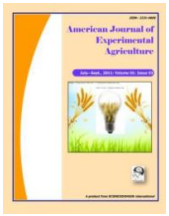

American Journal of Experimental Agriculture
6(5): 317-325, 2015, Article no.AJEA.2015.090
ISSN: 2231-0606

SCIENCEDOMAIN *international*
www.sciencedomain.org

Utilization of Dicalcium Phosphate and Different Bone Meals as Dietary Phosphorus Supplement in the Diets of *Clarias gariepinus* Fingerlings

I. A. Adebayo[1*] and G. H. Akinwumi[1]

[1]Department of Forestry, Wildlife and Fisheries Management, Faculty of Agricultural Sciences, Ekiti State University, Ado Ekiti, Ekiti State, Nigeria.

Authors' contributions

This work was carried out in collaboration between both authors. The two authors IAA and GHA designed and participated in the research work. Author IAA supplied the glass aquaria and bought the ingredients used for the experiment. The feed formulation and fish procurement at the commencement of the experiment were done by author IAA. Feed administration to fish for 70 days and weighing of fish were carried out by author GHA. The statistical analysis of data generated from the experiment was jointly done by the two authors. The manuscript was written by author IAA, while the literature searches was done by author GHA.The two authors read the final manuscript and approved its submission for publication.

Article Information

DOI: 10.9734/AJEA/2015/9118
Editor(s):
(1) Md. Yeamin Hossain, Department of Fisheries, Faculty of Agriculture, University of Rajshahi, Bangladesh.
Reviewers:
(1) Anonymous, Nigeria.
(2) Anonymous, India.
(3) Anonymous, Nigeria.
(4) Anonymous, Thailand.
(5) Anonymous, Brazil.
Complete Peer review History: http://www.sciencedomain.org/review-history.php?iid=867&id=2&aid=7579

Original Research Article

Received 21st January 2014
Accepted 20th March 2014
Published 31st December 2014

ABSTRACT

Phosphorus is most limiting mineral in the diet of fish and must be supplied in the right quality and quantity to prevent its deficiency or toxicity in fish. A 70 day feeding trial was conducted to evaluate the utilization of Dicalcium phosphate and different bone meals as phosphorus supplement in the diets of *Clarias gariepinus* fingerlings. At the start of the experiment, ten glass aquaria of size 70cm x 45 cm x 40 cm/each, filled with Well water up to 70L of its volume were stocked with one hundred (100) fingerlings (mean weight 6.00±0.02) g/one at 20 fish per treatment replicated twice using a complete randomized design. Five experimental diets (D_1-D_5) were formulated to be isocaloric

**Corresponding author: Email: dayadeisrael@yahoo.com;*

(11.1kcal/kg) and isonitrogeneous (40%Crude Protein). D_1 (control) was without Phosphorus (P) supplementation and P (0.44%) deficient, while D_2-D_5 were supplemented with Dicalcium phosphate (DCP), Chicken bone meal (CHBM), Clarias bone meal (CLBM) and Cattle bone meal (CABM) at 1.46%, 1.43%, 1.46% and 1.54% respectively based on the available P in each supplement to give 0.8% available P in the diets. The results showed that fish fed with D_3 (CHBM) was significantly ($p<0.05$) had the best growth performance in terms of Final Mean Weight Gain (FMWG) 14.40±0.14 g; Specific Growth Rate (SGR) 1.75±0.05 and Feed Conversion Ratio (FCR) 1.15±0.01. Phosphorus in the carcass of fish after the experiment was significantly ($p<0.05$) highest in D_5 (26.05±0.21)mg/g and least in fish fed D_1 (12.98±0.20) mg/g. The feeding trial established the necessity for phosphorus supplementation in the diets of *Clarias gariepinus* fingerlings for better growth and body mineralization.

Keywords: Dicalcium phosphate; bone meals; phosphorus; diets; growth and Clarias gariepinus.

1. INTRODUCTION

Quantitatively, 40 nutrients have been identified as necessary for the normal metabolic function of fish [1]. Minerals, especially phosphorus are important for optimal growth of cultivated fish. It guards against diseases such as lordosis (deformed backs and heads) as a result of abnormal calcification of bone [2]. Phosphorus is needed to help balance vitamins and other minerals which include vitamin D, iodine, magnesium and zinc. It is a vital component of organic phosphate such as adenosine triphosphate phospholipids, co-enzymes and DNA, which have major roles in metabolism of carbohydrates, fats and amino acids [3]. Phosphorus is essential for many intracellular processes notably; glycolysis, membrane maintenance, oxygen transport, muscle contraction and protein from oxidative damage [4].

