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EFFECT OF SUPPLEMENTAL MICROBIAL PHYTASE ON GROWTH PERFORMANCE OF NILE TILAPIA (*Oreochromis niloticus*)

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ABSTRACT

This study was conducted at fish research farm, poultry production Department, Faculty of Agriculture, Assiut University, Egypt. This experiment was designed to study the effect of addition microbial phytase on growth performance of Nile tilapia (*Oreochromis niloticus*). Healthy, Nile tilapia (*Oreochromis niloticus*) with an average initial body weight of 13±1g were adapted for two weeks in cylindrical tanks (1.5m X 2m depth). All experimental fish were fed on basal diet three times daily (9:00 AM; 12 PM and 3 PM) at a rate of 3% live body weight. At the end of adaptation period, a total number of 600 healthy fish were individually chosen, weighed and measured for the total length, randomly distributed into six experimental groups (100 fish each). The first group was fed on the basal diet and considered as a control (C). The 2nd (T2), 3rd (T3), 4th (T4), 5th (T5) and 6th (T6) groups were fed on the basal diet supplemented with 500, 750, 1000, 1250, 1500 unites of commercial phytase, respectively. During the experimental period (12 weeks), fish weight and length were measured individually every two weeks. The quantity of food was re-adjusted biweekly according to change in live body weight. Results showed that the fourth group (1000 units) had the highest body weight and body weight gain as compared to the control and other groups. Meantime, the same group was higher than the control in the specific growth rate, body length increment and condition factor (CF). Insignificant increase ($P>0.05$) in the body length was observed in first, second, third and fourth groups as compared to the control. Generally, there was an improve in fish growth performance in all studied parameters as a result of adding microbial phytase to the diet and this improvement was clear in the fourth group (1000 units) compared to the others.

Key words: Growth performance, Nile tilapia, Microbial phytase

INTRODUCTION

The shortage of human dietary protein can be provided by fish protein, particularly in developing countries. However, this approach requires good quality fish meals which represent 40-60% of total operating costs in intensive aquaculture enterprises (FAO, 1983). Recently microbial phytase products are commercially available in animal feeds (Jackson *et al*., 1996), and it could used for Nile tilapia feed containing high levels of plant ingredients in order to reduce the waste of phosphorus. After mid-1990s, many studies related to the effects of supplemental phytase on nutrient utilization or growth of fish was done in common aquaculture species (Schaefer *et al*., 1995; Robison *et al*., 2002; Liebert and Portz, 2005).

Phosphorus in feedstuffs presents in the form of phytate which is unavailable to fish and other monogastric animals that lack intestinal phytase (Storebakken *et al*., 1998). Addition of phosphorus to diets has implications on water quality and phosphorus pollution and it can reduce the bioavailability of several mineral ions such as calcium, magnesium, zinc, manganese, copper and iron (Papatryphon *et al*., 1999) as well as feed proteins (Liu *et al*., 1998 and Sugiura *et al*., 2001).

One of the best ways to decrease phytic acid content in high plant protein diets is the addition of phytase enzyme (Vielma *et al*., 2000). The roles phytase enzyme is reduce the incidence of aquaculture pollution, enhancement growth performance and minimizing the cost of feed formulation for fish. Also, it increases phytate digestibility and bioavailability of phosphorus (P) in cereal due to high absorption of calcium (Ca), magnesium (Mg), zinc (Zn), manganese (Mn), iron (Fe) and copper (Cu) divalent cations (Sardar *et al*., 2007). Moreover, it reduces the supplemented inorganic phosphorus (P) amount needed to reach high levels of growth, bone mineralization, (Cao *et al*., 2008), improve the utilization of dietary protein and energy (Cheng and Hardy 2004).

This study aimed to determine the effects of phytase supplementation to vegetable diet (corn-soy diet) on growth performance of Nile tilapia, *Oreochromis niloticus*.

MATERIALS AND METHODS

Experimental fish:

Nile tilapia *(Oreochromis niloticus)* with an average initial body weight of 13±1.0g and total length of 9±1.0cm were used in this experiment. Fish were reared in cylindrical tanks (1.5m x 2m depth) with water flow 8L/h and aerated by using electric pump. The average water temperature was29±1.0 ^{o}C, (measured three times daily). The experimental fish were adapted for two weeks in the previous conditions before starting. Thereafter, a total number of 600 healthy fish were chosen, weighed and the total length was measured, then divided randomly into six experimental groups (100 fish each).

