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Effect of Varied Levels of Lysine Supplemented with Phytase on the Growth, Nutrient Digestibility, Phosphorus and Nitrogen Load of all Male Nile Tilapia, *Oreochromis Niloticus*

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Abstract

Phytate can chelate amino acids in plant feedstuffs and with lysine being the first limiting amino acid in wheat meals that can replace fish meal in low cost and environmentally friendly fish feed production. The study evaluated the effects of different levels of lysine (Lys-HCl) and a dose of phytase (0.2 g kg^{-1} diet) in wheat meal based diets on the production of all male Nile tilapia. Six treatments (T1-T6) were used including, diet 1 the control with no phytase supplement, and diets 2-6 supplemented with (0.2 gkg^{-1}) phytase; 2.96 g Lys kg^{-1}; 2.96 g Lys kg^{-1} + (0.2 gkg^{-1}) phytase; 5.82 g Lys kg^{-1}; and 5.82 g Lys kg^{-1} + (0.2 gkg^{-1}) phytase, respectively. The diets were fed to the fish for 56 days. Fish grew well from the initial weight of 6.75g to weight gain of 25.1[c], 27.4[c], 32.3[bc], 35.2[ab], 37.4[ab] and 40.5[a] g in treatments 1-6, respectively, increasing the weight gain by between 73 and 84%. The trend showed increasing performance with increasing levels of dietary Lys. Treatments 4, 5 and 6 produced the same (P>0.05) growth performance which was higher (P<0.05) than the performance from fish in T1 and T2. Fish in T1, T2 and T3 had the same growth performance suggesting availability of same levels of Lys. Lysine reduced P, N and ash loadings by between 6.23 and 19.6%; 7.1 and 7.6% and 5.93-30.2%, respectively. The study demonstrated that phytase liberated more Lys in the diets which improved fish growth performance and nutrient utilization; and that when Lys is sufficient in the diets, supplementation of phytase may not be necessary.

Keywords: Growth, Nutrient Digestibility, Tilapia, Fish, Treatments.

Introduction

In the face of declining capture fisheries production due to forces of overfishing, climate change and environmental pollution, aquaculture remains the only viable alternative to increasing fish production to feed the world. Meanwhile, aquaculture must be responsible in regulation of phosphorus and nitrogen discharges, environmental pollution; and production of affordable food fish and fish products. Fish meal as the conventional animal protein used in fish diets (Sugiura et al.

2001), is scarce and expensive and sometimes high quality product is not guaranteed. Cho and Bureau (2001) stated that limited supply and increased price of fishmeal for fish feeds has becomes a driving force for substitution strategies. Besides, fish meal contains undigestible phosphorus (P) that is leached into the water environment with pollution problems. Because of high costs of fish meal and pollution issues, minimal quantity is suggested to be incorporated into fish feeds, and where possible to produce fish feed without fish meal (Oishi et al, 2010). Plant proteins may not serve as complete alternative to fish meal but can be used in partial replacement of fish meal to reduce the total costs of feeds in fish production. Incidentally the plant feed ingredients are deficient in essential amino acids; and over 70% of the phosphorus (P) in them occurs in the form of phytate which is not bio-available for fish utilization. Phytate also chelates the divalent metals (K, Mg, Ca, Mn, Fe, Zn, Lopez et al., 2002); proteins and amino acids (Sugiura et al. (2001) and make them unavailable for fish. Replacing fishmeal with plant feed ingredients, there would be need to grapple with the fact that they are limiting in essential amino acids needed for fish feed and nutrition. Lysine is the first limiting amino acids in feed ingredients such as wheat gluten meal, wheat bran, wheat flour and barley that are regularly used in fish nutrition. Similarly, not all lysine in protein is biologically available, since some are linked to sugars or other amino acids through their side chain amino group which are not hydrolyzed by digestive enzymes (Bender and Bender 2005). Diets deficient in lysine can slow down protein synthesis and result in poor growth and repair of muscle tissue (Longe 2005). Rostagno et al., (1995) and Koch et al. (2016) stated that missing the requirements of just one limiting EAA can result in drastically poorer performance of an alternate diet. Ahmed and Khan (2005) and Zhao et al.(2012) reported that histidine deficiency reduced growth and feed conversion ratio in *Cirrhinus mrigala* and Jian carp respectively. Therefore the mechanism for the realization of the laudable concept of production of low cost and environmentally friendly diets from plant feed ingredients must apply the means for balancing the deficient individual essential amino acids and the hydrolysis of the phytate in them to liberate the phytate P and other bound minerals needed for improved fish growth performance. This project was then designed to determine the effect of supplemental Lysine (Lys-HCl) and phytase (Natuphos) to high plant feed diets on the production of all male Nile tilapia *Oreochromis niloticus*. The main objective was to test if inclusion of phytase in the diets could liberate more lysine in the treatments with deficient or low Lys contents so that such treatments can produce the same results as in the treatments with sufficient Lys contents. And to determine the relevance of phytase when supplemented to diets sufficient in lysine.

