Effects of dietary phosphorus restriction on phosphorus balance in rainbow trout *Oncorhynchus mykiss*

Shozo SUGIURA

**Abstract:** The present study examined responses of fish to dietary phosphorus (P) restriction. Two groups of rainbow trout (mean body weight 129 and 115 g) were fed for 32 and 53 days, respectively, with either the low-P (LP) or high-P (HP) diet. Some fish were starved concurrently. Fish fed the LP diet (LP fish) gradually decreased plasma P from 93 (day 0) to 17 mg/l (day 32), whereas those fed the HP diet (HP fish) and the starving fish maintained normal plasma P levels (81-130 mg/l). Plasma P levels decreased markedly after feeding the LP, but not HP, diet. When fish were fed glucose, the plasma glucose level increased markedly for both LP and HP fish. The LP fish apparently absorbed P from water (~0.2 mg P/kg fish/day), whereas starving fish excreted P (1.8 mg P/kg fish/day). Waterborne P (5 and 50 mg P/l) had little effect on fish plasma P levels regardless of the fish P status. The LP fish had lower ash (65%), P (67%) and calcium (39%) contents compared to the HP fish on a whole body basis. Calcium-wasting is most characteristic in dietary P deficiency.

**Key words:** Rainbow trout; Environment; Fish feeds; Phosphorus

Phosphorus (P) is an essential nutrient for plants and animals. Animals, including fish, meet all or most of their P requirements by dietary sources (Maynard and Loosli 1962; NRC 2011). Commercial fish feeds contain P in amounts much higher than the dietary requirement of fish. Then, the fish excrete the excess portion of dietary P to the environment (Sugiura et al. 2000). In most freshwater ecosystems, P limits primary production (Mainston and Parr 2002). Thus, P discharged from aquaculture systems directly contributes to eutrophication of nearby waters, resulting in algal bloom, hypoxia and, in extreme cases, azoic zones (Baeverfjord et al. 1998).

World aquaculture production is increasing rapidly, which contributes corresponding amounts of P in the effluents. However, at the other end of the scale, environmental awareness is also increasing rapidly. The continuous increase of aquaculture production is important for better human nutrition and poverty alleviation around the world (Desai 2002). It is, therefore, imperative to improve technologies that can reduce environmental burden, especially P load, associated with aquaculture production (NRC 2011).

With this background, the use of low-P (LP) feeds has been increasingly common in aquaculture management (NRC 2011). Although reduction in P in aquaculture feeds and effluents are beneficial to the environment, it increases the risk and incidence of clinical P deficiencies in cultured fish, including growth depression and bone malformation (Sugiura et al. 2004). Further reduction of effluent P will require better understanding of P nutrition in fish.

Hence, the present study was conducted to examine the nature of P deficiency in fish. Specific questions studied were: (1) Does plasma...
P level accurately indicate the P status of fish? (2) Does P deficiency induce glucose intolerance? (3) Do fish absorb P from water when they are severely deficient in P? (4) What is the most pronounced sign of P deficiency? This study aimed at increasing our understanding of P deficiency in cultured fish in order to advance environmentally sustainable technology in aquaculture production.

**Materials and Methods**

**Experimental diets**

Two diets were made: a low-P (LP) diet and a high-P (HP) diet. Ingredient compositions of these diets (g/kg) were: wheat gluten 300; corn gluten 150; blood meal 100; soybean meal 150; fish oil 200; carboxymethyl cellulose 20; NaCl 10; MgO 2; KCl 16; α-cellulose 12 (LP diet only); KH₂PO₄ 28 (HP diet only); vitamin premix 30; choline chloride 5; myoinositol 2; ascorbyl-polyphosphate 3; trace mineral premix 1; SiO₂ 5. Compositions of the vitamin and the trace mineral premixes were reported previously (Sugiura et al. 2000).

The diets had the following analytical compositions (g/kg dry diet): total P, 2.66 (LP diet) and 9.38 (HP diet); crude protein, 528 (LP diet) and 528 (HP diet). The apparent availability (net absorption) of P determined in vivo (Sugiura et al. 2003) were 53.8 ± 0.68 (% mean ± SE, n = 12) for the LP diet and 86.3 ± 0.33 for the HP diet. Hence, the available-P contents (g/kg dry diet) were 1.43 (LP diet) and 8.09 (HP diet) (cf. Dietary P requirement is 7.0 g available-P/kg dry diet, NRC 2011). Apparent digestibility of protein were 96.1 ± 0.18 (LP diet) and 96.2 ± 0.17 (HP diet). The ingredient composition of the diet used for the glucose tolerance test was as follows (g/kg diet): glucose 500; gelatin 100; fish oil 200; α-cellulose 200; distilled water 200. All diets were cold-extruded, air-dried, and stored at 0-4°C until use.

