Available online at www.sciencedirect.com
ScienceDirect
Aquaculture 267 (2007) 292–299
Aquaculture
www.elsevier.com/locate/aqua-online

Different sources of microbial phytase in plant based low phosphorus diets for Nile tilapia *Oreochromis niloticus* may provide different effects on phytate degradation

Frank Liebert [a,*], Leandro Portz [b]

[a] *Institute for Animal Physiology and Animal Nutrition, Georg-August-University Goettingen, Kellnerweg 6, D-37077 Goettingen, Germany*
[b] *Universidade Federal da Bahia, Escola de Agronomia, Departomento de Zootecnia, 44380-000 Cruz das Almas, BA, Brazil*

Received 13 November 2006; received in revised form 17 January 2007; accepted 18 January 2007

Abstract

Conclusive experimental data regarding different sources of supplemented microbial phytase in tilapia feed are very scarce. An experiment was conducted using plant based diets (24.5% soybean meal; 11.5% wheat gluten; 22.5% corn; 32.5% wheat) containing graded supply (500, 750, 1000, 1250 U/kg) of microbial phytase from two different sources (experimental phytase SP 1002; Ronozyme®P). Additionally, a negative control diet (0.41% total P; 0.15% non-phytate P; no phytase added) and a positive control diet (0.88% total P; 0.62% non-phytate P; no phytase added) were examined. Apparent nutrient digestibility (0.3% titanium dioxide as marker) was measured using six sedimentation systems (0.15 m^3/system) with external sedimentation column. Triplicate groups of 10 fish (average BW 110 g) fed 3 times a day and feces collection was conducted within 4 h following each feeding period. Chyme of the proximal small intestine (hepatic loop) was sampled for measuring phytate P. In a subsequent study, blood sampling was conducted from the caudal peduncle vein (triplicates from 9 fish per diet; average BW 125 g) for monitoring of inorganic P concentration in plasma at 2, 4, 8 and 12 h following the last meal. Phytase supplementation significantly improved digestibility of protein, calcium and phosphorus. Disappearance of phytate P from the hepatic loop was significantly improved due to phytase SP 1002 and the course of inorganic plasma P concentration indicated superior release of phytate P from the plant based diet under study.

Keywords: Phytase; Phosphorus; Protein; Digestibility; Utilization; Tilapia

1. Introduction

Generally, replacing fish meal by plant protein sources in fish nutrition is of increasing interest (Fontainhas-Fernandes et al., 1999; Mabahinzireki et al., 2001). However, this substitution strategy may enhance the dietary level of plant antinutritional factors like phytic acid and its salts (Denstadli et al., 2006). Approximately 70% of the total phosphorus in plant feedstuffs is present as phytate phosphorus (Lall, 1991; Eeckhout and De Paepe, 1994) and due to the absence of intestinal phytase activity (Jackson et al., 1996; Hughes and Soares, 1998) not available for fish (NRC, 1993). Furthermore, several mineral ions like calcium, magnesium, zinc, manganese, copper and iron (Papatryphon et al., 1999) as well as feed proteins (Liu et al., 1998;

* Corresponding author. Tel.: +49 551 39 3331; fax: +49 551 39 3343.
E-mail address: flieber@gwdg.de (F. Liebert).

0044-8486/$ - see front matter © 2007 Elsevier B.V. All rights reserved.
doi:10.1016/j.aquaculture.2007.01.023

Sugiura et al., 2001) can be reduced in their bioavailability. Insolubility of phytate–protein complexes has been observed *in vitro* (Ravindran et al., 1995).

Excretion of dietary phosphorus contributes to eutrophication effects (Persson, 1991; Einen et al., 1995; Cho and Bureau, 2001) and consequently it is of special interest to achieve phytate degradation during passing through the fish gut. The native phytase activity from different feedstuffs used in fish nutrition has limited importance due to low activity in the feed ingredients and inactivation by heat treatment during feed processing. In addition, commercial phytase sources may differ in their efficiency for phytate degradation in fish (Liebert and Portz, 2005). Generally, from varying physiological conditions in species dependent digestive systems, the question arises if more adapted types of microbial phytase are needed in fish nutrition. Development of a neutral phytase with improved thermal stability (Luo et al., 2006) is an important step for better adapted phytase in fish nutrition. Insufficient adaptation of phytase characteristics to fish physiology may result in failed effects as reported for common carp (Nwanna and Schwarz, 2006).

