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OPTIMIZING ALL PLANT-BASED DIETS UTILIZATION FOR NILE TILAPIA (*Oreochromis niloticus*) USING EXOGENOUS MULTI-ENZYMES MIXTURE (NATUZYME®)

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Abstract

The present study was conducted to evaluate the efficiency of different exogenous multi-enzymes complex, Natuzyme® (NZ) levels to enhance soybean meal (SBM) based diets utilization and the potential replacement of fishmeal (FM) entirely by SBM in Nile tilapia diets. Five isonitrogenous and isocaloric diets were formulated to provide 28% protein and 425 kcal/100 g diets. The treatments were: (1) positive control diet (FM-based), (2) negative control diet (SBM-based), (3) SBM-based+ 0.50 g NZ/kg, (4) SBM-based+ 1.0 g NZ/kg and (5) SBM-based+ 1.50 g NZ/kg. Each diet was offered twice daily to apparent satiation in triplicate fish groups (2.52 g/fish) for 12 weeks. The results showed that the highest significant means ($p \leq 0.05$) of growth performance and survival rate values and the best values of feed conversion ratio, protein efficiency ratio, protein productive value%, and energy retention% were achieved by fish fed FM-based diet and SBM-based diet supplemented with 1.5 g NZ / kg compared to the other treatments. Insignificant differences ($p > 0.05$) were found in body composition among treatments groups. The results suggested that the Natuzyme® supplement at a rate of 1.5 g / kg SBM-based diets can improve the nutrient utilization and growth performance of Nile tilapia. Also, it has an economic return and can be considered as an efficient means to formulate cost effective diets.

Keywords: Nile tilapia, soybean meal, plant diets, exogenous enzymes, growth performance, nutrient utilization.

INTRODUCTION

Tilapia aquaculture is rapidly expanding with a global production of about 3.95 million metric tons in 2011 and estimated to increase to 9.2 million metric tons by the year 2030 (FAO, 2014).

Fish meal (FM) is considered to be the most ideal major protein source in aquaculture feed industry, ranging from 30 to 50% (Hardy, 1995 and Trushenski *et al.*, 2006). However, increasing demand, uncertain availability, and high price made it necessary to search for alternative protein sources for aqua feeds. (Ai *et al*., 2007). Expanded aquaculture production will require more fish feed, which will in turn require higher quantities of alternate plant protein sources to reduce the dependency on fishmeal and provide more economic and environmentally friendly aquaculture (Amer *et al.*, 2015). Nile tilapia can efficiently utilize fishmeal free feeds (Siti-Norita *et al.*, 2015). Soybean meal is the most promising alternative source of plant protein in compound aquatic feed due to its high protein content, excellent amino acid profile, low cost, availability and steady supply as compared to the other plant protein sources (Bhosale *et al.*, 2010; Castillo and Gatlin, 2015).

Phytate, protease-inhibitors and indigestible carbohydrates such as non-starch polysaccharides (NSP) are important anti-nutritional factors often present in plant ingredients (Azarm and Lee, 2014). Therefore, the identification and destruction of anti-nutritional factors that inhibit nutrient utilization is imperative for successful use of plant-based protein for fish feed (Hlophe-Ginindza *et al.*, 2016). Although soybean meal may provide good sources of nutrients, their full potential is generally not exploited by fish due to its high significant NSP content. Viscosity of NSP interferes with the rate of diffusion and acts as a physical barrier preventing or slowing down the access of endogenous enzymes to nutrients thereby reducing nutrient digestion and absorption (Bedford and Cowieson, 2012; Francis *et al*., 2001). This can lead to a lack of feed utilization and low fish growth rates (Watanabe, 2002).

Exogenous enzymes preparations can hydrolyze connective tissue and skin where it is difficult for fish to digest broken down fiber and certain carbohydrates found in protein sources from grains and oil seeds. Also, they improve the activities of endogenous enzymes and the efficacy of the digestion process (Hlophe-Ginindza *et al.*, 2016), protein and amino acids digestibility and growth performance (Vielma *et al.*, 2004) and reduce viscosity of NSP

(Bedford and Cowieson, 2012) and nutrient excretion of plant-based fish diets (Castillo and Gatlin 2015). A certain number of studies have reported a positive effect of using enzymes on the bioavailability of nutrients and minerals, protein and amino acids digestibility, growth performance and reduction of anti-nutritional factors for non-ruminants (Kung *et al*., 2000).

