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Effects of alternating feeding regimes with varying dietary phosphorus levels on growth, mineralization, phosphorus retention and loading of large rainbow trout (*Oncorhynchus mykiss*)

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Abstract – Excessive phosphorus (P) levels in freshwater aquaculture effluents are a major environmental problem in certain receiving water bodies. This study aimed to test an approach alternating that alternating feeding P deficient and P sufficient diets and measure P loading from rainbow trout (*Oncorhynchus mykiss*) culture. Three experimental practical diets consisting of P-deficient (0.4% P, P04), optimum level of P (0.6% P, P06) and P-sufficient as control diet (0.8% P, P08) were formulated. Six different feeding regimes of P-sufficient diet continuously (P08), P-deficient diet continuously (P04), optimum dietary level of P (P06) continuously, one week P-deficient/one week optimum level of P diet (P04/P06.1), 2 weeks P-deficient/2 weeks optimum level of P diet (P04/P06.2) and 4 weeks P-deficient/ 4 weeks optimum level of P diet (P04/P06.4) were tested. Fish were fed twice daily to apparent satiation level 16 weeks. Fish fed all alternating regimes showed growth rate (weight and length) comparable to those of continuous feeding with P08 and P06 diet. The feed conversion ratios (FCR) for all alternating regimes were comparable to that of the P08 and P06 continuous feeding regime. Neither the thermal unit growth coefficient (TGC) nor condition factor (K) significantly influenced by feeding regimes. Vertebrae P, ash and whole body ash content did not differ among regimes. Except fish fed continuous P04 diet, the ash and P content in opercula and whole body total P content were not significantly different among each other in a continuous feeding and alternating feeding schedule.

Fish fed all alternating regimes showed significantly lower P consumption than those fed continuously fed with P08 and P06. Different feeding regimes had no effect P retention. Significantly higher P loading (solid and dissolved) was noted in fish fed continuously with P08 diet, in contrast P loading values were lower for all alternating feeding regimes. The study demonstrated that growth and tissue mineralization of fish maintained on alternating feeding regimes with P04 and P06 diet were comparable to those continuously fed with diet of P08. These results demonstrate that it is possible to reduce P intake by 34% and reduce P loading 52% by adopting alternating feeding regimes compared to P08 diet. This study provides evidence that alternating feeding of P deficient and optimum dietary levels using practical ingredients can be adopted as a means of reducing P loading from rainbow trout culture without compromising growth.

Key words: Alternating feeding regime / Trout / Phosphorus / Loading / Growth

1 Introduction

In rainbow trout culture, discharge of phosphorus (P) into the environment arise from feed that is not ingested by the fish, non-digestible P or from absorbed P that exceeds physiological requirements. Excessive P levels in feed, results in excretion of excess or unavailable P as inorganic phosphate mainly in the urine or in the feces (Bureau and Cho 1999; Rodehutscord et al. 2000; Sugiura et al. 2000; Hua et al. 2008; Sarker et al. 2009; Bureau and Hua 2010). Intensive aquaculture can generate environmental P loadings that may contribute to eutrophication of sensitive receiving water bodies. In the province of Quebec (Canada), the limit for P loading from freshwater trout production is now regulated with a maximum permitted of 4.2 kg ton^{-1} of fish produced (TFAEDQ 2003). A number of technologies based on bioengineering, biotechnological, biological and chemical cleaning have been reported to reduce effluent P from aquaculture operation (Summerfelt et al. 1995; Dumas et al. 1998; Hussenot et al. 1998). Many of these technologies are subject to high initial capital investment, difficulty in controlling critical operational parameters and are not particularly applicable for cage culture operations

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Table 1. Ingredients and proximate composition of the experimental diets.

