Effect of Dietary Soybean Meal and Phytase Supplementation on Digestibility and Phosphorus Discharge in Red Sea Bream

Amal Kumar Biswas1,*, Seung-Chul Ji1, Manabu Seoka1 and Kenji Takii1

Abstract: This preliminary study was designed to investigate the influence of partial replacement of fish meal with soybean meal on digestive function of red sea bream, Pagrus major with or without phytase supplementation. Five isocaloric diets were formulated: F (70% fish meal), S15 (55% fish meal + 15% soybean meal), S25 (45% fish meal + 25% soybean meal), S45 (25% fish meal + 45% soybean meal) and S25 + P (S25 + 3,000 FTU phytase/kg diet). Fish weighing about 78 g were fed these diets for 6 weeks at 21 ± 1°C. The partial replacement of fish meal with soybean meal resulted in a lower apparent digestibility coefficient (ADC) of nutrients and energy which resulted in lower food consumption and final body weight in red sea bream. However, the ADC of protein and energy, feed consumption, and final body weight in S25 + P were higher than those of fish fed with S25. Fish fed with S25 + P showed remarkably lower phosphorus discharge than those fed with other diets (P < 0.05). The results demonstrated that fish meal replacement by soybean meal reduced the ADC of nutrients and energy; however, phytase supplementation in soybean meal diet increased the ADC of those parameters in red sea bream. In addition, phytase supplementation could help to reduce phosphorus discharge in the environment.

Key words: Pagrus major; Apparent digestibility coefficient; Fish meal replacement; Phytase

The alternative protein sources have been studied intensively during the last few decades because of the unavailability and high cost of fish meal, and to formulate diets which minimize phosphorus excretion (Lall 1991). An increasing trend in the global production of soybean meal over the past 25 years made it the most promising alternate protein source for fish feeds in terms of future availability (Hardy 1995). However, about two-third of phosphorus in soybean meal is present as phytate (inositol hexaphosphate) which is not efficiently digestible and utilizable for fish (Ketola and Harland 1993; NRC 1993; Sajjadi and Carter, 2004). Moreover, some antinutritional factors are present in soybean products (NRC 1993). In spite of these drawbacks and its adverse effects on growth performance in some species (Satoh et al. 1989; Pfeffer and Beckmann-Toussaint 1991; Rumsey et al. 1994; Stickney et al. 1996; Davies and Morris 1997), soybean meal has been used to replace 25 – 40% of the fish meal protein with no decrease in weight gain in others (Dabrowski et al. 1989; Oliveira et al. 1994; Sanz et al. 1994; Kaushik et al. 1995, 2004; Muzinic et al. 2004). It is also noteworthy, however, that these drawbacks can be minimized through the addition of phytase to the diet (Simons et al. 1990; Rodehutscord and Pfeffer 1995). It has also been demonstrated that the addition of phytase to the diet has potential to improve the nutritive value of plant products (Forster et al. 1999), apparent digestibility coefficient (ADC) of nutrients and minerals (Cheng and Hardy 2003; Yoo et al. 2005), and to reduce phosphorus discharge (Lanari et al. 1998; Forster et al. 1999; Vielma et al. 2002; Sajjadi and Carter 2004). However, there is no sufficient information on this regard in red sea bream, Pagrus major, which is one of the most...
important cultured fish in Japan. This study was therefore aimed at investigating the effect of fish meal replacement by soybean meal with phytase supplementation on the ADC of nutrients and energy and phosphorus discharge in red sea bream.

Materials and methods

Diet preparation

Fish meal and soybean meal were used as protein source and were supplied by Itochu Feeds Inc. (Tokyo, Japan). Sardine oil and \( \alpha \)-potato starch were used as lipid and carbohydrate sources, respectively. Vitamin and mineral mixtures were those of Halver (1957).

Five isocaloric diets (F, S15, S25, S45 and S25 + P) were formulated and the composition and chemical analyses of the diets are shown in Table 1. Chromic oxide (0.5%) was included in all diets as an inert marker. The diets were as pellets of 3 mm diameter by a laboratory pellet machine after mixing 100 parts of ingredients with 30 parts of tap water. The diets were freeze dried and stored in a freezer at \(-20\)°C until used.

