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Effect of Dietary Fructooligosaccharide on Bacterial Infection, Oxidative Stress and Histopathological Alterations in Nile Tilapia (*Oreochromis niloticus*)

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Abstract: Nile tilapia, *Oreochromis niloticus* (*O. niloticus*) of average body weight (24.5±1.6 g) were fed basal diet supplemented with fructooligosaccharide (FOS) at a concentration of 0, 10, 20 and 30 g/kg diet for 49 days (42 days before and 7 days post-challenge). At 42 days of feeding, fish of all groups were challenged intra-peritoneal with 0.2 ml *Aeromonas hydrophila (A. hydrophila*) (1.5×10^8 CFU ml^{-1}). Serum and tissue samples were collected on eighth day post infection. Results showed that cortisol level, liver enzymes (alanine aminotransferase (ALT) and aspartate aminotransferase (AST)) and urea level were significantly decreased ($P < 0.05$) with dietary FOS in comparison with infected group fed basal diet. Furthermore, malondialdehyde (MDA) level as well as superoxide dismutase (SOD), glutathione peroxidase (GPX) and glucose 6 phosphate dehydrogenase (G6PD) revealed significant decrease in the treated groups fed 20 g dietary FOS over the infected group fed basal diet. Moreover, total protein and globulin level as immune component were significantly increased ($P < 0.05$) in the group treated with 20 g dietary FOS. Feeding *O. niloticus* supplemented diet with FOS increased survivability after challenging with *A. hydrophila*. Histopathological examination of infected *O. niloticus* revealed various pathological alterations in spleen, liver and kidneys represented mainly in hemorrhage, necrosis, with inflammatory reaction. The severity of these changes was reduced in the infected fish groups fed different dietary concentrations of FOS with variable degrees especially at both concentrations of (20 and 30 g FOS/ kg diet). In conclusion, these findings indicated that dietary supplementation of FOS has a protective role in challenged *O. niloticus* with *A. hydrophila* and could minimize the physiological alterations, increase host immune system and relieve oxidative stress as well as renewal of tissue histological structures.

Key words: Prebiotic · Oxidative Stress · Cortisol · Histopathology · Nile Tilapia

INTRODUCTION

With the fast development of aquaculture, the infectious diseases particularly those caused by viruses and bacteria became a major problem in aquaculture resulting in severe economic losses to fish farmers [1]. These diseases occurred due to adverse environmental conditions and unmanaged fish culture practices that affect the fish health leading to production losses. *Aeromonas hydrophila* is one of the major opportunistic bacterial pathogens affecting wide variety of freshwater fish species. It has scientific and economic interest in Egypt as well as in other countries as it causes massive mortalities in aquaculture [2]. *A. hydrophila* is able to produce many extracellular proteins such as enterotoxins, aerolysin and hemolysin in addition to other factors such as adhesin and mucinase production which are considered the main virulence factors that responsible for the pathogenicity of *Aeromonas* species [3].

The use of alternative strategies such as the incorporation of prebiotics in aquafeeds have been received great attention for controlling the infectious diseases [4] instead of antibiotics which have been widely banned for their negative impacts that includes tissue residues, development of antibacterial resistant strains and immunosuppression of host immune system [5].

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Fructooligosaccharides (FOS) is one of the prebiotics that has been shown beneficial effects in aquaculture. It improved the immune response; growth and survivability of fish and shellfish [6- 8] by selectively stimulate the growth and metabolism of health-promoting bacteria present in the host gut such as *Bifidobacteria* and *Lactobacilli* [9]. The present study was carried out to investigate the protective role of dietary FOS supplement against *A. hydrophila* infection in *O. niloticus* through evaluating some biochemical parameters, antioxidant enzyme activities and histopathological examination.

MATERIALS AND METHODS

Fish: A total number of two hundred and eighty apparently healthy Nile tilapia *O. niloticus* of average body weight (24.5±1.6 g) was obtained from private fish farm at Kafr El-Sheikh Governorate, Egypt at Late November 2014. Fish were transported in polyethelene bags enriched with oxygen to the Lab of Fish Diseases and Management at Fac. of Vet. Med, Benha University, Egypt and divided into four groups in fiberglass tanks in duplicate (35 fish/ tank). Fish were allowed to acclimate for 15 days to the laboratory conditions and fed a basal diet two times per day.

Bacterial Strain: A well identified *A. hydrophila* strain was kindly obtained from Central Laboratory for Aquaculture Research, Abbassa, Sharkia Governorate, Egypt and kept at 4 °C. A loopful was taken aseptically on tryptic soy agar and incubated at 28 °C for 24 hr. The pure culture was harvested and suspended in sterile saline. The pre-intended concentration of pathogenic bacteria (1.5×10^8 CFU ml^{-1}) was estimated using McFarland's 0.5 standard tubes.

