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Effect of Adding Commercial Phytase to DDGS Based Diets on the Performance and Feed Utilization of Nile Tilapia (*Oreochromis niloticus*) Fingerlings

A.M. Tahoun, H.A. Abo-State and Y.A. Hammouda

Department of Animal Production,
Agriculture and Biological Research Division, National Research Center, Cairo, Egypt

Abstract: This study was conducted to assess the effect of graded levels of a commercial phytase preparation (Ronezyme) added to a DDGS based diet on growth performance and feed utilization of Nile tilapia (*Oreochromis niloticus*) fingerlings. Five different levels of phytase (0, 75,150, 225 and 300 mgkg^{-1} were used in triplicates of the five treatments. Fingerlings were accommodated in 15 hapas with the dimensions of 3x 6x 0.5 meters. 36 fingerlings were housed in every hapa for 82 days, feed was offered 3 times daily at the rate of 5% of body weight during the first 30 days and then decreased to 3% for six days a week until the termination of the experiment. The results showed that diets with added levels of 75 and 150 mg / kg phytase recorded higher final body weight, weight gain, average daily gain and specific growth rate. Increasing the phytase level markedly reflected on lower values for the abovementioned parameters. The highest feed intake was recorded for the control group, followed by T2, T4 and T5 with no significant differences between groups. The addition of 75 mg phytase / kg feed resulted in a better feed conversion (1.7), Protein efficiency ratio, protein productive value and energy utilization. There was no significant differences (P<0.05) among treatments in survival rate. The results suggested that the supplementation of phytase (75 mg / kg) and (150 mg / kg) in DDGS based diets can significantly improve growth and feed utilization parameters in Nile tilapia (*O. niloticus*) fingerlings.

Key words: Distiller's dried grain with solubles % Commercial phytase % Growth performance % Feed utilization % Nile tilapia (*Oreochromis niloticus*)

INTRODUCTION

There are increasing interests in the inclusion of DDGS as a plant protein to replace other costy vegetable protein sources such as soybean meal and corn gluten meal [1].The presence of phytate phosphorus is a major problem limiting the use of grain products in fish feeds, as phytate phosphorus is indigestible and hence not available to fish [2]. Based on that, available P in all plant protein diets will not meet the requirements of fish and hence, such diets supplemented with inorganic phosphorus to fish diets and undigested phosphorus will be excreted in feaces contributing to the environmental pollution. However, phosphorus is essential for growth, skeletal development and reproduction of fish [3-5]. The inclusion of DDGS in fish diets to replace soybean meal will reduce the phosphorus level in the diet as DDGS content of phosphorus is low which might be reflected on growth performance. Like plant protein feedstuffs, about 2/3 of phosphorus is in the form phytate which makes phosphorus unavailable for fish and interfere with the availability of other minerals [6-8].

Tilapia lacks the intestinal enzyme phytase that hydrolyses phytate [9]. Some studies found that the addition of phytase had positive effect on weight gain of rainbow trout [10, 11]. Channel catfish [12, 13], Carp [14, 15], Korean rock fish [16] and Tilapia [9, 17, 18]. Other studies detected no effects for rainbow trout and channel catfish [19, 20].

Many Studies have demonstrated that the addition of phytase to fish feeds can improve the utilization of phosphorus [11, 21]. However, phosphorus content of all plant diets may not meet the requirement of phosphorus for tilapia which was considered by NRC (National Research Council) [2] as 5 g / Kg and by [22, 23] as g / kg.

The objective of this study was to determine the effect of graded levels of commercial phytase when added to DDGS based diet on growth performance and survival rate of Nile tilapia *(Oreochromis niloticus)* fingerlings.

Corresponding Author: Dr. H.A Abo-State, Department of Animal Production, Agriculture and Biological Research Division, National Research Center, Cairo, Egypt

MATERIALS AND METHODS

Experimental Fish: A total number of (1000) mono sex Nile tilapia *(Oreochromis niloticus)* fingerlings of an initial average body weight of 3.81 g. Were obtained from commercial fish farm located in Kafr El- Sheikh Governorate Egypt. The fish were adapted for 15 days to the new environment until starting the experiment which lasted for 82 days, thereafter, fish were allotted randomly into 15 hapas (3 x 6 x 0.50m^3)giving net volume of 9m^3 for each hapa of 36 fish / hapa (4 fish/m^2) in earthen pond. They represented five treatments each in 3 replicates.

