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EFFECTS OF DIETARY PHYTASE AND DICALCIUM PHOSPHATE ON PHOSPHORUS BIOAVAILABILITY IN THE NILE TILAPIA *OREOCHROMIS NILOTICUS*

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Key words: Nile tilapia, *Oreochromis niloticus*, phosphorus, phytase, dicalcium- phosphate, phosphorus utilization and phytic acid .

ABSTRACT

The efficiency of supplemented diets with dicalcium phosphate (DCP) and phytase (500 and 1000 units) replaced with dietary fish meal instead of soybean meal or full fat soybean at two replacing levels of 50% or 75% for Nile tilapia was investigated.

The results of Nile tilapia (initial weight 12.10 ± 0.6 gm) revealed that the average growth performance of fish fed diet supplemented with phytase (500 and 1000 units) had improved feed conversion ratio and specific growth rate. However, it was also shown that the replacing 50% of the fish meal with soybean meal or soybean full fat in tilapia diet were successful and had no adverse effect on growth.

The diet supplemented with DCP or phytase (500 or 1000 units) significantly ($P<0.01$) increased body protein content, body fat, body ash and calcium concentration in both vertebral column and the whole body than control. An inverse relationship between phytase dose and fat content was seen in the present results. However, fish fed the control diet or diet contained 50% of the protein from either soybean full fat or soybean meal gave the highest body protein content, crude ash and the lowest value of ether extract and gross energy content. Calcium content of whole body was higher in the fish fed the control diet than in fish fed any diet containing plant protein sources. The same trend was observed for calcium in the vertebral column.

The data demonstrated that, supplementing diets with dicalcium phosphate or phytase either 500 or 1000 unit had increased the apparent digestibility of phosphorus, phosphorus retention and phosphorus concentration in the vertebral column and in the whole body than fish fed the control diet (unsupplemented). Whereas, diet supplemented with phytase either 500 or 1000 units caused a significant ($P<0.001$)) decrease in phosphorus excretion and a accumulation of phosphorus in water than the control diet or diet supplemented with DCP.

In conclusion dietary phytase (500 units/kg) has potential to improve the nutritive quality of plant protein sources of Nile tilapia and to concomitantly minimize phosphorus discharge into the aquatic environment.

INTRODUCTION

Success of intensive fish culture depends to a large extent on supplemental feeding (El–Sayed, 1999). Phosphorus (P) is an essential mineral in diets fed to fish, but (P) in aquaculture effluents is considered a source of pollution by many regulatory agencies (Lall, 1991). Uneaten ration and unavailable dietary (P) in feces are the two primary contributors in fish farm effluents (Bergheim *et al.*, 1991).

Common feedstuffs contain a considerable amount of phosphorus; however, about 2/3 of phosphorus found in plant feedstuffs is 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 should be considered when formulating diets. One of the safe ways to decrease phytic acid content in the diets containing high amount of plant protein sources is the supplementation of the diets with enzyme phytase (Vilema *et al.*, 2000).

Microbial phytase products are commercially available in Europe, Canada and USA for use in animal feeds (Jackson *et al.*, 1996). If cost is effective, microbial phytase may be a useful supplement in Nile tilapia feed containing high levels of plant ingredients to reduce waste phosphorus. Therefore, this study was conducted to evaluate efficiency of phytase and dicalcium phosphate for improving phosphorus bioavailability in diets containing soybean meal or full fat soybean fed to Nile tilapia fish.

MATERIALS AND METHODS

This experiment was carried out in the Fish experimental unit of Central Laboratory for Food and Feed, Agriculture Research Center, Ministry of Agriculture to determine the efficiency of supplemented diets with dicalcium phosphate (DCP) and phytase (500 and 1000 units) replaced with dietary fish meal instead of soybean meal or full fat soybean at two replacing levels of 50% or 75% , on feed utilization , phosphorus bioavailability, phosphorus apparent digestibility, excretion and retention of Nile tilapia *(Oreochromis niloticus)*.This study lasted for 58 days .

Experimental units:

An open system design was used to carry out the experiment. This system was composed of 24 tanks (from fiberglass) with a volume 85Leach tank. Another 24 fiberglass tanks were used to store water for 48h to get rid of chlorine ion, since the water used was tap water. Water temperature was about 27±1°C. All the experimental treatments were conducted under a synthetic photoperiod equal to natural light/darkness period (14h light: 10h-darkness). The tanks were individually aerated from a main compressor and had individual inlet and outlet for water.

Feed ingredients and experimental diets

Experimental diets were formulated from fish meal, soybean meal and full fat soybean as basic protein sources in diets. The proximate compared of the experimental feed ingredients are shown in Table (1), while, Table (2) illustrated the phytate phosphorus content of ingredients as percent of total phosphorus as compramison with the results of Ravindran *et al.* (1994) as a reference percentage.

Twenty experimental diets were formulated in this study. Five basal rations were made , the first served as control and contained only fish meal as animal protein source, the second and third diets contained full fat soybean as plant protein replacing 50% or 75% of the fish meal protein present in the first diet.The fourth and fifth diets contained soybean meal as another source of plant protein and were formulated to contain the same percentage used in the diets containing full fat soybean (50% or 75% of fish meal protein). These five basal

formulated to contain the same percentage used in the diets containing full fat soybean (50 % or 75 % of fish meal protein). These five basal diets were formulated without adding any source of inorganic phosphorus as a control diet. Similar basal five diets were supplemented with either 500 or 1000 units of commercial phytase (2500 FYT/g phytasonovo CT brought from Novo Nordisk Denmark), according to Rodehutscord & Pfeffer (1995); Schäfer, *et al.* (1995) and Jackson *et al.* (1996). Another group of previous five basal experimental diets were formulated and supplemented only with dicalcium phosphate to raise the level of dietary available phosphorus according to the NRC requirements for Nile tilapia (0.9% available phosphorus) (NRC, 1993).

Table (3) illustrates the proximate analysis of the basal experimental diets. The diets were formulated to contain 30% crude protein and 3440 ± 58 Kcal digestible energy at level of protein/energy ratio of (87mg /Kcal) according to NRC (1993).Soybean oil was added to the ration containing soybean meal to adjust the dietary energy requirement. Vitamin , amino acid and mineral mixture were added at 2% of the formulated diet (except in the diet containing 75% full fat soybean the mixture was added at 3% of the formulated diet) to prevent deficiency in these nutrients. The mineral mixture that was added to the diets was free from any source of phosphorus. Dicalcium phosphate and phytase were added to the diets according to Viola *et al.* (1988) and Jackson *et al.* (1996).