**Fish and rearing procedure**

Two feeding trials were conducted. In Trial 1, rainbow trout *Oncorhynchus mykiss* (initial mean body weight 129 ± 2.7 g, SE) were fed for 32 days with either the LP or HP diet. A third group of fish was kept in starvation for 32 days. In Trial 2, rainbow trout (initial mean body weight 115 ± 1.2 g, SE) were fed either the LP or HP diet for 53 days. At the start of the feeding trials, fish were randomly stocked into duplicate (Trial 1) or triplicate (Trial 2) tanks per treatment to contain 20 fish (Trial 1) or 26–28 fish (Trial 2) per tank (100 l in size). For both trials, fish were fed once daily at 1% (dry basis) of their body weight. Well water was continuously supplied at 2 l/min to each tank. The water temperature, dissolved oxygen, and inorganic P (Pi) concentrations were 15 ± 0.5°C, 8-9 mg/l, and 0.008-0.014 mg/l, respectively. A 14 h light/10 h dark diurnal photoperiod was maintained throughout the feeding trials using fluorescent lighting.

**Sampling and analytical procedures**

In Trial 1, blood plasma of each fish was collected at day 0, 8, 17, and 32, and analyzed for Pi, glucose, calcium (Ca) and alkaline phosphatase. To collect blood samples, each fish was anesthetized with tricaine methane sulfonate (100 mg/l; Sigma Chemical Co., St Louis, MO, USA), and the blood was collected from caudal vessels into a heparinized syringe 24 h after feeding. The blood samples were kept on ice, and the plasma was separated (1,500 ″g, 5 min) within 1 h of blood collection. The plasma was dispensed into 1.5 ml microtubes and stored at -20°C until analyses. Non-fecal 24 h excretions of P were quantified with the LP, HP, and starving fish at day 0, 5, 12, and 28 using a water recirculation system and by analyzing the recirculating tank water (Sugiura et al. 2000). Using the same system, non-fecal 24 h excretion of ammonium-N was determined at day 28. Water samples collected at 24 h were apparently clear and free of suspended matter. Portions of the water samples were stored at -20°C until analysis for Pi. Other portions of the same water samples were acidified with sulfuric acid, and analyzed for Kjeldahl-N. Preliminary tank tests showed no measurable loss of P and ammonia during the 24 h recirculation period. The net absorptions of waterborne P by fish
were estimated by balance using the same recirculation system except that Pi was dissolved in tank water at 0.793 mg P/l. The Pi absorption rates were estimated as P present in tank water at 0 h + P excreted by fish during 24 h period (determined separately) - P recovered in tank water at 24 h. It was assumed that fish excreted the same amount of P when they were in low-P water (0.008–0.014 mg P/l) and when they were in P-plus water (0.793 mg P/l). The recirculation system was thoroughly cleaned before each use. Water temperature (15 ± 0.5°C) and dissolved oxygen (5–9 mg/l) were maintained during the 24 h recirculation period. A glucose tolerance test was conducted at day 32 with the LP and HP fish. To do this, fish were starved for 48 h, lightly anesthetized with tricane, and force-fed individually with the glucose diet at 1% of the body weight of each fish using a polished glass plunger. The blood samples were collected at 0 (precibal), 1, 2, 4, 8, 14 and 24 h after feeding. Blood samples were collected no more than once from the same fish.

In Trial 2, effects of water P concentrations (0, 5, and 50 mg P/l) on fish plasma P, Ca, and glucose levels were studied at day 53 with the LP and HP fish using the same water recirculation system. The other protocols were the same as above except that the fish were starved for 3 days before the experiment. The same fish were analyzed for whole body ash, P and Ca contents. To do this, each fish was dissected and the contents of stomach, intestine and pyloric caeca were carefully removed. The emptied digestive tract was replaced to the fish. Then, the whole body of each fish was ground into paste using a food processor. The fish paste was individually placed into a plastic bag, and kept at -20°C until analyses.

