

The Growth and Mineral Utilization of *Clarias Gariepinus* Fingerlings Fed Phytase-Supplemented Toasted Lima Bean (*Phaseolus lunatus*) Diets

Orisasona O* and Ajani EK

Department of Aquaculture and Fisheries Management, University of Ibadan, Ibadan, Nigeria

Abstract

Toasting causes slight reduction in the phytate content of Lima bean, thus requiring further treatment for effective utilization when incorporated into fish feed. The growth, mineral utilization and liver response of *Clarias gariepinus* fingerlings fed Toasted Lima Bean Meal (TLBM) diets supplemented with phytase was investigated. An isonitrogenous diet (40% crude protein) was formulated with TLBM as a plant protein source. Phytase was added to diets post pelleting at 0 FTU (F1), 2500 FTU (F2), 5000FTU (F3), 7500FTU (F4) and 10,000FTU (F5). Feed were fed to triplicate groups of 15 fish ($1.43 \text{ g} \pm 0.0012 \text{ g}$) stocked in 25 liter capacity plastic tanks, cultured at a mean dissolved oxygen, pH and temperature of 5.37mg/l, 7.2 and 25.8°C respectively for 56 days. Mean weight gain and Feed conversion Ratio (FCR) were significantly ($P < 0.05$) higher in fish fed diets F3 and F4. Fish fed diets F4 had the highest Specific Growth Rate (SGR) 3.31, Protein Efficiency Ratio (PER) 1.78 and least FCR 1.41. The control (F1) gave the lowest SGR 2.79, PER 1.57 and highest FCR 1.6. There is increased phosphorus utilization with increased enzyme inclusion. Bone ash and phosphorus of fish showed mark increase with increasing level of phytase. Histopathological examination of fish revealed no negative effect of phytase on the liver of fish.

Keywords: *Clarias gariepinus*; Phytase; Lima bean; Phosphorus utilization

Introduction

Aquaculture is growing more rapidly than all other animal food-production sectors [1]. The current increase can be partly attributed to the wide availability and utilization of aqua-feeds, which has a growth rate in excess of 30 percent per year [2]. This growth is highly based on the utilization of fishmeal and fish oil, which contributes to the increasing cost of fish feed. The major challenge facing aquaculture industry is to identify economically viable and environmentally friendly alternatives to fishmeal and fish oil on which many present aqua-feeds are largely based [3]. The viable utilization of plant feedstuffs is therefore an essential requirement for future development of aquaculture.

Lima bean is an important source of plant protein, with a production capacity in excess of 2000 kg/h reported [4]. It is well cultivated all across Nigeria and some African countries [5]. The utilisation of Lima bean in feed production like any other plant protein ingredient, is however limited with the presence of some anti-nutritional factors, notably phytate. Over 60% of phosphorus in plant feed stuffs are in the form of phytate [6], which is not available to monogastric animals because they lack intestinal phytase for its digestion. Inorganic phosphorus is thus required to be supplemented in diets to prevent deficiency. In addition to increasing cost of feed, the excretion of excess phosphorus in fish waste is also a major concern.

A number of processing methods have been employed to improve nutrient availability and utilization in fish, in order to expand the ingredients base for fish feed. Orisasona et al. [7] observed that Boiled Lima Bean Meal (BLBM) successfully replace 50% SBM in the diets of *C. gariepinus*, though; boiling only slightly reduced the phytate content of BLBM. Other method(s) to ensure the complete elimination of phytate is therefore required. A method currently applied to overcome the challenge of phytate, is the use of phytase in breaking down the phytate-nutrient complexes to enhance release for the target organism [8]. Phytase have been applied into diets to liberate phytate phosphorus and make more utilizable phosphorus available for fish growth. Van Weerd et al. [9] reported a positive effect of phytase treatment using

Soy bean meal (SBM), particularly on phosphorus digestibility, retention and consequently its conversion efficiency and budget in African catfish. In the report, utilization was no however affected. This is however contrary to the observations of Rodehutsord and Pfeffer [10] and Schaeffer et al. [11] where positive effect of phytase on phosphorus utilization for rainbow trout and common carp were reported. A combination of some processing methods and phytase treatment to improve efficacy on SBM have been reported [12].