Based on commercial sources of microbial phytase, adding between 500 and 1500 U/kg feed improved calcium and phosphorus availability, growth performance, bone mineralization and protein digestibility in tilapia (Furuya et al., 2001). Supplementing of microbial phytase at 1000 U/kg feed resulted in growth and mineral utilization similar to a plant based diet supplemented with inorganic phosphorus (Portz et al., 2003; Portz and Liebert, 2004; Liebert and Portz, 2005). Effects of different sources of microbial phytase in fish species are mostly unknown, but have been reported for tilapia (Liebert and Portz, 2005). Based on identical diets, the current study investigated phytate degradation in the gut of Nile tilapia along with corresponding inorganic blood phosphorus concentrations.

2. Materials and methods

2.1. Experimental diets

Plant based diets (Table 1) were formulated according to the recommendations for tilapia (Santiago and Lovell, 1988; NRC, 1993) and according to Liebert and Portz (2005). Before final diet formulation, feed ingredients were analyzed for proximate composition, calcium, phosphorus and phytate-P content. Two sources of microbial phytase (provided by the former Roche Vitamins Ltd., Basel, Switzerland) were utilized. The experimental phytase SP1002 (phytase A) is a *Consensus* phytase produced by a genetically modified strain of the yeast *Hansenula polymorpha*. The production strain is carrying a synthetic gene coding for 3-phytase (IUB, 3.1.3.8.). Phytase A has an elevated pH optimum (>5) and higher stability against proteolytic enzyme activity and heat treatment. The phytase Ronozyme® P5000 (Phytase B) is a commercial product containing 6-phytase (IUB, 3.1.3.26) from *Peniophora lycii*, produced by submerged fermentation of a genetically modified *Aspergillus oryzae* strain. The experimental diets were supplemented with microbial phytase at similar graded levels, based on the actual phytase activity (Table 2). The control diet (0.88% total P; 0.62% non-phytate P) contained mono-sodium-phosphate (NaH_2PO_4; 1.5% of the diet) to meet the total P requirement (Miranda et al., 2000). For evaluating the phytase effects on P-utilization, non-phytate P concentration in the experimental diets was kept below the recommendations. Precision laboratory mixing systems (Co. Loedige, Germany) were applied for diet preparation. Finally, the mixtures were moistened with 15% distilled water and immediately pelleted (Co. Lister, England) to 2.5 mm granules. The pelleting

Table 1
Composition (%, as-fed basis) of the basal diet for tilapia

Ingredients	(%)
Soybean meal (49.95% crude protein)	24.50
Wheat gluten	11.50
Corn	22.50
Wheat	32.50
Soybean oil	4.00
Vitamin and mineral mix [a]	1.00
L-Lysine-HCl	1.10
DL-Methionine	0.90
Calcium carbonate	1.00
Carboxymethylcellulose	1.00
Total	100.00
Proximate analysis	
Crude protein	30.23
Crude fat	8.19
Ash	5.12
Calcium	0.53
Gross energy (MJ/kg)	19.68
Total P	0.41
Non-phytate P	0.15

[a] Vitamin and mineral mix (per kg of diet): $MnSO_4$, 40 mg; MgO, 10 mg; K_2SO_4, 40 mg; $ZnCO_3$, 60 mg; KI, 0.4 mg; $CuSO_4$, 12 mg; Ferric citrate, 250 mg; Na_2SeO_3, 0.24 mg; Co, 0.2 mg; retinol, 40000 IU; cholecalciferol, 4000 IU; α-tocopherolacetat, 400 mg; menadione, 12 mg; thiamin, 30 mg; riboflavin, 40 mg; pyridoxine, 30 mg; cyanocobalamin, 80 mcg; ;nicotinic acid, 300 mg; folic acid, 10 mg; biotin, 3 mg; pantothenic acid, 100 mg; inositol, 500 mg; ascorbic acid, 500 mg.

Table 2
Supplementation of microbial phytase and analyzed total phytase activity in the diets

Phytase supplementation (U[a]/kg)		Diet	Total dietary phytase activity analyzed (U[a]/kg)
	0	NC[b]	289
Phytase A	500	A 500	767
	750	A 750	1107
	1000	A 1000	1438
	1250	A 1250	1753
Phytase B	500	B 500	941
	750	B 750	1080
	1000	B 1000	1455
	1250	B 1250	1731
	0	PC[c]	253

[a] Phytase activity units according to the method of Engelen et al. (1994).
[b] Negative control diet.
[c] Positive control diet with 1.5% mono sodium phosphate (NaH_2PO_4) and 1.0% calcium carbonate ($CaCO_3$) supplemented in exchange with wheat.

temperature was held below 40 °C. The feed drying was conducted to prevent inactivation effects on the added sources of microbial phytase (ventilated oven for 24 h at 45 °C). A significant phytate degradation during this short period of moistening can be excluded.

