ISSN: 0975-8585

Research Journal of Pharmaceutical, Biological and Chemical Sciences

Ameliorative Effect Of Betaglucan Diet In Oreochromis Niloticus Against Aeromonas Hydrophila.

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ABSTRACT

Fish culture is an important element of many rural development programs in areas suffering from animal protein shortages. Tilapias are one of the most popular fish for culture and have been introduced into many countries around the world. In recent years, attention has been focused on developing tilapia culture. We studied the effects of a dietary supplementation of beta-glucans on Nile tilapia (Oreochromis niloticus). Two-hundred-seventy fingerlings (mean mass ± SD =40.7 ± 0.4 g) were separated into six groups of 270 fish; G1 (control normal) was fed a basal diet, whereas G2 (control infected). G3 (betaglucan 0.5% normal), G4 (betaglucan 1%normal), G5 (betaglucan 0.5% infected), G6 (betaglucan 1% infected). Each group was fed for 12 weeks to evaluate growth performance, and to evaluate immune status and disease resistance and some biochemical and histopathological parameters. The best growth and feed utilization were observed in G4. Serum IgM values were significantly higher in G4 than G1.Fish that were fed the betaglucan had better relative percent survivability after challenge with Aeromonas hydrophilla. We could recommend that dietary supplementation with β-G improves the performance of Nile tilapia and possesses an immunostimulating effects.

Keywords: β-glucan; Nile Tilapia; Aeromonas hydrophilla; Immunostimulant effects.

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ISSN: 0975-8585

INTRODUCTION

Among the wide variety of tilapias, Nile tilapia (Oreochromis niloticus) is the most common in aquaculture and the need for a systematic effort to secure and to further improve the genetic quality (Bentsen et al., 1998).

The Nile tilapia, Oreochromis niloticus, is the most widely cultured tilapia in the world because of its rapid growth, early age of sexual maturity and planktivorous feeding habits. It is the most common fish cultured in Egypt (Abdelghany and Ahmed, 2002).

Betaglucans, polymers of glucose classified as biological response modifiers are structural components of the bacterial cell membrane that have been found to stimulate immunity by increasing resistance to infectious pathogens. In addition, b-glucan-based products have been used commercially to increase productivity and immunity in aquaculture. (Debaulny et al., 1996).

Aeromonas hydrophila, considered as one of the most important bacterial pathogens that causes a great economic losses to fish of either fresh or marine fish due to a high mortality with decreasing the fish weight. Also (**Dhayanithi et al., 2010)** reported that the Aeromonas hydrophila considered as one of the most important stress related diseases that causes a great loss among fish.

Thus , The present study was conducted to evaluate the effect of addition of beta-glucan to nile tilapia diet on growth and health and assess the effect of beta-glucan on some biochemical and immune response of nile tilapia challenged with Aeromonas hydrophila.

MATERIALS AND METHODS

Fish

Two hundred and Seventy (270) Nile tilapia (Oreochromis niloticus) weighing 40± 0.4 gm were obtained from the fish farm in Abbassa, Sharkia, Egypt.

The fish were acclimatized for two weeks in indoor cement tanks supplied with dechlorinated tap-water with continuous aeration. The pH was 7.1 and total hardness 0.95 mM.

The fish were randomly stocked at a rate of 10 fish per 120 L aquarium.

Fish were fed twice daily with standard commercially prepared pellets at 3% of their body weight throughout the period of the experiment.

Betaglucan

They are sugars that are found in the cell walls of bacteria, fungi, yeasts, algae, lichens and plants such as oats and barley. They are used sometimes as medicine, it is available in the diet A powder by using (star fix) commercial product imported by best choice pharma and manufactured by I.C.C company, Brazil contain 210gm/kg (1,3-1,6) Betaglucan.

Diets used for experimental fish:

A standard commercial ration containing approximately 30% crude protein and 5.6% lipid
The commercial diet, vitamins and minerals met the basic dietary requirements of Nile tilapia, according to National Research Center (NRC).

The ingredients were mixed mechanically by the horizontal mixer (Hobarts model D300-T, Troy, OH, USA).
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Induction of Pathogen

Aeromonas hydrophila was previously isolated from naturally infected fish (Oreochromis niloticus) and identified according to the standard bacteriological tests.

It was cultured in nutrient broth (Oxoid) for 24 h at 37 C. The broth culture was centrifuged for 10 min at 3000 r p m.

