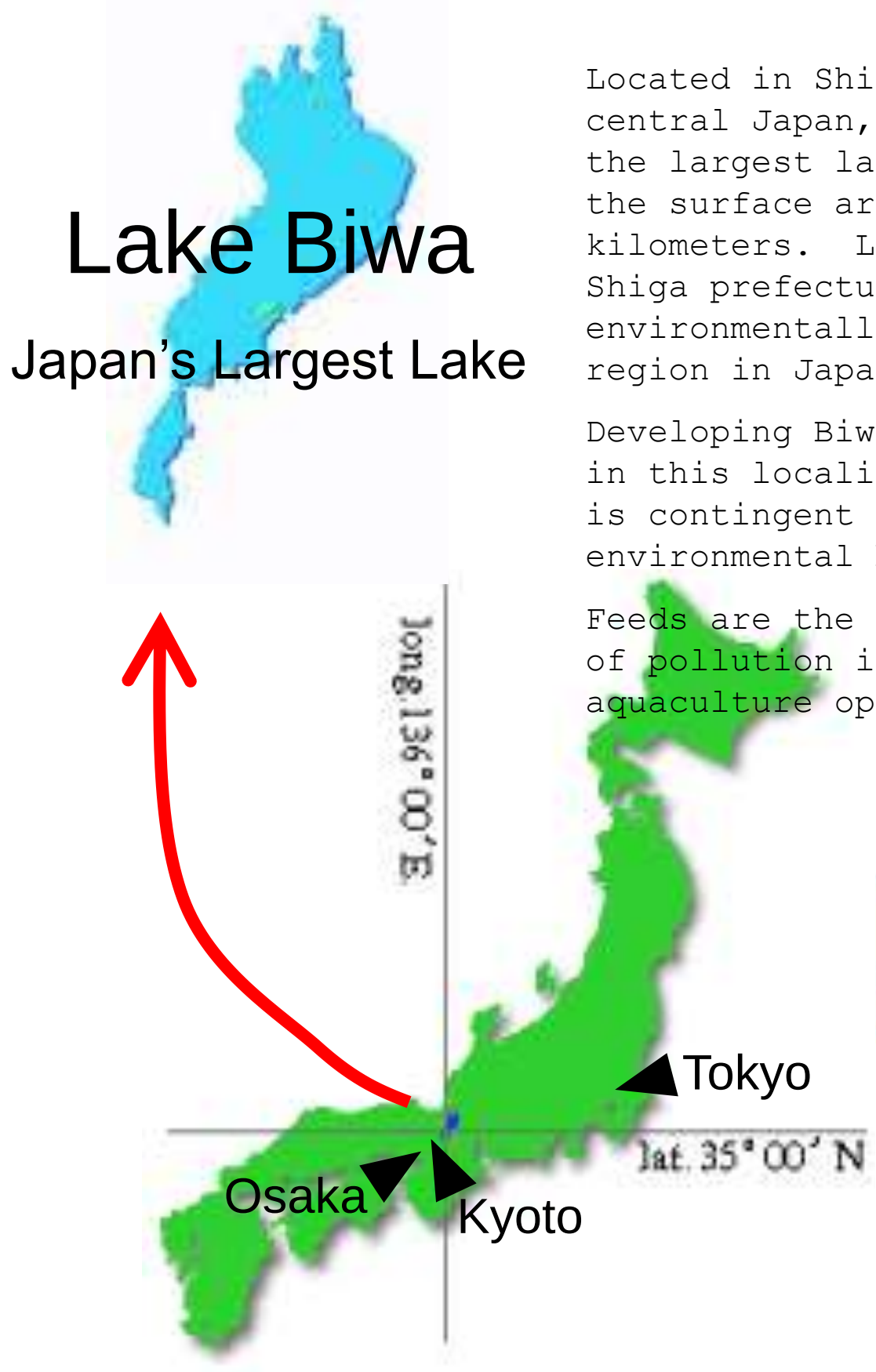
# Use of low-phosphorus feed for Biwa salmon aquaculture

Shozo Sugiura[1]*, Takashi Taguchi[1], Akira Yamaoka[2], Hidetomo Tanaka[3], Kunihiko Kuwamura[3]
[1] Department of Bioresources Management, University of Shiga Prefecture, Hikone, Shiga, 522-8533, Japan
[2] Matsuya Inc., Kinomoto, Shiga, Japan
[3] Shiga Fisheries Experiment Station, Hikone, Shiga, Japan *E-mail address*: sugiura@ses.usp.ac.jp (杉浦 省三)

**Abstract:** Biwa salmon, *Oncorhynchus masou rhodurus*, is endemic to Lake Biwa, and is considered one of the tastiest fishes among salmonids. However, aquaculture of this species is still in its infancy. Thus, we conducted the present study to develop eco-friendly feed for Biwa salmon aquaculture. Biwa salmon (1.5 year-old, Samegai strain, initial mean body wt. 277g) were reared in FRP tanks (1mt ton) and also in concrete ponds for 3 months. Fish were fed either LP feed (low- phosphorus (P), fish meal-free diet; total P 0.73%, CP 39%, fat 16%) or control feed (commercial feed for Masu salmon; total P 1.61%, CP 45%, fat 6%) once daily to apparent satiation. Availability of dietary P was determined *in vivo* in separate trials. Both groups of fish showed comparable feed consumption on an energy basis; however, toward the end of feeding duration feed intake decreased due to sexual maturation. Final mean body wt was 370g and 392g for LP and control groups, respectively (p>0.05). Both groups of fish showed similar feed efficiency (48-61%). Carcass P content was similar for both groups of fish, and was within a normal range. Fat content of fish fillets was 8.9% (LP) and 7.6% (control)(p<0.05). Both EPA and DHA contents were significantly higher in LP than control fish. Gonad- somatic index (GSI: 3.2-3.6%) and Visero- somatic index (VSI: 4.8-5.2%) were similar for both groups of fish (p>0.05). The mRNA expression of phosphate-transporter (NaPi2b) in the colon was higher in control than LP fish (p<0.05). Fish fillets were evaluated by young panels (n=68) and senior panels (n=43) using sensory and hedonic ratings. Both panels tended to give higher ratings for LP fish on taste, favor, texture and overall quality, though not significant (p>0.05). Effluent P concentration was 10.7-18.2ppb in LP tanks, and 23.8-35.9ppb in control tanks (p<0.05). However, using a stagnant water system, net P excretion over 6 hours became 0ppb in LP and 118.3ppb in control tanks (p<0.01). Apparent availability of dietary P was 50.8% (LP) and 28.8% (control). Estimated P excretion per kg of feed consumed was 3.5g (LP) and 12.0g (control). When the LP feed was pre-treated with phytase, fecal P excretion decreased by 33%. These results indicate several merits of using LP feed in Biwa salmon aquaculture.