2.2. Experimental conditions

Metabolism studies were conducted with all male *Oreochromis niloticus* obtained by mating yy-males with normal females (Kronert et al., 1989; Müller-Belecke and Hörstgen-Schwark, 2000). The studies were divided into three parts:

(1) Digestibility trial by the sedimentation procedure
(2) Direct sampling of intestinal chyme using $Ti0_2$ as marker
(3) Blood sampling depending on post-feeding time for monitoring of plasma inorganic P.

Fish were acclimated to six sedimentation systems (0.15 m^3 per system) as described by Meyer-Burgdorff and Rosenow (1995). Each system was equipped with continuous aeration and water exchange by recirculation. Titanium dioxide (0.3%) was utilized as the indigestible marker. Triplicates of 10 fish per diet were fed three times a day.

2.3. Sample collection

In the digestibility study (average individual BW 110 g), following the adaptation period the feces were collected for 4 h during a day collection period after each feeding. Feces were centrifuged and lyophilized before analysis. At the end of the digestibility experiment, 2 h following a single meal three animals per sedimentation system (nine fish per diet) were sacrificed by an anesthetic overdose of ethylene–glycol–monophenyl–ether for sampling of intestinal chyme. The chyme of the hepatic loop (Tengjaroenkul et al., 2000) was collected semi quantitatively and frozen for analysis of phytate phosphorus.

In a subsequent study (average individual BW 125 g) using similar diets, blood was sampled from 3 fish per diet at 2, 4, 8 and 12 h post-feeding of a single meal. For blood sampling, fish were anesthetized and blood was removed from the caudal peduncle vein.

2.4. Analytical procedures

The analyses of ingredients and diets run in duplicate according to German standard methods (Naumann and Bassler, 1976–1997). Crude protein was determined by Dumas-method ($N \times 6.25$) with a nitrogen auto-analyzer (FP-2000, Leco). Total phosphorus and phytate-P in feed, feces and digesta samples were determined in duplicate using the vanadium-molybdate method (total-P) and ammonium molybdate method (phytate-P) due to the modified AOAC-method 986.11 (AOAC, 1990), respectively. Absorbance measurement was conducted at 430 nm and at 640 nm using a UV–VIS spectrophotometer (Specord S100, Carl Zeiss, Jena, Germany). Inorganic phosphorus content in blood plasma was detected

Table 3
Phytate phosphorus concentration in freeze dried feces and chyme of the hepatic loop of Nile tilapia fed plant based diets with graded levels of two different sources of microbial phytase

Diets	Content of phytate phosphorus (%) *	
	Feces	Chyme of hepatic loop
NC**	0.50^{a}	0.17^{a}
A 500	0.33^{c}	0.12^{abc}
A 750	0.26^{de}	0.10^{bc}
A 1000	0.25^{e}	0.09^{c}
A 1250	0.25^{e}	0.10^{bc}
B 500	0.39^{b}	0.16^{a}
B 750	0.29^{d}	0.16^{a}
B 1000	0.26^{de}	0.14^{ab}
B 1250	0.27^{de}	0.14^{ab}
PC***	0.49^{a}	0.16^{a}

* As freeze-dried substance.
** Negative control, low phosphorus diet.
*** Positive control, phosphorus adequate diet.
[a, b, c, d, e] Means within the same column with different superscript letters are significantly different ($P<0.05$).

Table 4
Apparent digestibility of crude protein, calcium and phosphorus in Nile tilapia ($n=3$) fed plant based diets with graded levels of two different sources of microbial phytase

Diets	Crude protein	Calcium	Phosphorus
	Apparent digestibility (%)		
NC*	91.8^{e}	31.0^{c}	43.1^{f}
A 500	93.2^{d}	44.2^{ab}	60.6^{d}
A 750	95.8^{a}	47.6^{a}	64.6^{cd}
A 1000	95.3^{ab}	48.4^{a}	70.8^{ab}
A 1250	95.6^{a}	49.6^{a}	75.7^{a}
B 500	93.7^{d}	37.6^{bc}	50.0^{e}
B 750	95.0^{ab}	48.0^{a}	65.0^{cd}
B 1000	95.4^{ab}	49.6^{a}	67.4^{bc}
B 1250	94.7^{bc}	50.7^{a}	73.0^{a}
PC**	93.9^{cd}	46.7^{a}	75.5^{a}
Pooled SEM	0.56	1.89	1.76
P-value	<0.0001	<0.0001	<0.0001