The supernatant was discarded and the pellets were resuspended in phosphate buffered saline at pH 7.4 (PBS 7.4) and the optical density (OD) of the solution was adjusted to 0.5 at 456 nM, which correspond to 1 _10^7 cells mL_1. This bacterial suspension was serially diluted using standard dilution technique with PBS 7.4 and used for the challenge experiment and bactericidal activity.

Experimental design

A total number of 270 (Oreochromis niloticus) with average body weight 40 gm were divided into six equal tri replicated groups,each replicate contains 15 fish kept in cages in the artificial cement pond for two weeks to be acclimatized before starting the experiment.

These groups included:

Group 1: Control, Group2: Fish fed on diet with Betaglucan 0.5 %, Group 3: Fish fed on diet with Betaglucan 1%. Group 4: Control +ve fish fed on a normal diet and will be challenged by Aeromonas. Group 5: Fish fed on diet with Betaglucan 0.5, and then exposure to Aeromonas infection. Group 6: Fish fed on diet with Betaglucan 1 %, and then exposure to Aeromonas infection.

- The experimental protocol of betaglucan administration was scheduled for 12 consecutive weeks, challenge with A. hydrophila was carried out at the end of the trial and the following parameters were measured:

Serum biochemical analysis:

For determination of Serum Glutamic Pyruvic transaminase (SGPT) ALT, Creatinine and Cortizol.

Evaluation of the immunological parameters:

Humoral immune response (IgM determination)

IgM was measured according to Laemmli (1970).

Histopathological examination was performed according to (Rober, 1989).

Statistical analysis

The obtained data were statically analyzed using analysis variance procedure in SAS (2011).

RESULTS

Survivability rate

The result demonstrated that, G2 showed survivability rate of (95%), while fish groups exposed to 0.5% and 1% betaglucan in the diet and control groups showed a similar survivability rate (100%) as shown in table (1).

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Biochemical and immunological analysis

G4, there was no significant change in ALT, Creatinine, IgM compared to G1, while G4, high in lysozyme, nitric oxide and cortizol than G1. The total leucocytic count was significantly higher in G4 than in G3 and G2.

Histopathological examination

Viewing section in gills of O. niloticus in G5 stained with H&E. showing slight congestion of gill lamellae was the only histopathological change observed in gill lamellae.

A section in the intestine of G2 stained with H&E. showing infiltration of the submucosa with inflammatory cells and eosinophilic granular cells

Section in liver of G2 and section in liver of G5 stained with H&E. showing vacuolar degeneration of hepatocytes.

Table (1): Effect of betaglucan and Aeromonas hydrophilla on survivability rate of Oreochromis niloticus:

Mortality / Groups	1-3	3-6	6-9	9-12	Total mortality no	Total mortality%	Total survivability
Control normal	0	0	0	0	0	0	100
Control infected	0	0	0	2	2	4	95
Beta 0.5 % normal	0	0	0	0	0	0	100
Beta 1 % normal	0	0	0	0	0	0	100
Beta 0.5 % infected	0	0	0	0	0	0	100
Beta 1 % infected	0	0	0	0	0	0	100

Table (2): The outcome of oral diet, supplementation of betaglucan (0.5% and 1%) in healthy and experimentally infected Nile tilapia with Aeromonas hydrophilla on Body weight (g) :N=15 Means± S.E.

Time / Groups	Week 1	Week3	Week6	Week9	Week12
Control normal	23 ± 1.25^{ab}	25.31 ± 1.2^{b}	27.66 ± 0.37^{a}	28 ± 0.99^{bc}	35 ± 0.89
Control infected	17.3 ± 1.66^{c}	20 ± 1.05^{c}	21.33 ± 1.20^{b}	24.6 ± 1.76^{c}	30.3 ± 1.30

ISSN: 0975-8585

Beta 0.5 % normal	20 ±0.67^{ab}	21.3 ± 0.13^{c}	28.66 ± 0.13^{a}	34.3 ± 0.33^{ab}	37 ± 0.87
Beta 1 % normal	25 ± 2.3^{a}	28 ± 1.73^{ab}	33 ± 2.30^{a}	35 ± 1.03^{a}	38.6 ± 1.85
Beta 0.5 % infected	18 ± 0.57^{c}	22.56 ± 0.33^{c}	29 ± 3.26^{a}	32 ± 3.26^{ab}	40 ± 3.51
Beta 1 % infected	26.67±1.05^{a}	30.86 ± 1.63^{a}	33.27±0.872^{a}	35 ± 1.13^{a}	40 ± 0.27

Means of different group within the column having different superscripts are significantly different ($p < 0.05$).

