Effect of Dietary Xylanase on Growth Performance, Digestive Enzymes and Physiological Responses of Nile Tilapia, *Oreochromis Niloticus* Fingerlings Fed Plant-Based Diets

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Abstract

A feeding study was conduct to investigate the effect of dietary supplementation of xylanase on growth performance, feed utilization, digestive enzymes, proximate composition, hematological and serum biochemical parameters of Nile tilapia, *Oreochromis niloticus* fingerlings fed plant-based diets for 70 days. Three isonitrogenous (293 g kg^{-1} crude protein) and isocaloric (18.42 MJ kg^{-1} gross energy) diets were formulated. Each diet was supplemented with xylanase at levels; 0 (control), 0.5 and 1 g kg^{-1} diet. After 70 days, the highest weight gain, specific growth rate, protein efficiency ratio, protein productive value, fat retention, energy retention, and the best feed conversion ratio were recorded in fish fed either 0.5 or 1 g xylanase kg^{-1} diet. As well as, the addition of xylanase up to 0.5 g kg^{-1} diet increases the activity of lipase, amylase, and trypsin. The addition of xylanase significantly improved hemoglobin, hematocrit, red blood cells, white blood cells, total protein, albumin, and globulin compared with the control diet. On the other hand, the addition of xylanase increased low-density lipoprotein cholesterol. The highest values of growth hormone and phosphorus were observed in fish fed diets supplemented with 0.5 g xylanase kg^{-1} diet. Based on the obtained findings, it could be conclude that the useful using of xylanase as feed additive up to 0.5 g kg^{-1} diet for significantly enhancement the growth performance, feed utilization, endogenous enzyme activity, and physiological responses of *O. niloticus*.

Keywords: Tilapia, Exogenous xylanase, Plant protein, Growth, Physiological responses.

Introduction

Aquaculture is one of the fastest-growing sectors in the world and is expected to continue growing rapidly in the foreseeable future **(FAO, 2018)**. The feed is the most expensive item in aquaculture industry subsequently, continuous growth and intensification of aquaculture production depends upon the development of sustainable cheap protein sources instead of fishmeal (FM) in aquafeeds that is widely used as a source of protein in the aquafeed industry (**Hassaan *et al.*, 2015; Hassaan *et al.*, 2018)**. Increasing the manufacturing cost of FM allows feed manufacturers to search for alternative sustainable cheap sources of proteins other than FM such as plant protein and their by-products (**Tacon and Metian, 2015**). In recent years, several alternative plant protein sources in tilapia diets have been investigated to reduce FM levels in diets, such as cottonseed meal (**El-Saidy and Gaber, 2004**), okara meal (**El-Saidy, 2011**), fermented soybean meal (**Hassaan *et al.*, 2015**), soy protein concentrate (**Ribeiro *et al.*, 2016**), Jatropha meal (**Hassaan *et al.*, 2016**), corn protein concentrate (**Khalifa *et al.*, 2018**) and fermented sunflower meal (**Hassaan *et al.*, 2018**), which were showed to be suitable as partial replacer of FM. However, total replacement of FM with plant protein has generally resulted in decrease in fish growth performance and feed utilization due to the presence of anti-nutritional factors (ANFs) like the non-starch polysaccharides (NSPs) (**Sinha *et al.*, 2011**) or imbalance in essential amino acids (**Cheng *et al.*, 2013**).

Exogenous enzymes are widely used in feed industry to mitigate the problem of ANFs especially in plant protein diets, thereby improving the nutritive value (**Forster *et al.*, 1999**), nutrients utilization (**Cheng *et al.*, 2013**), and animal growth (**Cheng *et al.*, 2013; Saputra *et al.*, 2016**). Furthermore, aquatic animals lack certain digestive enzymes like xylanase, so supplement plant protein diets with xylanase improve nutrients utilization through their ability to degrade NSPs in plant cell walls into xylose accordingly improve the efficiency of feed utilization (**Ghosh and Mukhopadhyay, 2006**). Xylanase has been widely used in the poultry (**Pirgozliev *et al.*, 2015**) and livestock (**Passos *et al.*, 2015**) industries to enhance energy and nutrient availability of plant ingredients, but little information are available about its application in aquafeed industry. Therefore, the present study was designed to evaluate the effect of the graded levels (0.5 and 1 g kg^{-1} diet) of xylanase on growth performance, endogenous enzyme activity, chemical composition, hematological, and serum biochemical parameters of Nile tilapia, *Oreochromis niloticus* fingerlings for 70 days.