Phosphorus must be provided in fish feed because of its low concentration in water [5]. According to [1] quantity of phosphorus that are available in water to fish are lower than 0.1ppm, as a result, the soluble phosphorus available to fish is less than 1% of what the fish actually needed to survive and for this reason phosphorus must be supply in the feed.

It has been demonstrated that phosphorus requirements are species-specific [5]. An aquaculturist must know the nutritional requirement of fish before compounding a feed. This information will help a farmer in formulating a feed that is nutritionally balanced for such fish particularly when they are cultured under intensive system [6]. However, bioavailability of dietary phosphorus is influenced by the digestibility of diet, particle size, and interaction with other nutrients, feed processing and water chemistry [7]. The optimal amount of phosphorus supplementation in commercial feed is important economically and environmentally and must carefully balance to prevent signs of deficiency and minimize the urinary and fecal discharge of phosphorus into natural water which causes deterioration of water quality [7].

Little information is available on the phosphorus availability and utilization of bone meal prepared separately from individual animal bone species. Historically, bone meal is prepared from mixture of bone meal from undefined sources [8]. The evaluation of bone meal phosphorus and utilization is important in order to ensure that fish are not deficient or have marginal levels of phosphorus under the assumption that bone meal phosphorus is available completely [9]. There are variations in the availability and subsequently utilization of Phosphorus from different bone meals. This study therefore investigates the utilization of available phosphorus in Dicalcium phosphate, chicken bone meal, Clarias bone meal and Cattle bone meal as P supplement in the diets of *Clarias gariepinus* fingerlings.

2. MATERIALS AND METHODS

2.1 Experimental Procedure

The study was conducted from February 1st to 12th April, 2013 at the Faculty of Agricultural Sciences Laboratory, Ekiti State University, Ado-Ekiti, Nigeria. Prior to the start of the experiment, one hundred and twenty (120) fingerlings of *Clarias gariepinus* were purchased from Success Fish Breeding and Poultry Farms Nig. Ltd., Akure, Ondo State, Nigeria. Fish were temporarily kept in already prepared indoor concrete tank of size 4 m × 4 m × 1.5 m for a week and fed with commercial catfish feed (Coppens) of size 2 mm and 40% Crude Protein to acclimatize them.

At the start of the experiment, ten glass aquaria tanks of size 70 cm × 45 cm × 40 cm/one were filled with clean Well water to 70 litres of their capacity. Fish were then counted (10/tank) and weighed (mean weight 6.00±0.02) g/one, using Digital Pocket Scale (Model: 1000 g/0.1) and randomly stocked in a complete randomized design. The five treatments, replicated twice according to the test diets including the control were fed for a period of 70 days.

2.2 Experimental Diets and Feeding Procedure

All the ingredients used in feed preparation were purchased from Metrovet Feed mill in Ado-Ekiti, Ekiti State, Nigeria. Ingredients were analyzed for proximate composition prior to feed formulation according to [10]. The gross composition of the experimental diets was presented in Table 1. Experimental diets contained different proportion of feed ingredients to achieve different Phosphorus (P) inclusion levels. The control diet (D_1) was P (0.44%) deficient (without supplementation), while D_2- D_5 were supplemented with Dicalcium phosphate (DP) $_{1.46\%}$; Chicken bone meal (CHBM) $_{1.43\%}$; Clarias bone meal $(CLBM)_{1.46\%}$ and Catlle bone meal $(CABM)_{1.54\%}$ to give 0.8% P level in the test diets according to Adebayo (2011). In preparing the diets, dry ingredients including the bone meals (plate 2-4) were ground to a powdery form in a willey mill to enhance optimum utilization and digestibility. Plate 1 is dicalcium phosphate (DCP) already in powdery form and there was no need for further milling before adding it to other ingredients to formulate D_2. Diets were thoroughly mixed with cod liver oil and pelleted using Hobart A 200 pelleting machine with a 2.0 mm die. They were immediately sun dried and packed in a labeled tight container and kept in the refrigerator prior to use. Isonitrogenous and isocaloric diets (40% CP) as recommended by Faturoti et al. [11] were used during the experimental study. Fish were fed to satiation twice daily at 09:00 and 16:00 for 70 days while the weights of the experimental fish were measured bi-weekly to calculate their response to feed.