* Negative control, low phosphorus diet (no phytase added).
** Positive control, phosphorus adequate diet (no phytase added).
[a, b, c, d, e, f] Means within the same column with different superscript letters are significantly different ($P<0.05$).

by using the commercial UV-test kit SYS 1 (Roche Diagnostics GmbH, Mannheim, Germany) based on the principle of inorganic phosphorus binding with ammonium–molybdate and forming the complex ammonium–phospho–molybdate (extinction measured at 340 nm). Titanium in feed and chyme samples was analysed with atomic absorption phase spectrophotometer (SpectrAA-55; Varian, Darmstadt, Germany) following wet ashing ($HN0_3$:H_2S0_4=1:1) in 30 ml teflon beakers (at 180 °C for 8 h) using a pressure digestion system (PDS-6; Loftfield's Analytical Solutions, Neu Eichenberg, Germany). In-feed analysis of phytase activity was carried out due to the procedure of Engelen et al. (1994). Established total phytase activity in the final feeds was generally in line with the expected graded dietary phytase supply (Table 2).

Digestibility data were analyzed as one-way completely randomized design, submitted to ANOVA ($P<0.05$) and Tukey's test. Plasma P data were submitted to multivariate ANOVA running with SPSS software package (Version 10.0 for Windows; SPSS Inc., Chicago, IL). According to equality or non-equality of variances (verified by Levene-test), the Fisher-LSD or Games–Howell post-hoc test was used.

3. Results

The phytate P concentration in the freeze-dried feces (Table 3) was generally higher than in the freeze-dried chyme of the hepatic loop. This observation indicates

Table 5
Inorganic phosphorus concentration [mg/L]* in blood plasma ($n=3$) depending on post-feeding time

Diet	Post-feeding time [h]				P-value**
	2	4	8	12	Time
NC	41.4±0.9^{a}	42.1±0.2^{a}	44.9±0.3^{ad}	44.3±0.8^{a}	<0.0001
A 500	41.4±1.4^{a}	48.7±0.4^{bc}	44.6±0.5^{ad}	42.5±1.0^{a}	<0.0001
A 750	41.7±0.9^{ac}	48.1±0.4^{bc}	51.6±1.1^{ce}	43.7±1.2^{a}	<0.0001
A 1000	43.7±0.6^{b}	45.4±1.6^{ac}	51.1±1.5^{ae}	40.4±1.9^{ab}	<0.0001
A 1250	43.7±1.6^{b}	47.4±0.6^{bc}	51.0±0.8^{ce}	49.5±0.4^{b}	<0.0001
B 500	41.9±0.4^{ac}	40.7±0.8^{a}	41.7±1.2^{df}	42.8±1.9^{ab}	0.279
B 750	43.3±0.8^{bc}	42.6±2.0^{ac}	41.8±0.5^{bf}	49.3±1.6^{ab}	0.002
B 1000	44.5±1.3^{b}	47.2±0.5^{bc}	49.1±0.7^{ce}	44.5±0.5^{a}	<0.0001
B 1250	43.7±0.7^{b}	49.1±0.5^{bc}	50.8±0.6^{ce}	43.8±0.9^{a}	<0.0001
PC	44.8±0.8^{b}	52.1±1.6^{b}	47.3±1.2^{aef}	44.8±1.7^{ab}	0.002
Pooled SEM**	0.2735	0.6503	0.7478	0.5858	
P-value** Diet	0.001	<0.0001	<0.0001	0.052	

P-value***		
	Phytase	<0.0001
	Level	<0.0001
	Time	<0.0001
	Phytase × Level	<0.0001
	Phytase × Time	<0.0001
	Phytase × Level × Time	<0.0001

* Multivariate and one-way ANOVA (SPSS for windows 10.0). Fisher-LSD or Games–Howell post-hoc test depending equality or non-equality of variances (verified by Levene-test).
** One-way ANOVA with all diets.
*** Multivariate ANOVA, diets NC and PC with no added microbial phytase excluded.
[a, b, c, d, e, f] Means within the same column with different superscript letters are significantly different ($P<0.05$).

the beginning of the digestive process in the hepatic loop and phytate P is still diluted by nutrients which will be digested and absorbed up to the end of the small intestine. Supplementing phytase A up to 750 U/kg diet, significantly reduced phytate P content of feces. Consequently, the phytate P concentration in feces decreased by approximately 50% as compared to the phytate P concentration in samples from fish fed the control diets (NC, PC). Higher activity of phytase A did not significantly improve the disappearance of phytate-P from fecal samples. The observed changes of phytate P concentration in the freeze-dried samples of the proximal intestinal chyme were similar. It was noted that only phytase A reduced the phytate P content in this part of the gut significantly.

