Evaluation of gastric evacuation and digestion of extruded pellet composed of a single protein source for juvenile red seabream Pagrus major

Ken Touhata¹*, Tomohiko Koshiishi², Shuichi Satoh³ and Noriko Ishida¹

Abstract: In order to characterize the properties of protein sources, we prepared extruded pellets (EPs) which were composed of only a single protein source: fish meal (FM), pork meal (PM), blood meal (BM), corn meal (CM), soybean protein concentrate (SP), or soybean meal (SM), evaluated the gastric evacuation rate (GER) of red seabream Pagrus major juveniles, and determined the degree of undigested protein of the test EPs with gut enzyme extract. GER was highest in SM diet among the test EPs in order SM>BMI>CM>FM>PM diets, and SP diet tended to have a high GER value, suggesting that the soybean protein advances gastric evacuation. BM and SM diets had a low degree of digestion with gut enzyme extract, and this was due to their inhibitory activities. These diets had higher GER, suggesting that a high amount of indigestible protein in the diet may promote gastric evacuation. The concentration of triglycerides and free amino acids in serum of red seabream fed test EPs was measured as an index of absorption of dietary nutrients. The triglyceride and free amino acid levels showed different patterns depending on the protein source. These results suggest that the protein sources influence protein and lipid digestion and absorption.

Key words: Pagrus major; Extruded pellet; Substitute protein source; Gastric evacuation rate

Fish meal is an important feed source for use in aquaculture diets because of its high nutritive value and palatability. Recently, increasing demand for making fish feeds for aquaculture has caused a remarkable rise in the international fish meal price, leading to an increasing demand for new diets containing low or no fish meal. There are many studies on partial substitution of fish meal. Utilization of a protein source derived from plants, for example corn meal, soybean meal and soybean protein concentrate, and other animal proteins have been examined in several fish species (Viyakarn et al. 1992; Watanabe et al. 1992; Pongmaneerat and Watanabe 1993a; Pongmaneerat and Watanabe 1993b; Watanabe and Pongmaneerat 1993; Aoki et al. 1996; Aoki et al. 1997; Watanabe et al. 1998; Aoki et al. 2000; Yamamoto et al. 2007). Mostly only low levels of plant protein can be substituted for fish meal without decreasing the growth performance of fish, and higher inclusions of plant proteins reduce growth (Pongmaneerat and Watanabe 1992; Watanabe et al. 1992; Pongmaneerat et al. 1993; Watanabe et al. 1998).

One of the reasons for the low growth performance is considered to be induced by the low diet intake. In fact, increasing the plant protein source content is reported to reduce the palatability resulting in low growth (Pongmaneerat 1993b; Watanabe and Pongmaneerat 1993).
and Watanabe 1993b; Watanabe et al. 1998; Supraydi et al. 1999). The other cause of the low diet intake has been considered to be slow recovery of appetite after prolonged retention of diet in the stomach. There is a close relationship between appetite revival and gastric evacuation times (Grove et al. 1985; Tekinay and Güner 2001). So, slow gastric evacuation of a diet causes slowly appetite recovery resulting in low diet intake. There are many reports that gastric evacuation of natural prey in fish are estimated and the gastric evacuation rate (GER) depends on temperature, prey species and friability of meals (Amundsen and Klemetsen 1988; Bromley 1988; Hopkins and Larson 1990; Andrade et al. 1996; Hayward and Weiland 1998; Temming et al. 2002; Kawaguchi et al. 2007; Bernreuther et al. 2009). A cultured fish feed formulated diets, and there are also reports in formulated diet and the GER depends on temperature, fish body weight, and meal size (Basimi and Grove 1985; Grove et al. 1985; Du et al. 2009; Azaza et al. 2010). The formulated diets often consist of several protein sources. In mammals, protein source in the diet is reported to influence the GER (Hara et al. 1992; Nishi et al. 2003). The protein source should affect the GER as well as in fish. But, there are few reports about the influence of the protein source to gastric evacuation in fish as far as we know. Another reasons for the low growth performance is undernutrition in the diet. The cause of undernutrition is deficiency of some essential amino acids (Ketola 1983; Watanabe et al. 1993) or containing anti-nutritional factors (Rumsey et al. 1995). For example, defatted soybean meal has a high trypsin inhibitor activity and a low protein digestibility, resulting that its feeding causes low growth in yellowtail (Shimeno et al. 1992).

