

## IMPROVING FEED EFFICIENCY OF PLANT DIETS BY EXOGENOUS ENZYMES ADDITIVE AND HEAT TREATMENT OF SOYBEAN FOR NILE TILAPIA *OREOCHROMIS NILOTICUS*

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### SUMMARY

This study was conducted to evaluate the effect of using exogenous digestive enzymes (Zymogen<sup>®</sup>) and heat treatment of soybean (cooked) in diets on growth performance and feed efficiency of Nile tilapia *Oreochromis niloticus* (4.07g). Six experimental diets were formulated to be isonitrogenous (27% crude protein) and isocaloric (4.29kcal/g) and used to feed triplicate groups of fish to apparent satiation for 11 weeks. Treatments were: T1 contain both fish meal and plant protein sources as FM control diet (FM), T2 contain only plant protein additives as a SBM control diet, T3 (SBM +1.0% zymogen) Z<sup>1</sup>, T4 (SBM +2.0% zymogen) Z<sup>2</sup>, T5 and T6 (SBM +1.0% or 2.0% zymogen level with cooked soybean treatment) Z<sup>1</sup>C, Z<sup>2</sup>C respectively. Results showed that supplemented fish diets with zymogen and cooked soybean enhanced fish performance over plant diet control. Fish maintained at FM, Z<sup>2</sup>, Z<sup>2</sup>C and Z<sup>1</sup>C diets were significantly higher (P<0.05) in all growth performance parameters and survival rate values than other treatments. Differences among FM, Z<sup>2</sup>, Z<sup>2</sup>C and Z<sup>1</sup>C treatments were not significant (P>0.05). Also, feed efficiency parameters were improved significantly (P<0.05) at fish maintained on FM, Z<sup>2</sup>C and Z<sup>1</sup>C diets. These results suggest that using exogenous digestive enzymes (zymogen) and cooked soybean treatment in all plant based diets may enhance the growth performance and the feed efficiency of Nile tilapia. Moreover, the economic efficiency in terms of feed utilization has been improved.

**Keywords:** Fishmeal, zymogen, cooked soybean, growth performance, feed efficiency, Nile tilapia.

### INTRODUCTION

With increasing the international prices of the feed ingredients used in aquaculture, there is a great need to enhance feed efficiency, growth performance and disease resistance of cultured fish species. Dietary supplementation of different feed additives usually in small quantities for the purpose of fortifying it with certain nutrients have been found to be beneficial for improving growth performance and feed efficiency of fishes (Ganguly *et al.*, 2012).

Fish meal (FM) represents an ideal nutritional source of dietary protein for fish. Increasing demand, unstable supply, and high prices of FM along with the continuous expansion of aquaculture are reasons for many nutritionists to realize that soon they will no longer be able to afford it as a major protein source in aquatic feeds. A current priority in the aquaculture industry and one of the challenges that fish nutritionists face is the need to partially or totally replace FM with less expensive, non-traditional animal or plant protein sources including plant-based protein ingredients to ensure its sustainability (Peng *et al.*, 2013, Castillo and Gatlin, 2015). Soybean has been recognized as one of the most appropriate alternative protein source for FM in aquatic feed because of high protein content, balanced essential amino acids, availability and its low cost, but its full nutritional value can be obtained only after inactivation of soy anti-nutritional factors such as protease inhibitor, anti-vitamin and lectin (Azarm and Lee, 2012, Storebakken, 2000). At present, commercial SBM produced can partially replace fishmeal, and we need to improve processing methods to develop new products that will be suitable for fish consumption and utilization (Mahmoud *et al.*, 2014). Feed ingredients exposed to varying degrees of heating during manufacturing and processing. Barrows *et al.*, (2007) reported that heat treatment of soybean is necessary

to de-nature endogenous trypsin inhibitors and maximize nutritional value. Moreover, El-Dahhar (2006) stated that heat treatments and pressure of the diet are expected to improve product quality and make the best nutritional use of the raw materials. It can also sterilize the product and give the flexibility to utilize a wider range of raw materials while maximizing their nutritional quality.

