RESEARCH ARTICLE

Phosphorus requirements of African catfish, *Clarias gariepinus*, based on broken-line regression analysis methods

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ABSTRACT: Phosphorus (P) is a critical nutrient vital for growth and buffer systems in the blood. However, excess supply can lead to toxic effects in fish and eutrophication and pollution problems in the culture environment. African catfish (*Clarias gariepinus*) juveniles were fed diets containing different levels of inorganic phosphorus supplements to determine its optimum requirements for fish growth. Eleven diets were used. Their P content varied from 3.9 g/kg diet (for the basal diet) to 13.5 g/kg diet. Weight gain, specific growth rate, feed conversion ratio, carcass protein, and fat improved significantly from fish fed without supplemental P to fish fed 8 g P/kg and then declined consistently in fish fed with a diet supplemented with larger amounts of P. The P and Ca contents of the fish whole body increased significantly and showed linear relationship with increasing dietary P levels, whereas the Mg and Zn contents of the fish indicated a decreasing trend. Broken-line analysis based on specific growth rate and total dietary P contents was used to determine the P requirement for the fish. Quadratic-linear method indicated an optimum P requirement for the growth of the fish as 8.20 g/kg. Quadratic-quadratic and linear-quadratic methods showed optimum P requirement as 7.00 and 6.70 g/kg, respectively. Therefore the study suggests P of 6.70–8.20 g/kg diet as the requirement for the growth of African catfish of 10.2 g weight.

KEYWORDS: broken-line analysis

INTRODUCTION

Phosphorus (P) is an essential and critical nutrient for animal production. It is vital for the formation of bone, energy transfer through ATP, and an essential component of buffer systems in the blood[1]. P is also involved in the control of appetite, weight gain, and feed efficiency[2]. Inadequate supply of P may lead to poor growth of fish, skeletal abnormalities, low feed efficiency, low ash in whole body and vertebrae, high lipid content, bone deformity, and deformity of the head[1, 3–5].

However, P is also an antinutritional factor and an environmental pollutant[6] because it forms insoluble soaps with calcium and lipids, and the insoluble soaps reduce the digestibility of several nutrients including fats, carbohydrates, and amino acids. Calcium-phosphate-phytate complexes also bind directly to starches[7] and inhibit alpha-amylase action[8] thereby lowering starch solubility and digestibility[9]. Additionally, aquaculture effluents high in P levels contribute to the pollution of the aquatic ecosystem and, eventually, cause the eutrophication of natural freshwater and unpleasant flavours in fishes[10–12]. The available dietary P must therefore be at a level that will neither compromise fish growth nor cause environmental pollution. It has been reported that P levels in the effluent can be reduced by lowering its levels in fish foods, and/or by improving their utilization by the fish[13]. This nutritional approach should conform to dietary P requirement levels and should consider the proportion of dietary P that is available to the fish. It should also include better knowledge of the P requirement by different species of fishes, formulating feeds closer to P requirements, and increase in plant P digestibility. It is critical to know precisely the dietary P requirement in order to minimize excess P without risking P deficiency in cultured fish[14].

African catfish *Clarias gariepinus* is an important aquaculture species that is cultivated world wide. However, there is a dearth of information on the P requirement of the fish. The requirement of hybrid African catfish (*C. gariepinus* × *Heterobranchus bidorsalis*) is 6–8 g P/kg of diet[15], while the estimated

P requirement for African catfish fry and fingerlings is 10–12 g/kg diet [16]. However, it is known that relative availability of P varies greatly with diet composition, P sources, fish species, and their sizes [17, 18]. Therefore the present study was carried out to investigate the effect of different levels of inorganic P as dicalcium phosphate ($CaHPO_4$) on the growth and mineralization in African catfish juveniles with a view to determining the optimum P requirements for the fish.

