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EFFECT OF DIETARY SUPPLEMENTATION (PREBIOTIC AND PROTEASE ENZYME) ON GROWTH PERFORMANCE AND FEED UTILIZATION OF RED TILAPIA *(Oreochromis* SP.) FED WITH DIETS FREE FISHMEAL

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ABSTRACT

The present study was carried out at Mari culture Research Center, Arish University, North Sinai, Egypt. This study aimed to examine the effects of total replacement of fish meal by a combination of prebiotics and protease enzyme on growth performance and feed utilization parameters of red tilapia. A total of 150 fish were equally distributed in 15 glass aquaria. Ten fingerlings per aquarium were stocked with an average initial weight 7.40 ± 0.05g and an average initial length 7.30 ± 0.24cm. The fish were acclimatized for two weeks at experimental condition. This study was conducted as four treatments with three replicates. The fish were fed a diet containing 30.14 ± 0.08% crude protein and isocaloric 4431.1 kcal kg^{-1} Kcal/kg twice a day at a rate of 3% of total body weight for 84 days. At the end of experiment body weight, length and whole-body composition were calculated to determine growth parameters and feed utilization. The results of growth parameters indicated that T4 was recorded the highest values of most growth parameters. But T3 recorded the highest condition factor. FCR and PER were not significantly differencing between all treatments. The highest feed and protein intake were recorded for control group and T4. The highest dry matter and ether extract of whole-body composition were recorded for T3. But the best protein value, gross energy, protein, and energy retained were recorded for T4. There were not significantly differences *(P>0.05)* at fat and ash retained. In conclusion, the increasing protease level with prebiotic can effectively improve the growth performance and feed utilization of red tilapia (*Oreochromis sp*.) in low levels of fish meal 20% or the absence of fish meal in diets.

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INTRODUCTION

Feed additives are supplemented in small amounts for a specific purpose, such as to improve the quality of fish as a final product, to preserve the physical and chemical quality of the diet or to maintain the quality of the aquatic environment **(Bie *et al.*, 2015)**. The gut microbiota of fish plays an important role in the health and performance of fish. The fish gut microbiota is a complex and dynamic ecosystem containing a large amount of microorganisms and it has been reported that genetic, nutritional and environmental factors modulate the gut microbiota **(Merrifield *et al.*, 2010; Nayak, 2010; Liewellyn *et al.*, 2014)**. Phytogenic are plant-derived products which are added to the diet to improve palatability of feeds or animal performance **(Encarnacao, 2009; Karaskova *et al*., 2015)**. These plant active ingredients can exert multiple effects on the organisms, including improvement of feed

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efficiency and digestion, reduction of nitrogen excretion and improvement of gut flora and health status. Phytogenic feed additives are an extremely heterogeneous group of feed additives originating from leaves (e.g. extract of *Moringa oleifera*) roots, tubers (e.g. garlic, *Allium sativum*; ginger, *Zingiber officinale* or fruits of herbs, spices or other plants.

They are either available in a solid, dried or ground form or as extracts or essential oils **(Metwally, 2009; Gbadamosi *et al.,* 2016)**. These natural plant products have various activities like antistress, appetizer, tonic, antimicrobial and immunostimulants **(Citarasu *et al.,* 2002; 2003)**.

Ginger increases the pancreatic and intestine lipase **(Platel and Srinivasan, 2000)**. Turmeric, *Curcuma longa*, is used for wound healing, inflammation and acidity **(Jyothi *et al.,* 2003; Kumar *et al.,* 2006)**. Fenugreek, *Trigonella foenum-graecum*, seed possesses aphrodisiac and digestive effects. It has high potential to be used as a cheap source of alternative protein supplement **(Nasri and Tinay, 2007)**. **El-Kholy (2012)** studied the effect of marjoram, *Majorana hortensis,* on hybrid tilapia. He found that the response of tilapia hybrid, *Oreochromis niloticus* × *O. aureus*, to dietary herbs plants supplement with respect to growth performance and feed utilization through feeding experiments was investigated. Most studies have evaluated the effect of different concentrations of protein, carbohydrates and lipids in feed formulations, correlating these results with enzyme activity and differences in enzyme quality profile may be related to nutrient levels in the diet **(Fountoulaki *et al.*, 2005)**.