Fish and experimental conditions

Juvenile red sea bream (a mean weight of 20 g) of Kinki University strain (Taniguchi et al. 1995; Murata et al. 1996) were obtained from the Fish Nursery Center of Kinki University, Uragami, Japan. One hundred fifty fish were randomly distributed among five 400-l conical bottom shaped tanks where they acclimated to the new rearing environment for 6 weeks. These tanks were used to rear the fish and collect feces from the fish and a simple diagram of the fecal collection column is given in Fig. 1. The fecal collection tank had a sloping bottom leading to a centrally located drainage slot, and the effluent water was directed first over a fecal collection column and then to waste. The photoperiod in all tanks was set to 12-h light: 12-h dark. Tanks were supplied with filtered seawater at 4 l/min and aerated to maintain the oxygen level near 100% saturation. The temperature was maintained at \(21 \pm 1\)°C. Fish were fed to apparent satiation with a commercial diet (protein 47.7%, lipid 10.7%, Marubeni Nisshin Feed Co. Ltd., Tokyo, Japan), twice a day at 09:00 and 15:00 during the acclimation period.

After conditioning for 6 weeks, the fish were starved for 24 h and the body length and weight were measured to the nearest 0.1 cm and 0.1 g, respectively. The stocking density was reduced to 20 fish (mean weight, 77.9 ± 10.9 g) per tank. Fish were fed to apparent satiation with the

Table 1. Formulation and proximate composition of the diets

<table>
<thead>
<tr>
<th>Ingredients (%)</th>
<th>F</th>
<th>S15</th>
<th>S25</th>
<th>S45</th>
<th>S25+P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fish meal</td>
<td>70</td>
<td>55</td>
<td>45</td>
<td>25</td>
<td>45</td>
</tr>
<tr>
<td>Soybean meal</td>
<td>15</td>
<td>25</td>
<td>45</td>
<td>25</td>
<td>25</td>
</tr>
<tr>
<td>Fish oil</td>
<td>10</td>
<td>10</td>
<td>10</td>
<td>10</td>
<td>10</td>
</tr>
<tr>
<td>( \alpha )-starch</td>
<td>10</td>
<td>10</td>
<td>10</td>
<td>10</td>
<td>10</td>
</tr>
<tr>
<td>Vitamin mixture*</td>
<td>5</td>
<td>5</td>
<td>5</td>
<td>5</td>
<td>5</td>
</tr>
<tr>
<td>Mineral mixture*</td>
<td>5</td>
<td>5</td>
<td>5</td>
<td>5</td>
<td>5</td>
</tr>
<tr>
<td>Phytase (FTU/kg)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>3,000</td>
</tr>
<tr>
<td>Proximate composition</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Crude Protein (%)</td>
<td>52.1</td>
<td>49.2</td>
<td>46.5</td>
<td>44.9</td>
<td>46.9</td>
</tr>
<tr>
<td>Crude fat (%)</td>
<td>15.8</td>
<td>15.3</td>
<td>14.4</td>
<td>13.2</td>
<td>14.2</td>
</tr>
<tr>
<td>Ash (%)</td>
<td>11.5</td>
<td>10.9</td>
<td>9.8</td>
<td>9.4</td>
<td>9.9</td>
</tr>
<tr>
<td>Phosphorus (%)</td>
<td>2.03</td>
<td>1.72</td>
<td>1.51</td>
<td>1.45</td>
<td>1.48</td>
</tr>
<tr>
<td>Gross energy (kJ/g)</td>
<td>20.3</td>
<td>20.2</td>
<td>20.3</td>
<td>20.0</td>
<td>20.2</td>
</tr>
</tbody>
</table>

*Halver (1957).
experimental diets, twice per day at 09:00 and 15:00, 6 days per week for 6 weeks. There was only one tank for each diet because of unavailable fecal collection column. An earlier study demonstrated that a single tank per treatment can be used to get reliable measurements for ADC (Biswas et al. 2007). Feces were collected for 3 days on 6th week. All possible care was taken during feeding when the feces were sampled so that no uneaten food settled on the tank bottom. Fecal samples were collected at 08:30 h before the first feeding, treated according to Bureau et al. (1999), and stored at -80°C for chemical analysis. At the end, fish were starved for 24 h and body length and weight were measured.

**Calculation and chemical analyses**

The data obtained was analyzed for the ADC of protein, lipid, energy and phosphorus, phosphorus retention efficiency, and phosphorus discharge. For energy determination, the initial and final whole body energy concentrations of fish were determined directly using an automated oxygen bomb calorimeter (IKA JAPAN Y. K., Nara, Japan). The minced whole body samples were freeze-dried for 24 h and used to determine the energy concentration, which was converted to calculate the whole body energy concentration on a wet weight basis (EC\text{wet}) using the following equation: 

\[
EC_{\text{wet}} (\text{J/g}) = \frac{[\text{freeze-dried sample weight (g)} \times \text{energy concentration in dry basis (J/g)}]}{[\text{samples weight before freeze drying (g)}]}
\]

Samples (diets, fish and feces) were analyzed for dry matter, protein and ash using AOAC method (AOAC 1995). Crude lipid was determined using a chloroform-methanol extraction procedure (Folch et al. 1957). Chromic oxide in the diet and feces was determined by a wet-acid digestion method (Furukawa and Tsukahara 1966). Phosphorus content of diet, fish whole body and feces was determined using the ammonium-molybdate method described by Baginski et al. (1982) after the digestion of samples with nitric and perchloric acids. All chemical analyses were performed in duplicate.