Materials and Methods

Experimental fish and culture technique

Mono-sex *O. niloticus* L. all-male fingerlings were obtained from a private farm (El-Sahaba hatchery, Tolmbat 7, Kafr Elsheikh Governorate, Egypt). Fish

were acclimated to the experimental conditions in a concrete pond (4×2×1.25 m) for two weeks in the Experimental Fish Farm, Faculty of Agriculture, Benha University, Egypt. During the acclimation period, fish fed a commercial control diet (30% crude protein) at a rate of 3% of the biomass, which was provided equal rations at 09:00 am and 3:00 pm to adapt to the artificial diet and the trial conditions. After the acclimatization period, the experimental fish were randomly distributed into six experimental fiberglass tanks (0.5 m^3 for each) representing three treatments studied. A total of 360 mono-sex *O. niloticus* fingerlings with an average initial weight of 10.3 ± 0.039 g were use in this trail. Fish randomly stocked with a rate of 20 fish per each tank (0.5 m^3 for each, 40 Fish/m^3), as two tanks (replications) for each treatment. Tilapia were hand-fed with the respective diet 3% of biomass three times daily at 09.00 am, 11.00 am and 3.00 pm. Fish were weighed every two weeks to adjusted the amount of feed fish according to the changes in body weight through the experimental period. The experimental tanks were housed in the green house and was supplied with underground water. Each tank supplied with an automatic heater (150 watts) to maintain the rearing fish water temperature at 26 – 28 ºC. About one-third of the water volume in each tank was daily replace by aerated freshwater after removing the accumulated excreta. All tested water quality criteria (temperature, pH value, dissolved oxygen (DO) and total ammonia) were suitable and within the acceptable limits for rearing Nile tilapia *O. niloticus* fingerlings **(Boyd, 1990)**.

Experimental diets

Three isonitrogenous (293 g kg^{-1} crude protein) and isocaloric (18.42 MJ kg^{-1} gross energy) experimental diets were formulated and the proximate chemical composition of the experimental diets presented in Table 1. The free xylanase basal diet was used as a control group. The other two diets supplemented with 0.5 and 1 g xylanase kg^{-1} diet (xylanase as a product of Huvepharma, Antwerp, Belgium). All ingredients of diets were blended for 5 mins and thoroughly mixed with soybean oil and made into dry pellets using a laboratory pellet mill (A California Pellet Mill, San Francisco, CA, USA) at the National Institute of Oceanography and Fisheries, Cairo Governorate, Egypt, the temperature of pellets in this stage did not exceed than 40 ºC. The pellets (2-mm die) dried for 4 h at opened air and stored at –20 °C until used.

Table 1. Ingredients (g kg^{-1} diet) and proximate composition of the experimental diets (% on dry matter basis)

Ingredients	Experimental diets		
	Control	0.5 g xylanase kg^{-1}	1 g xylanase kg^{-1}
Soybean meal (44% CP)	450	450	450
Corn gluten	60	60	60
Yellow corn	170	170	170
Wheat bran	150	149.50	149
Rice polishing	100	100	100
Soybean oil	40	40	40
Lysine	5	5	5
Methionine	5	5	5
Vit. & Mine.[1]	15	15	15
Vitamin C	5	5	5
Xylanase (g kg^{-1})	0	0.5	1
Chemical analysis %			
Dry matter	89.53	89.03	89.01
Crude protein (CP)	29.32	29.30	29.22
Crude lipid	6.06	6.06	6.03
Ash	5.07	5.06	5.03
Crude fiber	6.47	6.40	6.35
NFE[2]	53.08	53.18	53.37
Gross energy (MJ kg^{-1})[3]	18.42	18.43	18.44