MATERIAL AND METHODS

Experimental diets

Diets were prepared with only 6% fish meal to obtain low P content. All raw ingredients used in diet preparation (Table 1) were purchased from fish feed vendors in Akure, Nigeria. This study was conducted in Alagbaka, Akure in a private fish farm owned by the second author. All the diets contained the same proportions of the same basic feed ingredients. The differences in the diets were the different levels of dicalcium phosphate P supplemented into the diets. The basal diet (D0) without supplemental P contained 40% crude protein and 0.39% P. The basal diet was supplemented with phosphorus at 0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7, 0.8, 0.9, and 1% to obtain diets D1–D10. The diets were prepared by thoroughly mixing the dry ingredients with cod liver oil and then adding cold water until a stiff dough resulted. The dough was then pelleted using a Hobart A-200 pelleting machine with a 2.0 mm die. After pelleting, the diets were immediately sun dried ($27 \pm 2\,°C$) and later broken mechanically into small sizes and packed in dry, airtight small containers prior to use. Inclusion of the different levels of inorganic P in the diets resulted in total dietary P of 3.9, 4.8, 5.9, 6.7, 8.0, 8.8, 9.7, 10.6, 11.5, 12.6, and 13.5 g/kg for diets D0–D10, respectively (Table 1).

Feeding experiment

The experiment was conducted in flow through systems with a flow rate of 1.5 l/min of water. Thirty three glass tanks of 70 l water capacity were used for the feeding trials. There were eleven treatments and each was performed in triplicate. Air stones were used to aerate the tanks throughout the feeding period. About 350 fingerlings (10.2 ± 0.11 g) of African catfish were purchased from a reputable fish farm in Ibadan, Nigeria. The fish were acclimatized in a 70 l tank for two weeks. Three hundred and thirty (330) fish were weighed individually and grouped into 10 fish per tank according to the eleven treatments. The fish were fed to satiation twice daily between 09:00–11:00 and 16:00–18:00, six days a week, for 84 days. Weight of the fish was measured bi-weekly and used to calculate the weight gain, specific growth rate (SGR) and feed conversion ratio. SGR = $100 \times (\ln$ [(final weight)/(initial weight)])/(culture period). Feed conversion ratio = (weight of feed fed)/(fish weight gain). Dissolved oxygen, temperature, and pH of the experimental tanks were measured weekly and the results obtained ranged between 6.3–8.0 mg/g, 25–27 °C, and 6.85–8.88 respectively.

Faeces collection

During the feeding experiment, after feeding the fish for a second time each day (16:00 and 18:00 h), all uneaten dissolved feed remains were removed from the tanks using rubber hose. Then the following morning from 07:00 h, faeces were collected from each of the tanks before the next feeding. The collection was done for the last four weeks before the end of the study.

Proximate and mineral analysis

At the end of the experiment fish were not fed for 24 h. The fish were weighed and an equal number of fish from each tank were collected. A total of twelve fish from each treatment were sacrificed. Three replicates of the fish carcass (whole body) and diets were analysed for proximate and minerals composition [19]. About 2.0 g of the samples were calcinated for 48 h at 480 °C and cooled to room temperature. Then, 6 ml of HCl was added and the mixture was brought to boiling point. The sample was allowed to cool at room temperature before another 2.5 ml of 6 N HCl was added and the mixture was warmed to dissolve all the solutes. The solution was cooled and diluted to 25 ml with distilled deionized water. The minerals (Mg, Ca, and Zn) were measured using an atomic absorption spectrophotometer. P content of the eleven diets, fish and faeces were analysed using the Vanadomolybophosphoric acid colorimetric method 4500-p with slight modifications. To 3 ml of the diluted solution of the sample, 3 ml of vanadomolybdate reagent was added and the P concentration was measured spectrophotometrically at 430 nm, after the reaction mixture was thoroughly mixed and allowed to stand at room temperature for 10 min.

Statistical and broken-line analysis

Data resulting from the experiment were subjected to a one-way analysis of variance (ANOVA) test using the SAS software package (2008 version). Individual differences ($p = 0.05$) among treatment means were separated using Duncan's multiple range test [20]. The

Table 1 Gross and chemical composition of experimental diets (g/kg DM).