	Diet		
Ingredients (g kg^{-1})	P04	P06	P08
Fish meal (SeaPro 75 01.011)	150	150	170
Blood meal (AP 301G)	100	100	0
Feather meal (Feather meal R1)	100	100	0
Wheat flour	110	100	0
Soybean meal (46%)	60	60	0
Corn gluten meal (Bi-Pro. SC 342)	170	170	376
Wheat gluten	50	50	0
Whey powder (Anilac)	50	50	80
Poultry byproduct meal (65%PB)	0	0	130
Lysisn HCL	0	0	14
Fish oil	170	170	190
CaHPO4	0	10	0
Vitamin-mineral premix[1]	6	6	6
Carophyll Pink (Astaxanthine)	2.5	2.5	2.5
Sipernat 50 (silicon dioxide marker)[2]	20	20	20
Water	11.5	11.5	11.5
Total	1000	1000	1000
Proximate composition (% as dry matter basis)			
Crude protein	49.1	49.5	49.2
Crude lipid	19.6	19.6	23.3
Ash	4.9	5.4	7.3
energy (MJ kg^{-1})	21	21.6	23.5
Total P	0.4	0.6	0.8
Available P[3]	0.2	0.3	0.4

[1] Supplied the following: (to provide mg kg^{-1} except as noted): retinyl acetate 2500 IU, cholecalciferol 2400 IU, tocopheryl acetate, 50; menadione, 10; thiamin, 1; riboflavin, 4; pyridoxine, 3; Ca-pantothenate, 20; vitamin B-12, 0.01; niacin, 10; biotin, 0.15; folic acid, 1; choline, 1000; inositol, 300; magnesium carbonate, 1.24 g; calcium carbonate, 2.15 g; potassium chloride, 0.90 g; sodium chloride, 0.40 g; potassium iodide, 0.4; copper sulfate, 30; cobalt sulfate, 0.2; ferric sulfate, 0.20 g; manganese sulfate, 30; zinc sulfate, 40; dibasic calcium phosphate, 5 g; sodium fluoride, 10.
[2]Sipernat 50: source of acid insoluble ash comprised of 98.5% SiO_2 with an average particle size of 50 μm.
[3]Available P = Total P in diet × ADC of P.

(Cripps 1994; Chevalier et al. 2000). Furthermore the most contentious component of the effluent is soluble P that is readily available to aquatic algae; effluent P in feces and large particulates can be minimized by the use of settling tank but not soluble P. Reducing outputs of these fractions, especially dissolved wastes, is considered a key element for the long term sustainability of aquaculture in many parts of the world.

To address issues related to excessive P loading, a number of nutritional approaches have been proposed including improved feed efficiency, reduction of P in feeds using ingredients with high P bioavailability (Lall 1991; Nutt 1991; Vandenberg 2001) and phase feeding strategies (Lellis et al. 2004). The approach of alternating phosphorus deficient and sufficient diets has the potential to reduce P excretion without loss in production (Hardy et al. 1993), however this approach has not been evaluated using practical ingredients. Fish on alternating protein feeding schedule (i.e., high-protein feed alternating with low-protein feed) exhibited growth performance, reduced significantly nitrogen loading and reduced feed cost in many studies (De Silva 1989; De Silva et al. 1993; De Silva 2006; Sevgili et al. 2006). This study was therefore aimed to test the validity of the alternate feeding regimes of P deficient diets and optimum P level in reducing P loading from rainbow trout.

2 Materials and methods

2.1 Experimental diets

Three experimental pelleted practical diets consisting of P-deficient (0.4% total P and 0.2% available P, coded as P04), optimum level of P (0.6% total P and 0.3% available P, coded as P06) and P-sufficient as control diet (0.8% total P and 0.4% available P, coded as P08) were formulated using practical ingredients (Table 1). Low-P ingredients were carefully selected and only those with relatively high P availability were retained. The composition of P06 diet was identical to P04 diet with 10 g$CaHPO_4$ kg^{-1} diet added to obtain optimum level of P for rainbow trout. $CaHPO_4$was added as this inorganic P source is readily available to the rainbow trout (Ogino et al. 1979; Lall 1991; Gregus 2000). The P content (0.6%) of the P06 diet was in the range of the dietary P requirement that have been reported for normal growth of rainbow trout (0.5–0.6%) equal to a digestible P to energy ratio (MJ g^{-1}) of 0.19–0.27 (NRC 1993; Rodehutscord 1996). The sufficient diet (P08) was formulated according to the open formulation developed University of Guelph, Canada with the fish meal being a low-ash deboned fraction. P deficient (P04) and optimum level of P containing diet (P06) were formulated with low level of fish meal (15%) with other low-P ingredients: blood meal (10%), feather meal (10%), corn gluten meal (17%), soybean meal (6%), and whey powder (5%). An inert marker (Sipernat 50^{TM}) was used as a source of indigestible marker a concentration of 2% in dry diets. The ingredients of each diet were mixed and steam pelleted using a California Pellet Mill, and pellets were dried in a forced-air oven (22 °C, 24 h), sieved and stored at −20 °C.