2.3 Water Quality Parameters

Water quality parameters such as pH, DO, temperature and nitrate were closely monitored throughout the experimental period. pH values of the water during the feeding were measured directly by electronic pH meter (Metler toledo 320 model) by dipping the electrode into each tank. The dissolved oxygen (DO) of the water in experimental tanks was measured using Standardized YSI Do meter (YSI Model 57). The water temperature of the rearing system was measured using a mercury thermometer calibrated from 0ºC 110ºC on a daily basis. Measurements were carried out by gently immersing the thermometer into the water at vertical position and left for about 2 – 5 minutes. It was quickly moved near the surface of the water and read. Nitrate was measured weekly.

Table 1. Gross composition of experimental diets

Ingredients	Diets (%)				
	D_1 (Ctrl)	D_2(DCP)	D_3(CHBM)	D_4(CLBM)	D_5(CABM)
Fishmeal (72%CP)	44.03	36.90	37.30	36.92	36.60
SBM (44%CP)	22.02	18.50	18.50	18.50	18.30
GNC (48%CP)	22.02	18.50	18.50	18.50	18.30
Yellow maize (10%CP)	2.94	2.50	2.50	2.50	2.42
Cod liver oil	5.00	5.00	5.00	5.00	5.00
Vitamin premix	3.00	3.00	3.00	3.00	3.00
Methionine	0.30	0.30	0.30	0.30	0.30
Carboxymethy cellulose	0.70	0.70	0.70	0.70	0.70
Dicalcium phosphate (CaHPO4)	-	1.46	-	-	-
Chicken bone meal (CHBM)	-	-	1.43	-	-
Clarias bone meal (CLBM)	-	-	-	1.46	-
Catttle bone meal (CABM)	-	-	-	-	1.54
Total (%)	100.01	100.00	100.10	100.02	100.02
%Crude protein	40.01	40.00	40.10	40.02	40.02
Available Phosphorus (Calculated) %	0.44	0.80	0.80	0.80	0.80

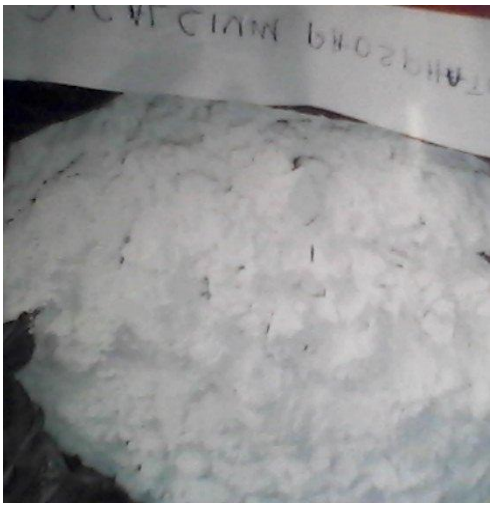

Plate 1. Dicalcium phosphate

Plate 2. Chicken bone meal

Plate 3. Clarias bone meal

Plate 4. Cattle bone meal

2.4 Growth and Nutrient Utilization Parameters

Growth performance was determined by measuring biweekly mean weight gain (MWG); specific growth rate (SGR) food conversion ratio (FCR), protein efficiency ratio (PER) and net protein utilization (NPU). These growth response parameters were calculated as follow:

MWG (g) = W_i-W_t
Where wt is the final weight of the fish at time (t), w_1 is initial weight of the fish at time 0
SGR (%) = (lnW_t –lnW_i)/T x100
Where W_t is weight of the fish at time t, W_i is weght of the fish at time 0, and T is the culture period in days.

FCR = total dry feed fed (g)/ total wet weight gain (g).
PER = wet weight gain (g)/ amount of protein fed (g)
NPU (%) = 100 x (protein gain/ protein consumed)

2.5 Proximate Composition and Mineral Analysis

Samples of experimental diets and fish (before and after experiment) in all treatments were analyzed for proximate composition and minerals (P and Ca) according to the method of AOAC (2000). Gross energy in kcal/kg of test diet was determined using ballistic oxygen bomb calorimeter (gallen kamp) as described by [12].