The fish were fed a pre-determined amount of feed (1.8% of body weight/day) using the method of Cho (1992). This amount was observed to be close to maximum voluntary feed intake of the fish.

Experimental fish :

One thousand Nile tilapia mono sex *(O. niloticus)* with an average weight 12.10 ± 0.6 gm, were stocked and equally divided into 40 tanks (25 fish in each), so each two tanks represent a diet from the previous twenty formulated diets. The experimental fish were carefully stocked into the experimental tanks. However the first 14 days of the experimental period were considered as an adaptation period and thereafter the growth trials were carried out for 58 days. All the diets contained 0.5% Cr_2O_3 as an inert indicator to determine the apparent digestibility of phosphorus. Diets were randomly assigned to the experimental units. Fish were hand fed the experimental diets for six days weekly, three times per day.

treatment. The samples were collected using siphon technique to determine the apparent digestibility of phosphorus according to (Riche and Brown, 1996).

Water phosphorus samples:

Three days before water samples collection, fish were fed twice a day the experimental diets. After the last meal (third day), a sample from water inlet (blank sample) and samples taken from the all experimental tanks were collected. Experimental water samples (100ml) were stabilized with 0.5ml concentrated sulfuric acid (Bureau and Cho, 1999). Total phosphorus content was determined in both blank sample and water samples taken from the tanks using the total phosphorus vanadomolybdophosphoric acid colorimetric method (APHA, 1985).

Blood samples:

At the end of the experiment, five fish were collected from each tank. Fish were bled from the caudal vein, blood was allowed to clot at room temperature for one hour. Serum was obtained by centrifuging coagulated blood at 10,000 xg for 20min. The samples were duplicate for each diet. They were stored at -18°C until analysis (Rodehutscord *et al.*, 2000). Inorganic phosphorus was determined colorimetricaly in serum without deproteinization using the method described in the test kit for determination of inorganic phosphorus of Bio Merieux vitek,Inc 595 Anglum Drive Hazelwood. (USA).

Chemical analysis:

Five fish at the beginning and end of experimental period were randomly taken from each tanks for proximate body composion analysis. Additional five fish were collected from each tank for vertebral analysis. The vertebral samples were composed of the main vertebral column. These samples were frozen at -20°C and before analysis the samples were thawed, boiled in distilled water for 10min. and adhering flesh was removed. The bones were dried for 2h at 100°C (Eya and Lovell, 1997).

Whole body samples were analyzed for protein, lipid, ash, calcium and phosphorus following methods of AOAC (1990). Bone samples were analyzed for calcium and phosphorus using AOAC, (1990), while zinc was analyzed using atomic absorption

spectrphotometer (Perkin – Elmer, Uberlingen, Germany). AOAC, (1990). Chromic oxide in both diet and fecal samples were determined according to the procedure of (Edwards and Gillis, 1959). Phytic acid was determined in both the ingredients and the formulated diets according to the procedure of Wheeler and Ferrel (1971).

The apparent digestibility coefficients of phosphorus (ADC%) were measured at the end of the trials using the results of analysis of fecal phosphorus , feed and detecting the concentration of the marker (Cr_2O_3) in both of them. The apparent digestibility cofficiences were calculated using the standard formula according to Carter and Hauler, (2000):

$$ADC\% = 100 - [100 (\%I_{diet} / \%I_{feces}) \times (\%N_{feces} / \%N_{diet})]$$

Where:
I: is the inert marker
N: is the nutrient.

Phosphorus excretion and retention were calculated according to Edwards and Gills (1959); Einen *et al.*, (1995) respectively. Retained phosphorus was calculated as mg phosphorus retained per gm of diet consumed, while phosphorus excretion was calculated using the following equation.

$$\text{Phosphorus excretion} = Fn\ (mg\ g^{-1}\ diet) \times (100\% - ADC\%)$$

Where:
Fn: is the dietary phosphorus concentration.
ADC: is the apparent digestibility coefficients of phosphorus.

Statistical analysis:

Data obtained from the experiment were analyzed by two way analysis of variance using the general linear model (GLM) of SAS (SAS Institute, 1991). Means were compared using Duncan's new multiple range test (Duncan, 1955). The percentages were transformed to their arc sign values before analysis. The original percentages though, are listed in the tables.

RESULTS AND DISCUSSION

Growth performance and feed utilization:

The average growth performance and feed utilization of tilapia fed different experimental diets are shown in Tables (4 ,5 and 6). Result in Table (4) showed the significant differences ($P<0.001$) for

final body weight ; feed conversion ratio and specific growth rate of Nile tilapia among different experimental treatments .

Irrespective of replaceing level (Table 5), the diets supplemented with 1000 units/kg phytase, dicalcium phosphate or 500 units/ kg phytase showed highest significant differences ($P<0.001$) values in final body weight when compared with the control diet (not supplemented). The diet supplemented with dicalcium phosphate had better feed conversion ratio (2.76) than the other experimental treatments. The specific growth had increased significantly ($P<0.001$) and followed the same order of final body weight. These results are in agreement with the findings of Dato – Cajegas and Yakupitiyage (1996) who reported that supplementation of diets for Nile tilapia reared under semi – intensive conditions with phosphorus improved growth and feed utilization. Schäfer *et al.* (1995) reported that supplementation of diets with either phoytase or mono-calcium phsphate (MCP) showed significant positive effects on growth and feed utilization of Atlantic salmon, irrespective of the level of phytase. Feed conversion ratio was the best for fish fed basal diet supplemented with phytase than fish fed the basal diet without supplemental phytase.

Irrespective of dietary supplementation with dicalcium phosphate or phytase, the fish fed diets contained either 100% fish meal (control), 50% full fat soybean or 50% soybean meal had the highest final body weight, the specific growth rate and the better feed conversion ratio than the other treatments (Table 6) . Many previous studies had considered soybean meal as a partial or total fish meal alternative for feeding with varying results. SBM could replace between 67 and 100% of fish meal, depending on fish species, size, dietary protein level, soy bean meal source, processing methods and culture systems employed (El-Sayed, 1999). Processed, solvent extracted SBM, with or without methionine supplementation successfully replaced up to 75% of FM in tested diets fed to Nile tilapia fry (Tacon *et al.*, 1983), *O. mossambicus* (Jackson *et al.*, 1982) and 67% in case of tilapia hybrids (Shiau *et al.*, 1989).

