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Development of New Fish Feed for Marine Fish with Special Reference to Alternate Ingredients*1

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Contents

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Introduction

Present Status of Marine Fish Culture in Japan with Special Reference to Fish Feed

Japan is an island country surrounded by the sea, and the fisheries industry has been developed as a provider of food, especially animal protein, to the people. Aquaculture began with the culture of "nori" seaweed in the 16th century, and commercial fish culture started in the 18th century with common carp (Cyprinus carpio) and rainbow trout (*Salmo gairdneri, Oncorhynchus mykiss*). *Later, commercial culture of marine species was initiated with yellowtail* (*Seriola quinqueradiata*) in the 1920's. Annual production of aquaculture in Japan, including both marine and inland culture, has steadily increased since the end of World War II, and was 1,349 thousand metric tons (TMT) and valued at 659 billion yen in 1996. Although aquaculture represents only 18.2% of the country's total fishery production, its value accounts for 30.0%, showing that these products are high value and important for fisheries industry.

Nowadays, fish culture, both marine and fresh water, has become one of the most important food production industry providing the high value fish in Japan. There seems to be an increasing number of different fish species for culture especially in mariculture, and now about 30 species of marine fish are cultured in net pen in coastal areas. The total production of cultured marine fish was 256 TMT and valued at 266 billion yen, and it accounts for 20.1 and 47.0% of the total production volume and value of mariculture in 1996, respectively. In marine fish culture, yellowtail is a major species accounting for 60% of the total fish production in 1996. Yellowtail culture experienced rapid growth in the seventies, from 43 TMT in 1970 to 149 TMT in 1980, due to the fish's high market price and advancements in culture techniques. Since 1980, however, the annual production has remained around 150 TMT. In recent years, production of cultured red sea bream (*Pagrus major*) and striped jack (*Caranx delicatissimus*), Japanese flounder (*Paralichthys olivaceus*), and tiger puffer (*Takifugu rubripes*) is on the rise. Many researchers have conducted the trials with these fish to obtain the information of adequate culture techniques.

Nursery and grow out diets for marine fish culture are divided into three categories: fresh raw fish, moist pellets (MP), and dry pellets (DP). Fish farmer has traditionally used inexpensive fresh fish such as sardines, anchovy, mackerel, and sand lance, which are caught in large amounts in offshore and/or coastal areas. This practice has restrained the demand for formulated feeds. However, there are some problems associated with using these raw fish as feed from a nutritional point of view: viz., 1) nutrient contents vary depend on the place of catch, season, and fish size, 2) fat oxidizes easily, 3) contents of some vitamins are very low. The feed performance, fish health, and flesh quality will deteriorate if fish are reared with only raw fish feed. Therefore, usually raw fish feed is frozen and thawed before feeding. When feeding, farmers must add eutrophic medicine or multi vitamin mixture to meet the fish's dietary requirements. Moreover, feeding raw fish, especially in the minced or chopped form, for intensive culture has resulted in the pollution of sea water and culture grounds, aptly termed "self pollution", due to feed loss. This frequently led to occurrence of red tide at many farming sites in Japan, and was thought to be one of the factors of disease outbreaks. Thus the raw fish had reach its limit of use as feed for marine fish culture.

On the other hand, trials to develop a formula feed for marine fish were started in the 1960's, but success was not readily achieved. In the early 1980's, moist type formulated feed composed of minced fresh fish and formulated mash were developed through intensive studies promoted by the Fisheries Agency. The MP was effective in reducing self pollution due to the feeding, and to control dietary energy level by addition of fish oil. However, MP was not widely accepted by the industry, because of some drawbacks like: 1) growth rate of cultured fish was slightly inferior to those fed a raw fish, 2) fluctuations in nutrient contents depending on the raw fish, 3) vitamin contents falling below the requirement level of marine fish, 4) the need for the costly