Lake Biwa
Japan's Largest Lake

Located in Shiga prefecture in central Japan, Lake Biwa is the largest lake in Japan with the surface area of 670 square kilometers. Lake Biwa or Shiga prefecture is the most environmentally conscious region in Japan.

Developing Biwa salmon farming in this locality, therefore, is contingent upon its environmental burden.

Feeds are the ultimate source of pollution in most intensive aquaculture operations.

滋賀県立大学
THE UNIVERSITY OF SHIGA PREFECTURE
滋賀県水産試験場
Shiga Fisheries Experiment Station
株式会社 松屋, Matuya Inc.
独立行政法人 科学技術振興機構
Japan Science and Technology Agency

Acknowledgments

Authors are grateful to Dr. Y. Fujioka, Mr. M. Ujiie and other stuff of the Shiga Fisheries Experiment Station and the Samegai Trout Hatchery for their invaluable advice and cooperation. Authors also thank Mr. H. Wakizaka of the New Industry Promotion Division, Shiga prefecture, and Mr. T. Kawase of the Business and Industry Promotion Section, Nagahama city for their help in planning of this study, and Mr. T. Saito of DSM Nutrition Japan, for providing phytase for the digestion trial. This study was partly supported by a grant administered through the Japan Science and Technology Agency (JST), under Comprehensive Support Programs for Creation of Regional Innovation.

Ingredient composition of Low-P diet

| Ingredients | % |
|---|---|
| Soybean meal (45%CP) | 35.00 |
| Corn gluten meal | 12.00 |
| Wheat gluten meal | 5.00 |
| Wheat middlings | 21.90 |
| Fish oil | 12.95 |
| Palm olein oil | 5.55 |
| L-lysine | 0.50 |
| DL-methionine | 0.50 |
| Betaine | 1.00 |
| Yeast extract | 1.00 |
| Garlic powder | 0.50 |
| Vitamin premix [a] | 0.30 |
| Vitamin E | 0.05 |
| ROVIMIX STAY-C 35 [b] | 0.05 |
| Choline chloride (50%) | 1.00 |
| CAROPHYLL Pink [c] | 0.10 |
| Mineral premix (P-free) [d] | 1.00 |
| Calcium carbonate | 1.00 |
| Sodium chloride | 0.60 |
| total | 100.00 |

[a] Manufacturer' premix (composition undisclosed)
[b] Stabilized vitamin C (L-Ascorbic acid phosphate, DSM Nutritional Products, Inc.)
[c] Stabilized astaxanthin (DSM Nutritional Products, Inc.)
[d] Supplied the following per kg diet: MgO 2.0 g, Fe(II)-fumarate 400mg, $ZnSO_4 \cdot 7H_2O$ 600mg, $MnSO_4 \cdot 5H_2O$ 100mg, $CuSO_4 \cdot 5H_2O$ 12mg, KI 3mg, $CoSO_4 \cdot 7H_2O$ 2mg, wheat flour as carrier 6.883g

Ingredient composition of Commercial diet [a]

| Ingredients | % |
|---|---|
| Fish meal | 52 |
| Wheat flour, bread crumb | 30 |
| Soybean meal, corn gluten meal | 7 |
| Rice bran, corn gluten feed | 7 |
| Feed-grade yeast, bamboo powder, calcium phosphate, salt, others [b] | 4 |
| total | 100 |

[a] Manufacturer' description
[b] Supplied the following: vitamins A, D3, E, K3, B1, B2, B6, niacin, pantothenic acid, biotin, folic acid, vitamin B12, choline, inositol, vitamin C, zinc carbonate, zinc sulphate, calcium iodate, cobalt sulphate, ferrous sulphate, copper sulphate, manganese sulphate, magnesium sulphate, potassium di-hydrogen phosphate, aluminium hydroxide, ferrous fumarate, astaxantine, ethoxyquin


Low-P, all-plant-protein diet

Analytical composition of Low-P and Commercial diets

| | | Low-P diet | Commercial diet |
|---|---|---|---|
| Protein | % | 39.1 | 44.9 |
| CS [a] | | 78 (63) | 67 |
| EAAI [a] | | 92 (86) | 85 |
| Fat | % | 16.3 | 6.1 |
| EPA [b] | % | 0.466<br>(5.88) | 0.273<br>(8.89) |
| DHA [b] | % | 0.711<br>(8.99) | 0.250<br>(8.13) |
| Carbohydrates [c] | % | 36.5 | 29.8 |
| Ash | % | 6.1 | 10.4 |
| P | % | 0.73 | 1.61 |
| Water | % | 2.0 | 8.8 |
| Energy [d] | kcal/100g | 449 | 354 |

CS (chemical score), EAAI (essential amino acid index)
[a] CS and EAAI were calculated based on ingredient compositions of diets and the literature values. Values in parentheses are the scores calculated without supplemental amino acids
[b] Percentages in diet. Values in parentheses are percentages in total fatty acids
[c] Carbohydrate content was determined by subtraction.
[d] Energy content was calculated based on Atwater's coefficients.

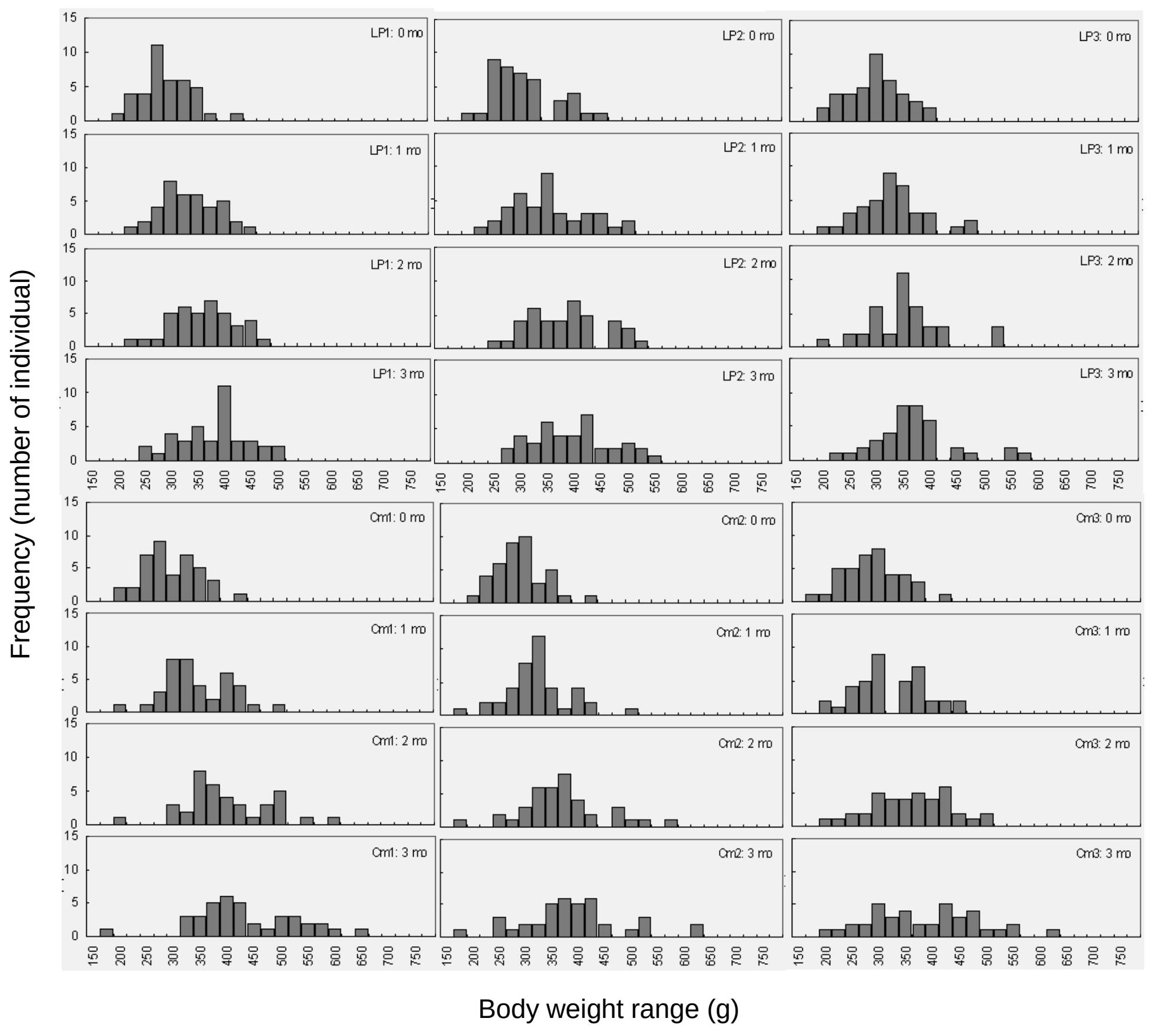
The Low-P diet was well accepted by the fish from the first day of feeding, suggesting that the dietary supplements effectively improved feed palatability.