[1] Each one Kg of vitamins and minerals mix. contains $MnSO_4$, 40 mg; Mg O, 10 mg; K_2SO_4, 40 mg; $ZnCO_3$, 60 mg; KI, 0.4 mg; $CuSO_4$, 12 mg; Ferric citrate, 250 mg; Na_2 SeO_3, 0.24 mg; Co, 0.2 mg; retinol, 40000 IU; cholecalciferol, 4000 IU; α-tocopherol acetate, 400 mg; menadione, 12 mg; thiamine, 30 mg; riboflavin, 40 mg; pyridoxine, 30 mg; cyanocobalamin, 80 mcg; nicotinic acid, 300 mg; folic acid, 10 mg; biotin, 3 mg; pantothenic acid, 100 mg; inositol, 500 mg.

[2] NFE (Nitrogen free extract) =100 - (crude protein + lipid + ash +fibre content).

[3]Gross energy calculated using gross calorific values of 23.63, 39.52 and 17.15 kj g^{-1} for protein, fat and carbohydrate, respectively according to **Brett (1973).**

Growth and feed utilization

Records of live body weight (g) was measured in all experimental fish for each pond and were registered every 14 days (two weeks) during the experimental period (70 days). Growth performance and feed utilization indices parameters were calculated by using the following equations: Weight gain (WG) = final body weight (g) – initial body weight (g); Specific growth rate (SGR % days^{-1}) = $\frac{lnW2-lnW1}{t} x100$, where: ln = the natural log; W_1 = first fish weight; W_2 = the following fish weight in grams; t = period in days. Feed conversion ratio (FCR) = feed intake (g)/weight gain (g); Protein efficiency ratio (PER) = weight gain (g)/protein ingested (g); Protein productive value (PPV) % = ((protein gain (g)/protein intake (g)) × 100; Fat retention (FR) % = Fat gain (g)/fat intake (g) ×100; Energy retention (ER) % = Energy gain (g)/ (energy intake) ×100.

Chemical composition

The proximate chemical composition of fish and diet samples was determined according to the procedures of **AOAC (1995)**. Dry matter (DM) was measured after drying the samples in an oven (105ºC) for 24 h. Ash estimated by incineration at 550 ºC for 12 h. Crude protein was determined by micro-Kjeldahl method, N% × 6.25 (using Kjeltechauto analyzer, Model 1030, Tecator, Höganäs, Sweden) and crude fat by Soxhlet extraction with diethyl ether (40 - 60ºC). Crude fiber content was determined using the method of **Van Soest *et al.* (1991)**. The nitrogen-free extract was compute by taking the sum of values for crude protein, crude lipid, crude fiber and ash content then subtracting this sum from 100.

Determination of digestive enzymes activity

Samples of intestine from three fish in each treatment were immediately homogenized in 10 volumes (w v^{-1}) of ice-cold physiological saline solution and centrifuged at 5000 g for 15 min. at 4ºC; then the supernatant was stored for endogenous enzymes activity analysis **(Furné *et al.*, 2008)**. Trypsin activity was measured by using methods of **Hummel (1959)**. Lipase activity was determine by a method described by **Zamani *et al.* (2009)**, titration method detailed by using olive oil-gum. Amylase activity estimated according to **Bernfeld (1951)** at 540 nm, starch used as a substrate.

