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# Nutritional Evaluation of Several Protein Sources for Yellowtail (*Seriola quinqueradiata*)

Toni RUCHIMAT[1,2], Toshiro MASUMOTO[1], Hidetsuyo HOSOKAWA[1], and Sadao SHIMENO[1]

[1] *Laboratory of Fish Nutrition, Faculty of Agriculture, Kochi University B200 Monobe, Kochi 783, Japan*
[2] *Central Research Institute for Fisheries Indonesia, Research Station for Coastal Fisheries, Gondol P.O Box 140, Singaraja, Bali 81101, Indonesia*

**Abstract**

Nutritional value of brown fish meal (BFM), soy protein concentrate (SPC) and corn gluten meal (CGM) for young yellowtail diets was evaluated by biological and biochemical methods. These proteins were incorporated at 30 - 50% of the diet as the only source of protein. The highest growth, feed efficiency, apparent protein digestibility, PER, NPU and BV were obtained from BFM-fed fish group while the lowest values were CGM-fed fish group, excluding BV which was similar between SPC and CGM. There was no apparent relationship between arginase or citrate synthase activities and protein sources. Blood urea nitrogen (BUN) and RNA concentrations of BFM- and SPC-fed fish groups tended to increase with increasing protein level, but those of CGM-fed fish group remained practically the same.

Low protein utilization of SPC and CGM are attributed to their unbalance amino acid profiles and that of CGM is also due to poor digestibility. Among the examined biochemical parameters, BUN may provide useful indication for protein quality evaluation, though clear results were not obtained. At present, NPU and BV are useful parameters because clear growth differences due to dietary nutrition are seen in a rapidly growing fish such as yellowtail.

**Key words** : Yellowtail, nutritional value, protein sources, biological, biochemical.

## INTRODUCTION

Feed represents a large part of production costs during intensive culture of fish, accounting for over half of the total variable operating cost. Protein component of feed is the most expensive portion and an important dietary nutrient in the formulation as it holds the key for a greater expansion in aquaculture (Tacon and Jackson, 1985). Thus, reducing cost for the dietary protein is an important strategy for the profitable operation of aquaculture.

The nutritional value of protein is generally defined as amino acid profile and digestibility. In this respect, fish meal is an excellent source of protein in the aquaculture feed. However, fish meal becomes less available because of poor landing and an increase demand due to rapid increase in aquaculture in the world. Therefore, it is necessary to reduce the fish meal component in a practical diet by replacing it partially or totally by another protein sources having good quality, low price and consistency in availability.

Numerous studies on the utilization of animal and plant proteins as partial or total replacement for fish meal in the fish feed have been conducted using various freshwater species, mostly salmonids, but relatively few studies for marine fish species. Therefore, further study on the utilization of animal or plant proteins in formulated feed for marine fish is necessary. Prior to the consideration of those partial or total replacement of fish meal in diet for marine

fish, the nutritional quality of those alternative protein sources should be evaluated.

The aims of the present study are to evaluate the quality of brown fish meal, full fat soybean meal and meat meal as a sole source of protein in yellowtail diet by biological and biochemical methods, and to identify the accurate and convenient parameters which exhibit nutritional value of protein.

## MATERIALS AND METHODS

*Experimental conditions and fish*

The experiment was conducted at Usa Marine Biological Institute, Kochi University and lasted for 3 weeks. Juvenile yellowtail (*Seriola quinqueradiata*) from natural stock were purchased from a commercial fish-farm in Uranouchi Bay, Kochi Prefecture, Japan. Prior to the feeding trial, the fish were reared in 1000 l fiber reinforced plastic (FRP) tanks for two weeks in order to acclimatize them to the laboratory conditions during which they were fed twice a day with a commercial diet (Marubeni Feed Co. Ltd., Hyogo, Japan). Initially, 5 fish were randomly

