Soybean Trypsin Inhibitors Inhibit Trypsin-like and Basic Proteinase Activities of Cultured-Fishes

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Soybean trypsin inhibitors (SBTI) inhibited trypsin and basic proteinase in the hapatopancreas, pyloric ceaca and intestine of cultured-marine and freshwater fishes. The highest trypsin inhibition by the SBTI was obtained in tiger puffer Takifugu rubripes, followed by rainbow trout Oncorhynchus mykiss, striped jack Pseudocaranx delicatissimus, red sea bream Pagrus major, Japanese striped knifejaw Oplegnathus fasciatus, grouper Epinephelus septemfasciatus, bluefin tuna Thunnus thynnus, purplish amberjack Seriola dumerili, yellowtail Seriola quinqueradiata. The highest basic proteinase inhibition by the SBTI was obtained in red sea bream, followed by rainbow trout, grouper, purplish amberjack, tiger puffer, Japanese striped knifejaw, bluefin tuna, striped jack, yellowtail. These results suggest that the dietary soybean meal utility, being somewhat higher in a rainbow trout, red sea bream, and tiger puffer than yellowtail, do not actually correlate to their sensitivity of trypsin-like and basic proteinase for SBTI.

Key words: soybean trypsin inhibitor, trypsin, basic proteinase, bluefin tuna, marine fish, rainbow trout

Studies on alternative proteins for dietary fish meal have promptly been conducted on several freshwater and marine cultured-fishes in this decade.1-9) These recommended soybean meal (SBM) as a more reasonable protein than several other plant proteins. The main reasons of higher dietary SBM utilization for several cultured-fishes have been considered its high protein contents, favorable balance of essential amino acids, and economical price.10) Otherwise, SBM contains some antinutritional factors, such as soybean trypsin inhibitors (SBTI), phytate, lectins, antigenic and estrogenic flavons, oligosaccharides, etc.11) Among these antinutritional factors, it is well known that the SBTI cause digestion and growth depression in mammals12) and fishes.12)

The SBTI consist of two types: Kunitz and Bowman-Birk inhibitors. The Kunitz inhibitor has a molecular weight between 20 K and 25 K and is the least heat and acid stable. The Bowman-Birk inhibitor has a molecular weight of about 8 K and a stable conformation even after disulfide bonds are broken by heating. The inhibitors inhibit trypsin as well as chymotrypsin.11) Krogdahl and Holm13) indicated that animals might be ranked as follows according to the sensitivity of caseinolytic activity in the pancreatic tissue for SBTI: trout Oncorhynchus mykiss > fox, chicken > pig > rat, cow > mink > man. They suggested from this result that a relatively low dietary SBM utility in rainbow trout resulted in their high sensitivities to the caseinolytic activity of SBTI.

The present study was conducted to identify the relationship between the sensitivities of trypsin-like and basic proteinase for SBTI and the dietary utility of SBM in several cultured-fishes. Higher or equivalent dietary utility of SBM appeared in rainbow trout, red sea bream Pagrus major, and tiger puffer Takifugu rubripes as compared with yellowtail Seriola quinqueradiata.13-14)

Materials and Methods

Fish and Enzyme Preparation

Details of fishes used in the present study appear in Table 1. Bluefin tuna Thunnus thynnus had been fed on raw fish, sardine Sardinops melanostictus and mackerel Scomber japonicus. Grouper Epinephelus septemfasciatus, Japanese striped knifejaw Oplegnathus fasciatus, purplish amberjack Seriola dumerili, rainbow trout, red sea bream, striped jack Pseudocaranx delicatissimus, tiger puffer, and yellowtail had been fed on artificial diets commercially available. Three fish in each fish-species were fasted for a day before the preparation of digestive enzyme solution. The marine fishes had been reared in net pens at water temperatures between 16 and 20°C, but freshwater rainbow trout had been reared in outdoor tanks at 11°C. The pyloric ceaca and intestine, which were obtained from bluefin tuna, grouper, Japanese striped knifejaw,
Table 1. Body weight, fork length, feed, and rearing water temperature of fishes used in the present study

<table>
<thead>
<tr>
<th>Species (Common name)</th>
<th>Body weight (g)</th>
<th>Fork length (cm)</th>
<th>Feed</th>
<th>Water temperature (°C)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Thunnus thynnus</em></td>
<td>37,400±710</td>
<td>117±3.80</td>
<td>RF*</td>
<td>19.6</td>
</tr>
<tr>
<td>(Bluefin tuna)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Epinephelus septem/fasciatus</em> (Grouper)</td>
<td>1,990±226</td>
<td>48.8±0.4</td>
<td>AD*</td>
<td>15.8</td>
</tr>
<tr>
<td><em>Oplegnathus fasciatus</em> (Japanese striped knifejaw)</td>
<td>130±4.10</td>
<td>18.3±0.1</td>
<td>AD</td>
<td>17.9</td>
</tr>
<tr>
<td><em>Seriola dumerili</em> (Purplish amberjack)</td>
<td>439±14.7</td>
<td>29.2±0.9</td>
<td>AD</td>
<td>18.0</td>
</tr>
<tr>
<td><em>Oncohysthus mykiss</em> (Rainbow trout)</td>
<td>1,120±252</td>
<td>43.5±0.4</td>
<td>AD</td>
<td>10.8</td>
</tr>
<tr>
<td><em>Piaractus major</em> (Red sea bream)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0*-year</td>
<td>74.6±1.20</td>
<td>14.6±0.3</td>
<td>AD</td>
<td>18.1</td>
</tr>
<tr>
<td>1*-year</td>
<td>919±13.5</td>
<td>35.5±0.6</td>
<td>AD</td>
<td>18.6</td>
</tr>
<tr>
<td>2*-year</td>
<td>1,460±50.0</td>
<td>40.8±3.3</td>
<td>AD</td>
<td>18.1</td>
</tr>
<tr>
<td><em>Pseudocaranx delicatissimus</em> (Striped jack)</td>
<td>295±1.50</td>
<td>25.1±0.5</td>
<td>AD</td>
<td>17.8</td>
</tr>
<tr>
<td><em>Takifugu rubripes</em> (Tiger puffer)</td>
<td>245±12.8</td>
<td>23.7±0.2</td>
<td>AD</td>
<td>17.9</td>
</tr>
<tr>
<td><em>Seriola quinququeradiata</em> (Yellowtail)</td>
<td>780±116</td>
<td>36.2±2.2</td>
<td>AD</td>
<td>16.2</td>
</tr>
</tbody>
</table>