The plasma samples were analyzed for Pi, Ca (Sugiura et al. 2000), glucose (Hyvarinen and Nikkila 1962), and alkaline phosphatase (Sigma Diagnostics Procedure #104). Nonfecal excretions of P and N were quantified according to Sugiura et al. (2000). Fish carcass (whole), feed, and feces were analyzed for ash, P, Ca, and acid-insoluble ash (Sugiura et al. 2000). The net absorptions of dietary P and nitrogen (N) were estimated based on the contents of acid-insoluble ash, P and N in feed and feces. The protein retention rates (% of intake) were calculated as N fed - N excreted in feces - N excreted in water. All analyses were at least duplicated per sample.

Statistical methods

Effects of fish P status (LP or P-deficient, HP or normal, and starved) on the levels of selected plasma and body components as well as on the net absorption of waterborne P were evaluated by the Newman-Keuls test following a single-factor ANOVA. Correlations among plasma and body measurements were examined using a linear regression analysis. Effects of waterborne P concentration and fish P status on fish plasma P and Ca levels were analyzed by a two-factor ANOVA. Effects of time, after feeding or stocking, were determined by Dunnett's test following a single-factor ANOVA. Differences between the LP fish (P-deficient) and HP fish (normal) were examined by a two-tailed t-test. Differences in P excretion rates between the LP fish and the starved fish were examined by a one-tailed t-test. The variance homogeneity was tested by Bartlett's test, and when heterogeneous, data were transformed before ANOVA or t-test. Computer software (Prism version 2.01, GraphPad, Inc., San Diego, CA) was used for statistical calculations. Treatment effects were considered significant at \( P < 0.05 \). Values are presented as mean ± SE (n) throughout the text, unless otherwise stated.

Results

Plasma P and Ca levels

Plasma P levels steadily decreased from 93.2 (day 0) to 16.6 mg/l (day 32) when fish were fed the LP diet (Table 1). Plasma P levels of the starving fish were slightly but significantly lower than those of the HP (normal) fish. At day 32, plasma P levels of the LP fish (P-deficient) were only ca. 14% of those of the HP (normal) fish, and ca. 20% of those of the starving fish. Within a day, plasma P levels decreased markedly when the LP fish were fed the LP diet
S. Sugiura

(30–50% of the precibal level), but not when the HP (normal) fish were fed the HP diet (Table 2). As for the glucose tolerance test, however, the plasma P levels increased within 1–2 h after feeding the high-glucose-P-free diet, but decreased thereafter to the precibal level (ca. 40 mg/l for LP, and ca. 90 mg/l for HP fish; Fig. 1).

Plasma Ca levels were similar among the LP, HP, and starving fish at all sampling days (Table 1). Within a day, however, plasma Ca levels tended to decrease after feeding for both the LP

### Table 1. Time course responses of rainbow trout to dietary phosphorus restriction and starvation: plasma components (Trial 1)

<table>
<thead>
<tr>
<th></th>
<th>Day</th>
<th>LP</th>
<th>HP</th>
<th>St</th>
<th>SE</th>
</tr>
</thead>
<tbody>
<tr>
<td>P (mg/l)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(93.2 ± 6.7)</td>
<td>8</td>
<td>56.6&lt;sup&gt;b&lt;/sup&gt;</td>
<td>129.9&lt;sup&gt;a&lt;/sup&gt;</td>
<td>98.6&lt;sup&gt;a&lt;/sup&gt;</td>
<td>10.2</td>
</tr>
<tr>
<td>Glu (mg/l)</td>
<td></td>
<td>8</td>
<td>695</td>
<td>789</td>
<td>779</td>
</tr>
<tr>
<td>(1230 ± 215)</td>
<td>17</td>
<td>33.5&lt;sup&gt;c&lt;/sup&gt;</td>
<td>109.1&lt;sup&gt;a&lt;/sup&gt;</td>
<td>80.7&lt;sup&gt;b&lt;/sup&gt;</td>
<td>7.8</td>
</tr>
<tr>
<td>Ca (mg/l)</td>
<td></td>
<td>32</td>
<td>609</td>
<td>116.4&lt;sup&gt;a&lt;/sup&gt;</td>
<td>82.0&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>AP (IU/l)</td>
<td></td>
<td>8</td>
<td>27.2</td>
<td>48.1</td>
<td>38.9</td>
</tr>
<tr>
<td>(75.8 ± 4.6)</td>
<td>17</td>
<td>15.4</td>
<td>33.3</td>
<td>34.8</td>
<td>15.2</td>
</tr>
</tbody>
</table>

Each value represents the average of three fish. Values in rows with common letters or without letters are not different (P > 0.05). Values in parentheses are the initial (day 0) values (mean ± SE, n = 3 fish). Fish had received a commercial diet until day 0. Plasma samples were collected and analyzed at day 8, 17, and 32 on test diets or starvation. LP, fish fed low-P diet; HP, fish fed high-P diet; St, starving fish; SE, pooled standard error of the mean; P, phosphorus; Glu, glucose; Ca, calcium; AP, alkaline phosphatase.