Materials and Methods

Experimental conditions

Studies were conducted in a semi-closed in-door water re-circulating system with 18 circular plastic tanks (320 L per tank). Each tank was continuously supplied with a mixture of freshwater (approximately 10%) and biologically filtered water. Water temperature (28 ±0.2°C) and photoperiod (12 h light: 12 h dark) were regulated. Water quality parameters were established weekly, and observed within the range reported by Plumb (1999). All male juvenile *O. niloticus*, originating from the lake Manzala (Egypt) population, were obtained by mating yy-males with normal females (Kronert et al. 1989; MullerBelecke and Horstgen-Schwark 2000) at the Institute for Animal Husbandry and Genetics of Goettingen University. Fish were acclimatized to experimental conditions for 2 weeks, using a standard feed. Following this period, the fish were individually weighed and selected according to similar body weight (BW) at the start of the experiment (BW 6.75-6.77 g). Three replicate groups for each diet (20 fish per tank) were utilized in a 56-day growth experiment. Fish were fed three times a day by hand feeding until apparent satiation.

Experimental diets

Diet formulation was according to the recommendations for Nile tilapia (Santiago and Lovell 1988; NRC 2011). Six diets of 33% crude protein each were prepared using the ingredients in Table 1. Diet 1 was deficient or contained minimal Lys; Diet 2 was deficient in Lys but supplemented with 0.2 g kg^{-1} phytase; Diet 3 contained low Lys; Diet 4 contained low Lys but was supplemented with 0.2g kg^{-1} phytase; Diet 5 contained sufficient Lys while Diet 6 contained sufficient Lys and supplemented with 0.2g kg^{-1} phytase. In diets 2, 4 and 6, the same quantity of phytase (0.2 g kg^{-1} diet) was added. TiO2 was added at 3g kg^{-1} diet to prepare diet for digestibility study. Diet mixing utilized precision laboratory systems (Loedige Co., Paderborn, Germany). Mixtures were moistened with 150 g $kg1^{-1}$ distilled water, pelleted (Lister-Petter Co., Gloucestershire, UK) to 2.2 mm granules at a temperature below 45 oC and dried in a ventilated oven at 50 oC for 24 h.

Parameters of the growth studies

Calculation of the performance data was according to Takeuchi (1988) and Tacon (1990). At the end of the experiment which lasted for 56 days, fish were counted and weighed. The growth parameters and feed utilization indices were calculated as follows: Weight gain = Final wt. – initial wt. Specific growth rate (SGR) = 100 (Ln W_2 – Ln W_1) / T; where W_1 and W_2 are the initial and final weight, respectively, and T is the number of days in the feeding period; Feed conversion ratio (FCR) = Feed intake (g)/Weight gain (g).

