American-Eurasian J. Agric. & Environ. Sci., 5 (4): 473-479, 2009
ISSN 1818-6769

Effect of Replacement of Soybean meal by DDGS Combined with Commercial Phytase on Nile Tilapia. *(Oreochromis niloticus)* Fingerlings Growth Performance and Feed Utilization

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

Department of Animal Production, Agric Research Division, National Research Center, Cairo, Egypt

Abstract: A feeding trail was conducted to evaluate the effect of different levels of DDGS replacing by soybean meal (0,25,50,75 and 100) with or without supplementation of Commercial phytase (Ronezyme) (0,150mg / kg) on juvenile tilapia (*Oreochromis niloticus*) in two factorial arrangements. Each diet was used in duplicate which means 10 treatments and 20 hapas (2x2x0.5m^3) at densities of 4 fish/m^2 (8 fish / hapa), fed 5-3% of wet body three times daily for 70 days. The results showed no significant differences ($P > 0.05$) observed in survival rate. Final weight, WG, ADG and SGR were superior in T_6, T_7 and T_8 (0,25, 50 DDGS with phytase supplementation 150mg kg^{-1} diet). Feed intake and WG were improved with phytase supplementation. The other parameters of feed utilization PER, PPV and energy utilization showed significant differences ($P<0.05$) between treatments where T6, T7 and T8 recorded higher values. The results suggested that DDGS replacing soybean meal and supplemented with exogenous phytase can significantly improve growth performance and feed utilization parameters in juvenile Nile tilapia (*Oreochromis niloticus*).

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

INTRODUCTION

Distiller's Dried Grains with soluble (DDGS) is a valuable feed ingredient and is one of the three co-products produced in dry grind ethanol plants, along with fuel ethanol and carbon dioxide [1,2]. Due to its reasonably high protein content, fairly low phosphorus content and low cost compared to fish meal [3] It was considered as a protein source that can replace expensive plant protein sources. The nutrient composition of distillers, depends on the type, variety and quality of the grains used, as well as the efficiency of starch conversion and the processing technique.

Colour and handling properties of DDGS can also change substantially between manufacturing plants [4] and drying conditions [5]. Up till now, only a little research has been carried out on utilizing DDGS as a protein source in aquaculture feed, limited work has investigated feeding Trout [6-9], Prawn [10-14]. Channel catfish [15-17] Carp [5] Tilapia [18-22].

These studies have found that DDGS in combination with other feed ingredients, could partially or even totally replace fishmeal as a protein source and that fish – growth performance could be maintained at acceptable levels depending on plant protein based diet.

Much work remains to improve and maximize the utilization of these co-products in aquaculture via growth performance trails and acceptability testing of these novel feed products [3].

Several Anti nutritional factor present in soybeans can be partially removed by proper heat treatment and extraction procedures [23]. However, phytate is relatively heat- stable. phytate bound P is not available to monogasteric animals including fish [24].

Furthermore, phytate may interfere with the availability of other minerals [23]and can bind trypsin and decrease protein availability in fish [25,26]. That may induce poor bone mineralization [27], increase carcass fat deposition [28] and decrease resistance to disease [29].

Phytatae-phosphorus in DDGS was 0.28%, which was about 36% of total phosphorus. This level of phytate- phosphorus was low compared to phytate-phosphorus in other cereal grain and oil seed meals. This might be due to the fermentation process used in producing ethanol, which could break the chemical bonds of the phytate [8].

However phytate is heat-stable and cannot be effectively removed without enzymatic reactions [6].

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

The objective of the present work was to study the the effect of replacement of soybean meal by DDGS combined with commercial phytase on Nile tilapia (*Oreochromis niloticus*) fingerlings growth performance and feed utilization.

MATERIALS AND METHODS

Experimental Fish: A total number of (800) mono sex Nile tilapia (*Oreochromis niloticus*) fingerlings of an initial average body weight of 2.0 gm 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 started the experiment. Experimental Fish were allotted randomly into 20 hapas ($2x2x0.5m^3$) giving net volume of $2m^3$ at a rate of (8) fingerling/ hapa (4 fish /m^3) in earthern pond representing ten treatments each in 2 replicates.