Effect of dietary supplementation with dicalcium phosphate or phytase on body composition:

The results presented in Tables (7 & 8) revealed that the supplemented diets with DCP or phytase (500 or 1000 units)

than the fish fed control diet (Table 8). These results conform to the suggestion of Lei *et al.*, (1993) who reported that diet supplemented with phytase might improve bioavailability of protein. Storebakken *et al.* (1998) and Vielma *et al.* (1998) found that plant protein utilization in practical diets had increased when supplemented with phytase. Schäfer *et al.* (1994) and Cain and Garling (1995), reported that the increase of whole body protein may be referred to the effect of phytase on phytin which could have prevented the formation of protein – phytin complexes and so prevented the reduction in protein bioavailability.

Irrespective of dietary protein sources ,supplementing of diets with DCP or with phytase (500 or 1000 units) reduced significantly ($P<0.01$) tilapia whole body fat compared with the fish fed the control diet (Table 8). An inverse relation between phytase dose and fat content was seen in the present results. It is clear from the present results that supplementation with phytase or dicalcium phosphate reduced fat deposition through increasing phosphorus availability which might have enhanced β - oxidation of fatty acids, increased glycogenesis or had a repartitioning effect, resulting in increased deposition of protein and reduced deposition of lipid (Sakamoto and Yone, 1978).

Lanari *et al.* (1998) and Elangovan and Shim (1998) observed that increasing available phosphorus caused a decrease in body energy deposition of juvenile Tiger barb and European sea bass. The tabulated data followed the same tendency, (Table 8). Where the values of the whole body energy content of tilapia fed diets supplemented with phytase 1000 units or 500 units and DCP were 5797.72, 5830.67 and 5812.87 Kcal/kg body, respectively than tilapia fed the control diet (5946.82 Kcal/kg body).

Supplementation with dicalcium phosphate or phytase 500 units or 1000 units, increased body ash percentage calcium concentration in both vertebral column calcium concentration and the whole body as compared to the control may be due to the Fact that freshwater fish can meet most of their calcium requirement by absorbing this element through the gills and skin (NRC, 1993 and Forster *et al.*, 1999), while the differences between Ca concentration in both of the vertebral column and whole body of fish fed diets supplemented with phytase and those fed the control diet were due to the effect of phytase on breaking phytic acid complexes with divalent ions and so increased Ca availability (Budavari *et al.*, 1989). The present results agree with the finding of Storebakken *et al.* (1998) who

present results agree with the finding of Storebakken *et al.* (1998) who reported an increase in Ca digestibility, availability and retention when diets were supplemented with phytase. These authors suggested that the increase in body Ca could be explained by the fact that phytic acid acts as a Ca binding agent in the proximal small intestine. Hydrolysis of phytate in the stomach as a result of phytase activity caused an increase in Ca digestibility and retention in the body. The significant increase of body Ca when diets were supplemented with dicalcium phosphate may be due to the increase of the available calcium.

Effect of partial replacement of fish meal with full fat soybean or soybean meal on tilapia body composition:

Fish fed the control diet or diets contained 50% of the protein from either full fat soybean or soybean meal showed the highest significant value of body protein content ,crude ash and the lowest ether extract (Table 9). The whole body gross energy content followed the same trend . The reduced carcass fat content with increasing SBM in diets up to 50% were mentioned with earlier findings of Reigh and Ellis (1992) and Olli *et al.* (1995). Olli and Krogdahl (1994) demonstrated that alcohol-soluble components of SBM comprise anti-nutrients, which negatively affect fat digestibility, particularly the long chain, saturated and mono unsaturated fatty acids in Atlantic salmon. This may also be one of the reasons for decreased carcass fat content in tilapia fed diets containing either full fat soybean or soybean meal up to level of 50% of the diets' protein. Moreover, the increase in body fat of Nile tilapia fish fed diet containing 75% of the protein from SBF may be referred to the high energy content of full fat soybean , as it was observed by Santinha *et al.* (1999) that the high inclusion of fat in the diet increased body fat content.

Effect of dietary supplementation with dicalcium phosphate or phytase on phosphorus utilization:

Significant interaction differences ($P<0.001$) was detected between supplementing diets with DCP or phytase and the percentage of replacement full fat soybean and soybean meal with fish meal (Table 10), on ash content of the body, inorganic phosphorus in serum, phosphorus retention and excretion. This could be explained by the effect of DCP on increasing phosphorus availability since it

covered the phosphorus needs for tilapia, thus increase the inorganic phosphorus in blood, retained phosphorus in body parallel to the decrease in the percentage of replacement. Although, phosphorus excretion increased by the increase in the percentage of replacement.

The data demonstrated that the supplementing diets with dicalcium phosphate or phytase either (500 or 1000 units) had increased the apparent digestibility of phosphorus, phosphorus retention and phosphorus concentration either vertebral column or whole body than fish fed the control diet (unsupplemented) (Table 11). Reddy *et al.* (1982) indicated that using microbial phytase was effective in improving bioavailability of phytate phosphorus, by hydrolyzing phytate to O-phosphate hence, increasing the available phosphorus and which could be utilized by fish and deposited in the body and in the vertebral column. Bureau and Cho (1999) reported that low level of dietary phosphorus had a weak effect on growth and feed efficiency, but with very significant effect on whole carcass and vertebral phosphorus content and phosphorus gain. This could explain the significant difference between the effect of supplementing the diet with dicalcium phosphate and phytase.

Fish fed diets supplemented with 1000 units phytase increased significantly ($P<0.001$) inorganic phosphorus in their serum (51.60 mg P/ dl serum)than the other treatment .These responses are more reflection of dietary concentration of phosphorus rather than the nutritional status of the fish. These results conform with the results of Eya and Lovell (1997) who reported that increasing dietary available phosphorus showed linear increase in serum phosphorus and suggested that the phosphorus concentration in the serum was a good reflection of phosphorus availability.