All experimental fish were healthy and free of disease and parasites at arrival and during the whole experimental period.

Experimental Diets: A basal diet (Table 1) was formulated to contain 35% CP and 3800 kcal /kg ME. Five different levels of dietary commercial phytase 0, 75, 150, 225 and 300 mg / kg (Rhonezyme) to obtain five treatments.

Table 1: Composition and proximate analysis of the experimental diet

Ingredients	Basal Diet
Fish meal (72% CP).	10.00
Soybean meal (44% CP).	21.00
DDGS (32% CP).	28.00
Corn gluten (62% CP).	13.00
Corn grain (9% CP).	22.00
Vegetable oil	3.00
Choline chloride	0.30
Vitamin C.	0.30
D L-Methionine	0.40
Minerals[1]	1.00
Vitamins[2]	1.00
Proximate composition	
Moisture	7.40
Dry matter (%)	92.60
Crude protein (%)	35.30
Ether extract (%)	8.21
Crude fiber (%)[3]	4.66
Ash (%)	5.56
Nitrogen free extract (%)	46.27
Metabolizable energy (KCal / Kg)[4]	3868.0
Protein energy ratio (mg protein/ K Cal)	91.26

- Mineral premix supplied per kilo-gram diet: calcium. 4.3g: phosphonus, 2.6g: copper, 5.0 mg: iron, 41 mg: mangenese, 120 mg, zink, 115 mg: iodine, 2.5mg, cobalt, 1.0 mg, sulfur, 153mg.
- The vitamin premix supplied per kilogram diet: vitamin A, 9900 international unit (IU), vitamin B-12, 0.014 mg, riboflavin (B2), 18.2 mg: niacin. 10.7 mg: pantothenic acid, 37 mg, choline, 715 mg: folic acid 6.1mg, biotin 0.17mg, ascorbic acid 220 mg: menadione (k3) 9mg, thiamine (B1) 16.2 mg.
- Crude fiber is not included in calculating ME of the diets.
- Metabolizable energy (ME):- calculated using values of 4.50, 8.15 and 3.49 K Cal for protein, fat and carbohydrate, respectively according to Pantha (1982)

Diets were fed to fish fingerlings at a feeding rate 5% of the total body weight all over the experimental period at the first month (30 days) then decreased to 3% at the start of 2nd month until the end of the experiment (52day) fingerlings were fed 3 times daily at 8am, 11am and 2pm for 6 days / week with feed amount adjusted at approximately 15 days intervals in response to weight gain.

Growth Performance and Feed Utilization Parameters: At the beginning of the feeding trail, 200 fish were sampled and stored at -20°C for the analysis of initial whole body composition (zero time). At the end of the experimental period (82 days), samples of 5 fish per hapa were withdrawn for analysis of whole body composition at the end of the experiment. Growth parameters which include: average weight gain (AWG), average daily gain (ADG), specific growth rate (SGR), feed conversion ratio (FCR), protein efficiency ratio (PER), protein productive value (PPV), energy utilization(EU) and survival rate were calculated according to the following equations:

- AWG (g/fish) = [Average final weight (g)-Average initial weight (g)].
- ADG (g/fish/day) = [AWG (g)/experimental period (d)].
- SGR (%day)= (Ln final weight (g)- Ln initial weight (g) experiment period (d)] x 100 where Ln = Natural Logarithm.
- FCR = FCR = Feed intake (g)/ live weight gain (g).
- PER = Live weight gain (g) / protein intake (g).
- PPV (%) = [Final fish body protein (g)-initial fish body protein (g)/crude protein intake (g)] x 100.
- EU (%) = (Retained energy Kcal /energy intake Kcal) x 100

Samples and Analytical Procedure: Experimental diet and fish samples were analyzed for their proximate composition in triplicate following the methods described by A.O.A.C. [24]. The Metabolizable energy (ME) content of the tested diets were calculated using values of 4.50, 8.15 and 3.49 Kcal for protein, fat and carbohydrate respectively according to Pantha [25]

Water Quality Parameters: Air and water temperature were determined four times weekly at 6.00 am and 2.00 pm. water dissolved oxygen (DO) content and water pH were measured weekly at 2.0 pm using a digital dissolved oxygen meter (Jenway model 9070) and a digital pH meter (model checker 1 produced by Hanna Instrument Co.), respectively. Water salinity (mg/L) was determined biweekly using a digital conductivity meter (Jenway model 4075). Water alkalinity and total ammonia nitrogen (TAN mg/L) were weekly determined following the methods described by Chattopadhyay [26].