Preparation of Experimental Diets: FOS (Nutraflora)® powder obtained from GTC nutrition company, USA were incorporated with the commercial basal diet (Trade Company, Egypt) (30% crude protein) at different concentrations 0, 10, 20 and 30 g/ kg basal diet. Approximately 250 ml of water per kg of diet was added to form soft dough which was re-pelleted using hand pelletizer. The experimental diets were left air dried and packed in clean plastic bag and then kept in refrigerator at 4°C until feeding.

Experimental Design: After acclimation, the first group was fed on FOS free diet (control). While, the second, third and fourth groups were fed basal diet containing 10, 20 and 30 g FOS/ kg diet respectively. At the 42 days from the beginning of feeding, a total of forty fish (ten fish/ treatment) were transferred to four well prepared glass aquaria (90×35×40 cm) and injected intra-peritoneal with 200 µl *A. hydrophila* (1.5×10^8 CFU ml^{-1}). All the infected groups were kept on the same feeding regime for additional 7 days. Fish were fed by hand, twice daily at a rate of 3 % of their body weight for 49 days. The water quality parameters were determined according to the guidelines of APHA [10]. The water temperature was adjusted at 28±1°C using thermostatic heaters and pH was maintained at 7.1±0.2 along the entire experimental period. Fish survivability was recorded in all experimental groups.

Biochemical Analysis: Blood samples were collected at 8th days post infection from the heart without anticoagulant using 3ml sterile syringe, then transferred to clean eppindroff tube and allowed to clot at room temperature for 2 hr. The blood samples were centrifuged at 3000 rpm at 4°C for 15 min and the resultants serum were pooled and stored at -80°C until analysis. The biochemical parameters of serum samples were determined as follow; cortisol level was measured according to Knobil [11], total protein [12] and albumin [13]. The globulin level was obtained by subtraction of albumin values from total protein level. Liver enzymes include alanine aminotransferase (ALT) and aspartate aminotransferase (AST) were estimated according to Reitman and Frankel [14]. Urea and Creatinine were determined according to Fawcet and Scott [15] and Husdan and Raporpot [16] respectively.

Lipid Peroxidation and Antioxidant Enzymes Assay: Immediately after blood collection, the liver was quickly removed and homogenized in cooled phosphate buffer saline pH 7.2 at a ratio 1: 10 (w/v) using electrical homogenizer. The procedure was performed on ice. The homogenates were centrifuged at 13,000 xg at 4°C for 15 min. The supernatants were collected and stored at -80°C until determination the following parameters which performed within one week after preparation. The malondialdehyde (MDA) level was assayed as a marker of lipid peroxidation following the procedure described by Ohkawa *et al*. [17]. The antioxidant enzyme activities were assayed as follow; superoxide dismutase (SOD) activity according to Nishikimi *et al*. [18], Catalase (CAT) [19], Glutathion peroxidase (GPX) [20] and Glucose 6-phosphate dehydrogenase (G6PDH) [21].

Histopathological Examination: Tissue specimen from liver, spleen and kidneys were removed and fixed in 10% neutral buffered formalin for 24 hr. The fixed tissues were

rinsed in tap water, dehydrated through graded series of alcohols, cleared in xylene and embedded in paraffin wax [22]. 5 µm thick sections were cut and stained with hematoxylin and eosin (H & E) and then the tissues were examined and evaluated by light microscopy. According to the severity and dissemination of pathological alterations, the detected pathological lesions were graded qualitatively according to Yardimci and Aydin [23] as following: (+) = the detected pathological alterations are at a mild degree; (++) = the identified pathological changes are at a moderate degree; (+++) = the demonstrated pathological lesions are at a severe degree.

Statistical Analysis: The obtained data were expressed as mean ± S.E and were statistically analyzed by One Way Analysis of Variance (ANOVA) and Duncan's multiple range tests to determine significant difference between groups using Statistical Package for the Social Sciences (SPSS) software (version 16.0). A value of $P < 0.05$ was considered significant.

RESULTS

Biochemical Analysis: As shown in Table 1. Serum cortisol was significantly decreased ($P < 0.05$) in the infected group fed different dietary levels of FOS especially at a dose of 20 g compared to the infected group fed basal diet. Moreover, total protein and globulin levels were significantly increased in infected group fed 20 g FOS/ kg diet, whereas there was no significant effect on serum total protein and globulin among groups fed 10 and 30 g FOS compared to the infected group (control). The albumin level exhibited no statistical difference among the infected treated groups and control.

Liver enzymes (AST and ALT) and kidneys function in all experimental groups were presented in Table 2. The infected groups fed different dietary levels of FOS revealed significant decrease ($P < 0.05$) of liver enzymes compared to the control. Urea level recorded significant decrease in the group challenged with *A. hydrophila* and fed 20 g FOS compared to other groups. There was no significance difference in serum creatinine of all groups either fed basal diet or FOS compared.