2.2 Experimental procedure and design

Rainbow trout, *Oncorhynchus mykiss* with an initial average mass 164 ± 2 g were randomly distributed into eighteen 160- L fiberglass-reinforced plastic (FRP) tanks, 32 fish per tank, with triplicate tanks for each feeding regime. There were 6 treatments utilizing the following feeding regimes: (1) continuously feeding with P-sufficient diet (P08); (2) continuously feeding with P-deficient diet (P04); (3) continuously feeding with optimum level of P diet (P06). Treatments 4–6 consisted of three alternating feeding regimes, in which the P04 was fed in an alternating fashion, with the P06 for a specified number of days. (4) One week P-deficient diet followed by one week optimum level of P diet (coded as P04/P06.1); (5) Two weeks P-deficient diet followed by two weeks optimum level of P diet (coded as P04/P06.2) and (6) Four weeks P-deficient diet followed by four weeks optimum level of P diet (P04/P06.4). Fish were hand-fed twice a day to apparent satiation level for 16 weeks, and care was taken to ensure no feed waste occurred.

All tanks were supplied with water from a recirculation system (95% recirculation) at the Regional Laboratory of Aquatic Sciences (LARSA) of Laval University. Suspended solids were removed using a sand filter and effluent ammonia was reduced using a trickling biofilter; ammonia and nitrite concentrations were monitored twice weekly to assess biofilter performance. Fish were held at 10 °C, dissolved oxygen was set at 10.0 mg L^{-1}, photoperiod was 18h light: 6h dark. The experimental protocol was conducted in accordance with the guidelines of the Canadian Council on Animal Care (1984) and with the permission of the Animal Protection Committee of Université Laval.

2.3 Biological sampling, tissue and feces collection

Fish were sampled at the start of the experiment and at four-week intervals during the 16-week feeding trial. At each sampling point, 5 fish were removed from each tank and rapidly euthanized in an anesthetic bath (0.2 ml L^{-1} Eugenol; Sigma-Aldrich, Oakville ON). The fish were then measured for fork length (cm) and body weight (g) to derive at the condition factor (K) and the thermal-unit growth coefficient (TGC). Prior to feeding trial 3 fish were killed, pooled, autoclaved, ground into a homogeneous slurry, freeze-dried, reground and stored at −20 °C until analyzed for the wholebody proximate analysis.

All fish were starved for 24 h prior to sampling. At the termination of the experiment (16 weeks), 10 fish were removed from each tank, euthanized as described above. Of the 10 fish, blood was withdrawn from 5 fish through caudal vessel punctured using 1 ml heparinzed syringes, and then centrifuged for 5 min at 10 000×g. The resultant plasma was used to measure inorganic P. The same fish were individually dissected to collect vertebrae and opercula for the estimation of P concentration. The remaining 5 fish were lyophilized, finely ground and stored at –20 °C until analyzed for the whole body analysis and P concentration.

Fecal samples were collected four times during the study period with four-week interval at week 4, 8, 12 and 16 of the feeding trial, into the bottom of an unstirred fecal collection column affixed to each tank Cho and Slinger (1979). Fecal collection was repeated for 3 days for each collection week and pooled to obtain three replicate measurements per tank.

