Evaluation of Dietary Yeast Autolysates for Red Sea Bream, *Pagrus major*

Kenji Takii¹, Shin-ichi Akiyama¹, Takashi Maōka², Kumi Hidaka², Kodo Otaka², Manabu Seoka¹ and Hidemi Kumai¹

Abstract: Nutritive values of yeast autolysates, *Candida utilis* (CA), *Hansenula anomala* (HA) and *Rhodotorula glutinis* (RA), and their mixtures, *C. utilis + R. glutinis* (CRA), *H. anomala + R. glutinis* (HRA) and *C. utilis + H. anomala* (CHA) as a single cell protein (SCP), were evaluated by substituting near half of fish meal in a diet for red sea bream, *Pagrus major*. Apparent protein digestibilities of diets including CA, HA, CRA and CHA were similar to a control fish meal diet, but those of diets including RA and HRA were significantly lower than the control diet. Apparent lipid digestibilities of diets including CA, HA and RA, apparent sugar and energy digestibilities of diets excepting the diet including CHA were significantly lower than the control diet. Fish given diets including CRA and HRA maintained higher daily intake but lower feed efficiency, protein efficiency ratio, energy efficiency and apparent protein and lipid retentions than fish given the control diet. Therefore, the CHA was evaluated as useful dietary SCP for red sea bream, due to comparable weight gain, growth performance and digestibility to fish given the control diet.

Key words: Red sea bream; Yeast autolysate; Dietary protein; Digestibility

Previous studies showed that red sea bream *Pagrus major* had some differences in feeding preference and nutritional performance for various dietary yeasts isolated from mandarin fields in Wakayama and Kyoto, Japan (Takii et al. 1999; Akiyama et al. 2001a, b). The yeasts did not remarkably differ in protein levels and essential amino acid contents and balances. Therefore, the differences of nutritional performance might result from other factors, such as low digestibility attributed to structural polysaccharides, chitin, β-glucan and β-mannan, and their mutual-complex constructions of cell wall (Robinson 1987; Gallaher and Schneeman 1996; Horiuchi 2002). Cell wall degradation has some possibility for improving yeast utility, as a dietary single cell protein (SCP) for the bream. Meanwhile, the digestive and absorptive functions of the bream with a stomach are more active than those of tiger puffer *Takifugu rubripes* without a stomach (Takii et al. 1997a). Gastric function of fish, as land mammals, promotes not only protein but also lipid and sugar digestibilities, and shortens time necessary for nutrient absorption (Takii et al. 1997b). Whereas, the bream do not utilize well dietary starch and glucose as energy source and metabolites (Shimeno 1974; Furuichi 1983). Blood glucose level reached ca. 400 mg/100 ml at 2–3 h after the injection of 167 mg glucose/100 g body weight, being markedly higher and later than healthy human beings (Takii et al. 2000). It is very interesting to investigate the response of the bream having these physiological traits to dietary yeasts with cell wall degradation by autolysis.

Yeast cell wall degradation has ordinarily and experimentally been conducted using chemical treatments and exogenous digestive
enzymes (Gorin et al. 1969; Freimund et al. 2003). These are highly expensive and unpractical methods for the extensive use of animal husbandry and fish aquaculture. Otherwise, it was reported that the mixing of two yeast species, especially a combination with Rhodotorula glutinis synergistically autolyses themselves under an incubation at 45°C (Otaka et al. 2002). This approach is a reasonable and costless for degrading yeast cell wall and may improve nutritional value of the yeast on the bream, as well as other culture-fishes. The objective of the present study, therefore, was stressed to identify a proper yeast combination for conducing good digestibility and growth performance of the red sea bream.

Materials and Methods

Yeast autolysis and diets

Three species of yeast, Candida utilis, Hansenula anomala and Rhodotorula glutinis, were cultivated at 28°C with a medium containing citrus-molasses (Brix 12), ammonium chloride, potassium phosphate dibasic, potassium phosphate monobasic and yeast extract (pH 4.5) for 24 h or 48 h, cited previously (Takii et al. 1999). After collection of yeast cells by centrifugation, yeasts and their mixtures, C. utilis (CA), H. anomala (HA), R. glutinis (RA), C. utilis + H. anomala (CHA), C. utilis + R. glutinis (CRA), and H. anomala + R. glutinis (HRA), were autolyzed. The autolysis was carried out by incubation of 10% yeast(s) suspension at 45°C, pH 4 – 5 for 27 h (Otaka et al. 2002). During the incubation, the autolysis achievement was monitored by the absorbance decreasing at 660 nm and was expressed as its decreasing percentage. The yeast(s) suspension was boiled for 10 min, and lyophilized and then submitted to various trials. Moistures of lyophilized autolysates were under 8%.

