Journal Home page : www.jeb.co.in ★ E-mail : editor@jeb.co.in

JEB
Journal of Environmental Biology

ISSN: 0254-8704
CODEN: JEBIDP

Effects of organic acids on growth and phosphorus utilization in rainbow trout, *Oncorhynchus mykiss*

Abhed Pandey[1]* and Shuichi Satoh[2]

[1]College of Fisheries, Guru Angad Dev Veterinary and Animal Sciences University, Ludhiana - 141 004, India
[2]Laboratory of Fish Nutrition, Department of Marine Biosciences, Tokyo University of Marine Science and Technology, Tokyo-108-8477, Japan
*Corresponding Authors Email : abhed@gadvasu.in

Publication Info

Paper received:
29 May 2013

Revised received:
18 September 2013

Accepted:
11 October 2013

Abstract

The present study aimed to investigate the effect of phosphorus and various organic acids supplementation on growth, nutrient retention and loading in rainbow trout fed on low fishmeal based diets. Five experimental diets were formulated, Diet D1 (0.5P) was positive control with 0.5% inorganic phosphorus addition and D2 (0P, negative control) without addition of inorganic phosphorus. D3, D4 and D5 were supplemented with 1% fumaric (FuA), formic (FoA) and acetic (AA) acids, respectively. All the diets were fed until satiation to duplicate groups of 25 fish for12 weeks. Feed conversion ratio (FCR) was recorded best with FoA diet and absorption of phosphorus was significantly higher ($P<0.05$) in all the diets supplemented with organic acids and was similar to positive control. The phosphorus and nitrogen retention with FoA diet was significantly high ($P<0.05$) as compared to 0P diet, in turn reducing their excretion. Hence, the present study demonstrated that, without additional phosphorus in the low fish meal-based diets, FoA supplement improved growth performance, absorption and retention of phosphorus and nitrogen and reducing excretion; thus it can be better incorporated to develop environment-friendly feed for rainbow trout.

Key words

Fish feed, *Oncorhynchus mykiss*, Organic acid, Phosphorus

Introduction

The increased production of aquatic organisms is being achieved by the use of more intensive and modern farming technologies. Excess deposition of phosphorus and nitrogen is harmful to both fish and aquatic ecosystem (Tucker, 1996). Since the ultimate source of aquaculture waste is the feed fed to fish, one effective way to reduce the waste load of fish farm effluent is to improve mineral utilization and feed quality, and achieve a reduction in excretion of phosphorus and nitrogen. Improvement of dietary phosphorus retention efficiency has become one of the current areas of research and main strategy to reduce the environmental impact of aquaculture (Lall, 1991; Sugiura and Hardy, 2000). Fish meal is the major protein source in fish feed; it contains more than 3% of the element in the form of tri-calcium phosphate which requires low pH for solubilization and is not easily available to fish. Fish meal phosphorous availability also depends on fish species, based on their anatomical and physiological features. An increase of fish meal in diet generally increases the phosphorous discharge (mainly through faeces), irrespective of their gut anatomy.

Formic acid increases apparent availability of phosphorus, calcium and magnesium in semi-moist diets containing fish protein concentrate and fishmeal in rainbow trout (Vielma and Lall, 1997). Numerous factors have been shown to affect gastrointestinal absorption of mineral elements in animals and fish modes of action of these supplements are diverse (Sugiura *et al.*, 1998). Since several factors are known to affect the bioavailability of dietary inorganic trace elements, continuous efforts have been made to improve their utilization. Cheng *et al.* (2003) reported that the weight gain, feed conversion ratio, apparent retention of protein and phosphorus were significantly improved in rainbow trout diet supplemented with methionine hydroxyl analogue. Citric acid supplementation to rainbow trout diet, chelates Ca and P, which increases the solubility of Ca and P and improving mineral utilization (Vielma *et al.,* 1999). Birds and mammals can digest nearly 100% of P in fish meal (Sugiura *et al.,* 2006). Addition of acids to fish feed is an attempt to aid the weak acidity of fish stomach. It has been known to improve phosphorus digestibility (Sugiura and Hardy, 2000). Advantages of using organic acid are the facts that it is easily available, cheap, required in lesser amount and can be easily mixed in the diet. These advantages gives great potential to develop cost effective method to produce environment friendly fish feed.