Although one particular enzyme may be the main component of multi enzyme preparation, it has been found that using purified enzymes does not bring about as good an improvement in performance as using a number of different enzymes together ('cocktails') (Clifford, 1989; Graham and Inborr, 1993). (Kumar *et al.*, 2011) reported that the supplementation of diets with exogenous enzymes can enhance feed utilization and reduce water pollution in fish culture.

The reported results of recent research concerning supplementation of exogenous enzymes to fish diets are inconsistent (Lemos and Tacon, 2015; Adeoye *et al.*, 2016). Therefore, the present study was conducted to determine the effects of commercially prepared exogenous multi enzyme preparations (Natuzyme®) on growth performance and feed efficiency of Nile tilapia (*Oreochromis niloticus*) fed all-plant based diets.

MATERIALS AND METHODS

Enzymes and diets formulation and preparation:

Natuzyme® used in the present work is a powdered micro-granulated multi-enzyme preparation commercially available feed additive produced by Bioproton Pty Ltd., Sunnybank, Queensland, Australia (Table 1).

Table 1. Composition of the exogenous multi-enzyme complex (Natuzyme®) used in this experiment.

Enzyme	**Activity* (unit/g) at 30°C, pH 7.2**
Cellulase	5000
Xylanase	10000
ß-glucanase	1000
Protease	6000
α- amylase	1800
Phytase	500
Pectinase	140

*Activity (unit/kg): the amount of the enzyme that catalyses the conversion of 1 μM of substrate per minute under specified conditions (temperature and pH).

Five isonitrogenous and isocaloric diets were formulated with natural ingredients to provide 28% protein and 425 kcal/100 g diet according to the known nutritional requirements of tilapia (NRC, 2011) (Table 2 and 3).

The treatments were: T_1 contained both herring fishmeal (FM) and soybean meal (SBM) as the main protein source (FM-based) to serve as a positive control. T_2 contained SBM as the main protein source in diet (SBM-based diet) served as a negative control. The last three treatments were SBM-based diets supplemented with different levels of exogenous multi-enzymes mixture, Natuzyme® (NZ) as follow: T_3 contained 0.50 g NZ/kg diet (SBM-based+NZ_1), T_4 contained 1.00 g NZ/kg diet (SBM-based+ NZ_2) and T_5 contained 1.50 g NZ/kg diet (SBM-based+ NZ_3). The dietary ingredients were homogeneously grounded to 500 μm and thoroughly mixed. Then sufficient amount of water (about 400 ml/kg diet) was added and mixed to obtain stiff dough which was passed through a 1.5 mm die mincer. The pelleted diets were air dried by electric fan at room temperature for 24 hrs.

Table 2. Composition of different experimental diets used in this experiment.

Ingredients	FM-based diet T_1	SBM-based diet T_2	SBM-based diet + Natuzyme® (g/kg)		
			T_3 NZ_1 (0.50)	T_4 NZ_2 (1.00)	T_5 NZ_3 (1.50)
Herring Fish meal	10.20	0.00	0.00	0.00	0.00
Soybean meal	35.00	55.00	55.00	55.00	55.00
Wheat bran	19.50	14.00	14.00	14.00	14.00
Yellow corn	26.00	20.00	20.00	20.00	20.00
Corn oil	3.30	5.00	5.00	5.00	5.00
Starch	3.00	3.00	2.95	2.90	2.85
Vitamins premix [1]	1.00	1.00	1.00	1.00	1.00
Minerals premix [2]	2.00	2.00	2.00	2.00	2.00
Natuzyme® (NZ) [3]	0.00	0.00	0.05	0.10	0.15
Total	100.00	100.00	100.00	100.00	100.00

[1]-Vitamins premix (per kg of premix): thiamine, 2.5 g; riboflavin, 2.5 g; pyridoxine, 2.0 g; inositol, 100.0 g; biotin, 0.3 g; pantothenic acid, 100.0 g; folic acid, 0.75 g; para-aminobenzoic acid, 2.5 g; choline, 200.0 g; nicotinic acid, 10.0 g; cyanocobalamine, 0.005 g; α-tocopherol acetate, 20.1 g; menadione, 2.0 g; retinol palmitate, 100,000 IU; cholecalciferol, 500,000 IU.

[2]-Minerals premix (g/kg of premix): $CaHPO_4.2H2O$, 727.2; $MgCO_4.7H2O$, 127.5; KCl 50.0; NaCl, 60.0; $FeC_6H_5O_7.3H_2O$, 25.0; ZnCO3, 5.5; $MnCl_2.4H_2O$, 2.5; $Cu(OAc)_2.H_2O$, 0.785; $CoCl_3..6H2O$, 0.477; $CaIO_3.6H_2O$, 0.295; $CrCl_3.6H_2O$, 0.128; $AlCl_3.6H_2O$, 0.54; Na_2SeO_3, 0.03.