Nowadays number of exogenous enzymes (e.g. phytase, carbohydrase, protease and lipase) are used in aquaculture feeds to overcome the negative effects of anti-nutritional factors, and to improve the digestion of dietary components and enhance growth of fish **(Lin *et al.,* 2007a; Ebru and Cengiz, 2016)**. Some proteases are stable and active under harsh conditions (high temperature and pH) and in the presence of oxidizing agents or surfactants **(Klomklao *et al.*, 2005)**. Proteases are primary enzymes which have been isolated and characterized from various parts of Nile tilapia digestive tract **(Tengjaroenkul *et al.,* 2000; Hinsui *et al.,* 2006)**. In this study we evaluate the effect of using protease and prebiotic mixtures as food additives on growth performance and feed utilization for red tilapia fed with diets free fishmeal.

MATERIALS AND METHODS

Experimental Fish and Study Technique

One thousand fingerlings of red tilapia, *Oreochromis sp.*, were obtained from El-kilo 21 Marine Fish Hatchery at Alexandria Governorate, Egypt. The fish were transferred to a cement pond for the experimental condition adaptation at Mari culture Research Center, North Sinai Governorate, Egypt. The fish were weighted and randomly distributed to the experimental glass aquaria (70 X 40 X 60) cm with total capacity of 100 liters.

A total of 150 fish were equally distributed in 15 glass aquaria. Ten fingerlings per aquarium were stocked with an average initial weight 7.40 ± 0.05g and an average initial length 7.30 ± 0.24 cm. The fish were acclimatized for two weeks and fed commercial diet. This study was conducted as four treatments beside control with three replicates. The fish were fed on a diet containing 30.14±0.08% crude protein and isocaloric 4431.1 ±0.5Kcal kg^{-1} twice a day at 10:00 am and 16:00 pm at a rate of 3% of total body weight for 84 days. All aquaria were siphoned once a day to remove fecal materials then replaced by aerated clean water. Each aquarium was supplied with compressed air. Total fish weight in each aquarium was determined every two weeks

to evaluate their growth and adjust the feeding rate. Water temperature, salinity, pH and dissolved oxygen (DO) were measured twice daily. Total ammonia nitrogen (TAN) was measured twice weekly. Water salinity and temperature were recorded using conductivity-temperature meter (SET model 315i, Weilheim, WTW GmbH, Germany). DO was measured by oximeter (SET model 315i, Weilheim, WTW GmbH, Germany). pH was measured using a pH-meter (SET model 315i, Weilheim, WTW GmbH, Germany). TAN was measured using ammonia nesslerization method **(Eaton *et al.,* 1992)**. During the experimental period, means of the aquaria water temperature were 28.3 ± 0.21°C, water salinity were 20.8 ± 0.26 mg L^{-1}, dissolved oxygen were 6.8 ± 0.05 mg L^{-1}, pH were 7.97 ± 0.04 and TAN were 0.16 ± 0.02 mg L^{-1}.

Feeding Diets

The feeding diets were composed of protease enzyme and prebiotic mixtures (125g fennel + 250g marjoram + 375g fenugreek + 250g ginger) per kilogram with diets free fish meal **El-Badwy (2016)**. The control group feeding diet without any additives. T1 was the group feeding on diet containing prebiotic mixtures without protease. Treatments of T2, T3 and T4 were fed on diets containing protease enzyme at 0.10, 0.15 and 0.20 g kg^{-1} plus plant mixture, respectively. The protease (5000 U g^{-1} product, supplied by Huvepharma, Antwerp, Belgium) was added to the diets to provide three concentrations of 500 (0.10 mg kg^{-1}), 750 (0.15 mg kg^{-1}) and 1000 (0.20 mg kg^{-1}) U protease kg^{-1} diet, the formulation and chemical composition of the experimental diets are presented in (Table 1). Activity of protease enzyme was assayed according to the method of **Committee on Food Chemicals Codex (1996).** One protease unit was the amount of enzyme that releases 1.0 µg of phenolic compound, expressed as tyrosine equivalents, from a casein substrate per minute at pH 7.5 and 40°C. The analyzed activity of protease was 4395 Ug^{-1}. Using a laboratory pellet mill (California Pellet Mill, San Francisco, CA, USA), the ingredients were mixed and manufactured as pellets and dried at 37°C overnight. The pellets were subsequently stored at −20°C until use **(Shi *et al.,* 2016)**.

Growth Performance and Feed Utilization

Growth performance and feed utilization were measured using the following equations: Weight gain (WG) = final weight (g) – initial weight (g); Gain % = (WG/W1) x 100; Condition factor (K) = (W/L^3) x 100, where, W is weight of fish in grams and L is total length of fish in cm; specific growth rate (SGR) = (LnW2 – LnW1)/ t X 100, Where, Ln is the natural log; W1 is initial body weight and W2 is the final body weight in grams and "t" is the experimental period in days; feed conversion ratio (FCR) = Feed intake (g)/Weight gain (g); Feed efficiency (FE %) = gain in weight (g) / feed intake (g) ; protein efficiency ratio (PER) = weight gain (g)/protein ingested (g); protein productive value (PPV%) = (retained protein/protein intake) X 100 and EPV% = energy retained / energy intake.