Lake Biwa harbors a strong culture of clean environment (Kumagai and Vincent 2003, Mitsch 2006). Thus, low-polluting feed is required for Biwa salmon aquaculture. We monitored the performances of Biwa salmon raised on low-polluting all-plant protein diet, and compared the performances with those raised on commercial fish meal-based diet.

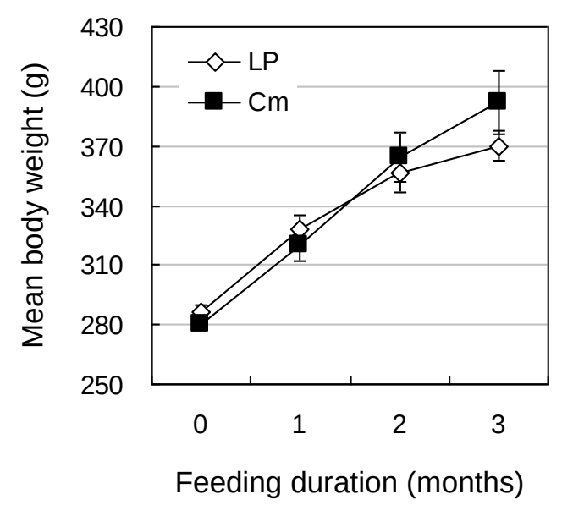
Additional aim of Biwa salmon aquaculture is to raise fish of high quality. High total fat content in fish fillets, especially in tunas and salmons, is a highly preferred attribute among Japanese consumers. Also, high EPA and DHA contents in fillets endow health-promotive quality (Sekikawa et al. 2008). To increase fat deposition in fish fillets, we increased the fat content of diet. In addition, we tried to increase fillet fat deposition, by inducing moderate P deficiency in fish by feeding low-P diet, since P-deficiency is reported to increase body fat deposition in fish (Sakamoto and Yone 1980, Takeuchi and Nakazoe 1981, Higashitani & Sugiura 2007).


Biwa salmon, *Oncorhynchus masou rhodurus,* 2 year old male (top) and female

Biwa salmon, Oncorhynchus masou rhodurus, is endemic in Lake Biwa, and is considered one of the tastiest fishes among salmonids (Kawanabe 2000). The total fisheries catch of Biwa salmon, however, is only about 20-40 metric tons per year (Takahashi 2009), raising the commercial value of this species. Farming of this species, therefore, could promote tourism-related industries through this local cuisine. Aquaculture of this species, however, is still in its infancy. Recently, a strain of Biwa salmon, whose growth rate is comparable to more domesticated rainbow trout, has been developed by selective breeding (Tanaka 2006). This strain of fish appear to grow over 1 kg in body weight in 22 months after hatch, compared with only 325g for the wild strain during the same period and under the same cultural conditions.

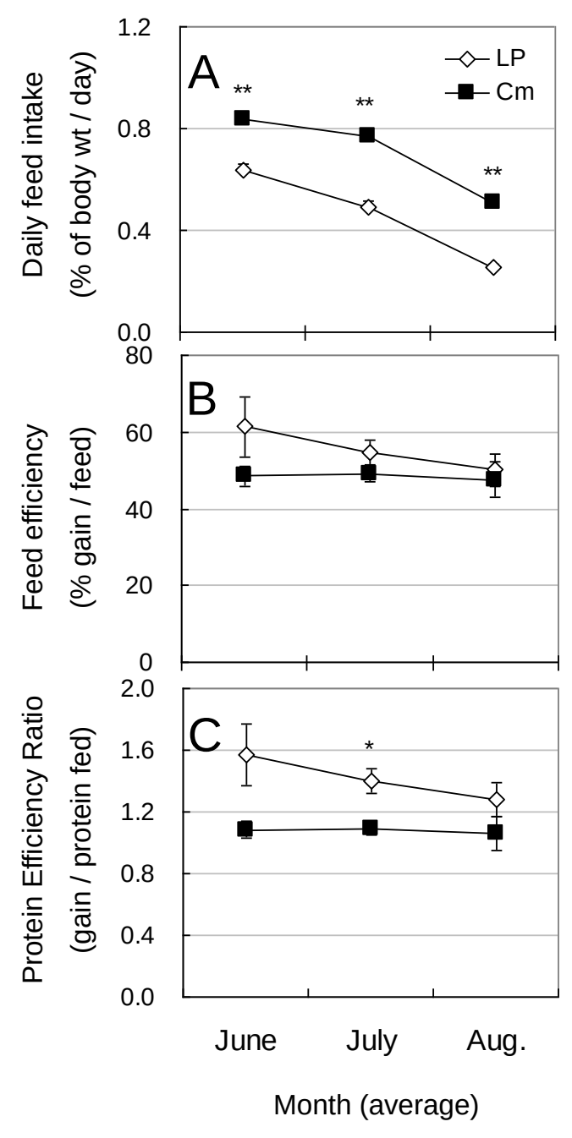


Growth pattern of Biwa salmon in each tank over the period of 3 months. LP1 (LP tank#1), LP2 (LP tank#2), LP3 (LP tank#3), Cm1 (Cm tank#1), Cm2 (Cm tank#2), Cm3 (Cm tank#3). Fish growth is more uniform in the groups fed LP diet compared with those fed Cm diets based on size distribution of each month.