Supplementing phytase B also significantly decreased the phytate P content in feces. However, phytase A and B tended not to reduce the fecal phytate-P content to a similar extent. In the intestinal chyme, phytase B did not significantly reduce the phytate P content. Due to phytase A supplementation, the phytate P concentration in the freeze-dried chyme of the hepatic loop was significantly lower, indicating a more pronounced phytate degradation and P disappearance in this part of the gut. The digestibility of P was significantly improved by both sources of microbial phytase (Table 4). However, the dose-response effects on phosphorus digestibility were not identical. The same observation was made for crude protein digestibility, but phytase supplementation significantly increased the apparent protein digestibility.

Additionally, the course of inorganic phosphorus concentration in blood plasma (Table 5), showed different responses depending on the microbial phytase used. This difference in response due to both sources of microbial phytase is more evident when pooled dietary phytase activity is applied (Fig. 1). The results indicate that differences may occur in the metabolic availability of inorganic P due to the supplemented microbial phytase sources. Following the feeding of the highest dietary phytase activity (A 1000, A 1250, B 1000, B 1250), blood sampling at 2 h showed a similar response on plasma P as the PC diet. Blood sampling at 4 h resulted in more pronounced phytase effects on plasma P concentration. Following the feeding of phytase A, no dose dependent effect on plasma P was observed. After the feeding of phytase B, dose-response effects were established. However, at both sampling times (2 and 4 h) the level of plasma P concentration following the feeding of diet PC (including inorganic phosphorus) was generally not achieved. Sampling 8 h post-feeding, the plasma P concentrations due to higher levels of dietary phytase activity tended to exceed the effect of feeding diet PC. The observed results at 12 h post-feeding are mostly inconsistent, reflecting the decreasing peak of phosphorus absorption with high variation. Generally, these preliminary results of blood sampling are useful as additional information corresponding to varying effects depending on the microbial phytase source fed.

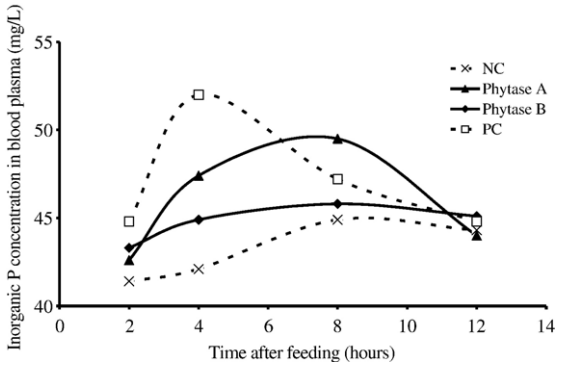

Fig. 1. Inorganic phosphorus concentration in blood plasma of Nile tilapia depending on diet (pooled dietary phytase activity), microbial phytase and time after feeding.

4. Discussion

The incorporation of microbial phytase into fish diets improves P availability and overall performance, while reducing P excretion in several fish species (Rodehutscord and Pfeffer, 1995; Schäfer et al., 1995; Jackson et al., 1996; Hughes and Soares, 1998; Papatryphon et al., 1999; Sugiura et al., 2001; Yan and Reigh, 2002; Portz and Liebert, 2004; Liebert and Portz, 2005). However, the examination of the specific effects of phytase in different fish species is insufficient. Furthermore, only limited data are available for different sources of microbial phytase, mainly in plant based tilapia diets. The results of the present study demonstrate that different effects on zootechnical parameters and nutrient deposition in juvenile tilapia (Liebert and Portz, 2005) are further explained by physiological data. The observed course of phytate P in feces and in chyme of the hepatic loop is in agreement with the observed utilization of total P and crude protein (Liebert and Portz, 2005). However, the current results give support to the assumption that the sources of microbial phytase used in this study were not equal due to the improvement of phytate degradation in the digestive tract. It is also indicated that this difference in efficiency was not compensated by the level of supplemented phytase activity which was in the range of efficient dosages for tilapia (Furuya et al., 2001; Portz et al., 2003; Portz and

Liebert, 2004) and for diets with high quantities of indigestible phytates (NRC, 1993; Cain and Garling, 1995; Francis et al., 2001; Riche et al., 2001).