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

Experimental Diets: Five experimental soybean meal based diets replaced with DDGS (0, 25, 50, 75 and 100%) were combined with two levels of phytase (0 and 150 mg^{-1} kg) diet to obtain 10 treatments, containing (35% CP and 3884 kcal/kg ME) as presented in Table 1 were fed to the fish at a feeding rate 5% of the total body weight at the first month (30 days) then decreased to 3% at the beginning of the 2nd month until the end of the experiment. The whole experiment period was 70 days. Fingerlings were fed 3 times daily at 2 pm and 8 pm for 6 days / week with feed amount adjusted approximately at 15 days intervals in response to weight gain.

Table 1: Composition and proximate analysis of the experimental diets

Diets	Diet 1	Diet 2	Diet 3	Diet 4	Diet 5
Ingredients					
Fishmeal (72 % CP).	10.00	10.00	10.00	10.00	10.00
Soybean meal (44% CP).	----	10.00	21.00	30.00	40.00
$DDGS_s$ (32%CP).	55.00	42.50	28.00	14.00	----
Corn gluten (62%CP).	15.00	13.00	13.00	12.50	12.00
Corn grain (9%CP).	14.00	18.00	22.00	26.70	30.40
Vegetable Oil	2.00	3.00	3.00	4.00	5.00
Choline Chloride	0.30	0.30	0.30	0.30	0.30
Vitamin C.	0.30	0.30	0.30	0.30	0.30
L-Lysine HCL 98%.	0.40	0.20	-	-	-
D L-Methionine	1.00	0.70	0.40	0.20	-
Minerals[1]	1.00	1.00	1.00	1.00	1.00
Vitamins[2]	1.00	1.00	1.00	1.00	1.00
Proximate composition					
Moisture	7.60	7.50	7.40	7.30	7.50
Dry matter (%)	93.40	92.50	92.60	92.70	92.50
Crude protein (%)	35.18	34.97	35.30	35.12	35.16
Ether extract (%)	8.09	8.21	8.21	8.17	8.20
Crude fiber (%)[3]	5.10	4.91	4.66	4.30	4.10
Ash (%)	5.72	5.62	5.56	5.60	5.59
Nitrogen free extract (%)	45.91	46.29	46.27	46.81	46.95
Metabolizable energy (KCal / Kg)[4]	3837.1	3854.0	3868.0	3876.0	3884.0
Protein energy ratio (mg protein/ K Cal)	91.68	90.74	91.26	90.61	90.53

1- 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.

2- 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.

3- Crude fiber is not included in calculating ME of the diets.

4- 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).

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 whole body composition as a initial (zero time). At the end of the experiment period (70 days), samples of 5fish per hapa were withdrawn for analysis of whole body composition at the end of the experiment. The growth parameters were recorded including, 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 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.
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) x100.

Samples and Analytical Procedure: Experimental diet and fish samples were analyzed for their proximate composition in triplicate following the methods of the AOAC [30]. The Metabolizable energy (ME) content of the tested diets calculated using values of 4.50, 8.15 and 3.49 kcal for protein, fat and carbohydrate, respectively according to Pantha [31].

Water Quality Parameters: Air and water temperature were determined four times weekly at 6.00 am and 2.00 pm by using a thermometer. Water dissolved oxygen (DO) content and water pH were measured weekly at 2.00pm 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 determind biweekly using a digital conductivity meter (jenway model 4075). Water alkalinity and total ammonia nitrogen (TAN mg/L) were weekly determind following the methods dscribed by Chattopadhyay [32].

Statistical Analysis: Statistical analysis of the experiment was done using SPSS package ver. 15 for windows Data were statistically analyzed in a factorial design procedure. Mean of treatments were compared by Duncan [33] 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 [34] and Khalfalla *et al.* [35]. Average values recorded were Temperature 27.5C°, pH 7.6, Do 7.5 gm/L, total ammonia nitrogen 0.040 mg/L and total alkalinity 175 mg/L, respectively.

Initial weight, final weight, WG, ADG, SGR and survival rate of fingerlings fed experimental diets for 70 days are presented in Table 2. there were no significant differences in fish initial weight ($P>0.05$).

After feeding period, significant differences occurred among fish fed different diets. Fish fed diets T1, T2 and T3 (replaced soybean meal by DDGS 0,25, 50%). Showed higher final weight, indicating that the fish grew faster than the other groups T4 and T5 (75, 100% replacement). The other parameters WG, ADG and SGR followed the same pattern similar to fish final weight that there were no significant differences between the first three treatments T1, T2 and T3 they superior than the last two treatments (T4 and T5).

