Effect of dietary fructooligosaccharide supplementation on feed utilization and growth performance of Nile tilapia (*Oreochromis niloticus*) fingerlings

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Abstract

The present study was conducted to investigate the effect of prebiotic fructooligosaccharide (FOS) on the feed utilization and growth performance of Nile tilapia fingerlings through evaluation the digestive enzymes activities and histological feature of the intestine. Fish were distributed into four groups in well prepared fiberglass tanks and were fed on basal diet without additive (control), 1%, 2%, and 3% FOS for 8 weeks. Results showed that the final weight, weight gain and the specific growth rate were significantly increased with 2% dietary FOS with lower feed conversion ratio compared to the control group. Digestive enzymes activities showed no significant effect ($P > 0.05$) of dietary FOS on intestinal amylase and lipase activities. While, protease activity was significantly higher ($P < 0.05$) especially in group fed 2 % (22.7 ± 2.28 U) dietary FOS compared to the other treated and control groups. Histological examination of the mid-intestine revealed significant increase of villi length and number of mucous cells with increased dietary FOS levels ($P < 0.05$) especially in groups fed 2 and 3% FOS. In conclusion, FOS supplemented diets could improve intestinal enzymes activities, absorptive ability, and histological feature of intestinal villi and consequently increase the feed utilization and growth performance of Nile tilapia fingerlings.

Keywords: Growth performance, Histology, Intestinal enzymes activities, Nile tilapia fingerlings, Prebiotics.

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Introduction

Prebiotics are non-digestible food ingredients that beneficially affect the host by selectively stimulating the growth of and/or activating the metabolism of health promoting bacteria in the gastrointestinal (GI) tract (**Manning and Gibson, 2004**). Recently, prebiotics have been used in aquaculture as an alternative strategy for vaccination and antibiotics administration to reduce the susceptibility of fish to various bacterial fish diseases. The prebiotics such as mannanoligosaccharides (MOS), fructooligosaccharides (FOS) and inulin has proven as preventive and environmentally friendly alternatives dietary supplement to antibiotics in aquaculture, especially for fishes (**Akrami *et al*., 2013**; **Song *et al*., 2014**). FOS is a short chain oligosaccharide that is made up of a glucose molecule linked to 2-4 fructose molecules and it occurs naturally in plants such as onion, chicory, garlic, asparagus and banana (**Sabater-Molina *et al*., 2009**).

Several studies have indicated that fructooligosaccharides (FOS) could improve growth performance and feed utilization of various fish species through improving the ultrastructure of the intestinal mucosa and hence increased gut absorptive area (**Soleimani *et al*. 2012**; **Wu *et al*., 2013**; **Zhang *et al*., 2014a**) as well as activate health promoting bacteria in the intestine (**Zhou *et al*., 2007**). Moreover, it could enhance non-specific immune responses and resistance to bacterial infections (**Buentello *et al*., 2010**; **Zhou *et al*. 2010**; **Zhang *et al*., 2014b**). Improvements in the intestinal morphology and activities of digestive enzymes caused by prebiotics are increasingly important, which contribute to improved growth and feed efficiency.

The strong demand for Nile tilapia, *Oreochromis niloticus* (*O. niloticus*) in our Egyptian market is stimulating the development of intensive tilapia culture due to its desirable characteristics for aquaculture such as rapid growth, tolerance of wide range of water quality parameters, disease resistance, good taste and high market value (**Barcellos *et al*., 1999**). Unfortunately, the rapid expansion and intensification of tilapia farming led to the occurrence of infectious disease causing considerable economic losses. Hence, it is recommended to improve aquafeed with

prebiotics in order to maximizing the nutrient digestibility and utilization and minimizing the fish mortality through the development of health-promoting diets which will lead to higher fish performance and health. Therefore, the present study aimed to investigate the effect of different levels of dietary FOS on the growth performance and feed utilization of *O. niloticus* fingerlings through evaluation the digestive enzymes activities and morphological changes of fingerlings intestine.