Table (3): The outcome of oral diet, supplementation of betaglucan(0.5% and 1%) in healthy and experimentally infected Nile tilapia with Aeromonas hydrophilla some hematological and biochemical analysis.N=15 means± S.E.

Group	ALT	Creatinine	IgM
Control + normal	24.00^{b} ± 0.58	0.21^{c} ± 0.09	0.15^{c} ± 0.20
control + infection	36.33^{a} ± 1.45	0.63^{a} ± 0.01	0.73^{b} ± 0.02
beta 0.5% + normal	25.33^{b} ± 0.88	0.22^{c} ± 0.22	0.24c± 0.12
beta 1% + normal	23.67^{b} ± 0.88	0.24^{c} ± 0.03	0.20^{c} ±0.06
beta 0.5% + infection	28.33^{b} ± 0.88	0.60^{b} ± 0.05	1.16^{a} ± 0.03
beta 1% + infection	26.00^{b} ± 1.73	0.42^{bc} ± 0.06	1.43^{a} ± 0.19

ISSN: 0975-8585

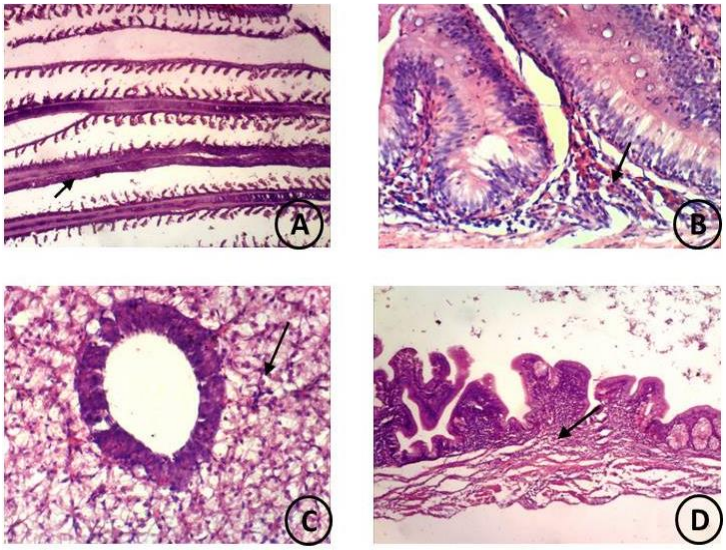

Fig.: (A) Photomicrograph of gills from group 4 showing fusion of gill lamellae (arrow) (H & E X 100).(B)Photomicrograph of intestine from group 1 showing infiltration of the submucosa with inflammatory cells and eosinophilic granular cells (arrow) (H & E X 400). (C) Photomicrograph of intestine from group 1 showing infiltration of the lamina propria and submucosa with inflammatory cells (arrow) (H & E X 100).(D)Photomicrograph of liver from group 2 showing vacuolar degeneration of hepatocytes (arrow) (H & E X 400).

DISCUSSION

B-glucans represent a diverse group of linear and branched polysaccharides functioning as structural or storage components in bacteria, fungi, algae and plants and widely acknowledged for their immunostimulatory capacities as reported in invertebrates, fish and mammals. In fish, a number of studies have demonstrated an immunostimulatory effect of orally administered b-glucan resulting in both increased innate and adaptive responses as well as increased resistance to experimental infection (Jakob Skov et al., 2012).

B-glucans are polymers of glucose found in the cell walls of plants, fungi and bacteria, which have been shown to have immunostimulatory activities in fish. The Nile tilapia recognize these polysaccharides, as foreign agents because of their similarity to fungal or bacterial gram-negative polysaccharides. Numerous studies have reported that b-glucans induce an increase in the resistance of fish to several bacterial pathogens through an increase in the levels of complement and lysozyme as well as an enhancement of the phagocytic activity (Chandra Kanta Misra et al., 2005).

B-glucans are widespread in nature, plant, algae, bacteria, yeast and mushrooms (Dalmo and Seljelid, 1995). They are non-antigenic in animals, but have been shown to be powerful activators of nonspecific defense mechanisms in a wide range of fishes (Kumari and Sahoo 2006 and Guselle et al., 2007).