Treated groups were distributed as follows: the control group (T1) fed the basal diet and five groups T2,T3, T4,T5, T6 which were fed on the basal diet supplemented with 500, 750, 1000, 1250 and 1500 units of commercial phytase respectively. Phytase is a feed additive manufactured by Multi Vita Animal nutrition St.14-4c Second Industrial Area, 6 October City, Giza. Plant based diets were formulated according to the recommendations for tilapia (Santiago and Lovell, 1988; NRC, 1993) and according to Liebert and Portz (2005).

Experimental diet:

Feed ingredients and proximate analysis of the experimental diet is shown in Table (1). Dry ingredients of the experimental diets were thoroughly grinded, mixed and compressed in a pelleted form (1.5-2.5 mm diameter). Diets were offered 3 times daily (9:00 AM; 12 PM and 3 PM) at rate of 3% body weight. Feed quantity was re-adjusted biweekly according to the change in body weight (BW).

Studied criteria:

Water quality parameters: Water temperature was measured three times daily (8.00 AM, 12.00 noon and 5.00 PM).

Growth parameters: The individual body weight (g) to the nearest 0.1g and total body length (cm) to the nearest 0.1cm for all fish (100 fishes) per treatment were recorded biweekly. Feed consumption of each treatment was recorded and readjusted according to the obtained biomass for every treatment biweekly.

Table (1): Composition and chemical analysis (on DM basis) of the experimental basal diets.

Ingredients	**(%)**
Soybean meal (49.95% crude protein)	31
Corn gluten	18
Corn	40
Soybean oil	6.00
Vitamin and mineral mix [a]	1.00
L-Lysine	1.10
DL-Methionine	0.90
Calcium carbonate	1.00
Carboxymethylcellulose	1.00
Total	100.00
Proximate analysis	
crude protein %	30.23
Crude fat %	8.19
Ash%	5.12
Calcium %	0.53
Gross energy (MJ/kg)	19.68
Total P	0.41
Non-phytate P	0.15

[a] Vitamin and mineral mix (per kg of diet): $MnSO_4$, 40 mg; MgO,10 mg; K_2SO_4, 40 mg; $ZnCO_3$, 60 mg; KI, 0.4 mg; $CuSO_4$, 12 mg; Ferric citrate, 250 mg; Na_2SeO_3, 0.24 mg; Co, 0.2 mg; Retinol,40000 IU; Cholecalciferol, 4000 IU; α-tocopherolacetat, 400 mg; menadione, 12 mg; Thiamin, 30 mg; Riboflavin, 40 mg; Pyridoxine,30 mg; Cyanocobalamin, 80 mcg; Inicotinic acid, 300 mg; Folic acid,10 mg; Biotin, 3 mg; Pantothenic acid, 100 mg; Inositol, 500 mg; Ascorbic acid, 500 mg.

Specific growth rate is calculated using the following formula:

$$(SGR) = \frac{\ln(Wf) - \ln(Wi)}{T} X100$$

Where:

SGR specific growth rate (% body weight gain per day)

$\ln(W_f)$ natural log of the mean final weight

ln(Wi) natural log of the mean initial weight

T time in days.

The body length increment (LI, cm) was estimated. Condition factor (K- Value) was calculated according to (Schreek and Moyle, 1990).

Statistical analysis:

Analysis of variance was conducted using the General Linear Models (GLM) procedure of SAS (1998). Duncan's multiple range tests was used to compare between treatment means (Steel and Torrie, 1980). The Model of analysis was as follows:

$Yij = \mu + Ti + Eij$

μ = the overall mean.

Ti= the effect of treatment. Where (i=1 to .6)

Eij = the random error.

RESULTS AND DISCUSSION

Growth performance:

The obtained results are presented in Table (2). The results showed that the fourth group (1000 units phytase/kg diet) had the highest body weight as compared to the control and other groups, the differences were insignificant (P>0.05).