However, information on the combination of processing and phytase supplement on LBM is inadequate; therefore, this study was conducted to determine the effects toasted lima bean meal diets supplemented with phytase on the growth performance and mineral utilisation of *Clarias gariepinus* fingerlings.

Materials and Methods

Diet preparation

Lima bean seeds were sundried for 3 days and a portion of it heated at 240°C for 15 minutes in an oven. The Raw and the toasted beans were milled into fine particles. Raw (RLBM) and toasted (TLBM) meals were analysed for proximate composition (Table 1). A 40% crude protein (CP) diet was prepared using TLBM and other ingredients including fish meal, yellow maize, wheat offal and vitamins premix (Table 2). Finely ground ingredients were mixed thoroughly in a Hobart A200 (Troy Ohio USA) pelleting machine. The homogenized masses were

*Corresponding author: Orisasona O, Department of Aquaculture and Fisheries Management, University of Ibadan, Ibadan, Nigeria, Tel: +2348186523096; E-mail: osasonagbenga@gmail.com

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Components	Raw	Toasted
% Dry matter	91.74	91.66
% Crude protein	23.56	23.76
% Crude fibre	5.86	4.97
% Ether extract	1.73	1.79
% Ash	3.96	3.91
% Moisture	8.26	8.34
NFE	56.63	57.23
% Calcium	0.47	0.38
% Phosphorus	0.36	0.28
Trypsin Inhibitor (TIU/mg Protein)	32.66	0.00
Tannin (g/kg)	9.12	4.12
Phytate (g/kg)	3.2	2.9

Table 1: Composition of Lima bean (*Phaseolus lunatus*).

Ingredient	DIETS				
	F1	F2	F3	F4	F5
Fishmeal	36.31	36.31	36.31	36.31	36.31
Lima bean meal	54.46	54.46	54.46	54.46	54.46
Yellow maize	2.06	2.06	2.06	2.06	2.06
Wheat offal	4.17	4.17	4.17	4.17	4.17
Vitamin premix	1.0	1.0	1.0	1.0	1.0
Oil	0.5	0.5	0.5	0.5	0.5
Salt	0.5	0.5	0.5	0.5	0.5
Binder	1.0	1.0	1.0	1.0	1.0
Total	100	100	100	100	100
Phytase (g)	0	0.5	1.0	1.5	2.0
Proximate composition %					
Crude Protein	39.93	39.95	39.81	39.82	39.90
Ether extract	3.89	3.91	3.91	3.92	3.90
Crude fibre	6.22	6.21	6.22	6.18	6.20
Ash	7.69	7.70	7.60	7.75	7.79
NFE	32.99	32.98	33.18	33.13	32.94
Phosphorus (mg/g)	4.12	4.31	4.67	4.40	3.94

Table 2: Gross and proximate composition level of experimental diets.

pelletized using starch paste as binder through a 2 mm die. Diets were divided into five (F1, F2, F3, F4 and F5) and 0.5, 1.0, 1.5 and 2.0 g/kg feed of granulated phytase {Ronozyme P 5000 (CT)} from *Peniophora lycii* were each dissolved in 40 ml of distilled water and sprayed on the diets (F2, F3, F4 and F5 respectively), while F1 (Control) was sprayed with 40ml of distilled water to ensure uniform moisture. Feed were sundried for three days, packed in separate polythene bags and labeled. Feeds were kept in a refrigerator until needed.