In a previous study (Pandey and Satoh, 2008) on rainbow trout several organic acids such as citric acid, lactic acid, methionine hydroxy analog and liquid trace elements were supplemented at 1% level in the experimental diets and it was found that citric acid improved the availability of some minerals in fish meal. This study aimed, to investigate the effect of phosphorus and various organic acids supplementation on growth, nutrient retention, and loading in rainbow trout fed on low fishmeal based diets.

Materials and Methods

Experimental diets : Fish diets were formulated with low level of fish meal (FM) and alternate low-phosphorus ingredients (defatted soybean meal, SBM; corn gluten meal, CGM; and feather meal, FM). Diet supplemented with 0.5% mono calcium phosphate (0.5P) was considered as positive control and diet without any phosphate (0P) was considered as negative control. Diets FuA, FoA and AA were supplemented with 1% fumaric acid, formic acid and acetic acid, respectively. The composition of vitamin and mineral premixes used were same as described previously (Satoh *et al.,* 2003). 1% chromic oxide premix (1Cr_2O_3 : 1dextrin) was added in all the five experimental diets to determine the digestibility and absorption of nutrients. The diets were pelleted using laboratory pelletizer (AFZ12M, Hiraga-Seikakusho, Kobe, Japan), and stored at 4^0C until used. The experimental set up was designed in a completely randomized design with two replication per treatment.

Fish, experimental conditions, and feeding : Thirty fish (average weight 17.6g) were randomly distributed in each well aerated 60 L glass tanks and duplicates were maintained for all five diets. Another 15 fish were randomly selected and used for initial whole body proximate and mineral analysis. The feeding experiment was conducted for 12 weeks, in a partially re-circulated system, with a permanent water supply at a rate of 0.6-1.0 l min^{-1} and adequate aeration. Average water temperature was 17.1 ºC and it ranged from 15ºC to 19ºC. The fish were hand-fed twice a day, 6 days a week to apparent satiation level.

Sample collection and chemical analyses : Initial weight data were obtained at the start of experiment and growth of fish was measured every 3 weeks subsequently. Upon termination of experiment, 10 fish from each tank were randomly selected for chemical analysis of the whole body. Whole body samples were pooled from 5 fish and minced in a centrifugal mill (Retsch ZM I00, Hann, Germany) fitted with 0.5 mm screen and stored at -20^0C until analyzed. Tokyo University of Fisheries (TUF) column system was used (Satoh *et al.,* 1992) for faeces collection with the aid of Cr_2O_3 as the indigestible marker. Proximate and mineral analyses in whole body samples and diets were made in three replicates, and the values obtained were employed for calculating phosphorus and protein retention in whole body, following standard procedures and according to the formula of Satoh *et al*. (1987, 1996).

(Final body nutrient - initial body nutrient / Nutrient intake) x 100

Digestion of the samples for mineral analyses was conducted with nitric acid using the MLS-1200 Mega Microwave Digestion System (Milestone, Bergamo, Italy). phosphorus concentration was analyzed using a spectrophotometer (UV 256 FW, Shimadzu, Kyoto, Japan) at a wavelength of 750 nm and mineral content were determined employing Atomic Absorption Spectrophotometer (Hitachi Z-5010, Tokyo, Japan). Feed performance was evaluated on the basis of weight increase, daily feed consumption, specific growth rate (SGR) and feed conversion ration (FCR). Nutrient retained in the fish body (%) was obtained using the formula presented by Watanabe (1988). Nutrient loading (kg t^{-1} fish produced) was calculated based on the intake and retention in whole body, on a weight gain basis using the formula given by Satoh *et al*. (2003).