MATERIAL AND METHODS

Experimental Fish

Nile tilapia, *O. niloticus* fingerlings (average weight 10.72 ± 0.45 g) were obtained from private fish farm at Kafer El Sheikh Governorate, Egypt. The fish were transported to the Lab of Fish Diseases and Management at Fac. of Vet. Med, Benha University, Egypt and maintained in fiberglass tanks provided with oxygenated dechlorinated tap water for 15 days for acclimation to the laboratory conditions. Fifty percent of the water tanks were changed and uneaten food was siphoned daily to maintain water quality parameters. During acclimation period, fish were fed twice daily to apparent satiation with a commercial diet containing 30% crude protein (Joe Trade Company, Kafer El Sheikh, Egypt).

Preparation of diets and experimental design

The FOS powder (Nutraflora)® used in this study was purchased from GTC Nutrition company (Westchester, USA). Commercial basal diet containing 30 % crude protein and 3.17 % crude lipid was used as the control. This basal diet was supplemented with one of three levels of FOS (0, 1, 2 and 3 %). The graded doses of FOS were dissolved in sufficient amount of water and added onto the crushed basal diet followed by mixing manually to form a stiff dough. The dough was then pelleted using manual mincer and allowed to dry in room temperature. After drying, the diets were broken up into appropriate size and stored in tight plastic bags at 4ºC until use. After acclimation period, fish were divided randomly into four equal groups (30 fish each) in duplicate. The first group was fed on basal diet containing 0% FOS and kept as control. The second, third and fourth groups were fedon diets containing 1, 2 and 3 % FOS

respectively. All experimental groups were fed by hand, twice daily at a rate of 4% of their body weight for 8 weeks. The water temperature was maintained at 28 ±1°C along the experimental period. Excreta and uneaten food were siphoned daily.

Growth performance

At the end of the feeding experiment (8 weeks), all fish were weighted to calculate final weigh, weight gain (WG), specific growth rate (SGR), feed conversion ratio (FCR) and feed efficiency (FE). Calculations were conducted using the following formulae:

WG (g) = FW-IW. Where FW is final weight (g), IW is initial weight (g)

SGR (%) = [(ln FW-ln IW)/T] 100. Where T is the duration of feeding experiment (days).

FCR = FI (g) / WG (g). Where FI is the dry feed intake.

FE = WG (g) / FI (g).

Digestive enzymes assays

Apart of the intestine was carefully taken from six fish per treatment and pooled together (n=3). The samples were homogenized on crushed ice with ten volumes (v/w) of cooled phosphate buffer saline (pH 7.2) using electrical homogenizer. The homogenate was centrifuged at 3000 rpm at 4 °C for 15 min. The supernatant was then collected and stored at -80 °C for subsequent analysis. Amyalse activity was quantified according to **Bernfeld (1995)** while lipase activity was determined according to manufacturer instructions of commercial kits obtained from (Biodiagnostic, Egypt)**.** Protease enzyme activity was measured following the procedures described by **Lowry *et al*. (1951)** using Folin phenol reagent.

Histological analysis

The mid part of intestine was taken for the histological examination. The tissue specimens were fixed in 10% neutral buffered formalin for 48 h, then dehydrated in alcohol, cleared in xylene, and embedded in paraffin wax. Sections were cut and stained with hematoxylin and eosin for general structure, periodic acid Schiff for neutral mucopolysaccharides, and alcian blue for acidic mucopolysaccharides. The fixation and staining

were done as outlined by **Bancroft and Gamble (2001)**. Microvilli lengths were measured with LAS image analysis software version 4.

Statistical analysis

The data obtained were analyzed using a one-way analysis of variance (ANVOA) and Duncan's multiple range tests using SPSS (version 16.0) software. Mean values ± SE were considered significantly different at $P < 0.05$.

RESULTS

The results revealed significant effect ($P < 0.05$) of the FOS administration on growth performance of *O. niloticus* fingerlings as presented in **Table 1**. Fish final weight, weight gain and SGR were significantly increased with 2 % dietary FOS compared to control. There is no significance difference of FCR and FE in all experimental groups compared to control, although 2% dietary FOS recorded lower FCR and higher FE than other groups.