2.6 Statistical Analysis

Data obtained from the experiment were subjected to analysis of Variance (ANOVA using SPSS version 15. Differences in means were separated using Duncan Multiple range test.

3. RESULTS

3.1 Proximate Composition of Experimental Diets

The Proximate compositions of experimental diets were presented in Table 2.T The crude protein level of the five diets (D_1-D_5) ranged from 40.07% to 40.19%.The same trend was observed for the nitrogen free extract (NFE) and ether extract. Ash content increased gradually from D_I (6.10%) and highest in D_4 (6.79). The P content (determined) of the diets ranged between 0.43 and 0.83%. This shows that the diets were formulated in line with the experimental objective. Therefore any changes in the performance of the fish would be attributed to the effect of the supplemented P.

3.2 Proximate Analysis of Experimental Fish after feeding Trial

The results of the proximate analysis are summarized in Table 3. There was no significant difference (P>0.05) in protein content of fish fed D_1 to D_5 when compared with the initial value before feeding trial with the reported values that ranged between (64.25% - 69.05%). Fish fed D_3 (CHBM) had the highest (69.05%) protein content. There was a slight increase in carcass ash with the highest value (15.10%) in fish fed D_5 (CABM). No fibre was detected but there was a gradual reduction in NFE values in fish carcass before and after the feeding trial as shown in the table.

3.3 Water Quality Parameters

Table 4 shows the mean values of water parameters recorded during the period of the experiment. Water temperature fluctuated within the range of 26.80 – 27.00ºC, while the Dissolved oxygen (DO) ranged from 5.5-6.8 mg/litre. pH values ranged between 6.7-6.9 and nitrate (0.22 – 0.23) mg/L in all the treatments. Though, there was no significant difference (P>0.05) in the values of the parameters analyzed.

3.4 Growth Performance and Nutrient Utilization of Experimental Fish

The growth parameters of the fish fed the various experimental diets were presented in Table 5. Mean weight gain significantly (P>0.05) showed similar pattern to that of SGR with the best values in fish fed D_3 (14.4g, 1.75) respectively. Feed utilization expressed as the feed conversion ratio (FCR) was not significantly (P>0.05) different in fish fed D_2 (1.60) and D_4 (1.53). While the FCR of fish fed D_1 (1.99) and D_5 (1.89) were the poorest. Analyzed values of P and Ca were significantly (P>0.05) highest in fish feed D_3 and D_5 (20.69, 23.79) mg/g respectively.

Plates (5-9) were the samples of fish fed (D_1 –D_5) respectively after the feeding trial. The size of fish in plate 5 (Ctrl) without P supplementation was smallest and biggest in plate 7 (D_3, supplemented with chicken bone meal). Growth of fish in plate 8 and 9 were relatively uniform.

Table 2. Proximate composition experimental diets

Parameters (%)	Diets (%)				
	D_I(Ctrl)	D_2(DCP)	D_3(CHBM)	D_4(CLBM)	D_5(CABM)
Moisture content	12.10	12.13	12.10	12.10	12.12
Crude protein	40.19	40.07	40.12	40.12	40.12
Ether extract	4.21	4.20	4.30	4.30	4.30
Ash	6.10	6.47	6.77	6.79	6.76
Crude fibre	2.25	2.30	2.21	2.23	2.20
NFE	37.40	37.13	36.71	36.69	36.70
phosphorus (determined)	0.43	0.78	0.75	0.82	0.83
Calcium (determined)	1.15	1.19	1.12	1.14	1.15
Ca/P ratio	1-1.1	1-1.2	1-1.2	1-1.1	1-1.2

Table 3. Proximate composition of experimental fish after feeding trial

Parameters(%)	Initial	D_1 (Ctrl)	D_2 (DCP)	D_3 (CHBM)	D_4 (CLBM)	D_5(CABM)
Moisture	11.21±0.15	11.11±0.02	11.20±0.10	11.33±0.06	11.11±0.30	11.34±0.50
Crude protein	64.25±0.07	66.25±0.01	68.03±0.01	69.05±0.07	68.60±0.50	68.01±0.60
Ether Extract	3.58±0.03	3.32±0.02	3.36±0.02	3.33±0.02	4.11±0.50	4.02±0.06
Ash	12.20±0.07	13.60±0.50	13.40±0.07	14.20±0.03	13.60±0.03	15.10±0.04
NFE	5.38±0.04	4.52±0.04	5.21±0.08	3.51±0.50	3.58±0.06	3.81±0.07