### Table 2. Effects of waterborne phosphorus (P) concentration on plasma P, calcium and glucose levels in low-P and high-P rainbow trout: time-course responses after feeding (Trial 2)

<table>
<thead>
<tr>
<th>Fish P</th>
<th>Water P</th>
<th>Low-P&lt;sup&gt;a&lt;/sup&gt;</th>
<th>High-P&lt;sup&gt;a&lt;/sup&gt;</th>
<th>SE</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>P</td>
<td>0 h&lt;sup&gt;c&lt;/sup&gt;</td>
<td>145</td>
<td>153</td>
<td>158</td>
<td>176</td>
</tr>
<tr>
<td></td>
<td>1 h</td>
<td>66&lt;sup&gt;<em><strong>&lt;/sup&gt; 82&lt;sup&gt;</strong></em>&lt;/sup&gt; 106</td>
<td>173</td>
<td>182</td>
<td>188&lt;sup&gt;***&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>4 h</td>
<td>71&lt;sup&gt;*<strong>&lt;/sup&gt; 92 60&lt;sup&gt;</strong>&lt;/sup&gt; 181</td>
<td>174</td>
<td>166</td>
<td>13.7</td>
</tr>
<tr>
<td></td>
<td>12 h</td>
<td>45&lt;sup&gt;*<strong>&lt;/sup&gt; 48&lt;sup&gt;</strong>&lt;/sup&gt; 52&lt;sup&gt;**&lt;/sup&gt; 180</td>
<td>156</td>
<td>160</td>
<td>9.1</td>
</tr>
<tr>
<td></td>
<td>24 h</td>
<td>46&lt;sup&gt;<em><strong>&lt;/sup&gt; 43&lt;sup&gt;</strong></em>&lt;/sup&gt; 68&lt;sup&gt;***&lt;/sup&gt; 159</td>
<td>149</td>
<td>152</td>
<td>7.2</td>
</tr>
<tr>
<td>Ca</td>
<td>0 h&lt;sup&gt;c&lt;/sup&gt;</td>
<td>163</td>
<td>159</td>
<td>157</td>
<td>149</td>
</tr>
<tr>
<td></td>
<td>1 h</td>
<td>134&lt;sup&gt;<em><strong>&lt;/sup&gt; 139&lt;sup&gt;</strong></em>&lt;/sup&gt; 155</td>
<td>138&lt;sup&gt;**&lt;/sup&gt;</td>
<td>142</td>
<td>134&lt;sup&gt;***&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>4 h</td>
<td>143</td>
<td>155</td>
<td>140&lt;sup&gt;*&lt;/sup&gt;</td>
<td>141</td>
</tr>
<tr>
<td></td>
<td>12 h</td>
<td>126&lt;sup&gt;<em><strong>&lt;/sup&gt; 137&lt;sup&gt;</strong></em>&lt;/sup&gt; 138&lt;sup&gt;**&lt;/sup&gt; 141</td>
<td>132&lt;sup&gt;***&lt;/sup&gt;</td>
<td>130&lt;sup&gt;**&lt;/sup&gt;</td>
<td>4.7</td>
</tr>
<tr>
<td></td>
<td>24 h</td>
<td>137&lt;sup&gt;<em><strong>&lt;/sup&gt; 125&lt;sup&gt;</strong></em>&lt;/sup&gt; 135&lt;sup&gt;***&lt;/sup&gt; 141</td>
<td>148</td>
<td>141</td>
<td>3.6</td>
</tr>
<tr>
<td>Glu</td>
<td>0 h&lt;sup&gt;c&lt;/sup&gt;</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>991</td>
</tr>
<tr>
<td></td>
<td>1 h</td>
<td>901</td>
<td>—</td>
<td>—</td>
<td>667&lt;sup&gt;**&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>4 h</td>
<td>865</td>
<td>—</td>
<td>—</td>
<td>792&lt;sup&gt;**&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>12 h</td>
<td>931</td>
<td>—</td>
<td>—</td>
<td>712&lt;sup&gt;**&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>24 h</td>
<td>865</td>
<td>—</td>
<td>—</td>
<td>915</td>
</tr>
</tbody>
</table>