Addition of exogenous enzymes in fish feeds can improve nutrient utilization, thereby reducing nutrient losses. Exogenous enzymes have been proven to improve nutritional value of feed and decrease environmental pollution. Tacon (1993) stated that presence of anti-nutritional factors within plant feed-stuffs limit their use in aquatic feeds. However, certain enzymes provide an additional powerful tool that can inactivate anti-nutritional factors and enhance the nutritive value of plant based protein in feeds. Nowadays, exogenous enzymes are extensively used all over the world as additives in fish diets to improve the nutritional value of fish feeds, especially with the raise of using plant proteins in aquafeeds and reduce water pollution (Godaet *al.*, 2012). SBM contains also non-starch polysaccharides that are not efficiently digested by most fish species. Lobo (1999) found that use of exogenous enzymes, such as xylanase,  $\alpha$ -galactosidase,  $\beta$ -glucanase and endo- $\beta$ -mannanase, make the oligosaccharide fraction more digestible in some species. Lin *et al.*, (2007) demonstrated that using of digestive enzyme (protease) in fish diet improved protein digestibility in hybrid tilapia.

The present study was conducted to evaluate the effect of using exogenous digestive enzymes supplementation (zymogen) with heat treatment of soybean (cooked) in fish diets on growth performance and feed efficiency of Nile tilapia *Oreochromis niloticus* fed on all based plant protein diets.

## MATERIALS AND METHODS

### *Diets* formulation and preparation:

Six practical diets were formulated from commercial ingredients to contain iso-nitrogenous (27% crude protein) and iso-caloric (4.29 kcal/g diets) based on feedstuff values reported by NRC (1993). The composition and chemical analysis of the experimental diets are presented in Table (1). Treatments were: T1 both fish meal and plant protein sources as a FM control, T2 all plant protein sources without any additives as a SBM control, T3 all plant protein sources supplemented with 1.0% zymogen level ( $Z^1$ ), T4 all plant protein sources supplemented with 2.0% zymogen level ( $Z^2$ ), T5 all plant protein sources supplemented with 1.0% zymogen level with cooked soybean ( $Z^1C$ ) and T6 all plant protein sources supplemented with 2.0% zymogen level with cooked soybean ( $Z^2C$ ). Cooked soybean was done by using an autoclave with a maximum pressure of 1.2 kg/cm<sup>2</sup>, for 15 minutes according to El-Dahhar and El-Shazly (1993). Exogenous digestive enzymes (Zymogen®) (manufactured by the Nile co. for pharmaceuticals and chemical industries, Cairo, Egypt) contain Lipase (800 USNF units), amylase (5000 USNF units), protease (5000 USNF units), pepsin 100mg, hemicellulase 40mg, Ox bile extract 20mg, dimethylpolysiloxane 20mg and vitamin B1 25mg. The ingredients of each diet were separately blended with additional 50% of warm water to make a paste of each diet. Vitamins and minerals mixtures were added to the diets after heat treatment. The pastes were separately passed through a grinder, and pelleted in a modified paste extruder to form the tested diets (1-mm diameter). Diets were dried in a drying oven model (Fisher oven 13–261–28A) for 24 hours at 65°C. Zymogen was mixed with some oil and sprayed onto the diets and homogenized well, then dried under room temperature and stored in plastic bags which were kept dry until they were used.

### *The Experimental Fish:*

Nile tilapia fry (*Oreochromis niloticus*) were obtained from the fish hatchery ponds, Central Laboratory for Aquaculture Research (CLAR) Abbassa, Abu-Hammad, Sharkiya Governorate, Egypt. Fish were kept in indoor tank for 2 weeks as an acclimation period to the laboratory conditions. Fish with an average initial body weight of (4.07 ± 0.03 g) were divided into 6 groups (3 replicates per treatment) each containing 20 fish/ glass aquarium (100 Liter capacity). Aquaria were supplemented with continuous aeration. Water was exchanged partially every day by stocked dechlorinated tap water. Fish were fed twice daily to apparent satiation and aquaria were cleaned every day before feeding. Fish feeding was offered 6 days/week. Fish were weighed at the beginning of the experiment and then biweekly for 11 weeks experimental period.