Experimental fish and culture condition

A total of 225 *C. gariepinus* (1.43 g ± 0.0012 g) obtained from a reputable fish farm were acclimatized for 7 days in Department of Aquaculture and Fisheries Management laboratory, University of Ibadan. Fifteen fish were randomly stocked into triplicate plastic aquaria (60 cm × 40 cm × 40 cm) for each of the five treatments. Fish were fed at 5% body weight. Feed was divided into two equal parts; these were fed to the fish twice daily between 8.30 hr-9.00 hr and 17.30 hr-18.00 hr, for 56 days. Feed adjustments were made biweekly, after weighing of experimental fish. Water temperature, dissolved oxygen and pH were monitored weekly. Growth and nutrient utilisation were calculated according to Castell and Tiews [13].

Chemical analysis

Raw and toasted Lima bean meals, diets and fish (whole body) were analyzed for proximate and mineral compositions, using methods described by Association of Official Analytical Chemists [14].

Histological preparations

Two fish taken per treatment was decapitated to observe the liver for gross lesions. The livers were subjected to Lynch's laboratory procedures as described by Raphael.

Statistical analysis

Data resulting from the experiment were subjected to one way analysis of variance using the SPSS (Statistical Package Computer, Software 1988 version Chicago Illinois, USA). Duncan's multiple range test was used to compare differences among individual means at P=0.05 [15].

Results

The results of the proximate, mineral and anti-nutritional factors in raw and toasted lima bean meals are presented in Table 3. Crude protein content was slightly higher in the toasted meal, while crude fibre and ash contents were marginally higher in the raw meal. Also there was a slight reduction in calcium and phosphorus content of toasted meal.

Trypsin inhibitor was completely eliminated from 32.66 TIU/mg protein in the raw meal to 0 TIU/mg protein in the toasted meal. However, toasting did not completely eliminate tannin and phytate components. Crude protein content of experimental diets ranged from 39.81 to 39.95% as shown in Table 4.

The carcass, proximate composition and mineral concentrations of experimental fish are presented in Tables 5 and 6. Crude protein was significantly higher (P<0.05) in all experimental fish when compared with the initial value. However, crude protein in F1 group was significantly lower (P<0.05) than other groups.

Phytase caused significant increase (P<0.05) in the ash contents of experimental fish. The lowest ash content of 6.75% was recorded in F1, while the highest values of 7.62% was observed in F4.

Fish fed diets F1 had similar zinc content as the initial that is significantly lower than fish fed phytase supplemented diets. With the exception of the statistical similarity in F1 and F3 groups, calcium was significantly higher and varied in fish fed diets F2, F4 and F5. Carcass manganese ranged from 19.63 to 27.36 µg/g.

Fish in F1 and F2 groups had similar phosphorus concentration, that were significantly lower (P<0.05) than F3, F4 and F5 groups.

The highest mean weight gain of 7.56 g was recorded in F4, with the control diet (F1) recording the lowest value of 5.66 g. Weekly average weight gained in experimental fish is shown in Figure 1. Fish fed diets with phytase-supplementation produced significantly higher (P<0.05) weight gain and SGR when compared with fish fed diet F1. The SGR and PER were better in F3 and F4 groups than other groups.

Bone ash and phosphorus were significantly higher (P<0.05) in treatments with phytase-supplement. Results of the water quality analysis revealed values within the range recommended for the culture of *C. gariepinus*. Liver response showed the moderate presence of macrophage hyperplasia in fish fed diets F1 and F5, while lymphatic