The body weight gain ranged from 4.95g/fish for the control to 5.2g for T4. These results are in agreement with the findings of Furuya *et. al*., (2001) who reported that phytase supplementation between 500 and 1500 U/kg fed with plant-based diets led to increase in weight for Nile tilapia (*O. niloticus*).

This increase in body weight could be due to improvement of digestive enzyme activities which improve digestibility of protein and lipid (Nwanna, 2004 and Nwanna et *al*., 2008) and releasing enough phytate-phosphorus for growth (Roehutscord and Pfeffer, 1995; Papatryphon *et al*., 1999; and Bai *et. al*., 2003).

In addition, the obtained results in Table (2) showed that the level of 1000 U/kg diet was the best level because it led to the highest body weight change while levels higher than that (1250 and 1500 U/kg diet) led to decrease of body weight. This could be due to increasing levels of phytase which showed negative effect on growth performance (Roehutscord and Pfeffer, 1995; and Papatryphon *et al*., 1999).

Contrary, some researchers (Robinson *et al*., 2002; Kornegay, 2001; Sajjadi and Carter (2004) and Nwanna, 2007) recorded no improvement on fish growth when fed with phytase. These results may be due to the phytase levels (Yoo *et al*., 2005; Biswas *et al*., 2007; and Nwanna and Schwarz, 2007) and/or fish species (Furuya *et al*., 2001; Cao *et al*., 2007; and Ayhan *et al*., 2008).

Data in Table (2) showed that the body weight gain of T3, T4 and T5 increased significantly (*P<0.05*) as compared to the control ones.

Table (2): Averages body weight and daily gain (g) of *O. niloticus* fed different levels of dietary phytase

Period (week)	Treatments (U Phytase)/kg diet					
	Control (0)	T2 (500)	T3 (750)	T4 (1000)	T5 (1250)	T6 (1500)
Initial	13.25±0.4684	13.26±0.4684	13.26±0.4684	13.26±0.4684	13.26±0.4684	13.25±0.4684
Final	18.20±0.4601	18.28±0.4601	18.34±0.4601	18.46±0.4601	18.41±0.4601	18.20±0.4601
B.W change	4.95	5.02	5.08	5.20	5.15	4.95
Daily gain	0.06	0.06	0.06	0.06	0.06	0.06

$\bar{x}$ = Means±SE.

These results were confirmed by Schafer *et al*., 1995; Jackson *et al*., 1996; Van Weerd *et al*., (1999) , Debnath *et al*., 2005, Yu and Wang (2000) and Yoo *et al*., (2005) who found that microbial phytase supplementation at 1000 units phytase/kg diets significantly (P<0.05) improved weight gain for juvenile Korean rockfish fed diet containing 30% soybean meal. On the other hand, Cain and Garling (1995), Eya and levell (1997), Vielma *et al*., (2000) and Sajjadi and Carter (2004) found no significant difference in the weight gain of Atlantic salmon fed diets with or without phytase.

Data in Table (3) indicated that addition of phytase increased significantly (*P<0.05*) the specific growth rate (SGR %) up to 1250 units phytase/kg. After that level (1250 units phytase/kg) increasing phytase in the diet decreased significantly

(*P<0.05*) the specific growth rate (SGR)%.

These results are in agreement with the findings of Riche *et al*., (2001); Heindl *et al*., (2004); Phromkunthong *et al*., (2004); Liebert and Portz (2007); Nwanna *et al*., (2007, 2008); Nwanna and Schwarz, (2007); Tudkaew, *et al*., (2008); Cao *et al*., (2008); Hassan *et al*., (2009); Huynh (2010); Kumar (2011) and Khajepour *et al*., (2012).

Table (3):Averages specific growth rate (SGR%/day) of *O. niloticus* fed different levels of dietary phytase.