pelleting machine and the labor to produce it. With regard to dry type feed, it was found that almost all of cultured fish species accept DP prepared using pellet mill, and grow normally, yellowtail being an exception. The reason why yellowtail dislike DP was thought to be because of the hardness and taste of the diet. The trial product was tested on yellowtail in the mid 1980's, but there were some problems still to be solved for practical use because it was costly and low in nutritional quality. Since then, Watanabe *et al.* (1991) have succeeded in developing a new type of dry pellet for yellowtail, so called "soft dry pellet (SDP)" prepared using a twin screw extruder. The SDP was a high calorie pellet that satisfies the nutritional requirement level of yellowtail, and was highly palatable and acceptable to this species. It has been proved that SDP is excellent in terms of growth rate, feed efficiency, and fish health for yellowtail, apart from lowering feeding related pollution, compared to MP used before (Watanabe *et al.* 1991). Another related development was the high calorie DP (steam pellet and/or extruded pellet) for red sea bream (Sakamoto 1994). It has been reported that growth performance of red sea bream fed the high energy diets was higher than those of fish on an ordinary DP and MP. At present, many kinds of commercial DP are manufactured for several fish species. Thus the formulated DP diet is recommended as an adequate feed form for sustainable development of marine fish culture.

However, the irony is that aquaculture feed for marine fish still depends greatly on the raw fish, which are locally caught and economically attractive. In 1997, total consumption volume of formulated feed (mash plus pellet) for yellowtail and red sea bream accounted for only about 10 and 30% (presumed values, data by Japan Fish Feeds Association) of total feed amount (wet value) for each fish, respectively.

The recent trend in the production of formulated diet for marine fish is shown in Fig. 1. Annual production of formulated feed for marine fish culture tends to increase in recent years. Production of pellet type feeds for yellowtail and red sea bream especially has showed a marked increasing trend. This is not related to an increase in aquaculture production but because farmers started depending on formula feed. They were left with few alternatives because of therapid decrease in the supply of raw fish catch and its spiraling cost. The catch of sardines largely dropped from maximum 4,500 TMT in 1988 to 300 TMT in 1996, and the catch level was lower than the raw fish requirement used as feed materials for mariculture in the past several years. Of course, the development of high quality DP contributed to the promotion and the increase of formulated feed consumption. Thus the use of formulated feeds will become more and more extensively for marine fish culture in future.

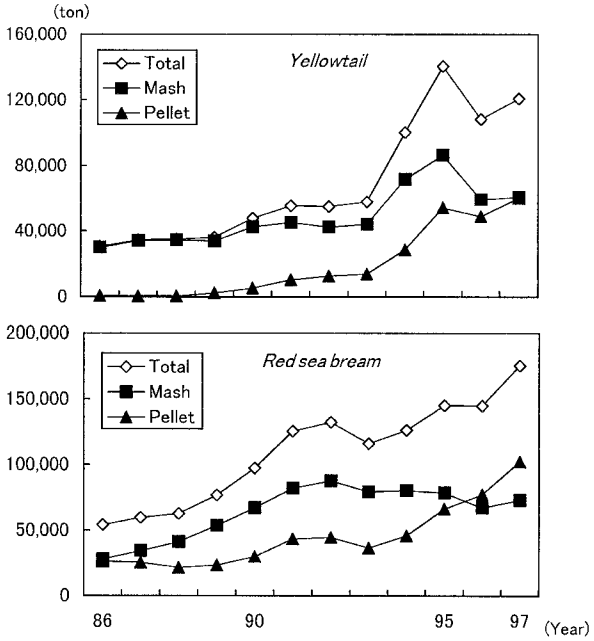

Fig. 1. Recent trends in the production of formulated diets for yellowtail and red sea bream.

Background and the present trends in the quest of alternative protein sources

Fish have a much higher dietary protein requirement than land animal. Therefore, fish feeds need large amount of protein rich ingredients. At present, fish meal is used as the main ingredient for dietary protein source; commercial marine fish feed in Japan generally approximately 55% of it. Other sources of protein ingredients are of plant origin such as defatted soybean meal (SBM) and corn gluten meal (CGM), both contributing only as little as 10% to the feeds. The reason for these choices is due to high nutritive value in