Tilapias are among the most important warm water fishes used for aquaculture production (Charo-Karisa et al., 2006). The adaptability of tolerance of tilapias to a wide range of environments and intense of cultivation systems has resulted in a rapid expansion of tilapia farming and introduction of these fish in many subtropical and temperate regions of the world.

Dhayanithi et al., (2010) reported that the Aeromonus hydrophilla considered as one of the most important stress related diseases that causes a great loss with a high mortality among fish.

The current study aims at scrutinization of the immunostimulant potentials of betaglucans at doses of 0.5% and 1% diet supplementation for 12 weeks on Nile tilapia in addition to growth performance indices and some histopathological changes after being challenged with Aeromonus hydrophilla.

Our results regarding mortality rate revealed that, infected non treated fish showed survivability rate (96%). While Fish groups exposed to 0.5% and 1% betaglucan in the diet and control groups showed a similar survivability rate (100%).

Absence of mortalities among treated groups with betaglucans could be attributed to that β-glucan enhanced non specific immunity and disease resistance. Cook et al., (2001), Kumari and Sahoo (2006), Selvaraj et al., (2006), Guselle et al., (2007) and Ai et al., (2007) recorded that β-glucan increase disease resistance in Pagrus auratus, Clarias batrachus, Oncorhynchus mykiss, Cypinus carpio and Pseudosciaena crocea respectively.

This bacterial infection causes heavy losses to the producer and health risk to the consumers (Lau et al., 2007). Paniagua et al. (1990) reported that Aeromonas affects both fish and shellfish causing mortalities, loss in body weight, decrease body weight gain, decreasing feed intake with higher feed conversion ratio.

The results concerning economical outcome revealed that, infected, non treated fish showed a significant decrease in body weight when compared to control fish. This nearly agreed with the result obtained by Elmurr (2011). The author mentioned that their was a significant decrease in body weights of fingerlings challenged with aflatoxin compared to normal fish.

Healthy fish, treated with betaglucan (0.5%) in (6, 9, 12) weeks showed a significant increase in body weight when compared to control fish. Interestingly, healthy fish, treated with betaglucan (1%) showed a significant increase when compared to control fish all over the experimental period.

Non infected fish, treated with betaglucan (1%) and infected group, treated with betaglucan (1%) showed the most improvement in body weight when compared to other tested groups.

Infected, non treated fish during (1-3, 3-6, 6-9, 9-12) weeks showed no significant change in body gain when compared to control fish .

Healthy fish, treated with betaglucan (0.5%) in (3-6, 6-9, 9-12) weeks showed significant increase in body weight gain when compared to control fish .

Healthy fish, treated with betaglucan (1%) in (1-3, 6-9) weeks showed no significant changes, when compared to control fish while in (3-6, 9-12) weeks showed significant increase when compared with control fish .

Infected fish, treated with betaglucan (0.5%) in (1-3 ,3-6 ,-6-9 ,9-12) weeks showed significant increase when compared to infected, non treated group. While the infected group, treated with betaglucan (1%) in (1-3 ,3-6 ,-6-9 ,9-12) weeks showed no significant change when compared to infected, non treated fish.

Wu et al., (1997) observed no significant effects of β-glucans in daily feed intake in weanling pigs. But Dritz et al., (1995) found that β-glucans incorporated at 0.025%, significantly improved the daily gain of weanling pigs than that of the control group.

Infected, non treated fish during (1-3, 6-9) weeks showed significant increase in body gain % when compared to control fish while in (3-6) weeks showed significant decrease when compared to control and in (9-12) weeks showed no significant change.

Healthy fish, treated with betaglucan (0.5% in (1-3, 3-6, 6-9)weeks showed significant increase compared to control fish while in (9-12) weeks showed significant decrease when compared to control fish.

Healthy fish, treated with betaglucan (1%) in (1-3 and 3-6) weeks showed significant increases when compared to control fish while in (6 -9, 9-12)weeks showed significant decrease when compared to control fish. There was an agreement with Dritz et al., (1995), they found that β-glucans incorporated at 0.025%, significantly improved the daily gain of weanling pigs than that of control group.

Infected fish, treated with betaglucan (0.5%) in (1-3 ,3-6, 6-9) weeks showed significant improvement in body weight gain when compared to infected, non treated fish.