Digestibility study

Digestibility was conducted by sedimentation using titanium dioxide (TiO_2) as marker according to the established procedure at the Department of Animal Nutrition and Physiology, Georg-August-University, Goettingen, Germany (Liebert and Portz 2007). The faecal samples were pooled for each treatment and analyzed for dry matter, crude protein, ash, P, N and titanium dioxide contents. Apparent digestibility coefficient (ADC) of nutrients was estimated as follows, according to the methods of (Furukawa and Tsukahara, 1966).

$$ADC\ 10^2 - \left(100 \times \frac{\%\ \text{titanium dioxide in diet}}{\text{titanium dioxide in faeces}} \times \frac{\%\ \text{nutrient in faeces}}{\%\ \text{nutrient in diet}}\right)$$

Sample collection

At the beginning of the experiment, 10 fish were sampled, processed and analysed for body composition. After finishing the experiment, three fish per tank were killed by anaesthetic overdose (ethylene-glycol-monophenyl-ether), autoclaved (110 oC, 3 h), mixed (laboratory mixer) and stored at -20 oC for subsequent analyses Hanau, Germany). A nitrogen auto-analyzer (LP-2000; LECO Instrument, Kirchheim, Germany) was utilized for crude protein determination, using the Dumas method, Netheler-Hinz, Hamburg, Germany) following acid hydrolysis with and without an oxidation step. Ether extract was determined due to extraction with petroleum ether according to the Soxhlet procedure.

Analytical procedures

Chemical analyses of feed ingredients, diets and fish were run according to German standard methods (Naumann and Bassler 1976–1997). Dry matter determinations used an oven at 110 °C (Memmert, Schwabach, Germany) until constant weight; crude ash was detected by 4 h ashing at 600 °C in a furnace muffle (Thermicon P; Heraeus Holding, (N * 6.25). Amino acid analyses (except tryptophane) of the diets were run by ion exchange chromatography (LC 3000; Biotronik, Eppendorf). Experimental data were statistically analysed as one-way completely randomized design, submitted to ANOVA ($P = 0.05$) using the Duncan test within the statistical package of SAS/STAT (1986) software.

Results

Table 2 presents the chemical composition of the experimental diets which highlighted that addition of lysine naturally increased the lysine levels, and so also the Met levels in the diets. The dry matter contents of the diets are high and other parameters remained almost the same.

In Table 3 is the growth and nutrient utilization of the fish fed diets supplemented with Lys and phytase. The mean weight gain was the same ($P>0.05$) in treatments 1 and 2 with lysine deficiency irrespective of treatment 2 being

Table 1. Gross composition of experimental diets (g kg^{-1} DM)

	Diet 1	Diet 2	Diet 3	Diet 4	Diet 5	Diet 6
Feed ingredients						
Fish meal	100	100	100	100	100	100
Wheat gluten	170	170	170	170	170	170
Corn gluten	110	110	110	110	110	110
Soybean meal	90.0	90.0	90.0	90.0	90.0	90.0
Wheat	165	165	165	165	165	165
Corn	210	210	210	210	210	210
Oil (fish:soy)	40.0	40.0	40.0	40.0	40.0	40.0
Premix	10.0	10.0	10.0	10.0	10.0	10.0
CMC	20.0	20.0	20.0	20.0	20.0	20.0
DL-Met	2.00	2.00	2.00	2.00	2.00	2.00
L-Thr	0.35	0.35	0.35	0.35	0.35	0.35
L-LysHCl	0.00	0.00	2.86	2.86	5.72	5.72
Phytase (g/kg)	0.00	0.20	0.00	0.20	0.00	0.20
TiO_2	3.00	3.00	3.00	3.00	3.00	3.00
Wheat starch	79.65	79.45	76.79	76.59	73.93	73.73

1 Mixture of soybean oil and fish oil (1 : 1 w/w). 2. Vitamin and mineral mix (provided per kg of diet): MnSO4, 40 mg; MgO, 10 mg; K2SO4, 40 mg; ZnCO3, 60 mg; KI, 0.4 mg; CuSO4, 12 mg; ferric citrate, 250 mg; Na2SeO3, 0.24 mg; Co, 0.2 mg; vitamin A, 4000 IU; vitamin B6, 30 mg; vitamin D3, 400 IU; vitamin E, 400 mg; vitamin B12, 80 l g; vitamin B1, 30 mg; vitamin B2, 40 mg; vitamin K3, 12 mg; folic acid, 10 mg; biotin, 3 mg; pantothenic acid, 100 mg; inositol, 50 mg; ascorbic acid, 500 mg.