{[FCR x nutrient in diet (g) - nutrient retained in fish (g)] / production (t)} x 1000

Statistical analyses : Results were analyzed using one-way ANOVA (Statistica Release 6.0 StatSoft. Inc., USA). Difference between treatments were compared by Duncan's Multiple Range Test. Level of significance was tested at P<0.05.

Results and Discussion

Growth and feed performance : Among all organic acids supplemented diets, the specific growth rate (SGR) value was tend to be high in FoA and low in AA group. FCR of the 0P diet was worst and improved with FoA supplementation. Among all treatments, apparent protein digestibility (APD%) was recorded significantly (P<0.05) low in AA diet group (Table 1).

Carcass proximate composition and mineral contents : Moisture and ash content was lower and lipid content was higher in all the experimental groups than in the initial fish. Although the proximate composition was not appreciably different between treatments, ash content was tend to be high in FuA, FoA and AA groups compared to 0P group. Among all organic acid supplemented groups, carcass phosphorus content was tend to high in FuA and AA groups whereas carcass calcium and zinc content tend to be high in AA group (Table 2).

Table 1 : Growth and feed performances of rainbow trout fed experimental diets for 12 weeks[†]

Components	Diet code				
	0.5P	0P	FuA	FoA	AA
Mean initial weight (g)	17.7±1.7	17.5±1.5	17.2±1.6	18.0±1.5	17.8±1.5
Mean final weight (g)	91.5±6.2^{a}	81.1±0.7^{a}	87.5±6.1^{a}	95.2±9.0^{a}	76.6±3.8^{a}
SGR (% per day)	2.00±0.1^{a}	1.87±0.1^{a}	1.98±0.1^{a}	2.03±0.1^{a}	1.78±0.0^{a}
FCR	0.98±0.0^{ab}	1.09±0.1^{a}	0.96±0.1^{ab}	0.91±0.2^{b}	1.07±0.0^{ab}
Survival (%)	96.0±0.0	94.0±2.8	98.0±0.7	98.0±0.7	94.0±0.7

[†]Values (mean ± standard deviation) in a row sharing same letter superscripts are not significantly different (*P*>0.05).

Table 2 : Proximate and mineral composition of rainbow trout after 12 weeks feeding[†]

Components	Initial	Diet code				
		0.5P	0P	FuA	FoA	AA
Moisture (%)	74.3	70.2±0.4^{a}	70.3±0.2^{a}	70.3±0.0^{a}	70.2±0.1^{a}	70.7±0.3^{a}
Crude ash (%)	2.72	2.25±0.0^{a}	2.05±0.0^{b}	2.15±0.0^{ab}	2.11±0.1^{ab}	2.12±0.0^{ab}
Crude protein (%)	16.2	16.5±0.2^{a}	16.2±0.6^{a}	16.5±0.3^{a}	16.6±0.1^{a}	16.4±0.1^{a}
Crude lipid (%)	6.12	10.3±0.4^{a}	10.8±0.5^{a}	10.8±0.4^{a}	10.8±0.2^{a}	10.5±0.0^{a}
P (mg g^{-1})	6.18	5.23±0.0^{a}	4.32±0.0^{c}	4.72±0.0^{c}	4.40±0.0^{c}	4.76±0.2^{b}
Ca (mg g^{-1})	5.31	4.32±0.0^{ab}	4.12±0.3^{ab}	3.85±0.2^{b}	3.79±0.0^{b}	4.53±0.2^{a}
Mg (mg g^{-1})	0.34	0.30±0.0^{a}	0.30±0.0^{a}	0.29±0.0^{a}	0.28±0.0^{a}	0.31±0.0^{a}
K (mg g^{-1})	3.46	3.44±0.1^{a}	3.63±0.2^{a}	3.47±0.0^{a}	3.40±0.0^{a}	3.36±0.2^{a}
Na (mg g^{-1})	1.14	0.99±0.1^{a}	1.14±0.2^{a}	1.14±0.0^{a}	1.14±0.1^{a}	1.12±0.0^{a}
Zn (µg g^{-1})	29.2	23.9±1.1^{b}	26.7±0.1^{a}	22.8±1.8^{b}	22.6±1.0^{b}	27.2±1.3^{a}
Cu (µg g^{-1})	2.46	2.75±0.1^{a}	2.97±0.4^{a}	2.80±0.1^{a}	2.68±0.1^{a}	2.52±0.3^{a}
Mn (µg g^{-1})	3.39	1.69±0.1^{a}	1.98±0.0^{a}	2.00±0.0^{a}	2.01±0.0^{a}	2.05±0.0^{a}
Fe (µg g^{-1})	48.2	39.5±0.7^{a}	49.2±10^{a}	50.4±12^{a}	40.8±10^{a}	50.1±2.3^{a}