The new type of dry pellet, extruded pellet (EP) is a porous diet which is prepared to become soft like a sponge by absorbing water. It has an advantage compared with moist pellet. The light weight of EP and stability of the feed quality improve the work of efficiency, and floating on water surface and suitable settleability reduce the amount of leftovers. In addition, EP has superior digestion and absorbency (Shimeno et al. 1997; Shimeno et al. 1999). So, EP is becoming a mainstream in aquaculture feed.

As the protein source in formulated diets involves food intake which is related to appetite recovery and in low growth performance with a low protein digestibility, it is necessary to characterize the properties of protein sources in EP. We prepared EPs which were composed of only a single protein source, and then evaluated the GER and determined the degree of digestion of the test EPs in gut enzyme extracts of juvenile of red seabream, Pagrus major. Furthermore, triglycerides and the free amino acids in the serum were measured after administration of the test EP as an index of absorption of protein and fat.

Materials and methods

The test EPs

Six test EPs were prepared as extruded pellets by Marubeni, Nisshin, Feed Co., Ltd. They were composed of only a single protein source, fish meal (FM), pork meal (PM), blood meal (BM), corn meal (CM), soybean protein concentrate (SP), or soybean meal (SM). The all ingredients were able to pass through 45 mesh screen. The composition of the test EPs and proximate compositions of the test EPs are listed in Table 1. Determination of the moisture content, crude protein content, crude fat content, and ash content of the test EPs were made by drying at 110°C, semi-micro Kjeldahl method (nitrogen-to-protein conversion factor, 6.25), hydrolysis and ethyl ether extraction method, and combustion at 600°C, respectively. As all test EPs were made on the same formulation, they were not formulated to be iso-nitrogenous or iso-caloric, thus the protein and lipid contents varied and were not the same in the test EPs.

Test fish

Juveniles of red seabream (body weight 82.0 ± 8.6 g) were obtained from a commercial supplier and transferred the National Research
Gastric evacuation and digestion of EP

Institute of Fisheries Science, Japan Fisheries Research and Education Agency (Yokohama, Kanagawa). The fish were initially stocked in an indoor 1 k\(l\) cylindrical tank supplied continuously with sand filtered seawater and fed a commercial diet at 20°C until the experiment. Photoperiod operated 12:12 hour light-dark cycles using an automatic timer and fluorescent lamps.

Gastric evacuation rate

Among the six test diets, two each of experiments was carried out in three times on different days. No diet was fed for 48 h to allow complete emptying of the fish stomach before the gastric evacuation experiments. The fish were anesthetized by MS222 (Acros Organics, USA, NJ) in 20 l tank and the same weight of the test EP (ca. 0.85 g/fish) was pushed into the fish stomach with a glass bar using minimum force, and then the fish was moved to a plastic tank (90 × 45 × 45 cm) started at 09:00, but in the case of the 16 h sampling groups, we performed the same procedure at 17:00 and then sampled the next morning (09:00). After 2, 4, 8, 16, and 24 h from the feeding, 4 fish were killed by pith using a needle as quickly as possible. Blood was taken using a heparinized syringe. The solid diets in the stomach were collected by forceps and residual diets were collected by gentle scraping with spatula. The collected gastric contents were pooled together and weighed. The quantity of gastric contents was converted from its crude protein content measured by the semi-micro Kjeldahl method. The residual diet was measured by percentile relative to the amount at feeding. Gastric evacuation was described using a linear model (Jones 1974; Olson and Boggs 1986; Zhang et al. 2000) and an exponential model (Persson 1982; Jobling 1987; Hossain et al. 2000). To compare GER among the test EPs, GER was calculated