Dietary formula for the estimation of apparent protein, lipid, sugar and energy digestibilities and the nutritional evaluation of diets including yeast autolysates are shown.

Table 1. Dietary formula used for digestibility of yeast autolysates (%)

<table>
<thead>
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<th>6</th>
<th>7</th>
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<td>-</td>
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<td>α-Potato starch</td>
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<td>3.5</td>
<td>3.5</td>
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<tr>
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<td>3</td>
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<td>-</td>
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Proximate composition

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<th>Crude lipid</th>
<th>Sugar</th>
<th>Crude ash</th>
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<td>18.8</td>
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<td>20.7</td>
<td>5.1</td>
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<td>50.1</td>
<td>14.0</td>
<td>16.8</td>
<td>5.2</td>
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<td>46.3</td>
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<td>22.0</td>
<td>5.7</td>
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<td>47.3</td>
<td>14.2</td>
<td>17.8</td>
<td>5.3</td>
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<tr>
<td>51.7</td>
<td>14.3</td>
<td>15.8</td>
<td>9.9</td>
<td></td>
</tr>
</tbody>
</table>

Gross energy*3

| kcal / 100 g | 499 | 482 | 481 | 476 | 474 | 474 | 462 | 479 |


*2 Halver’s mixtures (Halver 1957).

*3 kcal / 100 g diet calculated by the following factors, 5.49 kcal / g protein, 9.09 kcal / g lipid and 4.10 kcal / g sugar (Takii et al. 1997b).
in Tables 1 and 2. In diets 1–8 for assaying digestibilities, diets 1 and 7 were control diets and included 60% and 55% brown fish meal (BFM) as a main protein source. Diets 2–6 and 8 were composed of 40% CA, HA, RA, CRA and HRA and 35% CHA, substituting nearly half of the dietary BFM. Other ingredients were wheat gluten, pollack liver oil, α-potato starch, Halver's vitamin and mineral mixtures (Halver 1957) and cellulose. Diet 8 alone included 10% blood meal for increasing dietary protein level up to that of diet 7. All diets were supplemented with 0.5% chromic oxide as an inert marker for assaying digestibilities. No remarkable differences were detected in dietary crude protein and lipid contents, ca. 50% and 14%, respectively. Crude sugar contents of diets 3, 4 and 6 were above 20%, higher than other diets 16–19%, and ash contents of diets 7 and 8 were above 9%, more than other diets ca. 5.5%. Gross energy contents of the diets showed slight differences between diets, from 499 kcal/100 g of diet 1 to 462 kcal/100 g of diet 7.

Diet 9 to 12 were formulated for evaluating the nutritional value of diets including yeast autolysates. A control diet 9 contained 55% BFM, 10% wheat gluten, 9% pollack liver oil and 13.5% α-potato starch. Diets 10–12 were respectively composed of 35% CRA, HRA and CHA, substituted more than half of the BFM in the control diet. Soy protein concentrate (Danpro-A: Bayer Japan, Tokyo) and blood meal were also added to the diets for adjusting dietary protein level.

**Fish and rearing protocol**

For assaying apparent protein, lipid, sugar and energy digestibilities of diets including yeast autolysates, the Fish Nursery Center, Kinki University, Uragami supplied red sea bream juveniles. Each 20 bream having a mean body weight of 12.7 g was accommodated into an indoor 400 l circular tank attached with a fecal trap column. The bream were given one of the diets 1–6, twice a day for 27 days. Feces were pooled for 5 or 6 consecutive days and lyophilized by the methods cited previously, preparing 3 pooled-samples for assay (Takii et al. 1997a). Water temperature and DO in the rearing period were 23.1±1.4°C and 6.8±0.8 mg/l (mean±SD, n = 27), respectively. Each 50 bream having a mean body weight of 14.1 g was then introduced into the same 400 l circular tank and fed either diet 7 or 8, twice a day for 11 days. Feces were pooled for 3 consecutive days and lyophilized, preparing 3 pooled-samples for digestibility assay (Takii et al. 1997b). Rearing water temperature and DO were 18.1±1.4°C and 6.1±0.8 mg/l (mean±SD, n = 11), respectively.