Parameter %	Initial	After Trials/Treatments				
		F1	F2	F3	F4	F5
Crude Protein	50.59 ± 0.00 ^a	51.28 ± 0.03 ^b	53.73 ± 0.00 ^e	51.92 ± 0.04 ^c	54.62 ± 0.00 ^f	52.61 ± 0.32 ^d
Crude fibre	1.83 ± 0.03 ^a	3.73 ± 0.05 ^b	4.95 ± 0.18 ^d	4.78 ± 0.00 ^d	3.97 ± 0.00 ^c	3.62 ± 0.00 ^b
Ether extract	7.22 ± 0.00 ^d	4.77 ± 0.00 ^b	4.92 ± 0.00 ^b	5.14 ± 0.01 ^c	4.38 ± 0.14 ^a	5.26 ± 0.01 ^c
Ash	9.44 ± 0.00 ^f	6.75 ± 0.00 ^a	6.93 ± 0.00 ^b	7.44 ± 0.05 ^d	7.62 ± 0.00 ^e	7.32 ± 0.00 ^c
NFE	20.78 ± 0.00 ^c	22.78 ± 0.00 ^e	20.24 ± 0.00 ^a	20.64 ± 0.00 ^b	20.65 ± 0.00 ^b	22.48 ± 0.89 ^e
Zinc (µg/g)	22.00 ± 0.00 ^a	23.01 ± 0.00 ^a	29.64 ± 0.68 ^d	27.03 ± 0.02 ^c	25.14 ± 0.07 ^b	31.33 ± 0.00 ^e
Magnesium (mg/g)	23.80 ± 0.00 ^d	17.62 ± 0.30 ^a	21.00 ± 0.01 ^c	19.37 ± 0.21 ^b	23.96 ± 0.04 ^d	26.75 ± 0.36 ^e
Calcium (mg/g)	17.31 ± 0.14 ^a	19.15 ± 0.06 ^b	19.67 ± 0.01 ^c	18.90 ± 0.10 ^b	23.97 ± 0.03 ^e	23.59 ± 0.04 ^d
Manganese (µg/g)	19.03 ± 0.03 ^a	19.63 ± 0.27 ^a	27.36 ± 0.37 ^c	21.38 ± 1.67 ^a	21.31 ± 0.24 ^a	25.00 ± 0.01 ^b
Phosphorus (mg/g)	18.51 ± 0.01 ^a	19.890.08 ^b	20.68 ± 0.55 ^b	21.62 ± 0.29 ^c	25.50 ± 0.21 ^d	22.07 ± 0.05 ^c

Means along the same row with same superscript are not significantly different (P>0.05)

Table 3: Proximate and mineral composition of experimental fish before and after feeding trials.

PARAMETERS	DIETS				
	F1	F2	F3	F4	F5
Mean initial weight (g)	1.50 ± 0.00	1.43 ± 0.01	1.43 ± 0.03	1.44 ± 0.02	1.45 ± 0.00
Mean final weight (g)	7.16 ± 0.00 ^a	7.73 ± 0.14 ^b	8.23 ± 0.00 ^c	9.00 ± 0.00 ^d	8.15 ± 0.16 ^c
Mean weight gain (g)	5.66 ± 0.00 ^a	6.30 ± 0.13 ^b	6.80 ± 0.03 ^c	7.56 ± 0.01 ^d	6.70 ± 0.16 ^c
Total feed fed/Fish (g)	9.10 ± 0.00 ^a	9.62 ± 0.03 ^b	9.87 ± 0.03 ^c	10.68 ± 0.02 ^e	10.29 ± 0.08 ^d
Food conversion ratio (FCR)	1.60 ± 0.00 ^c	1.52 ± 0.03 ^b	1.45 ± 0.00 ^a	1.41 ± 0.00 ^a	1.53 ± 0.02 ^b
Specific growth rate (SGR)	3.10 ± 0.00 ^a	3.29 ± 0.03 ^b	3.43 ± 0.00 ^c	3.62 ± 0.00 ^d	3.40 ± 0.04 ^c
Protein efficiency ratio (PER)	1.45 ± 0.00 ^a	1.56 ± 0.00 ^b	1.74 ± 0.01 ^c	1.78 ± 0.00 ^d	1.56 ± 0.00 ^b
% Bone Ash	1.38 ± 0.00 ^a	1.67 ± 0.00 ^b	1.76 ± 0.00 ^d	1.80 ± 0.01 ^e	1.69 ± 0.00 ^c
Bone Phosphorus (mg/g)	47.00 ± 1.00 ^a	50.66 ± 0.88 ^{bc}	50.00 ± 0.57 ^b	53.00 ± 0.57 ^c	50.66 ± 0.88 ^{bc}
Parked cell volume (PCV)	33.33 ± 0.33 ^b	38.40 ± 0.70 ^c	35.03 ± 0.03 ^b	27.30 ± 0.30 ^a	32.66 ± 1.45 ^b
Survival (%)*	96.00	98.00	100.00	98.00	89.00

Means with same superscript on same row are not significantly different (P<0.05)

*No statistical analysis.