terms of protein content and essential amino acid composition in relation to the necessary requirements of several fish, and palatability. The recent rapid rise in formula feed production has accelerated the demand for fish meal. However, fish meal production has also been affected by the rapid reduction in catch of sardines which are used as raw material. The domestic production of fish meal largely decline with the decrease of the wild meal grade fish catch since 1989 (Fig. 2). In 1995, while about 590 TMT of fish meal was imported from Chile, Peru and other countries, domestic fish meal production was only 10 TMT, for feed ingredients of farm animal and fish, and manure. High grade fish meal produced from jack mackerel having an excellent quality of freshness imported from Chile is mainly used for aquaculture. Thus, at present Japanese feed companies depends entirely on the imports from foreign countries as the main source of fish meal. This situation will lead to instability in farm management practices because of the undue influence of the fluctuations in fish meal market price. To cope with this crisis, the need is to reduce the reliance on fish meal component in practical feeds by using the alternative protein sources without impairing growth and feed efficiency. The use of less expensive protein ingredients is important not only from the point of view of contributing to the stable supply of feed, but also from that of reduction of the feed cost. Therefore, it is now necessary to explore the alternative protein ingredients for fish meal.

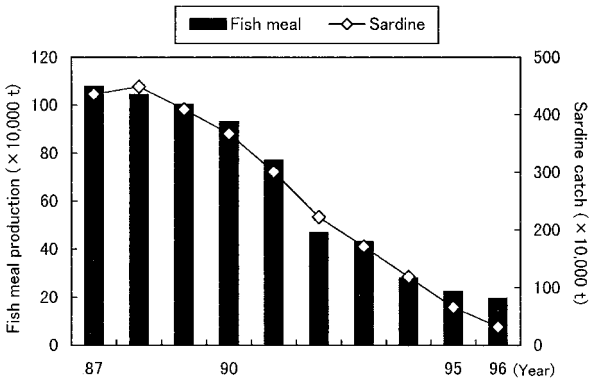

Fig. 2. Recent trends of fish meal production and sardine catch in Japan.

The potential alternate protein sources in aquaculture feeds has to satisfy several criteria for practical incorporation, viz., 1) inexpensive and available in large amounts, 2) have constant nutrient composition, 3) be of no harm to fish and humans, 4) contain a certain level of protein, 5) good amino acid composition to satisfy fish, 6) the digestibility of the protein should be high for fish, and 7) contain no anti nutritional factor (Akiyama 1994). The alternative protein ingredients that are already known can be generally classified as animal by products, single cell proteins, oil seeds, grain legumes and cereal grains (Tacon 1994). As concrete examples, meat meal (**MM**), meat and bone meal (**MBM**), blood meal (**BM**), poultry feather meal (**PFM**), etc. are animal products that have been tried. The potential plant protein sources are **SBM**, **CGM**, cottonseed meal, canola meal, peanut meal, rapeseed meal, sunflower seed meal, leucaena leucocephala leaf meal, and malt protein flour (**MPF**). The crude protein content in animal proteins is high compared with most plant ingredients, but both have poorer balance of essential amino acid (EAA) than fish meal, suggesting the inferiority in nutritional quality. From an economical point of view, plant protein is generally cheaper than animal source. Moreover, in evaluating the availability of plant ingredients, the adverse effects of anti nutritional factor should be considered. It was stated that enzyme inhibitors, anti vitamins, saponins, polyphenols, lectins, tannins, phytic acid, and gossypol are the most important antinutrients that causes negative effects on the physiological condition of an animal, and have also been found to bring to similar adverse effect in many fish (Kaushik 1989, Krogdahl 1989, Vohra and Kratzer 1991, Elstner 1996). However, almost all of anti nutritional factors can be fortunately destroyed through thermal treatment. Therefore, extrusion cooking is effective for inactivating several of the anti nutritional factors.

Among the alternative protein sources, defatted SBM is considered to be one of the most favored source that can practically be used in fish feeds, due to nutritional quality, greater availability, and lower costs. The world production of SBM is 99.7 million metric tons (**MMT**), and this material is the major protein meal from oilseed (1997 data). In contrast the production of fish meal and CGM is 6.1 and 13.7 **MMT**, respectively. It is available

in the world market at a price usually much below that of the fish meal. The crude protein content of SBM is about 45%, and SBM has a favorable EAA profile as compared to other plant protein sources, though it is deficient in certain amino acid like methionine. Moreover, SBM is palatable to many species of fish and shrimp, and digestibility of this ingredient protein is also high (Akiyama 1998, Watanabe *et al.* 1996). Therefore, SBM has received more attention than other plant proteins, and its potential use in fish feed has been tried out intensively.