Lipid Peroxidation and Antioxidant Enzyme Activities: As seen from Table 3. The infected group fed 20 g FOS/ kg diet recorded the lowest MDA level ($P < 0.05$) compared to other experimental groups. SOD, GPX and G6PDH activities showed significant decrease in the infected group fed basal diet supplemented with 20 g FOS in comparison with the infected group fed basal diet. CAT activity exhibited no significant difference ($P > 0.05$) among all experimental groups.

Survivability after Challenge with *A. hydrophila*: *O. niloticus* fed FOS especially at a concentration of 20 and 30 g/ kg diet revealed the highest survival rate 70 and 80 % respectively after challenging with *A. hydrophila*. While, fish fed low dose 10 g FOS/ kg diet showed 40 % survivability. The infected group fed basal diet showed congestion at the base of caudal and dorsal fins, abdominal distension, detachment of scales and darkness of the skin (Plate, 1A). Internally, congestion of liver, darkness of spleen and enlargement of kidneys as well as congested intestine and distended gall bladder were observed (Plate, 1B). While the infected treated groups with FOS at different dietary levels revealed slight congestion on the skin and caudal fin.

Histopathological Examination: The severity of histopathological changes that were seen in the splenic, hepatic and renal tissue of the infected group was alleviated to variable degrees in the challenged groups fed dietary FOS as shown in Table (4).

Spleen of infected *O. niloticus* fed basal diet showed extensive subcapsular edema (Fig. 1A) with marked congestion of splenic blood vessels. Diffuse splenic hemorrhage with marked lymphoid depletion where eosinophilic meshes free of lymphocytes was detected (Fig. 1B&C). Furthermore, inhibition of melanomacrophage center (MMC) was noticed where there were decrease in size, pigmentation and cellular density. The splenic tissues of treated group with 10 g dietary FOS has hemorrhage in the red pulp with marked congestion of splenic blood vessels. Moreover, lymphoid depletion with mild degeneration of melanomacrophage center was demonstrated (Fig. 1D). On the other side, group treated with 20 g dietary FOS revealed congestion of splenic blood vessels with extensive improvement in the lymphocytic population with well-developed melanomacrophage center but to some extent still have mild degeneration in some examined fish (Fig. 1E). Moreover, mild congestion of splenic blood vessels was observed in few cases. Meanwhile, there were well developed melanomacrophage center and increase in the lymphocytic cell population was demonstrated in Nile tilapia challenged with 30 g dietary FOS (Fig. 1F).

Table 1: Effect of dietary FOS on some biochemical parameters in *O. niloticus* challenged with *A. hydrophila*.

Parameters	G1	G2	G3	G4
Cortisol (µg/dl)	164.0±6.66[a]	114.33±3.48[b]	50.67±5.24[d]	87.67±5.69[c]
Total protein (g/dl)	2.50±0.34[b]	3.13±0.01[ab]	4.46±0.18[a]	3.23±0.09[b]
Albumin (g/dl)	1.68±0.31[a]	1.97±0.06[a]	1.81±0.23[a]	2.09±0.13[a]
Globulin (g/dl)	0.82±0.17[b]	1.16±0.07[b]	2.65±0.39[a]	1.14±0.06[b]

Mean values ± (S.E) in the same raw with different superscript letters are statistically different (P < 0.05). G1: infected fed basal diet (control); G2, infected fed 10 g FOS/ kg diet, G3: infected fed 20 g FOS/ kg diet, G4: infected fed 30 g FOS/ kg diet.

Table 2: Effect of dietary FOS on liver and kidney functions in *O. niloticus* challenged with *A. hydrophila*.

Parameters	G1	G2	G3	G4
AST (U/L)	137.0±5.77[a]	106.67±3.76[b]	89.33±3.93[c]	107.0±2.52[b]
ALT (U/L)	79.0±5.86[a]	29.0±0.6[c]	36.0±4.16[bc]	46.67±6.74[b]
Urea (mg/dl)	21.65±2.17[a]	17.60±0.39[ab]	17.20±0.51[b]	17.93±0.81[ab]
Creatinine (mg/dl)	0.93±0.02[a]	0.66±0.04[a]	0.75±0.06[a]	0.79±0.15[a]

Mean values ± (S.E) in the same raw with different superscript letters are statistically different (P < 0.05). G1: infected fed basal diet (control); G2, infected fed 10 g FOS/ kg diet, G3: infected fed 20 g FOS/ kg diet, G4: infected fed 30 g FOS/ kg diet.

Table 3: Effect of dietary FOS on lipid peroxidation and antioxidant enzyme activities in *O. niloticus* challenged with *A. hydrophila*.

