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Evaluation of phytases of three *Bacillus* spp. in the diet of sex-reversed *Oreochromis mossambicus* fingerlings on growth, feed efficiency and mineral deposition

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ABSTRACT

This study aims to evaluate dietary phytases from three Bacillus spp. for Oreochromis mossambicus on growth, feed utilization and nutrient deposition. Plant-based diets were supplemented with 500 FTU kg^{-1} of B. pumilus, B. megaterium and B. licheniformis phytases (Bpum, Bmeg and Blic, respectively) while diet without supplementation and the commercial diet (NoP and ComD, respectively) served as negative and positive controls, respectively. Bmeg, Blic and Bpum diets did not have marked effect on growth performance. Carcass ash was significantly the highest in the Bmeg and Blic groups while carcass P and Ca were significantly increased by all supplemented diets. Scale ash was increased significantly by both the Bmeg and Blic diets while scale P and Ca by all three supplemented diets. Bone P and Mg were significantly the highest in Bmeg and Blic diet groups; bone Ca were highest in the Bmeg group. P retention was significantly increased in the three supplemented diets. P load were significantly the lowest in all supplemented diets. All the supplemented diets were effective in significantly reducing fecal P. In conclusion, the Bmeg and Blic phytases were most effective in hydrolyzing phytate P and in ameliorating water quality.

Key words: Bacillus, phytases, Oreochromis mossambicus, mineral deposition, growth

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INTRODUCTION

Most scientific work on phytases have been done employing microbes as sources, specifically those from filamentous fungi [1]. Some plants such as wheat and barley contain an endogenous phytase but because of their narrower pH spectrum of activity, the enzyme is less effective than microbial phytases [2]. Bacteria are also a source of phytase and *Bacillus subtilis* is the most studied species in this respect. We have previously compared the biochemical characteristics of four other *Bacilli* phytases, namely *B. pumilus*, B. *megaterium*, B. *coagulans*, and B. *licheniformis* [3]. The crude phytases were optimally active between pH 5.5 and 7.0 at 37^{o}C, with high activity retention at temperatures up to 80^{o}C, and with remarkably high thermo- and pH stability. These properties indicated that the *Bacillus* phytases appear to be suitable for animal feed supplementation in aquaculture to improve the bioavailability of phosphorus.

Bacterial phytases from *Bacillus* phytases are an alternative to fungal enzymes because of their high thermal stability, calcium-phytate complex substrate specificity, pH profile, and proteolytic resistance [4; 5; 6]. Unlike the *Aspergillus* phytases, *Bacillus* phytases are specific for phytate. Thus, non-phytate phosphate compounds remain available for animal uptake [7]. In addition, the phytases from *Bacillus* are suitable as feed additives for animals with neutral digestive tracts, such as some aquatic species.

P is an essential nutrient for growth, skeletal development [8] and reproduction [9] in fish. Phosphate uptake from water is negligible in fish and dietary P are more important than water to satisfy P requirement. Also, P is a critical pollutant in bodies of water. Excessive P levels are the most common cause of eutrophication of rivers, lakes and reservoirs [10]. Researches on incorporating microbial phytases in fish diets are driven by the need to reduce P excretion and its loss into the environment, where P pollution threatens water quality.

Microbial phytase hydrolyses the phytate-mineral complex and increases the availability of the minerals [11; 12; 13] leading to increased mineral utilization in the body. Adeola *et al.* [14] and Hauler and Carter [15] report that addition of microbial phytase to diets increases phytate hydrolysis and availability of P and other minerals that may be chelated by phytic acid. Supplementation of complete diets with phytase has generally enhanced P utilization in rainbow trout *O. mykiss* [16], common carp *Cyprinus carpio* [17] and channel catfish *Ictalurus punctatus* [18; 19]. Furuya *et al.* [20] have observed that phytase supplementation in Nile tilapia diets between 500 and 1500 FTU kg^{-1} improves Ca and P availability, growth performance, bone mineralization and protein digestibility. Phytase supplementation of 1000 FTU kg^{-1} results in growth rates and mineral utilization similar to a plant-based diet supplemented with P_i [21].

The amount of inorganic phosphorus (P_i) released from phytate depends on many factors like phytate source and its solubility, type of phytase and phytase activity as well as physiological conditions in the gut of different fish species. Knowledge of the effects of different sources of bacterial phytase in fish is lacking. The present study aims to compare the effects of phytases from various *Bacillus* species on growth, feed efficiency and deposition of minerals in *Tilapia mossambica*. To our knowledge, this is the first time that comparison of efficacy of phytases from three *Bacillus* species was done in fish.