Proximate Analysis

Chemical analysis of diets and five individual fish from each aquaria was carried out according to **AOAC (2000)**. Moisture, crude protein (CP), either extract (EE) and ash were performed as follow: Dry matter (DM) was determined after drying the samples in an oven (105°C) for 24 h. Crude protein was determined by Micro Kjeldahl method, N × 6.25 (Model VELP Scientifica, UDK127) and crude fat by Soxhlet extraction with diethyl ether (40 – 60°C). Ash was estimated by fish incineration at 550°C for 12 h. Crude fiber was determined using the method of **Van Soest *et al.* (1991)**. Nitrogen free extract (NFE) was calculated as [100 – (crude protein + ether extract + fiber + ash)].

Table 1. Formulation and proximate analysis of the experimental diets on dry matter basis

	Control	T1	T2	T3	T4
Fish meal (60%)	**10**	**0**	**0**	**0**	**0**
Soya bean meal (45%)	**28**	**27**	**27**	**27**	**27**
Rice bran (14.4%)	**16**	**15**	**15**	**15**	**15**
Yellow corn (8.5)	**13**	**9**	**9**	**10**	**10**
Wheat bran (16.4)	**13**	**17**	**16.9**	**14.85**	**14.8**
Gluten (60%)	**10**	**20**	**20**	**21**	**21**
Prebiotics[(1)]	**0**	**2**	**2**	**2**	**2**
Protease Enzyme[(2)]	**0**	**0**	**0.1**	**0.15**	**0.2**
Linseed oil	**2**	**2**	**2**	**2**	**2**
Sun flower oil	**2**	**2**	**2**	**2**	**2**
Fish oil	**2**	**2**	**2**	**2**	**2**
Vitamin and minerals mixture[(3)]	**4**	**4**	**4**	**4**	**4**
Proximate analysis (% on dry matter basis)					
Crud protein	**30.14**	**30.20**	**30.02**	**30.20**	**30.19**
Either extract	**2.73**	**2.19**	**2.18**	**2.07**	**2.06**
Crude fiber	**5.62**	**5.93**	**5.92**	**5.69**	**5.69**
Ash	**5.63**	**4.56**	**4.55**	**4.43**	**4.43**
NFE	**55.88**	**57.12**	**57.33**	**57.61**	**57.63**
GE[(4)]	**4433.2**	**4433.9**	**4432.1**	**4435.3**	**4420.9**
DE[(5)]	**3712.0**	**3712.7**	**3703.8**	**3706.3**	**3708.5**
ME[(6)]	**1358.2**	**1359.4**	**1361.2**	**1369**	**1409.9**
P/E[(7)]	**222.3**	**222.2**	**220.5**	**220.6**	**213.8**

[1]Prebiotics (125g fennel + 250g marjoram + 375g fenugreek + 250g ginger)/kg according to **El-Badwy (2016).**

[2]The protease (5000 U g^{-1} product, supplied by Huvepharma, Antwerp, Belgium).

[3]Vitamin and minerals kg^{-1} of mixture contains: 4800 IU Vit A, 2400 IU cholecalciferol (Vit D), 40g Vit E, 8g Vit K, 4g Vit B12, 4g Vit B2, 6g Vit B6, 4g pantothenic acid, 8g nicotinic acid, 400mg folic acid, 20mg biotin, 200mg choline, 4g copper, 0.4g Iodine, 12g Iron, 22g manganese, 22g zinc, 0.04g selenium folic acid, 1.2mg niacin, 12mg d-calcium pantothenate, 26mg pyridoxine HCl, 6mg riboflavin, 7.2mg thiamin HCl, 1.2mg sodium chloride (NaCl, 39% Na and 61% Cl), 3077mg ferrous sulfate ($FeSO_4.7H_2O$, 20% Fe), 65mg manganese sulfate ($MnSO_4$, 36 % Mn), 89 mg zinc sulfate ($ZnSO_4.7H_2O$, 40 % Zn), 150mg copper sulfate ($CuSO_4.5H_2O$, 25 % Cu) and 28mg potassium iodide (KI, 24 % K and 76 % I).