The effect of dietary FOS on the digestive enzyme activities (amylase, lipase and protease) were shown in **Fig 1**. Amylase and lipase activities showed no significance difference ($P > 0.05$) between the FOS treated groups and control. While, protease activity was significantly higher ($P < 0.05$) especially with fish fed 2 % FOS (22.7 ± 2.28 U) and 3 % (15.01 ± 3.53 U) compared to the control group (8.77 ± 1.74 U).

Histological examination of the mid-intestine of Nile tilapia fingerlings revealed significant increased of intestinal villi length with dietary supplementation of FOS (**Fig 2**). The highest average intestinal villi length was recorded among group fed 2 % (47.02 ± 0.5 μm) and 3% (37.74 ± 0.74 μm) compared to the control group (28.92 ± 1.19 μm). General histological features of the mid-intestine of the experimental groups showed significant changes compared to control. The length of villi was highest in fish fed on 2% and 3% FOS (**Figs. 3C, D**). Moreover, fish fed 3% FOS, had branched intestinal villi. The intestinal goblet cells in all groups showed weak PAS reaction (**Fig. 4**) but they were strongly alcian blue positive (**Fig. 5).**

Table 1. Growth performance of Nile tilapia fingerlings fed different dietary levels of FOS for 8 weeks.

Parameters	Control	1 % FOS	2 % FOS	3 % FOS
Initial weight (g)	9.92 ± 0.82	10.34 ± 0.79	11.64 ± 0.93	10.99 ± 0.98
Final weight (g)	30.0 ± 2.85^{b}	30.40 ± 2.65^{b}	40.47 ± 2.55^{a}	36.45 ± 2.19^{ab}
Weight gain (g)	20.08 ± 2.30^{b}	20.06 ± 2.31^{b}	29.43 ± 2.12^{a}	25.8 ± 2.22^{ab}
SGR (%)	1.94 ± 0.1^{b}	1.89 ± 0.13^{b}	2.37 ± 0.13^{a}	2.29 ± 0.2^{ab}
FCR	0.93 ± 0.09^{ab}	0.99 ± 0.11^{a}	0.66 ± 0.06^{b}	0.83 ± 0.13^{ab}
Feed efficiency	1.21 ± 0.11	1.19 ± 0.13	1.61 ± 0.17	1.58 ± 0.21

Values (mean $\pm$ S.E) in the same row with the same or no superscript letters denote non significance difference ($P > 0.05$).
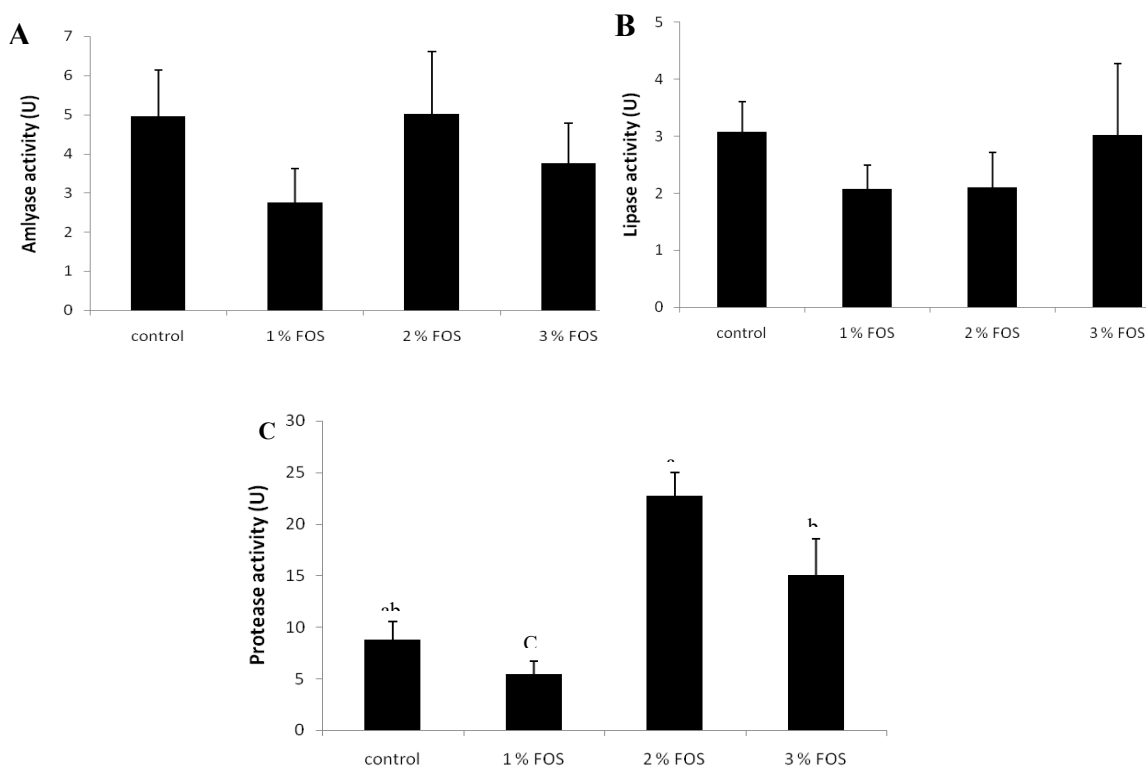