2.4 Analytical methods

Frozen feces, vertebrae, opercula and whole body samples were freeze-dried, ground using a 1-mm screen, and stored at –30 °C until required for analysis. Using standard methods ....(AOAC 1990), feed, vertebrae, opercula, whole body and feces were analyzed for dry matter (drying in a vacuum oven at 65 °C for 18 h) and ash (incineration at 550 °C for 18 h). Crude protein (% $N \times 6.25$) was quantified using the semi-automatic Kjedahl method (Foss Electric, Denmark; AOAC method 7.B01-7.B04), lipid in the feed via ethyl ether extraction (Soxtec System HT12, Foss Tecator AB; Hoganas, Sweden) and energy using a adiabatic bomb calorimeter (Parr Instrument Co., Moline IL, USA). Phosphorus was determined following ash digestion in 10% nitric acid solution, filtered (Whatman paper No.1), using a Technicon AutoAnalyzer®apparatus (Technicon Corporation, TarryTown, NY, USA) employing the vanadate/molybdate analytical method of analysis ....(Varley 1966). Ammonia and nitrite concentrations in water were determined following the methods described in APHAAWWA-WPCF (1989). The plasma P concentration was determined by spectrophotometry using the Inorganic Phosphorus (IP) Reagent (CataChem, Bridgeport, CT). Fish performance was evaluated on the basis of weight gain, final length, feed conversion ratio (FCR), TGC (thermal-unit growth coefficient), and condition factor (K). Indices were calculated as follows: Weight gain = (final weight – initial weight/initial weight) × 100%; FCR, feed conversion ratio= feed intake/ weight gain; The TGC refers a measure of fish growth rate on a linear scale for the typical production phase of most fishspecies (Iwama and Tautz 1981; Cho 1990; Lankford and Weber 2006) and is calculated in relation to the amount of growth per temperature per day. Calculation of TGC is as follows, modified from Iwama and Tautz (1981) by Cho (1990): TGC, thermal-unit growth coefficient = 100 × [(final wight$^{1/3}$ – initial weight$^{1/3}$)/Σ(water temperature × number of days)]; K, condition factor = (10^5 × final weight in g)/(fork length in cm)^3(Barnham and Baxter 1998).

- P retained in the fish body (%) was obtained using the formula presented by Vielma et al. (2002), as follows: Phosphorus retention (%) = Phosphorus deposited/Phosphorus fed.

Total P loading estimated based on solid and dissolved P loading (solid P load + dissolved P load).

- Solid and dissolved Phosphorus load was calculated using the following formula: Solid P load (kg ton^{-1} fish) = {1-apparent P digestibility coefficient × P intake (kg ton^{-1} fish)};

Table 2. Growth performance of rainbow trout maintained on continuous or alternating feeding regimes for 16 weeks[1].

Parameters	Feeding regimes						Pooled SEM
	P08	P04	P06	P04/P06.1	P04/P06.2	P04/P06.4	
Initial body weight (g)	165	163	166	162	166	162	6
Final body weight (g)	567[a]	496[b]	531[ab]	529[ab]	549[a]	545[a]	25
Initial body length (cm)	20	19	19	21	20	19	1
Final body length (cm)	33[a]	29[b]	33[a]	32[a]	32[a]	32[a]	1
FCR[2]	1[b]	1.14[a]	1.04[ab]	1.08[ab]	1.1[a]	1.07[ab]	0.12
TGC[3]	0.23	0.2	0.22	0.22	0.22	0.23	0.03
K[4]	1.47	1.54	1.47	1.47	1.46	1.49	0.18

[1]Mean values (Pooled SEM) within a row having the same superscript letters are not significantly different at $p > 0.05$.
[2] FCR, feed conversion ratio= feed/gain.
[3]TGC, thermal-unit growth coefficient= 100 ×(final weight$^{1/3}$- initial weight$^{1/3}$)/sum of daily water temperature.
[4] K, condition factor = $(10^5 \times \text{final weight})/(\text{fork length})^3$.

Dissolved P load (kg ton^{-1} fish) = [P apparent P digestibility coefficient × {P intake (kg ton^{-1} fish) -retained P (kg ton^{-1} fish)}]

Acid insoluble ash in diets and feces were determined according to the official methods (Naumann and Bassler 1976). The apparent digestibility coefficients (ADCs) of P were calculated as described by Cho and Slinger (1979) using following formula:

ADC = 100 × [1- (% nutrient of feces/% nutrient of diet) × (% marker in diet/% marker in feces)].

2.5 Statistical analysis

Data were analyzed as a completely randomized design with six regimes and three replicates. Results were analyzed using one-way ANOVA (SAS, version 1, 2004). Means were compared using Fisher's protected least significant difference (LSD). Values were considered significant at $p < 0.05$.

