diets over the control (Table 4). However, there was increasing trend in the serum protein in pre- and post-challenged groups for 12 weeks for group that received 5 g kg^{-1} XOS diet over the control. Fish fed supplemented diets for 8 weeks had shown significantly higher protein content over the control.

Immunological parameters

Serum immunoglobulin

Compared with the control, there was a significantly higher level of serum immunoglobulin in the groups that were fed XOS at 5 g kg^{-1} diet and 10 g kg^{-1} diet for 8 weeks. The immunoglobulin was significantly

Table 3 Haematological parameters of Tunisian sea bass fingerlings fed commercial extruded diets with different levels of XOS inclusion and a control diet

Parameters	Days of feeding (weeks)		Control	XOS 5 g kg^{-1} diet	XOS 10 g kg^{-1} diet
RBC (10^6/mm^3)	8	Pre-challenged	3.72 ± 0.10^a	4.35 ± 0.12^b	4.20 ± 0.23^b
		Post-challenged	3.54 ± 0.35^a	4.01 ± 0.15^b	3.83 ± 0.13^b
	12	Pre-challenged	3.89 ± 0.10^a	4.42 ± 0.21^b	4.35 ± 0.09^b
		Post-challenged	3.62 ± 0.01^a	4.05 ± 0.81^b	3.84 ± 0.15^b
WBC (10^3/mm^3)	8	Pre-challenged	11.52 ± 0.13^a	14.68 ± 0.08^b	13.94 ± 0.76^b
		Post-challenged	20.03 ± 0.64^c	16.14 ± 0.32^a	18.09 ± 0.32^b
	12	Pre-challenged	13.23 ± 0.72^a	15.02 ± 0.09^c	14.30 ± 0.13^b
		Post-challenged	19.01 ± 1.27^b	16.71 ± 0.66^a	17.43 ± 0.54^a
Hb (g/dl)	8	Pre-challenged	9.86 ± 0.76^a	11.88 ± 0.10^b	11.49 ± 0.13^c
		Post-challenged	7.89 ± 0.17^a	13.38 ± 0.42^c	12.33 ± 0.24^b
	12	Pre-challenged	13.38 ± 0.15^a	14.31 ± 0.42^b	14.66 ± 0.61^b
		Post-challenged	8.79 ± 0.19^a	10.67 ± 0.45^b	11.01 ± 0.35^b
PCV %	8	Pre-challenged	39.87 ± 0.13^a	40.31 ± 1.15^{ab}	42.56 ± 2.54^b
		Post-challenged	29.64 ± 1.74^a	31.92 ± 1.04^a	37.34 ± 1.65^b
	12	Pre-challenged	40.30 ± 2.53^a	43.89 ± 1.67^b	45.91 ± 3.64^b

Different letters within a line denote significant differences ($P < 0.05$). Control = 0 g kg^{-1} diet; XOS = 5 g kg^{-1} diet; XOS = 10 g kg^{-1} diet. Values expressed in mean ± SD. Values in the same line indicated by different superscript letters are significantly different ($P < 0.05$) from the control and each other

Table 4 Serum total protein content (mg mL^{-1}) and immunoglobulin (mg mL^{-1}) in blood serum and of Tunisian sea bass fingerlings *D. labrax* fed 8 and 12 weeks with different levels of XOS inclusion and a control diet

Parameters	Treatments days (weeks)		Control	XOS 5 g kg^{-1} diet	XOS 10 g kg^{-1} diet
Protein	8	Pre-challenged	2.13 ± 0.03^a	2.14 ± 0.21^a	2.32 ± 0.02^b
		Post-challenged	2.35 ± 0.01^a	2.32 ± 0.24^a	2.25 ± 0.13^a
	12	Pre-challenged	2.26 ± 0.02^b	2.57 ± 0.56^b	2.15 ± 0.02^a
		Post-challenged	2.14 ± 0.07^a	2.52 ± 0.26^{ab}	2.08 ± 0.04^a
Immunoglobulin	8	Pre-challenged	2.64 ± 0.40^a	3.12 ± 0.03^b	3.03 ± 0.10^{ab}
		Post-challenged	2.73 ± 0.22^a	3.43 ± 0.35^b	3.06 ± 0.32^b
	12	Pre-challenged	2.41 ± 0.12^a	3.61 ± 0.13^c	3.14 ± 0.17^b
		Post-challenged	2.93 ± 0.26^a	3.32 ± 0.01^b	3.03 ± 0.16^a

Data expressed as mean ± SD. Values in the same line indicated by different superscript letters are significantly different ($P < 0.05$) from the control and each other ($n = 3$)

($P < 0.05$) increased in the 12-week fed groups over the control. The infection of *D. labrax* with 5 g kg^{-1} XOS diet led to showing an increase in trend of serum immunoglobulin (Table 4).