[3]-Natuzyme®: Multi-enzyme preparation containing cellulose, xylanase, ß-glucanase, protease, α- amylase, phytase and pectinase (Table 1).

All diets were packed in sealed plastic bags and kept stored at 4 °C until use. The chemical composition of the experimental diets is presented in Table (3).

Table 3. Proximate analysis of experimental diets used in this experiment.

Proximate analyses%	FM-based diet T_1	SBM-based diet T_2	SBM-based diet + Natuzyme® (g/kg)		
			T_3 NZ_1 (0.50)	T_4 NZ_2 (1.00)	T_5 NZ_3 (1.50)
Dry matter	89.73	89.11	89.25	89.25	89.12
Crude protein	28.19	28.19	28.20	28.19	28.21
Crude fat	7.72	7.35	7.38	7.36	7.32
Crude fiber	6.80	7.43	7.51	7.50	7.54
Ash	9.91	10.00	10.21	10.10	10.12
NFE *	47.38	47.03	46.70	46.85	46.81
GE (kcal/100g) **	425.28	424.73	424.82	424.68	424.85

*NFE is nitrogen free extract and ** GE is gross energy.

Fish and husbandry conditions:

The experiment was performed at the department of fish nutrition, Central Laboratory for Aquaculture Research (CLAR) Abbassa, Abu-Hammad, Sharkiya governorate, Egypt. Nile tilapia (*Oreochromis niloticus*) fry were obtained from CLAR hatchery ponds. Fish were held in an indoor tank and fed the basal diet (T_1) for two weeks as an acclimation period to the laboratory conditions prior to the trial. Twenty fish with an average initial body weight of (2.52±0.08 g) were weighed and stocked into each of 100 L glass aquaria (3 replicates of 5 treatments). One half of water in each aquarium was changed daily to avoid accumulation of the metabolites. Each aquarium was supplied with an air stone for continuous aeration using an electrical air pump to maintain oxygen level. All fish were fed to apparent satiation, twice a day, 6 days/week for 12 weeks. During the course of the experiment, all fish were collected from each aquarium every two weeks and collectively weighed.

Sampling, Analytical Procedure and Measurements:

Fish were sampled at the beginning and at the end of the trial from each tank, dried and immediately stored at −20 °C pending analyses. Diets and carcass samples were submitted to proximate composition analysis according to the standard methods of AOAC (1990) for moisture, crude protein, total lipids, and ash. Moisture content was estimated by drying the samples at 85 °C in a drying oven (GCA, model 18EM, Precision Scientific group, Chicago, Illinois, USA) until a fixed weight was achieved. Crude protein was estimated by multiplying the nitrogen content which was determined using a micro-Kjeldahl apparatus (Labconco Corporation, Kansas, Missouri,USA) by 6.25. Lipid content was determined by petroleum ether extraction in a Soxhlet apparatus (Lab-Line Instruments, Inc., Melrose Park, Illinois,USA) at 40-60 °C for 16 h. Ash was determined by combusting dry samples in a muffle furnace (Thermolyne Corporation, Dubuque, Iowa, USA) at 550 °C for 6 h. Crude fiber was estimated according to Goering and Van Soest (1970) and the nitrogen free extract (NFE) was calculated as:

NFE (%) =100 − (% crude protein + % crude lipid + % crude fiber + % ash). Gross energy was calculated according to NRC (1993).

Growth performance parameters:

The following equations were used for the weight gain, daily weight gain, specific growth rate and survival rate, respectively.

Weight gain (WG) = W_1-W_0.

Daily weight gain (DWG) = (W_1-W_0)/T.

Specific growth rate (SGR%/day) = [(Ln W_1− Ln W_0)/T] × 100.

Where, Ln = natural log, W_0 = Initial body weight (g), W_1= Final body weight (g) and T= Time (day).

Survival rate (%) = 100 × (fish No. at the end/ fish No. stocked at the beginning).

Feed utilization parameters:

The following equations were used for the feed intake, feed conversion ratio, protein efficiency ratio, protein productive value and energy retention, respectively.

Feed intake (FI) = total feed consumed over 12 weeks (g)/ number of fish.

Feed conversion ratio (FCR) = feed intake (g)/body weight gain (g).

Protein efficiency ratio (PER) = total weight gain (g)/protein intake (g).

Protein productive value (PPV %) = 100 (protein gain/protein intake).