Blood sampling for hematological and biochemical indices

At the end of the experiment, three fish were randomly selected from each treatment and euthanized with tricaine methanesulfonate 1 g L^{-1} for 5 minutes to collect the blood samples from the caudal vein of fish in all treatments and were divided into two portions. The first portion was collected with anticoagulant 10% ethylene diamine tetraacetate (EDTA) to determine the hematocrit (Htc), hemoglobin (Hb), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH) and mean corpuscular hemoglobin concentration (MCHC), red blood cells (RBCs) count and total white blood cells (WBCs) count were done according to standard methods as described by **Rawling *et al.* (2009)**. The second portion of the blood sample collected without anticoagulant, allowed to clot at 4 °C, and centrifuged at 3000 rpm for 10 min. to obtain the blood serum. The non-hemolyzed serum was collected and stored at −20 °C until used for measuring the serum biochemical parameters. Serum total protein and albumin were determined according to **Henry (1974)** and **Wotton and Freeman (1982)**, respectively. However, globulin was calculated by subtracting albumin from total protein according to **Coles (1974)**. Serum growth hormone (GH) was measured by a radioimmunoassay (RIA) kit for Tianjin Nine Tripods Medical and Bioengineering Co., Ltd. (Tianjin, China), following the manufacturer's protocol. Serum total cholesterol, triglyceride, high-density lipoprotein cholesterol (HDL-C), low-density lipoprotein cholesterol (LDL-C) and phosphorus were spectrophotometrically measured using commercial kits produced by Pasteur labs (Egyptian American Co. for Laboratory Services, Egypt).

Statistical analysis

All the obtained data were statistically analyzed by using SAS software (version 9.1) (**SAS, 2004**). All data submitted to a one-way analysis of variance (One-way ANOVA). Duncan's multiple range test was used to compare differences between treatment means when significant values were observed **(Duncan 1955)**, at ($P < 0.05$) level.

RESULTS

Growth performance and feed utilization

The growth performance and feed utilization of *O. niloticus* fed the experimental diets are illustrated in **Table 2.** Fish fed diet supplemented with 1 g xylanase kg^{-1} had the highest final body weights (FBW), WG and SGR. Feed intake was increased significantly with increasing the level of xylanase. Addition of xylanase to the feed also produced the better FCR, PER, PPV, FR, and ER with significantly ($P = 0.0001$) values higher than those un-supplemented diet with xylanase (control), more specifically in case of fish treated with 1 g xylanase kg^{-1} diet.

Table 2.Growth performance and feed utilization of *O. niloticus* fed plant-based diets supplemented with xylanase

Items	Treatments			± SEM	*P*-values
	Control	0.5 g xylanase kg^{-1}	1 g xylanase kg^{-1}		
IBW (g/fish)	10.30^{a}	10.50^{a}	10.52^{a}	0.0398	0.3176
FBW (g/fish)	22.20^{b}	26.30^{a}	28.15^{a}	0.2589	0.0001
WG (g/fish)	11.90^{b}	15.80^{a}	17.63^{a}	0.2259	0.0001
SGR (% $days^{-1}$)	1.10^{b}	1.42^{a}	1.40^{a}	0.0110	0.0001
FI (g/fish)	25.78^{b}	27.52^{a}	27.56^{a}	0.2121	0.0001
FCR	2.16^{a}	1.74^{b}	1.56^{c}	0.0385	0.0001
PER	1.81^{b}	2.10^{a}	2.03^{a}	0.0115	0.0001
PPV (%)	26.12^{b}	35.25^{a}	34.41^{a}	0.5611	0.0001
FR (%)	35.57^{b}	47.88^{a}	45.24^{a}	0.8087	0.0001
ER (%)	13.97^{b}	18.20^{a}	18.56^{a}	0.3052	0.0001

- Values (± SEM, *n* = 3). Mean in the same row sharing the same superscript are not significantly different (P > 0.05).

Endogenous enzymes activity

Table 3 showed that the supplementation of xylanase in the plant-based diets of Nile tilapia significantly increased the digestive enzymes. The highest activity of lipase, amylase, and trypsin at a level of 0.5 g xylanase kg^{-1} diet.

Table 3. Lipase, amylase, trypsin and chymotrypsin activities of *O. niloticus* fed plant-based diets supplemented with xylanase

Digestive enzyme	Treatments			± SEM	*P*-values
	Control	0.5 g xylanase kg^{-1}	1 g xylanase kg^{-1}		
Lipase U g^{-1}	3.75^{b}	5.30^{a}	3.40^{c}	0.1415	0.0001
Amylase U g^{-1}	4.25^{b}	6.55^{a}	4.20^{b}	0.1814	0.0007
Trypsin U g^{-1}	26.00^{c}	41.50^{a}	29.40^{b}	0.8540	0.0008

- Values (± SEM, *n* = 3). Mean in the same row sharing the same superscript are not significantly different (P > 0.05).