Period (week)	Treatments (U Phytase)/kg diet					
	Control (0)	T2 (500)	T3 (750)	T4 (1000)	T5 (1250)	T6 (1500)
2	0.15±0.0153	0.16±0.01534	0.16±0.0153	0.18±0.01534	0.17±0.0153	0.15±0.0153
4	0.51^{b}±0.0206	0.55^{ab}±0.0206	0.57^{ab}±0.0206	0.56^{a}±0.0206	0.57^{ab}±0.0206	0.51^{b}±0.0206
6	0.41±0.0237	0.41±0.0237	0.44±0.0237	0.46±0.0237	0.45±0.0237	0.41±0.0237
8	0.62^{a}±0.0172	0.59^{ab}±0.0172	0.54^{bc}±0.0172	0.53^{c}±0.0172	0.53^{c}±0.0172	0.62^{a}±0.0172
10	0.33±0.0134	0.34±0.0134	0.34±0.0134	0.33±0.0134	0.34±0.0134	0.33±0.0134
12	0.24^{b}±0.0114	0.26^{ab}±0.0114	0.26^{ab}±0.0114	0.29^{a}±0.0114	0.29^{a}±0.0114	0.24^{b}±0.0114
Overall mean	0.38	0.38	0.39	0.39	0.39	0.38

Means within the same row significantly differ (P<0.05) when superscripts are different

On the other hand, some researchers showed that the addition of phytase to diets had no significant effect on specific growth rate of the fish (Pham *et al*., 2008); Phromkunthong *et al*., (2010) and Dechavez and Serrano (2012). However, the effect of phytase supplementation on specific growth rate of the fish may be affected by fish species, level of phytase and also source of phytase (Yoo *et al*., 2005; Biswas *et al*., 2007; and Nwanna and Schwarz, 2007; Furuya *et al*., 2001; Cao *et al*., 2007; and Ayhan *et al*., 2008).

Data presented in Table (4) showed that there were no significant (*P>0.05*) differences in body total length of the fish fed different levels of dietary phytase. Although the differences were not significant (*P>0.05*) treatment with 1000 units phytase/kg diet enhanced the total length. These results are in harmony with the findings of Barnes *et al*., (2012 a,b) who reported that the addition of phytase had no significant (*P>0.05*) effect on body length of rainbow trout.

On the other hand, Khalafalla *et al*., (2010) recorded that the addition of amecozyme® (phytase) to the diet insignificantly (*P<0.05*) improved body length of Nile tilapia.

Results in Table (5) showed that phytase supplementation significantly (*P<0.05*) enhanced the length increment of Oreochromis *niloticus*. It is remarkable that the addition of 1000 units phytase/kg diet was superior in the length increment as compared to other treatments. Similar results were found by Khalafalla *et al*., (2010) who recorded that addition of phytase (amecozyme®) in the diet significantly (P<0.05) improved length increment for Nile tilapia fingerlings.

Dietary phytase supplementation in higher quality diets of fish species other than rainbow trout showed no positive effect on growth and feed conversion ratio (Denstadli *et al*., 2007; Sajjadi and Carter 2004), except in common carp *Cyprinus carpio* (Nwanna and Schwarz 2007; and Nwanna *et al*., 2007) and Nile tilapia *Oreochromis niloticus* (Liebert and Portz 2005).

Table (4) : Average body total length (cm) of *O. niloticus* fed different levels of dietary phytase.

Period (week)	Treatments (U Phytase)/kg diet					
	Control (0)	T2 (500)	T3 (750)	T4 (1000)	T5 (1250)	T6 (1500)
Initial 0	9.19±0.0981	9.19±0.0981	9.18±0.0981	9.19±0.0981	9.19±0.0981	9.19±0.0981
2	9.21±0.0947	9.25±0.0947	9.25±0.0947	9.27±0.0947	9.26±0.0947	9.20±0.0947
4	9.45±0.1077	9.46±0.1077	9.49±0.1077	9.56±0.1077	9.55±0.1077	9.45±0.1077
6	9.70±0.1037	9. 71±0.1037	9.71±0.1037	9.74±0.1037	9.72±0.1037	9.68±0.1037
8	9.97±0.0898	9.98±0.0898	10.01±0.0898	10.03±0.0898	10.01±0.0898	9.96±0.0898
10	10.06±0.0830	10.05±0.0830	10.10±0.0830	10.11±0.0830	10.10±0.0830	10.05±0.0830
12	10.14±0.0845	10.09±0.0845	10.21±0.0845	10.24±0.0845	10.22±0.0845	10.13±0.0845
Overall mean	9.67	9.67	9.71	9.73	9.72	9.67

$\bar{x}$ = Means±SE.