Table 4. Mean of water quality parameters recorded during the experimental period

Parameters	D_1 (CTRL)	D_2 (DCP)	D_3 (CHBM)	D_4(CLBM)	D_5 (CABM)
Temperature (ºC)	27.00±0.11	26.80±0.21	27.00±0.21	26.80±0.15	26.80±0.20
pH	6.7±0.15	6.9±0.21	6.5±0.20	6.7±0.22	6.7±0.22
DO (mg/L)	6.6±0.20	5.2±0.20	5.1±0.20	4.8 ± 0.22	5.8 ±0.21
Nitrate (mg/L)	0.22±0.20	0.25±0.20	0.24±0.20	0.22±0.20	0.23±0.20

Table 5. Growth performance of *Clarias gariepinus* fed phosphorus supplemented diets

Parameters	D_1(Ctrl)	D_2(DCP)	D_3(CHBM)	D_4(CLBM)	D_5(CABM)
Mean initial body weight(g)	6.00±0.02	6.04±0.01	6.02±0.03	6.01±0.01	6.02±0.03
Mean final body weight(g)	17.67±0.14^b	18.75±0.12^b	20.42±0.04^a	18.75±0.11^b	18.50±0.05^b
Mean weight gain(g)	11.67±0.03^b	12.71±0.20^b	14.4±0.14^a	12.74±0.02^b	12.48±0.05^b
Average body weight gain daily(g)	0.17±0.01	0.18±0.06	0.21±0.02	0.18±0.03	0.18±0.11
Specific growth rate (SGR)	1.54±0.05^b	1.62±0.07^b	1.75±0.05^a	1.62±0.04^b	1.62±0.03^b
Protein efficiency ratio (PER)	0.29±0.02^b	0.32±0.03^b	0.36±0.14^a	0.32±0.06^b	0.31±0.02^b
Food conversion ratio (FCR)	1.99±0.02^c	1.60±0.05^b	1.25±0.01^a	1.53±0.05^b	1.89±0.22^c
Net protein utilization (NPU)	1.87±0.05	1.89±0.01	1.89±0.12	1.90±0.04	1.88±0.02
Phosphorus (Analyzed) mg/g	12.98±0.20^c	16.06±0.20^b	20.69±0.60^a	16.26±0.21^b	16.05±0.50^b
Calcium (Analyzed) mg/g	17.60±0.05^b	17.37±0.21^b	23.79±0.21^a	22.68±0.20^a	23.80±0.22^a
Ca/p ratio (analyzed)	1.2:1^b	1:1^c	1.1:1^c	1.5:1^a	1.2:1^b

Mean values with similar superscript are not significantly different (P>0.05)

4. DISCUSSION

Evolving recent research works implicate inadequacy of dietary mineral supplementation in fish. It is assumed that fish in their natural habitat should meet the requirements for all the mineral elements. However, the intensive culture of certain fish species in man-made ponds together with dependence on artificial feeding makes it necessary to incorporate adequate quantities of mineral nutrients in the feed. Di-calcium phosphate is an inorganic source of phosphorus, while Chicken bone meal, *Clarias* bone meal and Cattle bone meal were the organic sources used in this experiment. There is dearth of information on the required level of different bone meals as phosphorus supplement in fish diets. [13] reported a supplementation level of 0.8% P for some selected clariid catfishes using Dicalcium phosphate with 80-90% available phosphorus. Also, [14] reported a supplementation level of 0.7%P using purified diets for *Heterobranchus bidorsalis* fingerlings. In this study, varying bone meal sources as phosphorus supplement in the test diets promoted the growth of *Clarias gariepinus* fingerlings with the best growth

Plate 5. Fish fed with the control diet (0% Phosphorus supplementation)

Plate 6. Fish fed with Di-calcium phosphate supplemented diet

Plate 7. Fish fed with Chicken bone meal supplemented diet

Plate 8. Fish fed with Clarias bone meal supplemented diet

Plate 9. Fish fed with cattle bone meal supplemented diet

performance in fish fed Chicken bone meal-supplemented diet.