Chemical composition of whole fish

As illustrated in Table 4, the addition of the graded levels (0.5 or 1 g kg^{-1} diet) of xylanase to plant-based diet for *O. niloticus* significantly decreased the dry matter (P = 0.0001) and the crude lipid (P = 0.0001) content. On the other hand, the opposite trend was observed for the crude protein (CP) (P = 0.0001) and ash (P = 0.0001) content where they were significantly increased.

Table 4. Proximate analysis (% on dry matter basis) of *O. niloticus* fed plant-based diets supplemented with xylanase

Proximate composition	Treatments			± SEM	*P*-values
	Control	0.5 g xylanase kg^{-1}	1 g xylanase kg^{-1}		
Dry matter	26.35^{a}	25.45^{b}	25.2^{b}	0.0612	0.0001
Protein	62.25^{a}	63.85^{b}	63.75^{b}	0.0540	0.0001
Lipid	18.55^{a}	17.45^{b}	16.85^{b}	0.0346	0.0001
Ash	15.65^{b}	16.90^{a}	17.10^{a}	0.0641	0.0001

- Values (± SEM, *n* = 3). Mean in the same row sharing the same superscript are not significantly different (P > 0.05).

Hematological parameters

Data in Table 5 showed that fish fed supplemented diet with (0.5 and 1 g kg^{-1} diet) of xylanase was significantly (P = 0.0001) increased the values of Hb, Htc, MCV, MCH, MCHC, RBCs, and WBCs count compared with the control group.

Table 5. Hematological indices of *O. niloticus* fed plant-based diets supplemented with xylanase

Hematology	Treatments			± SEM	*P*-values
	Control	0.5 g xylanase kg^{-1}	1 g xylanase kg^{-1}		
Hemoglobin (g/dL^{-1})	5.55^{c}	11.35^{a}	9.55^{b}	0.0254	0.0001
Hematocrit (%)	18.25^{b}	30.65^{a}	31.40^{a}	0.6858	0.0001
MCV (fl)	122.5^{c}	145.50^{a}	135.50^{b}	0.3469	0.0001
MCH (g/dL)	57.50^{c}	60.50^{a}	58.50^{b}	0.2886	0.0001
MCHC (pg)	39.50^{c}	41.50^{b}	43.00^{a}	0.2500	0.0001
RBCs ($\times 10^{6}$ µL)	1.84^{c}	3.65^{a}	3.27^{b}	0.0259	0.0001
WBCs ($\times 10^{3}$ mm^{-3})	87.70^{b}	125.80^{a}	125.60^{a}	0.2031	0.0001

- Values (± SEM, *n* = 3). Mean in the same row sharing the same superscript are not significantly different (P > 0.05).

Biochemical blood parameters

According to the obtained data of Table 6, values of the serum of total protein (P = 0.0022), albumin (P = 0.0005) and globulin (P = 0.0001) were significantly affected by the addition of different levels of xylanase (0.5 or 1 g kg^{-1} diet). On the other hand, addition of xylanase with both tested levels significantly decrease the total cholesterol (P = 0.0001), and HDL-C (P = 0.0002), but significantly increase triglyceride (P = 0.0001), and LDL-C (P = 0.0001) compared with the control group. As noted in Figures 1 and 2 serum GH, and phosphorus were significantly (P < 0.05) increased by the addition of different levels of xylanase (0.5 or 1 g kg^{-1} diet) compared with the control group, and the highest levels were obtained in fish fed diet supplemented with 0.5 g xylanase kg^{-1} diet.