**Table (1). Formulation of feed ingredients and proximate chemical composition (%; on dry matter basis) for the experimental diets.**

Ingredients	FM	SBM	Z <sup>1</sup>	Z <sup>2</sup>	Z <sup>1</sup> C	Z <sup>2</sup> C
Herring Fish meal	10.00	0.00	0.00	0.00	0.00	0.00
Soybean meal	33.00	51.00	51.00	51.00	51.00	51.00
Corn meal	30.00	25.00	25.00	25.00	25.00	25.00
Wheat bran	18.00	14.00	14.00	13.00	14.00	13.00
Zymogen	0.00	0.00	1.00	2.00	1.00	2.00
Corn oil	4.00	5.00	5.00	5.00	5.00	5.00
Starch	2.00	2.00	1.00	1.00	1.00	1.00
Vitamins premix <sup>1</sup>	2.00	2.00	2.00	2.00	2.00	2.00
Minerals premix <sup>2</sup>	1.00	1.00	1.00	1.00	1.00	1.00
Total	100	100	100	100	100	100
Dry matter%	89.85	89.76	89.74	89.77	89.69	89.71
Protein %	27.16	27.24	27.22	27.19	27.22	27.24
Fat %	8.51	8.24	8.21	8.23	8.32	8.26
Fiber %	6.11	6.32	6.31	6.34	6.28	6.32
Ash %	10.35	10.63	10.56	10.59	10.61	10.49
NFE % <sup>3</sup>	47.87	47.57	47.70	47.65	47.57	47.69
GE (kcal/g) <sup>4</sup>	4.30	4.27	4.27	4.27	4.28	4.28

<sup>1</sup>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;  $\alpha$ -tocopherol acetate, 20.1 g; menadione, 2.0 g; retinol palmitate, 100,000 IU; cholecalciferol, 500,000 IU.

<sup>2</sup>Minerals premix (g/kg of premix): CaHPO<sub>4</sub>.2H<sub>2</sub>O, 727.2; MgCO<sub>4</sub>.7H<sub>2</sub>O, 127.5; KCl 50.0; NaCl, 60.0; FeC<sub>6</sub>H<sub>5</sub>O<sub>7</sub>.3H<sub>2</sub>O, 25.0; ZnCO<sub>3</sub>, 5.5; MnCl<sub>2</sub>.4H<sub>2</sub>O, 2.5; Cu(OAc)<sub>2</sub>.H<sub>2</sub>O, 0.785; CoCl<sub>3</sub>.6H<sub>2</sub>O, 0.477; CaIO<sub>3</sub>.6H<sub>2</sub>O, 0.295; CrCl<sub>3</sub>.6H<sub>2</sub>O, 0.128; AlCl<sub>3</sub>. 6H<sub>2</sub>O, 0.54; Na<sub>2</sub>SeO<sub>3</sub>, 0.03.

<sup>3</sup>NFE (%) = 100 - (% crude protein + % crude lipid + % crude fiber + % ash)

<sup>4</sup>Gross energy (GE) was calculated according to NRC (1993) as 5.65, 9.45, and 4.11 kcal/g of protein, lipid, and carbohydrates, respectively.

#### **Chemical analysis of diets and fish:**

The tested diets and whole-fish body from each treatment at the beginning and at the end of the experiment were analyzed according to the methods of AOAC (1990) for moisture, crude protein, total lipids, and ash. Moisture content was estimated by drying the samples to constant weight at 85 °C in a drying oven (GCA, model 18EM, Precision Scientific group, Chicago, Illinois, USA). Nitrogen content was determined using a microkjeldahl apparatus (Labconco Corporation, Kansas, Missouri, USA). Lipid content was determined by ether extraction in a multi-unit extraction Soxhlet apparatus (Lab-Line Instruments, Inc., Melrose Park, Illinois, USA) for 16 h. and 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). Gross energy was calculated according to NRC (1993).