**Table 1.** Formulation and proximate composition (%) of experimental diets

| Diet : | BFM 30 | BFM 35 | BFM 40 | BFM 50 | CGM 30 | CGM 35 | CGM 40 | CGM 50 | SPC 30 | SPC 35 | SPC 40 | SPC 50 | PFD |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Brown fish meal[1] | 44.0 | 51.0 | 58.0 | 71.0 | | | | | | | | | |
| Corn gluten meal | | | | | 45.0 | 52.0 | 60.0 | 73.0 | | | | | |
| Soy protein concentrate[2] | | | | | | | | | 46.0 | 53.0 | 61.0 | 74.0 | |
| Pollock liver oil | 20.0 | 17.0 | 14.0 | 9.0 | 20.0 | 17.0 | 14.0 | 9.0 | 20.0 | 17.0 | 14.0 | 9.0 | 20.0 |
| $\alpha$ - corn starch | 8.5 | 8.5 | 8.5 | 8.5 | 8.5 | 8.5 | 8.5 | 8.5 | 8.5 | 8.5 | 8.5 | 8.5 | 38.5 |
| Dextrin | | | | | | | | | | | | | 17.5 |
| Others[3] | 8.5 | 8.5 | 8.5 | 8.5 | 8.5 | 8.5 | 8.5 | 8.5 | 8.5 | 8.5 | 8.5 | 8.5 | 8.5 |
| $\alpha$ - cellulose | 19.0 | 15.0 | 11.0 | 3.0 | 18.0 | 14.0 | 9.0 | 1.0 | 17.0 | 13.0 | 8.0 | 0.0 | 15.5 |
| Total | 100.0 | 100.0 | 100.0 | 100.0 | 100.0 | 100.0 | 100.0 | 100.0 | 100.0 | 100.0 | 100.0 | 100.0 | 100.0 |
| Feeding stimulant[4] | 0.5 | 0.5 | 0.5 | 0.5 | 0.5 | 0.5 | 0.5 | 0.5 | 0.5 | 0.5. | 0.5 | 0.5 | 0.5 |
| Freshwater | 50.0 | 50.0 | 50.0 | 50.0 | 50.0 | 50.0 | 50.0 | 50.0 | 50.0 | 50.0 | 50.0 | 50.0 | 50.0 |
| | Proximate composition of nutrient content[5] | | | | | | | | | | | | |
| Crude protein | 30.3 | 36.3 | 41.3 | 51.7 | 30.4 | 36.0 | 41.8 | 50.5 | 30.6 | 36.2 | 41.3 | 51.6 | 0.8 |
| Crude fat | 24.5 | 21.8 | 19.4 | 15.7 | 25.4 | 20.4 | 19.2 | 16.0 | 22.1 | 19.3 | 18.0 | 10.4 | 20.4 |
| Crude sugar | 11.2 | 11.4 | 9.8 | 9.4 | 14.5 | 16.4 | 17.3 | 18.1 | 16.6 | 17.5 | 17.9 | 19.2 | 44.6 |
| Crude ash | 9.1 | 10.3 | 11.6 | 13.7 | 2.3 | 2.5 | 2.6 | 2.9 | 4.8 | 5.4 | 5.6 | 6.5 | 2.2 |
| Energy (kcal/kg diet) [6] | 3637.1 | 3696.7 | 3684.9 | 3845.7 | 3806.0 | 3711.2 | 3901.4 | 4059.3 | 3609.8 | 3663.0 | 3799.7 | 3691.6 | 2838.2 |
| Calorie/protein ratio[7] | 120.0 | 101.8 | 89.2 | 74.4 | 125.2 | 103.1 | 93.3 | 80.4 | 118.0 | 101.2 | 92.0 | 71.6 | - |

[1] Steam-dried Chilean fish meal (Marubeni Feed Co. Ltd., Hyogo, Japan).

[2] Danpro A (Aarhus Olie Co. Ltd., Denmark).

[3] Vitamin and mineral mixtures : 5.5% (Shimeno et al., 1992), CMC Na 2.5%, guar gum 0.5%.

[4] Supplied (g/kg) : L-Proline + L-Alanine + 5-IMP = 354 + 232 + 414 g (Takeda, 1981).

[5] Expressed as % dry weight.

[6] Digestible energy (kcal/g) : protein, 4,5; lipid, 8.0; carbohydrate, 2.8 (Shimeno et al., 1992).