Energy retention (ER %) = 100 (gross energy gain/gross energy intake).

Water quality analysis:

Water samples were collected biweekly throughout the experimental period from each aquarium. Water temperature and dissolved oxygen were measured in each aquarium with an YSI model 58 oxygen meter (Yellow Spring Instrument Co., Yellow Spring, Ohio, USA). While the pH was measured using a pH–meter (Digital Mini-pH Meter, model 55, Fisher Scientific, USA). Unionized ammonia, total alkalinity and total hardness were determined according to Boyd and Tucker (1992).

Statistical Analyses:

Data were submitted to one-way ANOVA and were expressed as the mean ± SD of the replicates. Differences were considered significant if P was less than 0.05. All statistical analyses were performed as described by Dytham (1999) using SPSS, version 10 (SPSS Inc. 1999). Significant differences ($p \leq 0.05$) among means were tested by the method of Duncan (1955).

Economical evaluation:

A simple economic analysis was conducted for different experimental treatments to estimate the cost of feed required to produce a unit of fish biomass. The estimation was based on local retail sale market price of all the dietary ingredients at the time of the study. These prices were as follows: herring fish meal, 17.00; soybean meal, 4.00; yellow corn meal, 2.50; wheat bran, 2.25; corn oil, 12.00; starch 6.00, vitamins mixture,10; minerals mixture, 4.50 and enzymes mixture, 120 LE/kg.

RESULTS

Water temperature ranged from 28.00 to 28.8°C, while pH ranged from 7.4 to 7.8. Dissolved oxygen level (DO) was higher than 5.85 mg DO/l, whereas, unionized ammonia concentration was lower than 0.2 mg NH_3/l throughout the study period. Total alkalinity and total hardness values ranged from 125 to 165 mg/l and 165-180 mg/l as $CaCO_3$, respectively. No significant differences were detected among the treatments in water quality parameters.

Growth performance parameters [final body weight (FBW), daily weight gain (DWG) and specific growth rate (SGR %)] of Nile tilapia, *O. niloticus*, fed at different experimental diets over 12 weeks period are presented in Table 4 and Figure 1. FBW, WG and SGR were significantly ($p \leq 0.05$) affected by different treatments. The significant highest growth performance values were found with fish fed FM-based diet and fish fed SBM-based diet supplemented with 1.50 g NZ/kg diet with insignificant differences ($p > 0.05$) between them. While fish fed SBM-diet (negative control) recorded the significant lowest growth performance parameters values ($p \leq 0.05$) (Table 4).

Table 4. Growth performance parameters (means ± SE) of Nile tilapia (*Oreochromis niloticus*) fry fed at different experimental diets.

Items	FM-based diet	SBM-based diet	SBM-based diet + Natuzyme® (g/kg)		
	T_1	T_2	T_3 NZ_1 (0.50)	T_4 NZ_2 (1.00)	T_5 NZ_3 (1.50)
FBW (g)	24.06±0.12^a	17.56±0.04^c	21.11±0.27^b	22.10±0.14^b	23.06±0.05^{ab}
WG (g)	21.54±0.12^a	15.04±0.05^c	18.59±0.28^b	19.58±0.14^b	20.54±0.04^{ab}
DWG (g)	0.31±0.00^a	0.21±0.00^c	0.27±0.00^b	0.28±0.00^b	0.29±0.00^a
SGR% /day	2.69±0.01^a	2.31±0.01^c	2.53±0.02^b	2.58±0.01^b	2.64±0.00^a
Survival rate%	96.67±1.67^a	93.33±1.67^c	93.33±1.67^c	95.00±0.00^b	96.67±1.67^a

Mean values with the different superscript along the same row are significantly different ($p < 0.05$).