The tabulated results (Table 11) revealed that the increase in phosphorus excretion by tilapia fed the diets supplemented with dicalcium phosphate may be due to the increase in the dietary phosphorus to cover the deficiencies in phosphorus caused by the presence of phytic acid and the low phosphorus content of soybean. Low amount of added dicalcium phosphate would increase the risk of developing phosphorus deficiency. However, supplementing diets with phytase had decreased phosphorus excretion due to phytase role in hydrolyzing phytate to O – phosphate and the consequent increase in phosphorus availability, retention and concentration in both vertebral column and the whole body which reduced excretion of phosphorus in feces.

Effect of partial replacement of fish meal with full fat soybean or soybean meal on phosphorus utilization:

Nile tilapia fed either control diet or the diets that contained 50% of their protein from full fat soybean or soybean meal had the highest phosphorus intake (Table 12). Lovell (1989) reported that the decrease in feed intake with increasing the inclusion of soybean meal was referred to poor palatability, while the decrease in feed intake by fish fed diets containing 75% of the protein from full fat soybean was due to high energy content and low palatability of the diet. The same trend was observed in phosphorus retention (5.3, 5.3 and 5.4 mg P/gm diet respectively).

Partial replacement of fish meal by soybean full fat or soybean meal had increased inorganic phosphorus in serum significantly ($P<0.001$) compared with fish fed the control diet (Table 12). However, from nutritional points of view the elevation in phosphorus concentration in serum of fish fed diets containing plant protein sources compared to the control diet could be due to the effect of the significant ($P<0.01$) interaction between the diets containing plant protein and the supplementation with phytase or dicalcium phosphate. This suggestion agree with results reported by Eya and Lovell (1997) who found that serum phosphorus showed a linear increase with supplementation with dicalcium phosphate due to the increase in dietary available phosphorus. Pretreatment of diets with phytase increased the available phosphorus with a consequent increase in phosphorus concentration in serum.

The fecal excretion of phosphorus by fish fed diets that contained 75% of their protein from full fat soybean or soybean meal was significantly ($P<0.001$) high. This could be due to the low phosphorus apparent digestibility and low palatability of soybean meal and full fat soybean. The results of phosphorus accumulation in water had different trend from fecal phosphorus excretion.This was explained by Rodehutscord *et al.* (2000) who found that fecal phosphorus excretion was affected at a basal phosphorus intake, while non – fecal phosphorus excretion was not affected till a high level of phosphorus intake was attained, then it started to change.

In conclusion addition of phosphorus to diets, however has implications on water quality and phosphorus pollution and should be considered when formulating diets. Supplemented diet with phytase

(500 units/ kg) has potential to improve the nutritive quality of plant protein sources of Nile tilapia and to concomitantly minimize phosphorus discharge into the aquatic environment .However it was also shown from the results that the replacing 50% of the fish meal with soybean meal or soybean full fat in tilapia diet were successful and supplemented diet with phytase (500 units / kg) had improved feed conversion ratio and specific growth rate.

Hence, additional studies are needed to determine the best strategies for optimizing the cost effectiveness of dietary phytase when tilapia are fed diets based upon plant protein which contain a considerable amount of phosphorus in the form of phytic acid.

REFERENCES

American Public Health Association (APHA); (1985). "Standard methods for examination of water and waste water", 15 *th* Ed. APHA, Washington, DC.

A. O. A. C., (1990). "Official methods of analysis of the Association of official Analytical Chemists",15 *th.* Ed. Vol. 1, published by the A. O. A. C., Benjamin Francklin station, Washington, DC.

Bergheim, A.; Aabel, J. P. and Seymour, E. A. (1991). Past and present approaches to aquaculture waste management in Norwegian net pen culture operations. In: C. B. Cowey and C. Y. Cho (Editors), "Nutritional Strategies and Aquaculture Waste". Proceedings of the First International Symposium on Nutritional Strategies in Management of Aquaculture Waste, 2 – 6 June 1990, Guelph, Canada. Univ. Guelph, Ont., Canada, pp. 117 – 136.

Budavari, S.; O'Neil, M. J.; Smith, A. and Heckelman, P. E. Eds. (1989). "The Merck Index". An Encyclopedia of Chemicals, Drugs and Biologicals, 11 *th* ed., Merck, Rahway, NJ, USA

Bureau, D. P. and Cho, C. Y. (1999). Phosphorus utilization by rainbow trout *Oncorhynchus mykiss*: estimation of dissolved phosphorus waste output. Aquacult., *179*: 127 – 140.

Cain, K. D. and Garling, D. L. (1995). Pretreatment of soybean meal with phytase for salmonid diets to reduce phosphorus concentrations in hatchery effluents. Prog. Fish - Cult., *57*: 114 -119.

Carter, C. G. and Hauler, R. C. (2000). Fish meal replacement by plant meals in extruded feeds for Atlantic salmon, Salmo solar L. Aquacult., *185*: 29 – 311.

Cho, C. Y. (1992). Feeding systems for rainbow trout and other salmonids with reference to current estimates of energy and protein requirements. Aquacult., *100*: 107 – 123.

Dato – Cajegas, C. R. S. and Yakupitiyage, A. (1996). The need for dietary mineral supplementation for Nile tilapia *Oreochromis niloticus* cultured in a semi – intensive system. Aquacult., *144*: 227 – 237.

Duncan, D. B. (1955). Multiple range and Mulliple Ftest. Biometrics., *11*: 1– 42.

Edwards, H. M., Jr. and Gillis, M. B. (1959). A chromic oxide balance method for determining phosphate availability. Poultry. Sci., 569 – 574.

Einen, O.; Holmefjord, I.; Asgard, T. and Tallbot, C. (1995). Auditing nutrient discharges from fish farms: theoretical and practical considerations. Aquacult. research., *26*: 701 – 713.

El-Sayed. A. F. M. (1999). Alternative dietary protein sources for farmed tilapia *Oreochromis* spp. Aquacult., *179*: 149 – 168.

Elangovan, A. and Shim, K. F. (1998). Dietary phosphorus requirement of juvenile tiger barb, *Barbus tetrazona* (Bleeker, 1855). Aquarium. Sci. conserv., *2* (1): 9-19.

Eya, J. C. and Lovell, R. T. (1997). Available phosphorus requirements of food-size channel catfish *Ictalurus punctatus* fed practical diets in ponds. Aquacult., *154*: 283-291.