Fig. 1. Digestive enzymes activities (U) of Nile tilapia fingerlings fed different levels of dietary FOS. (A) amylase activity, (B) lipase activity and (C) protease activity. Data represent the mean $\pm$ S.E. Bars with no or have the same superscript letters are considered none significantly differed ($P > 0.05$).
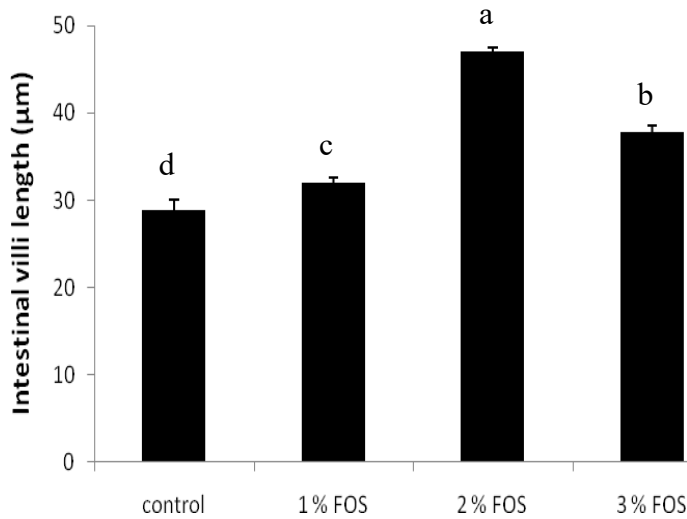
Fig. 2. The villi length in the mid-intestine of Nile tilapia fingerlings fed different dietary levels of FOS (Means ± S.E). Bars with the same superscript letters are not significant ($P > 0.05$).
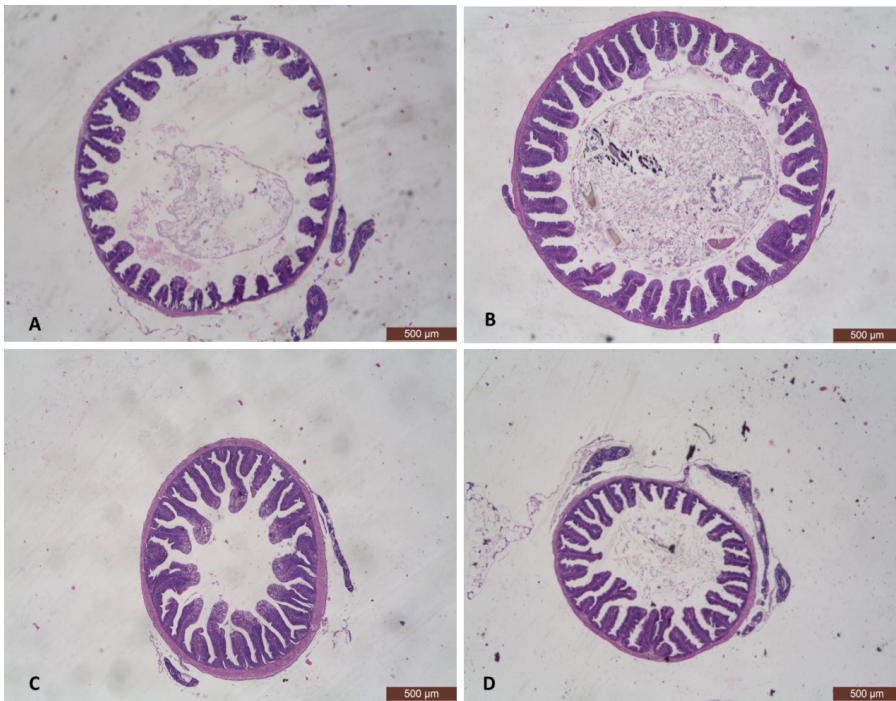