Supplementing phytase A at 750 U/kg diet was adequate for maximal improvement of phytate degradation under the conditions used in this study. The supplementation of at least 1000 U/kg from phytase B provided similar effects on the decrease of fecal phytate P. Additional effects of both sources of microbial phytase have been discussed elsewhere (Liebert and Portz, 2005). The improved protein digestibility due to phytase addition may explain the effects on enhanced protein utilization. Similar to warm blooded animals (Sebastian et al., 1997; Martin et al., 1998; Selle et al., 2006), the observed effects of supplemental phytase on protein utilization in fish are still contradictory. Lanari et al. (1998) and Yan and Reigh (2002) did not observe significant effects of supplemented phytase on protein utilization. In contrast, several studies using plant based diets have observed improved protein utilization in fish (Storebakken et al., 1998; Vielma et al., 1998, 2004; Sugiura et al., 2001). However, improved protein digestibility was not correlated with higher lysine utilization in rainbow trout (Vielma et al., 2004).

In addition, the digestibility of calcium was significantly enhanced by supplementing both sources of microbial phytase. Calcium seems to be more associated with the phytate complex than other minerals (Papatryphon et al., 1999), but, in general, phytase also effects on mineral availability (Hossain and Jauncey, 1991). Based on current observations on calcium digestibility, we were unable to detect a difference between the two sources of microbial phytase used. A trend indicates enhanced efficiency of phytase A due to the low level of supplementation. However, the post-feeding time course of inorganic P in blood plasma provides evidence for a faster phosphorus uptake in the gut of juvenile tilapia due to supplementation of phytase A (SP 1002). Multivariate ANOVA established the type of added microbial phytase as a significant factor of influence on inorganic P concentration in blood plasma.

Reported data are generally in accordance with our observed effects of microbial phytase on nutrient deposition and nutrient utilization (Liebert and Portz, 2005). Both sources of microbial phytase under study improved the dietary phosphorus supply for tilapia, thereby reducing phosphorus excretion. However, the efficacy of the phytase sources was not similar, indicating that the type of added microbial phytase and the gut conditions may result in varying effects depending on fish species. Similar to warm blooded animals (Liebert et al., 1993; Selle et al., 2006), the current knowledge about this physiological background in fish species is extremely scarce.

In conclusion, more basic research is needed on "working conditions" for supplemental feed enzymes in the digestive tract of fish species because structure and physiology of digestive systems in fish species are different. Developing of specific types of microbial phytase which are well adapted to the gut conditions in fish will be of growing importance, possibly also in combination with several other feed enzymes (Selle et al., 2006). Conclusive studies depending on species, physiology of the digestive tract and different dietary sources of plant phytate are needed. Experiments using a graded supply of model substances like sodium phytate (Denstadli et al., 2006) need completion by investigating native phytates. Additionally, the role of native phytase activity from feed ingredients needs more attention.

Acknowledgement

The authors thank the former Roche Vitamins Ltd, Basel for providing the microbial phytase, in-feed analyses of phytase activity and financial support of the study.

References

Association of Official Analytical Chemists, 1990. Method No. 986. 11. Official Methods of Analysis, 15th ed. Association of Official Analytical Chemists, Washington, DC.

Cain, K.D., 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.

Cho, C.Y., Bureau, D.P., 2001. A review of diet formulation strategies and feeding systems to reduce excretory and feed wastes in aquaculture. Aquac. Res. 32, 349–360.

Denstadli, V., Skrede, A., Krogdahl, ., Sahlstrøm, S., Storebakken, T., 2006. Feed intake, growth, feed conversion, digestibility, enzyme activities and intestinal structure in Atlantic salmon (*Salmo salar* L.) fed graded levels of phytic acid. Aquaculture 256, 365–376.

Eeckhout, W., De Paepe, M., 1994. Total phosphorus, phytate-phosphorus and phytase activity in plant feedstuffs. Anim. Feed Sci. Technol. 47, 19–29.

Einen, O., Holmefjord, I., Åsgård, T., Talbot, C., 1995. Auditing nutrient discharges from fish farms: theoretical and practical consideration. Aquac. Res. 26, 701–713.

Engelen, A.J., Van der Heeft, F.C., Randsdorp, P.H.G., Smith, E.L.C., 1994. Simple and rapid determination of phytase activity. AOAC Int. J. 77, 760–764.