Parameters	G1	G2	G3	G4
MDA (nmol/g)	74.30±3.24[a]	73.50±1.84[a]	54.47±6.71[b]	72.47±4.9[a]
SOD (U/mg)	403.33±3.93[a]	310.33±6.36[c]	287.0±4.72[d]	341.0±5.77[b]
CAT (U/g)	66.33±3.48[a]	55.67±5.61[ab]	46.33±0.88[b]	65.67±6.17[a]
GPX (mU/mg)	173.46±7.2[a]	139.72±5.48[b]	132.08±5.49[b]	136.78±7.94[b]
G6PD (mU/mg)	16.16±1.05[a]	14.38±2.03[ab]	10.91±1.08[b]	15.72±0.41[a]

Mean values ± (S.E) in the same raw with different superscript letters are statistically different (P < 0.05). G1: infected fed basal diet (control); G2, infected fed 10 g FOS/ kg diet, G3: infected fed 20 g FOS/ kg diet, G4: infected fed 30 g FOS/ kg diet.

Table 4: Lesions degree of various histopathological alterations in different experimental groups

Lesions	G1	G2	G3	G4
		Spleen		
Subcapsular edema	+++	+	-	+
Congestion of splenic blood vessels	+++	++	+	+
Splenic hemorrhage	+++	++	+	+
Lymphocytic depletion	+++	++	-	-
Degeneration of MMC	+++	++	+	+
		Liver		
Congestion of blood vessels	+++	++	+	+
Hemorrhage	+++	+	-	-
Leukocytic infiltration	+	+	-	-
Degenerative changes	++	++	+	+
Necrosis of hepatic cell	+++	+	-	+
Hyperplasia of bile duct	+	+	-	-
Periductal cirrhosis	+	-	-	-
Degeneration of pancreatic aciner cell	++	++	-	+
		Kidneys		
Congestion of renal blood vessels	+++	++	+	+
Hemorrhage	+++	+	-	+
Perivascular edema	++	+	-	+
Cellular degeneration	+++	++	+	+
Tubular epithelial necrosis	++	+	-	+
Hyaline casts	++	+	-	-
Leukocytic infiltration	++	+	-	+
Hyalinization of glomerular tuft	++	+	-	-

(-) None lesion, (+) Mild degree, (++) Moderate degree, (+++) Severe degree. G1: infected fed basal diet (control); G2, infected fed 10 g FOS/ kg diet, G3: infected fed 20 g FOS/ kg diet, G4: infected fed 30 g FOS/ kg diet

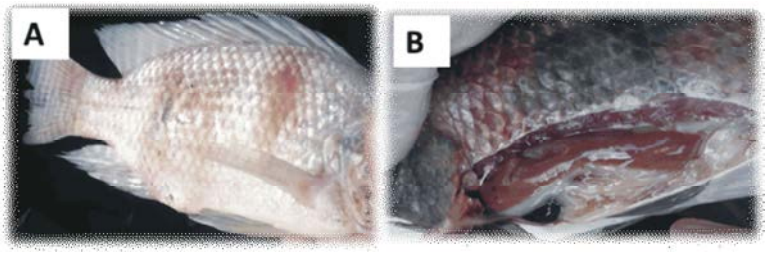

Plate 1: Experimentally infected *O. niloticus* with *A. hydrophila* showing A) congestion of fins and skin, ulcer, depigmentation and detachment of scales; B) congestion of liver