Survival rate showed no significant differences ($P> 0.05$) among fish fed different diets. Survival rate was approximately 100% for fish fed all diets.

Feed intake, feed conversion ratio, protein efficiency ratio, protein productive value and energy utilization of fish fed diets containing different levels of DDGS regardless of phytase supplementation are shown in Table 3. The results indicated that there were no significant differences in feed intake ($P>0.05$) between treatments. The best values of FCR was recorded for fish fed T3. Recorded values for PER and Eu showed significant differences between treatments ($P<0.05$) the best values recorded in T1, T2 and T3 than T4 and T5. the protein productive value had nearly the same trend of PER and Eu.

Table 4 showed the effect of different levels of DDGS and phytase level on growth parameters of *O. niloticus* fingerlings. The data demonstrated that all diets from

Table 2: Effect of different levels of DDGS regardless of dietary phytase on growth parameters 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	1.965^{a}	22.950^{a}	20.988^{a}	0.300^{a}	3.513^{a}	99.500^{a}
T2	1.975^{a}	22.600^{a}	20.625^{a}	0.295^{a}	3.478^{a}	99.500^{a}
T3	2.050^{a}	22.625^{a}	20.575^{a}	0.295^{a}	3.433^{ab}	100.00^{a}
T4	1.988^{a}	21.000^{b}	19.125^{b}	0.270^{b}	3.368^{bc}	100.00^{a}
T5	2.025^{a}	20.515^{b}	18.490^{b}	0.265^{b}	3.308^{c}	100.00^{a}

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 DDGS regardless of dietary phytase on feed and protein utilization of *Oreochromis niloticus* fingerlings

Treatments	Feed intake (g)	Feed conversion ratio	Protein efficiency ratio	Protein productive value (%)	Energy utilization (%)
T1	36.438^{a}	1.673^{b}	1.790^{a}	31.143^{a}	21.568^{a}
T2	36.840^{a}	1.718^{ab}	1.730^{a}	30.318^{ab}	21.043^{a}
T3	35.328^{a}	1.640^{b}	1.790^{a}	31.258^{a}	21.805^{a}
T4	36.355^{a}	1.823^{a}	1.625^{b}	28.240^{bc}	19.323^{b}
T5	35.375^{a}	1.808^{a}	1.595^{b}	27.478^{c}	18.918^{b}

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

Table 4: Effect of different levels of DDGS and dietary phytase on growth parameters 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	1.975	21.450^{bc}	19.475^{bc}	0.280^{b}	3.490^{bc}	99.00
T2	1.975	21.350^{c}	19.375^{b}	0.280^{b}	3.410^{c}	100.00
T3	2.050	21.700^{c}	19.650^{b}	0.280^{b}	3.375^{cd}	100.00
T4	1.975	20.450^{cd}	18.475^{bcd}	0.256^{bc}	3.340^{d}	99.00
T5	2.000	19.630^{d}	17.630^{d}	0.255^{c}	3.260^{d}	100.00
T6	1.950	24.450^{a}	22.500^{b}	0.320^{a}	3.615^{a}	100.00
T7	1.975	23.850^{a}	21.875^{a}	0.310^{a}	3.555^{ab}	100.00
T8	2.050	23.550^{a}	21.500^{a}	0.310^{a}	3.490^{b}	100.00
T9	2.000	21.550^{bc}	19.550^{b}	0.275^{b}	3.395^{c}	100.00
T10	2.050	21.400^{bc}	19.350^{ab}	0.275^{b}	3.375^{cd}	100.00

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

Table 5: Effect of different levels of DDGS and dietary phytase on feed and protein utilization parameters of *Oreochromis niloticus* fingerlings

Treatments	Feed intake (g)	Feed conversion ratio	Protein efficiency ratio	Protein productive value (%)	Energy utilization (%)
T1	36.925^{a}	1.810^{b}	1.625^{bcd}	27.755^{cb}	19.100^{bcd}
T2	38.965^{a}	1.915^{abc}	1.525^{cd}	26.215^{c}	18.060^{cd}
T3	36.765^{a}	1.775^{abc}	1.640^{abc}	27.755^{cb}	19.365^{abc}
T4	38.460^{a}	1.915^{a}	1.485^{d}	25.585^{c}	17.145^{d}
T5	36.000^{a}	1.915^{ab}	1.495^{cd}	25.225^{c}	17.365^{d}
T6	35.950^{ab}	1.535^{cd}	1.925^{a}	34.530^{a}	24.035^{a}
T7	34.715^{b}	1.520^{d}	1.935^{a}	34.420^{a}	24.025^{a}
T8	33.950^{b}	1.505^{d}	1.940^{a}	34.335^{ab}	24.245^{a}
T9	34.250^{b}	1.665^{cd}	1.770^{ab}	30.895^{b}	21.500^{b}
T10	34.750^{b}	1.700^{abcd}	1.695^{abc}	29.730^{b}	20.470^{bc}