Fontainhas-Fernandes, A., Gomes, E., Reis-Henriques, M.A., Coimbra, J., 1999. Replacement of fish meal by plant protein in the diet of Nile tilapia: digestibility and growth performance. Aquac. Int. 7, 57–67.

Francis, G., Makkar, H.P.S., Becker, K., 2001. Antinutritional factors present in plant-derived alternate fish feed ingredients and their effects in fish. Aquaculture 199, 197–227.

Furuya, W.M., Gonçalves, G.S., Furuya, V.R.B., Hayashi, C., 2001. Phytase as feeding for Nile tilapia (*Oreochromis niloticus*). Performance and digestibility. Rev. Bras. Zootec. 30, 924–929.

Hossain, M.A., Jauncey, K., 1991. The effects of varying dietary phytic acid, calcium and magnesium levels on the nutrition of common carp, *Cyprinus carpio*. In: Kaushik, S.J., Luquet, P. (Eds.), Fish Nutrition in Practice. INRA, Paris, pp. 705–715.

Hughes, K.P., Soares Jr., J.H., 1998. Efficacy of phytase on phosphorus utilization in practical diets fed to striped bass *Morone saxatilis*. Aquac. Nutr. 4, 133–140.

Jackson, L., Li, M.H., Robinson, E.H., 1996. Use of microbial phytase in channel catfish *Ictalurus punctatus* diets to improve utilization of phytate phosphorus. J. World Aquac. Soc. 27, 309–313.

Kronert, U., Hörstgen-Schwark, G., Langholtz, H.-J., 1989. Prospects of selecting for late maturity in tilapia (*Oreochromis niloticus*). Aquaculture 77, 113–121.

Lall, S.P., 1991. Digestibility, metabolism and excretion of dietary phosphorus in fish. In: Cowey, C.B., Cho, C.Y. (Eds.), Nutritional Strategies and Aquaculture Waste. University of Guelph, Guelph, Ontario, Canada, pp. 21–36.

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

Liebert, F., Portz, L., 2005. Nutrient utilization of Nile tilapia *Oreochromis niloticus* fed plant based low phosphorus diets supplemented with graded levels of different sources of microbial phytase. Aquaculture 248, 111–119.

Liebert, F., Wecke, C., Schöner, P.-J., 1993. Phytase activities in different gut contents of chickens as dependent on levels of phosphorus and phytase supplementations. Proceedings of the 1st Symposium Enzymes in Animal Nutrition. Kartause Ittingen, Switzerland, pp. 202–205.

Liu, B.L., Rafing, A., Tzeng, Y.M., Rob, A., 1998. The induction and characterization of phytase and beyond. Enzyme Microb. Technol. 22, 415–424.

Luo, H.Y., Wang, Y.R., Yao, B., 2006. Overexpression of neutral phytase. In: Kaushik, S. (Ed.), XII International Symposium Fish Nutrition & Feeding 28 May-1 June 2006, Biarritz, France, p. 286.

Mabahinzireki, G.B., Dabrowski, K., Lee, K.-J., El-Saidy, D., Wisner, E.R., 2001. Growth, fed utilization and body composition of tilapia (*Oreochromis* sp.) fed with cottonseed meal-based diets in a recirculating system. Aquac. Nutr. 7, 189–200.

Martin, E.A., Nolan, J.V., Nitsan, Z., Farrell, D.J., 1998. Strategies to improve the nutritive value of rice bran in poultry diets: IV. Effects of addition of fish meal and a microbial phytase to duckling diets on bird performance and amino acid digestibility. Br. Poult. Sci. 39, 612–621.

Meyer-Burgdorff, K.-H., Rosenow, H., 1995. Protein turnover and energy metabolism in growing carp. J. Anim. Physiol. Anim. Nutr. 73, 113–122.

Miranda, E.C., Pezzato, A.C., Pezzato, L.E., Graner, C.F., Rosa, G.J., Pinto, L.G.Q., 2000. Availability of calcium:phosphorus ratio in diets for Nile tilapia (*Oreochromis niloticus*). Rev. Bras. Zootec. 29, 2162–2171.

Müller-Belecke, A., Hörstgen-Schwark, G., 2000. Performance testing of homozygous lines in *Oreochromis niloticus*. Aquaculture 184, 67–76.

Naumann, C., Bassler, R., 1976–1997. VDLUFA-Methodenbuch. Die chemische Untersuchung von Futtermitteln, vol. III. Neumann-Neudamm, Darmstadt.

NRC (National Research Council), 1993. Nutrient Requirements of Fish. National Academy Press, Washington D.C., USA.