Table 6. Blood biochemical parameters of *O. niloticus* fed plant-based diets supplemented with xylanase

Items	Treatments			± SEM	*P*-values
	Control	0.5 g xylanase kg^{-1}	1 g xylanase kg^{-1}		
Total protein (g dl^{-1})	4.85^{c}	6.40^{a}	5.60^{b}	0.0803	0.0022
Albumin (g dl^{-1})	1.25^{b}	1.35^{a}	1.35^{a}	0.0289	0.0005
Globulin (g dl^{-1})	3.65^{b}	4.15^{a}	4.05^{a}	0.0288	0.0001
Cholesterol (mg dl^{-1})	198.00^{b}	178.00^{c}	208.00^{a}	0.5204	0.0001
Triglyceride (mg dl^{-1})	$140.\ 00^{c}$	$185.5.00^{a}$	189.00^{a}	0.4356	0.0001
HDL-C (mg dl^{-1})	38.00^{a}	38.50^{a}	35.50^{b}	0.3938	0.0002
LDL-C (mg dl^{-1})	88.50^{b}	108.00^{a}	103.00^{a}	0.3469	0.0001

- Values (± SEM, *n* = 3). Mean in the same row sharing the same superscript are not significantly different (P > 0.05).

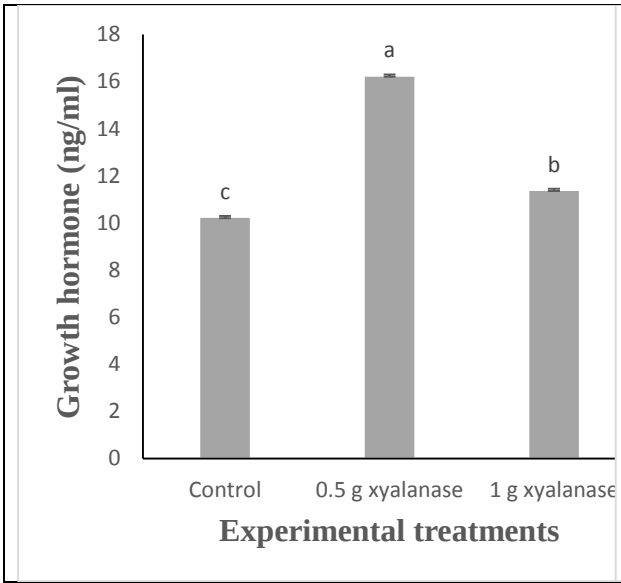

Fig. 1. Serum growth hormone (GH) of O. niloticus fed plant -based diets supplemented with xylanase (0.5 and 1 g kg^{-1} diet

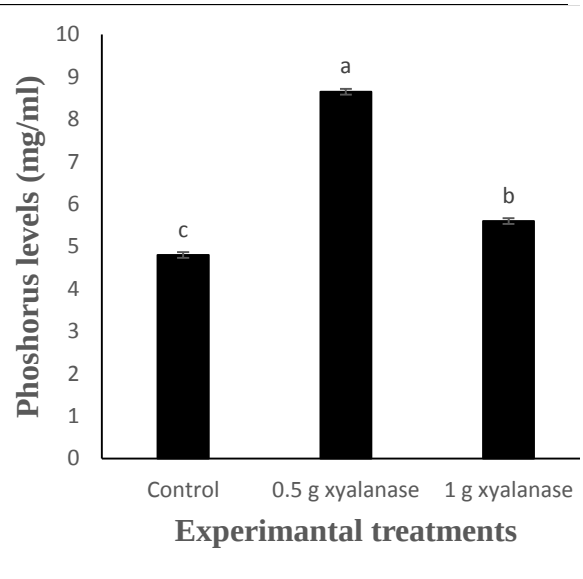

Fig. 2. Serum phosphorus (P) of *O. niloticus* fed plant -based diets supplemented with xylanase (0.5 and 1 g kg^{-1} diet