Each value represents the average of five fish. Values in columns with asterisks are significantly different from control (0 h, pooled): *P < 0.05; **P < 0.01. Low-P, P-deficient fish; High-P, P-sufficient fish; SE, pooled standard error of the mean; P, phosphorus; Ca, calcium; Glu, glucose.

<sup>a</sup> P status of fish is shown in Table 3.

<sup>b</sup> P level in water was either low (0 mg P/l water), mid (5 mg P/l water), or high (50 mg P/l water).

<sup>c</sup> Hours after feeding (0 h: right before feeding).
Dietary phosphorus deficiency in trout and HP fish (Table 2). Plasma alkaline phosphatase activities were similar among the LP, HP, and starving fish at any sampling days, but were considerably high at day 0 (Table 1).

Concentrations of each plasma component (mg/l, n = 50 fish) ranged: 27-207 for P, 116-200 for Ca, and 542-1420 for glucose. A linear regression analysis of these variables showed that the low P level was associated with the low Ca level ($P = 0.001, r = 0.45$), but not with the glucose level ($P = 0.30, r = -0.15$).

**Plasma glucose level**

Plasma glucose concentrations ranged from 602 to 893 mg/l (mean values), and were similar among the LP, HP, and starving fish at all sampling days (Table 1). Within a day, plasma glucose levels significantly decreased 1-12 h after feeding for HP but not LP fish (Table 2). Plasma glucose levels returned to the precibal levels 24 h after feeding for both the LP and HP fish.

For the glucose tolerance test, a high glucose diet markedly elevated plasma glucose levels from ca. 1000 mg/l (0 h) to 8000 mg/l (8 h) for both the LP and HP fish (Fig. 1). However, HP fish had significantly lower plasma glucose levels ($P = 0.02$) than LP fish at 14 h after feeding (Fig. 1). At other sampling points, there was no significant difference in plasma glucose levels between the LP and HP fish.

**Endogenous P excretion**

The starving fish excreted P in the amount ca. 0.4 mg (P/kg fish/day) at day 5 and 12, whereas the amount increased up to 1.2-2.4 mg at day 28 (Fig. 2). The LP fish, however, excreted little or no P at day 5 and 12. At day 28, the LP fish apparently absorbed a small amount of P from water (Fig. 2). The obligatory loss of N by the starving fish at the end of experiment (day 28) was 94.7 ± 5.96 mg (N/kg body weight/day, $n = 4$).

**Absorption of waterborne P**

Both the LP and HP fish, and to a lesser degree, the starving fish apparently absorbed P from tank water (Fig. 3). The estimated

![Fig. 1. Effects of oral glucose overdose on plasma glucose (A) and plasma phosphorus (B) levels in low-phosphorus (P) (○) and high-P (●) rainbow trout (Trial 1). Each point represents the mean (± SE as error bar) of 5 fish. Asterisks indicate significant differences between low-P and high-P fish: *$P < 0.05$; **$P < 0.01$. Hours after feeding vs. diurnal time were as follows: 0 h (8:00), 1 h (9:00), 2 h (10:00), 4 h (12:00), 8 h (16:00), 14 h (22:00), 24 h (8:00), lighted daily from 6:00 to 20:00.](image)

![Fig. 2. Non-feecal excretions of inorganic phosphorus (P) by low-P (○) and starving (×) rainbow trout at day 0, 5, 12, and 28 (Trial 1). Fish had been given a commercial trout diet until day 0. Each treatment had duplicate tanks containing 20 fish/tank. Asterisks indicate significant differences between low-P and starving fish: *$P < 0.05$. The tank water (initial) contained 0.008-0.014 mg P/l. Values lower than zero indicate that P concentration in tank water after a 24 h recirculation period was lower than the initial (0 h) concentration. (Excretion of P by fish fed the high-P diet was mainly dietary excess P, and was between 10-17 mg/kg fish/day during day 5-28).](image)
amounts of waterborne P absorption were ca. 5 mg (P/kg fish/day) for the LP and HP fish, and ca.3 mg for the starving fish (when the tank water contained 0.793 mg P/l). The degree of P deficiency of the fish had little or no effect on the amount of P absorbed from water.