[7] Digestible energy (kcal/kg diet)/protein (g/100 g diet).

collected and sacrificed for proximate analysis. Fish with initial mean weight of 31.7 g were assigned to 13 FRP tanks with holding capacity of 800 l at a density of 25 fish per tank. The tanks were supplied with well-aerated seawater at a flow rate of 10 - 12 l per min, temperature of 25.4 ± 1.2℃ and salinity of 30.8 ± 1.7. All tanks were located indoors and lighting was not provided overnight. The fish were fed twice a day at 5% level of total body weight. Individual fish in each group was weighed at an interval of 7 days to monitor overall growth and adjusting feeding rate. The fish were treated with sodium nifurstyrenate (Ueno Fine Chemicals Industry Ltd., Tokyo, Japan) after weighing to prevent bacterial infections caused by handling. Mortality was checked daily and the number and weight of dead fish were recorded. At the end of the feeding trial, 3 fish from each group were sacrificed and stored at -20℃ for further analysis of body composition. Blood samples were drawn from 5 fish from each group through the caudal vein. The serum were separated by centrifugation at 10,000 rpm for 5 minutes and then kept at -80℃ until assayed for blood urea nitrogen (BUN). These fish were then sacrificed, the liver and lateral muscle were immediately removed and frozen in liquid nitrogen, then stored at -80℃ until assayed for liver arginase, muscle citrate synthase (CS) and ribonucleic acid (RNA) content. The remaining fish were used for digestibility study.

**Table 2.** Amino acid composition of proteins used

| Protein source : | BFM | CGM | SPC |
|---|---|---|---|
| | A/E ratio of essential amino acid (mg/1000 mg)[1] | | |
| Threonine | 101.7 | 77.7 | 95.5 |
| Valine | 100.6 | 88.4 | 93.6 |
| Methionine | 73.6 | 62.0 | 33.9 |
| Isoleucine | 82.3 | 74.2 | 89.5 |
| Leucine | 169.4 | 401.6 | 181.8 |
| Phenylalanine | 93.6 | 152.4 | 120.7 |
| Lysine | 183.1 | 37.4 | 151.6 |
| Histidine | 55.4 | 39.3 | 57.0 |
| Arginine | 140.3 | 67.0 | 176.5 |
| Tryptophan | 25.0 | 12.0 | 29.0 |
| EAAI2 | 100.0 | 64.8 | 89.7 |

[1] Amino acid/ total essential amino acid.

[2] Essential amino acid index.

*Experimental diets*

Brown fish meal (BFM), soy protein concentrate (SPC) and corn gluten meal (CGM) were used as a sole source of protein. The feeds were formulated to contain crude protein levels of 30 - 50% from each protein source (Table 1). Protein-free diet (PFD) was also provided in order to determine net protein utilization (NPU). Amino acid composition of these protein sources are presented in Table 2. All dry ingredients of each experimental diet were mixed in a mixer. Lipid soluble vitamins were dissolved in pollock liver oil, blended with the dry ingredients and finally mixed well with 500*ml* freshwater. The moist mixture were pelleted (diameter 3.0 - 4.5 mm), then stored at -20℃ until used.

*Digestibility study*

Following feeding trial, the remaining fish in each group were further fed to apparent satiation with the same experimental diets containing 0.5% chromic oxide. After being fed the respective diets for 5 days, the fish were starved for 1 day in order to evacuate previous diet. The fish were fed in the following morning. After two hours, 5 fish from each experimental group were randomly transferred into a feces collector tank. Feces collections were conducted at 2, 4, 6, 10, and 24 h after the commencement of experiment. The collected feces were centrifuged at 5000 rpm for 5 min, freeze-dried and then kept in desiccator until used.

*Determination and analytical methods*

Amino acid composition of protein source was determined with auto - amino acid analyzer (Hitachi, Model 835, Hitachi Ltd., Tokyo, Japan), equipped with a column (no. 2619, 4 x 150 mm) and lithium buffer system. Prior to the analysis, the samples were hydrolyzed with 6 N HCl at 110℃ for 22 h. Cysteine was determined as cystine after oxidation of the sample with 0.1 N NaOH for 4 h at room temperature. Proximate compositions of the diets and whole body were assayed according to standard methods (AOAC, 1984). Protein digestibility was determined by the method of Furukawa and Tsukahara (1966). Net protein utilization (NPU) and biological value (BV) were calculated by the equation of Miller and Bender (1954).

Arginase (EC. 3.5.3.1) activity in liver was measured by a modification of the method of Schwartz (1971). Citrate synthase (EC. 4.1.3.7) activity in muscle was assayed according to the method of Pelletier et al. (1993). The RNA concentration in muscle was determined by a modification of the Schmidt-Thannhauser method (Bukley, 1979). Blood urea nitrogen concentration was determined with dinabot test kit on auto analyzer Dainabot VP super system (Abbot Co., Texas, USA).