Serum lysozyme

Serum lysozyme in fish groups fed with XOS and control is represented in Fig. 3. Application of XOS resulted in an increase in lysozyme level over the control. A higher lysozyme activity occurred in fish that were given 5 g kg^{-1} XOS-supplemented diet followed by 10 g kg^{-1} XOS diet in both 8 and 12-week fed groups. Serum lysozyme level showed an increase in the post-challenged fish that fed control diet over the pre-challenged fish (Fig. 3).

Histological in liver tissue studies

Figure 4 shows that in liver samples taken after 12 weeks of the feeding trial, no macroscopic observations were considered XOS feeding related.

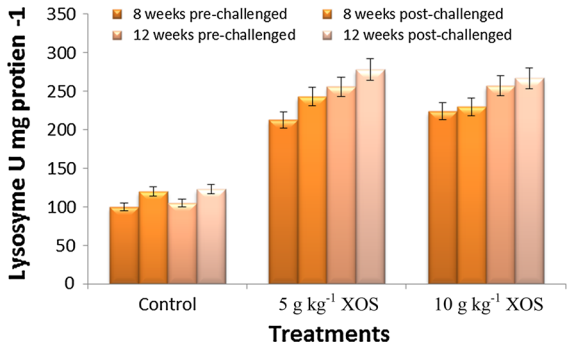

Fig. 3 Pre-challenged and post-challenged lysozyme levels of the sea bass (*D. labrax*) fingerlings XOS at different levels (values are mean ± SD)

Histological examination did not identify any differences between the animals fed the basal diet and those fed the XOS prebiotic diet at the end of the treatment. In fact, despite the slight epithelium swelling detected in control group, characterized by cytoplasm vacuolization and nuclei displaced to periphery cellular, morphological analyses for fish fed diets containing XOS resulted qualitatively in a regular-shaped morphology of the hepatocytes around sinusoidal spaces when compared with controls. Thus, these changes were not considered XOS treatment related in this study because these microscopic changes were commonly observed in untreated fish and were of comparable incidence and severity between treatment group and control group.

Morphometric analysis

According to Table 5, no significant differences were found between control and XOS-treated groups of fish in regard to the liver weight as well as to the liver somatic index (LSI).

Discussion

To the best of our knowledge, there are few studies which investigate the effects of xylooligosaccharide on *Dicentrarchus labrax* fingerlings. It has been already established that prebiotics effectively improve immune response, resistance, intestinal microbiota communities and performance of fish and shellfish species (Gibson 2004). This study demonstrated that feeding XOS significantly reduced the mortality rate of *D. labrax* and bestowed resistance to challenge with *A. hydrophila*. The data clearly demonstrated that