MATERIALS AND METHODS

Experimental diet

The experimental diets used contained common feed ingredients from the locality as recommended for tilapia [22; 23] with some modification (Table 1). Prior to formulation, all feed ingredients were analyzed for proximate composition as well as P, Ca and Mg content. Pure isolates of the 4 strains of *Bacillus* spp. were sub-cultured and their phytases were assayed and prepared as described by Dechavez *et al.* [3]. *Bacillus* phytase in solution was sprayed just before feeding at 500 FTU kg^{-1} diet. One unit of enzyme activity (FTU) was defined as the amount of enzyme hydrolyzing 1 µmol of P_i min^{-1} under the assay conditions.

Four plant-based diets of the same composition were formulated, three of which were incorporated with 500 units of phytase (FTU) from *Bacillus pumilus* (Bpum), *Bacillus megaterium* (Bmeg) and *Bacillus licheniformis* (Blic) while a diet without bacterial phytase (NoP) and a commercial feed (ComD; PRIZE CATCH, San Miguel Corp., Iloilo, PHL) served as a negative control and positive control diets, respectively.

Table 1. Formulation and proximate compositions of plant-based and commercial diets for the sex-reversed *Oreochromis mossambicus* (g kg^{-1} DM)

Ingredient	NoP	Bpum	Bmeg	Blic	ComD[1]
Fish meal (Peruvian meal)	150.0	150.0	150.0	150.0	-
Soybean meal	410.9	410.9	410.9	410.9	-
Corn meal	349.1	349.1	349.1	349.1	-
Cassava leaf meal	50.0	50.0	50.0	50.0	-
Cassava starch (binder)	50.0	50.0	50.0	50.0	-
Cod liver oil	20.0	20.0	20.0	20.0	-
Vitamins-mineral mix [2]	20	20	20	20	-
Microbial phytase (FTU kg^{-1})					
Bacillus pumilus	-	500	-	-	-
Bacillus megaterium	-	-	500	-	-
Bacillus licheniformis	-	-	-	500	-
Proximate Composition (analyzed)					
Dry matter	956.6	956.6	956.6	956.6	943.7
Crude Protein	352.4	352.4	352.4	352.4	336.6
Crude Fat	41.1	41.1	41.1	41.1	47.1
Crude fiber	27.6	27.6	27.6	27.6	39.4
Ash	33.3	33.3	33.3	33.3	36.5
Nitrogen Free Extract	602.6	602.6	602.6	602.6	540.4
ME (kJ g^{-1})[3]	16.7	16.7	16.7	16.7	16.6
Phosphorus	3.0	3.8	3.5	3.9	5.4
Calcium	9.0	7.1	7.2	7.7	6.3
Magnesium	2.9	5.3	6.0	4.7	7.6

[1] *PRIZE CATCH floating feeds (B-Meg, San Miguel Foods, Inc., Iloilo, Philippines)*
[2]*Vitamin-mineral premix (IU or mg kg^{-1} diet): Vitamin A, 1.11 mg; Vitamin D, 0.44 mg; Vitamin E, 2,222 IU; Vitamin K, 889 IU; Riboflavin, 110 mg; Niacin, 890 mg; Vitamin B_{12}, 2.67 mg; Biotin, 4.44 mg; Folic acid, 66.7 mg; thiamine hydrochloride, 400 mg; Pyridoxine hydrochloride, 111.1 mg; Calcium pantothenate, 120 mg; Mn, 2,170 mg; I, 33 mg; Co, 18 mg; Fe, 890 mg; Cu, 66.7 mg; Zn, 890 mg*
[3]*Metabolizable energy (ME) was computed using the following energy values: 17 kJ.g^{-1} protein and nitrogen-free extract; 37.6 kJ.g^{-1} fat.*

Experimental fish and conditions

The experimental set up consisted of 15 circular concrete tanks (500-L capacity) in a flow- through system provided with sufficient aeration. Physico–chemical parameters like dissolved oxygen, pH, and temperature were monitored three times a week, while ammonia and P of the water were measured weekly. Standard methods for water analysis were as described by Strickland and Parsons [24].

Three hundred seventy five (375) fingerlings of sex reversed tilapia *O. mossambicus* (3.6 g ABW) were acclimatized for two weeks to the diet and experimental conditions. Prior to stocking, similar-sized fish were gathered and randomly divided into 15 tanks. Each experimental diet was fed to triplicate tanks for 60 days. Fish were fed twice daily (0900 h and 1600 h) at a daily feeding rate of 5% body weight adjusted accordingly following sampling.