*Statistical analysis*

In the prevailing circumstances, it was not possible to allot more than a single tank for each treatment diet. Therefore, significant differences between treatment means were compared using Duncan's new multiple-range test (Gomez and Gomez, 1983), if data were appropriate. The effect of tanks was minimized by changing the tank for each treatment when the fish were weighed.

## RESULTS AND DISCUSSION

*Growth and feed efficiency*

Changes in average body weight of yellowtail fed the test diets are shown in Fig.1. Although all fish accepted their respective amount of the experimental diets, reduction in feeding activity and loss of appetite were observed in fish receiving SPC30 and CGM30-40 diets after 2 weeks of feeding. Survival rate was 100% except in PFD which was 95%. The growth rate of BFM-fed fish group was highest followed by SPC- and CGM-fed fish groups. The lowest average weight gain was found in CGM group (Table 3). Ketola (1982) reported that trout fed with diet containing CGM without amino acid supplementation, gained only about 6% of body weight compared to control fish which fed herring meal diet. The growth of yellowtail was also reduced significantly when 50% of fish meal was replaced by CGM (Shimeno et al., 1993a). Soy protein gave a better growth than that of CGM but lower than BFM. The reason behind the low growth of CGM- and SPC-fed fish could be attributed to unbalance amino acid profile of CGM and SPC compare to that in BFM diet. CGM has low lysine, arginine and tryptophan, while SPC low in methionine content (Table 2). Improvement of its lacking amino acid or as a