3 Results

The fish reared on alternating regimes, P04/P06.1, P04/P06.2 and P04/P06.4 showed final weight comparable to those fed P08 (control) and P06 (optimum level) diet continuously (Table 2). The changes of weight of fish over time from six regimes remained similar during the initial 56 days (Fig. 1). After this period, the fish fed continuously with the P04 (P-deficient) diet demonstrated a reduced rate for growth; those fed other diets demonstrated increased growth rates until the end of the experiment. However, the changes of weight over time period were not statistically significant ($P > 0.05$). Finally fish fed alternating regimes showed growth rate comparable to those of continuous feeding with P08 and P06 diet, whereas P deficient (P04) diet continuously showed the lowest growth. Average fish length showed the same trend among treatments (Fig. 2). The FCR varied between 1.00 and 1.14. The FCRs for all alternating regimes were comparable to that of the P-sufficient control diet (P08) and P06 continuous feeding regime. Neither TGC nor K was significantly influenced

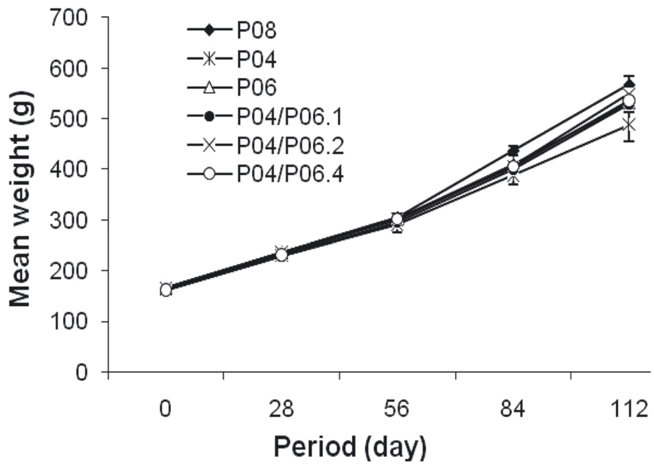

Fig. 1. Weight gain of rainbow trout maintained on continuous or alternating feeding regimes for 112 days. Each point represents means ±SEM of three tanks for each feeding regime (n = 3).

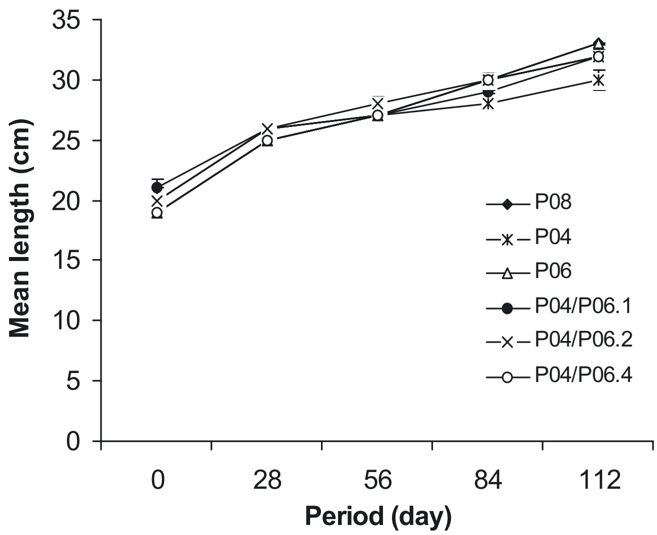

Fig. 2. Length of rainbow trout maintained on continuous or alternating feeding regimes for 112 days. Each point represents means ±SEM of three tanks for each feeding regime (n = 3).

Table 3. Vertebrae, opercula and whole body ash (% of dry matter) and P (% of dry matter); serum inorganic phosphorus concentration of rainbow trout fed continuous and alternating feeding regimes for 16 weeks[1].