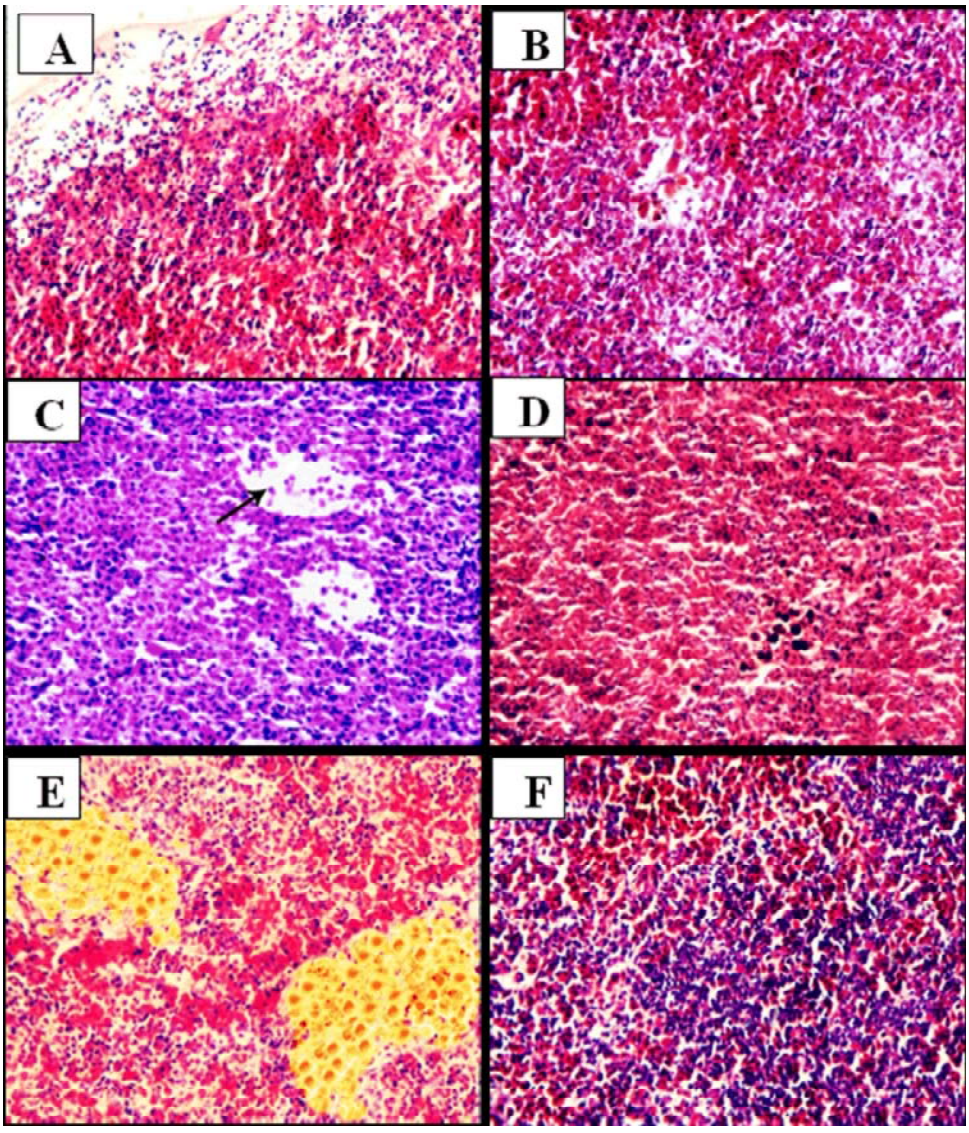

Fig. 1: Light micrograph of spleen (A-C) of *O. niloticus* infected with *A. hydrophila* showing (A) subcapsular edema (x200), (B) splenic hemorrhage with inhibition of melanomacophage center (MMC) (x200), (C) marked lymphoid depletion (arrow, x200), (D) spleen of *O. niloticus* challenged with 10g FOS showing splenic hemorrhage with degenerated MMC (x200), (E) spleen of *O. niloticus* challenged with 20g FOS showing well developed MMC (x200), (F) spleen of *O. niloticus* challenged with 30g FOS showing improvement in lymphocytic population (x200), H&E stain

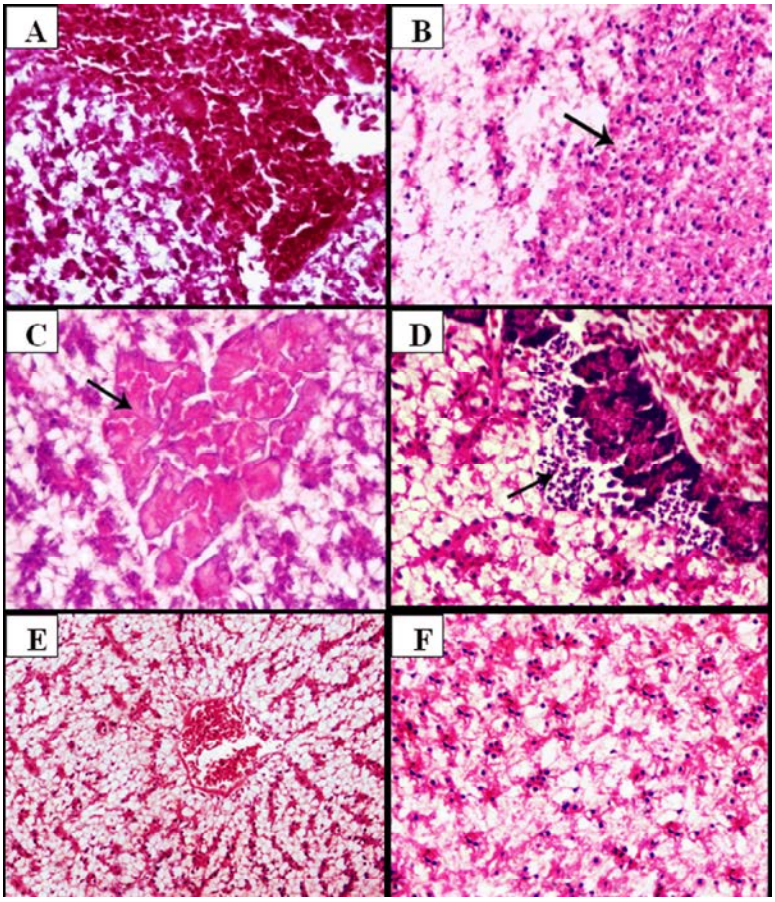

Fig. 2: Light micrograph of liver (A-C) of *O. niloticus* infected with *A. hydrophila* showing (A) hemorrhage in hepatic parenchyma (x200), (B) hepatic cell necrosis (arrow, x200), (C) necrosis of pancreatic acinar cell (x400), (D) liver of *O. niloticus* challenged with 10g FOS showing congestion of hepato-portal vein with peripancreatic leukocytic infiltrations (arrow, x200), (E) liver of *O. niloticus* challenged with 20g FOS showing mild congestion of central vein (x200), (H) (F) liver of *O. niloticus* challenged with 30g FOS showing necrosis of some hepatocytes (x200). H&E stain