**Statistical analyses**

All statistical analyses were carried out using the SPSS program for Windows (v. 10.0). There was only one value for each treatment at each sampling point, making post hoc tests impossible. For the post hoc test, all chemical analyses at each sampling point were performed in duplicate, which were considered as two cases per group for the post hoc test. For ADC, three data for feces from 3-days samples during the 6th week were considered as one set of data for each group when the variation was compared among treatments. Data were expressed as the mean ± S.D. The means among treatments were compared using Tukey’s test of multiple comparison with a 95% significance level.

**Results**

There was no significant difference in ADC of lipid, but significant differences were existed in protein, energy and phosphorus among the treatments (Table 2). Fish fed with S25 + P showed significantly higher ADCs of protein and energy than those fed with S25. Phosphorus ADC was significantly higher in fish fed with S25 + P than those fed with other diets (P < 0.05). Similarly, fish fed with S25 + P showed significantly higher phosphorus retention efficiency than those fed with other diets (P < 0.05, Table 2). Inversely, fish fed with S25 + P showed remarkably lower phosphorus discharge than those fed with other diets (P < 0.05, Fig. 2).

Although fish meal replacement reduced the final body weight in fish fed with S25 and S45, phytase supplementation to a soybean meal diet (S25 + P) increased the final body weight comparable to those fed with diet F (Table 2). Daily feeding rate was reduced remarkably when fish fed with S45; however, there was no major variation in feeding rates among other treatments.

**Discussion**

Although fish meal replacement with soybean meal resulted in significantly lower ADC of protein and energy, phytase supplementation in diet S25 + P significantly improved the
Table 2. Apparent digestibility and retention efficiencies in fish under different treatments

<table>
<thead>
<tr>
<th>Parameters†</th>
<th>F</th>
<th>S15</th>
<th>S25</th>
<th>S45</th>
<th>S25+P</th>
</tr>
</thead>
</table>
| Apparent digestibility coefficient (%)  
Protein              | 96.6 ± 0.9a| 95.1 ± 1.0ab| 92.4 ± 0.5b| 91.8 ± 0.8b| 96.4 ± 1.0a |
| Lipid               | 95.3 ± 0.5 | 95.4 ± 0.8 | 94.1 ± 0.6 | 93.8 ± 0.9 | 95.0 ± 0.6 |
| Energy              | 88.7 ± 0.2a| 88.1 ± 0.8ab| 85.9 ± 0.7b| 86.0 ± 0.5b| 88.7 ± 0.3a |
| Phosphorus          | 61.7 ± 0.6b| 56.2 ± 0.4c | 52.9 ± 1.2ed| 48.7 ± 1.5d| 75.6 ± 1.14a|
| Retention efficiency (%)  
Phosphorus            | 25.4 ± 0.4b| 24.5 ± 0.6b | 24.2 ± 1.3b| 23.1 ± 0.6b| 31.6 ± 0.7a |

1 Values in a row with different letters are significantly different (P<0.05).
2 Apparent digestibility coefficient (%) = 100 × [dietary Cr2O3/fecal Cr2O3] × [fecal nutrients or energy/dietary nutrients or energy].
3 Phosphorus retention efficiency (%) = 100 × (final whole body phosphorus - initial whole body phosphorus)/total phosphorus intake.
4 Feeding rate (g/day/100g fish) = 100 × [total feed intake (g) / {number of day × average of initial and final body weight (g) × average of initial and final survival fish}].

Fig. 2. Phosphorus discharge in red sea bream fed with experimental diets. Statistical analysis could not be performed because of a single value for each treatment. [Phosphorus discharge (g P/kg weight gain) = (phosphorus fed (g) - phosphorus deposited (g))/weight gain (kg)].

Although fish meal replacement significantly reduced the ADC and retention efficiency of phosphorus in fish fed with S25, both parameters were increased significantly by 22.7 and 7.4%, respectively, in fish fed with S25 + P. This may be attributed to the activity of phytase to dephosphorylate the phytic acid and phytate phosphorus to increase the phosphorus availability (Rodehutscord and Pfeffer 1995; Lanari et al. 1998; Storebakken et al. 1998). This resulted in higher ADC and retention efficiency of phosphorus in diet S25 + P, which is consistent with the findings in other studies (Rodehutscord and Pfeffer 1995; Jackson et al. 1996; Storebakken et al. 1998; Papatryphon et al. 1999; Sugiura et al. 2001; Vielma et al. 2002; Cheng and Hardy 2003; Sajjadi and Carter 2004; Yoo et al. 2005).

