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Alternative phosphorus sources for formulated fish feed

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Abstract

An experiment was conducted to evaluate the possibility of using inorganic fertilizer triple super phosphate (TSP), inorganic fertilizer 16:20 (a 16:20 grade fertilizer contains 16 percent N and 20 percent P_2O_5), rice-bran and duck-manure as phosphorus sources in formulated fish feed for Nile tilapia (*Oreochromis niloticus*). Experiment was conducted for a period of 2 months in net-cages suspended in fertilized earthen ponds and all male sex-reversed Nile tilapia (9.39 – 10.37 g) were used in the experiment. Seven treatments including one non-feed treatment were used in this experiment. Treatment 1 (non-feed), treatment 2 (-P) where fish fed with phosphorus non-supplemented diet acted as control 1 and treatment 3, 4, 5, 6 and 7 where fish fed with 3% di-calcium phosphate (DCP), 3% triple supper phosphate (TSP), 7% 16:20 inorganic fertilizer, 30% rice-bran and 30% duck-manure supplemented diet, respectively. Results showed that the TSP and 16:20 grade inorganic fertilizer supplementation in diets as phosphorus sources were equivalent to DCP (Di-calcium phosphate) supplementation in terms of growth performance, feed utilization efficiency and final body composition of Nile tilapia. Rice-bran and duck-manure were not found as good phosphorus sources.
Key words: Phosphorus, Di-calcium phosphate, Triple super phosphate, Duck-manure

Introduction

Phosphorus is an essential mineral required for normal life processes including growth, reproduction, health and bone mineralization of fish. Fish can absorb phosphorus at low rate from water through gills (Nose and Arai 1979, Mullins 1950 and Phillips 1953 *et al.* cited in Heper 1984) but not sufficiently to satisfy their phosphorus requirements. Because phosphorus concentration is very low in both freshwater and seawater with a range of 0.005 – 0.05 ppm (Kim and Oh 1985). Even in ponds fertilized with phosphate, the concentration of phosphorus does not increase much for long period above its normal low level due to adsorption to soil colloids and precipitation as insoluble compounds (Hepher 1984). Therefore, the diet remains the main source of phosphorus for fish. Diet deficient in phosphorus can suppress the appetite, normal food conversion ratio and growth, under extreme circumstances affect bone formation and lead to death of fish (Lall 1979).

Increased demand of fish due to rapid growth of population has been forced to the process of intensification of fish culture in many Asian countries using supplementary diets which may be deficient in phosphorus or contain in an unavailable form to fish. Then addition of phosphorus in supplementary diets may be more appropriate to obtain better growth of fish. Pure di-calcium phosphate and tri-calcium phosphate are rich source of phosphorus which have been found to be supplemented in the fish diet as phosphorus sources. But unfortunately, these ingredients are much expensive and sometimes unavailable to rural fish farmers. Thus lack of cheap phosphorus sources become one of the major limiting factor for cost effective small scale feed manufacturing and intensification of fish culture in most resource poor regions in Asia. To overcome this problem, inorganic fertilizer such as triple super phosphate, 16:20, rice-bran and duck-manure which are good source of phosphate could be used as alternative phosphorus sources in fish diet.

Therefore, the study reported herein was designed to evaluate the possibility of using inorganic fertilizer TSP, inorganic fertilizer 16:20 (a 16:20 grade fertilizer contains 16 percent N and 20 percent P_2O_5), rice-bran and duck-manure as phosphorus sources in the formulated feed for Nile tilapia (*Oreochromis niloticus).*

Materials and methods

The experiment was conducted for a period of 2 months from February to April'95 in 21 net-cages each measuring 2mx2mx1m suspended in three fertilized earthen ponds each measuring 200 m^2 at the campus of Asian Institute of Technology, Bangkok, Thailand. Seven treatments including one non-feed treatment were used in this experiment. Treatment 1 (non-feed) acted as control 2 and Treatment 2 (-P) where fish fed with phosphorus non-supplemented diet acted as control 1. In treatment 3 (+DCP), 4 (+TSP), 5 (+16:20), 6 (+RB) and 7 (+DM) fish were fed with 3% DCP, 3% TSP, 7% inorganic fertilizer 16:20, 30% rice-bran and 30% duck-manure supplemented diets, respectively. Six isonitrogenous and isocaloric experimental diets were formulated and prepared with DCP, TSP, 16:20, rice-bran and duck-manure supplementation as phosphorus sources. Diet 1, 2, 3, 4, 5, and 6 were fed to fish in treatment 2 (-P), 3 (+DCP), 4 (+TSP), 5 (+16:20), 6 (+RB) and 7 (+DM), respectively. Composition, nutrient and energy content of diets used in the experiment are given in Table 1.