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

(T6 to T10) were supplemented with commercial phytase (150 mg / kg) had significantly ($P<0.05$) higher values compared with the other treatments which were not supplemented with the phytase (T1 to T5).

Data obtained from (T6 to T10) revealed that all parameters concerning final W, WG, ADG and specific growth rate showed no significant difference between T6, T7 and T8 which recorded higher values than the other diets T9 and T10.

Feed intake, feed conversion ratio, protein efficiency ratio, protein productive value and energy utilization of fish fed diets containing different levels of DDGS with phytase are shown in Table 5. Feed intake decreased with phytase supplementation.The best values of FCR was recorded with fish fed T6,T7 and T8. There were improvement in PER, PPV and Eu with phytase supplementation. The best values where recorded for T6, T7 and T8.

The growth performance and FCR of Nile tilapia after the 70 day growth trial was improved significantly ($P<0.05$) when diets were supplemented with phytase compared with the unsupplemented diets.Phytase improved the nutritional quality of the diets [36,37]. Similar results was observed in rainbow trout [26,38,39] Channel catfish [40], Carp [41]. However, the inclusion of phytase did not improve the growth of juvenile korearn rock. Fish *(sebastes schlegeli)* fed diets containing soybean meal [42].

The results of this work had support to the previous studies which showed that DDGS is a good protein source in fish diets that could replace soybean protein based diets with acceptable levels. Trout [6-8, 12-14] Channel catfish [15-17], Carp [5], Tilapia [18-22]).

The level of phytate – phosphorus in DDGS was low compared to phytate phosphorus in soybean meal due to the fermentation process used in producing ethanol, which could break the Chemical bonds of phytate [9] suggested that diets containing DDGS mainly supplemented with phytase increased deposition of P in the whole fish body, indicating that P load to the environment was reduced. Therefore, 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. [8,37,43] which reflects on improving growth performance and feed utilization up to 50% replacement along with 150mg kg^{-1} diet supplementation of phytase. Information obtained from this study will allow flexibility in least- cost feed formulation leading to lower feed costs, increased profits for fish producers, as well as increase the market demand for the increased supply of DDGS.

REFERENCES

1. Shurson, J., 2003. Feeding new generation DDGS to swine. Feed Mgmt.,54(5): 17-20.
2. US Grains Council 2008. Use of DDGS in Aquaculture diets DDGS user Handbook. http: //www.grains. org/galleries/DDGS %20 user %20 Handbook /60% 20% use 20% of 20% DDGS% 20 IN %20 Aquaculture % 20 Diets. pdf8.
3. Rosentrater, K.A., Muthukumarappan and S. kannadhason, 2009. Effects of ingredients and extrusion parameters on Aquafeeds containing DDGS and potato starch. J. of Aquaculture feed science and Nutrition,. Department of Agriculture and food Government of Western Australia.
4. Bonnardeaux, J., 2007. Potential Uses For Distillers Grains. Department of Agriculture and Food Government of Western Australia, pp: 1-11.
5. Tangendjaja, B., 2008. Update on DDGS quality consideration 16th Annual ASA- IM sea feed technology and Nutrition workshop Singapore.
6. Vielma, J., T. Makinen, P. Ekholm and J. Koskela, 2000. Influence of dietary soy and phytase levels on performance and body composition of large rainbow trout *(Oncorhynchus mykiss)* and algal availability of phosphorus load Aquaculture, 183: 349-362.
7. Cheng, Z.J., R.W. Hardy and M. Blair, 2003. Effects of supplementing methionine hydroxy analogue in Soybean meal and distiller's dried grain – based diets on the performance and nutrient retention of rainbow trout (*Oncorhynchus mykiss* (Waibaum). Aquaculture Research, 34: 1303-1310.
8. Cheng, Z.J. and R.W. Hardy, 2004 a. Effects of microbial phytase supplementation in corn distiller's dried grain with solubles on nutrient digestibility and growth performance of rainbow trout, *Oncorhynchus mykiss*. J. of Applied Aquaculture, 15(3/4): 83-100.
9. Cheng, Z.J. and R.W. Hardy, 2004b. Nutritional value of diets containing Distiler's dried grain with soluble for rainbow trout, *Oncorhynchus mykiss*. J. of Applied Aquaculture, 15(3/4): 101-113.
10. Tidwell, J.H., C.D. Webster, J.A. Clark and L.R. D'Abramo, 1993a. Evaluation of Distillers dried grains with soluble as an ingredient in diets for pond culture of the freshwater prawn *Macrobrachium rosenbergii*. J. of the World Aquaculture Society, 24(1): 66-70.