#### **Parameters of growth performance:**

Growth performance was determined, and feed utilization efficiency parameters were calculated using the following equations:

$$\text{Weight gain} = W_2 - W_1$$

$$\text{Specific growth rate (SGR; \% g/day)} = 100 (\ln W_2 - \ln W_1) / T$$

Where W<sub>1</sub> and W<sub>2</sub> are the initial and final fish weights, respectively, and T is the experimental period in days.

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

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

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

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

**Water quality:**

Water temperature and dissolved oxygen were measured daily using a YSI Model 58 oxygen meter (Yellow Springs Instrument, Yellow Spring, OH, USA). Total ammonia, nitrite and nitrate were measured twice weekly using a DREL 2000 spectrophotometer by the method of Golterman *et al.* (1978), pH was monitored using an electronic pH meter (pH pen; Fisher Scientific, Cincinnati, OH, USA). Unionized ammonia was measured using DREL/2 HACH kits (HACH Co., Loveland, Colorado, USA).

**Statistical analysis:**

The obtained data were subjected to one-way ANOVA (complete randomized design). Differences between means were tested at the 5% probability level using Duncan's new multiple range test by Duncan (1955). All the statistical analyses were done using SPSS program version 10 (SPSS, Richmond, USA) as described by Dytham (1999).

**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 (in LE/kg) were as follows: herring fish meal, 18.00; soybean meal, 4.00; yellow corn meal, 2.50; wheat bran, 2.25; corn oil, 10.00; starch 6.00, vitamins mixture, 10; minerals mixture, 7.00 and zymogen 48.0LE/kg.

**RESULTS AND DISCUSSION**

In the present study, water temperature ranged from 26.0 to 27.0°C, dissolved oxygen concentration range was 4.7 to 5.5 mg/L, total ammonia concentration ranged from 0.13 to 0.19 mg/L and pH from 7.3 to 7.8. These water quality parameters are in acceptable ranges for normal growth of Nile tilapia during the experimental period (Boyd, 1984).

Data of Table (2) show that final body weight (FBW), weight gain (WG), daily weight gain (DWG), specific growth rate (SGR %) and survival rate of Nile tilapia (*Oreochromis niloticus*) were improved significantly ( $P < 0.05$ ) with zymogen supplementation in fish diet. Fish maintained on diets containing zymogen exhibited significantly ( $P < 0.05$ ) the greatest growth performance parameters compared to fish fed

**Table (2). Growth performance values (means ± SE) of Nile tilapia fed diets containing different levels of zymogen and cooked soybean.**

Item	FM	SBM	Z <sup>1</sup>	Z <sup>2</sup>	Z <sup>1</sup> C	Z <sup>2</sup> C
IBW, g	4.09±0.31	4.07±0.04	4.05±0.04	4.05±0.04	4.04±0.04	4.10±0.06
FBW, g	27.46±0.06 <sup>a</sup>	19.47±0.11 <sup>c</sup>	22.59±1.03 <sup>bc</sup>	26.93±1.00 <sup>a</sup>	25.34±1.91 <sup>ab</sup>	26.06±1.72 <sup>ab</sup>
WG, g	23.37±0.37 <sup>a</sup>	15.40±0.15 <sup>c</sup>	18.54±1.02 <sup>bc</sup>	22.88±0.96 <sup>a</sup>	21.30±1.88 <sup>ab</sup>	21.96±1.78 <sup>ab</sup>
DWG, g	0.33±0.01 <sup>a</sup>	0.22±0.00 <sup>c</sup>	0.26±0.01 <sup>bc</sup>	0.33±0.01 <sup>a</sup>	0.30±0.03 <sup>ab</sup>	0.31±0.03 <sup>ab</sup>
SGR, %/d	3.02±0.11 <sup>a</sup>	2.48±0.02 <sup>c</sup>	2.73±0.07 <sup>b</sup>	3.00±0.04 <sup>a</sup>	2.91±0.11 <sup>a</sup>	2.93±0.13 <sup>a</sup>
Survival%	96.66±1.98 <sup>a</sup>	91.67±1.98 <sup>c</sup>	93.33±3.96 <sup>bc</sup>	95.00±2.22 <sup>ab</sup>	95.00±2.22 <sup>ab</sup>	96.66±1.98 <sup>a</sup>