Collection of samples and analysis

At the start of the experiment, 10% of the total fish were sacrificed for body composition analysis which included moisture, ash, crude protein and crude fat. Sampling of scales was done by removing them from both lateral surface of the fish and dried in an oven for 2 h at 110^{o}C. Fish were steamed for about 15 min until the vertebrae could be excised, then defatted in ethyl alcohol for one week, oven dried for 3 h at 110^{o}C and ground for mineral analysis. Scale, bone and fish carcass were separately pooled by treatment, ashed and pulverized for P, Ca and Mg analyses.

Fecal P was determined before the growth trial by collecting fecal samples daily for one week, dried for 24 h at 110^{o}C. P was determined according to the method by [25] and [26].

Analytical procedure

Chemical analyses of ingredients, diets and fish were done in three replicates. Moisture content was measured by drying in an oven at 110^{0} C to constant weight [27]; crude ash was analyzed by combusting the sample at 550^{0} C for 4 h; N was determined and translated to crude protein content by multiplying by 6.25. Crude fat was determined using Soxhlet extraction with petrol ether. Chromium analysis was determined using an acid digestion method [28]. Metabolizable energy (ME) was estimated using physiological fuel values of 17.0 kJ g^{-1} protein or NFE and 37.6 kJ g^{-1} lipid.

Ca and Mg in fish body, scales and vertebral bone and formulated feeds were analyzed using flame atomic absorption spectrometer (SpectrAA 55B, Varian Australia Pty. Ltd., Victoria, Australia) after wet ashing and acid digestion. P content in the fish carcass, scales, bones, feces and feeds were determined in three replicates using the ammonium-molybdate method [25; 26; 27]; the optical density of the solution due to ammonium-molybdophosphorus complexes was read at 430 nm.

Calculations and statistical analysis

A number of biological parameters were computed as follows:

Weight gain, WG (g) = (ABW_f, g) – (ABW_i, g)

Specific Growth Rate (SGR, unit d^{-1}) = 100 * [(ln Wf)) - (ln Wi)]*(t^{-1})

Protein efficiency ratio (PER) = [(wet WG (g) x (crude protein fed (g)$^{-1}$]

Feed conversion efficiency (FCE) = [(dry weight diet, g) x (wet wt. gain, g)$^{-1}$]

Protein gained, PG (g kg^{-1}) = [(ABW_f x CP)-(ABW_i x CP)]*[Crude protein fed, kg]$^{-1}$

Nutrient retention (g kg^{-1}) = [(ABW_f x N_f)–(ABW_i x N_i) x (feed intake, kg) x N_{diet})$^{-1}$

Nutrient load = [(Nutrient fed, g) – (nutrient deposited, g)]*[WG (kg)]$^{-1}$

Where ABWf and ABWi are final and initial average body weights, N_f and N_i are final and initial concentration of the nutrient in question in fish or diet.

Data were analyzed using one-way analysis of variance (ANOVA), followed by Tukey's test if there are significant differences, for the means in WG, SGR, FCE , PER, protein retention (%) and mineral contents in final body, scales, bone and vertebra of sex reversed *O. mossambicus* [29]. Data were tested for homogeneity of variance and normality of data prior to ANOVA. Results were considered significant at 5% level of significance ($P < 0.05$).

RESULTS

There were no significant differences in the initial and final ABW, feed intake, weight gain, specific growth rate, survival rate and protein gained of tilapia fed the experimental diets (Table 2). Highest feed utilization efficiencies (FCE and PER) were recorded in the Bmeg group but were not significantly different from the Blich and ComD groups. Lowest was recorded in the NoP group which did not vary significantly from the Bpum group. The data (Table 2) showed that adding *B. megaterium* phytase to the plant-based diet significantly increase the FCE and PER by 10.6 and 10.4%, respectively.

Table 2. Growth performance and feed utilization of sex reversed *O. mossambicus* fingerlings fed commercial diet, plant-based diet alone or supplemented singly with a *Bacillus* phytase for 60 days