Table 1. Composition of the diets used for administration to red seabream

<table>
<thead>
<tr>
<th>Ingredients (%)</th>
<th>Test diets</th>
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<tbody>
<tr>
<td></td>
<td>FM</td>
</tr>
<tr>
<td>Fish meal(^a)</td>
<td>70</td>
</tr>
<tr>
<td>Pork meal(^b)</td>
<td>0</td>
</tr>
<tr>
<td>Blood meal(^c)</td>
<td>0</td>
</tr>
<tr>
<td>Corn gluten meal(^d)</td>
<td>0</td>
</tr>
<tr>
<td>Soybean protein concentrate(^e)</td>
<td>0</td>
</tr>
<tr>
<td>Defatted soybean meal(^f)</td>
<td>0</td>
</tr>
<tr>
<td>Tapioca starch(^g)</td>
<td>20</td>
</tr>
<tr>
<td>Fish oil(^h)</td>
<td>10</td>
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Proximate composition (%)

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<tr>
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<tr>
<td>Moisture</td>
<td>5.6</td>
<td>4.0</td>
<td>4.4</td>
<td>5.1</td>
<td>5.8</td>
<td>3.1</td>
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<tr>
<td>Crude Protein</td>
<td>46.1</td>
<td>48.0</td>
<td>60.7</td>
<td>44.9</td>
<td>46.7</td>
<td>34.5</td>
</tr>
<tr>
<td>Crude lipid</td>
<td>15.4</td>
<td>15.1</td>
<td>9.9</td>
<td>18.4</td>
<td>13.7</td>
<td>14.3</td>
</tr>
<tr>
<td>Crude Ash</td>
<td>12.3</td>
<td>11.2</td>
<td>4.6</td>
<td>1.0</td>
<td>4.7</td>
<td>4.7</td>
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Diameter of diet (mm)\(^i\)

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<tr>
<td>5.2</td>
<td>3.9</td>
<td>5.1</td>
<td>5.2</td>
<td>4.4</td>
<td>4.2</td>
<td></td>
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</tbody>
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Number of diet particles (/fish)\(^j\)

<table>
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<tr>
<th></th>
<th>FM</th>
<th>PM</th>
<th>BM</th>
<th>CM</th>
<th>SP</th>
<th>SM</th>
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<tbody>
<tr>
<td>9-10</td>
<td>14-16</td>
<td>14-16</td>
<td>9-11</td>
<td>19-21</td>
<td>17-19</td>
<td></td>
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</table>

Swelling ratio at 8 h (%)\(^k\)

<table>
<thead>
<tr>
<th></th>
<th>FM</th>
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<th>BM</th>
<th>CM</th>
<th>SP</th>
<th>SM</th>
</tr>
</thead>
<tbody>
<tr>
<td>336.2</td>
<td>480.0</td>
<td>332.1</td>
<td>261.9</td>
<td>480.4</td>
<td>402.5</td>
<td></td>
</tr>
</tbody>
</table>

\(^a\)Fish meal was made from anchovy as the main raw material, imported from Peru.
\(^b\)Kagoshima Pure Foods Co., Ltd.
\(^c\)APC Japan Co., Ltd.
\(^d\)Japan Corn Starch Co., Ltd.
\(^e\)Soycomil; Archer Daniels Midland Japan Ltd.
\(^f\)Nisshin Oillio Co., Ltd.
\(^g\)Sanguan Wongse Industries Co., Ltd.
\(^h\)Tsuji Oil Mills Co., Ltd.
\(^i\)Mean value of particle size (diameter).
\(^j\)Particle number of test EP administrated to fish.
\(^k\)Swelling ratio=protein content of test EP (%) / protein content of gastric content at 8 h after feeding (%) × 100.
as linear model in the present study:

$$\text{GER} = - \frac{dW}{dT} \text{(% volume / hour)}$$

where \( W \) is the relative value of residual diet in the stomach and \( T \) (h) is a time after administration of the test EPs. GER means the percent decrease of the start amount of the test EPs per hour and the test EP with high value of GER evacuated from stomach quickly.

The degree of absorbance of the water in the test EPs was estimated as the swelling ratio as below.