Ingredients	D0[a]	D1	D2	D3	D4	D5	D6	D7	D8	D9	D10
Danish fish meal (72% CP)	60.0	60.0	60.0	60.0	60.0	60.0	60.0	60.0	60.0	60.0	60.0
Casein (94.6%CP)	305.0	305.0	305.0	305.0	305.0	305.0	305.0	305.0	305.0	305.0	305.0
Gelatin (97% CP)	70.0	70.0	70.0	70.0	70.0	70.0	70.0	70.0	70.0	70.0	70.0
Dextrin	461.7	449.3	437.2	424.7	412.3	399.8	387.4	375.1	363	351	339
Cod liver oil	50.0	50.0	50.0	50.0	50.0	50.0	50.0	50.0	50.0	50.0	50.0
Vitamin min-premix[b]	30.0	30.0	30.0	30.0	30.0	30.0	30.0	30.0	30.0	30.0	30.0
Carboxyl methyl cellulose	15.0	15.0	15.0	15.0	15.0	15.0	15.0	15.0	15.0	15.0	15.0
Phosphorus ($CaHPO_4$)	0	10.4	20.8	31.2	41.6	52.0	62.4	72.8	83.2	93.6	104.0
$CaCO_3$	8.32	10.3	12	14.1	16.1	18.2	20.2	22.1	23.8	25.4	27
Chemical composition											
Crude protein (%)	40.1	40.1	40.0	40.1	40.1	40.1	40.0	40.0	40.3	40.0	40.2
Fat (%)	6.55	6.44	6.64	6.88	6.03	6.04	6.25	6.02	6.49	6.84	6.75
Ash (%)	4.13	4.15	4.21	4.26	4.35	4.41	4.18	4.13	4.16	4.23	4.08
Fibre (%)	7.92	7.09	7.14	7.06	7.19	7.94	7.16	7.47	7.04	7.2	7.08
Nitrogen free extract (%)	40.4	40.4	40.8	40	40.4	40.5	40.5	40.5	40.5	40.4	40.1
P (g/kg)	3.90	4.80	5.90	6.72	8.00	8.80	9.70	10.6	11.5	12.6	13.5

[a] D0 (diet without supplemental P); Dx (diet supplemented with x g P/kg)

[b] Kg/diet: Vit. A 1 000 000 IU; Vit. D_3 600 000 IU; Vit. E 12 000 IU; Vit. K_3 15 mg; Vit. C 12 500 mg; Vit. B_1 250 mg; Vit. B_2 1750 mg; Vit. B_6 875; Vit. B_{12} 2500 mg; Ca-D-pantothenate 5000 mg; Nicotinic acid 3750 mg; Folic acid 250 mg; Co ($CoSO_4$) 24 999 mg; Cu ($CuSO_4$) 1999 mg; Fe ($FeSO_4$) 11 249 mg; Se ($Na_2SeO_3 \cdot 5H_2O$) 75 mg; I (KI) 106 mg; antioxidant 250 mg.

"broken-line model" was used to calculate the P requirements[21].

RESULTS

Chemical concentration of the experimental diets (Table 1) showed closely related values of protein, fat, ash, fibre, and nitrogen free extract. The major difference is the dietary P levels which increased from D0 to D10 because of the differences in the supplemental levels. Therefore changes in the performance of the fish may be ascribed to differences in the dietary P composition.

The growth parameters (Table 2) indicated that weight gain and specific growth rate increased significantly with increase in dietary phosphorus levels up to 8.0 g/kg in fish fed D4 and then declined consistently to dietary level of 13.5 g P/kg in fish fed D10. Feed conversion ratio (FCR) improved ($p < 0.05$) from fish fed D0 without supplemental P to fish fed D4 and worsened towards fish fed D10. High FCR ($p < 0.05$) in fish fed D0, D1, and D2 are indications of dietary P deficiency. High values in fish fed D8, D9, and D10 could be attributed to the toxic effect of excess P in the diets. The broken-line analysis (Fig. 1) highlighted the optimum P requirement for the growth of the fish as 8.20 g/kg diet. The linear-quadratic method (Fig. 2) indicated that P of 6.70 g/kg diet should be adequate for the growth of the fish. Similarly,
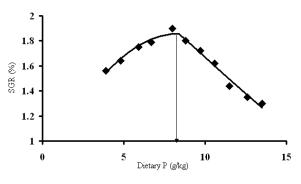

Fig. 1 Total P requirement for growth of *Clarias gariepinus* (quadratic-linear method).

quadratic-quadratic methods showed the requirements as 7.00 g P/kg diet. This means that the P requirement for the growth of African catfish is in the range of 6.70–8.20 g/kg diet, by using dicalcium phosphate as the P source.

Chemical composition of the fish after the experiment (Table 3) showed that addition of inorganic P in the diets significantly increased the body protein, fat, and ash contents in the fish. The moisture content of the fish showed a decreasing trend, indicating the effect of P deficiency.