All sex reversed male Nile tilapia were used in the experiment and stocked with 25 fish per net-cage. The initial and the final individual length and weight of fishes were measured and recorded. For the analysis of initial carcass proximate composition and bone phosphorus thirty fishes were sacrificed and for the analysis of final carcass proximate composition and bone phosphorus fifteen fishes per replicate were also sacrificed at the end of the experiment. Earthen ponds were fertilized one week before stocking of fish and after that regularly weekly basis with inorganic fertilizer at the rate of 4 kg Urea-N/h/day and 2 kg TSP-P/h/day. In the experiment fish were fed at the rate of 3% body weight (dry feed/wet fish weight) and feeds were given twice daily in the feeding trays suspended in water column. The ration was adjusted bi-weekly intervals according to batch weight after every sampling. Water samples were taken at weekly and bi-weekly intervals and analyzed for assessing temperature, dissolved oxygen, pH, ammonia, nitrogen, total alkalinity, total phosphate, nitrite, total suspended solid (TSS), chlorophyll-*a*, phaeophytin-*a* and plankton biomass.

Table 1. Composition, nutrient and energy content of experimental diets (g/100g dry weight basis)

Ingredients	Diets					
	1	2	3	4	5	6
Soybean meal	60	60	60	60	55	55
Fish meal	5	5	5	5	5	5
Cassava starch	29	26	26	22	4	4
Corn oil	4	4	4	4	4	4
Vitamin premix	2	2	2	2	2	2
DCP[1]	0	3	0	0	0	0
TSP[2]	0	0	3	0	0	0
16:20[3]	0	0	0	7	0	0
Rice-bran	0	0	0	0	30	0
Duck-manure	0	0	0	0	0	30
Total	100	100	100	100	100	100
Proximate composition						
% Dry mater	91.01	89.82	90.12	89.63	89.63	88.88
% Protein	31.52	31.83	32.44	31.66	33.09	32.83
% Lipid	5.02	4.97	5.11	5.07	8.57	4.96
% Crude fiber	4.99	7.89	8.53	6.62	8.81	9.36
% Ash	5.78	8.65	8.03	8.68	11.24	10.24
% NFE	52.69	46.65	45.89	47.97	38.29	42.69
% Phosphorus	0.57	1.14	1.18	1.15	1.09	0.94
Gross energy (kj/g)	18.68	17.65	17.74	17.91	17.46	17.25
P.E ratio (mg/kj)	16.87	18.01	18.28	17.67	18.95	19.03

[1] DCP = Di-calcium phosphate [2] TSP = Triple super phosphate

[3] 16:20= A 16: 20 grade fertilizer contains 16 percent N and 20 percent P_2O_5

To evaluate the fish growth performances mean length and weight, daily weight gain, relative growth rate indices were used, and to assess feed utilization efficiency, apparent food conversion ratio (AFCR), true food conversion ratio (TFCR), apparent protein efficiency ratio (APER), true protein efficiency ratio (TPER), apparent protein conversion ratio (APCR) and true protein conversion ratio (TPCR) were used in the experiment. The formula for AFCR, TFCR, APER, TPER, APCR and TPCR are given below:

$$\text{AFCR} = \frac{\text{Total dry feed fed}}{W_2 - W_1}$$

Where W_2= Mean final weight of fish
W_1= Mean Initial weight of fish

$$TFCR = \frac{\text{Total dry feed fed}}{(W_2 - W_1) - (N_2 - N_1)}$$

Where N_2= Mean final weight of non-feed fish
N_1= Mean Initial weight of non-feed fish