[4]Gross energy (Kcal/kg) = 5.65 (CP %) + 9.45 (EE %) + 4.0 (NFE %) according to **Viola *et al.* (1981).**

[5]Digestable energy (Kcal/kg) = 5 (CP %) + 9 (EE %) + 3.5 (NFE %) according to **NRC (1993).**

[6]Metabolizable Energy (Kcal/kg) = 3.9 (CP %) + 8 (EE %) + 1.6 NFE %) according to **philips and Brockway (1959).**

[7]P/E (mg/ Kcal) = (mg Protein/Metabolizable energy Kcal) X 100 according to **Wee and Tuan (1988).**

Statistical Analysis

Data were tested using the analysis of variance one way (ANOVA) using SAS **(SAS, 2004)**. Where a significant difference was observed for a measured value, mean separated using Duncan's multiple range test **(Duncan, 1955)** at the 5% level.

RESULTS

Growth Performance and Feed Utilization

Data of initial body weight, final body weight, gain in weight (GW), average daily gain (ADG) and specific growth rate (SGR% /day) are presented in Table 2. The analysis of variance of these data indicated that there is no significantly differences (*P>0.05*) among treatments in gain in weight, average daily gain and specific growth rate. The highest values recorder for T4; 14.8 ± 0.50, 0.17 ± 0.01 and 1.13 ± 0.03, respectively. Initial body length and final body length values show not significantly differences (*P>0.05)* between treatments. The highest value of final body length was recorded for T1 and the lowest value of final body length was recorded for T3. The highest condition factor was recorded for T3 but the lowest value was recorded for T1. No significantly differences were recorded in survival rate among all groups (Fig. 1).

Feed, protein, fat and energy intake of the experimental diets are presented in Table 3. The analysis of variance shows that there were significantly differences (P≤0.05) among all treatments. Control and T4 groups were recorded the highest feed, protein, fat and energy intake. The lowest values of feed, protein, fat and energy intake were recorded for T1 group.

There were no significantly differences (P>0.05) among treatments of FCR and FE. The highest FCR and FE were recoded for T4 group and the lowest values were recorded for T1 group. PER of tested diets was not significantly different (P>0.05) between treatments. Table 3 shows the percentage of protein productive value (PPV %) of diets. The highest value of it was recorded for T4 group and the lowest one was recorded for T1. The highest EPV % was recorded for T3 group. Furthermore, the least EPV % value was recorded for T1 group.

Body Composition and Energy Content of Whole-Body Fish

Analysis of whole-body composition on DM basis, crude protein CP, either extract EE, ash and gross energy content for red tilapia at initial sample and the end of the experimental period are presented in Table 4.

There were significantly differences *(P≤0.05)* between treatments in dry matter. The highest value of DM was recorded for T3 group and the lowest one was recorded for initial sample. There were significantly differences *(P≤0.05)* between treatments in (CP). The highest value of CP was recorded for T4 group and the lowest value was recorded for T1 group. The highest values in either extract was recorded for T3 group and the lowest one was recorded for initial sample. Ash content also significantly differences *(P≤0.05)* between treatments. The highest value of ash content was recorded for initial sample and the lowest value was recorded for T1 group.

Data in Table 5 show protein, fat, ash and energy retained. There were no significantly differences *(P>0.05*) of fat and ash retained between all treatments. But there were significantly different (*P≤0.05)* of protein and energy retained between treatments. The highest values of protein retained, and energy retained were recorded for T4 group. The lowest protein retained value was recorded for T1 group.

Table 2. Growth performance of red tilapia as affected by addition of protease and prebiotic mixtures with diets free fishmeal

	Experimental Diets				
Items*	**Control (T0)**	**T1**	**T2**	**T3**	**T4**
Initial body weight (g fish^{-1})	7.43 ± 0.06	7.33 ± 0.06	7.47±0.06	7.33 ± 0.06	7.40 ±0.00
Final body weight (g fish^{-1})	21.57 ±3.25	18.53 ± 2.78	19.50 ±0.46	20.50 ±1.51	22.20 ±0.50
WG (g fish^{-1})	14.13 ±3.28	11.20 ± 2.81	12.03 ± 0.45	13.16 ± 1.55	14.80 ± 0.50
ADG (g fish^{-1})	0.258 ±0.04	0.22 ± 0.03	0.232 ±0.01	0.244 ±0.02	0.26 ± 0.01
Gain %	190.17	152.80	161.04	179.54	200.00
SGR (% day^{-1})	1.26 ±0.18	1.09 ±0.18	1.14 ± 0.03	1.22 ± 0.10	1.31 ± 0.03
Initial length (cm)	7.32 ± 0.93	7.18 ± 0.81	7.42 ± 0.89	7.20 ±1.14	7.17 ±1.22
Finial length (cm)	9.53^{a}±1.49	9.78^{a}±1.32	9.12^{ab}±0.82	8.57^{b}±2.09	9.22^{ab}±1.89
Condition factor (K)	2.49^{bc}±0.41	1.98^{c}±0.22	2.58^{abc}±0.21	3.30^{a}±0.59	2.85^{ab}±0.35

*Means followed by different letters in each row are significantly different *(P≤0.05)*.