Experimental diets were formulated to have minimal or to be deficient in lysine (Lys-HCl) (diets 1 and 2); to have low lysine (diets 3 and 4) and to have sufficient lysine (diets 5 and 6) Diets 1-6 make up treatments 1-6. Diets 1 and 2 are the same, but phytase was added to diet 2 to see if that could liberate more lysine for fish growth and physiological functions. Diets 3 and 4 are the same that contained low lysine, but phytase was added to diet 4 to understand whether that could liberate more lysine in comparison with the lysine sufficient diets. Diets 5 and 6 are the same that are sufficient in lysine, but phytase was supplemented to diet 6 to observe any additional advantage over diet 5.

supplemented with phytase, showing that when lysine is insufficient, phytase cannot work. Supplemental Lys marginally increased the mean weight gain in treatment 4 over the value in treatment 3 with the same low Lys content. This is an improvement in the effect of phytase when compared with the effect on the treatment with minimal or deficient Lys content. The trend observed in treatments 5 and 6, showed that with lysine sufficient diet, addition of phytase may not be necessary. Comparing the mean weight gain of fish in treatments 4 5 and 6, it is clear that low lysine diet supplemented with phytase is as good as a lysine sufficient diet, a good evidence that phytase capably liberated more lysine in the diet. This also means that phytase was able to liberate about 2.86g kg^{-1} of Lys in diet 4 to meet up with effect of 5.72 g kg^{-1} Lys in diets 5. The trend of the result of the mean weight gain of the fish is the same with the specific growth rate (SGR). Food conversion ratio (FCR) was the same in treatments 1 and 2 with lysine deficiency despite

Table 2. Chemical composition of experimental diets (% DM)

	Diet 1	Diet 2	Diet 3	Diet 4	Diet 5	Diet 6
Dry matter	94.1	92.7	93	92.9	93	93.1
Ash	5.39	5.26	5.25	5.16	5.1	5.19
Nitrogen	6.13	6.09	6.03	6.04	6.11	6.04
Protein	38.3	38.1	37.7	37.8	38.2	37.8
Lipid	8.37	8.37	8.37	8.37	8.37	8.37
Crude fibre	1.89	1.88	1.87	1.88	1.87	1.89
% phytate-P	0.20	0.20	0.20	0.20	0.20	0.20
% phytate	0.81	0.81	0.81	0.81	0.81	0.81
Total Phosphorous (%)	0.61	0.59	0.58	0.56	0.58	0.69
Calcium (%)	0.76	0.77	0.78	0.79	0.78	0.79
Methionine	2.21	2.22	2.45	2.51	2.40	2.37
Lysine	2.96	2.97	3.49	3.57	6.50	6.70

Table 3. Growth and nutrient utilization of all male Nie tilapia fed experimental diets

	Diet 1	Diet 2	Diet 3	Diet 4	Diet 5	Diet 6
Initial mean wt (g)	6.75	6.75	6.75	6.75	6.75	6.75
Final mean wt (g)	31.9^{c} ±1.11	34.1^{c} ±4.95	39.0^{bc} ±1.57	42.0^{ab} ±4.92	44.1^{ab} ±3.39	47.3^{a} ±0.51
Mean wt gain	25.1^{c} ±1.11	27.4^{c} ±4.95	32.3^{bc} ±1.57	35.2^{ab} ±4.92	37.4^{ab} ±3.39	40.5^{a} ±0.51
SGR	2.77^{d} ±0.06	2.87^{cd} ±0.26	3.13^{bc} ±0.07	3.26^{ab} ±0.20	3.35^{ab} ±0.14	3.48^{a} ±0.02
Feed intake/fish	10.6^{b} ±0.43	11.1^{b} ±0.12	14.0^{ab} ±3.75	15.2^{a} ±2.31	17.6^{a} ±0.20	17.7^{a} ±1.81
FCR	1.30^{a} ±0.05	1.27^{a} ±0.02	1.22^{a} ±0.13	1.21^{a} ±0.03	1.21^{a} ±0.03	1.19^{a} ±0.02

supplementing phytase to treatment 2, another confirmation that phytase did work on diet deficient in Lys. Again treatment 3 had the same (P>0.05) FCR with that of treatment 4 despite supplementing phytase to treatment 4. The same trend was also observed in treatments 5 and 6.