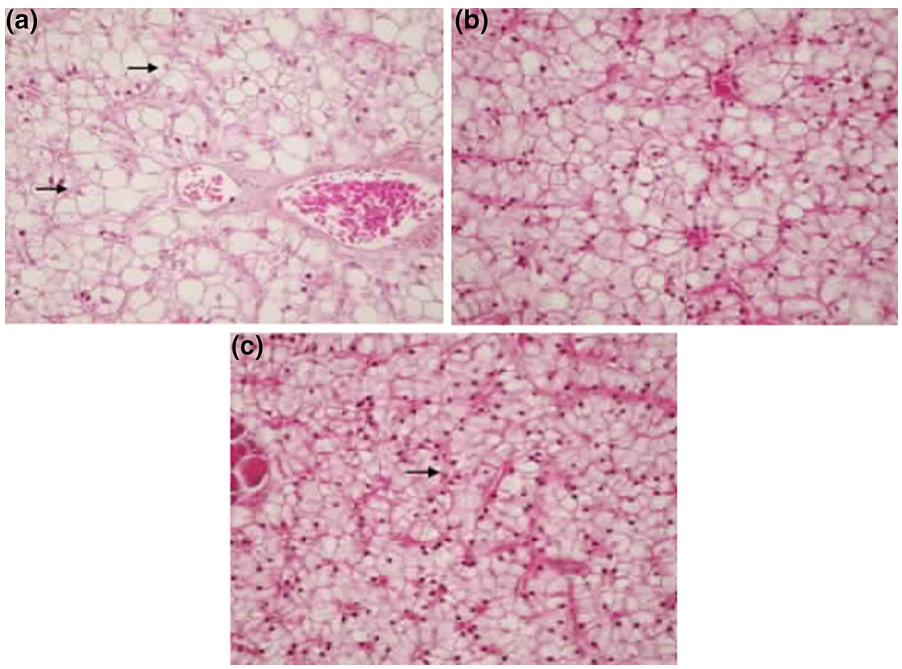

Fig. 4 Hepatocytes sections (H&E. ×400) from Mediterranean sea bass (*D. labrax*) fingerlings fed: **a** control diet; **b** group fed with 5 g kg^{-1} XOS-supplemented diets showing no signs of serious lesions; and **c** group fed with 10 g kg^{-1} XOS-supplemented diets showing slight B cell infiltration

Table 5 Morphometric analysis of Tunisian sea bass fingerlings Liver *D. labrax* fed with different levels of XOS inclusion and a control diet at the end of the treatment

	Control	XOS 5 g kg^{-1} diet	XOS 10 g kg^{-1} diet
Liver weight (g)	1.65 ± 0.73[a]	1.89 ± 0.01[a]	2.01 ± 0.86[a]
Liver somatic index (LSI)	4.85 ± 0.28[a]	4.70 ± 0.44[a]	5.04 ± 0.09[a]

Different letters within a line denote significant differences ($P < 0.05$). Control = 0 g kg^{-1} diet; XOS = 5 g kg^{-1} diet; XOS = 10 g kg^{-1} diet. Values expressed in mean ± SD. Values in the same line indicated by different superscript letters are significantly different ($P < 0.05$) from the control and each other

XOS significantly exerted a positive effect on survival performance of *D. labrax* after challenge with *A. hydrophila*. The highest survival rates in the XOS-treated group could also be attributed to their increased potential response providing favourable conditions in the rearing tanks. The protective effect of the XOS-supplement diets might also be featured to their sugar contents which are reported to increase disease resistance by stimulating non-specific immune system (Burgents et al. 2004). In this study, several fish were found with a localized ulcer-type lesion at the site of injection of induced pathogen; this could be associated with the higher pathogenicity of the *A. hydrophila* which has shown advanced stage of infection. This infection healed up after 1 week in survivors of treated groups which indicate that the XOS in the diet had wielded a possible protective prophylactic role against *A. hydrophila* and thus enhanced the survival of fish.

Alike to survival performance, feeding the XOS diets to *D. labrax* demonstrated beneficial effects in terms of higher weight gain and improved FCR over the control. Compared to progress made with fish, research on the dietary additives (probiotics and prebiotics) for fish is still in its infancy. Improved growth may be related to the enhanced amino acid absorption as it has been shown in chicken (Iji et al. 2001). The enhanced growth observed in the present study was parallel to previous reports feeding oligosaccharides to *Channa striata* fingerlings (Talpur et al. 2014), European sea bass (*Dicentrarchus labrax*) (Torrecillas et al. 2007), Caspian white fish (*Rutilus frisii kutum*) fry (Hoseinifar et al. 2014) and juvenile turbot, *Scophthalmus maximus* (Li et al. 2008). Furthermore, the addition of XOS to fish diet improved growth in *Channa striata* fingerlings (Talpur et al. 2014). The results of XOS diet were similar to those of the study of Geraylou et al. (2013), who find effects on growth after feeding juvenile Siberian sturgeon (*Acipenser baerii*) with arabinoxylan-oligosaccharides (AXOS) supplement diets. Xu et al. (2009) found also elevated growth performance, digestive enzymes activity and feed utilization of crucian carp (*Carassius auratus gibelio*) fed 50 mg kg^{-1} or 100 mg kg^{-1} XOS and administration of 0.4 g kg^{-1} XOS in the diet of juvenile turbot (*Scophthalmus maximus* L.) significantly improved growth (Li et al. 2008). It seems that the type of prebiotic, routes of administration, dosage, fermentability, the intestinal microbiota communities, species and developmental stages are the main factors that determine the efficacy of dietary prebiotic on fish growth and feed utilization (Hoseinifar et al. 2010).