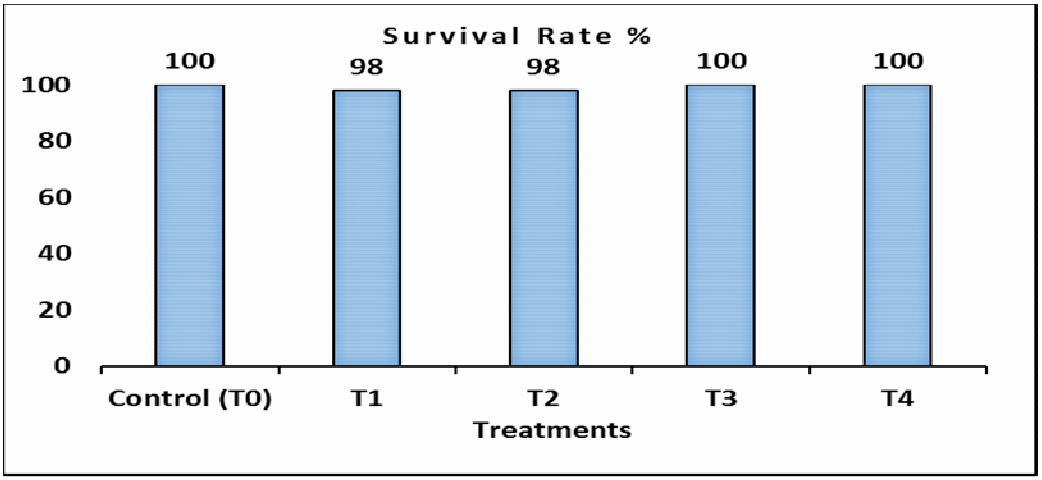

Fig. 1. Survival rate of red tilapia through the experimental period

Table 3. Feed utilization of red tilapia as affected by addition of protease and prebiotic mixtures with diets free fishmeal

	Experimental Diets				
Items*	**Control (T0)**	**T1**	**T2**	**T3**	**T4**
Feed intake (g fish^{-1})	30.85^{a}±2.82	26.03^{b}±1.91	27.54^{ab}±0.86	27.68^{ab}±1.53	29.8^{a}±0.83
Protein intake(g fish^{-1})	9.29^{a}±0.85	7.81^{b}±0.57	8.3^{ab}±0.26	8.36^{ab}±0.46	9.01^{a}±0.25
Fat intake (g fish^{-1})	0.84^{a}±0.08	0.57^{b}±0.04	0.57^{b}±0.02	0.61^{b}±0.04	0.62^{b}±0.02
Energy intake (Kcal kg^{-1})	136.39^{a}±2.46	115.37^{ab}±8.47	122.09^{b}±3.82	122.77^{ab}±6.80	132.17^{a}±3.70
FCR[1]	2.18 ± 0.34	2.34 ± 0.06	2.28 ± 0.07	2.10 ± 0.12	2.00 ± 0.03
FE[2]	45.00 ±2.82	42.00 ±2.17	43.86 ±2.7	47.62±2.59	50.00 ±1.23
PER[3]	1.52±0.12	1.41 ±0.14	1.45 ±0.01	1.56±0.06	1.65 ±0.01
PPV%[4]	31.17	22.01	32.63	33.33	37.43
EPV%[5]	19.74	16.62	19.32	21.94	21.66

*Means followed by different letters in each row are significantly different $P \leq 0.05$. 1- FCR= feed conversion ratio, 2- FE= feed efficiency, 3- PER= protein efficiency ratio, 4- PPV= protein productive value, EPV= energy productive value.