The P concentrations of tank water had no apparent effect on plasma P levels of the LP and HP fish (Table 2). For the LP fish, regardless of the P concentrations of tank water, plasma P levels remained low even at 24 h after feeding. Plasma Ca levels slightly, but significantly decreased after feeding in both the LP and HP fish at all water P concentrations (Table 2).

**Body Ca content**

The LP fish (P-deficient) had markedly lower ash (65%), P (67%) and Ca contents (39%) on a whole body basis compared with the HP fish (100% as control) (Table 3). However, the P contents of the ash portion were higher for the LP than HP fish. The P/Ca ratios of the whole body were much higher for the LP (1.215) than HP fish (0.705).

**Protein balance**

The apparent protein digestibility did not differ significantly among different sampling time and between the LP and HP fish (overall mean 96.1%, n = 24). However, the protein retention rates (% of intake) measured at day 32 were 42.0 ± 1.35 (LP fish) and 46.6 ± 1.25 (HP fish) (P < 0.05, n = 4 tanks). The difference was due to higher excretion of nonfecal N in the LP than HP fish.

**Discussion**

Fish fed the LP diet had very low plasma P levels (Trial 1). This well-established response suggests that the plasma P level may be a useful indicator to evaluate the fish P status. However, at the end of Trial 1, when fish were fed the glucose diet, the plasma P level changed

![Fig. 3. Net absorption rates of waterborne phosphorus (P) by low-P (○), high-P (●), and starving (×) rainbow trout at day 5, 12, and 28 (Trial 1). Fish had been given a commercial trout diet until day 0. Each treatment had duplicate tanks containing 20 fish/tank. Net P uptake was measured using tank water to which P was dissolved at 0.793 mg P/l. No significant difference was detected among treatments at any sampling points.](image)

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**Table 3.** Ash, phosphorus (P) and calcium contents of the whole body of rainbow trout fed a low-P or high-P diet for 53 days (Trial 2)

<table>
<thead>
<tr>
<th></th>
<th>Low-P (g/kg)</th>
<th>High-P (g/kg)</th>
<th>SE</th>
<th>P-value</th>
<th>% of normal</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ash/wet matter</td>
<td>15.6</td>
<td>24.0</td>
<td>0.38</td>
<td>0.0001</td>
<td>65</td>
</tr>
<tr>
<td>Ash/dry matter</td>
<td>48.6</td>
<td>80.3</td>
<td>2.31</td>
<td>0.0006</td>
<td>61</td>
</tr>
<tr>
<td>P/wet matter</td>
<td>2.85</td>
<td>4.24</td>
<td>0.05</td>
<td>0.0000</td>
<td>67</td>
</tr>
<tr>
<td>P/dry matter</td>
<td>8.89</td>
<td>14.19</td>
<td>0.33</td>
<td>0.0003</td>
<td>63</td>
</tr>
<tr>
<td>P/ash</td>
<td>183</td>
<td>177</td>
<td>1.12</td>
<td>0.02</td>
<td>103</td>
</tr>
<tr>
<td>Ca/wet matter</td>
<td>2.36</td>
<td>6.04</td>
<td>0.19</td>
<td>0.0002</td>
<td>39</td>
</tr>
<tr>
<td>Ca/dry matter</td>
<td>7.35</td>
<td>20.24</td>
<td>0.86</td>
<td>0.0005</td>
<td>36</td>
</tr>
<tr>
<td>Ca/ash</td>
<td>151</td>
<td>251</td>
<td>5.05</td>
<td>0.0002</td>
<td>60</td>
</tr>
<tr>
<td>P/Ca ratio</td>
<td>1.215</td>
<td>0.705</td>
<td>0.04</td>
<td>0.0003</td>
<td>172</td>
</tr>
</tbody>
</table>

Each value represents the average of three tanks (Three fish from each tank were individually analyzed and averaged). Fish were free of the GI contents. Mean body weights of fish analyzed were (g): 141 (P-def), 149 (P-suf), 7.9 (pooled SE).

Low-P, P-deficient fish; High-P, P-sufficient fish; SE, pooled standard error of the mean.