Means with different superscripts in the same row are significantly different ( $P < 0.05$ ).

on the control SBM based diet. Moreover, results indicated that differences among fish maintained on diets containing Z<sup>2</sup>, Z<sup>1</sup>C, Z<sup>2</sup>C and fish fed on FM diet were insignificant ( $P > 0.05$ ). These results agree with those found by several authors who reported that exogenous enzymes improved nutrients digestibility of plant-based fish diets and leading to better growth performance and feed efficiency (Tovar *et al.*, 2002; Mohapatra *et al.*, 2012; Ray *et al.*, 2012; Dawood *et al.*, 2014; Mahmoud *et al.*, 2014). Moreover, these results are in agreement with those found by El-Dahhar (2006) who indicated that exogenous enzymes supplementation in fish diet improved the weight gain, final biomass and the survival rate of grey mullet larvae. Also, Castillo and Gatlin (2015) stated that exogenous enzymes supplementation improved nutrients digestibility and reduced nutrient excretion of plant-based fish diets. Dalsgaard *et al.* (2014) indicated that plant-based feed ingredients typically contain remnants of dietary fibers that have

various anti nutritive effects of fish. Exogenous enzymes improve the apparent digestibility coefficients of plant-based diets presumably by assisting in the breakdown of non-starch polysaccharides and alleviating some of the anti nutritive effects. Moreover, Ghomiet *et al.* (2012) found that optimum growth performance in great sturgeon fingerlings can be obtained by adding 250 mg of exogenous multienzyme/kg diet.

On the other side, fish fed on the control SBM based diet was the worst in the growth performance and feed utilization values (Table 2). Similar results have been reported in previous studies (Merrifield *et al.*, 2009; Chen *et al.*, 2011) who reported that reduced growth response and feed utilization could be explained by many factors as suboptimal amino acid balance, inadequate levels of phosphorus, inadequate levels of energy, lower feed intake caused by palatability or presence of endogenous anti-nutrients. Francis *et al.* (2001) reported that drawback in the use of soybean protein in fish diets is the presence of phytate which cannot be inactivated by heat treatment. Mahmoud *et al.* (2014) reported that the negative effect of total replacement of soybean meal on growth could be mainly explained by a general decline in feed intake combined with reduced nutrient availability. However, lower growth of fish fed on SBM based diet without any addition in the present study may have been caused by one or more of previous mentioned factors.

Data of Table (2) showed that fish fed on cooked soybean diet were higher in growth performance parameters than fish fed on non cooked soybean diets. The highest significant values ( $P < 0.05$ ) of growth performance parameters were recorded for fish fed on diet containing FM, Z<sup>2</sup>, Z<sup>1</sup>C and Z<sup>2</sup>C, where differences among last four groups were not significant ( $P > 0.05$ ). Soybeans contain several anti nutritional factors that are important considerations in fish nutrition. Present results agree with those by Peres *et al.* (2003) who found that autoclaving the defatted raw soybean meal (heated in an autoclave using the dry cycle at 130°C and 22 psi for 40 min) lowered the trypsin inhibitor and protein dispensability index, improved the nutritional value of SBM and increased plasma lysozyme, protein and resistance of fish to *E. ictaluri* challenge.

Studies on the nutritional value of processed plant proteins in various fish species have consistently shown an improvement of digestibility and growth compared to feeding unprocessed ingredients (Drew *et al.*, 2007). However, Barrows *et al.* (2007) indicated that heat treatment of soybeans is necessary to denature endogenous trypsin inhibitors and maximize nutritional value, but overheating can damage protein and reduce nutritional value. Also, they demonstrated the importance of extruder processing conditions on fish performance, and indicated that high temperature (127°C) and short time in the extruder barrel results in the greatest weight gain of rainbow trout. The improving in growth parameters in the present study may be due to the positive effect of the exogenous enzymes supplementation or cooked soybean treatment on improving diet quality by make the best nutritional use of raw materials which increase the Nile tilapia capability to utilize all plant based diets.