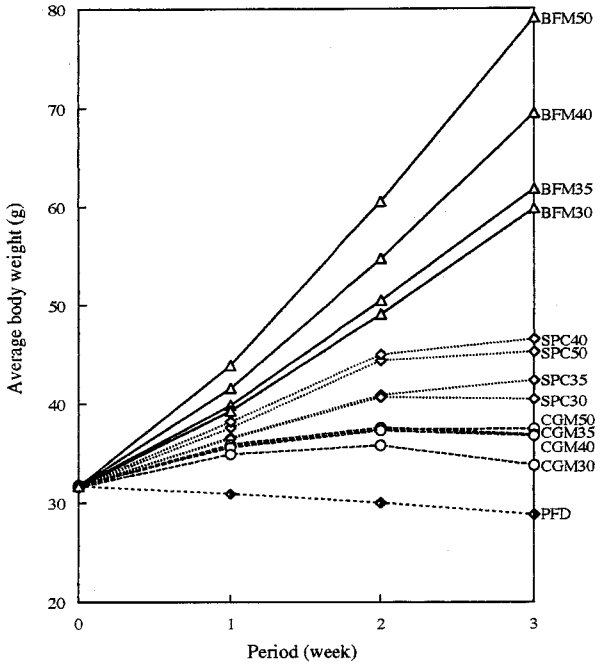


**Fig. 1.** Changes in average body weight of yellowtail fed the test diets for 3 weeks.

**Table 3.** Performance of yellowtail fed the test diets for 3 weeks

| Diet | Final mean weight (%)[1] | Weight gain (%) | DGR[2] (%) | DFR[3] (%) | Feed Efficiency (%) | PER | Protein digestibility (%) | NPU (%) | BV (%) | Nitrogen gain (mg/100g BW/day) |
|---|---|---|---|---|---|---|---|---|---|---|
| BFM30 | 59.8 | 88.6 | 2.92 | 4.47 | 65.5 | 2.16 | 86.9 | 49.2 | 56.6 | 41.6 |
| BFM35 | 61.8 | 95.0 | 3.07 | 4.43 | 69.1 | 1.90 | 87.1 | 45.5 | 52.2 | 39.3 |
| BFM40 | 69.5 | 119.2 | 3.56 | 4.47 | 79.5 | 1.92 | 88.7 | 44.6 | 50.3 | 39.7 |
| BFM50 | 79.1 | 145.9 | 4.07 | 4.13 | 98.6 | 1.91 | 90.2 | 41.3 | 45.8 | 37.3 |
| CGM30 | 33.8 | 7.0 | 0.32 | 4.96 | 4.7 | 0.15 | 36.0 | 11.6 | 32.2 | 2.2 |
| CGM35 | 36.8 | 16.1 | 0.69 | 4.92 | 14.1 | 0.39 | 38.0 | 13.6 | 35.8 | 5.7 |
| CGM40 | 36.9 | 16.4 | 0.72 | 4.84 | 14.2 | 0.34 | 47.1 | 11.3 | 24.0 | 4.7 |
| CGM50 | 37.5 | 18.3 | 0.80 | 4.83 | 16.3 | 0.33 | 48.9 | 11.2 | 22.9 | 5.6 |
| SPC30 | 40.5 | 30.9 | 1.16 | 4.78 | 24.2 | 0.79 | 73.4 | 22.6 | 30.8 | 13.8 |
| SPC35 | 42.4 | 33.8 | 1.38 | 4.64 | 29.7 | 0.82 | 76.4 | 24.2 | 31.7 | 16.7 |
| SPC40 | 46.5 | 46.7 | 1.80 | 4.60 | 39.2 | 0.95 | 83.2 | 24.6 | 29.9 | 18.4 |
| SPC50 | 45.3 | 42.9 | 1.68 | 4.63 | 36.2 | 0.70 | 82.2 | 18.7 | 22.7 | 13.7 |
| PFD | 28.8 | -9.1 | - | 4.71 | - | - | - | - | - | -18.6 |

[1]Initial mean weight was 31.7 g for all treatments.

[2]Daily growth rate.

[3]Daily feeding rate.

partial replacing of fish meal, enabled the use of CGM or SPC as a protein source in yellowtail diet. As a partial replacement of fish meal up to 20%, CGM gave a comparable growth to that of 100% fish meal diet (Shimeno et al., 1993a). Takii et al. (1989) showed a comparable growth to that of 100% fish meal diet when SPC was used as a partial replacement up to 20% combined with amino acid supplementation.

Feed efficiency of all experimental diets increased with increasing dietary protein level. However, a slight decrease was found in fish fed SPC50. The highest feed efficiency obtained among the protein sources was BFM and the lowest was CGM.

**Table 4.** Proximate composition of whole body of yellowtail fed the test diets[1]

| Diet | Moisture | Crude protein | Crude fat | Crude sugar | Crude ash |
|---|---|---|---|---|---|
| BFM30 | 70.6 | 18.8 | 6.1 | 0.5 | 3.2 |
| BFM35 | 71.4 | 19.5 | 5.7 | 0.4 | 3.3 |
| BFM40 | 70.9 | 19.6 | 5.8 | 0.4 | 3.1 |
| BFM50 | 71.0 | 19.1 | 4.8 | 0.4 | 3.3 |
| CGM30 | 71.0 | 18.2 | 4.5 | 0.5 | 3.9 |
| CGM35 | 70.5 | 17.9 | 4.8 | 0.7 | 4.1 |
| CGM40 | 69.8 | 17.7 | 4.8 | 0.5 | 3.4 |
| CGM50 | 71.2 | 18.2 | 4.1 | 0.5 | 3.8 |
| SPC30 | 70.3 | 18.2 | 3.3 | 0.4 | 4.0 |
| SPC35 | 72.3 | 18.9 | 4.0 | 0.5 | 3.7 |
| SPC40 | 72.1 | 18.7 | 3.9 | 0.4 | 3.6 |
| SPC50 | 73.3 | 18.7 | 2.4 | 0.4 | 3.6 |
| PFD | 75.0 | 17.7 | 2.1 | 0.4 | 4.7 |

[1] Pooled samples of 3 fish from each treatment. Samples were taken at the end of experiment.
Initial body composition was 73.8% moisture, 18.4% crude protein, 3.3% crude fat, 0.5% crude sugar and 3.8% crude ash.

*Body composition*

Body composition was not greatly affected by protein source and dietary protein level (Table 4). Within BFM group, fish fed the lowest dietary protein level tended to have a slightly lower protein and moisture content, but higher in lipid content. Moisture and lipid levels of BFM group appeared to be inversely related which is in agreement with an earlier experiment by Shimeno et al. (1980). However, such a relation was not found in CGM and SPC groups. The low protein content of fish fed low protein or PFD has also been previously reported in other species (Atack et al., 1979; Jauncey, 1982). In the same protein source group, the body ash content was unaffected by dietary protein levels.