For evaluating the nutritive value of diets including yeast autolysates, CRA, HRA and CHA utilities as SCP, the Fish Nursery Center, Kinki University, Uragami also supplied the bream. Before the rearing trials, the bream were fed the control BFM diet and acclimated to the rearing conditions for a week. In 1st trial, each 12 bream having a mean body weight of 13.4 g was accommodated into an indoor 200 l rectangular tank and fed one of diets 9–11, and was named as diet 9, 10 and 11 groups. Otherwise, in 2nd trial, each 15 bream having a mean body weight of 12.1 g in an indoor 300 l rectangular tank was fed either diet 9 or 12, and was named as diet 9 and 12 groups. Fish were

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**Table 2.** Dietary formula used for dietary utility of yeast autolysates (%)

<table>
<thead>
<tr>
<th>Component</th>
<th>Diet no. 9</th>
<th>Diet no. 10</th>
<th>Diet no. 11</th>
<th>Diet no. 12</th>
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<tr>
<td>BFM*</td>
<td>55</td>
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<td>25</td>
<td>25</td>
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<tr>
<td>CRA*</td>
<td>-</td>
<td>35</td>
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<td>-</td>
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<tr>
<td>HRA*</td>
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<td>-</td>
<td>35</td>
<td>-</td>
</tr>
<tr>
<td>CHA*</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>35</td>
</tr>
<tr>
<td>Soy protein concentrate</td>
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<td>2</td>
<td>-</td>
</tr>
<tr>
<td>Blood meal</td>
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<td>10</td>
</tr>
<tr>
<td>Wheat gluten</td>
<td>10</td>
<td>10</td>
<td>10</td>
<td>8</td>
</tr>
<tr>
<td>Pollack liver oil</td>
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<td>11</td>
<td>11</td>
<td>11</td>
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<tr>
<td>α-Potato starch</td>
<td>13.5</td>
<td>2.5</td>
<td>2.5</td>
<td>2.5</td>
</tr>
<tr>
<td>Vitamin mixture*</td>
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<td>3</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td>Mineral mixture*</td>
<td>2.5</td>
<td>3.5</td>
<td>3.5</td>
<td>3.5</td>
</tr>
<tr>
<td>Cellulose</td>
<td>7</td>
<td>5</td>
<td>5</td>
<td>2</td>
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</table>

Proximate composition

<table>
<thead>
<tr>
<th>Component</th>
<th>49.7</th>
<th>50.2</th>
<th>48.9</th>
<th>51.7</th>
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<tr>
<td>Crude protein</td>
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<td>15.8</td>
<td>15.3</td>
<td>14.3</td>
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<tr>
<td>Crude lipid</td>
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<td>15.9</td>
<td>17.7</td>
<td>15.8</td>
</tr>
<tr>
<td>Crude ash</td>
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<td>8.8</td>
<td>9.0</td>
<td>9.0</td>
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<tr>
<td>Gross energy*</td>
<td>478</td>
<td>484</td>
<td>480</td>
<td>479</td>
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</table>

* Shown in the footnote of Table 1.
fed diets twice a day, 6 days a week, for 56 days. These rearing trials were conducted with three replicates. Rearing water temperature and DO of the trial 1 and 2 were $23.2 \pm 1.2^\circ\text{C}$ and $8.2 \pm 1.0 \text{mg/l}$ and $18.1 \pm 1.6^\circ\text{C}$ and $7.3 \pm 0.6 \text{mg/l}$ (mean ± SD, $n = 56$), respectively.

The indoor-photoperiod of rearing trials for the digestive and nutritional value of diet including yeast autolysates was set at 12L:12D with fluorescent tubes.

**Assay and statistical analysis**

Apparent digestibilities of diets including yeast autolysates were assayed by the indirect method using Cr$_2$O$_3$ as a inert marker (Furukawa and Tsukahara 1966). Moisture, crude protein, crude lipid and ash contents of feces, diets, fish carcass and the hepatopancreas were measured by the method of A.O.A.C. (A.O.A.C. 1984). Crude sugar contents of feces and diets were assayed by the phenol-sulfuric acid method (Hodge and Hofreiter 1962).

The data were statistically analyzed by one-way ANOVA to determine the effects of dietary treatments ($P<0.05$). If treatment effect was significant, differences between means were identified by Duncan’s Multiple Range Test ($P<0.05$) (Harter 1960).

**Results**

**Yeast autolysis**

Autolysis achievements of yeasts and their mixtures are shown in Fig. 1. The autolysis of CRA, HRA, RA, CHA, CA and HA were 65%, 40%, 25%, 15%, 10%, and 10% after 27h-incubation. The singular RA and mixture with others had a increasing trend in autolysis with the incubation time, but those of CHA, CA, and HA were stayed at low level.