Parameters	Tissue	Feeding regimes						Pooled SEM
		P08	P04	P06	P04/P06.1	P04/P06.2	P04/P06.4	
Ash (%)	Vertebrae	54.8	50.8	55	51.8	52.1	52	5.6
	Opercula	37.1^{a}	33^{b}	37.5^{a}	36.7^{a}	34.6^{ab}	36.1^{ab}	4.4
	Whole body	6.8	6.2	6.9	6.7	6.5	6.5	1.3
P (%)	Vertebrae	10.2	9.5	10.2	9.6	9.7	9.5	1.5
	Opercula	6.9^{a}	6.2^{b}	6.9^{a}	6.8^{a}	6.5^{ab}	6.7^{ab}	0.7
	Whole body	0.33^{ab}	0.31^{b}	0.35^{a}	0.33^{ab}	0.32^{b}	0.33^{ab}	0.04
iP (mg dl^{-1})	Serum	18.9^{ab}	17.2^{b}	19.6^{a}	18.9^{a}	19.1^{a}	18.6^{ab}	2.4

[1]Mean values (pooled SEM) within a row having the same superscript letters are not significantly different at $p > 0.05$.

Table 4. Apparent digestibility coefficient of P, P retention, solid and dissolved P loading in rainbow trout maintained on continuous and alternating feeding regimes for 16 weeks[1].

Parameters	Feeding regimes						Pooled SEM
	P08	P04	P06	P04/P06.1	P04/P06.2	P04/P06.4	
P ingestion (g kg^{-1} fish)	7^{a}	4^{d}	5.2^{b}	4.6^{c}	4.6^{c}	4.6^{c}	0.7
ADC of P	0.56^{b}	0.48^{ab}	0.58^{ab}	0.41^{b}	0.41^{b}	0.44^{ab}	0.06
P retention (g kg^{-1} fish)[2]	2.8 (40%)	2.4 (60%)	2.9 (57%)	2.9 (62%)	2.4 (53%)	2.7 (59%)	0.8
Dissolved P load (g kg^{-1} fish)[3]	2.4^{a}	0.8^{c}	1.3^{b}	0.7^{c}	0.9^{c}	1^{bc}	0.5
Solid P load (g kg^{-1} fish)[4]	1.8^{a}	0.8^{c}	1^{bc}	1^{bc}	1.3^{b}	0.9^{bc}	0.4

[1]Mean values ($n = 3$ tanks per diet) in a row with similar superscripts are not significantly different ($p > 0.05$).
[2] P retention (%)= P deposited / P fed.
[3]Dissolved P load (kg ton^{-1} fish) = [P apparent P digestibility coefficient×{P intake (kg ton^{-1} fish) -retained P (kg ton^{-1} fish)}].
[4]Solid P load (kg ton^{-1} fish)= {1-apparent P digestibility coefficient ×P intake (kg ton^{-1} fish)}.

by the continuous feeding of P08, P06 diet and all alternate feeding regimes.

There was no significant difference in serum P content in fish continuously fed with P08, P06 and all alternating feeding regimes (P04/P06.1, P04/P06.2 and P04/P06.4) (Table 3). With the exception of fish fed continuous P04 diet, the ash and P content in opercula and whole body total P content, vertebrae P, ash and whole body ash content did not differ significantly ($p < 0.05$) among treatments.

Fish fed all alternating regimes showed significantly ($p < 0.05$) lower P consumption than those fed continuously with P08 and P06. Among the treatments, significantly ($p < 0.05$) higher ADC of P was found with continuous feeding with P06 than alternate feeding regime of P04/P06.1 and P04/P06.2. The ADC of P with alternate feeding regime of P04/P06.4 did not differ with other continuously fed with P08, P04 and P06 diet. The calculation of the P retention at the end of the experiment gave values that had no effect on P retention as P intake varied more widely with different feeding schedules. Significantly higher P loading (solid and dissolved) was calculated in fish fed continuously with P08 diet. In contrast, P loading values were much lower for all alternate feeding regimes and those are not significantly different from the values obtained fish fed continuously with P04 and P06 diet (Table 4 and Fig. 3).