Values of feed conversion ratio FCR, protein efficiency ratio PER, protein productive value PPV% and energy retention ER % of Nile tilapia (*Oreochromis niloticus*) are shown in Table (3). Results indicated that fish fed on FM, Z<sup>2</sup>C, Z<sup>1</sup>C and Z<sup>2</sup> diets had significantly ( $P < 0.05$ ) better FCR than other treatments, however, differences among the last treatments were not significant. The worst ( $P < 0.05$ ) FCR was found in fish maintained on the control SBM based diet. Results of (Table 3) indicated that highest

**Table (3). Feed utilization values (means ± SE) of Nile tilapia fed diets containing different levels of zymogen and cooked soybean.**

Item	FM	SBM	Z <sup>1</sup>	Z <sup>2</sup>	Z <sup>1</sup> C	Z <sup>2</sup> C
Feed intake	37.74±0.26 <sup>a</sup>	28.85±0.28 <sup>c</sup>	32.26±1.85 <sup>b</sup>	37.73±1.14 <sup>a</sup>	35.06±3.21 <sup>ab</sup>	35.51±3.15 <sup>a</sup>
FCR	1.61±0.02 <sup>c</sup>	1.87±0.01 <sup>a</sup>	1.74±0.01 <sup>b</sup>	1.65±0.03 <sup>c</sup>	1.65±0.01 <sup>c</sup>	1.62±0.02 <sup>c</sup>
PER	2.29±0.03 <sup>a</sup>	1.96±0.01 <sup>d</sup>	2.11±0.01 <sup>c</sup>	2.18±0.04 <sup>b</sup>	2.23±0.01 <sup>ab</sup>	2.27±0.02 <sup>a</sup>
PPV	36.42±0.46 <sup>a</sup>	33.50±0.63 <sup>c</sup>	33.79±0.37 <sup>c</sup>	35.40±0.59 <sup>b</sup>	35.55±0.25 <sup>ab</sup>	36.18±0.34 <sup>ab</sup>
ER	20.68±0.21 <sup>a</sup>	19.01±0.31 <sup>b</sup>	19.20±0.26 <sup>b</sup>	20.20±0.33 <sup>a</sup>	20.48±0.18 <sup>a</sup>	20.22±0.23 <sup>a</sup>
ER	20.68±0.21 <sup>a</sup>	19.01±0.31 <sup>b</sup>	19.20±0.26 <sup>b</sup>	20.20±0.33 <sup>a</sup>	20.48±0.18 <sup>a</sup>	20.22±0.23 <sup>a</sup>

Means with different superscripts in the same row are significantly different ( $P < 0.05$ ).

significant ( $P < 0.05$ ) values of PER and PPV were found in fish maintained on FM followed by Z<sup>2</sup>C and then Z<sup>1</sup>C diets, without any significant differences ( $P > 0.05$ ) among last treatments. Similarly, results indicated that highest significant ( $P < 0.05$ ) value of ER% were found in fish maintained on FM followed by Z<sup>1</sup>C, Z<sup>2</sup>C and then Z<sup>2</sup> diets, while the lowest significantly values of PER, PPV and ER% were found in