Table 4: Growth Performance and Nutrient Utilization of *Clarias gariepinus* Fed Phytase Supplemented Lima Bean.

Parameters	F1	F2	F3	F4	F5	MEAN
Dissolved Oxygen (mg/l)	5.40	5.30	5.44	5.34	5.41	5.37
pH	7.20	7.10	7.20	7.30	7.20	7.20
Temperature (°C)	25.50	26.00	26.00	25.50	26.00	25.80

Table 5: Mean value of water quality parameters in experimental units.

Observations	Treatment				
	F1	F2	F3	F4	F5
Macrophage hyperplasia	+	-	-	-	+
Lymphotic infiltration	+	+	-	+	-
Congestion	-	-	-	+	-

- Not Present

+ Moderately Present

Table 6: Liver Response of Fish to Different levels of Phytase-supplemented Lima Bean diet of *C. gariepinus*.

infiltration moderately occurred in fish in F1, F2 and F4 groups, indicating no particular effect of phytase on organ.

Discussion

The crude protein content of raw and toasted lima bean of 23.56% and 23.76% respectively are similar to results reported by Falaye et al. [16] and Adeparsi and Olute [17]. The complete elimination of trypsin inhibitor by toasting agreed with the report of Ologhobo [18] where heat treatments such as toasting, autoclaving and cooking were shown

to destroy heat-labile anti-nutritional factors. However, toasting only resulted in about 10% reduction in phytate content, which is similar to the 9.3% reduction reported in Falaye et al. [16].

The proximate composition of the experimental fish showed an increase in crude protein with increasing level of phytase enzyme. This may be attributed to improved availability of nutrients caused by phytase activities. This assertion is supported by Nwanna et al. [12] who reported a high crude protein in fish fed soybean meal diet supplemented with 8000 phytase units.

Zinc, magnesium and calcium deposit in fish fed phytase supplemented diets were significantly higher than in F1 group. Similarly, phosphorus content in fish increased with increasing level of phytase, however, manganese only showed a marginal increase. The result of this present study may be attributed to the efficacy of phytase in breaking the phytate-nutrient bond, causing the availability of more nutrients that were readily utilized by fish. Improved minerals deposition in fish as a result of phytase pretreatment or supplementation in diets have been reported [9,10,19,20]. Bone ash and phosphorus concentrations were higher in fish fed phytase supplemented diets, however, increasing the level of supplement did not significantly increase bone phosphorus.

The growth indices showed significantly superior values with phytase supplement. Phytate forms complexes with protein [21] causing depression of protein and amino acids digestion in fish. However the ability of phytase to break such complexes is demonstrated in this present study. This is in consonant the reports of increase in weight,

length and protein utilization in various fish species fed various plant protein sources supplemented with microbial phytase [10,22-24].

Packed cell volume did not show any particular pattern resulting from phytase supplement, but values recorded fall within the normal ranges reported for *C. gariepinus* [25-27].

From the results of this present study, it can be deduced that phytase supplementation in fish feed will enhance feed digestibility, thus increasing nutrient availability which will in turn increase growth of cultured fish. The effective utilization of phosphorus by fish fed phytase diets will surely reduce the level of phosphorus released as fecal wastes into the environment, thereby reducing eutrophication in ponds and adjoining water bodies. It could be concluded that the inclusion of 7500 phytase unit in diets with Lima bean (*Phaseolus lunatus*) as the plant protein source will be beneficial in terms of fish growth and survival.

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