In regard to feed intake, there was no significance in all tested groups as all fingerlings show a similarity in their feed intake all over the experimental time. Wu et al., (1997) supported our work as they observed no significant effects of β-glucans in daily feed intake in weanling pigs.

Infected, non treated fish during (1-3, 6-9) weeks showed significant decrease in feed conversion ratio when compared to control fish while in (3-6 , 9-12) weeks showed no significant change when compared to control. This was in agreement with the result obtained by Elmurr (2011), who mentioned that there was a non-significant changes feed conversion ratio of fingerlings challenged with aflatoxin compared to normal fish.

Healthy fish, treated with betaglucan (1%) in (1-3, 3-6) weeks showed significant decrease in feed conversion ratio when compared to control fish while in (6-9) weeks showed no significant changes and in (9-12) weeks showed significant increase when compared to control fish. On a similar ground, Selim and Redaa (2015) found that after 30 days of betaglucan administration, it had a significantly higher final body weight, weight gain, and specific growth rate than the control group. The feed conversion ratio after 30 d was significantly lower than in control fish.

Meanwhile, infected fish, treated with betaglucan (0.5%) in (1-3,-6-9) weeks showed significant decrease in F.C.R. when compared to infected, non treated group. While in (3-6) weeks showed significant increase when compared to infected, non treated fish and in (9-12) weeks no significant changes.

Infected group, treated with betaglucan (1%) in (1-3 ,3-6) weeks showed a significant decrease in feed conversion ratio when compared to infected, non treated fish while in (6-9, 9-12) weeks showed significant increase when compared to infected, non treated.

Concerning overall growth performance, infected, non treated fish showed a significant decrease in body weight, feed intake when compared to control fish, while there is a significant increase in body gain, body gain % and no significant changes in feed conversion ratio. Healthy fish, treated with betaglucan (0.5% and 1%) showed a significant increase in all growth performance parameters when compared to control fish, except no significant changes in feed conversion ratio.

Welker et al., (2012) reported that Nile tilapia given diets supplemented with 0.1% β-G showed improvements in weight gain and feed utilization efficiency.

Infected fish, treated with betaglucan (0.5%) showed a significant increase in all growth performance parameters when compared to infected, non treated, except no significant changes in feed conversion ratio. Infected group, treated with Betaglucan (1%) showed a significant increase in all growth performance parameters compared to infected, non treated, except there is a significant decrease in body gain.

Serum transaminases represented in ALT had showed a significant elevation in infected, non-treated fish when compared to healthy ones. This goes hand in hand with the results of Elmurr (2011), who marked a significant increase in serum AST and ALT follows aflatoxin intoxication. This elevation could be attributed to hepatic injury. Healthy fish, treated with betaglucan (0.5% and 1%) showed no significant changes when compared to control fish.

Serum creatinine levels showed a significant elevation in infected, non-treated fish when compared to healthy ones. This was in near disagreement with the results of Elmurr (2011), who marked a non-significant increase in serum creatinine follows aflatoxin intoxication. Our suggestion is Aeromonas hydrophilla may cause an observed renal damage resulting in creatinine elevation. Healthy fish, treated with betaglucan (0.5% and 1%) showed no significant changes regarding serum creatinine levels when compared to control fish.

Infected fish, treated with betaglucan (0.5% and 1%) showed also the same results as no significant changes occurred compared to infected, non treated group.

Infected, non treated fish showed a significant increase in serum IgM levels when compared to control fish.

Healthy fish, treated with betaglucan (0.5%) showed a nonsignificant increase when compared to control fish. The group which administered betaglucan (1%) as an oral diet supplementation for 12 weeks showed a significant increase in serum IgM levels when compared to control fish. Meanwhile, Aeromonas hydrophilla infected groups, treated with betaglucan (0.5% and 1%) showed a significant increase in serum IgM levels compared to infected, non treated.

A diet containing 0.5 g b-1.3/1.6-glucan/100 g of pellets was fed to rainbow trout (Oncorhynchus mykiss) daily for a week and were immunized by immersing them in anti-Yersinia ruckeri vaccine. This resulted in an increased number of antibody-secreting cells (ASC) and specific Ig levels in serum, thus enhanced the effectiveness of Yersinia ruckeri vaccine in fish (Siwicki et al., 2004). However, feeding them with 0.1 % b-glucan for 4 weeks and exposing to 2 h of transportation stress showed an elevated innate immune response (phagocytosis and oxidative radical production) in treated fish and helped to prevent negative effects of stress and protection against Flexibacter columnaris.