Swelling ratio (%) = protein content (%) of the test EP / protein content (%) of gastric contents at 8 h after feeding \times 100

**Degree of undigested protein**

Estimates of the degree of digestion of the test EPs were performed by the method of Eid and Matty with some modification (Eid and Matty 1989). Four red seabream were killed at 2 h after feeding. The intestinal content was washed away with distilled water and washed intestines were homogenized with 10 times volume of 5 mM Tris-HCl buffer pH 8.0 using a Potter-Elvehjem homogenizer. The homogenate was centrifuged at 10,000 g for 15 min. The supernatant was filtrated with a 0.45 \( \mu \)m syringe filter to avoid contamination by microorganisms.

The minced test EPs, which contained 100 mg protein, were incubated with the enzyme solution in 20 ml of 0.1 M Tris-HCl buffer pH 9.5 at 30°C for 24 h. Enzyme blanks were prepared by incubation under the same condition without enzyme solution. After incubation, the mixtures were added 4 ml of 40% trichloroacetic acid (TCA), mixed well, and then kept for 15 min on ice. After centrifugation at 10,000 g for 10 min, the precipitates were collected by filtration and then washed with cold 5% TCA. The crude protein content of collected solids was measured described above.

**Inhibitor assay**

The test EPs were homogenized with 5 times volume of 5 mM Tris-HCl buffer pH 8.0 using a Polytron homogenizer. The homogenate was centrifuged at 10,000 g for 15 min. Thirty \( \mu \)l of the supernatant, 30 \( \mu \)l of 0.4 M Tris-HCl buffer pH 9.5, 30 \( \mu \)l of 20 mg/ml azocasein, and 30 \( \mu \)l of enzyme solution described above were mixed and incubated for 1 h at 30°C. After incubation, 120 \( \mu \)l of 10% TCA was added to the mixture, mixed well, kept for 15 min on ice, and then centrifuged at 10,000 g for 10 min. Zero time blanks were prepared without incubation. The supernatant was diluted with the same volume of 1 N NaOH and the absorbance was measured at 440 nm. Enzyme activity contained the supernatant was expressed as a percentage of the sample blank. Inhibitory activity was expressed as below.

Inhibitory activity (%) = 100 – enzyme activity (%) contained the supernatant

**Biochemical analysis of blood**

Plasma triglyceride (TG) was measured with triglyceride E-test Wako (Wako Pure Chemical Industries, Ltd., Osaka, Japan). Plasma free amino acid (FAA) content was measured as amino groups by the TNBS (2, 4, 6-trinitrobenzenesulfonic acid; Wako Pure Chemical Industries, Ltd., Osaka, Japan) method (Fields 1972).

**Results**

**The test EPs**

The test EPs were prepared with only one protein source in the same content. The particle size of test EP was varied, so the number of EP administrated to fish was different among test EPs (Table 1). The protein contents of FM, PM, SP, and CM diets were approximately constant regardless of the source. On the other hand, protein contents of BM and SM were higher and lower than those of the other the test EPs, respectively. Because of the different sources of the test EPs, their physical properties, size and swelling, varied (Table 1). EP swells by absorbing water and then softens in the fish stomach. The swelling ratio at 8 h after feeding varied among the test EPs. The value of SP was highest among the test EPs and value of PM was almost same in order SP \( \geq \) PM > SM > FM > BM > CM.
Gastric evacuation and digestion of EP diets (Table 1). Because the degree to absorb water varied among the test EPs, the residual diets in stomach were calculated from the protein content instead of its weight (wet).