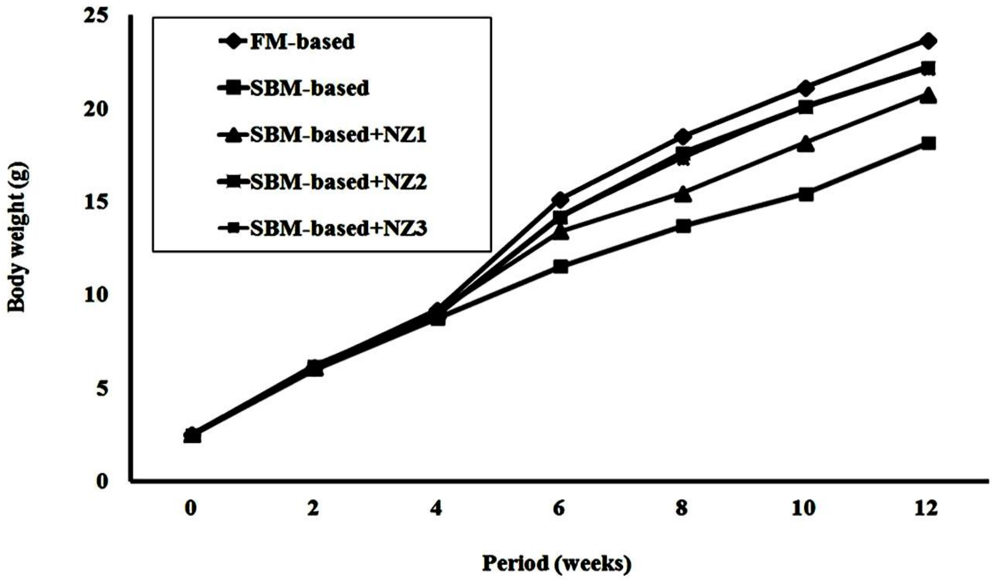

Figure 1. Changes in mean body weight of Nile tilapia fed fishmeal-based diet and soybean meal-based diets supplemented with Natuzyme® (NZ) levels (0.5, 1, 1.5) for 12 weeks.

High survival rate % was recorded in all the treatments (Table 4). At the end of the experiment, survival rate showed significant differences ($p \leq 0.05$) among treatments. The best ($p \leq 0.05$) survival rate (96.67 %) was observed when fish was maintained at each FM-based diet or SBM-based+1.5g NZ/kg diet, while the lowest value of survival rate (93.33%) was found with fish maintained at the SBM-based diet (negative control).

The results of feed intake (FI), feed conversion ratio (FCR), protein efficiency ratio (PER), protein productive value (PPV %) and energy retention (ER %) of fish fed diets supplemented with graded levels of commercial exogenous enzyme mixture are shown in Table 5. With regard to FI, results revealed that, the highest FI value (33.69 g) was recorded for fish fed FM-based diet. This which differed significantly from those for the other diets ($p \leq 0.05$), followed by fish fed SBM-based+NZ_2 diet (32.23 g). The significant lowest FI value ($p \leq 0.05$) was recorded for fish fed SBM-based diet (T_2).

Table 5. Feed and nutrient utilization parameters (means ± SE) of Nile tilapia (*Oreochromis niloticus*) fry fed at different experimental diets.

Items	FM-based diet T_1	SBM-based diet T_2	SBM-based diet + Natuzyme® (g/kg)		
			T_3 NZ_1 (0.50)	T_4 NZ_2 (1.00)	T_5 NZ_3 (1.50)
FI (g)	33.69±0.05[a]	26.83±0.14[d]	30.49±0.08[c]	32.23±0.01[b]	32.22±0.03[b]
FCR	1.56±0.01[c]	1.78±0.01[a]	1.64±0.02[b]	1.65±0.01[b]	1.57±0.00[c]
PER	2.26±0.02[a]	1.99±0.01[c]	2.16±0.03[b]	2.15±0.02[b]	2.26±0.00[a]
PPV%	35.86±0.42[a]	31.97±0.32[c]	35.04±0.48[a]	34.24±0.51[b]	34.75±0.58[a]
ER%	21.39±0.22[a]	19.57±0.27[b]	20.91±0.44[a]	21.62±0.25[a]	21.37±0.33[a]

Mean values with the different superscript along the same row are significantly different ($p<0.05$).

The overall mean FCR values were significantly better (lower) (1.57) with both FM-based diet and SBM-based diet+NZ_3 than that in the other treatments. No significant differences ($p>0.05$) were found between T_1 and T_5 groups. The poorest FCR value ($p\leq0.05$) was determined as 1.78 with fish fed at SBM-based diet (T_2). Concerning the PER, the results showed that the highest PER value (2.26) was obtained in both fish fed FM-based diet and SBM-based diet+NZ_3 with insignificant differences ($p>0.05$) between them, while the lowest ($p\leq0.05$) PER value (1.99) was recorded in fish maintained at the SBM-based diet (negative control). The same trend was observed in PPV%. With regard to ER% as shown in Table 5, no significant differences ($p>0.05$) were observed in ER values among fish fed FM-based diet and SBM-based diet gropes supplemented with all NZ levels. The highest ER value (21.62%) was recorded with SBM-based+NZ_2 diet. The lowest ER value (19.57%) was recorded with SBM-based diet (T_2) without enzymes supplementation.