*Protein utilization parameters*

Apparent protein digestibility, PER, nitrogen gain, NPU, and BV are shown in Table 3. The apparent protein digestibility was highest in BFM and lowest in CGM groups. The value changed according to the protein level, increased with increasing dietary protein level, except for SPC50 which slightly decreased. However, Pongmaneerat and Watanabe (1991), found that protein digestibility in the carp was not affected by dietary protein level, except a small de-

crease in the low dietary protein level due to the high influence of metabolic fecal nitrogen excretion. The digestibility value for BFM ranged from 87 - 90%. This value was higher than an earlier study in yellowtail (Shimeno et al., 1993b), but comparable to the result for carp (Pongmaneerat and Watanabe, 1991). The differences may partly be due to different techniques used for feces collection and the raw material which frequently vary in quality. Until now, only few report regarding the digestibility of CGM and SPC as a sole source of protein in yellowtail diet are available. Apparent digestibility for CGM and SPC ranged from 36 - 49% and 73 - 82%, respectively. These results are in agreement with our previous study (Masumoto et al., 1996), but for CGM, it was lower than that for carp (Ogino and Chen, 1973; Pongmaneerat and Watanabe 1991).

Nitrogen gain was highest in BFM-fed fish followed by SPC- and CGM-fed fish respectively. Concerning PFD-fed fish, they lost body nitrogen of 18.6mg-N/100g BW/day. This value is higher than that reported for carp (Pongmaneerat and Watanabe, 1991), but lower than that for rainbow trout (Nose, 1971). These differences might be attributed to the difference in species and energy levels in their test diets.

The PER value for BFM slightly decreased with decreasing protein level, however, that tendency was less obvious in CGM and SPC groups. It is well known that PER decreases as dietary protein level increases (Takeuchi et al., 1978; Mazid et al., 1979, Murai et al., 1985). However, it was not clearly observed in the present experiment.

**Table 5.** Relative values of NPU and BV parameters

| Protein source | NPU(%) | | BV(%) | |
|---|---|---|---|---|
| | a | b | a | b |
| BFM | 100.0 | 100.0 | 100.0 | 100.0 |
| CGM | 27.6 | 27.1 | 63.3 | 50.0 |
| SPC | 50.0 | 59.6 | 56.0 | 64.6 |

a: The best value within the group.

b: The best value at the best growth.

The NPU and BV values of BFM tended to decrease with increasing protein level. This tendency was also found in earlier investigations in other fish species (Jauncey, 1982; Watanabe and Pongmaneerat, 1991; Pongmaneerat and Watanabe; 1991). This tendency was less obvious in CGM and SPC groups. The NPU values of CGM was the lowest among the proteins. It did not improve much at the low dietary protein levels of CGM and SPC. Biological value of BFM was the highest in all protein levels, while the values for SPC group were comparable with those of CGM group. Generally, NPU and BV as well as other parameters used in biological estimation of proteins are considered to be an absolute number. In practical situations, it would be convenient if these values are expressed as relative value with respect to a reference protein. Therefore, in this study BFM was used as a reference protein. As shown in Table 5, the relative NPU value of SPC was better than CGM while for BV value was comparable each other. From these point of view, it could be concluded that SPC has better nutritional value than CGM for yellowtail diet.

*Biochemical Evaluation*

Arginase is one of amino acid metabolizing enzymes which play an important role in the ami-

no acid degradation. In the present experiment, SPC-fed fish had high arginine content compared with BFM and CGM groups (Table 1). However, as shown in the results (Table 6), the liver arginase activity in SPC-fed fish tended to decrease as dietary protein level increased. Meanwhile, CGM group which is considered to have a low content of arginine, had higher arginase activity. Therefore, the arginine content in the diets did not affect the arginase activity. Meanwhile, for BFM fed fish, the value increased as protein level increased. Though there was no significant correlation between arginase activity and growth rate, it could be considered that protein degradation of SPC and CGM were high since these proteins are not utilized for protein synthesis. On the contrary, the activity in BFM group was low until protein became excess in the body.