Liver of *Oreochromis niloticus* infected with *Aeromonas hydrophila* showed marked congestion of hepatic blood vessels and sinusoids with perivascular mononuclear leukocytic cellular infiltrations including lymphocytes, macrophage and plasma cells were demonstrated. Injury of the endothelial lining of blood vessels with perivascular hemorrhage and thrombosis of portal blood vessels was noticed. As well, scattered hemorrhage in the hepatic parenchyma with diffuse vacuolation of hepatocytes (Fig. 2A), proliferation of the lining epithelium of bile duct with periductal fibrosis was observed. Multiple focal areas of coagulative necrosis of hepatic cell that characterized by pyknotic nuclei with retention of tissue architecture with loss of cellular detail and cytoplasmic hypereosinophilia (Fig. 2B). Pancreatic acinar cell necrosis was demonstrated (Fig. 2C) with peripancreatic leukocytic cellular infiltrations was also noticed. The liver of infected fish treated with 10g FOS/kg basal diet showed congestion of hepatic blood vessels and blood sinusoids with necrosis of hepatocytes. Degeneration of the pancreatic acinar cell with periportal edema admixed with mononuclear leukocytic cellular infiltration was also detected (Fig. 2D). Meanwhile, congestion of hepatic blood vessels and blood sinusoids (Fig. 2E) in association with mild degenerative changes in hepatocytes was seen in challenged fishes with 20g of FOS/kg basal diet. Interestingly, beside congestion of hepatic blood vessels and blood sinusoids, coagulative necrosis of some hepatocytes was also observed in the hepatic parenchyma (Fig. 2F). Furthermore, degeneration of pancreatic acinar cell was noticed in the liver of few fish treated with 30 g FOS.

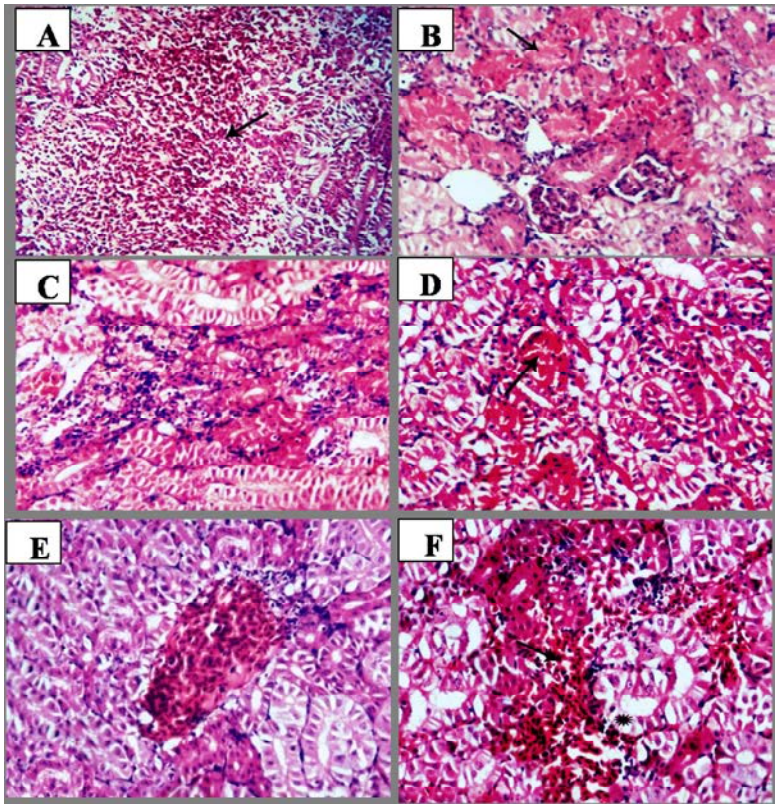

Fig. 3: Light micrograph of kidneys (A-C) of *O. niloticus* infected with *A. hydrophila* showing (A) interstitial hemorrhage (arrow, x200), (B) necrobiotic changes of the surrounding renal tubular epithelium with the presence of eosinophilic hyaline cast in the lumen of some renal tubules (arrow, x200), (C) focal interstitial infiltration of mononuclear cells with necrosis of the surrounding renal tubular epithelium (x200), (D) kidneys of *O. niloticus* challenged with 10g FOS showing vacuolation of the surrounding renal tubular epithelium with hyalinization of glomerular tuft (arrow, x200), (E), kidneys of *O. niloticus* challenged with 20g FOS showing congestion of interstitial blood vessels (x200), (F) kidneys of *O. niloticus* challenged with 30g FOS showing mild vacuolation of the lining epithelium of some renal tubules with mild intertubular hemorrhage (arrow, x200). H&E stain.