Discussion

The presence of ANFs in the plant-based diets reduced the utilization of nutrients of feed staff, which bind nutrients and reduce their bioavailability. Exogenous enzyme supplementation could be mitigated the effects of ANFs and enhance the nutrients assimilation (**Farhangi and Carter, 2007; Lin *et al.*, 2007; Soltan, 2009**). Xylanase was incorporated to feed contained a high amount of plant protein with the purpose of increasing NSPs, thus enabling improved carbohydrate utilization (**Adeola and Cowieson, 2011; Hassaan *et al.*, 2019a**) which has been shown to have beneficial effects on growth and physiological state of fish. In the present study, all parameters of growth performance; FBW, WG and SGR of Nile tilapia fed plant protein diet were improved by the addition of xylanase (0.5 g or 1 g kg^{-1} diet). The improved in growth and feed utilization of fish fed plant-based diets supplemented with xylanase could be contributed to the degradation of NSPs to the level that the viscosity property of these fractions is largely reduced (**Jiang *et al.*, 2014; Hassaan *et al.*, 2019a**). The suppression of nutrients utilization by the addition of exogenous enzymes also due to their role for eliminating the ANFs effect (**Farhangi and Carter, 2007; Lin *et al.*, 2007; Soltan, 2009**). The improved growth performance of Nile tilapia was showed with

Natuzyme50® supplementation was attributed to the presence of enzymes such as cellulase, xylanase and phytase in the cocktail that are not naturally produced by fish (**Hlophe-Ginindza *et al.*, 2016**). **Yildirim and Turan (2010)**, **Ghomi *et al*. (2012)**, and **Zamini *et al.*, (2014)** found a positive effect of various commercial multi-enzyme complexes (phytase, xylanase, β-glucanase, β-amylase, cellulase and pectinase) on the growth performance and feed efficiency of African catfish (*Clarias gariepinus*), great sturgeon (*Huso huso*) and Caspian salmon (*Salmo trutta*), respectively. However, no specified plant-based feedstuffs were used in any of these studies. In contrast, **Ogunkoya *et al.* (2006**) indicated that a commercial enzymes cocktail (xylanase, amylase, cellulase, protease, and β-glucanase) supplementation in soybean based diets did not affect the growth performance of rainbow trout.

Digestive enzymes play an important role in digestion of the nutrients for fish (**Wen *et al.*, 2009; Adeoye *et al.*, 2016; Hassaan *et al.*, 2019b**). The present findings showed that the highest activities of digestive enzymes recorded in diet supplemented with 0.5 g xylanase kg^{-1} diet. This improved in digestive enzymes attributed to inhibit ANFs, high levels of fiber, and NSPs in plant-based diet, which increased the digestion (**Hassaan *et al.*, 2019b**). Several authors showed that exogenous enzymes improved the activity of endogenous enzymes (**Lin *et al.*, 2007; Wei *et al.*, 2010; Jiang *et al.*, 2014; Hlophe-Ginindza *et al.*, 2016**). This enhancement in the activity of endogenous enzymes may be attributed to the role of xylanase in the degradation products of arabinoxylans, hydrolyze cell wall components in the plant material, thereby it reduced the molecular size characteristics of NSPs content of the plant materials (**Sinha *et al.*, 2011; Ganguly *et al.*, 2013**).

Hematological indices regularly monitoring the information of physiological responses and nutritional status affecting aquatic animals (**NRC, 2011**). The current findings showed that Hb, Htc, RBCs and WBCs was significantly improved in fish fed plant-based diet supplemented with xylanase may be associated with decrease the ANFs binding iron and amine group of amino acids which in turn lowers their availability in the blood and increases the erythrocytes (**Soltan, 2005**). According to the increased number of RBCs multiplies the concentration of Hb ultimately resulting in a higher capacity for oxygen carrying in fish. While, **Zamani *et al.* (2009)** noted that no significant ($P > 0.05$) difference of Htc, Hb, RBCs, MCV, MCH and MCHC ($P > 0.05$) in Caspian salmon (*Salmo trutta caspius*) fed supplemented diet with exogenous enzymes (Natuzyme® and Hemicell®). The highest WBCs count also indicated that multi-enzymes including β-mannans, xylanase, in Hemicell and Natozyme crossing the intestinal mucosa are potent stimulators of the innate immune system, resulting in increased proliferation of macrophages and monocytes and resultant cytokine production (**Ehsani and Torki, 2010**).

The dietary xylanase was used in the present study caused elevated total protein, albumin and globulin levels in serum. Albumin and globulin are essential for a healthy immune system (**Tahmasebi-Kohyani *et al.*, 2011**). No data are available to discuss the effect of exogenous enzymes supplementation on the blood biochemical parameters, thus further studies are potentially required.