Body weight gain of Biwa salmon fed LP or Cm diets for 3 months. Each plot indicates mean ± SE (as error bar) of 3 tanks. Mean values were not significantly different between LP and Cm groups at any month.

One year and seven month-old Biwa salmon were stocked in six 1-ton plastic tanks (20 fish per tank, 3 tanks per diet). Fish were hand-fed respective diets, once daily at 900 h, 5 days a week, to apparent satiation for 3 months from June 1 to Aug. 31, 2009. Before the initiation of experiment, fish were acclimated to the tank environment for one month. Each tank was continuously supplied with spring water at ~20L/min. The spring water had the following water quality parameters: water temperature 12 ± 0.5℃; pH 7.3-8.2; suspended solids ≤ 0.1 mg/L; COD 0.29-0.49 mg/L; total N 0.77-0.89 mg/L; total P 0.026-0.030 mg/L; chloride ion 4.51-4.95 mg/L. Dissolved oxygen was near saturation at all times. Natural photoperiod was applied; approximate daylight hours were 830-1700. All tanks were located in a quarantined indoor facility.

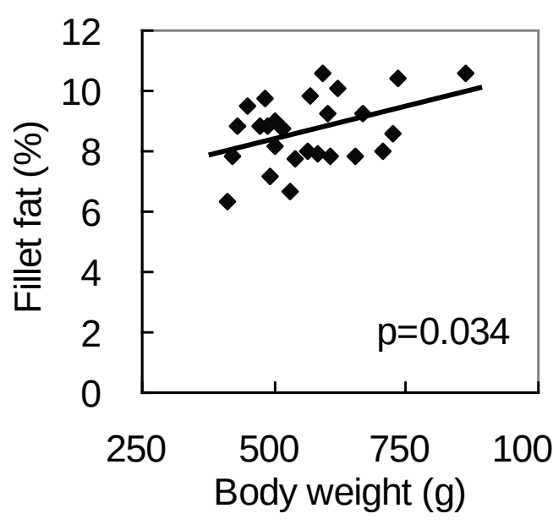


Daily feed intake of Biwa salmon at satiation feeding (A), feed efficiency (B), and protein efficiency ratio or PER (C). Each plot indicates mean ± SE as error bar of 3 tanks. Asterisks show statistical significance between LP and Cm groups (* p<0.05, ** p<0.01).

Body, fillets, and bone analyses of Biwa salmon raised on LP or Cm diet

| | Initial | LP | Cm | P value |
|---|---|---|---|---|
| *body* | | | | |
| BW (g) | 288 ±29 | 558 ±35 | 574 ±26 | 0.718 |
| VSI (%) [a] | 7.48 ±0.69 | 5.18 ±0.22 | 5.03 ±0.14 | 0.566 |
| GSI (%) | 0.21 ±0.04 | 3.89 ±0.34 | 3.71 ±0.47 | 0.761 |
| *Fillet* [b] | | | | |
| Fillet fat (%) | 10.15 ±0.62 | 9.48 ±0.23 | 7.87 ±0.23 | **0.000** |
| EPA / fillet (%) | 0.157 ±0.015 | 0.154 ±0.006 | 0.112 ±0.005 | **0.000** |
| EPA / TFA (%) | 2.66 ±0.24 | 2.79 ±0.05 | 2.44 ±0.08 | **0.002** |
| DHA / fillet (%) | 0.428 ±0.045 | 0.464 ±0.023 | 0.345 ±0.022 | **0.001** |
| DHA / TFA (%) | 7.22 ±0.52 | 8.39 ±0.30 | 7.45 ±0.35 | 0.053 |
| *Bone* [c] | | | | |
| Ash / bone (%) | 48.8 ±4.11 | 48.3 ±1.49 | 52.4 ±0.81 | **0.025** |
| P / bone (%) | 9.50 ±0.000 | 8.97 ±0.28 | 10.03 ±0.19 | **0.004** |

Values are means ± SE of 3 fish (for Initial samples) or 13 fish (for LP and Cm): 2 fish were sampled from each plastic tank and 7 fish were sampled from each concrete pond
LP: fish fed low-P diet; Cm: fish fed commercial diet; BW: body weight of fish; VSI: viscero-somatic index; GSI: gonado-somatic index; TFA: total fatty acids
[a] Visceral weight to calculate the VSI did not include gonad
[b] Including skin and belly flap
[c] Fat-free dry basis