Statistical Analysis: Using SPSS package ver. 15 for windows. Data were statistically analyzed in a one- way analysis of variance. Mean of treatments were compared by Duncan [27] multiple range test. Duncan test (P<0.05) was used to compare means and (F<0.05) was considered for the variance analysis.

RESULTS AND DISCUSSION

All values of the water quality parameters measured were suitable for the normal growth of tilapia and warm water fish as mentioned by Tahoun [28] and Khalfalla *et al*. [29]. Average values recorded for temperature, pH, Do, total ammonia nitrogen and total alkalinity were: 27.5°C, 7.6, 7.5, 0.04 and 175 mg/L, respectively.

Initial weight, final weight, WG, ADG, SGR and Survival rate of fingerlings fed diets for 82 days are presented in Table 2. There were no significant differences (P>0.05) in initial weight and survival rate among fish fed different diets. Survival rate was approximately 100% for fish fed all diets.

Growth performance in terms of final mean weight, weight gain and average daily gain was better in the treatments supplemented with (75, 150 mg / Kg phytase), intermediate with (0, 225 mg / Kg phyase) and the lowest value was recorded in T5 (300 mg/kg phytase). SGR showed no significant differences (P>0.05) between the treatments supplemented with phytase (0, 75,150 and 225 mg / Kg) in the diets. (P>0.05), but significantly different (P>0.05) with T5 (300 mg / Kg) which showed low SGR than the other treatments.

The results of feed intake, feed conversion ratio, protein efficiency ratio, protein productive value and energy utilization of fish fed diets supplemented with graded levels of commercial phytase are shown in Table 3. Feed intake decreased with phytase supplementation until T3 (150 mg / Kg) phytase, then increased gradually in T4 and T5. FCR values were significantly different between (P>0.05) treatments (P<0.05), the best FCR was recorded in T2 (75 mg / Kg phytase). The poor FCR was recorded in T5 (300 mg / Kg) phytase.

As for in PER the best values were recorded in T2 and T3 which represent the higher PER than the other treatments. The same trend was observed in PPV and Eu. The results suggested that the supplementation of phytase (75 mg / Kg) and (150 mg/Kg). In DDGS based diets can significantly improve growth and feed utilization parameters in Nile tilapia *O. niloticus* fingerlings.

Table 2: Effect of graded levels of dietary commercial phytase on growth performance and survival rates of *Oreochromis niloticus* fingerlings

Treatments	Average initial weight (g)	Average final weight (g)	Average weight gain (g)	Average daily gain (g / day)	Specific growth rate (% / day)	Survival rates (%)
T1 (0 mg/kg)	$2.983^{a}\pm0.167$	$35.00^{bc}\pm0.451$	$32.017^{bc}\pm0.442$	$0.390^{b}\pm0.005$	$3.003^{a}\pm0.013$	$99.667^{a}\pm0.167$
T2 (75 mg/ kg)	$2.983^{a}\pm0.167$	$36.500^{a}\pm0.493$	$32.050^{a}\pm0.524$	$0.408^{b}\pm0.006$	$3.034^{a}\pm0.029$	$100.667^{a}\pm0.333$
T3 (150 mg/ kg)	$3.000^{a}\pm0.029$	$36.667^{a}\pm0.273$	$33.467^{a}\pm0.252$	$0.410^{a}\pm0.003$	$3.053^{a}\pm0.008$	$99.833^{a}\pm0.167$
T4 (225 mg/ kg)	$3.033^{a}\pm0.017$	$35.033^{bc}\pm0.426$	$32.050^{bc}\pm0.431$	$0.391^{a}\pm0.006$	$3.004^{a}\pm0.018$	$99.833^{a}\pm0.167$
T5 (300 mg/ kg)	$3.033^{a}\pm0.017$	$33.633^{c}\pm0.689$	$30.600^{c}\pm0.673$	$0.373^{b}\pm0.008$	$2.934^{b}\pm0.019$	$100.00^{a}\pm0.167$

a,b,c.etc: Means in the same column with different superscripts are significantly different (P<0.05)