On the other hand, Cheng and Hardy (2004) and Barnes *et al.*, (2012 a,b) showed that the addition of phytase to dietary high protein distillers dried grain with soluble (HPDDG) had no significant (P>0.05) effect on length increment of rainbow trout. Lanari *et al.*, (1998), Vielma *et al.*, (1998) and Dalsgaard *et al.*, (2009) reported that phytase enhanced the length increment of rainbow trout when included in soybean-based diets.

Phytase supplementation had no effect on growth or feed conversion in the fish fed diets containing high protein distillers dried grain with soluble (HPDDG). However, these results are difficult to interpret because it is unknown how much phytate (phytic acid salts or esters that interfere with phosporous absorption) was present in the HPDDG, the experimental diets were likely not phosphorous- limited, the efficiency of phytase may be somewhat dependon the method used to incorporate it into the feed, Denstadli *et al*., (2007) and the phytase may have been deactivated during extrusion Cao *et al*., (2007). Cheng and Hardy (2004) observed that there was no improvement in growth or feed conversion in rainbow trout receiving diets containing 15% DDGS and varying amounts of phytase. Rainbow trout growth was also unaffected by phytase supplementation in diets containing soy, Vielma *et al.*, (2000) or canola protein concentrate Forster *et al*., (1999).

Table (5): Averages of length increment (cm) of *O. niloticus* fed different levels of dietary phytase.

Period (week)	Treatments (U Phytase)/kg diet					
	Control (0)	T2 (500)	T3 (750)	T4 (1000)	T5 (1250)	T6 (1500)
2	0.02^{c}±0.0048	0.06^{b}±0.0048	0.07^{ab}±0.0048	0.08^{a}±0.0048	0.07^{ab}±0.0048	0.01^{c}±0.0048
4	0.24^{c}±0.0137	0.22^{c}±0.0137	0.24^{c}±0.0137	0.29^{ab}±0.0137	0.30^{a}±0.0137	0.25^{bc}±0.0137
6	0.25^{a}±0.0177	0.24^{a}±0.0177	0.22^{a}±0.0177	0.18^{b}±0.0177	0.17^{b}±0.0177	0.23^{a}±0.0177
8	0.27±0.0127	0.27±0.0127	0.30±0.0127	0.29±0.0127	0.29±0.0127	0.28±0.0127
10	0.09±0.0077	0.07±0.0077	0.09±0.0077	0.09±0.0077	0.09±0.0077	0.09±0.0077
12	0.08^{b}±0.0076	0.04^{c}±0.0076	0.12^{a}±0.0076	0.12^{a}±0.0076	0.11^{a}±0.0076	0.08^{b}±0.0076
Overall mean	0.16	0.15	0.17	0.18	0.17	0.16

Means within the same row significantly differ (P<0.05) when superscripts are different.

Results in Table (6) showed that there were significant (P<0.05) differences in condition factor (CF) of *O. niloticus* between the treatments and control. The achieved results of dietary phytase supplementation on condition factor of *O. niloticus* are supported by (Ayhan *et al*., 2008; Nwanna *et al*., 2008 and Khalafalla *et al*., 2010) who found that the addition of phytase into diets improved the condition factor. On the other hands, Barnes *et al*.,(2012 a,b) reported that the addition of phytase with dietary high protein distillers dried grain with soluble (HPDDG) had no significant (P>0.05) effect on condition factor for rainbow trout.

In conclusion, dietary phytase slightly improve growth performance of *Oreochromis niloticus*. It is obvious that 1000 units phytase/kg diet is useful in enhancement of growth performance for Nile tilapia, *Oreochromis niloticus* in intensive production. However, further studies are still needed.

Table (6) : Averages of condition factor of O. niloticus fed different levels of dietary phytase.