**Apparent digestibilities for diets with yeast autolysates**

Nutrient and energy digestibilities for diets including yeast autolysate(s) (Table 3), had a reverse tendency as compared with the order of yeast autolysis achievement (Fig. 1). Protein digestibility of diets 2, 3 and 5 was similar to control diet 1, but higher than diets 4 and 6. Lipid digestibility of diets 2, 3 and 4 was significantly lower than diets 1, 5 and 6. Sugar and energy digestibilities of diets 2−6 were commonly lower than diet 1. No significant differences were found between the diet 8 and control diet 7 in apparent protein, lipid, sugar and energy digestibilities.
**Growth performance**

There were no significant differences in final mean body weights, survivals and weight gain from diet 9 to 11 groups, in the 1st trial (Table 4). The diet 10 and 11 groups showed higher daily feed intake but lower feed efficiencies, protein efficiency ratios, energy efficiencies and apparent protein and lipid retentions than those of the diet 9 group.

All variables relating growth performance including final mean body weight showed no differences between the diet 9 and 12 groups, in the 2nd trial.

**Digestive organ-somatic indices and proximate compositions**

In the 1st trial, the diet 10 and 11 groups had significantly lower hepatopancreas- and higher intestine-somatic indices than the diet 9 group, but not in stomach-somatic index (Table 5). Hepatopancreatic proximate composition indicated relatively low moisture content of 55% (Table 5).

---

**Table 4. Growth performance of the bream fed diets including various yeast autolysates for 56 days**

<table>
<thead>
<tr>
<th>Diet group (Trial 1)</th>
<th>SEM</th>
<th>Diet group (Trial 2)</th>
<th>SEM</th>
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<td>Survival (%)</td>
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<td>94.4</td>
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<tr>
<td>Mean BWt (g)</td>
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<td>Final</td>
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<td>Daily</td>
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<tr>
<td>Weight gain (%)</td>
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<td>4.8</td>
<td>5.0</td>
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<tr>
<td>Feed intake (%)</td>
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<td>Feed efficiency (%)</td>
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<td>79.6b</td>
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<td>EE*3</td>
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<td>Apparent retention (%)</td>
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<td>Lipid</td>
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**Table 5. Organ somatic indices (%) and proximate compositions (%) of carcass and the hepatopancreas of the bream fed diets including various yeast autolysate for 56 days**

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<th>Diet group (Trial 1)</th>
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<tr>
<td>Hepatopancreas</td>
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<td>2.5b</td>
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<td>17.7</td>
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<td>Crude lipid</td>
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<td>10.8</td>
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<tr>
<td>Crude ash</td>
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<td>5.2</td>
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<td>59.1</td>
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<td>Crude ash</td>
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</tbody>
</table>

*1 Trials were all conducted by three replicates. Mean water temperature of 1st (diets 9, 10 and 11) and 2nd (diets 9 and 12) trials were 23.2 ± 1.2°C and 18.1 ± 1.6°C, respectively.

*2 The same superscript in a row indicates no significant difference (P > 0.05).

*3 PER: protein efficiency ratio and EE: energy efficiency = weight gain ÷ 100 / energy intake.

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*1 n = 5.

*2 The same superscript in a row indicates no significant difference (P > 0.05).

*3 Pooled sample from 5 individuals.
in the diet 10 group and the high crude lipid content of ca. 20% in the diet 10 and 11 groups, as compared with 60% and 15% of the bream given diet 9, respectively (Table 5).

No significant differences in digestive organosomatic indices were found between diet 9 and 12 groups in the 2nd trial. Meanwhile, excepting the group 12 with slightly lower final carcass moisture than the diet 9 group, carcass proximate compositions indicated no marked differences among the dietary treatments (Table 5).