Diets	ABW_i (g) [1]	ABW_f (g) [2]	FI (g) [3]	WG (g) [4]	SGR (% d^{-1}) [5]	SR (%)[6]	FCE[7]	PER[8]	PG (g kg^{-1}) [9]
NoP	3.50 ± 0.36	24.20 ± 0.87	29.5±1.9	20.70 ± 0.91	3.2 ± 0.8	96.0±6.9	80.9 ± 1.3^{b}	2.30 ± 0.12^{b}	485.8±1.3
Bpum	3.50 ± 0.57	26.48 ± 1.08	27.3±4.0	22.92 ± 0.98	3.3 ± 0.4	93.3±4.6	82.1±1.0^{b}	2.33 ± 0.11^{b}	494.2±1.6
Bmeg	3.56 ± 0.16	26.51 ± 0.93	27.6±0.8	22.95 ± 0.75	3.4 ± 1.5	98.6±2.3	89.5 ± 1.0^{a}	2.54 ± 0.05^{a}	528.4±2.2
Blich	3.61 ± 0.36	25.47 ± 1.23	28.4±2.5	21.81 ± 0.90	3.3 ± 1.0	98.7± 4.0	82.7± 1.0^{ab}	2.35 ± 0.14^{ab}	489.0±2.3
ComD	3.56 ± 0.13	26.49 ± 0.43	29.5±0.8	22.93 ± 0.32	3.4 ± 0.5	100 ±0.00	83.3 ± 1.0^{ab}	2.36 ± 0.06^{ab}	471.0±1.7
P	NS	NS	NS	NS	NS	NS	P<0.05	P<0.05	NS

Values are mean ± SEM of triplicate groups, values in the same column not sharing a common superscript showed significant difference (P<0.05)
[1]ABW_i **(**initial average body weight, g)
[2]ABW_f (final average body weight, g)
[3]FI **(**Feed intake, g**)**
[4]WG (weight gain, g) = AW_f - ABW_i
[5]SGR (Specific growth rate, unit day^{-1}) = [(lnW_i – lnW_f)/ day] x100
[6]SR **(**Survival rate, %)
[7]FCE (Feed conversion efficiency, %) = [(wet weight gain, g)/(dry feed intake, g)] x 100
[8]PER (Protein efficiency ratio) : wet wt gain(g)/protein intake (g)
[9]PG (Protein gained,g kg^{-1}) = [(W_f x CP) – (W_i x CP) / (kg Protein consumed)]

There were no significant differences in the carcass moisture, crude fat, and Mg of fish fed the test diets (Table 3). Body protein, however, was significantly the lowest in the ComD group and the rest of the groups exhibited higher values that did not vary significantly. Final levels of body P and Ca were significantly the lowest in the NoP group while the Bmeg group exhibited the highest values but did not vary significantly from the Blic, Bpum and ComD groups. Data showed that supplementation of dietary phytase from *B. megaterium* increased the body P and Ca by 28.4 and 42.8%, respectively. Body Mg was not affected by the dietary treatments.

Table 3. Body composition (g kg^{-1} DM) of sex reversed *O. mossambicus* fingerlings fed commercial diet, plant-based diet alone or supplemented singly with a *Bacillus* phytase for 60 days.

Diets	Moisture	Crude protein	Crude Fat	Ash	P	Ca	Mg
NoP	750.5 ± 7.2	626.7 ± 18.3^{ab}	222.3 ± 5.0	126.4 ± 3.2^{b}	13.4 ± 0.4^{b}	28.5 ± 0.9^{b}	2.26 ± 0.10
Bpum	745.9 ± 3.6	626.1 ± 10.4^{ab}	220.9 ± 9.9	125.1 ± 2.6^{b}	14.6 ± 0.3^{ab}	39.0 ± 2.9^{a}	2.44 ± 0.10
Bmeg	754.3 ± 10.2	627.9 ± 2.7^{a}	221.2 ± 6.7	132.7 ± 2.7^{a}	17.2 ± 2.2^{a}	40.7 ± 0.7^{a}	2.63 ± 0.20
Blic	744.5 ± 8.3	622.9 ± 8.4^{ab}	222.0 ± 9.4	129.6 ± 4.4^{ab}	16.2 ± 1.5^{ab}	39.5 ± 1.1^{a}	2.51 ± 0.10
ComD	733.9 ± 7.6	601.9 ± 1.7^{b}	239.5 ± 9.3	127.3 ± 0.5^{b}	14.2 ± 0.1^{ab}	39.0 ± 1.8^{a}	2.40 ± 0.30
P	NS	P<0.05	NS	P<0.05	P<0.05	P<0.05	NS

Values are mean ±SEM of triplicate groups, values in the same column not sharing a common superscript showed significant difference (P<0.05).

Highest scale ash and P were exhibited by tilapia fed the Bmeg diet while those fed the negative control diet (NoP) exhibited the lowest (Table 4). Supplementation of *B. megaterium* phytase increased terminal scale ash and P by 9.1 and 35.2%, respectively, relative to the negative control diet. The highest scale Ca was recorded in the Bmeg group and the highest scale Mg was in the Blic group; the lowest values were recorded in the control diet NoP. These represented an increase of 13.4% in the scale Ca by the Bmeg group and 27.1% in the scale Mg by the Blic group, respectively.