Table 3 also showed that P, Ca, and Mg contents of the fish increased linearly with increasing dietary P levels so that fish fed D10 with the highest P

Table 2 Growth parameters of *Clarias gariepinus* fed diets with different levels of inorganic P.

	D0	D1	D2	D3	D4	D5	D6	D7	D8	D9	D10
Initial mean weight (g)	10.0	10.1	10.2	10.3	10.2	10.2	10.2	10.3	10.1	10.0	10.3
	± 0.01	± 0.02	± 0.08	± 0.10	± 0.20	± 0.03	± 0.40	± 0.01	± 0.08	± 0.02	± 0.00
Final mean weight (g)	37.7^e	40.0^{de}	44.0^{bcd}	47.4^{ab}	50.6^a	45.8^{abc}	42.0^{cde}	37.2^{de}	34.5^f	31.1^f	30.3^f
	± 0.14	± 1.58	± 0.41	± 1.86	± 1.74	± 0.40	± 0.88	± 5.11	± 1.00	± 1.04	± 1.23
Weight gain (g)	27.7^{ef}	29.9^e	34.0^{de}	37.4^e	40.5^a	35.7^b	32.0^c	27.2^c	24.5^{cd}	21.1^{cd}	20.1^{cde}
	± 0.15	± 1.58	± 0.42	± 1.87	± 1.73	± 1.41	± 0.88	± 5.11	± 1.00	± 1.04	± 1.23
SGR	1.59^e	1.64^{de}	1.75^{bcd}	1.87^{ab}	1.90^a	1.81^{abc}	1.72^{cde}	1.63^{de}	1.44^f	1.37^f	1.30^f
	± 0.03	± 0.05	± 0.01	± 0.05	± 0.04	± 0.04	± 0.03	± 0.03	± 0.05	± 0.02	± 0.01
FCR	1.77^{cd}	1.88^c	1.68^{cde}	1.47^e	1.47^e	1.57^{de}	1.80^{cd}	1.63^{cde}	2.22^{ab}	2.15^b	2.47^a
	± 0.08	± 0.10	± 0.02	± 0.07	± 0.06	± 0.07	± 0.05	± 0.05	± 0.02	± 0.04	± 0.01

Means of triplicate values in the same row with the same superscript are not significantly different ($p > 0.05$).

Table 3 Chemical composition of *C. gariepinus* (whole body) fed diets with different levels of inorganic P.

	D0	D1	D2	D3	D4	D5	D6	D7	D8	D9	D10
Protein (% DM)	61.8^j	63.1^h	63.5^e	67.9^d	69.6^a	68.9^b	68.1^c	63.4^f	63.2^g	63.1^h	62.6^i
	± 0.08	± 0.34	± 0.10	± 0.03	± 0.15	± 0.46	± 0.45	± 1.00	± 0.53	± 0.09	± 0.12
Fat (%)	1.68^g	1.83^e	1.84^e	2.22^b	2.32^a	2.05^c	2.03^c	1.89^d	1.88^d	1.87^d	1.71^f
	± 0.64	± 0.58	± 0.60	± 0.40	± 0.54	± 0.41	± 0.32	± 0.01	± 0.51	± 0.30	± 0.49
Ash (%)	10.5^h	10.5^h	10.6^h	10.8^g	11.1^f	11.4^e	12.0^d	12.8^c	12.9^b	12.9^b	14.5^a
	± 0.00	± 0.00	± 0.01	± 0.00	± 0.01	± 0.00	± 0.00	± 0.01	± 0.01	± 0.02	± 0.03
Moisture (%)	13.0^a	12.9^b	12.9^b	12.8^c	12.0^d	11.4^e	11.1^f	10.7^g	10.6^h	10.5^h	10.5^h
	± 0.02	± 0.01	± 0.19	± 0.02	± 0.03	± 0.09	± 0.01	± 0.40	± 0.40	± 0.07	± 0.02
P (mg/g DM)	14.3^k	16.5^j	18.5^i	19.9^h	21.5^g	22.7^f	23.8^e	25.0^d	27.2^c	28.5^b	29.8^a
	± 0.04	± 0.62	± 0.05	± 0.00	± 0.03	± 0.06	± 0.06	± 0.05	± 0.01	± 0.05	± 0.04
Ca (mg/g DM)	22.1^k	25.1^j	28.3^i	30.2^h	35.3^g	36.2^f	36.6^e	36.9^d	37.5^c	37.8^b	40.8^a
	± 1.08	± 0.94	± 1.10	± 0.73	± 0.25	± 0.86	± 0.65	± 1.00	± 0.83	± 1.09	± 0.72
Mg (mg/g DM)	3.86^k	4.25^j	4.47^h	5.14^f	7.25^a	6.70^b	6.58^c	6.03^d	5.58^e	5.05^g	4.40^i
	± 0.05	± 0.06	± 0.08	± 0.02	± 0.06	± 0.06	± 0.10	± 0.03	± 0.05	± 0.05	± 0.06
Zn (μg/g DM)	42.6^i	45.9^g	49.5^e	55.8^b	60.1^a	53.3^c	50.7^d	47.7^f	45.4^h	42.5^j	40.6^k
	± 0.14	± 0.39	± 0.37	± 0.59	± 0.30	± 0.45	± 0.06	± 0.07	± 0.10	± 0.20	± 0.24
Ca:P ratio	1.55	1.52	1.53	1.52	1.64	1.59	1.54	1.48	1.38	1.33	1.37