Nwanna, L.C., Schwarz, F.J., 2006. Effect of phytase on the availability of phosphorus for common carp (*Cyprinus carpio* L.). In: Kaushik, S. (Ed.), XII International Symposium Fish Nutrition & Feeding, 28 May-1 June 2006, Biarritz, France, p. 296.

Papatryphon, E., Howell, R.A., Soares Jr., J.H., 1999. Growth and mineral absorption by striped bass *Morone saxatilis* fed a plant feedstuff based diet supplemented with phytase. J. World Aquac. Soc. 30, 161–173.

Persson, G., 1991. Eutrophication resulting from salmonid fish culture in fresh and salt waters: Scandinavian experiences. In: Cowey, C.B., Cho, C.Y. (Eds.), Nutritional Strategies and Aquaculture Waste. Proceedings of the First International Symposium on Nutritional Strategies in Management of Aquaculture Waste. University of Ghelph, Ontario Canada, pp. 163–185.

Portz, L., Liebert, F., 2004. Growth, nutrient utilization and parameters of mineral metabolism in Nile tilapia *Orechromis niloticus* (Linnaeus, 1758) fed plant based diets with graded levels of microbial phytase. J. Anim. Physiol. Anim. Nutr. 88, 311–320.

Portz, L., Benkendorff, K., Liebert, F., 2003. Growth and mineral absorption of Nile tilapia *Oreochromis niloticus* fed a plant based diet supplemented with microbial phytase. In: Cooksey, J. (Ed.), Proceedings of the World Aquaculture Society, Salvador, Brazil, vol. 1, p. 592.

Ravindran, V., Bryden, W.L., Kornegay, E.T., 1995. Phytates: occurrence, bioavailability and implications in poultry nutrition. Poult. Avian Biol. Rev. 6, 125–143.

Riche, M., Trottier, N.L., Ku, P.K., Garling, D.L., 2001. Apparent digestibility of crude protein and apparent availability of individual amino acids in tilapia (*Oreochromis niloticus*) fed phytase pretreated soybean meal diets. Fish Physiol. Biochem. 25, 181–194.

Rodehutscord, M., Pfeffer, E., 1995. Effects of supplemental microbial phytase on phosphorus digestibility and utilization in rainbow trout (*Oncorhynchus mykiss*). Water Sci. Technol. 31, 141–147.

Santiago, C.B., Lovell, R.T., 1988. Amino acid requirements for growth of Nile tilapia. J. Nutr. 118, 1540–1546.

Schäfer, A., Koppe, W.M., Meyer-Burgdorff, H., Günther, K.D., 1995. Effects of a microbial phytase on the utilization of native phosphorus by carp in a diet based on soybean meal. Water Sci. Technol. 31, 149–155.

Sebastian, S., Touchburn, S.P., Chavez, E.R., Lague, P.C., 1997. Apparent digestibility of protein and amino acids in broiler chickens fed a corn-soybean diet supplemented with microbial phytase. Poult. Sci. 76, 1760–1769.

Selle, P.H., Velmurugu, R., Wayne, L.B., Scott, T., 2006. Influence of dietary phytate and exogenous phytase on amino acid digestibility in poultry: a review. J. Poult. Sci. 43, 89–103.

Storebakken, T., Shearer, K.D., 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*. Aquaculture 161, 365–379.

Sugiura, S.H., Gabaudan, J., Dong, F.M., Hardy, R.W., 2001. Dietary microbial phytase supplementation and the utilization of phosphorus, trace minerals and protein by rainbow trout *Oncorhynchus mykiss* (Walbaum) fed soybean meal-based diets. Aquac. Res. 32, 583–592.

Tengjaroenkul, B., Smith, B.J., Caceci, T., Smith, S.A., 2000. Distribution of intestinal enzyme activities along the intestinal tract of cultured Nile tilapia, *Orechromis niloticus* L. Aquaculture 182, 317–327.

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

Vielma, J., Ruohonen, K., Gabaudan, J., Vogel, K., 2004. Top spraying soybean meal-based diets with phytase improves protein and mineral digestibilities but not lysine utilization in rainbow trout, *Oncorhynchus mykiss* (Walbaum). Aquac. Res. 35, 955–964.

Yan, W., Reigh, R.C., 2002. Effects of fungal phytase on utilization of dietary protein and minerals, and dephosphorylation of phytic acid in the alimentary tract of channel catfish *Ictalurus punctatus* fed an all-plant-protein diet. J. World Aquac. Soc. 33, 10–22.