11. Tidwel, J.H., C.D. Webster, D.H. Yancey and L.R. D' Abramo 1993 b. Partial and total replacement of fish meal with soybean meal and distiller's by-products in diets for pond culture of the fresh water prawn (*Macrobracium rosenbergii*). Aquaculture, 118: 119-130.
12. Coyle, S. and T. Najeeullah, 1996. A preliminary evaluation of Naturally occurring organisms, Distillery by- products and prepared diets as food for juvenile freshwater prawn *Macrobrachium rosenbergii*. J. of Applied Aquaculture, 6(2): 57-66.
13. Coyle, S., J.H. Tidwell, Van A. Arnum and L.A. Bright, 2003. A comparison of two feeding technologies in freshwater prawns, *Macrobrachium rosenbergii*, Raised at high Biomass Densities in temperate ponds J. Applied Aquaculture, 14(1/2): 123-135.
14. Coyle, S., J.H. Tidwell, L.A. Bright and D.Ya sharian 2004. Effect of different feeding strategies on production and economic returnes for fresh water prawn, *Macrobracium rosenbergii*, raised in earthen ponds in a temperate climate J. Applied Aquaculture, 16(1/2): 147-156.
15. Webster, C.D., J.H. Tidwell and L.S. Goodgame, 1993. Growth, body composition and organoleptic evaluation of channel catfish fed diets containing different percentages of Distillers grains with soluble. The Progressive Fish Culturist, 55: 95-100.
16. Lim, C., M. Aksoy and P. Klesius, 2007. Growth response and resistance to *Edwardiella ictaluri* of Channel catfish (*Ictalurus punctatus*) fed diets containing Distillers, dried grains with soluble. Journal of the World Aquaculture Society, 2: 231-237.
17. Robinson, E.H. and M.H. Li, 2008. Replacement of Soybean meal in Channel catfish, *Ictalurus punctatus* diets with cottonseed meal and Distiller's Dried Grains with Soluble. J. of the World Aquaculture Society., 39(4): 521-527.
18. WU, Y.V., R. Rosati, D.J. Sessa, D.J. and P. Brown, 1994. Utilization of protein – rich ethanol coproducts from corn in tilapia feed. Journal of the American Oil Chemists Society, 71(9): 1041-1043.
19. Wu, Y.V., R.R. Rosati and P.B. Brown, 1996. Effect of diets containing various levels of protein and ethanol co products from corn on growth of tilapia fry. J. Agric. Food Chem., 44: 1491-1493.
20. WU, Y.V., R.R. Rosati and P.B. Brown, 1997. Use of corn- derived ethanol co products and synthetic lysine and tryptophan for growth of tilapia (*Oreochromis niloticus*) fry. J. Agric. Food Chem., 45: 2174-2177.
21. Tidwell, J.H., S.D. Coyle, A. Vanarnum, C. Weibel and S. Harkins, 2000. Growth, Survival and Body composition of cage-Cultured Nile tilapia *Oreochromis niloticus* fed pelleted and unpelleted Distillers grains with solubles in polyculture with freshwater prawn *Macrobrachium rosenbergii*. J. of the world Aquaculture Society, 31(4): 627-631.
22. Shelby, R.A., C. Lim, M. Yildrim and P.H. Klesius, 2008. Effect of distillers dried grains with soluble-incorporated diets on growth, immune function and disease resistance in Nile tilapia (*Oreochromis niloticus L.*). Aquaculture Research, 39(12): 1351-1353.
23. Liener, I.E., 1994. Implications of antinutrational components in soybean foods CRC crit Rev. Food Sci.. Nutr., 34: 33-67.
24. NRC (National Research Council) 1993. Nutrient Requirements of Fish National Academy press, Washington, D.C. USA, pp: 114.
25. Singh, M. and A.D. krikorian, 1982. Inhibition of trypsin activity in vitro by phytate. J. Agric. Food Chem., 30: 799-800.
26. Spinelli, J., C.R. Houle and J.C. Wekell, 1983. The effect of phytase on the growth of rainbow trout (*Salmo gairdner*) fed purified diets containing varying quantities of calcium and magnesium. Aquaculture, 30: 71-83.
27. Vielma, J. and S.P. Lall, 1998. The control of phosphorus homeostasis of Atlantic salmon, Salmo solar L., in fresh water. Fish Physiol. Biochem., 18: 83-93.
28. Eya, J.C. and R.T. Lovell, 1997. Available phosphorus requirements of food-size channel catfish (*Ictalurus punctatus*) fed practical diets in ponds. Aquaculture, 154: 283-291.
29. Eya, J.C. and R.T Lovell, 1998. Effects of dietary phosphorus on resistance of channel catfish to *Edwardsiella ictaluri* J. Aqua. Anim. Health, 10: 28-34.
30. AOAC. 1995. Official Methods of Analysis of the Association of Official Analytical Chemists. International, 16th edn. Association of official Analytical Chemists, Arlington, VA.
31. Pantha, B., 1982. The use of soybean in practical feeds for *tilapia niloticus*. M.Sc. Thesis University of Sterling.
32. Chattopadhyay, G.N., 1998. Chemical Analysis of Fish Pond Soil and Water. Daya publishing House. New Delhi, India.
33. Duncan, D.B., 1955. Multiple Range and Multiple F-test Biometrics, 11: 1-42.