Table 4 contains the apparent digestibility coefficient of the fish fed experimental diets. Dry matter, protein and nitrogen (N) digestibility were closely related in all the treatments showing no effects of lysine or phytase. Ash digestibility was higher in other treatments than in the treatments 1 and 2 with Lys deficiency.

Table 4. Apparent digestibility coefficient of all male Nile tilapia fed experimental diets

	Diet 1	Diet 2	Diet 3	Diet 4	Diet 5	Diet 6
Dry matter	71.2^{a} ±1.02	71.3^{a} ±1.00	65.3^{ab} ±2.11	65.4^{ab} ±1.67	63.0^{ab} ±2.01	63.1^{ab} ±1.56
Ash	16.6^{c} ±1.65	16.7^{c} ±2.02	24.4^{a} ±0.87	24.5^{a} ±2.42	22.9^{ab} ±2.34	23.0^{ab} ±2.10
Nitrogen	86.7^{a} ±0.88	86.8^{a} ±0.67	85.3^{a} ±1.02	85.4^{a} ±0.98	85.5^{a} ±1.22	85.7^{a} ±2.01
Protein	86.8^{a} ±2.24	86.9^{a} ±3.02	85.3^{a} ±4.03	85.4^{a} ±3.43	85.3^{a} ±4.61	85.5^{a} ±4.48
Total Phos (%)	15.8^{a} ±1.32	15.7^{a} ±2.04	16.7^{a} ±1.64	16.6^{a} ±1.48	15.1^{ab} ±1.76	15.2^{ab} ±2.00

Table 5. Chemical composition of fish before and after the experiment

	Start	Diet 1	Diet 2	Diet 3	Diet 4	Diet 5	Diet 6
Dry matter	23.5	27.1^{a}± 2.00	26.4^{a} ±2.01	26.2^{a} ±1.86	26.9^{a} ±2.11	26.3^{a} ±1.98	25.9^{a} ±2.11
Ash	16.3	11.5^{a}± 1.23	11.2^{a} ±0.89	11.2a ±0.89	11.2^{a} ±1.08	10.6^{a} ±1.65	10.5^{a} ±1.72
Nitrogen	9.72	8.59^{a}± 0.76	8.44^{a} ±0.87	8.93^{a} ±1.01	8.68^{a} ±2.01	9.14^{a} ±1.32	9.39^{a} ±0.78
Protein	60.7	53.7^{a}± 3.66	52.7^{a} ±3.86	55.8^{a} ±4.01	54.2^{a} ±3.54	57.1^{a} ±4.11	58.7^{a} ±4.00
Total Phos (%)	2.94	2.00^{a}± 0.23	1.92^{a} ±0.26	1.94^{a} ±0.16	2.14^{a} ±0.15	1.74^{ab} ±0.54	1.72^{ab} ±0.59
Ca (%)	4.91	3.31^{a}± 0.58	3.47^{a} ±0.65	2.99^{a} ±0.32	3.04^{a} ±0.44	2.87^{ab} ±0.65	2.76^{ab} ±0.76

The carcass composition of the fish before and after feeding them with the experimental diets is given in Table 5, which indicated that apart from the lower dry matter in the initial fish sample, the ash, N, CP total P and Ca composition of the initial fish sample were marginally higher than the values after the feeding trials. Then the values of the carcass DM, ash, N, CP, P and Ca of fish in all the treatments were very closely related. Similarly, while phytase appeared to improve Ca deposition in fish fed lysine deficient or low lysine diet, phytase did not affect the Ca deposition in the fish fed high or sufficient lysine diet. This is an indication that phytase actually liberated more Ca in those diets leading to corresponding deposition in the fish fed the diets. It also shows that supplemental phytase may not be necessary when Lys is sufficient in the diet.