fish maintained on the control SBM based diet. These results suggesting that plant diets supplementation with zymogen and cooked soybean meal improve the protein and energy utilization in diet. Same trends were observed by Goda *et al.* (2012) who concluded that the use of exogenous digestive enzymes (pepsin, papain and  $\alpha$ -amylase) as a feed additive for Nile tilapia is recommended to stimulate growth performance, feed conversion and nutrient utilization. Also, this result is nearly similar to what found by Ginindza *et al.* (2015) who found that all groups of *Oreochromis mossambicus* fed diets supplemented with exogenous enzyme were higher in growth performance, protein digestibility and digestive enzyme activities compared to those fed on control diet. Moreover, they explained that improvement in the growth performance was attributed to the presence of cocktail of enzymes such as cellulase, xylanase and phytase which are not naturally produced by fish. Oliveira *et al.*, (2007) reported that the inclusion of exogenous enzyme (0.05%) containing cellulose, protease and amylase in Nile tilapia diet enhanced feed conversion rate and nutrient digestibility. Also, Moura *et al.* (2014) indicated that the inclusion of enzymes in Nile tilapia diets may be an alternative to reduce anti nutritional factors found in ingredients of plant origin and increase the availability of nutrients. Moreover, Tomáset *al.* (2005) reported that apparent digestibility coefficient of dry matter, protein, and lipid were high due to the process used for preparing diets in which pellets heating might have inactivated the trypsin inhibitor.

With respect to body composition of Nile tilapia in (Table 4), results showed that no significant differences ( $P > 0.05$ ) were observed between treatments in dry matter of fish body contents. Results of fish body protein content showed that highest values ( $P < 0.05$ ) were found in fish maintained on Z<sup>2</sup>C followed by Z<sup>1</sup>C and then FM diet, without any significant differences between the last treatments, while the lowest values ( $P < 0.05$ ) were found in the fish maintained on Z<sup>1</sup> diet. Results of fish body lipid contents showed that fish maintained on Z<sup>1</sup> and Z<sup>2</sup> diets were significantly ( $P < 0.05$ ) higher than other treatments, while the lowest ( $P < 0.05$ ) value was found in fish maintained on FM diet. These results are in agreement with those found by Mahmoud *et al.* (2014) where crude protein content of Nile tilapia was increased significantly with exogenous multi enzyme supplementation. Ogunkoya *et al.* (2006) observed a positive effect on the coefficients of apparent digestibility of crude protein and lipids by investigating the inclusion of soybean meal and an enzyme complex containing xylanase, amylase, cellulase, protease and  $\beta$ -glucanase in fish diet. On the other hand, Ghomiet *al.* (2012) revealed that body protein contents decreased slightly while fat increased when fish fed on enzyme-supplemented diets. However, Stone *et al.* (2003) did not find any effect on the apparent digestibility of dry matter, energy or protein contents in Silver Perch (*Bidyanus bidyanus*) when fed on enzyme complex diets. This may be explained as body composition of fish is primarily influenced by diet composition, feeding practices, fish size, and can be controlled through nutrition.

**Table (4). Body composition (Means  $\pm$  SE) % on dry weight basis of Nile tilapia fed diets containing different levels of zymogen and cooked soybean.**

Item	FM	SBM	Z <sup>1</sup>	Z <sup>2</sup>	Z <sup>1</sup> C	Z <sup>2</sup> C
Dry matter	24.39 $\pm$ 0.04	25.88 $\pm$ 0.33	24.57 $\pm$ 0.27	25.85 $\pm$ 0.06	24.96 $\pm$ 0.04	24.86 $\pm$ 0.01
Crude protein	66.25 $\pm$ 0.04 <sup>a</sup>	64.65 $\pm$ 0.27 <sup>b</sup>	63.72 $\pm$ 0.37 <sup>b</sup>	64.26 $\pm$ 0.03 <sup>b</sup>	66.32 $\pm$ 0.13 <sup>a</sup>	66.58 $\pm$ 0.35 <sup>a</sup>
Lipid	18.53 $\pm$ 0.03 <sup>c</sup>	19.37 $\pm$ 0.09 <sup>b</sup>	21.71 $\pm$ 0.42 <sup>a</sup>	20.67 $\pm$ 0.28 <sup>a</sup>	19.06 $\pm$ 0.02 <sup>b</sup>	18.70 $\pm$ 0.12 <sup>c</sup>

Means with different superscripts in the same row are significantly different ( $P < 0.05$ ).