Table 4. Final concentration (g kg^{-1} DM) of ash, P, Ca and Mg in scale and bone of sex reversed *O. mossambicus* fingerlings fed commercial diet, plant-based diet alone or supplemented singly with a *Bacillus* phytase for 60 days.

Diet	Scale				Bone			
	Ash	P	Ca	Mg	Ash	P	Ca	Mg
NoP	328.8 ± 5.0^{c}	31.0 ± 0.8^{c}	118.3±2.7^{b}	4.40 ± 0.3^{b}	448.3 ± 7.6	37.3 ± 1.8^{c}	153.7 ± 3.8^{d}	6.82 ± 0.2^{b}
Bpum	341.3 ± 10.8^{bc}	37.6 ± 1.9^{ab}	123.4±3.2^{ab}	4.62 ± 0.3^{b}	455.8 ± 20.0	44.9 ± 2.5^{b}	164.1 ± 1.5^{c}	7.06 ± 0.3^{b}
Bmeg	358.6 ± 1.1^{a}	41.9 ± 2.4^{a}	134.1±6.1^{a}	4.78 ± 0.1^{b}	480.6 ± 32.0	50.3 ± 1.5^{a}	189.7 ± 1.0^{a}	8.32 ± 0.1^{a}
Blic	356.6 ± 6.9^{ab}	40.3 ± 2.3^{a}	131.7 ± 2.2^{a}	5.59 ± 0.1^{a}	479.2 ± 14.1	48.4 ± 1.9^{ab}	182.1 ± 1.3^{b}	8.08 ± 0.1^{a}
ComD	344.1 ± 4.5^{abc}	33.2 ± 3.3^{bc}	114.5 ± 6.9^{b}	4.60 ± 0.0^{b}	450.5 ± 21.8	46.2 ± 0.4^{ab}	158.8 ± 2.2^{cd}	6.93 ± 0.3^{b}
P	P<0.05	P<0.05	P<0.05	P<0.05	NS	P<0.05	P<0.05	P<0.05

Values are mean ±SEM of triplicate groups, values in the same column not sharing a common superscript showed significant difference (P<0.05).

Bone ash was not affected by the dietary treatments. The highest values for bone P, Ca and Mg were recorded in the Bmeg group but did not vary significantly from the Blic group; the lowest were recorded in the control group (Table 4). Supplementation of *B. megaterium* phytase to diet increased bone P, Ca and Mg by 34.9, 23.4 and 22.0%, respectively.

N retention was not significantly affected by the dietary treatments. Highest P retention was observed in the Bpum group but was not significantly different from the Bmeg and Blic groups. Fish fed the commercial diet exhibited the lowest P retention while those fed the negative control diet exhibited intermediate values (Table 5). Adding *B. pumilus* or *B. megaterium* phytase in the diet increased P retention by an average of 16.2% relative to the negative control diet.

Lowest P load was recorded in the Bpum group that did not vary significantly from the Bmeg and Blic groups. The significantly highest P load was recorded in the ComD group followed by the NoP group. Adding phytase of *B. pumilus* or *B. megaterium* phytase to the plant-based diet decreased P load by an average of 62%. N load was lowest in fish fed the Bmeg diet which did not vary significantly from those fed the ComD diet. Highest N load was observed in the Bpum group and was not significantly different from the Blic and NoP groups. Supplementing the diet with phytase of *B. megaterium* decreased N load by 9.7% relative to the negative control diet.

Table 5. Nutrient retention, nutrient load and fecal P of sex-reversed *O. mossambicus* fed commercial diet, plant-based diet alone or supplemented singly with a *Bacillus* phytase for 60 days.

Diet	Nutrient retention (g kg^{-1})		Nutrient load (g kg^{-1})^{a}		Fecal P (g kg^{-1})
1	N	P	P load	N load	
NoP	381.3±17.2	775.4±30.9^{b}	4.2±0.7^{b}	46.9±2.5^{b}	13.5 ± 0.6^{c}
Bpum	383.3±7.8	901.4±23.7^{a}	1.5±0.4^{a}	47.8±2.2^{b}	10.5 ± 0.2^{ab}
Bmeg	400.3±18.8	900.7±40.3^{a}	1.7±0.7^{a}	42.4±2.0^{a}	8.7 ± 1.4^{a}
Blic	387.2±4.7	851.9±49.1^{ab}	2.8±0.9^{ab}	47.0±2.9^{b}	9.3 ± 0.7^{a}
ComD	403.0±10.3	577.6±12.3^{c}	10.4±0.5^{c}	43.8±1.2^{a}	12.2 ± 0.3^{bc}
P	NS	P<0.05	P<0.05	P<0.05	P<0.05

[a]*Data are means of three replicate tanks. Values not sharing the same superscripts are significantly different at P<0.05*
[b]*Nutrient retention (%) = nutrients deposited/nutrient fed*

Lowest fecal P was recorded in fish fed Bmeg diet which was not significantly different from those fed the Bpum and Blic diets. Highest value was observed in the NoP group which did not vary significantly from the ComD group. Supplementing the diet with phytase from either *B. megaterium* or *B. licheniformis* reduced the fecal P by an average of 33.3% relative to the negative control diet.