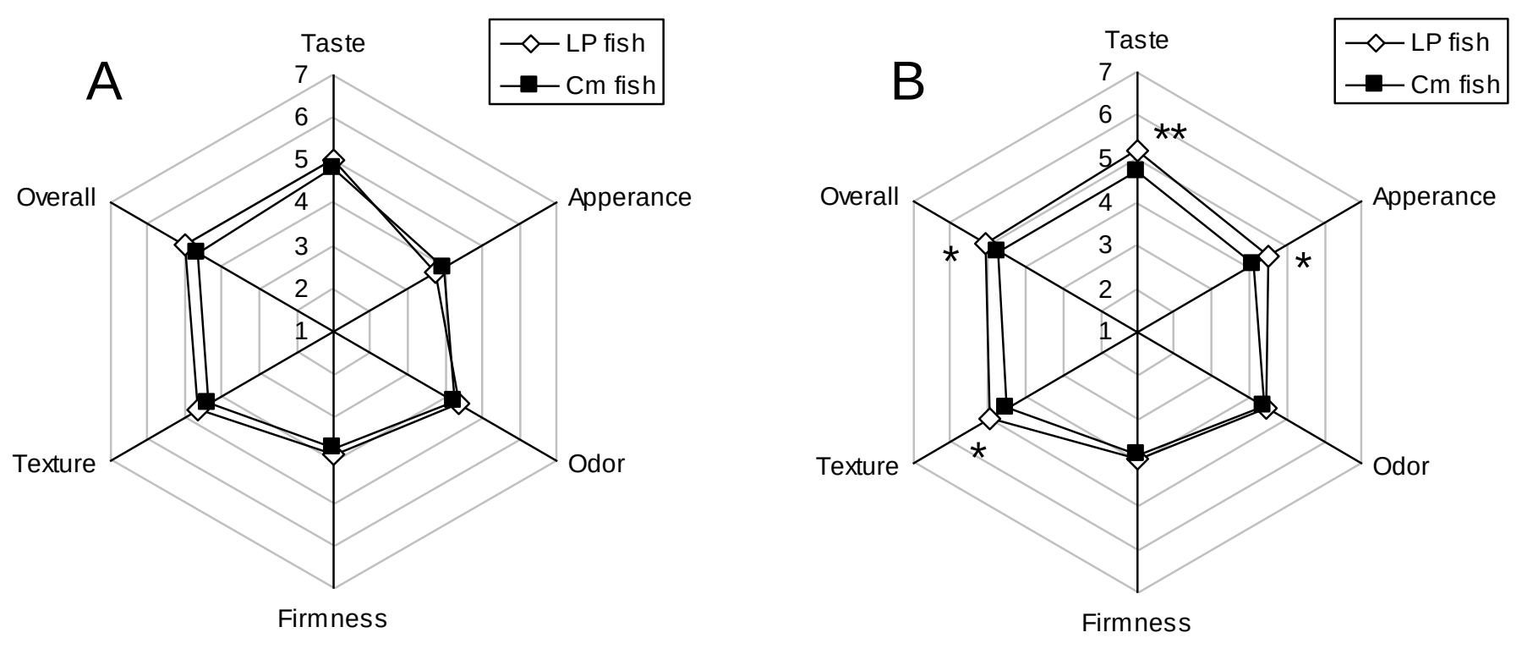


Correlation between fish body weight and fillet fat content in Biwa salmon. The larger the size of the fish, the more fat it tends to retain. Fish body weight and fillet fat content were significantly and positively correlated, confirming similar observations in Atlantic salmon (Jobling and Johansen 2003).

Body, fillets, and bone analyses of Biwa salmon: Comparison between female and male

| | Female | Male | P value |
|---|---|---|---|
| *body* | | | |
| BW (g) | 535 ±25 | 609 ±35 | 0.092 |
| VSI (%) [a] | 5.18 ±0.18 | 5.01 ±0.19 | 0.522 |
| GSI (%) | 4.41 ±0.39 | 2.97 ±0.25 | **0.009** |
| *Fillet* [a] | | | |
| Fillet fat (%) | 8.68 ±0.29 | 8.67 ±0.37 | 0.987 |
| EPA / fillet (%) | 0.138 ±0.007 | 0.126 ±0.009 | 0.293 |
| EPA / TFA (%) | 2.62 ±0.09 | 2.61 ±0.09 | 0.984 |
| DHA / fillet (%) | 0.425 ±0.022 | 0.377 ±0.034 | 0.233 |
| DHA / TFA (%) | 8.03 ±0.28 | 7.77 ±0.44 | 0.617 |
| *Bone* [a] | | | |
| Ash / bone (%) | 50.88 ±0.75 | 49.63 ±1.97 | 0.517 |
| P / bone (%) | 9.50 ±0.15 | 9.49 ±0.43 | 0.977 |

Values are the means ± SE of 15 fish (for female) or 11 fish (for male): 2 fish were sampled from each tank and 7 fish were sampled from each concrete pond.
[a] See footnotes of Table 2



Organoleptic qualities of Biwa salmon: Comparison between fish raised on low-P diet (LP) and those raised on commercial diet (Cm). Figures A and B show results of senior panels (n=41 people, average age 48) and young panels (n=68 people, average age 22), respectively. Asterisks indicate statistical significance (* p<0.05, ** p<0.01).

LP fish tended to show higher ratings than Cm fish in sensory scores by both senior and young panels. These differences may be attributed to the higher fat content in LP than Cm fish. High fat content of fillets is a consumer-preferred trait in Japan for many fish species including salmonids and tunas (Miyake et al. 2003, Kiyokawa & Ioka 2007). Thus, in future studies with Biwa salmon, further increase in fish body weight will be essential in order to increase fillet fat content and thereby to increase organoleptic ratings of the fillets.

Body composition of Biwa salmon: Correlations between two variables [a]

| | BW (g) | VSI (%) | GSI (%) | fillet fat (%) | EPA / fillet (%) |
|---|---|---|---|---|---|
| VSI (%) | 0.34 | --- | | | |
| GSI (%) | 0.18 | 0.01 | --- | | |
| Fillet fat (%) | **0.42** | 0.31 | -0.02 | --- | |
| EPA / fillet (%) | 0.00 | 0.11 | 0.11 | **0.63** | --- |
| DHA / fillet (%) | -0.05 | 0.22 | 0.11 | **0.47** | **0.84** |

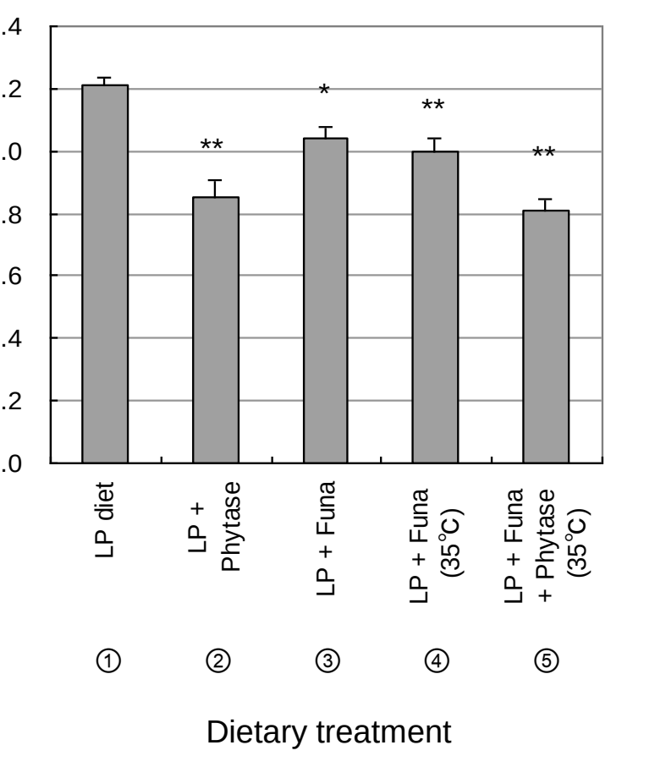
[a] Negative values are inverse correlations. Values in bold are significant (p<0.05).

P digestibility was higher in LP than Cm diets. Fecal P content was lower in fish fed LP diet than those fed Cm diet. Amount of P excreted per kg diet consumed was 3.51g with LP diet and 12.01g with Cm diet. Amount of P absorbed per diet consumed (%) was 0.361 in fish fed LP diet, and 0.486 in fish fed Cm diet. Amount of P excretion per kg of fish produced was 6.33g with LP and 24.8g with Cm diets.