The effects of the experimental diets on the P and N load in the Nile tilapia culture systems are presented in figures 1 and 2, which indicated that

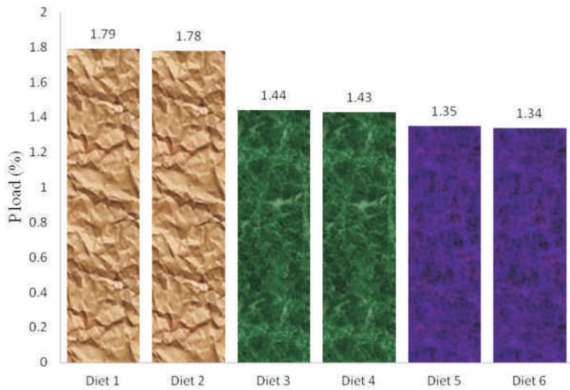

Figure 1. Faecal P load of Nile tilapia fed diets supplemented with Lys and phytase

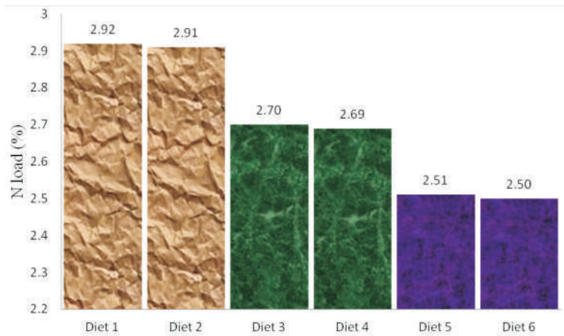

Figure 2. N load of Nile tilapia fed diets supplemented with Lys and phytase

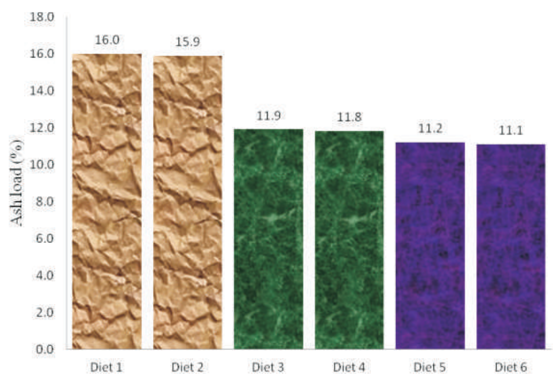

Figure 3. Faecal ash load of Nile tilapia fed diets supplemented with Lys and phytase

P and N loadings reduced with increasing levels of Lys. In reducing faecal P loadings, the effect of diet 5 sufficient in Lys and the same diet 5 supplemented with phytase were the same, suggesting that it is not necessary to supplement phytase. Treatment 3 low in Lys produced the same faecal P load as when the same treatment 3 or (D4) was supplemented with phytase. In treatments 1 and 2 with lysine deficiency, phytase did not affect the P loading. This result also explains that supplemental phytase is not necessary either when the diet is deficient or sufficient in Lys. Similar explanation is applicable to faecal N load. In treatments 3 and 4 with low lysine, and phytase, the N load was reduced to the same level. Treatments 5 and 6 with high or sufficient Lys reduced the faecal N load more than treatments 3 and 4 with low lysine. So far the results explained that when the lysine requirement of the fish is met/sufficient, that supplementation of phytase does not have any additional advantage in terms of reduction of P and N loadings in the environment.

The effect of the experimental diets on ash leachate into the environment is presented in Figure 3 which clearly showed that ash leachate reduced with increasing levels of Lys in the diets. For instance, Lys reduced ash leachate by about 13.2% in comparison with treatments 5 and 3. Similarly, in comparison with treatments 1 and 3, lysine reduced ash leachate by 25.8%; while in relation to treatments 1 and 5, Lys reduced ash leachate by 30.2%.