**Table 6.** Response of biochemical parameters of yellowtail fed the test diets for 3 weeks[1]

| Diet | Arginase ($\mu$mol/min/g tissue) | Citrate synthase ($\mu$mol/min/g tissue) | RNA ($\mu$g/g tissue) | BUN (mg/100 ml) |
|---|---|---|---|---|
| BFM30 | 46.80 ± 12.23$^{d}$ | 2.71 ± 0.19$^{a}$ | 2.19 ± 0.19$^{a}$ | 19.60 ± 1.56$^{b}$ |
| BFM40 | 48.49 ± 3.90$^{d}$ | 1.38 ± 0.19$^{b}$ | 2.26 ± 0.15$^{a}$ | 27.99 ± 1.50$^{ab}$ |
| BFM50 | 80.63 ± 24.18$^{bcd}$ | 1.45 ± 0.39$^{b}$ | 2.62 ± 0.21$^{a}$ | 30.18 ± 3.28$^{a}$ |
| CGM30 | 88.48 ± 19.50$^{bc}$ | 1.31 ± 0.45$^{b}$ | 2.19 ± 0.12$^{a}$ | 14.73 ± 3.12$^{b}$ |
| CGM40 | 67.57 ± 11.06$^{cd}$ | 1.11 ± 0.24$^{b}$ | 2.19 ± 0.21$^{a}$ | 14.87 ± 1.66$^{bc}$ |
| CGM50 | 87.78 ± 6.12$^{b}$ | 1.45 ± 0.41$^{b}$ | 2.24 ± 0.13$^{a}$ | 16.27 ± 2.10$^{bc}$ |
| SPC30 | 129.30 ± 8.60$^{a}$ | 1.62 ± 0.19$^{b}$ | 1.97 ± 0.12$^{a}$ | 19.04 ± 1.79$^{b}$ |
| SPC40 | 107.25 ± 6.41$^{ab}$ | 1.24 ± 0.06$^{b}$ | 1.99 ± 0.18$^{a}$ | 20.54 ± 3.56$^{b}$ |
| SPC50 | 107.69 ± 9.30$^{ab}$ | 1.36 ± 0.10$^{b}$ | 2.28 ± 0.15$^{a}$ | 29.46 ± 1.18$^{a}$ |
| PFD | 91.26 ± 17.87$^{abc}$ | 2.51 ± 1.02$^{ab}$ | 1.55 ± 0.07$^{a}$ | 9.91 ± 3.77$^{c}$ |

[1] All values are means ± SD of 5 fish per each treatment. Values sharing the same superscripts in the same column are not significantly different ($P>0.05$).

Muscle is a major body mass in animal, therefore it contributes most on increasing body mass. Change in nutritional status and growth rate can also modify the metabolic organization of fish musle. In this respect, citrate synthase (CS), which is mitochondrial enzyme, was chosen in order to know whether its activity is affected by protein source. Pelletier et al. (1993) found that growth rate is positively correlated with CS activity. In the present study, muscle citrate synthase activities for all protein sources were almost comparable, whereas growth rate was different among the protein sources.

It has been known that there was a correlation between protein synthesis and growth rate (Mathers et al., 1992). Since RNA serves as both template and organizer for protein synthesis, the low RNA results in a decrease in the potential maximum rate of protein synthesis and may consequently limit growth (Houlihan et al., 1989). However, the relation between RNA content and growth was unclear, because the RNA value for BFM which gave the highest growth was only slightly higher than SPC and CGM.

Blood urea nitrogen (BUN) responded differently according to various protein sources and levels. The value for BFM and SPC increased as dietary protein level increased, while for CGM, it remain practically constant. These results may reflect metabolic state of protein in the animal. Since BUN measurement is easy to perform, it will be useful if biochemical meanings of BUN shifts are understood.

There are still some shortcomings in biological estimation. This requires a series of protein

levels for one protein source in feeding experiment thus, requiring very large space. This disadvantage can be overcome in biochemical estimation. Only one level of protein may be enough to determine the quality of a protein after feeding study. Moreover, biochemical changes which reflect dietary protein quality may appear faster than growth response. This should be another advantage from the practical viewpoint. Although clear results were not obtained, BUN may provide useful indication. However, further studies are needed for practical applications. Since feeding with PFD is accepted by fish, NPU and BV are useful parameters. Therefore, biological evaluation may be better in evaluating proteins at present, because for a rapidly growing fish such as yellowtail, growth response is clearly differentiated and strongly affected by dietary nutrition in a shorter time.

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