Digestibility of experimental diets and fecal P excretion by Biwa salmon

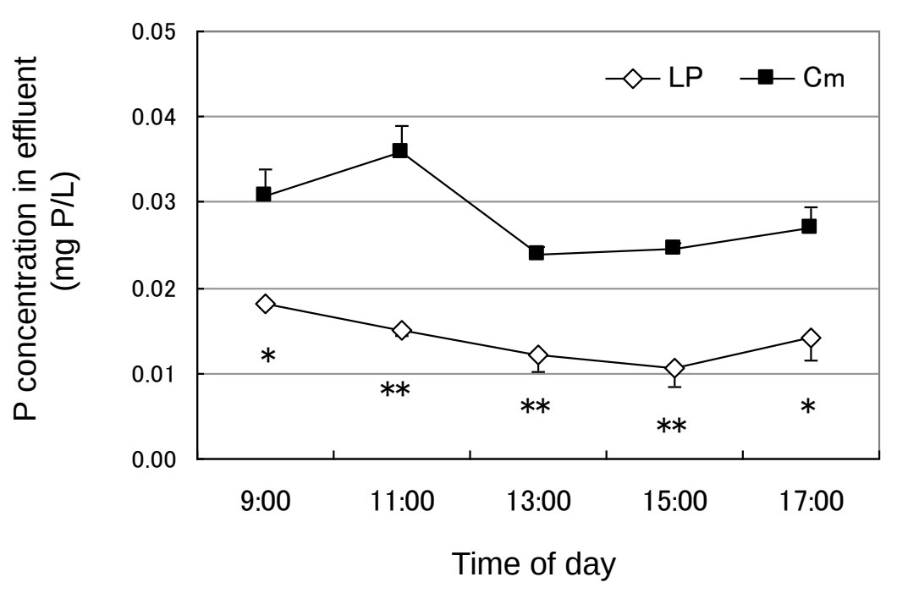
| | LP diet | | Cm diet | | *P* value |
|---|---|---|---|---|---|
| Dry matter digestibility (%) | 70.6 | ± 1.8 | 61.1 | ± 1.5 | 0.002 |
| P digestibility (%) | 50.8 | ± 4.9 | 28.8 | ± 2.7 | 0.003 |
| P content in feces (%, dry feces) | 1.219 | ± 0.141 | 3.110 | ± 0.164 | 0.000 |
| P excretion (g / kg diet consumed) | 3.51 | ± 0.35 | 12.01 | ± 0.46 | 0.000 |
| P absorbed (%) per diet consumed | 0.361 | ± 0.035 | 0.486 | ± 0.046 | 0.054 |
| P excretion (g) per kg fish produced | 6.33 | ± 0.62 | 24.80 | ± 0.94 | 0.000 |

Values are mean ± SE (n = 6 fish)



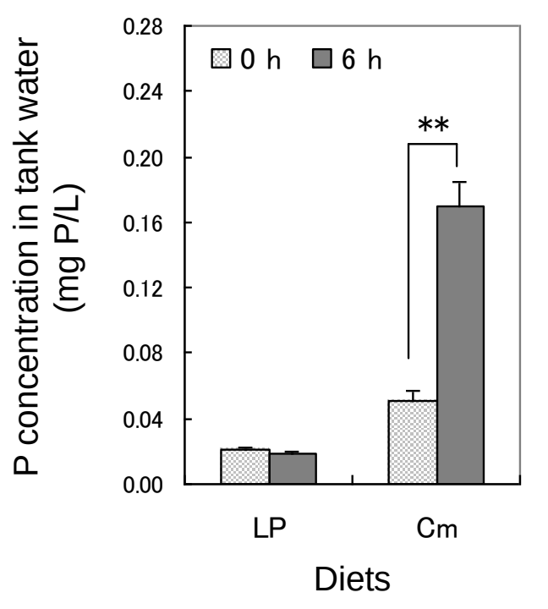
Fecal P content of Biwa salmon fed LP diet treated with phytase and/or fermented rice paste.

Total P content (%, dry basis) of each diet was as follows: diet① 0.75; diet② 0.77; diet③ 0.78; diet④ 0.78; diet⑤ 0.79. Funa: fermented rice paste. 35℃: incubated at 35 ℃ for 24h. Fish used were one-year-old Biwa salmon. Each column represents average and SE (as error bar) of 6-8 fish. Significant effects of treatment or supplement are indicated as asterisks (* p<0.05, ** p<0.01). Amount of phytase supplemented was 150mg/kg diet; the amount of Funa or fermented rice paste used was 5% (dry basis). Incubation conditions were 35 ℃-24h with 36% water content in the dough. Funa or fermented rice paste was used to acidify the dough as phytase is active only at acidic pH.



P concentration in effluent water: Biwa salmon were fed either LP or Cm diet (n=3 tanks, * p<0.05; ** p<0.01). Fish were fed at 830-900. P concentration in inflow water was 0.024 ± 0.002 (SD)

Results of this analysis showed that the effluent P concentration from tanks of LP diet was similar to P concentration of influent water, i.e., net P excretion was around zero.



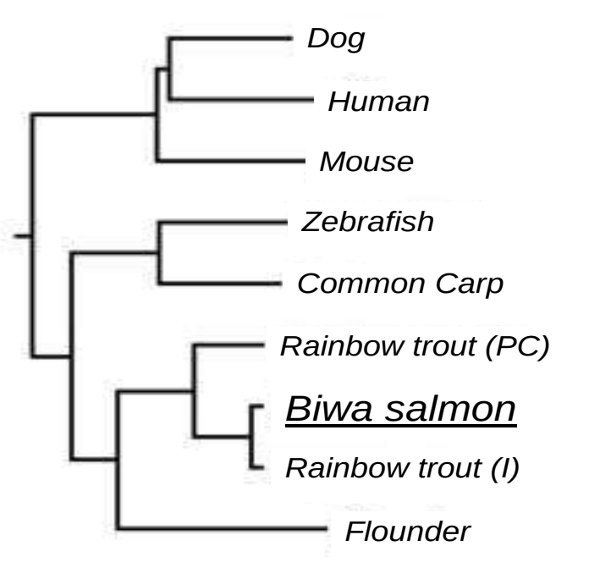
Cumulative P concentration in tank water: Biwa salmon were fed either LP or Cm diet (each bar indicates mean ± SEM as error bar, n=3 tanks, ** p<0.01). After feeding, water supply to each tank was withheld for 6 hours. P concentration in water of each tank was determined at 0h and 6h.

The cumulative 6 hour net P excretion was zero in LP tanks, whereas it was high (0.12mg/L or 0.12g P) in Cm tanks (Fig.2). At 0 hour period, P concentration in Cm tanks was ~2 times higher than that in LP tanks.