$$APER = \frac{W_2 - W_1}{\text{Total protein intake}}$$

$$TPER = \frac{(W_2 - W_1) - (N_2 - N_1)}{\text{Total protein intake}}$$

$$APCR = \frac{PF_2 - PF_1}{\text{Total protein intake}}$$

Where PF_2= Mean final carcass protein of fish
PF_1= Mean Initial carcass protein of fish

$$TPCR = \frac{(PF_2 - PF_1) - (PN_2 - PN_1)}{\text{Total protein intake}}$$

Where PF_2= Mean final carcass protein of non-feed fish
PF_1= Mean Initial carcass protein of non-feed fish

Proximate composition of ingredients, diets and fish carcass were analyzed according to the analytical methods of AOAC (1984) and phosphorous were analyzed by molybdate–vandate and spectrophotometric (420nm) method.

The multi-factor analysis of variance (ANOVA) and paired t-test at 95% confidence level ($P<0.05$) was used for statistical analysis of the experimental data.

Results

Growth performance of fishes in different experimental treatments were evaluated and compared their treatment mean of final weight, length, daily weight gain (DWG) and relative growth rate (RGR). The results of growth performance are given in Table 2.

Table 2. The mean growth performance of fish (± S.E)

Growth indices	Treatment						
	1 (No-feed)	2 (-P)	3 (+DCP)	4 (+TSP)	5 (+16:20)	6 (+RB)	7 (+DM)
Initial Length (cm)	8.06 ± 0.04	8.18 ± 0.08	8.21 ± 0.10	8.21 ± 0.14	8.13 ± 0.09	8.21 ± 0.03	8.36 ± 0.09
Initial Weight (g)	10.00 ± 0.36	10.04 ± 0.20	10.02 ± 0.20	10.21 ± 0.30	9.81 ± 0.24	10.06 ± 0.14	10.37 ± 0.11
Final Length (cm)	15.60^{c} ± 0.69	17.57^{b} ± 0.21	18.56^{a} ± 0.60	17.56^{a} ± 0.22	18.22^{a} ± 0.44	18.86^{b} ± 0.5	16.77^{b} ± 0.48
Final weight (g)	60.17^{c} ± 5.33	84.50^{b} ± 2.54	106.90^{a} ± 7.47	102.7^{a} ± 3.94	100.9^{a} ± 5.45	86.69^{b} ± 4.01	85.92^{b} ± 3.87
DWG[1] (g/day)	0.80^{c} ± 0.09	1.18^{b} ± 0.04	1.54^{a} ± 0.12	1.47^{a} ± 0.07	1.44^{a} ± 0.08	1.22^{b} ± 0.06	1.20^{b} ± 0.06
RGR[2] (mg/g/d)	79.53^{c} ± 10.09	116.8^{b} ± 1.48	153.6^{a} ± 11.56	144.4^{a} ± 10.69	147.1^{a} ± 8.88	121.1^{b} ± 7.75	118.5^{b} ± 7.49
% Survival rate	98	99	99	96	99	95	96

[1] DWG = Daily weight gain,

[2] RGR (Relative growth rate)= $\frac{\text{Mean final weight} - \text{Mean initial weight}}{\text{Mean initial weight x days}} \times 1000$

** Letters in superscript denotes significance level (a= P<0.05, b=P>0.05 and c=P<0.01) when tested in ANOVA and compared by paired t-test against control (treatment 2-P).

The supplementation of phosphorus with DCP, TSP, 16:20, rice-bran and duck-manure in formulated diets for Nile tilapia showed varied growth response. Significant differences in mean final weight and length of fish were observed both among the treatments (P<0.01) and blocks (P<0.001). The growth performance in treatment 3 (+DCP), 4 (+TSP) and 5 (+16:20) where fish fed with DCP, TSP and 16:20 supplemented diet respectively were homogenous and significantly (P<0.01) different from all other treatments. The growth performance in treatment 6 (+RB) and 7 (+DM) where fish fed with rice-bran and duck-manure supplemented diet respectively were also homogenous and not significant. The growth performance in treatment 1 (non-fed) where fish fed with no supplementary feed was significantly lower than all other diet-fed treatments.