**Gastric evacuation rate**

To investigate the effect of particle size of the test EPs to GER, the fish under anesthetic were administered the different particle sizes (ca. 5 and 8 mm) of FM diet. As a result, there was no significant difference between two different particle sizes of the test EP (3.64 and 3.51, respectively). Because the fish did not intake the test EPs, the pellets were pushed into the stomach with a glass bar under anesthesia. As many fish in 24 h has no remaining diet in their stomachs, GER was calculated from 2 h to 16 h of gastric content after administration. Among the test EPs, the linear model was well fitted in the case of FM, SP, BM and PM from the conventional $r^2$ values. On the other hand, exponential model was fitted in the case of SM and CM. To compare GER among the test EPs, GER was calculated as absolute value of the slope of regression line (linear model). The higher value of GER means faster evacuation of diet from the stomach. The GER value was highest in SM diet and ranged between 5.80 and 3.30 in order SM-BM-SP-CM-FM-PM diets (Fig. 1 and Table 2). GER value of SP diet was higher than those of FM and PM diets, indicating that GER of EP derived from soybean has a tendency for higher value. The feces of the fish given BM were apparently diarrhea states and the diet transported into intestinal tract as not chyme like but pellet in some fish (data not shown).

**Degree of undigested protein**

For estimation of the ease of digestion of the test EPs, the amount of undigested protein by gut enzyme extract was measured. As a result, the amount of undigested protein was decreasing in order BM-SM-CM-FM-SP-PM diets (Fig. 2). The degree of digestion of BM was remarkably lowest among all test EPs. On the other hand, PM diet was best digested among

<table>
<thead>
<tr>
<th>Gastric evacuation rate (Vol%/h)</th>
<th>FM</th>
<th>PM</th>
<th>BM</th>
<th>CM</th>
<th>SP</th>
<th>SM</th>
</tr>
</thead>
<tbody>
<tr>
<td>FM</td>
<td>3.43</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PM</td>
<td>3.30</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>BM</td>
<td>5.47</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CM</td>
<td>4.05</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>SP</td>
<td>5.24</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SM</td>
<td>5.80</td>
<td></td>
<td></td>
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</tbody>
</table>

**Fig. 1.** Change in gastric content of red seabream. Solid lines and dotted lines indicate regression line from 2 h to 16 h after pushing the EPs into the stomach. (A) The test EPs derived from animal proteins. Samples are identified by the following symbols: ○, fish meal EP; ●, pork meal EP; □, blood meal EP. (B) The test EPs derived from plant proteins. Samples are identified by the following symbols: ○, corn meal EP; ●, soybean protein concentrate EP; □, soybean meal EP.

**Fig. 2.** Degree of undigested protein of the test EPs. Solid, light shadowed and dark shadowed boxes represent extract, digested and undigested fractions, respectively. FM, fish meal EP; PM, pork meal EP; BM, blood meal EP; CM, corn meal EP; SP, soybean protein concentrate EP; SM, soybean meal EP.
the test EPs, and SP diet was digested to a similar degree as PM diet.

**Inhibitory assay**

To investigate the inhibitory effects in the test EPs, we performed inhibitor assays of extracts of the test EPs by using gut enzyme extract. As a result, BM and SM diets had an inhibitory activity, and SP diet also had a slight effect (Fig. 3). On the other hand, there were no inhibitory activities in the other test EPs.

**Biochemical analysis of blood**

The concentration of triglyceride and free amino acid in serum was measured as an index of digestion and absorption of dietary nutrients. The results of plasma TG concentration are shown in Fig. 4. The peak of TG in FM, SP, and SM diets was 16 hours after administration. In contrast, the concentration in PM, BM, and CM diets increased until 24 hours after administration. Thus, the maximum time of TG was different among EPs. In addition, the level of TG was also different among EPs. The maximum TG level of CM was lower than that of SM, SP, FM and PM diets, although the CM diet had the highest lipid content among the test EPs.

The results of plasma FAA concentration are shown in Fig. 5. As well as in TG, the change in FAA level was different among the test EPs. In SM and SP, which were made from soybean, FAA level showed a maximum in 8 h after administration, and formed a clear peak. On the other hand, FAA levels of other EPs gradually increased from 2 h later.