The results of the whole body composition of tilapia fed the experimental diets are displayed in Table 6. There were no significant differences ($p>0.05$) in the body moisture (ranged between 74.11% and 75.41%), protein (ranged between 64.57% and 65.70%) and body lipid content (ranged between 20.31% and 21.73%) of Nile tilapia fed the dietary treatments.

Table 6. Body composition (Means ± SE) of Nile tilapia (*Oreochromis niloticus*) fry fed at different experimental diets.

Treatments	Moisture%	Protein%	Fat%
T1	75.41±0.55	64.82±2.16	20.66±0.24
T_2	74.97±0.56	65.00±2.47	21.54±1.01
T_3 (0.50 g NZ/kg)	75.18±0.06	64.57±3.52	20.31±0.97
T_4 (1.00 g NZ/kg)	74.11±0.76	64.95±2.99	21.73±1.54
T_5 (1.50 g NZ/kg)	74.88±0.40	65.70±1.65	20.98±1.74

Economical efficiency calculations of the SBM-based diets based on cost one kg gain in weight of Nile tilapia in comparison with the FM-based diet are shown in Table (7). It's clear that, NZ supplementation at 1.5 g /kg level in SBM-based diets reduced feed price from 4.97 LE /kg to 4.07 LE/ kg which in turn reduced the feed cost to produce one kg fish gain by 17.55% compared to fish fed control diet (FM-based diet).

Table 7. Economic efficiency for production of one kg gain of Nile tilapia (*Oreochromis niloticus*) fry fed at different experimental diets.

Items	FM-based diet T_1	SBM-based diet T_2	SBM-based diet + Natuzyme® (g/kg)		
			T_3 NZ_1 (0.50)	T_4 NZ_2 (1.00)	T_5 NZ_3 (1.50)
Price/ kg feed (L.E)	4.97	3.98	4.01	4.04	4.07
FCR (kg feed/kg gain)	1.56	1.78	1.64	1.65	1.57
Feed cost/kg gain (L.E)	7.75	7.08	6.58	6.67	6.39
Reduction in feed cost/ kg gain (L.E)*	0.00	0.67	1.17	1.08	1.36
Reduction in feed cost/ kg gain%**	0.00	8.65	15.10	13.94	17.55

* Reduction in feed cost per kg gain (L.E.) = feed cost per kg gain of FM-based treatment (L.E.) - feed cost per kg gain of SBM-based treatments (L.E.).

** Reduction in feed cost per kg gain (%) = 100 [Reduction in feed cost per kg gain (L.E.) / feed cost per kg gain of FM-based treatment (L.E.)].

DISCUSSION

All values of the water quality parameters measured were within the acceptable range for the normal growth of tilapia as mentioned by Boyd (1984).

In the present study, the response of Nile tilapia fry to plant diets supplemented with exogenous multi-enzymes, Natuzyme® (NZ) was investigated. Nile tilapia as an omnivorous fish able to digest plant material better than carnivorous fish (Siti-Norita *et al.*, 2015). Therefore, soybean meal was expected to be suitable to replace fishmeal in fish diets to reduce the cost of fish feed by the addition of enzyme. However, this study clearly demonstrated that 100% of the FM protein could be replaced by SBM protein in Nile tilapia diets by inclusion of exogenous enzymes.

Supplementation of SBM-based diets with NZ maintained a similar growth rate as the rate provided by the FM-based diet. This may be attributed to the improvement in protein digestion, amino acids absorption and decreasing in majority of anti nutritional factors (ANFs) at significant level in SBM-based diets. Nile tilapia fed the NZ supplemented diets performed significantly better ($p<0.05$) than those fed control SBM-based diet.

The results of the present work suggested that the supplementation of NZ (1.50 g/Kg) in SBM-based diets can significantly improve growth and feed utilization parameters of Nile tilapia *O. niloticus*. The higher growth performance parameters (FBW, WG, DWG and SGR %) were observed with fish groups maintained at FM-based diet and SBM-based diet supplemented with NZ_3. This is in agreement with previous studies. Hlophe-Ginindza *et al.* (2016) observed a significantly improved growth performance in tilapia (*Oreochromis mossambicus*) fed plant-based diet supplemented with an exogenous enzyme cocktail, Natuzyme® was added to a plant-based diet. Our results were also agreed with Khalafalla and El-Hais (2013) who reported that, Nutrasexylam© enzyme supplementation had a significant effect ($p\leq0.05$) on all growth parameters of Nile tilapia fry.