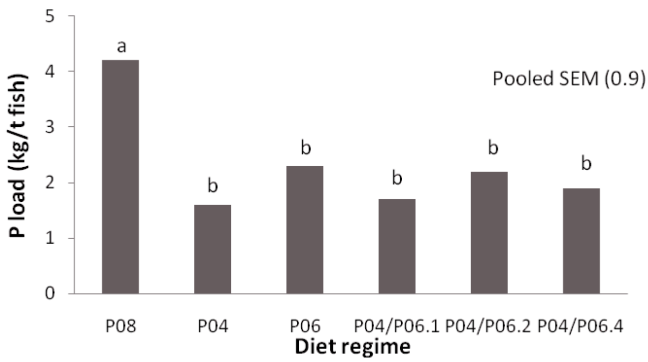

Fig. 3. Total P loading (kg ton^{-1}fish) of rainbow trout maintained on continuous or alternating feeding regimes for 112 days. Total P loading was estimated based on solid and dissolved P loading (solid P load + dissolved P load). Solid P load (kg ton^{-1} fish)={1-apparent P digestibility coefficient ×P intake (kg ton^{-1} fish)}. Dissolved P load (kg ton^{-1} fish)=[P apparent P digestibility coefficient×{P intake (kg ton^{-1} fish) -retained P (kg ton^{-1} fish)}].

4 Discussion

A major goal of the freshwater trout aquaculture industry in certain regions is to find an economically viable method of lowering effluent phosphorus without impacting levels of fish production. Alternating feeding regimes of P deficient and P-sufficient diets may help meet this goal; the results of the present feeding trial clearly demonstrate that the growth performance of a P-deficient (P04) alternating with an optimal level of P diet (P06) comparable with a P-sufficient diet (P08) and P06 continuously. Earlier studies on trout (Sevgili et al. 2006), carp and common carp (Nandeesha et al. 1993; Nandeesha et al. 1994), tilapia (Patel and Yakupitiyage 2003) using mixed feeding schedules in which low- and high-protein-based diets were fed in an alternating fashion resulted in higher growth and this was considered as a possible way of reducing feed cost and nitrogen waste from aquaculture operation. It is interesting to note that after 56 days the visual growth depletion (not statistically) in fish fed continuously with P04 (P-deficient diet) was noticed, this is likely due to the fact that fish have tissue reserves P and until that period they could draw upon these reserves, as dietary P intake was insufficient. Although there were no significant difference in TGC among treatment groups, there were difference in final weight and significant poor growth rate in terms of final weight exhibited by trout fed constantly with the P04 is probably a consequence of an insufficient intake of P in the diet and this agrees with the results of previous investigators (Cho and Kaushik 1990; Kim and Kaushik 1992; Sugiura et al. 2000; Rodehutscord 2000). Moreover, at the end of the trial it has been observed few visual deficiencies signs i.e. bone deformities in tail region and lethargic in fish fed continuously with the P-deficient diet (P04); similar clinical deficiency sign was also observed by Lake et al. (2010). Sarker et al. (2009) reported that despite the fish body P status change rapidly, the appearance of clinical P-deficiency signs takes longer even in smaller size fish. Lellis et al. (2004) found that the signs of P deficiency appeared after 18 weeks in large trout (300 g) fed P-deficient diet.

Feeding daily at a recommended P level and a constant feeding rate is the most prevalent practice in fish farming. The concept of alternating feeding regimes was based on the observation that protein utilization of feed varies from day to day, following an apparent cyclic pattern (De Silva 1985). Subsequently, De Silva (1985) demonstrated that alternating feeding regimes, where a high protein was alternated with a low protein, was more efficient in term of growth and nutrient utilization compared with feeding Nile tilapia with a high protein continuously. Based on this concept, it is reasonable to hypothesis that P metabolism might be the same cyclic pattern in fish and continuously high or optimal level of P feeding may be wasteful as on a day when the P utilization of the fish is high, relatively lower P intake would be sufficient to fulfill the requirement and vice versa.

Vertebrae and opercula ash levels are sensitive indicators of P status and bone mineralization (Lall 1991); however, in this study the magnitude of change in opercula ash and P was more pronounced because neither vertebrae P nor ash was influenced by treatments. Opercula ash, P and whole body P content were numerically lower in fish fed continuously a P-deficient diet (P04). Likewise, Helland et al. (2005) found that the fish fed low-P were apparently suffering from P deficiency, while the fish fed high-P were sufficiently mineralized to maintain bone integrity. The opercula ash and P levels in all alternative regimes are comparable with P08 and P06 diet. Lower plasma phosphate concentrations in fish fed continuously a P-deficient diet (P04), indicated that physiological mechanisms conserve body P when P intake is marginal to meet the metabolic needs of the fish.