Forster, I.; Higgs, D. A.; Dosanjh, B. S.; Rowshandeli, M. and Parr, J. (1999). Potential for dietary phytase to improve the nutritive value of canola protein concentrate and decrease phosphorus *output* in rainbow trout *Oncorhynchus mykiss* held in 11°c fresh water. Aquacult., *179* : 109-125.

Hepher, B.; Liao, I. C.; Cheng, S. H. and Haseih, C. S. (1983). Food utilization by red tilapia. Effect of diet compostion, feeding level and temperature on utilization efficiency for maintenance and growth. Aquacult., *32*: 255 – 272.

Jackson, A. J.; Capper, B. S. and Matty, A. J. (1982). Evaluation of some plant proteins in complete diets for the tilapia *Sarotherodon mossambicus*. Aquacult., *27*: 97 – 109.

Jackson, L. S.; Li, M. H. and Robinson, E. H. (1996). Use of microbial phytase in channel catfish Ictalurus punctatus diets to improve utilization of phytate phosphorus. J. World Aquacult. Soc., *27*(3): 309 – 313.

Lall, S. P. (1991). Digestibility, metabolism and excretion of dietary phosphorus in fish. In: C. B. Cowey and C. Y. Cho (editors), "Nutritional strategies and Aquaculture waste". Proceedings of the first International Symposium on Nutritional Strategies and Aquaculture Waste, 2-6 June (1990). Guelph, Canada. University of Guelph, Ont., Canada, pp. 21-36.

Lanari, D.; D' Agro, E. and Turri, C. (1998). Use of nonlinear regression to evaluate the effects of phytase enzyme treatment of plant protein diets for rainbow trout *Oncorhynchus mykiss*. Aquacult., *161*: 345 – 356.

Lei, X.; Pao, K. K.; Miller, E. R.; Ullery, D. E. and Yokoyama, M. T. (1993). Supplemental microbial phytase improves bioavailability of dietary zinc to weaning pigs. J. Nutrition, *123*: 1117 – 1123.

Lovell, R. T. (1989). Nutrition and feeding of fish .Auburn Univ. Van Nostrand Reinhold, New York.

NRC (National research council). (1993). Nutrient requirements of fish. National Academy Press, Washington D.C., 114pp.

Olli, J. J. and Krogdahl, A., (1994). Nutritive value of four soybean products as protein sources in diets for rainbow trout *Oncorhynchus mykiss* reared in fresh water. Acta. Agric. Scand. Sect. A. Anim. Sci., *44*: 185 – 192.

Olli, J. J.; Krogdahl, A. and Vabeno, A., (1995). Dehulled solvent extracted soybean meal as a protein source in diets for Atlantic salmon Salmo salar L. Aquacult. Res., *26*:167– 174.

Ravindran, V.; Ravindran, G. and Sivalogan, S. (1994). Total and phytate phosphorus contents of various foods and feedstuffs of plant origin. Food Chem., *50*: 133.

Reddy, N. R.; Sathe, S. K. and Salunkhe, D. K. (1982). Phytates in legumes and cereals in: "Advances in Food Research" Chichester, C.O.; Mark, E.M. and Stewart G.F. editors. Academic press. NewYork, USA.

Reigh, R. C. and Ellis, S. C. (1992). Effects of dietary soybean and fish-protein ratios on growth and body composition of red drum *Sciaenops ocellatus* fed isonitrogenous diets. Aquacult., *104*: 279 – 292.

Riche, M. and Brown, P. B. (1996). Availability of phosphorus from feedstuffs fed to rainbow trout, *Oncorhynchus mykiss*. Aquacult., *142*: 269 – 282.

Rodehutscord, M. and Pfeffer, E. (1995). Effects of supplemental microbial phytase on phosphorus digestibility and utilization in rainbow trout *Oncorhynchus mykiss*. Wat. Sci. Tech., *31*(10): 143 – 147.

Rodehutscord, M.; Gregus, Z. and Pfeffer, E. (2000). Effect of phosphorus intake on fecal and non-fecal phosphorus excretion in rainbow trout *Oncorhynchus mykiss* and the

consequences for comparative phosphorus availability studies. Aquacult., *188*: 383-398.

Sakamoto, S. and Yone, Y. (1978). Effects of dietary phosphorus on chemical composition of red sea bream. Bull. Jpn. Soc. Sci. Fish., *44*: 227-229.

Santinha, P. J. M.; Medale , F.; Correze, G. and Gomes, E. F. S. (1999). Effect of the dietaey protin: lipid ratio on growth and nutrient utilization in githead seabream *Sparus aurata.* Aquacult. Nutr., *5*(3) .

SAS, (1991). SAS / STAT User's guide. SAS Institute, Cary, NC, USA, 1028.

Schäfer, A.; Koppe, W. M.; Meyer – Burgdorff, K. H. and Günther, K. D. (1994). Effects of a microbial phytase on the utilization of native phosphorus in a soybean meal based diet in carp. Proc. of the 2nd International Symposium on Nutritional Strategies in Management of Aquaculture Waste, 24-27 April 1994, Aalborg, DK. Univ. of Guleph, Ontario, Canada, pp. 32-33.

Schäfer, A.; Koppe, W. M.; Meyer – Burgdorff, K. H. and Günther, K. D. (1995). Effects of a microbial phytase on the utilization of native phosphorus by carp in diet based on soybean meal. Wat. Sci. Tech., *31*(10): 149 – 155.

Shiau, S.- Y.; Kwok , C. C.; Hwang , J. Y.; Chen, C . M. and Lee, S. L. (1989). Replacement of fish meal with soybean meal in male tilapia *Oreochromis niloticus* O. aureus* fingerling diets at a suboptimal level . J. World Aquacult. Soc., *20* (4): 230 –235.

Storebakken, T.; Shearer, K. D. and Roem, A. J. (1998). Availability of protein, phosphorus and other elements in fish meal, soy – protein concentrate and phytase – treated soy – protein concentrate – based diets. to Atlantic salmon *Salmo salar*. Aquacult., *161*: 365 – 379.