DISCUSSION

The interest of phytase supplementation in aquaculture research is to make available P and other nutrients bound to phytate and also to minimize the excretion of P to the water environment so as to minimize pollution and eutrophication. Adding phytase topically or online has been reported to be effective in making phytate P bio-available to fish [30].

The hydrolysis of phytate resulting in the release of P in the stomach of tilapia was the major factor that could manifest in growth performance and feed utilization efficiency. Growth performance was statistically the same among the dietary groups probably due to the absence of P deficiency in the diets. The first indication of P sufficiency in the diet was the body fat that did not differ significantly among treatments. Body fat is expected to increase if there was dietary P deficiency [31; 18; 32]. The second indication was bone ash which was not affected by the diets. Bone ash is a more sensitive indicator of the phosphorus status in fish than growth rate [31; 33]. Even if P deficiency existed, it would only manifest in growth rate when the whole-body P content falls below a critical level [34].

Cao *et al*. [35] have reviewed growth performance responses to phytase supplementation in different species and have observed that these are somewhat inconsistent. Positive results are observed in channel catfish [36], common carp [37], African catfish [38], striped bass [39]), rainbow trout [40] and Atlantic salmon [41]. In agreement with the present study are findings of no significant differences in feed intake and growth performance in pond-raised channel catfish [11]. Cao *et al*. [2] recommends further research to confirm whether this conclusion indicates that the function of phytase relates to diet formulation, fish size, development status of fish digestive system, or the content of endogenous phytase in fish digestive system.

Inconsistent effects of phytase supplementation in fish in various studies are probably due to two factors: the supplementation level and the incorporation method into diets, as pointed out by Ai *et al*. [42]. Previous studies show that the level of phytase required for maximum growth and carcass P deposition ranges from 500 to 1,000 IU kg^{-1} [43; 36]. Ai *et al*. [30] maintain that supplementing 500 IU kg^{-1} phytase to the diet is not adequate to improve the growth and protein utilization for Japanese sea bass. Results of the present study agreed with this observation on no growth enhancement having also used 500 IU kg^{-1} *Bacillus* phytases but differed on the observation on no enhancement of protein utilization. The enhanced feed and protein utilization (i.e. FCE and PER) in the present study by *Bacillus* phytase supplementation could be due to the topical method of application which is considered more effective in improving feed utilization than does pre-extrusion application[43; 44; 45; 40].

The dietary *Bacillus* phytases in the present study, especially the *B. megaterium* and *B. licheniformis* phytases probably made the chelated phytate-P more available to fish resulting in the enhanced utilization rate of P. Cao *et al*. [2] observe that apparent P digestibility and bone mineralization are considered the most sensitive criteria for assessing the influence of phytase on P utilization. This was borne out by the results of the present study of significant increases in both the final content of P in the scale and bone as well as the retained P in the body of tilapia fed the Bmeg and Blic diets.

The highest final levels of body ash, Ca and Mg in tilapia fed the plant-based diet supplemented with *B. megaterium* and *B. licheniformis* phytases in the present study demonstrated the these bacterial phytases increased body mineralization. This showed that the two *Bacillus* phytases were more efficient in degrading phytate and releasing the bound minerals in the diets which were well utilized by tilapia for increase in their tissue mineralization than did the *B. pumilus* phytase.

Davis and Robinson [46] observe that scales are the source of reserve P in fish. Thus, available P will first be utilized by the bone for proper mineralization and then utilized for scale growth and storage. Scale P apparently are easily mobilized for utilization in the body when needed and thus closely reflects dietary P status [47]. Supplementation of either one of the three *Bacillus* phytases increased final scale P in tilapia more than did the negative control diet or the commercial diet; this was another indication that phytate P were made more bioavailable to the fish equally by the *Bacillus* phytases. The same trend was observed in the final scale Ca levels of experimental tilapia in the present study.

Bone P and Ca levels were both the highest in the Bmeg diet group indicating that the *B. megaterium* phytase was the most effective enzyme for bone mineralization in tilapia and *B. pumilus* appeared to be the least.