The mean weight of fishes in all treatment at bi-weekly growth monitoring are graphically represented in Fig. 1.

In the experiment no significant differences were found in feed utilization indices against experimental treatments. The results of feed utilization indices in different treatments are given in Table 3.

The final fish carcass in different treatments reflected the significance alteration in proximate composition. The initial and final mean proximate compositions of fish carcass are given in Table 4.

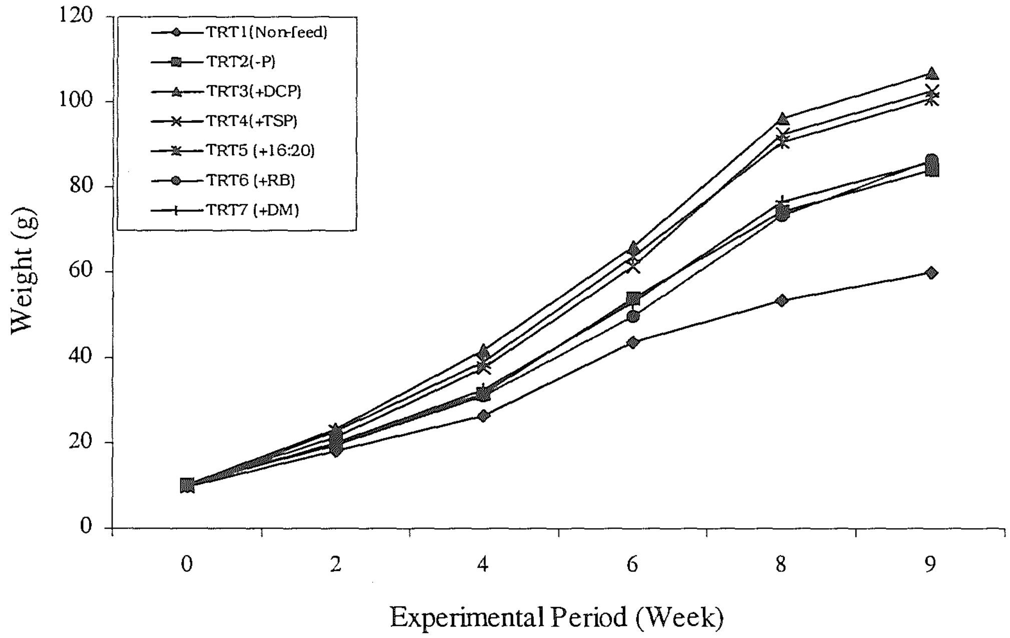

Fig. 1. Mean weight of fish at bi-weekly growth monitoring in different treatment during the experimental period.

Table 3. The mean feed utilization indices (± S.E)

Indices	Treatment					
	2	3	4	5	6	7
AFCR[1]	0.88 ±0.02	0.83 ±0.01	0.81 ±0.03	0.80 ±0.04	0.83 ±0.06	0.86 ±0.01
TFCR[2]	1.88 ±0.02	1.43 ±0.09	1.46 ±0.07	1.45 ±0.13	1.77 ±0.18	1.85 ±0.11
APER[3]	3.62 ±0.09	3.79 ±0.06	3.39 ±0.12	3.96 ±0.21	3.69 ±0.27	3.54 ±0.05
TPER[4]	1.66 ±0.04	2.21 ±0.14	2.14 ±0.08	1.89 ±0.14	1.75 ±0.19	1.66 ±0.01
APCR[5]	24.01 ±0.80	26.43 ±2.41	20.04 ±2.16	26.99 ±1.81	24.37 ±1.97	21.10 ±2.65
TPCR[6]	12.70 ±0.84	17.91 ±2.25	10.51 ±1.58	16.55 ±1.01	13.21 ±1.86	10.25 ±3.38

[1]AFCR = Apparent food conversion ratio [2]TFCR = True food conversion ratio
[3]APER = Apparent protein efficiency ratio [4]TPER = True protein efficiency ratio
[5]APCR = Apparent protein conversion ratio[6]TPCR = True protein conversion ratio