Lipid metabolism are usually express to triglyceride and lipoprotein in aquatic animal (**Ma *et al.*, 2016**). HDL-C aids in removal of cholesterol from the periphery for delivery to the liver and excretion into the bile (**Norata *et al.*, 2006**) and LDL-C is the major cholesterol carrier in circulation and its physiological function is to convey cholesterol to the cells (**Deng *et al.*, 2010**). In the present study, serum total cholesterol and triglyceride content of Nile tilapia slightly increased with increasing the level of dietary xylanase; also lower content of HDL-C currently detected with xylanase supplementation due to hypocholesterolemic effects of high ANFs in plant-based protein. Since, more studies are necessity to clarify the effect of xylanase supplementation on lipid metabolism of plant-based diets of fish.

The dietary graded levels of xylanase was used in the present study increased the serum GH levels of Nile tilapia fed plant-based diet. The few data that have been published on the effect xylanase supplementation on the plant-based diets did not contained any information about of GH, thus more studies well be needed.

Conclusion

It could be conclude that, using of xylanase up to 0.5 g kg^{-1} diet improved the growth performance, feed utilization, endogenous enzyme activity, hematological and blood biochemistry parameters of *O. niloticus* fingerlings. Yet, further studies are actually required for sharply determine the optimum level of xylanase not only for *O. niloticus* fingerlings, but also for other fish species or live stages. Additionally, the effects of xylanase on physiological or immune responses of fish and understanding the mechanisms of these effects are also necessity needed for advanced studies.

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تأثير إضافة الزيليناز على أداء النمو والأنزيمات الهاضمة والإستجابات الفسيولوجية في أسماك البلطى النيلى (اوريوكرومس نيلوتيكس) المغذاه على علائق نباتية البروتين

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1 كلية الزراعة – جامعة بنها

2 كلية الزراعة – جامعة المنصورة

أجريت تجربة تغذية لمدة 70 يوم لدراسة تأثير إضافة انزيم الزيليناز على أداء النمو والإستفادة من الغذاء ونشاط الإنزيمات الداخلية والتركيب الكيماوى لجسم الأسماك وصفات الدم البيوكيميائية لإصبعيات البلطى النيلى المغذاه على علائق نباتية البروتين. تم تكوين ثلاث علائق متساوية فى محتواها من البروتين (293 جرام/ كجم بروتين خام) والطاقة (18.43 ميجا جول/كجم علف طاقة كلية) وتم إضافة 3 مستويات من إنزيم الزيليناز وهى صفر (الكنترول) و0.5 و 1 جرام زيليناز/ كجم عليقة. وكانت النتائج المتحصل عليها كالتالى: سجلت الأسماك المغذاه على 0.5 أو 1 جرام زيليناز/ كجم عليقة أعلى وزن مكتسب ومعدل نمو نسبي ومعدل كفاءة للبروتين وقيمة إنتاجية للبروتين وأعلى قيم دهن وطاقة محتجزة بالجسم كما أعطت أفضل كفاءة لتحويل الغذاء. كما أدت إضافة الزيليناز بمستوى 0.5 جم/ كجم عليقة الى زيادة نشاط إنزيمات كلا من الليبيز, الأميليز والتربسين. أدت إضافة الزيليناز بمعدل 0.5 أو 1 جم/ كجم إلى تحسين قيم الهيموجلوبين , الهيماتوكريت , كرات الدم الحمراء, البروتين الكلي , الألبيومين , الجلوبيولين , عنصر الفوسفور معنويا مقارنة بالمجموعة الكنترول. سجلت الأسماك المغذاه على علائق مزودة الزيليناز بمعدل 0.5 جم/ كجم أعلى قيم لهرمون النمو ويمكن أن نستنتج أن الإستخدام الأمثل للزيليناز كإضافات غذائية حتى 0.5 جرام/ كجم عليقة يحسن من أداء النمو , الإستفادة من الغذاء ونشاط الإنزيمات الداخلية والإستجابات الفسيولوجية المختلفة في أسماك البلطى النيلى.