Fig.3. Cross sections in the mid-intestine of Nile tilapia fingerlings stained with H&E for viewing the general histological features of the intestine. (A) Control group; (B) group fed 1% FOS; (C) group fed 2% FOS; (D) group fed 3% FOS. Note presence of branched villi in Figs. D and higher villi length in Figs. C, D
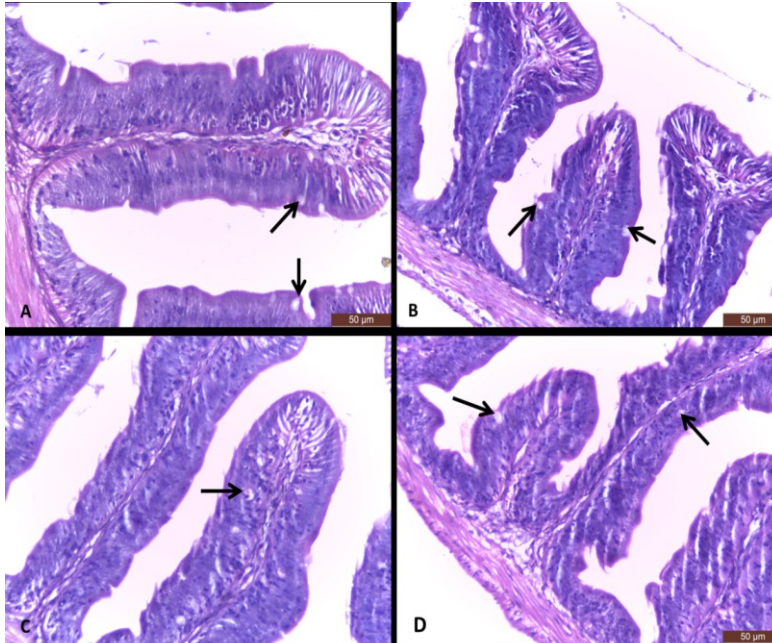
Fig. 4. Cross sections in the mid intestine of Nile tilapia fingerlings stained with PAS technique. (A) Control group; (B) group fed 1% FOS; (C) group fed 2% FOS; D: fish fed 3% FOS. Note weak PAS positive reaction in goblet cells (arrows).
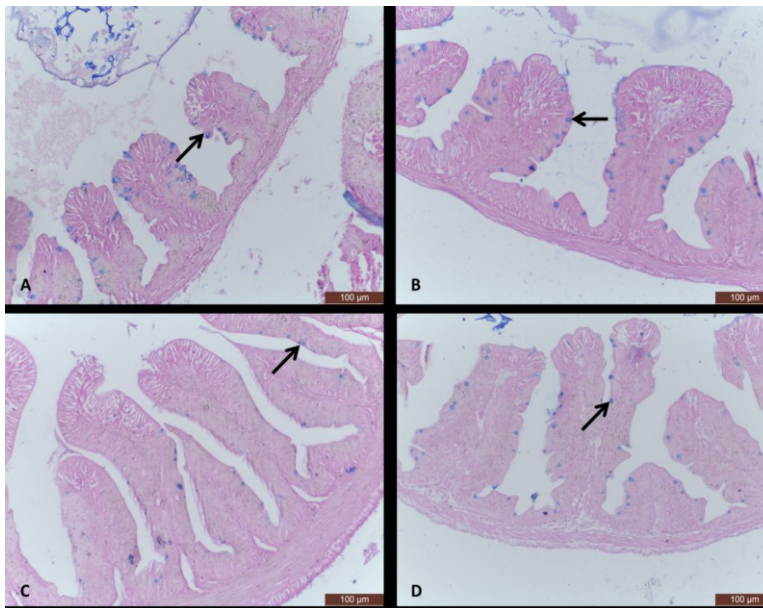