Table 4. The initial and final mean proximate composition of fish carcass (± S.E)

composition	Initial	Final in treatments						
		1	2	3	4	5	6	7
% Moisture	78.67 ±1.13	79.39 ±0.28	75.83 ±0.46	74.77 ±1.13	75.56 ±1.81	76.10 ±0.83	76.78 ±0.85	76.24 ±0.50
% protein	60.28 ±0.55	62.61 ±0.22	65.23 ±0.33	67.12 ±0.49	65.18 ±0.59	66.50 ±0.56	65.33 ±0.28	64.80 ±0.62
% Lipid	9.56 ±0.42	9.60 ±0.27	16.12 ±0.51	15.48 ±0.28	15.22 ±0.40	14.51 ±0.30	14.37 ±0.33	12.20 ±0.51
% Ash	17.51 ±0.62	21.55 ±0.60	15.45 ±0.37	16.66 ±0.58	16.81 ±0.67	19.26 ±0.36	17.38 ±0.55	19.98 ±0.26

Discussion

In the present study phosphorus were supplemented in experimental diets with 3% di-calcium phosphate (DCP), 3% triple super phosphate (TSP), 7% inorganic fertilizer 16:20, 30% rice-bran and 30% duck-manure. Phosphorus supplemented diets with these supplements contained total phosphorus ranged from 0.94-1.18% of diet was enough to fulfil the absolute phosphorus requirements of Nile tilapia for normal growth and bone mineralization and showed higher growth performance. On the other hand, the diet without phosphorus supplementation containing (phosphorus) 0.56% (supplied by dietary ingredients alone) was not enough to satisfy phosphorus requirements of Nile tilapia. It indicates that soybean meal based diet alone cannot satisfy all phosphorus requirements of Nile tilapia as it may be deficient in phosphorus or contains in an unavailable form to Nile tilapia. So, in this circumstances phosphorus supplementation in diets was imperative. In the present study higher growth of tilapia was found with the supplemention of 3% DCP, 3% TSP, and 7% inorganic fertilizer 16:20 in diets as phosphorus sources. Viola (1986) and Hung (1989) also found the similar results where ration with soybean meal required 3% di-calcium phosphate for fast growth of tilapia.

Phosphorus content in DCP, TSP, 16:20, rice-bran and duck-manure supplemented diets were similar (0.094-1.18%), even then varied growth response and feed utilization efficiency were observed. It indicates that the digestibility and bio-availability of phosphorus from DCP, TSP, 16:20, rice-bran and duck-manure were not equal to Nile tilapia. Bio-availability of dietary phosphorus from supplements is influenced by several factors such as chemical form, digestibility of diet, particle size, solubility and interaction with other nutrient (Heper 1984). Final carcass phosphorus data showed that Nile tilapia can utilize DCP, TSP, 16:20, rice-bran and duck-manure phosphorus around 79, 38, 48, 59 and 41% respectively and the rest of the phosphorus are released to the aquatic environment which may be adsorbed to soil colloid and precipitated as insoluble compound. Higher and significant growth performance and feed utilization efficiency were obtained in DCP, TSP and 16:20 supplemented diets. Supplementation of rice-bran and duck-manure in diets as phosphorus sources did not improve growth and feed utilization efficiency of Nile tilapia compared to phosphorus non-supplementation in diet. It indicates that the bio-availability of phosphorus from DCP, TSP and 16:20 were higher than rice-bran and duck-manure to Nile tilapia. Phosphorus content of rice-bran mostly exists organically bound as phytate. This phytate is hard to be assimilated by simple stomached fish like Nile tilapia due to lack of enzyme phytase in the gastrointestinal tract (Lall 1979). On the other hand, phosphorus content of duck-manure was already indigestible to duck and was also hard to digest by Nile tilapia. Perhaps these were the possible causes of poor availability of rice-bran and duck-manure phosphorus to Nile tilapia.