Table 4. Chemical composition and energy content of whole body of red tilapia as affected by different diets on dry matter basis

		Experimental diets				
Items*	**Initial Sample**	**Control (T0)**	**T1**	**T2**	**T3**	**T4**
Dry matter	29.10^{b} ± 0.10	30.26^{ab} ± 0.71	30.29^{ab} ± 0.68	30.25^{a} ± 0.97	31.17^{a} ± 0.64	30.26^{ab} ± 0.97
Crude protein	70.52^{b} ± 0.39	67.67^{c} ± 0.23	57.36^{d} ± 2.13	70.61^{b} ± 0.44	67.08^{c} ± 0.83	72.72^{a} ± 0.70
Either Extract	6.23^{c} ± 0.07	18.83^{a} ± 0.69	19.91^{a} ± 0.96	16.50^{b} ± 0.69	20.19^{a} ± 1.53	16.76^{b} ± 0.98
Ash	23.24^{a} ± 0.73	13.5^{bc} ± 0.91	22.72^{c} ± 0.38	12.89^{bc} ± 0.67	12.73^{c} ±1.80	10.5^{ab} ± 0.07
Gross energy	5442.2	5156.5	5105	5418.7	5384.6	5598.2

*Means followed by different letters in each row are significantly different $P \leq 0.05$.

Table 5. Protein, fat, ash and energy retained of whole-body composition for red tilapia at the end of the experiment

	Experimental diets				
Items*	**Control (T0)**	**T1**	**T2**	**T3**	**T4**
Protein retained (g)	2.90^{a} ± 0.72	1.72^{b} ± 0.53	2.71^{b} ± 0.04	2.79^{a} ± 0.46	3.37^{a} ± 0.30
Fat retained (g)	$1.1\,0^{a}$ ± 0.22	0.99^{a} ± 0.19	0.86^{a} ± 0.04	1.16^{a} ± 0.21	0.99^{a} ± 0.08
Ash retained (g)	0.54^{a} ± 0.33	0.95^{a} ± 0.59	0.44^{a} ± 0.32	0.48^{a} ± 0.26	0.37^{a} ± 0.20
Energy retained (g)	26.92^{ab} ± 6.17	19.18^{b} ± 4.83	23.59^{ab} ± 0.51	26.93^{ab} ±4.57	28.63^{a}±2.22

*Means followed by different letters in each row are significantly different $(P \leq 0.05)$.

* Protein, fat, Ash and energy retained were determined using the following equation. {[(Final body weight x final nutrient concentration) - (Initial body weight x initial nutrient concentration)]/ Total nutrient consumed throughout the study (g)} x 100.

DISCUSSION

Growth Performance and Feed Utilization

By increasing the level of enzyme and prebiotic mixtures the average body weight is increasing as shown in Table 2. The improved growth might come from the hydrolysis of protein, the degradation of dietary anti-nutritional factors these results in agreement with **Lin *et al.* (2007b)** who reported that the addition of a commercial enzyme complex of neutral protease, beta glucanase and xylanase improved growth performance. **Adeoye *et al.* (2016)** fed Nile tilapia with probiotics, a mix of enzymes containing phytase, protease and xylanase, and the combination of enzymes and probiotic. Tilapia fed diets supplemented with enzymes plus probiotics performed better than tilapia fed only the probiotic supplemented diets in terms of final body weight, feed conversion ratio and protein efficiency ratio.

Red hybrid tilapia fed diets gave positive effects of exogenous protease on growth performance, the same findings were reported in black carp *Mylopharyngodon piceus* by **Chen *et al.* (2009)**, crucian carp **(Shi *et al.*, 2016)**, red tilapia (*Oreochromis niloticus* × *O. aureus)* and Pacific white shrimp **(Song *et al.*, 2017)**. These results disagreement with those reported by **Ng and Chong (2002)**. Palm kernel meal did not improve growth and feed utilization

when a combination of protease, cellulase, glucanase, pectinase and pure mannanase when added to the diets. Our results demonstrated that there are significantly differences between groups for WG, ADG and SGR. The highest values were recorded for T4 group. It might be due to the digestive enzymes' response can be also influenced by the feeding period, as changes in protein synthesis and enzyme activity in fish which can be observed after a long feeding period. These findings are in agreement with **Krogdahl *et al*. (1994) and Kaushik *et al*. (1995).**

Protease supplementation can improve 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. These authors **(Yigit *et al.*, 2016)** stated that no 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. In the present study, there are improvement of growth including FBW, GW, G% and SGR of fish fed diets containing protease and prebiotic mixtures as supplementation diets. This may be inferring the improved nutrient bioavailability to minimize anti-nutritional effects on nutrient digestibility of proteins. Based on the nutritional properties of most plant derived ingredients however, combing several ingredients supplemented with exogenous feed additives such as amino acids are more effective in replacing fish meal **(Gatlin *et al.*, 2007)**. **Carter *et al.* (1994)** reported no effects of dietary supplementation with combinations of enzymes on the apparent digestibility of nitrogen in Atlantic salmon, however the specific growth rate and feed efficiency were significantly improved.