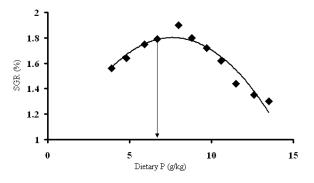Means of triplicate values in the same row with similar superscript are not different ($p > 0.05$).

Fig. 2 Total P requirement for growth of *Clarias gariepinus* (linear-quadratic method).

content had the highest body P and Ca concentration. However, Mg and Zn concentrations were highest in fish fed D4 that had the best growth performance. Zn concentration in the fish also showed increasing trend up to fish fed D4, and then reduced continuously towards fish fed D10 with the highest dietary P content. It was difficult to calculate the optimum P requirement for mineralization using broken-line analysis because of the linear relationship between the dietary P levels and the P deposited in the fish whole body. However, it is clear that the optimum P level for mineralization is definitely more than that obtained as optimum for the growth of the fish. This shows that more P is required for mineralization than is required for growth. The Ca:P ratio of the fish whole body was in the range of 1.33–1.64 and showed a positive correlation with the
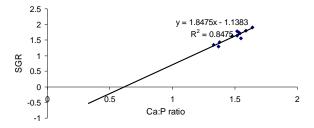
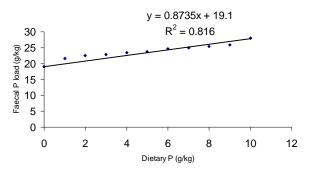**Fig. 3** Relationship between SGR and whole fish body Ca:P ratio.

Fig. 4 Faecal phosphorus load from *Clarias gariepinus* fed diets with different P levels.

growth of the fish (Fig. 3). Hence the fish with the highest body Ca:P ratio of 1.64 had the best growth performance. This is an indication that a particular Ca:P balance is needed for optimum growth of the fish.

Fig. 4 shows the faecal P load as a result of increasing levels of dietary P. It indicates a linear relationship between faecal P load and dietary P levels, with regression equation Faecal P $= 0.874x + 19.1$ and high correlation $R^2 = 0.816$, indicating high dependence of P load to total dietary P level. This emphasizes the fact that a minimum level of P must be fed to the fish as P is a factor of eutrophication and pollution. Besides, feeding fish with excess P amounts to a waste of resources in terms of extra costs and reduced/poor performance of the fish fed diets with excess P.

DISCUSSION

Suboptimal mineral supply leads to biochemical changes in the metabolism and inadequate storage of minerals without showing clinical symptoms[22], while over supply of minerals at subtoxic levels can cause metabolic changes without leading to external symptoms[18]. The study investigated the effect of different levels of phosphorus (P) ($CaHPO_4$) on growth and mineral concentration in African catfish, *Clarias*

gariepinus with a view to determining the optimum P requirement for the growth of the fish. The result obtained from the study indicated that suboptimal or subtoxic levels of dietary P can negatively affect the growth performance of the fish.