The kidney of infected fish showed extensive congestion of renal glomerular tuft and the interstitial blood vessels with degenerative changes in the blood vessels wall and perivascular edema admixed with erythrocytes and leukocytes. Marked interstitial hemorrhage (Fig. 3A) and subcapsular hemorrhage with subcapsular leukocytic cellular aggregations mainly lymphocytes and macrophage was demonstrated as well as in the interstitial tissues. Eosinophilic hyaline casts in the lumen of some renal tubules with hyaline droplets in the lining epithelium of some tubules (Fig. 3B) and hyalinization of the glomerular tuft was seen. Necrobiotic changes of the renal tubular epithelium characterized by pyknosis and chromatolysis and focal interstitial infiltrations of mononuclear cells were also demonstrated (Fig. 3C). However, the microscopical examination of fish treated with 10g dietary FOS revealed clear congestion of the interstitial blood vessels with perivascular and interstitial hemorrhage was detected. Vacuolar degeneration of the renal tubular epithelium with hyalinization of the glomerular tuft was seen in some examined fish (Fig. 3D). Necrosis of the surrounding renal tubular epithelium was noticed only in few cases. Overall, mild congestion of the renal blood vessels was seen (Fig. 3E) in the kidney of fish treated with 20g FOS. Interestingly, the lining epithelium of renal tubules of this treated group is nearly normal in most cases, except only mild vacuolar degeneration of the lining epithelium of some renal tubules was demonstrated in few examined cases. In the meantime, congestion of the renal blood vessels in association with vacuolation of the lining epithelium of renal tubules and inter-tubular hemorrhage was noticed in the renal tissues of the fish treated with 30g FOS/kg basal diet (Fig. 3F).

DISCUSSION

The survivability is considered a valuable indicator for monitoring fish health and determining the efficacy of the immunostimulants [8]. In the present study, *O. niloticus* fed dietary FOS especially at a concentration of 20 and 30 g / kg diet showed higher survival rate after challenging with *A. hydrophila*. This could be attributed to enhancement of phagocytosis by the stimulation of fructose binding lectin [24]. Another possible explanation may be that FOS inhibits the growth of pathogenic bacteria in the intestine through the production of short chain fatty acid by lactic acid bacteria [25].

Regarding physiological status, the infected group fed dietary FOS especially at a concentration of 20g/kg diet revealed significant decrease of serum cortisol compared to the control group. This result agrees with Luo *et al*. [26] and Zhang *et al*. [27] who observed a reduction in cortisol level following stress in fish fed prebiotics. The decrease of cortisol level could be attributed to the protective effect of FOS and its ability to relive stress and hence reduce the release of corticosteroid hormones, cortisol and catecholamines [28]. In the present work, the spleen of infected Nile tilapia exhibited marked lymphoid depletion with decrease in size, pigmentation and cellular density of melanomacrophage center. Whereas, splenic tissues revealed an improvement in the lymphocytic population with well developed melanomacrophage center especially in group fed basal diet containing 20g FOS/kg basal diet.

Serum total protein and globulin of treated groups with different dietary level of FOS recorded significant increase especially with 20g FOS/ kg diet which indicates stronger innate immune response and enhancement of disease resistance [29]. These results were supported by the obtained histopathological finding of spleen which considered one of the fish immune organs responsible for phagocytosis [30]. The concentration of serum proteins including albumin and globulin are considered as a basic index for fish health status [31] through the role of acute phase proteins in limiting the dispersal of infectious agents by repairing tissue damage and killing micro-organisms [32]. Moreover, globulins are considered the major proteins which play a significant role in the immune response [31]. Our result come in accordance with Talpur *et al*. [33] who observed increase in serum total protein in post challenged snakehead, *Channa striata* fingerlings with *A. hydrophila* and fed prebiotic supplemented diet. Similarly, serum globulin level recorded significant increase in crucian carp, *Carassius auratus gibelio* experimentally infected with *A. hydrophila* and fed mannan oligosaccharide [34]. Furthermore, enhancement of fish immune responses by dietary FOS has been reported in previous studies [8, 35]. The data of this study advocate that decrease of total protein in the infected Nile tilapia might be owing to impairment of protein synthesis by the liver [36] as a result of pathological alterations induced by *A. hydrophila* in liver including thrombosis of portal blood vessels and multiple focal areas of coagulative necrosis of hepatic cell with pyknotic nuclei associated with loss of structural integrity.