* % of normal: Low-P/ High-P.
from hour to hour (Fig. 1). Similar fluctuations in plasma P levels were observed in Trial 2 (Table 2). Hence, the plasma P level could be inaccurate or even misleading as a diagnostic indicator for fish P status, unless the sampling protocol is carefully controlled (e.g., postcibal hour, feeding rate). Other researchers reported similar observations in salmonids (Baeverfjord et al. 1998; Vielma and Lall 1998). The present results also suggested that the plasma P level of the LP fish can increase by starvation. Kramer et al. (1931) also noted in rachitic rats that the serum P level increased from 24 to 102 (mg/l) after 21 h of starvation. Fasting-induced recovery of plasma P in fish may be due to bone resorption to replenish plasma P for miscellaneous metabolic needs (Gaasbeek and Meinders 2005), rather than due to the absorption of waterborne P as there was no significant effect of waterborne P on postcibal plasma P (Table 2). Unlike plasma P, plasma Ca levels of the present fish were stable regardless of the P status of fish, which corresponds to the response in humans (IOM 1997).

In higher animals, a chronic P-deficiency is associated with glucose intolerance (Knochel 2000). In the present study, although plasma glucose levels of fish surged in response to oral glucose overload, there was no marked difference between the LP (P-deficient) and HP (normal) fish in plasma glucose levels. The HP fish, however, had lower \( (P = 0.02) \) plasma glucose levels than the LP fish at 14 h after feeding. Also, when fish were fed the LP or HP diet, there was a significant decrease in plasma glucose levels with the HP fish but not LP fish (Table 2). These results suggest that a P-deficiency in fish may be associated with impaired glucose use as in mammals.

In both the LP and HP fish, plasma P levels increased slightly in response to glucose feeding, and then decreased to the precibal level or slightly lower (Fig. 1). This response is different from mammals that show acute hypophosphatemia in response to glucose intake, i.e., reactive-hypophosphatemia (Berner and Shike 1988). However, in Trial 2 (Table 2), the plasma P levels decreased sharply after feeding the LP diet. Since the LP diet was low in glucose, the postcibal decrease of plasma P cannot be attributed to dietary glucose. The present study showed a feeding-induced, and not glucose-induced, hypophosphatemia. The mechanism of fish response to dietary glucose is currently under investigation.

Trial 1 was also intended to compare “urinary” P excretion between the LP and the starving fish. Fecal P excretion was not determined since it had been studied before (Sugiura et al. 2000). The present study showed that there was a small amount of P excreted at day 0, which was due to commercial trout feed that the fish had received until day 0. After day 0, the starving fish continued to excrete P in the amount more than the LP (P-deficient) fish. The difference was small until day 12, but large at day 28 (Fig. 2). In mammals, P loss from the body is known to be less on a P-free diet than during fast (Maynard and Loosli 1962), which is in agreement with the present results in fish.

Perhaps, at an early stage of starvation, N excretion, especially ammonia excretion, is low due to preferential use of body fat over protein (muscle) as an energy source. However, as starvation extends and the fat store begins to deplete, the fish will need to start using body protein as an energy source, and this shift can be seen as an increase of ammonia excretion by the fish. In the present study, the obligatory loss of N by the starving fish was measured only at the end of experiment (day 28), which was \( 94.7 \pm 5.96 \) (mg N/kg body weight/day, \( n = 4 \)). Since P and protein contents in muscles are fairly constant and are about 1.20 g and 200 g/kg, respectively (Shearer 1984, 1994), the loss of N mentioned above was likely accompanied by the simultaneous loss of 3.55 mg P/kg body weight/day from waning muscle tissues during prolonged starvation. However, the amount of P excreted by the starving fish at day 28 was only about a half of this theoretical amount (Fig. 2). This discrepancy might be due to high experimental error (Fig. 2) or due to the forms of excreted P by the fish that might be partly organic forms not quantified by the present method. In this study, only Pi was measured to avoid errors associated with fecal P.
Both LP and HP fish absorbed P from water, but there was no detectable difference between the two groups of fish (Fig. 3). In addition, LP fish did not increase the absorption of water-borne P over time. These observations suggest that dietary P deficiency does not induce compensatory ability to absorb waterborne P in fish; i.e., waterborne P may not be absorbed actively. Starving fish excreted P as discussed above (Fig. 2). However, as in Fig. 3, they absorbed a small amount of P from water. The difference might be due to higher waterborne P concentration in the latter experiment. In the latter experiment, diffusive or passive absorption of P might be significant.