The positive effect of the exogenous enzymes on fish growth performance in the present study may be due to some factors: firstly, their ability to break the bonds between phytate-minerals and phytate-protein in SBM-based diets and increase the availability of amino acids (Kumar *et al.*, 2011). Secondly, reducing the viscosity of NSP and increasing endogenous enzymes activities in the gut of fish which enhance the hydrolysis of the diets and nutrient liberation and digestibility (Hlophe-Ginindza *et al.*, 2016). Thirdly, the experimental diets in the present study were not exposed to any heat treatments that may have led to enzymes being highly active. In addition, Hassaan *et al.* (2013) reported that the balance of Ca:P ratio in the experimental diets may improve the bioavailability and utilization of plant phosphorus by fish, bone mineralization and protein digestibility.

The effect of enzymes in the bioavailability of nutrients and minerals, protein digestibility, amino acids utilization, growth performance and reduction of anti-nutritional factors of plant-based fish diets have been reported by a certain number of studies (Castillo and Gatlin, 2015; Lin *et al.*, 2007; Mahmoud *et al.*, 2014). It was hypothesized that protease supplementation can improve protein digestibility and growth performance by degrading complex proteins in the diet into usable amino acids and peptides (Vielma *et al.*, 2004). In contrary, (Yigit *et al.*, 2016) reported that the addition of protease or phytase as mix to soybean meal-based diet could not increase growth and nutrient digestibility in trout. The authors attributed the non – response of trout to exogenous enzymes supplementation to a variety of dietary factors such as the concentration and sources of protein in the diet and enzymes which were affected by different temperatures and pH levels.

The results on fish survival showed better survival rate among those fish fed FM-based diet and SBM-based + NZ_3 with insignificant differences ($p>0.05$) between them. While fish fed SBM-based diet (negative control) achieved the significant lowest ($p>0.05$) survival rate values. The exogenous enzymes supplementation did not exert any negative effect on the survival rate of tilapia among treatments in the present study. Similarly, Mahmoud *et al.*

(2014) reported that inclusion of commercially prepared exogenous multi-enzyme preparations (Pan Zyme and Phytase-plus broiler 500) didn't affect survival rates of Nile tilapia fed SBM-based diets.

The results showed that feed intake was higher among fish fed FM-based diet. This may be due to the high palatability of FM-based diets more than all-plant diets. While when SBM-based diets were supplemented with 1.00 and 1.50 g NZ/kg, feed intake showed higher levels compared with the control SBM-based diet due to enhanced release of nutrients of plant based diets by breaking down the bonds of phytate-protein and phytate-minerals complexes (Vielma *et al.*, 2004). Therefore, the exogenous multi-enzymes may enhance the palatability of the plant diets (Deguara *et al.*, 1999).

It is clear that supplementation of SBM-based diets with NZ positively affected feed efficiency parameters such as FCR, PER, PPV% and ER%. In the present study, NZ supplementation significantly improved feed intake and FCR of *O. niloticus* fry fed on plant based diets. These results agree with those found by several authors who reported that exogenous enzymes can improve nutrient digestibility of plant-based fish diets and result in better growth performance and feed efficiency (El-Tawil, 2015; Mahmoud *et al.*, 2014). The enhancement of FCR in fish fed diets containing enzymes in the present work coincided with the increased protein utilization. This is in consistence with the findings of the other studies which evaluated the inclusion of enzymes in diet containing high level of plant sources (Vielma *et al.*, 2004; Vahjen *et al.*, 2005). The better FCR, PER and PPV% in the fishes fed multi-enzyme treated SBM-based diets can be attributed to the enhancement of the protein availability to the fishes and the presence of anti nutritional factors in permissible limit in the test diets. Hlophe-Ginindza *et al.* (2016) showed that the presence of protease and alpha-amylase in Natuzyme50®, improves the protein and starch digestibility.

The body composition of fish is primarily influenced by diet composition, feeding practices, fish size, and can be controlled through nutrition (Burtle, 1990). However, the results of body composition in the present study didn't reveal any significant differences ($p>0.05$) when FM was completely replaced by SBM with or without supplementation with exogenous enzymes. This is in accordance with Amer *et al.* (2015) who concluded that FM can be completely replaced by SBM in Nile tilapia diets by the inclusion of L-carnitine at 300 g/kg without any significant differences in body composition. Also, Khalafalla and El-Hais (2013) and Lin, *et al.* (2007) didn't find any significant differences in whole body composition of Nile tilapia fry fed diets supplemented with exogenous multi-enzyme preparations. Similar trend was detected by Ng and Chong (2002) who indicated that exogenous multi-enzyme Superzyme® supplementation in the diets did not have any effect on the whole body composition of tilapia. Mahmoud *et al.* (2014) indicated that substituting FM with SBM did not affect the moisture, ash and gross energy. While crude protein was significantly higher in fish fed SBM-based diet supplemented with phytase compared to the FM-based diet. On the contrary, Hassaan *et al.* (2013) indicates that phytase supplementation positively affected chemical composition of body and vertebra when combined with 0.6 Ca/P ratio.This inconsistency may be attributed to differences in feed ingredients, nutritional quality of plant ingredients, water quality, fish species, size and culture and experimental conditions.