Regarding to liver functions, the infected groups fed different dietary levels of FOS showed significant decrease of ALT and AST which indicated the positive effect of FOS in enhancing and protecting liver cells and this supported by the improvement of pathological changes in liver tissue especially in groups fed 20 and 30 g FOS. The elevation of ALT and AST in the infected non treated group could be attributed to the utilization of amino acid for protein synthesis to produce energy for overcoming the induced stress and hence maintain health and survivability of fish [37]. Serum ALT and AST are considered a sensitive indicator to hepatocellular damage [38]. This is confirmed by the severe histopathological changes of liver obtained in the present study as thrombosis of portal blood vessels, diffuse vacuolation of hepatocytes with mononuclear leukocytic cellular infiltrations and multiple focal areas of coagulative necrosis of liver cell. These findings agree with Yardimci and Aydin [23] and Noor El Deen *et al*. [39].

Nile tilapia Challenged with *A. hydrophila* and fed 20 g dietary FOS showed significant decrease of urea level compared to the control, indicating that FOS has protective effect on kidney tissue. This result confirmed by the histopathological examination of kidneys, which revealed improvement and renewal of renal tissue at a concentration of 20g FOS. Serum creatinine and urea could be used as a rough index of the glomerular filtration rate [40]. The significant increase of urea level in the infected group fed basal diet could be due to pathological alteration induced by *A. hydrophila* in *O. niloticus* kidneys; this explanation supported by extensive vacuolar degeneration of the renal tubular epithelium with hyalinization of the glomerular tuft and formation of hyaline casts or may be due increased protein catabolism. These results come in agreement with Aly *et al*. [41] and Noor El Deen *et al*. [39]. Although, serum creatinine revealed improvement with dietary FOS, but this was not statistically differed from the control. High creatinine level

indicated that kidneys not functioning probably [42]. Therefore, histopathology may be considered an important diagnostic tool detects the alterations induced by infectious microorganisms [43].

In the current study, MDA level recorded significant increases in the infected Nile tilapia which coincide with previously findings obtained by Adeyemi [44] and Zhang *et al*. [27] whereas, the dietary FOS could relieve oxidative stress induced by *A. hydrophila* evidenced by the significant decrease of MDA level especially in group fed 20g dietary FOS. Similarly to the present study, reduced liver MDA was recorded in blunt snout bream fed on FOS and exposed to ammonia stress [27]. The decreased MDA level indicated the increased enzymatic antioxidants of the defense system due to the fact that increased of MDA content is considered a direct evidence of the toxic processes caused by the free radicals as a result of stress [45]. Furthermore, it has been reported that under a sustained compromised situation, the activities of antioxidant enzymes may be saturated, thus leading to excessive accumulation of MDA [46].

SOD and GPX enzymes activities of infected *O. niloticus* fed 20 g dietary FOS recorded significant decrease compared to the infected group fed basal diet. These results indicated that dietary FOS could relieve oxidative stress in challenged Nile tilapia and keep the healthy status of fish. The improvement of antioxidant enzyme activities with dietary FOS has been previously studied by Guerreiro *et al*. [7] and Zhang *et al*. [8]. CAT activity showed no significance difference among all experimental groups. This is possibly due to the inability of CAT enzymes to overcome the extremely high levels of ROS and in turn excessive ROS could inactivate CAT activity [47]. SOD, GPX and CAT are considered the first line of antioxidant enzymatic defense [48] and serve as biomarkers of oxidative stress which occurs due to an imbalance between the generation and removal of ROS [49].

In this study, the infected group fed dietary FOS showed reduced G6PDH activity than infected group fed basal diet. This indicated that dietary FOS could be able to relieve stress and effectively remove free radicals. This was supported by the fact that G6PDH is considered to be essential for the activity of the H_2O_2 scavenging pathways [50]. G6PDH plays a crucial role in modulating antioxidant defenses and maintaining the redox state of the cell through the NADPH generated in the reaction, an important cofactor necessary to recycle reduced glutathione through glutathione reductase activity, in order to minimize the oxidative stress condition [51]. In the present study, it was observed that inclusion level 30 g FOS could not reduce ROS production in the stressed Nile tilapia and hence some biochemical parameters and antioxidant enzyme activities were still high. These results could be attributed to excessive accumulation of FOS in the intestine, which may be have adverse effect on the enterocytes and subsequent not absorbed effectively [52, 53].

The findings of the present study demonstrated that dietary FOS has protective effect and could minimize the negative impacts induced by *A. hydrophila* in Nile tilapia. This effect is not a dose –dependent since 30 g dietary FOS did not result in better effect, therefore supplementation of basal diet with 20 g FOS/kg is more recommended for Nile tilapia farms which could improve the physiological status and relieve stress of fish. Moreover, it reduced the deleterious effect of *A. hydrophila* in spleen, liver and kidney tissues.

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