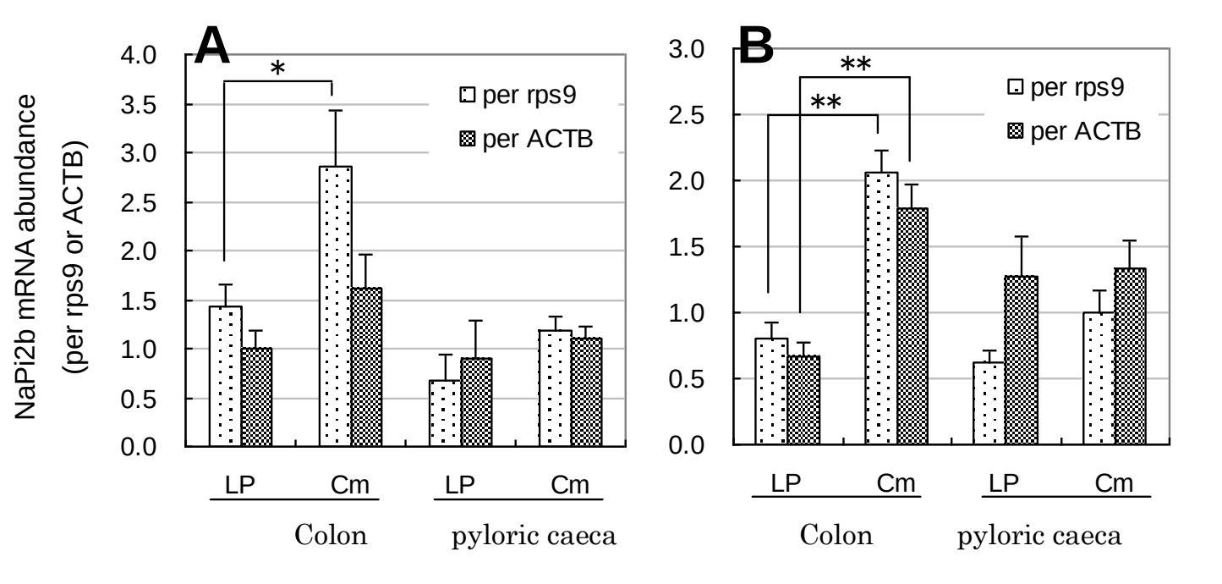
Expression of phosphate transporter type 2b gene (NaPi2b or SLC34A2) in the digestive tract of fish was examined in order to diagnose P status of fish. To do this, the mRNA sequences of NaPi2b, beta-actin (ACTB) and ribosomal protein s9 (RPS9) of Biwa salmon were determined. PCR primers (see below) were designed based on known sequences of taxonomically closely related species. The mRNA were extracted from the colon and the pyloric caeca. Based on the determined sequences, primers for qPCR were designed (see below).

PCR primers used for cloning and qPCR of Biwa salmon sequences

| gene | use | fwd (5'---3') | rev (5'---3') | amplicon (bp) |
|---|---|---|---|---|
| NaPi2b | cloning | tcctgagctctgctttccagctr | tagatcactgcgaaccagcggta | 1246 |
| | qPCR | tgaccttcatcgttcagagcag | agcagtggtagttgtgccgata | 131 |
| ACTB | cloning | aagatgaaatcgccgcactggt | agaagcatttrcggtggacgat | 1122 |
| | qPCR | ctcttccagccctccttcctc | cggatgtccacgtcacacttc | 89 |
| RPS9 | cloning | gttgccaggagttgggtttgtc | ctcytcbtcatcagckccycca | 570 |
| | qPCR | ctggatgagaaggaccccaag | caggcccaggatgtaatcgag | 114 |



Phylogenetic tree of phosphate transporter type 2b mRNA. Unlike rainbow trout, Biwa salmon appear to share only one type of NaPi2b in the GI tract. (*PC*: pyloric caeca; *I*: intestine)



Expression of phosphate transporter (NaPi2b) mRNA in the colon and pyloric caeca of Biwa salmon fed LP or Cm diets for 3 months. A: pond study; B: tank study. NaPi2b mRNA abundance was normalized based on rps9 and ACTB. Each bar indicates mean ± SE (as error bar) of 6-7 fishes. *p<0.05, **p<0.01.

## Discussion

### Effluent P

The 6 hour excretion of P increased in Cm tanks, but not in LP tanks. Since soluble P excretion is highest after feeding (Sugiura et al. 2000), the present data suggest no excretion of soluble P by fish fed LP diet. This zero excretion of soluble P is similar to our previous observations in rainbow trout. Zero excretion, however, suggests that fish were potentially deficient in P since soluble P, that is urinary origin, is excreted only when fish are fed excess P. Indeed, the amounts of P absorbed per diet consumed (see Table) is lower than the dietary P requirement in both LP and Cm groups of fish, especially LP group. However, since both LP and Cm diets were low in feed efficiency (gain/feed), the data need to be corrected based on respective feed efficiency (Sugiura et al. 2000). The corrected values are 0.60-0.72% for LP diet and 0.97-1.1% for Cm diet. These values show the amount of P absorbed (%) per diet consumed when feed efficiency is 100%. The values of LP diet are slightly higher than the dietary P requirement estimated with rainbow trout of 400g in body weight, suggesting that LP fish received just marginal amount of available P and Cm fish received sufficient amounts of available P in respective diets. These calculations, however, does not account for low feed efficiency due to feed loss or uneaten feeds. Thus, if the amount of uneaten feeds was considerable, the LP fish ought to become P deficient. Confirming this point, the bone P content of LP fish is marginally normal or slightly deficient. This implies that the low feed efficiency is at least partly attributed to some feed wastage by hand feeding.

### P digestibility and fecal P excretion

Digestibility of P in LP diet was 51%, which is unexpectedly high. The major sources of P in LP diet are soybean meal and corn gluten meal, and the digestibility of P in these ingredients determined with rainbow trout are 14% (Yamamoto et al. 1997) or 22% (Sugiura & Hardy 2000) for soybean meal, and 2-11% (Yamamoto et al. 1997) or 9% (Sugiura & Hardy 2000) for corn gluten meal. The high digestibility of LP diet is probably due to the extrusion process in feed manufacturing as extrusion at 150℃ has been shown to increase P availability of soybean meal by ~20% (Satoh et al. 2002). Estimated fecal P excretion was considerably different between LP and Cm diets. The difference was 3.4-fold, meaning that Cm diet is 3.4 times more polluting than LP diet. In other words, by replacing Cm diet with LP diet, fecal P burden to the freshwater environment is reduced by 71%.

In Digestion trial 2, fecal P content decreased by including fermented rice paste in LP diet. P contained in the fermented rice paste was presumably highly available because of its long fermentation process, adequate acidity and endogenous supply of phytase. Thus, the effect of fermented rice paste might be due to relative reduction of unavailable phytate-P content in the diet. However, compared with our former study, in which microbial phytase was capable to digest over 90% of phytate in diet (Sugiura et al. 2001), the effect of phytase in the present study was considerably low. This is because the basal LP diet was supplemented with calcium carbonate. Phytate or free phytic acid forms insoluble precipitates with calcium and/or magnesium (called phytin), which is known to be resistant to phytase digestion (Angel et al. 2002). Thus, reducing calcium and magnesium content in diet can be construed as further reduction of fecal P excretion. In the present study, the LP diet was supplemented with calcium in order to induce P-deficiency in fish rather than minimizing fecal P excretion.