Economic evaluation of the experimental diets is shown in Table (5). Results showed that fish maintained on zymogen supplementation diets with or without cooked soybean meal were more economic

**Table 5. Economic efficiency for production of one kg gain of Nile tilapia fed diets containing different levels of zymogen and cooked soybean.**

Items	FM	SBM	Z <sup>1</sup>	Z <sup>2</sup>	Z <sup>1</sup> C	Z <sup>2</sup> C
Price/ kg feed P.T	5.06	3.83	4.29	4.77	4.31	4.79
FCR kg feed/kg gain	1.61	1.87	1.74	1.65	1.65	1.62
Feed cost / kg gain P.T	8.15	7.16	7.46	7.87	7.11	7.76
Reduction cost in kg gain%	0.00	12.15	8.41	3.44	12.74	4.79

Means with different superscripts in the same row are significantly different ( $P < 0.05$ ).

and reduced the feed cost. The reduction in feed cost to produce one kg fish gain ranged between 3.44 to 12.74% compared to fish fed on FM control diet. One of the aims of present study was to evaluate the effect of zymogen supplementation on cost analysis of production. Totally replacing fish meal protein by soybean meal protein led to reduce the final cost of production by 12.74% compared to the fishmeal based diet. Similar results were observed by Dawood *et al.* (2014) who indicated that dietary supplementation of exogenous enzyme would increase the profitability of rabbit fish by 20.4%.

## CONCLUSION

In conclusion, the results of the present study show that dietary zymogen supplementation with cooked soybean meal in fish diet had beneficial effects on improving growth performance, feed utilization and survival rate of Nile tilapia fed all plant based diets. Also, it is more economic and sharply reduced the fish feed cost used to produce unit fish. It is clear that studies on exogenous enzyme supplementation in aquaculture nutrition are not extensive. According to these results and favorable effects found in aquaculture species studied to date, it may be significant to increase research on this subject because it could be a useful tool to improve commercial aquaculture.

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## تحسين الكفاءة الغذائية للعلائق النباتية للبلطي النيلي بالمعاملة الحرارية لفول الصويا وإضافة الإنزيمات الهاضمة

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أجريت هذه التجربة لدراسة تأثير استخدام الإنزيمات الهاضمة الخارجية (الزيموجين) والمعاملة الحرارية لفول الصويا في الغذاء على أداء النمو وكفاءة استخدام الغذاء لأسماك البلطي النيلي ذات الوزن الإبتدائي (4.07 جم). تم عمل ستة علائق متساوية في نسبة البروتين (27%) والطاقة (4.29 كيلو كالوري لكل جم غذاء)، تم تغذية الأسماك حتى الإشباع لمدة 11 إسبوع. وكانت المعاملات كالتالي: العليقة الأولى تحتوي على مسحوق السمك وبروتين نباتي كمصدر أساسي للبروتين، العليقة الثانية (نباتية) تحتوي على مصادر بروتين نباتي فقط كمصدر للبروتين، العليقة الثالثة (نباتية) مضافا إليها 1% زيموجين، العليقة الرابعة (نباتية) مضافا إليها 2% زيموجين، العليقة الخامسة والسادسة (نباتية) مضافا إليهما 1 أو 2% زيموجين مع المعاملة الحرارية لفول الصويا علي التوالي. أوضحت النتائج تحسن أداء الأسماك بإضافة الزيموجين مع المعاملة الحرارية لفول الصويا بالمقارنة بالعليقة النباتية، وأن الأسماك التي تغذت علي العلائق الأولى والرابعة والخامسة والسادسة كانت الأفضل معنويا بالنسبة لكل النتائج المتحصل عليها الخاصة بالنمو ومعدل إعاشة الأسماك وكفاءة استخدام الغذاء عن باقي المعاملات بدون أي فرق معنوي بينهم. تشير النتائج التي تم الحصول عليها في هذه التجربة إلى أن إضافة الإنزيمات الهاضمة الخارجية (الزيموجين) والمعاملة الحرارية لفول الصويا للعلائق النباتية يحسن من نمو الأسماك وكفاءة استخدام الغذاء لأسماك البلطي النيلي كما انه أوفر إقتصاديا و يخفض من التكلفة الكلية للغذاء.