In summary, this study revealed that when there is minimal or enough Lys-HCl in the diet, supplemental phytase is not necessary.

Discussion

The study determined the effects of supplementing lysine (Lys) and phytase to diets with minimal, low and sufficient Lys content on the growth performance and nutrient load of all male Nile tilapia (*Oreochromis niloticus*). Observation on the growth performance showed that phytase had no effect on the diet with minimal Lys, but improved growth performance by 30% in the diet with low Lys level. Similarly, addition of phytase to low Lys diet produced the same performance as when the fish was fed diet sufficient in Lys, indicating that phytase might have liberated more Lys in the diet with low Lys. Similarly, phytase improved growth marginally by 13% in the treatment sufficient with Lys. The ability of Lys in the present study to significantly improve the growth of all male Nile tilapia supports the works of Cheng et al. (2003) on rainbow trout (*Oncorhynchus mykiss*) fed different levels of Lys diets; and Deng et al. (2014) and Jiang et al. (2015) who disclosed that leucine has beneficial effects on intestinal antioxidant capacity and structural integrity, and thus improved growth and feed efficiency in grass carp (*Ctenopharyngodon idella*). Similarly, Nwanna et al. (2012) reported that addition of DL-Met to a methionine deficient diet significantly increased feed consumption with better conversion ratio which culminated into significant improvements in weight gain and feed efficiency of common carp (*Cyprinus carpio*). Gao et al, (2016) also described linear relationship in the growth of juvenile grass carp *Ctenopharyngodon idella* fed increasing levels of dietary histidine. In Ren et al. (2015) isoleucine significantly increased the growth of juvenile blunt snout bream *Megalobrama amblycephala*, just as Mukhopadhyay and Ray (2001) and Sardar et al. (2009) showed that dietary Met+Cys significantly improved weight gain, specific growth rate and feed gain ratio in Rohu (*Labeo rohita*) fingerlings. Supplemental phytase improved the growth performance of fish in the present study by increasing the dietary Lys levels. Positive impact of phytase on growth of fish has also been reported by a number of authors including Papatryphon and Soares (2001) in striped seabass, Vielma et al. (2000) in rainbow trout, Debnath et al. (2005) in Pangus catfish, Liebert and Portz (2005) in Nile tilapia, Nwanna et al. (2005; 2007) in common carp and Baruah et al. (2007) in rohu. This is attributed to ability of the phytase to degrade phytate in the diets and release the bound nutrients that promoted feed consumption, feed efficiency and the growth of the fish. Reduction in the growth and feed conversion ratio of fish fed amino acids deficient diets have been observed in several fish species, such as in chum salmon (Akiyama et al., 1985), in Labeo rohita (Murthy and Varghese, 1995), in *Cirrhinus mrigala* (Ahmed and Khan, 2005) and in Jian carp (Zhao et al.,2012). These observations are in line with the results from the present study, and the reason may be as discussed by (Deng et al.; 2014; Jiang et al., 2015) that amino acids have beneficial effects on intestinal antioxidant capacity and structural integrity, which thus improved growth and feed efficiency in fishes. Ahmed and Khan (2006); Khan and Abidi (2007); Di et al. (2009) and Zhao et al. (2012) have also demonstrated that isoleucine deficiency resulted in reduced growth performance and feed utilization in several fish species. Similarly, Liebert and Benkendorff (2007) explained that diets limiting in Threonine provided significantly lower effects on final body weight, specific growth rate and feed intake of Nile tilapia, *O. niloticus*.

Apparent digestibility of the dry matter, N and protein was closed related in all treatments indicating little or no influence of either Lys or phytase. Nwanna et al. (2012) reported that supplemental DL-Met marginally improved the organic matter digestibility in common carp, but in the present study dry matter digestibility was marginally higher in the fish fed diet with minimal Lys content than in those fed higher levels of Lys. The findings from the present study supports the report of (Mambrini et al. 1999) which stated that dietary methionine did not affect protein digestibility in common carp; but is in disagreement with the observation that dietary methionine significantly improved protein digestibility in common carp (Nwanna et al. 2012).