This study confirms that significant amount of total P (ingested and excreted) can be reduced by alternating feeding regimes; fish receiving alternating regimes (P04 and P06) demonstrated a significantly lower amount of ingested P compared to fish fed continuously with the P08 and P06 diets. The results of the present experiment indicate that it is possible via alternatively feeding diets P04 and P06 to reduce P intake by more than 13% and 34% compared to continuously feeding with P06 and P08 diets, respectively. This effect further translates into reduced P loading.

The retention of P remained unchanged for the six regimes used in this study. However, in terms of percentage compared to the amount of P ingested, the values of retention differed according to mode of feeding and numerically lower retention is noted for the P08 diet. Significantly lower (52%, 2.0 g P kg^{-1} diet) P loading of alternating regimes (P04 and P06) versus the P08 control diet (4.2 g P kg^{-1} diet) confirms that this feeding strategy would be an avenue for achieving the objective of reducing P loading.

Solid P load is estimated based on the apparent digestibility of nutrients, whereas dissolved (soluble) is estimated based on digestible P intake and deposition (Cho et al. 1994). Collecting feces after being voided permits leaching of P and can lead to an overestimation of digestibility, a number of studies have demonstrated that nutrient leaching increased digestibility of nutrients when feces are collected from the collection column (Spyridakis et al. 1989; Hajen et al. 1993; Vandenberg et al. 2001). However, Cho et al. (1985) found that nutrient leaching is minimal when the feces remain undisturbed and unbroken fecal pellets are collected. In our study, there are several reasons why fecal matter did not interfere with the estimation of P digestibility and eventually calculation of P loading. Comparison across studies is difficult due to the differences in experimental and environmental conditions; difference is also existed even in vivo and in vitro digestibility trial (Hua et al. 2006; Morales et al. 2010). Rainbow trout fecal pellets were well intact and rapidly settled into the bottom of an unstirred fecal collection column on each tank. In support of this system recently some investigators (Cho and Kaushik 1990; Satoh et al. 1992; Sarker et al. 2007; Sarker et al. 2009) have also recommended that collection facilities, which ensure rapid settlement of feces, will reduce the fecal nutrient loss. Furthermore, Coloso et al. (2003) stated that P leaching from feces is negligible because the fecal matter started to accumulate after the sharp peak of soluble P in the effluent water occurred. However, one limitation exists in this study regarding the fecal sample collection with four-week interval (week 4, 8, 12 and 16) that seems prevent theprecision of loading calculations (as this was not measured directly) especially for alternate feeding regimes of P04/P06.1 and P04/P06.2. This interval of fecal sampling allowed to measure digestibility when fish were fed one of the two diets. It might be one of the reasons why the ADC of P estimated unrealistically low values (numerically) for alternate regime of P04/P06.1 and P04/P06.2. This was likely because ADC was assumed to be under estimated, in every sampling period for P04/P06.1 and P04/P06.2 feeding regime considered only one of the two diets. Nonetheless, in the case of P04/P06.4 alternate feeding regime, the fecal collection sampling with four-week interval allowed to measure digestibility when fish were fed both diets that is reflected the rhythmic variation in the P digestibility of rainbow trout. This study suggests that it would be necessary to sampling feces in both periods in order to derive the proper digestibility coefficient for fish fed on alternate feeding regime. This is especially important as P loading was not measured directly by derived based on the digestibility coefficients.

Three types of alternating feeding regimes (1, 2 and 4-week intervals) with P04 and P06 diet gave good growth and tissue mineralization that is comparable to those continuously fed with diet of P08. Furthermore, it is possible to reduce P input and P loading by 34% and 52% respectively by adopting alternating feeding regimes (P0/P06.1, P0/P06.2 and P04/P06.4) compared to P08 diet. Thus, this study provides evidence of the potential applicability of feeding schedule using alternating feeding of P06 (optimal level of P) and P04 (deficient P level) as a means of reducing P loading from rainbow trout culture without compromising growth. In alternate feeding regime, feces collection for digestibility estimation should be taken into consideration when fish maintained on both periods of diets that ultimately help to accurate calculation of P loading. Further definition of optimal feeding period for altering diets as well the reduction formulation costs is currently being studied.

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