Fig. 5. Sections of the mid intestine of Nile tilapia fingerlings stained with alcian blue stain. (A) Control group; (B) group fed 1% FOS; (C) group fed 2% FOS; D: fish fed 3% FOS. Note alcian blue positive goblet cells (arrows).

DISCUSSION

Prebiotics, which are functional dietary supplements, have shown promise for improving growth performance and feed utilization of fish and shellfish **(Song *et al*., 2014).** In the present work, the dietary supplementation of FOS exerted beneficial effects on the growth performance of Nile tilapia fingerlings. Fish fed diet supplemented with 2% FOS obtained higher WG and SGR as well as lower FCR compared to the control. This improved growth could be ascribed to the digestibility enhancement and morphological changes in the intestine (**Zhang *et al*., 2014a)** through modulation of gut microbiota and/or increased enzymes activities. In this study, protease activity as well as the intestinal villi length were significantly increased with 2 % dietary FOS, which confirmed the result of growth performance. This result coincides with (**Soleimani *et al*., 2012; Wu *et al*., 2013; Zhang *et al*., 2014a).** The enhanced protease activity might be beneficial to the digestion of dietary protein, which might in turn contribute to the better feed utilization of Nile tilapia fingerlings. This supported by the fact that the activities of digestive enzymes as well as length of intestinal villi are positively correlated with fish growth performance which facilitates intestinal digestion and absorption (**Furne *et al*., 2005**). In addition, the improved growth could be attributed to the fact that dietary FOS can indirectly modify fish metabolism through gut fermented end products mainly short chain fatty acids which absorbed by the enterocytes and stimulate the growth of beneficial bacteria, *Bacillus spp*. in the host intestine (**Mahious *et al*., 2006**). In contrast to the previous findings, reports from other studies on different fish species (**Grisdale-Helland *et al*., 2008; Ai *et al*., 2011**; **Guerreiro *et al*., 2015a**) showed that FOS had no beneficial effects on growth performance and feed utilization. This difference may due to fish species, FOS inclusion level, FOS resource, fish size, culture condition and feeding duration (**Wu *et al*., 2013**). It was noted in the present study that, high inclusion level 3 % of dietary FOS has no significant effect on growth performance of Nile tilapia fingerlings compared to the control group, these results were in keeping with those from **Wu *et al*. (2013)** who found that inclusion level of dietary FOS 8

g/kg diet for 8 weeks, decreased growth performance of blunt snout bream, *Megalobrama amblycephala* fingerlings compared with that of 4 g/kg diet. This indicated that dietary supplementation of high level of FOS could reduce its efficacy due to the inability of intestinal microbiota to ferment excessive levels and subsequently accumulated in the intestine, which may be deleterious to the enterocytes (**Hoseinifar *et al*., 2011**).

Concerning to intestinal amylase and lipase activities, fish fed supplemented diet with FOS showed no significant effect compared to control fed basal diet. These results agree with (**Ye *et al*., 2011; Wu *et al*., 2013; Zhang *et al*., 2014a**). In contrast, other studies recorded significant increase of amylase and lipase activity (**Soleimani *et al*., 2012; Guerreiro *et al*., 2015b**). This difference may be attributed to fish species, size and dietary dose. This attribution supported by **Wu *et al*. (2013)** who reported that amylase activity of blunt snout bream fingerlings exhibited no significance difference with low dietary inclusion of FOS at a level of (0.5- 2 g/kg diet) while at a dose level 4 and 8 g/kg diet revealed significant increase in amylase activity compared with the control.