Tacon, A. G. J.; Jauncey, K.; Falaye, A.; Pantah, M.; Mac-Gowen, I. And Stafford, E. (1983). The use of meat and bone meal, hydrolyzed feather meal and soybean meal in practical fry and fingerling diets for *Oreochromis niloticus*. In: Fishelson, J.; Yaron, Z. (Eds). Proc. 1st Int. Symp. on tilapia in Aquacult. Tel Aviv Univ. Press. Israel., pp. 356 – 365.

Vielma, J.; Lall, S. P.; Koskela, J.; Schöner, F-J. and Mattila, P. (1998). Effects of dietary phytase and cholecalciferol on phosphorus bioavailability in rainbow trout *Oncorhynchus mykiss*. Aquacult., *163*: 309-323.

Vielma, J., Mäkinen, T., Ekholm, P. and Koskela, J., (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. Aquacult., *183*: 349- 362.

Viola, S.; Arieli, Y. and Zohar, G. (1988). Animal – protein – free feed for hybrid tilapia *Oreochromis niloticus* x *O.aureus* in intensive culture. Aquacult., *75*: 115 – 125.

Wheeler, E. L. and Ferrel, R. E., (1971). A method for phytic acid determination in wheat and wheat fractions. American Association of Cereal Chemistry., *48*: 312 – 320.

Table (1) : Proximate analysis composition of diet – ingredients .

Ingredients	Fish meal	Full fat soybean	Soybean meal	Wheat bran[1]
Crude protein	69.20	38.10	48.00	12.70
Ether extract	9.94	20.50	1.77	2.13
Crude fiber	0.97	11.90	5.00	3.50
NFE [2]	7.49	22.14	34. 50	78.97
Ash	12.40	7. 36	6.73	2.70
Calcium %	2.40	0. 59	0. 50	0.13
Phosphorus%	2.00	0.71	1. 01	0.49

[1] Wheat bran by product less than 4% fiber.

[2]NFE, *nitrogen free extract* = 100 – (crude protein % + crude fiber % + ether extract % + ash %)

Table (2): Phytate phosphorus (P) content in the ingredients and the percentage of phytate phosphorus from total phosphorus compared with the results obtained by Ravindran *et al.* (1994).

Ingredients	Phytate (P) %	Total phosphorus[1]	Phytate (P) % of total phosphorus	hytate (P) % of total phosphorus by Ravindran
Soybean meal	0.44	1.01	62.56	60.00
Soybean full fat	0.45	0.71	71.00	-
Wheat bran [2]	0.25	0.49	65.77	71.00
Fish meal	-	2.00	-	-

[1] Total phosphorus determined on dry matter bases.

[2] Wheat bran by product less than 4% fiber.

Table (3): Formulation and chemical composition of the basal experimental diets.

	Experimental treatments				
Basal ingredient	Fish meal	Soybean full fat		Soybean meal	
Animal protein: Plant protein	100 : 0	50 : 50	25 : 75	50 : 50	25 : 75
Fish meal	29	18.5	10.3	15.5	9.0
Soybean full fat	-	19.0	30.7	-	-
Soybean meal	-	4.0	7.0	21.5	31.5
Wheat bran	68.5	56.0	48.5	59.5	55.5
Soybean oil	-	-	-	1.0	1.5
Premix (Vit. ,Min.&A.A.)	2.0	2.0	3.0	2.0	2.0
Cr_2O_3	0.5	0.5	0.5	0.5	0.5
Chemical composition (DM bases)					
Crude protein	29.8	29.7	29.4	30.1	30.1
Ether extract	4.34	7.00	8.46	4.19	4.14
Crude fiber	2.68	4.60	5.80	3.31	3.61
NFE [1]	55.00	50.53	48.07	55.70	56.17
Crude ash	8.18	8.17	8.27	6.70	5.98
Total phosphorus	0.887	0.787	0.662	0.748	0.650
Phytate phosphorus	0.264	0.302	0.318	0.297	0.320
Total calcium	0.787	0.656	0.529	0.559	0.447
Digestible energy (Kcal/kg)[2]	3387	3491	3542	3431	3452
Protein energy ratio(mg/kcal)[3]	87.98	85.06	83.05	87.73	87.20

[1]NFE, nitrogen free extract = 100 – (crude protein % + crude fiber % + ether extract % + ash %).

[2] Gross energy content was calculated using the values 5.65, 4.2 and 9.45 Kcal/ gm for protein, carbohydrate and lipid, respectively and applying the coefficient of 0.75 to convert gross energy to digestible energy according to Hepher *et al.*, (1983).

[3] Protein energy ratio (P/E ratio) = crude protein x 10000 / digestible energy , according to Hepher *et al.*, (1983).

Table (4): The growth performance and feed utilization of tilapia fed different experimental diets

Protein sources	AP:PP[1]	Source of supplementation	Initial body weight	Final body weight	Feed conversion ratio[2]	Specific growth rate[3]
Fish meal	100: 0 [4]	Control	12.25	16.39	2.660	0.502
		Dicalcium phosphate	12.21	15.50	2.740	0.500
		500 unit phytase	12.21	15.50	2.880	0.500
		1000 unit phytase	12.10	15.83	2.950	0.500
Soybean full fat	50 : 50	Control	12.44	14.35	6..000	0.270
		Dicalcium phosphate	11.87	16.22	2.340	0.626
		500 unit phytase	11.65	15.50	2.520	0.570
		1000 unit phytase	12.23	16.50	2.840	0.570
	25 : 75	Control	12.47	14.04	6.955	0.205
		Dicalcium phosphate	12.51	15.57	3.420	0.438
		500 unit phytase	11.50	13.91	3.860	0.377
		1000 unit phytase	11.68	14.30	4.120	0.365
Soybean meal	*50 :50*	Control	12.71	14.95	5.535	0.300
		Dicalcium phosphate	11.88	16.33	2.210	0.636
		500 unit phytase	11.57	15.66	1.960	0.595
		1000 unit phytase	12.62	17.40	2.480	0.593
	25 :75	Control	12.00	13.28	7.305	0.175
		Dicalcium phosphate	12.00	15.33	3.080	0.490
		500 unit phytase	11.82	14.28	3.820	0.399
		1000 unit phytase	12.33	16.15	3.170	0.461

1 AP:PP animal protein source : plant protein source.
2 Feed conversion ratio (%).
3 Specific growth rate (%).
4 Control diet unsupplemented with dicalcium phosphate or phytase.

Table (5): Effect of supplementation with dicalcium phosphate or phytase on growth performance of tilapia irrespective of AP: PP1 in the diet.