The current study results show that not significantly differences *(P>0.05*) were recorded in survival rate among all fish groups. These results in agreed with **Amer (2017)** who 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 multienzyme preparations (Pan Zyme and Phytase-plus broiler 500) didn't affect survival rates of Nile tilapia fed SBM-based diets.

Data in Table 2 show that the highest condition factor was recorded for T3 group as compared with control. Higher condition factors indicated good health with an isometric growth, which is desirable for fish. The condition factor is an important factor to determine the relative degree of robustness and nourishment in fish **(Mortuza and Al-Misned, 2013)**. This condition factor might be influenced by sex, age, species, maturity, and environmental conditions **(Anyanwu *et al.*, 2007)**. Condition factor for all the experimental diets were indicating that fish were above the average condition with good health during the entire period of the experiment.

Data in Table 3 shows that increasing the levels of (protease enzymes and plant mixture) gave not significantly difference between all groups in FI, PI, EI, FE and PER. This means that the enzyme and prebiotic mixtures may help fish to get their protein needs from diets free fish meal. Consequently, the enzymes may be make up the fish for absent fish meal. These results agreed with the findings reported by **Li *et al.* (2016)** in (*Oreochromis niloticus* × *Oreochromis aureus*). These authors **(Li *et***

***al*., 2016)** reported that the increased digestibility of dry matter and crude protein was observed by the supplementation of protease and the improvements in feed utilization which can be due to by increasing of free amino acids in the diets by enzyme. **Dalsgaard *et al*. (2012)** reported that supplementing protease to soybean containing diets for rainbow trout significantly increased the apparent digestibility of protein, lipid, phosphorus and dry matter. **Dalsgaard *et al*. (2012)** explained an improved nutrient intake in fish fed soybean meal containing diets by targeting protein anti nutrients or hydrolyzing antigenic proteins. The exogenous multi-enzymes may enhance the palatability of the plant diets **(Deguara *et al.*, 1999)**.

Our results show that the lowest FCR for T4 might be due to the proteases has the potential to increase utilization of crude proteins from plant ingredients by increasing crude protein digestibility. **Hiophe-Ginindza *et al*. (2016)** reported that *Oreaochomis mosambicus* fed a kikuyu-based diet supplemented with a multi enzyme complex composed of cellulase, xylanase and phytase had improved growth, lower feed conversion rate values.

Bairagi *et al*. (2004) reported that diets formulated with 40% leucaena, *Leucaena leucocephala*, leaf meal inoculated with enzyme-producing fish intestinal bacteria *Bacillus subtilis* enhanced feed efficiency ratio, protein efficiency ratio, in tilapia (*Oreochromis niloticus* × *O. aureus*). Apart from the potential of exogenous (enzymes and plant mixture) might be due to promote growth and nutrient utilization in agreement with **Adeola and Cowieson (2011)**. Also this mixture of plant and proteases expected to substrates availability for specific populations of gut microbes agreed with **Ogunkoya *et al*. (2006)** and **Jiang *et al*. (2014)** they found no effect on growth and feed efficiency with the addition of graded levels of enzyme to rainbow trout offered a soybean meal based diet. These improvements in digestibility may be due to the increase of free amino acids in the diets by the enzyme becoming active with the help of moisture and temperature during processing.

PER of tested diets led to not significantly differences ($P>0.05$) between treatments. So these results agreed with PER which increased with supplemented enzymes as seen in **Tudkaew *et al*. (2008)** and **Lin *et al*. (2007a)** they found that the addition of a commercial enzyme complex improved growth performance but no effect was in apparent digestibility of protein, lipid and gross energy in (*Oreochromis niloticus* × *O. aureus)*. On contrary to **Hiophe-Ginindza *et al*. (2016)** who reported that *O. mosambicus* fed a kikuyu-based diet supplemented with a multi-enzymes increased protein efficiency, higher protein digestibility. In essence protease inhibitors are natural antimetabolic proteins which interfere with the digestive processes and protein utilization, similar to the effects seen with phytate by **Alarcón *et al.* (1999)**. The supplementation of 250 mg kg^{-1} protease in diet containing rapeseed and peas significantly improved the apparent digestibility of dry matter, crude protein, lipid and energy **(Drew *et al*., 2005)**. Digestibility of crude protein and crude lipid were significantly improved in juvenile Gibel carp, *Carassius auratus gibelio,* **(Liu *et al*., 2018)** fed incremental increases in dietary protease up to 300 mg kg^{-1}.