34. Tahoun, A.M.A., 2007. Studies on some factors affecting the production and reproduction of Nile tilapia. Ph. D. Dissertation. Fac. of Agriculture, Kafr El- Sheikh University, Egypt.
35. Khalfalla, M.M., Y.A. Hammouda, A.M. Tahoun and H.A.M. Abo- state, 2008. Effect of Brood stock sex ratio on growth and Reproductive performance of Blue tilapia *Oreochromis aureaus* (Steindachner) reared in hapas. 8th International Symposium on Tilapia in Aquaculture (CICC) Egypt, I: 115-125.
36. McClain, W.R. and D.M. Gatlin, 1988. Dietary zinc requirement of *Oreochromis aureus* and effects of dietary calcium and phytate on zinc bioavailability. J. World Aquaculture Society, 19: 103- 108.
37. Liebert, F. and L. Portz, 2005. Nutrient utilization of Nile tilapia *Oreochromis niloticus* fed plant based low phosphorus diets supplemented with graded levels of different sources of microbial phytase. Aquaculture 248: 111 -119.
38. Cain, K.D. and D.L. Garling, 1995. Pretreatment of soybean meal with phytase for salmonid diest to reduce phosphorus concentrations in hatchery effluents. Prog. Fish Cult, 57: 114-119.
39. Riche, M. and P.B. Brown, 1996. Availability of phosphorus from feedstuffs fed to rainbow trout (*Oncorhynchus mykiss*) Aquaculture, 142: 269-282.
40. Satoh, S., W.E. Poe and R.P. Wilson, 1989. Effect of supplemental phytata and/or tricalcium phosphate on weight gain, feed efficiency and zinc content in vertebrae of channel catfish.Aquaculture, 80: 155-161.
41. Hossain, M.A. and K. Jauncy, 1993. The effects of varying dietary phytic acid, calcium and magnesium levels on the nutrition of common Carp. *Cyprinus carpio*. In Fish nutrition in practice IVth International symposium on Fish nutrition and feeding Biarritz, France, June 24-27,. pp 705, INRA, Paris, France.
42. Yoo, G.Y, X.J. Wang, S.M. Choi, K.M. Han, J.C. Kang and S.C. Bai, 2005. Dietary microbial phytase increased the phosphorus digestibility in juvenile Korean rockfish *Sebastes schlegeli* fed diets containing soybean meal. Aquaculture, 243: 315-322.
43. Vielma, J., S.P. Lall, J. Koskela, F. Schoner and P. Matila, 1998. Effects of dietary phytase and cholecaleiferal on phosphorus bioavailability in rainbow trout (*Oncorhyn chusmykiss*). Aquaculture, 163: 309-323.