## Discussion

### Diet composition

Fish meals are generally very high in P content, and need to minimize its use in LP formulations. However, feed ingredients low in P are generally low in both protein content and protein quality. Only a few special ingredients are low in P and still high in protein, but they generally bear other difficulties. For example, wheat gluten meal is low in P, high in protein, and high in palatability, but high in price, low in certain amino acids and low in pellet-forming quality. Blood meal is also low in P and high in protein, and fairly good in palatability, but low in certain amino acids and the safety is not certain. To minimize P content of diets, protein content in the diet will have to be reduced. Low dietary protein content, however, is generally construed as low N-wastes in effluents, which is of course highly preferred. To compensate for the low protein content in LP diets, dietary fat content will need to be increased. Fats are very low in P, which enables further reduction of P in diets. Thus, low-P diets have a common profile, that is, low protein and high fat. The LP diet used in the present study was relatively low in fat content (16% fat). In future studies, dietary fat content should be increased.

### Fish growth, feed intake, and feed efficiency

Fish growth was similar between LP and Cm groups. This result is rather amazing since LP diet was free of animal protein sources. In Atlantic salmon, Espe et al. (2006) also reported comparable growth of fish between fish meal-based reference diet and all-plant protein diets. Feed intake was high at the beginning of feeding duration, especially in May (i.e., acclimation period); while it decreased gradually toward the end of feeding duration. This decrease could be attributed to the onset of sexual maturation since the fish had fairly high GSI values at the termination of experiment. The low fillet fat content compared with the initial fat content, also is indicative of sexual maturation. Feed efficiency was considerably low in both LP and Cm diets. Since this is unrelated to the month or the amount of feed intake, the low feed efficiency could be attributed to the diets or to Biwa salmon, which is far less domesticated than rainbow trout.

### Fillet and bone analyses

Higher fillet fat content in LP fish than Cm fish may be attributed to high dietary fat content in LP than Cm diets. However, since LP fish were marginally P-deficient (as diagnosed from bone ash and P contents), the effect of P-deficiency to increase body fat deposition could also contribute to high fat content in LP fish. Unfortunately, both LP and Cm fish had lower fillet fat content than the initial fish due presumably to sexual maturation. In Kojima (1990), wild Biwa salmon caught in July had 12.2% fat in flesh, while that caught in September had only 6.6% fat in flesh. Our present data agree well with these values. Regarding the EPA and DHA contents in fillets, EPA increased 38% and DHA 34% in fish fed LP diet compared with those fed Cm diet. However, these valuable fatty acids could be further increased by inducing frank P-deficiency or by simply increasing their contents in diets. In the present study, palm olein was used as an alternate fat source to reduce feed cost. In finishing diets, however, fish oil may be used as a sole lipid source to maximize EPA and DHA retention in the fillets (Ng et al. 2007). If P-deficiency is induced during this finishing stage, the deposition of dietary EPA and DHA will be more efficient. Bone ash content was similar between the initial fish (48.8%) and LP fish (48.3%), while slightly but significantly higher in Cm fish (52.4%). These values, however, appear to be normal compared with the ash content reported in other salmonids: 48-52% in Atlantic salmon (Vielma and Lall 1998) and 50-51% in rainbow trout (Ogino et al. 1979). Bone P content was also slightly but significantly lower in LP than Cm fish, but their values are normal or close to normal compared with normal bone P contents in closely related species: 9.1-9.6% in Atlantic salmon (Vielma and Lall 1998), and 9.0-9.7% in rainbow trout (Ogino et al. 1979). Based on these values, fish fed LP diet were marginally normal or slightly deficient in P.

### Taste test

The higher sensory quality of LP fish might also be attributed to the plant-based composition or various supplements used in LP diet as compared with Cm fish that consumed fish meal- based diet. In the US, catfish raised on plant-based feeds are free of fishy flavor, whereas those fed on fish meal-based feeds or feeds containing large amounts of fish oil are known to be fishy and unpleasant (Ploeg 1991). Since young generations tend to dislike fishy flavor (Mineki et al. 2005), minimizing the use of fish meal or fish oil in aquafeeds may be one approach to making fish of mild flavor, and thereby to help young people consume more fish. Also, in trout, high vitamin E content in diet appears to counteract with fishy odor in high fish oil diet (Chaiyapechara et al. 2003). The LP diet of the present study was supplemented with vitamin E in the amount ca.17 times the requirement for trout and salmon. Also, plant ingredients, including palm oil, are generally high in vitamin E content.

### Future studies

Our LP diet was not very high in fat content compared with commercial LP diets used for salmonid farming in northern Europe (typically 25-35% or higher, Riaz 2009). Increasing dietary fat content could further improve fish fillet quality, not to mention effluent profile. In addition to feed quality, the present study suffered from sexual maturation in fish. Biwa salmon, both sexes, generally mature at 2 years of age under aquaculture conditions (Tanaka 2006), while in the wild they grow slower and mature older (Fujioka 1990). As the maturation consumes body fat, the fillet quality deteriorates inevitably. This is obvious from the fat content of fish, in both dietary treatments, which was higher at the initial than at the end of trial. In commercial aquaculture production, it will be necessary to prevent fish maturation either by controlling daylight length or by using all-female triploid fish (Lincoln and Scott, 1983). Also, to make high-fat-high-quality fish, it will be necessary to grow fish larger since fish size and the fillet fat content apparently correlate to one another.

Presently, there is no feed commercially available for Biwa salmon aquaculture. Commercial feed for closely related species, such as amago salmon and rainbow trout, have been used to feed Biwa salmon. However, in Biwa salmon aquaculture, minimizing its environmental impacts appears to be no less important than maximizing fish growth due to environmentally oriented cultures surrounding Lake Biwa or Shiga prefecture (Itakura et al. 1999). Environmentally friendly low-P feeds are commercially available in western countries. Effluent profile of one of those feeds (EP diet manufactured and used in the US) was examined previously using rainbow trout (Sugiura et al. 2006), which showed ~40% less pollution compared with conventional feeds. However, our LP diet evaluated in the present study was even less polluting than the commercial LP diet. Also, former studies comparing experimentally prepared low-P diets and commercial diet (or experimentally prepared normal-P diet) reported better (less polluting) results (Hernandez et al. 2004) or slightly more polluting results (Satoh et al. 2003) than the commercial LP feed.

The LP fish meal-free diet supported normal fish growth that was comparable to Cm diet. However, the fillet quality has not been improved sufficiently by the LP diet, probably because dietary P content in LP diet was not low enough to induce fat deposition in fillets. Increasing body fat deposition is a known metabolic shift caused by dietary P deficiency. In future studies, reducing dietary P content (per feed efficiency) will be essential to increase body fat deposition and to enhance fillet quality. In our present study, feed efficiency (gain/feed) was low compared with commercial LP feeds. This indicates that P-excretion, when expressed as per kg of fish produced, will be higher in our LP diet than the commercial LP diet or low-P diets evaluated by other researchers. However, since Biwa salmon is far less domesticated than rainbow trout, the low feed efficiency and low feed intake in the present tank experiment might not be so surprising. Lastly, the present study did not thoroughly consider the cost of LP diet. Since, in commercial trout production, feed is the largest variable cost, much effort should focus on reducing the feed cost in future studies.