The relative availability of P varies greatly with fish species, diet composition and the form of P[18]. Dietary P requirements ranging from 0.5 to 0.8% have been reported for rainbow trout[23], Atlantic salmon[24], Chum salmon[25], Carp[26], and sea bream[27]. The requirement of hybrid African catfish (*C. gariepinus* × *Heterobranchus bidorsalis*) is 6–8 g P/kg diet[16]. The P requirement for African catfish fry and fingerlings are between 10–12 g P/kg[17]. The present study indicates the P requirement of *C. gariepinus* juveniles as 6.70–8.20 g/kg diet. These values agree very well with those in Ref. 16 but are lower than other reported values[17]. This discrepancy could be attributed to the sizes of fish used, ingredients combination, and even the methods used in estimating the P requirement. In a related study[18], some differences in the P requirement of common carp previously reported in the literature were compared with results obtained by using broken-line regression analysis. The slight differences between the results obtained from the present study and that in Ref. 16 could be ascribed to species differences or the sizes of fish used. P requirements do vary from about 0.25–1.0% which shows that the P requirement is species specific[28].

Increasing the concentrations of the available dietary P from 0.24% to 0.88% modestly enhanced the growth of rainbow trout[13] and a total P of 0.88% and 0.58% estimated available P was recommended as the optimum for the fish. This observation is in line with the result of the present study which showed increase in growth performance of *C. gariepinus* as a result of increase in dietary P content of the diets up to 8.0 g/kg. The consistent decrease in the growth of the fish beyond the optimal P requirement as obtained from the study is in consonance with the observation that the biomass gain per amount of P fed to rainbow trout was significantly lower in fish fed with the diet containing highest supplemental P[13], indicating an important trade off. The decrease in the weight gain and specific growth rate of the fish fed diets deficient in P is an indication that the native P was poorly utilized by the fish. Dietary deficiencies in P also cause a reduction in growth rate and body content of Ca and P of the fish[29]. In a related study[30], channel catfish (*Ictalurus punctatus*) fingerlings fed a low amount of P exhibited reduced growth, poor feed efficiency, and low bone ash. Diet P deficiency resulted in a reduced growth and high conversion ratio

in American cichlid, *Cichlasoma urophthalmus*[28].

The positive relationship between the Ca:P ratio and the growth of the fish found in the present study is line with the observation that Ca and P requirements per unit of growth and Ca and Ca:P ratio are positively correlated[28].

Enhanced carcass fat and reduction in water contents observed from the present study as dietary P deficiency symptoms supports the results of increase in fat content and decrease in carcass water of fish[26, 31]. P deficiency in carp is accompanied by accumulation of lipid in muscle and viscera, and an increase in the activity of the hepatopancreatic enzyme[32]. Carp fed a diet deficient in P show high lipid deposits[33]. Similarly, there is an inverse relationship between carcass lipid levels and dietary P[28]. Increase in carcass ash as observed from the study corroborates work which found that bone ash and scale mineralization in American cichlid are highly correlated with P level in the diet[28]. P deficiency produced lower bone and ash in channel catfish while Ref. 24 described that supplementary P in the diets significantly increased bone ash content in Atlantic salmon[30]. Similarly, dietary P levels greatly affected the ash content of the bones of chum salmon[25].

Due to the function of P in the bone structure, an increasing supply of P significantly increases the P content of the bone and its mineralization, leading to concomitant increases in ash and Ca[34]. In the present study, increasing levels of P similarly increased the P, Ca, and ash contents of the fish. Experiments with chum salmon showed that dietary P levels significantly affected the Ca and P contents of the fish bones[25]. Carcass P and Ca in rainbow trout has a positive correlation with dietary P levels[14]. Reduction in the carcass Zn levels of fish fed diets with high dietary P contents as observed from the present study is in consonance with observation that an excess amount of dietary P inhibited Zn utilization in rainbow trout[35]. P in diets also increases the Zn requirement in animals[36], and there is an inverse relationship between high dietary P levels and Zn mineralization in common carp *Cyprinus carpio*[18, 37, 38].

Increasing faecal P loadings due to increase in dietary P levels supports work in which faecal P concentrations tended to increase with dietary P concentrations[13]. In trout-fed practical diets containing CaHPO$_4$, soluble P in the effluent increased as the digestible dietary P increased[39]. Similarly, soluble P production per kg fish is a linear function of dietary P and is independent of the type of diet used (purified (egg white-based), semipurified or practical) and apparently on the size of fish[13]. The increasing

level of faecal P is also in agreement with previous work[14, 40, 41]. As P is a factor of eutrophication, pollution and off-flavour in fishes[12] the amount of P in animal feeds must be restricted to the requirement by the particular animal.

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