[†]Values (mean ± standard deviation) in a row sharing same letter superscripts are not significantly different (*P*>0.05).

Bone mineral contents : Bone phosphorus and calcium contents were recorded lowest in 0P diet and tend to be high with organic acids supplementation and recorded best with FoA diet. Among all organic acid diets, bone zinc content was recorded significantly (*P*<0.05) low with FuA diet and high with AA diet group (Table 3).

Absorption, retention and excretion of phosphorus and nitrogen : Absorption of phosphorus was recorded lowest in 0P diet without any phosphorus supplement and it increased significantly (P<0.05) in all the diets supplemented with organic acids whereas absorption of nitrogen increased significantly (P<0.05) in FoA and AA groups as compared to 0P group. Retention of phosphorus and nitrogen was recorded lowest in 0P group and it increased significantly (P<0.05) with FoA supplementation as compared to 0P group. Excretion of phosphorus and nitrogen was tend to be high in 0P diet and it reduced significantly (P<0.05) with FoA supplementation, as compared to 0P group (Table 4).

In continuation to previous study (Pandey and Satoh, 2008), where citric acid, lactic acid, methionine hydroxy analog and liquid trace elements were supplemented at 1% level and citric acid showed better results, this experiment was conducted again at 1% supplemental level to observe the effect of other organic acids like fumaric, formic and acetic acids**.** The present study showed that enhanced growth of fish, with addition of phosphorus and FoA, compared to the group without phosphorus supplement indicates that in FoA group, FoA might have liberated adequate inorganic phosphorus from tri-calcium phosphate (is from the fish meal) to allow fish growth. Previous studies also revealed that the growth of rainbow trout might be affected by chemical form of trace minerals (Satoh *et al.,* 2001); in red sea bream (Sarker *et al.,* 2005) and phosphorus absorption varied from feed ingredients in rainbow trout during growing stages (Satoh *et al.,* 2002). Improvement of digestibility of nutrients and energy depends on the level of organic acid used (Partanen and Mroz, 1999). The improved performance and digestibility because of organic acids supplementation may be due to lowered pH resulting in a higher dissociation of mineral compounds, reduced rate of gastric emptying and formation of chelated mineral complexes that can be easily absorbed. These results were in agreement with previous study of Hernández *et al.* (2005) where 0.5% mono-calcium phosphate addition to the same basal diet was sufficient for optimal growth in rainbow trout; observations in the present study on weight gain indicate that if supplemented with FoA, additional phosphorus was not required.