Phillips et al. (1954, 1958) and Yoshii et al. (1955) studied the uptake of waterborne P by fish using an isotope tracer, $^{32}$P. They concluded that P uptake from water was very small and the absorption was passive or diffusive in nature. Unfortunately, these researchers used starving fish of apparently normal P status, suggesting that the fish were not deficient in P at the time of experiment. Perhaps, those fish did not absorb P from the water because they had enough P store in the body. The present study, on the other hand, used severely P-deficient fish, and showed that there was little flow of P from water to fish. Nonetheless, the decrease of P in tank water where LP fish were stocked (Fig. 2) indicates that fish are capable of absorbing a small amount of P from water by some active mechanism; i.e., a high-affinity-low-capacity transport system. Sustaining this hypothesis, Pi transporters have been reported in the gill and skin of aquatic vertebrates (Sugiura et al. 2003; Mobjerg et al. 2007; Sugiura 2009; Schultz et al. 2014). The present study, however, indicates that the physiological importance of these transporters might be minor.

Regardless of their physiological P-status, fish appear to absorb similar amounts of P from water containing 0.793 mg P/l. Estimated waterborne P absorption (~6 mg/kg fish/day) by fish is only about 0.14% of the whole body P content; however, this is about 14% of the daily P accretion (when feed intake is 1% of body weight, and feed efficiency is 100%). Similar absorption rates between the LP and HP fish suggest that the absorption is passive in nature. The quantity of P absorbed passively depends on the P concentration in water. In natural waters, however, the waterborne P concentration is low (e.g., 0.012–0.024 mg P/l for mesotrophic lakes), and therefore the contribution of waterborne P to fish physiology must be negligible.

The LP or P-deficient fish became markedly low in ash, P and Ca contents compared with HP or P-sufficient fish (Trial 2). However, by far the most altered component was not P, but Ca. Similar observations were reported in Atlantic salmon (Baeverfjord et al. 1998). In humans, some 99% of Ca in the body is present in the skeleton, whereas for P, only 85% is in the skeleton and the remaining 15% are distributed in the extracellular fluid and soft tissues (IOM 1997). In trout, hard tissues other than the skeleton; e.g., scales and fins, also contain Ca and P. However, in soft tissues, the content of P is much higher than that of Ca (Shearer 1984). The higher P content in the ash portion of the LP fish indicates that in P-deficiency both P and Ca be withdrawn simultaneously, as hydroxyapatite, from the hard tissues in order to supply P for the growing soft tissues. The superfluous Ca is subsequently excreted from the body. Thus, during dietary P restriction, fish lose little P but lose much Ca from the body. The present study reports the whole body Ca content, rather than P content, as a sensitive and reliable P-deficiency indicator that could predict a forthcoming growth reduction. In fact, Day and McCollum (1939) reported many decades ago that P-restricted rats grew normally for 2–4 weeks, then the animals gradually became inactive and died in 7–9 weeks. “The most striking effect of the P deficiency”, they wrote, “was on Ca—The loss of Ca was so much greater than of P.”

Protein retention rates measured in Trial 1 at day 32 were higher in the HP than LP fish (46.6% vs. 42.0%). This small difference may be difficult to detect by weighing fish. But, this is a clear evidence of different growth rates between the P-sufficient and P-deficient fish.
References


**Japanese Text**

ニジマスのリン収支における飼料リンの影響

杉浦省三

本研究は低リン（LP）飼料と高リン（HP）飼料を用いてニジマス（平均体長129および115 g）を飼育し、リン制限下でのリン収支について調べた。同時に数尾の魚を無給餌で蓄養し比較した。LP飼料を給餌した魚（LP魚）では血漿リン濃度が次第に低下した（0日目93 mg/l；32日目17 mg/l）。一方、HP飼料を給餌した魚（HP魚）と無給餌の魚は正常値を維持した（81-130 mg/l）。血漿リン濃度はLP飼料給餌後、顕著に低下した。LP魚は水中的溶存リンを少量吸収したが、無給餌魚は少量のリンを排泄した。LP魚、HP魚共に、溶存リンは血漿リン濃度に影響しなかった。LP魚はHP鱼と比べて、全魚体あたりの灰分（65%）、リン（67%）およびカルシウム（Ca；39%）含量が顕著に低下した。魚体Ca含量がリン欠乏症の診断に有効と考えられた。