The replacement of fishmeal with extensively available plant or grain by-products is getting increased attention for the development of low-cost fish feed (Gatlin *et al.*, 2007). Better protein economy of plant-based aqua feeds by enzymes would increase the interest of the feed industry towards this relatively new feed additives (Felix and Selvaraj, 2004; Gabriel *et al.* 2007).

The low FCR value obtained with Natuzyme® supplemented groups lowered final cost of production than FM-based or SBM-based diet. SBM-based diet supplemented with 1.5g Natuzyme® provides the fish culturist to save 0.21 kg feed per kg body weight gain, which is 11.80% less than the feed required for the SBM-based diet group (negative control). Also, the results clearly showed that inclusion of 1.5 g Natuzyme® in SBM-based diet reduced feed cost by 17.55% compared to the FM-based diet. That would save 8.78% of fish culture costs because feeding cost accounts for over 50% of production costs of aquaculture (Amer *et al.*, 2015).

The present findings suggest that supplementation of commercially available exogenous multi-enzymes at a rate 1.5 g Natuzyme® / kg gives the possibility of total replacement of fishmeal by soybean meal in Nile tilapia diets by improving the utilization of soy bean meal-based diets. The inclusions of Natuzyme® in Nile tilapia feed enhance the nutrient utilization and improve fish performance which will have an economic return through its success in providing the formulation of cost effective diets.

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تحقيق أفضل استفادة من العلائق النباتية لأسماك البلطى النيلى باستخدام خليط متعدد الإنزيمات الخارجية (ناتوزيم)

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الملخص العربــى

تم إجراء هذه التجربة لدراسة كفاءة إضافة مستويات مختلفة من خليط متعدد الإنزيمات (ناتوزيم) فى تحسين الاستفادة من العلائق النباتية المعتمدة على مسحوق فول الصويا كمصدر رئيسى للبروتين. وكذلك إمكانية الاستبدال الكلى لمسحوق السمك بمسحوق فول الصويا فى علائق البلطى النيلى. تم إعداد خمس علائق متساوية فى محتوى البروتين (٢٨٪) والطاقة (٤٢٢ كيلو كالورى/ ١٠٠ جم غذاء). كانت المعاملات كالتالى: العليقة الأولى احتوت على مسحوق السمك (كنترول ١)، العليقة الثانية (نباتية) احتوت على مسحوق فول الصويا (كنترول ٢)، العليقة الثالثة والرابعة والخامسة (نباتية) مضافاً إليها ناتوزيم بمستويات ٠.٥ ، ١.٠ ، ١.٥ جم ناتوزيم/كجم بالترتيب. غُذيت الأسماك مرتين يومياً إلى حد الإشباع فى ثلاث مكررات من مجموعات الأسماك (متوسط وزن السمكة ٢.٥٢ جم) لمدة ١٢ أسبوع. أوضحت النتائج أن أعلى قيم ذات دلالة إحصائية لأداء النمو ومعدل الإعاشة و كذلك أفضل قيم لمعدل تحويل للغذاء ونسبة كفاءة البروتين والقيمة الإنتاجية للبروتين واحتجاز الطاقة تم تسجيلها مع الأسماك التى غُذيت على العليقة المحتوية على مسحوق السمك والعليقة النباتية المضاف إليها ١.٥ جم ناتوزيم/كجم بالمقارنة مع باقى العلائق. لوحظ أيضاً عدم وجود فروق ذات دلالة إحصائية فى تركيب الجسم بين المعاملات المختلفة. تشير النتائج إلى أن إضافة ناتوزيم بمعدل ١.٥ جم/كجم فى العلائق النباتية المحتوية على مسحوق فول الصويا يمكن أن يحسن الاستفادة من الغذاء وكذلك أداء نمو أسماك البلطى النيلى. كما أن له عائد اقتصادى و يعتبر وسيلة فعالة لتكوين علائق منخفضة التكلفة.