Table 3: Effect of different levels of dietary commercial phytase on utilization parameters of *Oreochromis niloticus* fingerlings

Treatments	Feed intake (g)	Feed conversion ratio	Protein efficiency ratio	Protein productive value (%)	Energy utilization (%)
T1 (0 mg/kg)	$58.705^{a}\pm1.183$	$1.833^{a}\pm0.017$	$1.669^{d}\pm0.015$	$27.621^{c}\pm0.254$	$18.787^{d}\pm0.173$
T2 (75 mg/ kg)	$56.875^{ab}\pm0.829$	$1.700^{C}\pm0.029$	$1.801^{b}\pm0.031$	$30.581^{ac}\pm0.515$	$21.306^{a}\pm0.349$
T3(150 mg/ kg)	$53.298^{b}\pm0.216$	$1.775^{b}\pm0.017$	$1.933^{a}\pm0.021$	$29.186^{ab}\pm0.311$	$20.372^{ab}\pm0.217$
T4(225 mg/ kg)	$55.001^{ab}\pm0.839$	$1.915^{a}\pm0.033$	$1.783^{bc}\pm0.035$	$28.856^{bc}\pm0.655$	$20.174^{bc}\pm0.430$
T5(300 mg/ kg)	$55.118^{ab}\pm2.069$	$1.915^{a}\pm0.029$	$1.701^{cd}\pm0.027$	$27.698^{bc}\pm0.447$	$19.270^{cd}\pm0.320$

a,b,c.etc: Means in the same column with different superscripts are significantly different (P<0.05)

The results obtained in this work are in good agreement with Schafer *et al*. [14] who found that using phytase in plant-based diets can reduce the need for inorganic P supplementation in diets, leading to the reductions of P discharged to the environment from fish farms.

Cao *et al*. [30] found that the indigestible phytate P was successfully converted to available P by phytase which mean improvement in phosphorus utilization. In this connection, Liebert and Portz [21] reported that the optimal growth of Nile tilapia was achieved by phytase supplementation at 750-1250 U / kg in plant based diets. Growth improvement was also observed in rainbow trout fed phytase supplemented diets [10, 31]. This indicated that the inclusion rate of phytase plays an important role in releasing phytate – phosphorus in plant protein - based fish diets and that the effectiveness of phytase varies with the plant ingredient source.

Cheng and Hardy [32] found that the effectiveness of supplementation of phytase in rainbow trout diets to release phosphorus and other minerals if DDGS is used as an alternative protein ingredient and the supplemental levels of trace minerals such as manganese and zinc may be reduced. This can be an advantage in fish production, especially at late stage of fish growth because most of the feed used in a production cycle is consumed at late growth stage and reducing water pollution.

These results are in good agreement with Sugiura *et al*. [33] and Forster *et al*. [34]. That's because the phytase had a pH optimum of 5.30 and an optimum temperature at 55 °C. The rate of enzyme hydrolysis activity was much faster at 37°C than at 15°C. This means that the efficacy of supplemental phytase in cold water fish could differ from warm water fish. Sugiura *et al*. [33] and Forster *et al*. [34] reported that the efficacy of dietary phytase to dephosphorylate phytic acid may be enhanced at higher water temperature than low water temperature.

However the results obtained in this particular study indicated that increasing levels of phytase had negative effect on growth performance and feed utilization parameters at higher levels, than 150 mg/kg which may be attributed to the feeding behavior of tilapia which may likely contributes to reduced availability from the phytase pretreated diets. Tilapia are equipped with palatine teeth and pharyngeal gil rakes allowing them to grind feed and fitter material before swallowing [35, 36]. This process creates small particles with high surface to volume ratio. Additionally, tilapias repeatedly draws in and expel their food before consumption [37]. These processes increase the potential for leaching of soluble components. Phytates decrease solubility of nutrients [38] and its removal may increase solubility of essential nutrients. Phytase may also serve in a protective capacity. Phytates bind to the globular proteins glycinin and B-conglycinin [38], both of which are ANFs in fish [39,40] phytates also may protect some of the more readily damaged AA, Such as lysine, from degradation during processing or pelleting therefore, increasing levels of phytase decreased the apparent availability of one or more of the IAA [9].

Results of this study suggest that supplementation with phytase at diets containing DDGS can improve growth and feed utilization parameters in Nile tilapia until 150 mg / kg but the highest levels of supplementation had adverse effect.

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