Histological examination of the mid-intestine which plays an important role in food absorption and fish immunity (**Ellis, 2001**), revealed that villi length and number of goblet cells of *O. niloticus* fingerlings were significantly increased with increased dietary FOS levels ($P < 0.05$) especially in groups fed 2 and 3% FOS. The increase of intestinal villi length could improve absorptive surface area, which resulted in better nutrient utilization of *O. niloticus* fingerlings. Similarly, it has been reported in previous studies that dietary supplementation of FOS improved intestinal microvilli length (**Wu *et al*., 2013; Zhang *et al*., 2014a**). However, **Guerreiro *et al*. (2015b)** recorded no difference in intestinal morphology of turbot, *Scophthalmus maximus* juveniles fed dietary FOS.

In this work, it was clear that intestinal goblet cells of *O. niloticus* fingerlings increased with dietary FOS, suggesting the important non specific immunity role of dietary FOS in the prevention of pathogen invasion (**Cain *et al*., 1996**) through the increased number of goblet cells

which contains immunological substances such as glycoprotein, cytokines, lysozyme, lipoprotein, complement, lectins (**Concha *et al*., 2003**; **Tsutsui *et al*., 2005**) as well as antibodies (**Cain *et al*., 2000**). Moreover, the goblet cell in the current study has acidic mucosubstances evidenced by strongly alcian blue positive stain. The acid mucosubstances are responsible for regulation the transfer of proteins or a fragment of them as well as of ions and fluids (**Petrinec *et al*., 2005**). This finding confirmed the significant improvement of feed utilization and growth performance of Nile tilapia fingerlings. In addition, acidic mucins have been proposed to protect the intestinal epithelium against the degradative actions of bacterial glycosidases (**Carrasson *et al*., 2006**).

CONCLUSION

It could be concluded that dietary supplementation of FOS has beneficial effects on feed utilization and growth performance of Nile tilapia fingerlings through improving the intestinal enzymes activities and intestinal villi length and number of goblet cells with a trend towards best result at a concentration of 2% dietary FOS in aquafeed.

ACKNOWLEDGMENTS

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تأثير الفركتواوليجوسكريد كمكمل غذائى علي الاستفادة الغذائية وأداء النمو فى اصبعيات البلطي النيلي

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¹ قسم امراض ورعاية الاسماك- كلية الطب البيطرى- جامعة بنها-مصر

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أجريت هذه الدراسة لمعرفة تأثير الفركتواوليجوسكريد على الاستفادة الغذائية واداء النمو فى إصبعيات البلطي النيلي من خلال تقييم نشاط الإنزيمات الهضمية والصورة النسيجية للأمعاء. تم توزيع الأسماك في احواض فيبركلاس مجهزة جيدا إلى أربع مجموعات ،وتم تغذيتها على العليقة الأساسية بدون اضافات (المجموعة الضابطة) و ١٪ و ٢٪ و ٣٪ الفركتواوليجوسكريد لمدة ٨ أسابيع. وأظهرت النتائج أن الوزن النهائي والوزن المكتسب و معدل النمو المحدد ازدادت بشكل ملحوظ مع جرعة الفركتواوليجوسكريد ٢٪ مع انخفاض نسبة التحويل الغذائي مقارنة بالمجموعة الضابطة. لم يظهر الفركتواوليجوسكريد أي تأثير معنوي على نشاط انزيم الأميليز والليباز المعوي بينما كان هناك تاثير معنوى على نشاط الأنزيم البروتيني وخصوصا في الاسماك التى تم تغذيتها على ٢% فركتواوليجوسكريد (٢٢,٧±٢,٢٨ U) مقارنة بالمجموعة الضابطة وباقى المجموعات المعالجة. كشف الفحص النسيجي للامعى الاوسط زيادة معنوية في طول الخملات المعوية و عدد الخلايا المخاطية مع زيادة جرعة الفركتواوليجوسكريد وخاصة في المجموعات المغذاة على ٢ و ٣٪ فركتواوليجوسكريد. وكان الاستنتاج أن العليقة المكملة غذائيا بالفركتواوليجوسكريد أدت الى تحسين نشاط الإنزيمات المعوية، والقدرة الامتصاصية، والصورة النسيجية للخملات المعوية، وبالتالي زيادة الاستفادة الغذائية ومعدل اداء النمو فى إصبعيات البلطي النيلي.