Experimental treatments				
Experimental Treatments	Initial body weight	Final body weight	Feed conversion ratio[2]	Specific growth rate[3] (% / day)
Control	12.37	14.64 b	5.69 a	0.29 b
Supplementation with dicalcium phosphate	12.09	15.79 a	2.76 b	0.54 a
Supplementation with 500 unit phytase	11.75	15.30 a	3.01 b	0.49 a
Supplementation with 1000 unit phytase	12.19	16.00 a	3.11 b	0.50 a
		P<0.001	P < 0.001	P < 0.001

1 AP: PP animal protein source: plant protein source
2 Feed conversion ratio (FCR) = feed intake/ weight gain
3 Specific growth rate (SGR) = 100 x [ln (final body wt) – ln (Initial body wt.)] x Day -1
-Values within column with different superscripts are significantly different (P<0.05).

Table (6): Effect of replacement of fish meal with soy full fat or soybean meal on growth performance of tilapia irrespective of dietary supplementation with dicalcium phosphate or phytase.

Experimental treatments				
AP:PP 1	Initial body weight	Final body weight	Feed conversion ratio[2]	Specific growth rate[3] (%/day)
FM : PP* (100 : 0)	12.19	15.81 a	2.807 c	0.448 a
FM : SBF ** (50: 50)	12.04	15.69a	3.426 bc	0.473 a
FM : SBF (75 : 25)	12.04	14.46 c	4.580 a	0.361 b
FM:SBM*** (50 :50)	12.19	16.09 a	3.046 bc	0.479 a
FM : SBM (25 : 75)	12.04	14.76 b	4.346 a	0.351 b
		P<0.01	P < 0.01	P < 0.01

* FM, fish meal. ** SBF, soybean full fat. ***SBM, soybean meal.
1AP: PP animal protein: plant protein source
2 Feed conversion ratio (FCR) = feed intake/ weight gain.
3 Specific growth rate (SGR) = 100 x [ln (final body wt) – ln (Initial body wt.)] x Day -1
- Values within column with different superscripts are significantly different (P<0.05).

Table(7) : Chemical composition (DM bases) calcium concentration of the hole body, calcium and zinein the vertebral column of tilapia fed different diets.

Protein sources	AP:PP[1]	Source of supplementation	DM(%)	Crude protein	Ether Extret	Crude ash	Ca (%)2	Ca (%)3	Zn (ppm)
Fish meal	100: 0	Control	26.66	68.7	19.75	10.7	5.59	5.88	83.64
		DCP	26.01	68.4	20.8	10.8	5.55	6.77	92.27
		500 unit phytase	26.8	68.6	19.8	10.7	5.55	6.77	83.6
		1000 unit phytase	27	68.5	20.6	10.8	5.8	6.8	83.6
Soybean full fat	50 : 50	Control	26.06	68	22	10	4.73	5.24	78.6
		DCP	25.84	68.3	19.8	11.9	4.96	7.25	100
		500 unit phytase	24.69	70.4	18.6	11.1	5.32	6.54	88.65
		1000 unit phytase	25.47	70.9	18.2	10.9	5.48	6.56	84.77
	25 : 75	Control	27.60	65.5	25.2	9.4	4.57	5.11	77.2
		DCP	24.90	67.4	20.9	11.7	6.17	6.91	85.8
		500 unit phytase	24.9	68.7	21.2	10.2	5.24	6.45	83.3
		1000 unit phytase	26.47	69.3	20.3	10.5	5.04	6.51	80.53
Soybean meal	50 :50	Control	26.00	68	21.7	10.3	4.89	5.23	74.64
		DCP	25.04	68.7	19.5	11.8	6.14	7.19	102.1
		500 unit phytase	25.46	68.6	20.7	10.6	5.36	6.58	84.83
		1000 unit phytase	25.95	70.8	18.7	10.5	4.81	6.68	75.01
	25 :75	Control	26.3	67.1	23.5	9.4	4.51	5.28	87.93
		DCP	24.1	67.8	20.4	11.8	6.1	7.1	83.31
		500 unit phytase	25.57	67.1	21.4	11.5	5.30	6.34	82.7
		1000 unit phytase	26.37	69.8	20	10.2	4.73	6.23	
SE±			0.55	1.01	2.11	0.65	0.54	1.15	0.84

* DCP, dicalcium phosphate

2 Ca(%) concentration in the whole body.

3 Ca(%) concentration in the vertebral column.

4 Control diet unsupplemented with dicalcium phosphate or phytase.

Table (8): Effect of supplementation with dicalcium phosphate or phytase on carcass chemical composition (on DM basis)of tilapia irrespective of AP:PP1 in the diet.

Experimental treatments							
Experimental Treatments	Crude protein	Ether extract	Crude ash	Gross energy (Kcal/kg)	Ca (%) 2	Zn (ppm)	Cu (%) 3
Control	67.42 b	22.46 a	9.96 c	5946.82a	4.86 c	77.79	5.35 c
Supplementation with dicalcium phosphate	68.11 a	20.79 b	11.10 a	5812.87b	5.99 a	93.61	7.04 a
Supplementation with 500 unit phytase	68.91 a	20.50 b	10.59 b	5830.67b	5.35 b	84.75	6.54 b
Supplementation with 1000 unit phytase	69.94a	19.53 b	10.53 b	5797.72c	5.17 b	83.00	6.57 b
	P <0.01	P <0.01	P <0.001	P<0.01	P <0.001		P <0.0(

[1]AP: PP animal protein: plant protein source

[2] Ca(%) concentration in the whole body.

[3] Ca(%) concentration in the vertebral column.

- Values within column with different superscripts are significantly different (P<0.05).

Table (9: Effect of replacement of fish meal by full fat soybean or soybean meal on carcass chemical composition of tilapia irrespective of supplementation with dicalcium phosphate or phytase .