Table 3 : Bone mineral composition of rainbow trout after 12 weeks feeding†

Components	Diet code				
	0.5P	0P	FuA	FoA	AA
P (mg g^{-1})	90.0±10^{a}	80.0±1.3^{b}	88.3±3.5^{ab}	90.9±2.2^{ab}	84.8±13^{ab}
Ca (mg g^{-1})	176±25^{a}	156±5.2^{a}	167±15^{a}	168±4.2^{a}	163±16^{a}
Mg (mg g^{-1})	3.91±0.3^{a}	3.36±0.0^{b}	3.31±0.4^{b}	3.57±0.1^{ab}	3.54±0.0^{ab}
K (mg g^{-1})	0.63±0.0^{a}	0.86±0.1^{a}	0.64±1.0^{a}	0.69±0.3^{a}	0.69±0.1^{a}
Na (mg g^{-1})	1.47±0.0^{a}	1.38±0.0^{a}	1.29±0.3^{a}	1.45±0.0^{a}	1.31±0.1^{a}
Zn (µg g^{-1})	237±11^{a}	228±0.1^{a}	207±9.2^{bc}	222±2.0^{ab}	225±1.0^{a}
Cu (µg g^{-1})	0.90±0.0^{a}	0.61±0.0^{a}	0.80±0.1^{a}	0.80±0.4^{a}	0.73±0.2^{a}
Mn (µg g^{-1})	19.6±0.4^{a}	20.0±1.3^{a}	17.0±2.0^{a}	17.8±0.7^{a}	16.0±2.0^{a}
Fe (µg g^{-1})	52.3±3.9^{a}	60.1±6.6^{a}	48.3±16^{a}	53.5±4.7^{a}	56.3±14^{a}

†Values (mean ± standard deviation) in a column sharing same letter superscripts are not significantly different (P>0.05).

Table 4: Retention and excretion of nitrogen and phosphorus in rainbow trout after 12 weeks of feeding

Diet code	Phosphorus absorption (%)	Phosphorus retention (%)	Phosphorus excretion(kg t^{-1})	Nitrogen absorption (%)	Nitrogen retention (%)	Nitrogen excretion (kg t^{-1})
0.5P	60.5±4.0^{a}	54.2±1.7^{ab}	4.07±0.2^{a}	91.7±0.2^{ab}	39.2±1.3^{b}	39.5±1.5^{ab}
0P	51.4±1.0^{b}	43.0±1.0^{b}	4.66±1.2^{a}	90.6±1.0^{b}	34.3±2.9^{b}	48.0±8.6^{a}
FuA	61.9±2.1^{a}	50.0±2.2^{ab}	3.54±0.4^{a}	91.4±0.8^{ab}	40.4±0.7^{ab}	37.6±2.1^{ab}
FoA	60.6±3.5^{a}	56.0±2.1^{a}	2.99±0.0^{a}	92.8±0.0^{a}	42.4±1.3^{a}	34.6±1.4^{b}
AA	63.3±1.8^{a}	53.0±0.6^{ab}	3.72±0.4^{a}	93.0±0.1^{a}	36.0±0.4^{b}	45.3±1.2^{ab}

Values are presented as mean ± standard deviation (n = pooled samples of 5 fish/tank); Values (mean ± standard deviation) in a row sharing same letter superscripts are not significantly different (P>0.05).

Dietary phosphorus requirement for optimum growth, feed utilization and bone mineralization ranging from 4 to 8 g kg^{-1} was reported rainbow trout and other fish (Chao-Xia *et al.,* 2006; Sugiura *et al., 2000*). The estimated available phosphorus level in basal diet employed in this study was approximately 3.6 mg g^{-1}, which was lower than the reported minimum requirement for normal growth of rainbow trout, since at phosphorus levels well below the requirement, bone mineralization responds to increments in available phosphorus supply in an almost linear pattern (Vielma and Lall, 1998).

Higher carcass mineral contents obtained in the diets supplemented with organic acids (OA) in this study suggests that OA enhanced the mineral utilization of fish meal. Dietary acidification by citric acid significantly increased whole body iron in fish (Vielma *et al.,* 1999). Sarker *et al.* (2005) reported that carcass calcium content significantly improved due to citric acid supplementation

In this trial, the absorption of P and N was higher in the experimental diets supplemented with FoA and AA is in agreement with the findings of Sugiura *et al.* (2006). Supplementation with formic acid enhanced the apparent absorption of phosphorus in fish meal-based diet in rainbow trout. Higher retention of phosphorus along with nitrogen with FoA diet indicated higher bioavailability of minerals to rainbow trout compared to inorganic source. This result could be attributed to two related factors *i.e.* effect of dietary acidification and solubilization and effect of subsequent chelation of released cations (Sarker *et al.,* 2005). Satoh *et al.* (2002) observed that, depending on the feed ingredients, phosphorus absorption varied as rainbow trout grew and phosphorus absorption from Pollock meal and sardine meal decreased slightly as body weight increased. The increased phosphorus and nitrogen retention with FoA fed group, and thereby lowered excretion in this experiment might have induced better growth.