In this trial, infected, non treated fish with Areomnas hydrophilla showed a significant increase in serum nitric oxide, when compared to control fish. Nitric oxide (NO) is an important effector molecule on antimicrobial and antitumor effects of macrophages. (1, 3)-beta-D-Glucan (beta-glucan) is well known to show various immunopharmacological effects such as antimicrobial effect and antitumor effect by activating various points of host defense mechanisms (Ohno et al., 1996).

Healthy fish,treated with betaglucan (0.5% and 1%) showed no significant changes in serum NO levels when compared to control fish. Infected fish, treated with betaglucan (0.5% and 1%) showed no significant changes when compared to control fish.

On the other hand, Selim and Redaa (2015) found that after 30 days of betaglucan administration, serum nitric oxide levels were significantly elevated when compared to the normal fingerlings.

Lysosomes contain active proteases, lipases and hydrolytic enzymes called lysozymes which can generate toxic oxidative compounds that assist in microbial degradation, and high levels of lysozyme can therefore be considered as an indicator that the fish is immunocompetent and has produced an immune response against an infection (Mock and Peters 1990, Roos and Winterbourn 2002).

Healthy fish, treated with betaglucan (0.5% and 1%) as oral diet supplementation for 12 consecutive weeks elicited a significant increase in serum lysozyme activites when compared to control fish. Bagni et al., (2005), Jorgensen et al., (1993) and Ai et al., (2007) reported that β-glucan had already significantly increased serum lysozyme levels in sea bass Dicentrarchus labrax, Salmo salar and Pseudosciaena crocea, respectively.

Engstad et al., (1992) found that Atlantic salmon had significant increases in serum lysozyme activity when the beta-glucans were included in their diet over a 3 week period. Studies such as Zhao (2015), which treated channel catfish with Actigen over a period of nine weeks, and Chen and Ainsworth (1992) which treated rainbow trout with beta-glucans for 9 weeks, have found an increased lysozyme activity and an enhanced immune response. Hung (2015) found that channel catfish fingerlings saw a significant increase in serum lysosome levels in those fish which were treated with the inclusion of Actigen to their diet after 10 weeks.

Infected, non treated fish showed a significant increase in serum lysozyme activities, when compared to control fish.

Infected fish, treated with betaglucan (0.5% and 1%) showed no significant changes in serum lysozyme activities when compared to infected, non treated group.

Infected, non treated fish showed a significant increase in serum cortizol levels when compared to control fish. Healthy fish, treated with betaglucan (0.5% and 1%) showed no significant changes in serum cortizol levels when compared to control fish.

Infected groups, treated with betaglucan (0.5% and 1%) showed a nonsignificant decrease in serum cortizol levels compared to infected, non treated group. Stress-induced elevated cortisol levels in plasma were lowest at 0.1 % fed b-glucan group (Jeney et al., 1997).

The histopathological alteration were recognized in the organs of tested O.niloticus was similar in which liver showed vacuolar degeneration of hepatocytes and congestion of hepatopancreas blood vessel.Nearly smilar results concluded by Elmurr (2011), who marked hydropic degenerations and vacuolation of hepatocytes were observed and liver showed portal areas with necrotic pancreatic acini and lymphocytes.

Intestine showed infiltration of the submucosa with inflammatory cells and eosinophilic granular cells and focal infiltration of the lamina propria with inflammatory cells and this agree with Hamilton(1990) in which intestine showed mucinous degeneration and leukocytic infiltration in the submucosa.

Gills showed congestion and fusion of gill lamellae and this agree with (Hamilton, 1990 and Elmurr, 2011) who showed a marked fusion of gill lamellae.

CONCLUSION

This trial has proven with no doubt that betaglucan possess an immunostimulating activity when administered to fish in diet and growth performances were markedly improved as shown in body gain, feed conversion ratio and feed efficiency.

Cultured O.niloticus fed on diet contained 0.5% or 1% betaglucan showed significant increase in average body weight, average body gain, body gain percent and significant increase of feed conversion ratio and has positive effect on some hematological, biochemical, immunological parameters and histopathological finding.

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