Phosphorus is an essential component of organic compounds involved in almost every aspect of metabolism. For optimum performance, P needs to be present in the diet at required levels. The dietary requirement for phosphorus ranges between 0.45 - 1% [5]. However, in some cases feeds do not contain sufficient amounts of P and

supplementation is necessary to optimize fish performance. Fresh water fish require dietary sources of phosphorus to meet their relatively high metabolic requirement since the water is deficient in phosphorus [15].

5. CONCLUSION

There is need for phosphorus supplementation in the diets of young indigenous catfish such as *Clarias gariepinus.* The study revealed poor growth performance at no P supplementation, while body phosphorus continued to increase above that required for maximum growth in fish fed cattle bone meal- supplemented diet. The study had shown that phosphorus is a growth promoter when supplied from the right source and at optimal concentration in fish feed which validated some of the early work done on warm water fresh fishes. Under practical condition, organic phosphorus sources should be included in the diets to prevent poor growth and symptoms of skeletal deformity often experienced in artificially raised fish. In addition, organic sources of phosphorus in fish feed are cheaper and easy to source and this will help reduce cost of feed formulation.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. NRC. National research council. Nutrient requirements of warm water fishes and shell fishes. National Academy Press, Washington DC, USA. 1993;114.
2. Kyeong-Jun Lee, Madison S, Powell, Frederick T, Barrows, Scott Smiley, Peter Bechtel, Ronald W. Hardy. Evaluation of supplemental fish bone meal made from Alaska seafood processing by products and dicalcium phosphate in plant protein based diets for rainbow trout (*Oncorhynchus mykiss*). Aquaculture. 2010;302:248-255.
3. Davis DA, Gatlin DM. Dietary requirements of fish and marine crustaceans. Rev. Fish Sci. 1996;4:75-99.
4. Rania S, Mabroke Azab M, Taboun Ashraf Suloma, Ehab R, El-Haroun. Evaluation of meat and bone meal and mono-sodium phosphate as supplemental dietary phosphorus sources for broodstocks nile tilapia (*Oreochromis niloticus*) under the condition of hapa-in- pond system. Turkish Journal of Fisheries and Aquatic Sciences 2013;13:11-18.
5. Lall SP. The minerals. In: Fish nutrition. 3rd edition. Halver JE, Hardy RW. eds. academic press, New York. 2002;264–274.
6. Omitoyin BO. Introduction to fish farm in Nigeria. University press. Ibadan, Nigeria; 2007.
7. Lall SP. Comparative mineral nutrition of fish sources and requirements. In: Nutritional biotechnology in the feed and food industries, Lyons TP, Jacques KA, JM Hower (Eds.). Nottingham University Press, Nottingham, UK. 2007;387-391.
8. Ye CX, Liu YJ, Tian LX, Mai KS, Du ZY, Yang HJ, Niu J. Effect of dietary calcium and phosphorus on growth, feed efficiency, mineral content and body composition of juvenile grouper. Epinephelus Coioides Aquacult. 2006;255:263-267.
9. Asgard T, Shearer KD. Dietary phosphorus requirement of juvenile Atlantic salmon, Salmon Salar. C. Aquaculture Nutr. 1997;3:17-23.
10. AOAC. Association of Analytical chemists. Official methods of analysis 16th edition. 2000;1141.
11. Faturoti EO, Balogun AM, Ugwu LLC. Nutrient utilization and growth responses of *Clarias* (Clarias lazera) fed different dietary protein levels. Nigerians Journal of Applied Fisheries and Hydrobiology. 1986;1:41-45.
12. AOAC. Association of official analytical chemists. Official methods of analysis. Washington DC. USA. 15th edition. 1990;200-210.
13. Adebayo IA. Growth and blood chemistry of selected clariid catfishes fed phosphorus supplemented diets. Journal of Fisheries and Aquatic Science. 2011;6(5):545-554.
14. Adebayo IA. Optimal phosphorus requirement of *Heterobranchus bidorsalis* using purified diet. International journal of Agriculture and forestry. 2012;2(5):195-198.

15. Albrektsen S, Hope B, Aksnes A. Phosphorus (P) deficiency due to low P availability in fishmeal produced from blue whiting (*Micromesistius poutassou*) in feed for under-yearling Atlantic salmon (*Salmo salar*) smolt. Aquaculture. 2009;296:3118-328.

Peer-review history:
The peer review history for this paper can be accessed here:
http://www.sciencedomain.org/review-history.php?iid=867&id=2&aid=7579