In the carcass composition of the present experiment, supplemental Lys and phytase had marginal effects on the ash, N, protein and total P deposition. This is unusual because phytase is known to greatly improve such nutrients. The data also showed a slight increase in Ca concentration as a result of increasing levels of Lys in the diets with minimal and low levels of Lys. Whole-body protein content is of particular concern in nutritional studies because of its association with product quality (Zehra and Khan, 2014). Ren et al. (2015) established that whole-body protein content of juvenile blunt snout bream significantly increased as dietary isoleucine levels increased up to a certain level and thereafter decreased. Khan and Abidi (2007) and Zehra and Khan (2013) have reported similar observations with catla carp and rohu carp respectively. However, these observations are contrary to the ones from the present study which revealed just marginal variations in the protein levels deposited in all male Nile tilapia. In Nwanna et al. (2012) supplemental DL-Met raised the dietary methionine content to 0.86% and significantly increased carcass protein and invariably the carcass quality. Bureau et al. (2000) also stated that leucine supplementation improved both protein and fat contents in the muscle of fish and shrimps, indicating that leucine has beneficial effects on the accretion of nutrients in the muscle. Gao et al. (2016) expressed that whole body protein and muscle protein in juvenile grass carp *Ctenopharyngodon idella* were not significantly affected by the increasing histidine levels, thus also supporting the findings from the present study.

Li et al. (2004) explained that discharge of high levels of soluble P from fish culture systems into open water environment stimulate phytoplankton growth, resulting in wide fluctuations in water quality parameters. The trend of nutrients loading from the present experiment depicted that Lys reduced P loading by between 6.23 and 19.6% as represented in treatments 3 and 5; and 2 and 4 respectively. However, the impact of supplemental phytase was not appreciable. The fundamental effect of phytase in high plant diets is reduction of P loadings (Vielma et al. 2002; Sajjadi and Carter 2004; Nwanna and Schwarz 2005; Nwanna et al. 2007). But the inability of phytase to perform this function in the present study could mean that the treatments had enough Lys, therefore additional effect of phytase could not reflect. This same observation was made with the growth performance of fish in the present study that when dietary Lys was sufficient, then no additional effect of phytase was observed.

Nitrogen (N) is a part of amino acids (AAs) that form *proteins* and all animals consume protein and AAs and then excrete various forms of N (Kumar et al. 2012). In the present study, Lys marginally reduced N loading by between 7.1 and 7.6 as in treatments 5 and 2, and 3 and 1, respectively. Also the effect of phytase was not recognizable. Liebert and Benkendorff (2007) expressed that dietary amino acid supply, according to the physiological need, improves feed efficiency, conversion of feed proteins into edible feed resulting in minimization of N pollution. This is in agreement with results from the present study.

In the present study, Lys reduced ash leachate by between 5.93 and 30.2% and there was no visible effect of phytase. Improper diet formulation leads to poor nutrient digestibility and utilization resulting to increase in nutrient load into the environment (Di et al. 2009 and Zhao et al. 2012). In the present study, In another study, Storebaken et al. (2000) attributed decreasing whole body ash and increasing ash load to potentially decreasing mineral uptake and lower P availability from diet ingredients that were either high in fiber or phytate content. Neto and Ostrensky (2015) stated that nutrient deficiency or imbalance caused incomplete diet utilization in Nile tilapia and led to increased nutrient leachate and eutrophication of adjacent water body. Schneider et al. (2004) made a similar observation with respect to whole body ash in Nile tilapia fed five alternative feed ingredients in practical diets.

Conclusion

Supplemental phytase has the ability to liberate more Lys in diets. However, when dietary Lys is sufficient, supplemental phytase does not produce any additional advantage with regard to fish growth promotion and reduction in nutrient leachate in the environment.

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