The growth of fish in AA group was tended to low and the apparent protein digestibility value was also significantly reduced but there were no significant difference in the apparent protein digestibility due to other organic acids supplementation.

Although the proximate composition was not appreciably different between treatments, ash content tended to be high in FuA, FoA and AA groups compared to 0P group. Among all organic acid supplemented groups, carcass phosphorus content tended to high in FuA and AA groups whereas carcass calcium and zinc content tended to be high in AA group. Bone phosphorus and calcium content were recorded lowest in 0P diet and tended to be high with organic acids supplementation and recoded best with FoA diet. Among all organic acid diets, bone zinc content was recorded significantly (P<0.05) low with FuA diet and high with AA diet group. It was reported that *Epinephelus coioides* fed with low phosphorus diet significantly had lower zinc level of vertebrae and scales (Chao-Xia *et al*., 2006). The increase in carcass mineral and bone mineral deposition suggests that the organic acids enhanced the mineral utilization of dietary fish meal and soybean meal by protecting the inhibitory action of dietary components. Similar results were observed by other researchers (Pandey and Satoh, 2008; Sarker *et al*., 2005). Pandey and Satoh (2008) reported reported that dietary acidification by citric acid significantly increased whole body iron in fish. Sugiura *et al*. (2006) also observed an increase in the apparent availability of Ca, P, Mg, Mn and Fe in rainbow trout fed fish meal based diets supplemented with citric acid.

Therefore, it can be concluded from the present study that addition of inorganic phosphorus supplementation is not necessary in low fish meal-based diets supplemented with FuA for juvenile rainbow trout and organic acids increased the apparent availability of phosphorus and several other minerals in fish meal. Addition of FuA improved phosphorus and nitrogen retention. Hence, the use of FuA can reduce the potential for environmental pollution in aquaculture waste; thus, it can be incorporated better to develop environment-friendly feed for rainbow trout.

Acknowledgments

We want to express our appreciation to the Okutama Branch of Tokyo Metropolitan Fisheries Experimental Station for providing the fish employed in this research and to the Japanese Ministry of Education, Culture, Sports, Science and Technology (MONBUKGAKASHO) for financially supporting this work.

References

Chao-Xia, Y., L. Yong-Jian, T. Li-Xia, M. Kang-Sen, D. Zhen-Yu, Y. Hui-Jun and N. Jin: Effect of dietary calcium and phosphorus on growth, feed efficiency, mineral content and body composition of juvenile grouper *Epinephelus coioides*. *Aquaculture*, **255**, 263-271 (2006).

Cheng, Z.J., R.W. Hardy and M. Blair: Effects of supplementing methionine hydroxyl analogue in soybean meal and distiller's dried grain-based diets on the performance and nutrient retention of rainbow trout, *Oncorhynchus mykiss*. *Aquacult. Nutr*., **34**, 1303-1310 (2003).

Hernández, A., S. Satoh and V. Kiron: Effect of monocalcium phosphate supplementation in a low fish meal diet for rainbow trout based on growth, feed utilization, and total phosphorus loading. *Fish. Sci*., **71**, 817-822 (2005).

Lall, S.P.: Digestibility, metabolism and excretion of dietary phosphorus in fish. In: Nutritional Strategies and Aquaculture Waste. *Proc. Ist Int. Symposium on Nutritional Strategies in Management of Aquaculture Waste* (Eds.: C.B. Cowey and C.Y. Cho), pp. 21–35. University of Guelph, Guelph (1991).

Pandey, A. and S. Satoh: Effects of organic acids on growth and phosphorus utilization in rainbow trout *Oncorhynchus mykiss*. *Fish. Sci*., **74**, 867-874 (2008).

Partenen, K.H. and Z. Mroz: Organic acids for performance enhancement in pig diets. *Nutr. Res. Rev*., **12**, 1-30 (1999).