![Fig. 3. Inhibitory activity in extracts of the test EPs. STI indicates sample blank and soybean trypsin inhibitor, respectively. FM, fish meal EP; PM, pork meal EP; BM, blood meal EP; CM, corn meal EP; SP, soybean protein concentrate EP; SM, soybean meal EP.](image1)

![Fig. 4. Change in triglyceride level of red seabream serum. (A) The test EPs derived from animal proteins. Symbols are the same as Fig. 1 identified by the following symbols: ○, fish meal EP; ●, pork meal EP; □, blood meal EP. (B) The test EPs derived from plant proteins. ○, corn meal EP; ●, soybean protein concentrate EP; □, soybean meal EP. Vertical bar denotes standard deviation. Means with the same superscripts in each group are not significantly different (Tukey-Kramer test, \( P < 0.05 \)).](image2)

![Fig. 5. Change in amino acid level of red seabream serum. (A) The test EPs derived from animal proteins. Symbols are the same as Fig. 1 identified by the following symbols: ○, fish meal EP; ●, pork meal EP; □, blood meal EP. (B) The test EPs derived from plant proteins. ○, corn meal EP; ●, soybean protein concentrate EP; □, soybean meal EP. Vertical bar denotes standard deviation. Means with the same superscripts in each group are not significantly different (Tukey-Kramer test, \( P < 0.05 \)).](image3)
Discussion

In the present study, GER varied among the test EPs, resulting in the protein source influencing gastric evacuation. SM and SP diets, which are derived from soybean, had a tendency of higher GER, suggesting that soybean protein advances gastric evacuation compared with fish meal and pork meal. As well as in mammals, it is reported that protein source influences the GER (Hara et al. 1992; Nishi et al. 2003). As appetite revival is closely related to gastric evacuation times (Grove et al. 1985; Tekinay and Güner 2001), the fish which fed on the soybean derived diets were found to have fast appetite recovery. It is assumed that the low growth performance in feeding soybean derived diets is not due to the loss of appetite by the full stomach.

Physical property of diet must influence gastric evacuation. Hopkins and Larson (1990) reported that friability, the ease of fragmentation of diets in the stomach, may be an important factor in determining evacuation patterns. Shimeno et al. (Shimeno et al. 1993) suggested that the formulated diet evacuates from stomach immediately when it became chyme. It is assumed that the diets absorbing water well is easy to become chyme. So, the swelling ratio was estimated as an index of the degree of water absorption. In the present study, SP diet had a high value of swelling ratio and evacuated from stomach rapidly, but PM diet was low GER in spite of the high degree of water absorption. This result suggests that the degree of water absorption does not affect the gastric evacuation in the case of EP.

The undigested protein level of BM diet was highest among the test EPs (Fig. 2). This is due to inhibitory activity. In addition, denatured protein in BM may have resistance for protease digestion. In fact, the feces of the fish given BM diet were apparently diarrhea states and the diet transported into intestinal tract as not chyme like but pellet in some fish. So, the diet which is mainly composed of BM is not suitable for fish culture. SM and SP diets have a tendency for a higher GER (Table 2). GER of SM diet was higher than that of SP and the amount of undigested protein of SM diet by the treatment with gut enzyme extract was higher than that of SP diet (Fig. 3). BM diet had also higher GER and was indigestible (Fig. 3 and Table 2). These results suggest that high amounts of indigestible protein in the diet may promote gastric evacuation.

The peak of TG in FM, SP and SM diets was 16 hours after administration. On the other hand, the TG level of PM, BM, and CM diets increased until 24 hours after administration. The peak of plasma TG concentration was different among the test EPs. This result suggests that the raw protein source in the diet influences lipid digestion and absorption in fish.

The FAA levels in plasma of SM and SP diets showed earlier peaks than those of other EPs. In mammals, the peak time of FAA level is reported to be different among protein sources (Boirie et al. 1997; Bos et al. 2003). Bos et al. (2003) reported that the peaks of FAA concentrations in soy protein meal occur earlier than those in milk protein meal. It is suggested that the protein source influences the protein digestion and absorption in fish as well as mammals.

In the present study, the GER and the degree of digestion varied among the test EPs which were composed of only a single protein source. The EPs derived from soybean have the ability to promote gastric evacuation and suggest recovering appetite earlier than the FM and PM diets. By composing of several protein sources, it would be able to make a diet which have high degree of digestion and recover appetite for fish rapidly, and thereby promote the growth of fish.

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References


Pongmaneerat, J., T. Watanabe, T. Takeuchi and S. Satoh