Discussion

In the present study, we ascertained that the autolysis achievement of yeast(s) was reverse against the dietary digestibility including them for the bream. The diet substituted nearly half of the BFM with CHA, that attained relatively lower autolysis achievement, showed high protein, lipid, sugar and energy digestibilities, comparable to those of the control BFM diet. The diets substituted with CRA, HRA and RA, that attained higher autolysis achievement, led relatively lower protein and sugar digestibilities. These results indicate that the autolysis does not necessarily improve the digestibility of yeast(s). Indigestible polysaccharides, such as glucan, mannan and chitin, mainly composing yeast cell wall, and their types and various constructions, particularly mannosidic linkages, well reflect to yeast taxonomic decisions (Barnett et al. 2000). Dietary fibers, representing as indigestible polysaccharides, have particular physical properties, such as water-holding capacity, viscosity, susceptibility to fermentation, bile acid binding and cation exchange capacity (Gallaher and Schneeman 1996). The reason for low digestibilities in the bream fed diets including RA is unclear from the present study. It may be reasonable that the indigestible polysaccharides of yeasts may induce the digestive and absorptive disorders of fish, more or less, as known in landed mammals (Gallaher and Schneeman 1996). Otherwise, it had already been indicated that RA have some amylase inhibitors and antibiotics (Robinson 1987). The previous studies also showed low growth performance of the bream given diets including R. rubra yeast (Akiyama et al. 2001). Genus Rhodotorula may commonly induce undesirable influence on digestion and assimilation of fish. Several fish species have high chitinolytic activities in their gastrointestinal tracts. It had been generally accepted that the chitinolytic activities was originated from enterobacteria in the gastrointestinal tract (Sera and Okutani 1968). However, recent research indicates the ability to produce chitinase in cod Gadus morhua and Japanese flounder, Paralichthys olivaceus (Danulat 1986; Kurokawa et al. 2004). We did not assay the indigestible polysaccharide contents of yeast autolysates in the present study. The lower digestibility of diets including CRA, HRA and RA might result in their glucan and mannan other than chitin, with regard to the reliable chitinase activity of gastrointestinal tract in the bream.

The growth performance, digestive organosomatic indices and carcass and hepatopancreatic proximate compositions of bream fed the diet substituted a half of BFM with CHA was equivalent to the control BFM diet. This result suggests that CHA is a valid yeast protein source as SPC for red sea bream diets, partly or half substituting for dietary BFM. The diet CRA and HRA groups appeared significantly lower growth performance and hepatopancreatic indices and higher hepatopancreatic crude lipid than the diet 9 group. The reason for getting the low growth performance of the CRA and HRA groups might also result from the disturbance of assimilative and metabolic mechanisms, due to low nutrient digestibilities of RA. Otherwise, the blood meal content of diet including CHA up to 10% resulted in no adverse influences on digestibilities and growth performance, in the present study. This also suggest that blood meal will be a useful additive protein source to develop a low fish meal diet for the bream, if there are the dissolution about problems of bovine spongiform encephalopathy.

Some yeasts of genus Saccharomyces and Candida are reliable and useful food stuff for
human beings, domestic animals and fishes (Kouno and Terashita 1993). The industrial facilities of yeast production need much investment as effective initial and running costs, besides their desirable possibility and utility. The present study suggests the autolysis of yeast(s) does not necessarily improve their digestibilities, nutrient availabilities and growth performance of the bream. Otherwise, Shimma et al. (1981) investigated the nutritive value of methanol-grown yeast (MGY), genus Candida prohibited using now in Japan, in diets of rainbow trout, Oncorhyncus mykiss. The diet including MGY sustained the good growth performance of the juvenile trout, as comparable to that fed the diet including fish meal mainly, but invited the lower growth performance of the matured trout. The MGY have lower sulfur amino acids and arginine contents than fish meal. This might induce the different growth performance between juvenile and matured rainbow trout (Shimma et al. 1982). Therefore, further studies are necessary to clear the nutritional values of CHA in diets for several stages of the red sea bream. Furthermore, Yano et al. (1991) and Fujiki et al. (1994) suggested 1,6-branched-𝛽-1,3-glucans of bacteria promoted immune response of carp, Cyprinus carpio. These reports also sustain some possibility for using yeast and/or yeast autolysate as a promoter for immune responses.

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References


マダイにおける自己消化酵母タンパク質の栄養価

澁井健二・秋山真一・篠岡孝至・日高久美・大高治堂・瀬岡 学・熊井英水

マダイに対する自己消化酵母の消化性と栄養価について調べた。自己消化率は *Candida utilis* + *Rhodotorula glutinis* (CRA) が最も高く、次で *Hasenula anomala* + *R. glutinis* (HRA), *R. glutinis* (RA), *C. utilis + H. anomala* (CHA), *C. utilis* (CA) および *H. anomala* (HA) の順に低下した。しかし、魚粉の半量近くをこれら自己消化酵母に代替した飼料の見掛けの消化率には、自己消化率と逆の傾向が得られ、CA, HA および CHA 飼料で高かった。また、魚粉の半量を CRA, HRA および CHA に代替した飼料を調製して56日間飼育したところ、CHA 代替飼料で魚粉飼料と同等の優れた飼育成績が得られた。これらの結果から、酵母の自己消化は栄養価の改善に直接結びつかなかったが、CHA はマダイに対して有望な SCP であることが示された。