Body Composition and Energy Content of Whole-Body Fish

The results of body composition in the present study revealed significant differences ($P<0.05$) when FM was completely replaced by corn gluten and supplementation with protease enzymes and plant mixture. The results presented in Table 4 show the lower dry matter, either extract and higher in protein and energy content of fish were recorded for T4 group. This might be due to enhanced feed utilization. These results in agreement with **Lei *et al*. (2016)** who

showed the digestibility of dry matter and crude protein was increased by the supplementation of protease. In contrary, **Amer *et al.* (2015)** concluded that FM can be completely replaced by SBM in Nile tilapia diets by the inclusion of L-carnitine at 300 g kg^{-1} without any significant differences in body composition. **Ng and Chong (2002)** 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.

There was no significantly differences between treatments in energy retention. These results in agreed with **Dalsgaard *et al.* (2012)** who observed no improvement in net energy retention when proteases were added to soybean meal containing diets for rainbow trout. Also, the type of enzymes and its concentration (relative to body weight) affects fish response to enzyme supplementation **(Lin *et al*., 2007a)**.

Conclusion

We concluded that the use of the prebiotic mixtures (fenugreek, fennel, marjoram and ginger) and protease enzyme in the diets free fish meal shows high significant improvement in the components of the diets and feed utilization, which led to enhancement the growth, survival rate and the chemical composition of red tilapia flesh.

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الملخص العربي

تأثير المكملات الغذائية (مخلوط نباتي وانزيم البروتيز) على أداء النمو والاستفادة من الغذاء لأسماك البلطي الأحمر *أوريوكروميس* المُغذاة على علائق خالية من مسحوق السمك

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تمت الدراسة بمركز بحوث الاستزراع البحري، جامعة العريش، شمال سيناء، مصر، تهدف هذه الدراسة إلي تقييم تأثير الإحلال الكلي لمسحوق السمك مع إضافة نسب مختلفة من مخلوط البريبيوتك وإنزيم البروتيز على أداء النمو والاستفادة من الغذاء لأسماك البلطي الأحمر. تم توزيع ١٥٠ سمكة في ١٥ حوض زجاجي بمعدل ١٠ سمكات للحوض بمتوسط وزن ابتدائي ٧,٤٠±٠,٠٥ جرام ومتوسط طول ابتدائي ٧,٣٠ ±٠,٢٤ سم. تم أقلمت الأسماك لمدة أسبوعين على ظروف التجربة، صممت هذه التجربة من ٤ معاملات بجانب المجموعة الضابطة بثلاث مكررات، غُذيت الأسماك على علائق تحتوي على ٣٠,١٤ ±٠,٠٨ % بروتين خام والطاقة الكلية (٤٤٣١.١ كيلو كالوري/كجم) مرتين باليوم بمعدل ٣% من الوزن الكلي للأسماك لمدة ٨٤ يوم. وفي نهاية التجربة، تم تقدير الوزن والطول النهائي وكذلك تم عمل تحليل كيميائي لجسم الأسماك وذلك لحساب معدلات النمو والاستفادة من الغذاء. وجد أن هناك فروق معنوية لكل النتائج بين المعاملات المختلفة، أوضحت بيانات النمو أن المعاملة رقم ٤ سجلت أعلى النتائج لأغلب قياسات أداء النمو. وسجلت المعاملة رقم ٣ أعلى معامل حالة، بينما لا توجد فروق معنوية بين المعاملات في نسبة التحويل الغذائي ونسبة كفاءة البروتين، وسُجل أعلى غذاء وبروتين مأكول للمجموعة الضابطة والمعاملة رقم ٤، وكانت أعلى نسبة رطوبة ونسبة دهون في جسم الأسماك في المعاملة رقم ٣. وكانت أفضل قيمة للبروتين والطاقة الكلية والبروتين والطاقة المترسبة في جسم الأسماك في المعاملة ٤، كما أنه لا توجد فروق معنوية بين المعاملات لكلا من الدهون والرماد المترسب في جسم الأسماك وعلى ذلك فان إضافة مخلوط البريبيوتك مع انزيم البروتيز يعمل على زيادة معدل النمو ورفع كفاءة استخدام الغذاء في علائق تحتوي على نسب قليلة من مسحوق السمك او خالية تماما من مسحوق السمك.

الكلمات الاسترشادية: البلطي الأحمر، إنزيم البروتيز، مخلوط نباتي، أداء النمو.

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