Sarker, M.S.A., S. Satoh and V. Kiron: Supplementation of citric acid and amino acid-chelated trace element to develop environment-friendly feed for red sea bream *Pagrus major*. *Aquaculture*, **248**, 3-11 (2005).

Satoh, S., T. Takeuchi and T. Watanabe: Availability to rainbow trout of zinc in white fish meal and of various zinc compounds. *Nippon Suisan Gakkaishi*, **53**, 595– 599 (1987).

Satoh, S., C.Y. Cho and T. Watanabe: Effect of fecal retrieval timing on digestibility of nutrients in rainbow trout diet with the Guelph and TUF feces collection systems. *Nippon Suisan Gakkaishi*, **58,** 1123-1127 (1992).

Satoh, S., N.K. Porn-Ngam, T. Takeuchi and T. Watanabe: Influence of dietary phosphorus levels on growth and mineral availability in rainbow trout. *Fish. Sci.*, **62**, 483–487 (1996).

Satoh, S., M.J. Apines, T. Tsukioka, V. Kiron, T. Watanabe and S. Fujita: Bioavailability of amino acids chelated and glass embedded manganese to rainbow trout *Oncorhynchus mykiss* fingerlings. *Aquacult. Res*., **32**, 18–25 (2001).

Satoh, S., M. Takenazawa, A. Akimoto, V. Kiron and T. Watabe: Changes of phosphorus absorption from several feed ingredients in rainbow trout during growing stages and effect of extrusion of soybean. *Fish. Sci*., **68**, 325-331 (2002).

Satoh, S., A. Hernández, T. Tokoro, Y. Morishita, V. Kiron and T. Watanabe: Comparison of phosphorus retention efficiency between rainbow trout (*Oncorhynchus mykiss*) fed a comercial diet and a low fish meal based diet. *Aquaculture*, **224**, 271-282 (2003).

Sugiura, S.H., F.M. Dong, C.K. Rathebone and R.W. Hardy: Apparent protein digestibility and mineral availability in various feed ingredients for salmonid feeds. *Aquaculture*, **159**, 177-202 (1998).

Sugiura, S.H. and R.W. Hardy: Environmentally friendly feeds. *In*: Encyclopedia of Aquaculture (Ed.: R.R. Stickney). Wiley-Interscience, New York, pp. 299-310 (2000).

Sugiura, S.H., F.M. Dong and R.W. Hardy: A new approach to estimating the minimum dietary requirement of phosphorus for large rainbow trout based on non-fecal excretions of phosphorus and nitrogen. *J. Nutr.,* **130,** 865–872 (2000).

Sugiura, S.H., P.K. Roy and R.P. Ferraris: Dietary acidification enhances phosphorus digestibility but decreases H^+/K^+ - ATPase expression in rainbow trout. *J. Exp. Biol.,* **209,** 3719–3728 (2006).

Tucker, C.S.: The ecology of channel catfish culture ponds in Northwest Mississippi. *Rev. Fish. Sci.*, **4**, 1-55 (1996).

Vielma, J. and S.P. Lall: Dietary formic acid enhances apparent digestibility of minerals in rainbow trout *Oncorhynchus mykiss* (Walbaum). *Aquacult. Nutr*., **3**, 265-268 (1997).

Vielma, J. and S.P. Lall: Control of phosphorus homeostasis of Atlantic salmon (*Salmo salar*) in fresh water. *Fish. Phys. Biochem*., **19**, 83–93 (1998).

Vielma, J., K. Ruohonen and S.P. Lall: Supplemental citric acid and particle size of fish bone-meal influence the availability of minerals in rainbow trout *Oncorhynchus mykiss* (Walbaum). *Aquacult. Nutr.*, **5**, 65-71(1999).

Watanabe, T.: Fish Nutrition and Mariculture. In: The General Aquaculture Course (Ed.: T. Watanabe). Kanagawa International Fisheries Training Centre, Japan International Co-operation Agency (JICA), Tokyo, p. 233 (1988).