AP:PP 1	Crude protein (%)	Ether extract (%)	Crude ash (%)	Gross energy (Kcal/ kg)	Ca (%) 2	Zn (ppm)	Cu (%) 3
FM : PP (100 : 0)*	69.51 a	19.73b	10.76 a	5791.8ab	5.62 a	84.87	6.57 a
FM : SBF (50: 50)**	69.41 ab	19.63 b	10.96 a	5776.7b	5.37 b	86.25	6.40 ab
FM : SBF (75 : 25)	67.74 b	21.84 a	10.42 b	5891.19a	5.25 b	81.13	6.25 b
FM:SBM (50 :50)***	69.03 ab	20. 16 b	10.81 a	5805.32ab	5.30 b	83.96	6.42 ab
FM : SBM (25 : 75)	68.25 b	21.30 a	10.45 b	5868.98a	5.17 b	81.44	6.23 b
	P <0.05	P<0.05	P <0.01	P<0.01	P <0.001		P <0.05

* FM, fish meal ** SBF, full fat soybean ***SBM, soybean meal

1AP: PP animal protein: plant protein source.

[2]Ca(%) concentration in the whole body.

3 Ca(%) concentration in the vertebral column.

- Values within column with different superscripts are significantly different (P<0.05).

Table (10): Estimation of phosphorus utilization by tilapia fed different diets.

	AP:PP[1]	Source of supplementation	(P) intake[3]	(P) excretion[4]	(P) in water[6]	(P) in serum[7]	ADC (%)	(P)[7] retention	(P)in body (%)	(P)in vertebral column (%)
Fish meal	100:0[2]	Control	23.0	3.75	0.187	31.00	59.35	5.3	2.65	3.13
		DCP	23.5	3.75	0.187	29.00	57.40	5.3	3.36	3.68
		500 unit phytase	23.5	3.75	0.187	29.50	57.40	5.3	3.36	3.68
		1000 unit phytase	25.5	3.75	0.187	28.70	58.25	5.3	3.34	3.43
Soybean full fat	50 : 50	Control	21.0	4.91	0.187	24.50	37.60	3.0	2.18	2.81
		DCP	34.7	3.57	0.179	38.25	70.30	8.4	3.31	3.35
		500 unit phytase	21.8	3.29	0.167	39.50	58.90	4.6	3.11	3.31
		1000 unit phytase	24.1	3.24	0.174	57.25	58.25	4.8	3.27	3.26
	25 : 75	Control	18.0	4.63	0.200	21.50	30.05	2.0	2.11	2.74
		DCP	36.0	5.07	0.196	37.15	58.50	7.2	3.40	3.24
		500 unit phytase	16.6	3.37	0.174	36.15	49.04	3.2	2.84	3.11
		1000 unit phytase	18.8	3.05	0.181	53.60	54.00	3.6	3.05	3.24
Soybean meal	50 :50	Control	20.9	4.40	0.196	26.00	41.15	3.1	2.24	2.81
		DCP	33.4	3.59	0.182	34.40	70.00	8.3	3.35	3.44
		500 unit phytase	19.2	2.36	0.171	39.60	68.32	5.1	2.91	3.12
		1000 unit phytase	23.6	2.48	0.163	63.75	66.80	5.1	2.95	3.33
	25 :75	Control	17.0	4.98	0.221	22.50	25.00	1.5	2.20	2.73
		DCP	34.7	4.84	0.217	32.50	59.50	7.1	3.40	3.37
		500 unit phytase	17.3	3.35	0.183	37.80	48.43	3.1	2.89	3.08
		1000 unit phytase	19.7	3.00	163	54.20	53.85	3.7	2.91	3.28

[1] AP:PP animal protein source : plant protein source.

[2] Control diet unsupplemented with dicalcium phosphate or phytase.

[3,4,5] mgP/gm diet.

[6] mgP/ml water.

[7] mgP/dl blood

Table (11): Effect of supplementation with dicalcium phosphate or phytase on phosphorus utilization by tilapia irrespective of AP:PP1 in the diet.

Experimental t treatment	(P) intake mg P/gm diet	(P) excretion (mgP/gm diet)	(P) accumulation in water (mgP/ml)	Inorganic (P) in serum (mgP/dl)	Apparent digestibility (%)	(P) retention (mgP/gm diet)	(P) in whole body(%)	(P) in vertebral column(%)
Control	19.98 b	4.53 a	0.198 a	25.10 c	38.63 c	3.00 c	2.27 c	2.84 c
Supplementation with dicalcium phosphate	32.46 a	4.16 a	0.192 a	34.26 b	63.14 a	7.30 a	3.36 a	3.42 a
Supplementation with 500 unit phytase	19.70 b	3.22 b	0.176 b	36.51 b	56.42 b	4.30 b	3.02 b	3.27 b
Supplementation with 1000 unit phytase	22.34 b	3.10 b	0.172 b	51.60 a	58.23 b	4.50 b	3.10 b	3.31 b
	P<0.05	P <0.001	P <0.001	P <0.001	P <0.001	P <0.001	P <0.001	P <0.001

[1]AP: PP animal protein: plant protein source

- Values within column with different superscripts are significantly different (P<0.05).

Table (12): Effect of replacement of fish meal by full fat soybean or soybean meal on phosphorus utilization of tilapia irrespective of supplementation with dicalcium phosphate or phytase.

Experimental treatments	(P) intake (mgP/gm diet)	(P) excretion (mgP/gm diet)	(P) accumulation in water (mgP/ml)	Inorganic (P) in serum (mgP/dl)	Apparent digestibility(%)	(P) retention (mgP/gm diet)	(P) in whole body(%)	(P)in vertebral column(%)
AP:PP 1								
FM : PP (100 : 0)*	23.8	3.75 b	0.187 b	29.55 b	58.10 a	5.30 a	3.29 a	3.36 a
FM : SBF (50: 50)**	25.4	3.75 b	0.177 b	39.88 a	56.26a	5.30 a	3.03 b	3.12 b
FM : SBF (75 : 25)	22.4	4.03 a	0.185b	37.10 a	47.90 b	4.00 b	2.92 c	3.00 c
FM:SBM (50 :50)***	24.3	3.21 c	0.178 b	40.94 a	61.57 a	5.40 a	3.00 bc	3.06 b
FM : SBM (25 : 75)	22.2	4.04 a	0.196 a	36.75 a	46.70 b	3.90 b	2.98 c	2.98 c
		P <0.001	P <0.05	P <0.001	P <0.01	P <0.001	P <0.001	P <0.001

* FM, fish meal ** SBF, soybean full fat ***SBM, soybean meal

[1]AP: PP animal protein: plant protei

- Values within column with different superscripts are significantly different (P<0.05).