Supplementation of phytases to plant-based diets in in various studies reduce P load to the environment by 30-50% in the Nile tilapia [20], salmon [48], rainbow trout [16], carp [37] and channel catfish [36]. Using either *B. megaterium* or *B. licheniformis* phytase resulted in a greater reduction in P load by 62% in the present study relative to the negative control diet. For the N load, the Bmeg and the ComD diets produced the least values. All diets supplemented with any *Bacillus* phytase resulted in the lowest fecal P. Thus, the species most beneficial in reducing N or P pollution were *B. megaterium* and *B. licheniformis*.

CONCLUSION

Supplementing plant-based diets with phytases from *B. megaterium*, *B. licheniformis* and *B. pumilus* did not have any effect on growth performance. However, *B. megaterium* phytase improved the feed utilization efficiency of the diet the most. All the *Bacillus* phytases improved the final carcass protein but did not have any effect on body fat, or Mg. Carcass ash was improved the most by *B. megaterium* and *B. licheniformis* phytases while carcass P and Ca were increased by all three *Bacillus* phytases. Scale ash was increased the most by both *B. megaterium* and *B. licheniformis* phytases while scale P and Ca by all three *Bacillus* phytases. Scale Mg was increased the most by *B. licheniformis*. Bone ash was unaffected by all the dietary treatments while bone P and Mg were increased the most by *B. megaterium* and *B. licheniformis* phytases; bone Ca was increased the most by *B. megaterium*. N retention was unaffected by the dietary treatments while P retention was increased by all three species. P load were the lowest when all *Bacillus* phytases were added to the diet while the N load was most reduced by the *B. megaterium* phytase. All the *Bacillus* phytases were effective in reducing the fecal P of tilapia. Thus, the *B. megaterium* and *B. licheniformis* phytases were most effective in hydrolyzing phytate P and in ameliorating water quality.

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REFERENCES

[1] H. Stefan, K. Anja, S. Edzard, B. Joerg, L. Markus, Z. Oskar, *Appl. Microbiol. Biotechnol.*, **2005**, 68(5), 588 - 597.
[2] L. Cao, W. Wang, C. Yang, Y. Yang, J. Diana, A. Yakupitiyage, *Enz. Microb. Technol.* , **2007**, 40, 497- 507.
[3] R. B. Dechavez, A. E. J. Serrano, S. Nunal, C. M. A. Caipang, *AACL Bioflux*, **2011**, 4, 394-403.
[4] D. H. Kim, B. C. Oh, W. C. Choi, J. K. Lee, T. K. Oh, *Biotechnol. Lett.*, **1999**, 20, 925 - 927.
[5] J. Keruvuo, I. Lappalainen, T. Reinikainen, *Biochem. Biophys. Re. Comm.*, **2000**, 268, 365 - 369.
[6] B. C. Oh, W. C. Choi, S. Park, Y. O. Kim, T. K. Oh, *Appl. Microbiol. Biotechnol.*, **2004**, 63, 362 - 372.
[7] M. Guerrero-Olazaran, L. Rodriguez-Blanco, J. G. Carreon-Trevino, J. A. Gallegos-lopez, J. M. Viader-Salvado, *Appl. Environ. Microbiol.*, **2010**, 76(16), 5601-5608.
[8] T. Asgard, K. D. Shearer, *Aquacult. Nutr.*, **1997**, 3, 17 - 23.
[9] R. W. Hardy, K. D. Shearer, *Can. J. Fish. Aquat. Sci.*, **1985**, 42, 181 - 184.
[10] D. L. Correll, *Poult. Sci.*, **1999**, 78, 674 - 682.
[11] E. H. Robinson, H. M. Li, B. B., A. Manning, *J. Appl. Aquacult.*, **2002**, 12.
[12] K. Baruah, A. K. Pal, N. P. Sah, K. K. Jain, S. C. Mukherjee, D. Debnath, *Aquacult. Res.* , **2005**, 36, 803-812.
[13] D. Debnath, S. N.P., P. A.K., J. K.K., S. Yengkokpam, M. S.C., *Aquacult. Res.*, **2005**, 36, 326-335.
[14] B. V. Adeola , A. L. Lawrence, T. R. Sutton, A. Cline, *J. Anim. Sci.* , **1995**, 73, 3384–3391.
[15] R. C. Hauler, C. G. Carter, In:(Ed.). Nutr. Soc. Aust., Australia, 139.
[16] M. Rodehutscord, E. Pfeffer, *Water Sci. Tech.*, **1995**, 31, 141-147.
[17] A. Schafer, W. M. Koppe, K. H. Meyer-Burgdorff, K. D. Gunther, *Water Sci.Technol.*, **1995**, 31, 149-155.
[18] J. C. Eya, R. T. Lovell, *J. World Aquacult. Soc.*, **1997**, 28, 286 - 391.
[19] M. H. Li, E. H. Robinson, *J. World Aquacult. Soc.*, **1997**, 27, 297 - 302.
[20] W. M. Furuya, G. S. Goncalves, V. R. B. Furuya, C. Hayashi, *Rev. Bras. Zootec.*, **2001**, 30, 924 - 929.
[21] L. Portz, F. Liebert, *J. Anim. Physiol. Anim. Nutr.* , **2004**, 88, 311-320.