Feed utilization data showed non-significant differences in apparent food conversion ratio (AFCR) and significant differences in true food conversion ratio (TFCR) among the treatments. Lower and similar TFCR found in DCP, TSP and 16:20 supplemented diets. Apparent food conversion ratio takes into account both natural food and supplementary feed, while true food conversion ratio takes into account supplementary feed alone with reference to non-fed fish. As a result higher TFCR (1.43-1.88) compared to AFCR (0.80-0.88) was observed in different experimental diets. Moreover, the plant based experimental diets caused lower digestibility. This could have lead to high true feed conversion ratio. Higher protein efficiency ratio was resulted in diets supplemented with DCP, TSP and 16:20 than rice-bran and duck-manure and phosphorus non-supplemented diets. It suggests that fish fed with DCP, TSP and 16:20 supplemented diets were more efficient in converting protein to weight gain. Protein efficiency ratio in DCP, TSP and 16:20 supplemented diets were analogous to each other. The differences in apparent and true protein conversion ratio among treatments were not significant, like wise protein level in final fish body in all treatments were not significant.

The final fish carcass in different treatments reflected the significant alteration in proximate composition. The highest body protein level was in fish fed DCP supplemented diet and the lowest body protein level was in phosphorus non-supplemented diet. Body protein and lipid content of fish in DCP, TSP and 16:20 supplemented diets were similar. The highest lipid content was in phosphorus non-supplemented diet. Hung (1989) and Wee and Shu (1989) found that supplementation of phosphorus in the diet causes decrease in the lipid content of the muscle and viscera and increase in muscle protein of fishes. Phosphorus supplementation in diets influenced the carcass and bone ash content and phosphorus level of fishes. The lowest ash content and phosphorus level in body carcass and vertebrae bone was found in fishes fed with phosphorus non-supplemented diet. Higher and coinciding ash content and phosphorus level in carcass and bone were found in fish fed with DCP, TSP and 16:20 supplemented diets. It indicates that there was a positive relationship between dietary phosphorus level and ash content in carcass and bone as well as phosphorus level in carcass and bone. Similar results were also found by Robinson *et al.* (1987) and Haylor *et al.* (1988). Analysis of body ash content, carcass and bone phosphorus level of non-fed fish suggests that natural food can meet the phosphorus requirements of Nile tilapia to support slow growth rate. Phosphorus supplementation also affected the phosphorus deposition in fish body carcass. The lowest phosphorus deposition was in fish fed with phosphorus non-supplemented diet. Higher and alike body phosphorus deposition observed in fish fed with DCP, TSP and 16:20 supplemented diets. It also indicates that phosphorus availability from DCP, TSP and 16:20 to tilapia were significant.

Natural food organisms, species composition and water quality parameters could have contributed to the variable response of fish in treatment replicates assigned to different ponds, making the effects of blocking significantly different in all growth indices. Treatment replicates allocated in pond with high abundance of natural food and better water quality showed better results compared to replicates allocated in pond with low abundance of natural food. Plankton bio-mass and chlorophyll-*a* level varied among the ponds and were found increasing during the experimental period that could have contributed to growth variation of fish between ponds. Variation in other water quality parameters such as alkalinity, total suspended solid, ammonia-nitrogen and total phosphate also could have contributed to growth variation of fish between ponds. Temperature, dissolved oxygen and pH level in different ponds were almost uniform, of course a little fluctuation in pH and dissolved oxygen were observed. Ammonia levels in all ponds increased with time. Indigestible portion of experimental diet was excreted by fish as faeces. Accumulation of faeces resulted in increased levels of ammonia.

Conclusions

In view of the above study it may be concluded that, phosphorus supplementation in soybean-meal based diets with 3% DCP, 3% TSP and 7% 16:20 grade inorganic fertilizer showed better growth performance and feed utilization efficiency of Nile tilapia. Growth performance and feed utilization efficiency of Nile tilapia fed with di-calcium phosphate (DCP), triple super phosphate (TSP) and a 16:20 grade fertilizer containing diets were comparable. Rice-bran and duck-manure inclusion in feeds as phosphorus sources did not improve growth of Nile tilapia. If DCP is not available in rural areas in Asia, triple super phosphate (TSP) and 16:20 grade inorganic fertilizer could be incorporated in formulated fish feed as alternative phosphorus sources.

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