* RF: raw fish and AD: artificial diet commercially available.

pulplish amberjack, rainbow trout, striped jack, and yellowtail, were inclusively homogenized using a glass homogenizer with iced deionized water. The hepatopancreas and the intestine of red sea bream and tiger puffer were also homogenized by the same method cited above. The homogenate was centrifuged at 10,000 ×g, at 4°C, for ten min. Resulting supernatant as enzyme solution was immediately frozen by liquid nitrogen and stored in a deep freezer at −80°C until assays. The activation of enzyme was conducted to incubate enzyme solution at 37°C for 60 min, immediately before assays. Porcine trypsin as a standard and SBTI from soybean were purchased from Wako Purechemicals (Osaka, Japan).

**Assays of SBTI Inhibitory Activities for Trypsin-like and Basic Proteinase**

The inhibitory activities of SBTI for trypsin-like (EC 3.4.21.4) and basic proteinase were assayed by the method of Kakade et al. and casein-Folin method, respectively. The inhibition of SBTI was done using an enzyme solution added to various levels of SBTI at 37°C for ten min. The substrates of trypsin-like and basic proteinase were N-benzoyl-L-arginine-p-nitro-anilide (BAPA) and casein, respectively.

The reaction of SBTI inhibition for trypsin-like proteinase was conducted at pH 8.2 and 37°C for ten min. The inhibitory activities of SBTI for trypsin-like proteinase, after confirming a linear dose response of SBTI, were expressed as trypsin inhibitory units (TIU)/mg SBTI. One TIU was defined as decreasing optical density of 0.01/tube under the assay condition.

**Statistical Analysis**

Data were analyzed by one-way ANOVA; if this was significant (p<0.05), differences between means were identified by Duncan’s multiple range test (p<0.05).

**Results**

**Inhibition for Trypsin-like Proteinase**

The inhibition of SBTI for trypsin-like protease of the fishes is shown in Fig. 1. The inhibitory activity of SBTI used in the present study was 6,700 TIU/mg SBTI for porcine trypsin as a standard. The highest inhibitory activity of 31,200 TIU/mg SBTI was obtained in tiger puffer, followed by 20,000 TIU/mg SBTI in rainbow trout, striped jack, 0*-year, and 2*-year red sea bream, 16,000 TIU/mg SBTI in Japanese striped knifejaw, and 12,000 TIU/mg SBTI in grouper, 1*-year red sea bream and bluefin tuna.

![Fig. 1. Inhibition of SBTI for trypsin-like enzyme of fishes.](image-url)
The inhibitory activity of SBTI for basic proteinase was obtained in purplish amberjack and yellowtail.

**Inhibition for Basic Proteinase**

The inhibition of SBTI for basic proteinase of the fishes is shown in Fig. 2. Some differences between the inhibition orders were found at each SBTI concentration. The highest inhibitory activity of 210,000 IU was detected in purplish amberjack and yellowtail.

The lowest activity about 8,000 TIU/mg SBTI was obtained in purplish amberjack and yellowtail.

**Discussion**

In the present study, SBTI was purchased from the Wako Purechemicals and came with no information about its purity and ratio of Kunitz to Bowman-Birk inhibitors. The SBTI were extracted and purified from soybean. It is suggested that, judging from growth performance, optimum substitutive levels of SBM for dietary brown fish meal were somewhat higher in rainbow trout, red sea bream, and tiger puffer than yellowtail, or of no conspicuous differences among these fishes. This discrepancy suggests the dietary utility of SBM in fishes is also affected by other antinutritional factors; phytate, lectin, antigenic and estrogenic flavons, oligosaccharides, etc, but not by only SBTI. Moreover, there is a possibility that fishes fed diets with some parts of fish meal substituted with SBM may adapt to promote their biosynthesis and secretion of SBTI-sensitive proteinase or SBTI-insensitive proteinase.

In the red sea bream, the sensitivities of trypsin and basic proteinase for SBTI differed among ages; 0+-, 1+-, and 2+-year fish. This also suggests that strains of red sea bream genetically have dissimilar enzymatic specificities of trypsin, chymotrypsin, and other basic proteinases, in each other. Or growth stages might shift the genotype of the proteinases. Further studies of the influence of dietary SBTI on digestion and absorption of red sea bream are now in progress.

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**References**