[22] C. B. Santiago, M. B. Aldaba, E. F. Abuan, *Fish. Res. J. Philipp.* , **1986**, 11, 5-12.
[23] SEAFDEC/AQD, Feeds and Feeding of Milkfish, Nile Tilapia, Asian Sea Bass and Tiger Shrimp, SEAFDEC Aquaculture Department, Tigbauan, Iloilo, Philippines, **1994**.
[24] J. Strickland, T. P. Parsons, *Fish Res. Board Can.*, **1972**, 310.
[25] R. T. Lovell, Laboratory Manual for Fish Feed Analysis and Fish Nutrition Studies, Auburn University, Auburn, Alabama, **1975**.
[26] D. Pearson, The Chemical Analysis of Foods, Chem. Pub. Co., N. Y., **1977**.
[27] AOAC, Official methods of analysis, Association of Official Analytical Chemists, Arlington Virginia, USA, **1990**.
[28] L. H. Furukawa, H. Tsukahara, *Bull. Japan, Soc. Sci. Fish.*, **1966**, 32, 502 - 506.
[29] SPSS, SPSS Statistics Base 17.0 User's Guide. Chicago, IL, SPSS, Inc., 640.
[30] Q. Ai, K. Mai, W. Zhang, W. Xu, B. Tan, C. Zhang, H. Li, *Comp. Biochem. Phys. A*, **2007**, 147, 502–508.
[31] M. Rodehutscord, *J. Nutr.*, **1996**, 126, 324-331.
[32] D. I. Skonberg, L. Yogev, R. W. Hardy, F. M. Dong, *Aquaculture*, **1997**, 157, 11 - 24.
[33] J. Vielma, S. P. Lall, *Fish. Physiol. Biochem.*, **1998**, 19, 83 - 93.
[34] S. Nordrum, T. Asgard, K. D. Shearer, P. Arnessen, *Aquaculture*, **1997**, 157, 51 - 61.
[35] L. Cao, W. Wang, C. Yang, Y. Yang, J. Diana, A. Yakupitiyage, Z. Luo, D. Li, *Enzyme Microbiol. Technol.*, **2007**, 40, 497 - 507.
[36] L. S. Jackson, M. H. Li, E. H. Robinson, *J. World Aquacult. Soc.*, **1996**, 27, 309-313.
[37] A. Schaefer, W. M. Koppe, *Water Sci. Technol.*, **1995**, 31(1), 149 - 155.
[38] J. H. Van Weerd, K. H. A. Khalaf, F. J. Aartsen, P. A. T. Tijssen, *Aquacult. Nutr.* , **1999**, 5, 135-142.
[39] E. Papatryphon, R. A. Howell, J. H. J. Soares, *J. World Aquacult. Soc.*, **1999**, 30, 161-173.
[40] J. Vielma, K. Ruohonen, M. Peisker, *Aquaculture*, **2002**, 204, 145-156.
[41] M. Sajjadi, C. G. Carter, *Aquaculture*, **2004**, 240, 417 - 431.
[42] Q. Ai, K. Mai, W. Zhang, W. Xu, B. Tan, C. Zhang, H. Li, *Comp. Biochem. Phys. A*, **2007**, 147, 502 - 508.
[43] K. D. Cain, D. L. Garling, *Prog. Fish-Cult.*, **1995**, 57, 114- 119.
[44] D. Lanari, E. D'Agaro, C. Turri, *Aquaculture*, **1998**, 161, 245-356.
[45] J. Vielma, T. Makinen, P. Ekholm, J. Koskela, *Aquaculture*, **2000**, 183, 349-362.
[46] D. A. Davis, E. H. Robinson, *J. World Aquacult. Soc.*, **1987**, 18, 128 - 136.
[47] K. Powers Hughes, J. H. J. Soares, *Aquacult. Nutr.*, **1998**, 4, 133 - 140.
[48] T. Storebakken, K. D. Shearer, A. J. Roem, *Aquaculture*, **1998**, 161, 363-377.