Phosphorus discharge in the environment is of increasing concern in aquaculture as it is the most important pollution source. Therefore, reducing phosphorus discharge is a critical factor in reducing environmental pollution from commercial fish production. As mentioned earlier, phytase supplementation in S25 + P significantly improved the phosphorus digestibility and retention efficiency which resulted in lower phosphorus discharge in the environment. In this study, phosphorus discharges in F and S25 were 36.2 and 24.0%, respectively, higher than that of S25 + P. The result of reduced phosphorus discharge from diet with phytase supplementation is agreed with the findings in other...
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studies (Lanari et al. 1998; Forster et al. 1999; Vielma et al. 2002; Sajjadi and Carter 2004), although the overall phosphorus discharge is higher in this study. This is mainly attributed to the difference in phosphorus content in the test diets used in different studies as phosphorus discharge is affected by factors like feed conversion ratio, ingredient digestibility and dietary phosphorus content. However, using a commercial diet with 18.1 g phosphorus/kg in rainbow trout diet, Satoh et al. (2003) demonstrated phosphorus discharge as high as 17.3 g/kg weight gain, which is comparable with the value observed in this study.

The lower ADC of protein and energy which resulted in lower feed consumption and consequently depressed the final weight in red sea bream fed with S25 and S45. Similar results were demonstrated in rainbow trout where the growth and feed utilization were reduced when fed diets with high levels of soybean meal because the digestible protein, carbohydrate and energy were too low (Rumsey et al. 1994; Stickney et al. 1996). On the contrary, it has been demonstrated that dietary fish meal levels can be considerably replaced by soybean meal without any adverse consequence in terms of somatic growth or nutrient utilization (Kaushik et al. 1995, 2004; Muzinic et al. 2004).

In this study, ADC of protein and energy were reduced by only 1.3 to 4.6% and 0.6 to 2.8%, respectively, in fish fed with S15, S25 and S45 compared with fish fed with F and S25 + P. Whereas, reduction in feed consumption, which was calculated by deducting the feed consumption in S15, S25 and S45 from those of F and S25 + P and converted into reduction in percentage, was as high as 1.5 to 15.6% in fish fed with S15, S25 and S45 compared with fish fed with F and S25 + P. One of the reasons of higher reduction in feed consumption than ADC of protein and energy in soybean meal fed groups may be attributed to the fact that red sea bream might be taking long time to digest properly the soybean meal diet. This was, in turn, resulted in higher digestibility of protein and energy but reduced feed consumption because the feed remain in the digest organs for long time. The similar final body weight and ADC of ingredients between S25 + P and F suggest that about 25% fish meal can be replaced by soybean meal without adverse consequences in red sea bream when phytase is supplemented. Similar results were observed in other studies where fish were fed either phytase-supplemented diets (Rodehutscord and Pfeffer 1995; Jackson et al. 1996; Papatryphon et al. 1999) or phytase pretreated ingredients (Cain and Garling 1995; Vielma et al. 2002).

In conclusion, although soybean meal reduced the ADC of nutrients and energy, phytase supplementation can be used for partial replacement of fish meal by soybean meal without compromising the ADC of nutrients and energy and final body weight in red sea bream. These findings also indicate that the phytase supplementation in soybean meal beneficially lowers the phosphorus discharge from red sea bream diets. The results therefore suggest that significant economical and ecological benefits could be achieved by supplying phytase in soybean meal for red sea bream, Pagrus major.

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References


**マダイの消化吸収に及ぼす飼料大豆粕とフィターゼの影響**

A. K. Biswas・池 承哲・瀬岡 学・渋井健二

大豆粕による魚粉代替とフィターゼ添加が消化吸収に及ぼす影響をマダイ（平均体重78 g）で検討した。魚粉（FM）70%（F）、FM55%+大豆粕（SM）15%（S15）、FM45%+SM25%（S25）、FM25%+SM45%（S45）およびS25+3000TFU フィターゼ（S25+P）の各飼料を、6週間給与して水温21℃で飼育した。SM 配合量が増加するのに伴って、タンパク質・エネルギー消化率とともに飼料量と飼育成績が低下した。しかし、S25+P 区の消化率、飼料量、終了時魚体重はF区に近いレベルにまで改善し、飼育水へのリン負荷量は他区より有意に低下した （P<0.05）。このように、FM のSM への代替は栄養素やエネルギーの消化率を低下させるが、フィターゼの添加で消化率とともにリン負荷量を改善できることが示唆された。