Understanding of the haematological parameters is a vital tool that can be used as an effective index to observe the physiological and pathological changes in fish to detect the fish health (De Pedro et al. 2005). The enhancement in RBC and WBC counts in the present study specifies the immunostimulant and potential effects and anti-infective properties of xylooligosaccharides. The findings conform to those by Hoseinifar et al. (2014) who reported that the number of RBC and WBC significantly increased ($P < 0.05$) in Caspian white fish (*Rutilus frisii kutum*) fry fed with a diet containing dietary xylooligosaccharides. Similarly, Talpur et al. (2014) reported increased RBC and WBC counts in *Channa striata* fingerlings after feeding with the galactooligosaccharide (GOS)- and mannanoligosaccharide (MOS)-supplemented diet. WBC serves as one of the front lines of body defence and is believed to increase quickly when infections occur. Increase in number of WBC in infected fish may serve as a protective barrier against pathogenic infection (Talpur and Ikhwanuddin 2013). An indication of low haematocrit (PVC %) targets on the anaemic condition of fish, and this situation occurs when the fish stop feeding as a result of any disease or

stress (Talpur and Ikhwanuddin 2013). In the present study, the haemoglobin content (Hb %) was significantly different in treated groups over the control, which correlates with the increase in numbers of RBC. Generally, there was a slight decrease in trend in protein content in post-challenged fish groups, but the level of protein was still remarkably higher than that of the control. The increase in protein content in challenged fish fed XOS diet is similar to Talpur et al. (2014) reporting a significant elevation of serum protein in the infected salmon compared with the healthy fish.

Immunoglobulins are the principal humoral components of the specific immune system, and their concentration has a significant importance with respect to understanding the fish physiology and pathology. In teleosts, the serum immunoglobulin level has been shown to vary between 2 and 7 mg mL^{-1} (Fuda et al. 1991). In the present study, the immunoglobulin level in *D. labrax* was estimated above 2.64 ± 0.4 mg mL^{-1} within the groups which was similar to the *C. striata* immunoglobulin level 3.48 ± 0.41 mg mL^{-1} reported by Talpur et al. (2014). The measured immunoglobulin level in the serum of *D. labrax* in this study had shown an increased trend with respect to control fish. The increased immunoglobulin activity in post-challenged fish indicates that a defence capability of fish against induced pathogen was eventually activated. Increase in serum immunoglobulin is likewise in line with previous work involving infected (*Channa striata*) fingerlings (Talpur et al. 2014) compared with the healthy fish.

The serum lysozyme activity is relevant insofar as it provides a defence line in preventing proliferation and colonization of pathogenic microbes as a consequence ensuing in the diminution of disease (Talpur and Ikhwanuddin 2013). In the present study, the lysozyme enzyme activity was enhanced in groups that were fed XOS over the control. These results are also similar to snakehead (*Channa striata*) fingerlings feeding with mannanoligosaccharides and galactooligosaccharides, showing an increase in serum lysozyme activity (Talpur et al. 2014). Similarly, Caspian white fish (*Rutilus frisii kutum*) fry after feeding with dietary xylooligosaccharide showed an increase in serum lysozyme activity (Hoseinifaret al. 2014). The rise in WBC, serum lysozyme and immunoglobulin levels in this study is believed to be related to a stronger innate immune response of fish, thus resulting in a reduced mortality of young fish facing bacterial infections.

To substantiate the biochemical findings, a histological examination of the liver was undertaken. In fact, histological pictures seen in the livers of treated fishes were characterized by a normal histoarchitecture. These results are supported by the morphometric analysis parameters as liver weight and hepatosomatic index were established. As a result, it has been observed that there is no significant alteration in the somatic index and in the liver weight from the animals exposed to XOS in the concentration of 5 and 10 g kg^{-1} (Table 5).

In summary, these results showed that dietary incorporation of XOS at 5 g kg^{-1} diet enhances Mediterranean Sea bass fingerling growth, activates its immune system and increases its resistance to a bacterial infection directly inoculated in the gut, one of the main sites of infection in fish (Zapata and Cooper 1990). Nevertheless, other mechanisms may be also implicated on the protection effect of XOS against pathogen infections in fish and deserve further studies. For instance, in poultry, probiotic and antibiotic dietary supplementation affects the intestinal bacterial populations (Smirnov et al. 2005). Further experiments must be conducted to clarify the action mechanisms of XOS, as well as the optimum feeding period and dose.

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