Period (week)	Treatments (U Phytase)/kg diet					
	Control (0)	T2 (500)	T3 (750)	T4 (1000)	T5 (1250)	T6 (1500)
Initial 0	1.71±0.0084	1.71±0.0084	1.71±0.0084	1.71±0.0084	1.71±0.0084	1.71±0.0084
2	1.73^{ab}±0.0078	1.71^{b}±0.0078	1.71^{b}±0.0078	1.71^{b}±0.0078	1.71^{b}±0.0078	1.74^{a}±0.0078
4	1.72^{a}±0.0081	1.73^{a}±0.0081	1.72^{a}±0.0081	1.69^{b}±0.0081	1.69^{b}±0.0081	1.72^{a}±0.0081
6	1.69±0.0086	1.69±0.0086	1.71±0.0086	1.71±0.0086	1.70±0.0086	1.70±0.0086
8	1.70±0.0097	1.70±0.0097	1.68±0.0097	1.68±0.0097	1.68±0.0097	1.70±0.0097
10	1.73±0.0093	1.74±0.0093	1.72±0.0093	1.71±0.0093	1.72±0.0093	1.73±0.0093
12	1.75^{bc}±0.0085	1.78^{a}±0.0085	1.72^{cd}±0.0085	1.72^{d}±0.0085	1.73^{bcd}±0.0085	1.75^{b}±0.0085
Overall mean	1.72	1.72	1.71	1.70	1.71	1.72

Means within the same row significantly differ ($P<0.05$) when superscripts are different.

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الملخص العربي

تأثير إضافة إنزيم الفيتيز الميكروبي على أداء النمو لأسماك البلطي النيلي

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أجريت هذه الدراسة بالمزرعة السمكية- التابعة لقسم إنتاج الدواجن - كلية الزراعة - جامعة أسيوط - مصر - لدراسة تأثير إضافة إنزيم الفيتيز على أداء النمو لأسماك البلطي النيلي.

استخدم في هذه الدراسة اسماك البلطي النيلي بمتوسط ١٣±١ جرام . تم جمع الأسماك وأقلمتها لمدة أسبوعين في التانكات حيث تم استخدام عدد ستة تانكات ذو أبعاد كل تانك ١,٥م X ٢م عمق ، الأسماك غذيت ثلاث مرات يوميا بمعدل ٣٪ من الوزن الحي. في نهاية فترة الأقلمة أخذت ٦٠٠ سمكة سليمة صحيا وتم أقلمتها وتم قياس الوزن والطول ثم وزعت عشوائيا إلى ستة مجاميع وزعت على التانكات بمعدل ١٠٠ سمكة بلطي نيلي/تانك.

تم دراسة ستة علائق الأول بدون إضافة إنزيم الفيتيز واعتبرت عليقه المقارنة والعلائق الأخرى تحتوى على ٥٠٠ ، ٧٥٠ ، ١٠٠٠، ١٢٥٠، ١٥٠٠وحدة /كجم من مادة العلف للعلائق من الثانية حتى السادسة على الترتيب.

استمرت الدراسة لمدة ١٢ أسبوع وتم وزن الأسماك وقياس طولها الكلى كل أسبوعين، وتم تعديل كمية الغذاء طبقا للتغيير في أوزان الأسماك ، وتم تغذية الأسماك خلال فترة الدراسة بمعدل ٣٪ من وزن الأسماك الحية ، وتم التغذية على هذه العلائق ثلاث مرات يوميا (٩ صباحا ، ١٢ ظهرا ، ٣ عصرا).

أوضحت النتائج المتحصل عليها أن إضافة الفيتيز إلى العليقة أدى إلى تحسن الزيادة في وزن الجسم و كانت المعاملة الرابعة (١٠٠٠ وحدة) متفوقة بالمقارنة للكنترول والمعاملات الأخرى ، وكذلك معدل النمو للمعاملة الرابعة (١٠٠٠ وحدة) أفضل بالمقارنة للكنترول ، وكذلك وجد أن طول الجسم للمعاملات الثانية والثالثة والرابعة والخامسة تزداد بدون أي تأثير معنوي ، وكذلك وجد أن متوسط الزيادة في طول الجسم في نهاية التجربة للمعاملة الرابعة أفضل في الزيادة بالمقارنة للكنترول ، وكذلك وجد أن معامل الحالة الجسمية للمعاملة الثانية والسادسة تزداد معنويا بالمقارنة للكنترول. و نخلص أن إضافة الفيتيز إلى العليقة أدى إلى تحسن في وزن الجسم والزيادة في وزن الجسم ومعدل النمو وطول الجسم والزيادة في طول الجسم ومعامل الحالة الجسمي.