Many researchers have attempted partial or complete reduction of fish meal by incorporation of cheaper animal and/or plant protein sources in feeds for several fish. Especially, many studies have been conducted for the fresh water fish because formulated feeds were developed earlier for them (Akiyama 1988, Kaushik 1989, Lim and Dominy 1991, Lovell 1991, Tacon 1993, 1994, Pongmaneerat 1994, Watanabe *et al.* 1996). The recent development of extruded pellets for marine fish has opened the possibility of using substitutive proteins for mariculture in Japan.

So far, among fresh water fish, utilization of SBM has been studied with the rainbow trout, chinook salmon (*Oncorhynchus tshawytscha*), coho salmon (*O. kistch*), common carp (*Cyprinus carpio*), tilapia (*Oreochromis mossambics, O. aureus, O. niloticus*×*O. aureus*), milk fish (*Chanos chanos*), and channel catfish (*Ictalurus punctatus*) (*Pongmaneerat 1994*). Most of the alternate protein studies have been carried out with the rainbow trout. Consequently, these and other results have demonstrated that approximately 20 40% SBM can be used to replace fish meal in aquaculture feed for several species. Also, other plant proteins like CGM, cottonseed meal, and rapeseed meal, and animal proteins like MM, MBM, and poultry by products have been successfully used as a partial protein source in diets for these species (Akiyama 1988, Watanabe *et al.* 1996, Pongmaneerat 1994).

On the other hand, availability of alternate protein sources for marine fish was studied with yellowtail, red sea bream, Japanese flounder, striped jack, and tiger puffer in Japan. The utilization of SBM combined with or without other ingredients in extruded pellet has been studied with yellowtail by the Laboratory of Fish Nutrition of the Tokyo University of Fisheries. In the first trial, Watanabe *et al.* (1992) evaluated the availability of SBM in a newly developed SDP to juvenile and adult yellowtail by feeding SDP containing 0 30% SBM. They observed that palatability and acceptability of fish were not affected by inclusion of SBM. They reported that SBM can be included up to 30% (substituting about 55% fish meal) as a fish meal replacer, although elevation of a protein and energy level will be needed to obtain feed performances comparable to a fish meal diet. In the second trial with juvenile and young yellowtail fed the SDP containing 0 50% SBM, Viyakarn *et al.* (1992) also found that palatability and acceptability were not influenced even when SBM in SDP was up to 50%. Growth and feed performances decreased corresponding to the elevation of SBM level, probably due to the reduced level of protein and energy in the SBM diets. They also confirmed that there was no apparent impairment of the performance parameters and health condition of yellowtail for dietary SBM levels up to 30%, as observed in the first trial. They also reported that a diet with 25% SBM and 15% CGM showed better performances than the 40% SBM, suggesting the potential of CGM as a protein source in combination with SBM. The availability of a highly purificated SBM product—the soy protein concentrate (SPC)— which has a protein content of about 70%, less carbohydrate and better EAA profile than SBM, was also evaluated in SDP, included at 50% with 5% fish meal, for juvenile and young yellowtail (Watanabe *et al.* 1995). Growth and feed performances of young fish were reduced after two months from the initiation of feeding, and the reason remained obscure. It was suggested that SPC cannot be used as a fish meal replacer at high proportions in SDP for yellowtail. Subsequently, Watanabe and co researchers examined the combinational use of alternate ingredients to yellowtail. The results from their experiments have shown that SBM in combination with CGM in some proportion can substitute more than 46% of fish meal in SDP for juvenile and young yellowtail (Watanabe *et al.* 1994). In another trial, they proved that fish meal can be replaced up to 62% by combinations of SBM, CGM, and MM in SDP for juvenile and young yellowtail without any adverse effect (Watanabe *et al.* 1995). Thus the recent investigation has shown that

combination of SBM with other protein sources was more effective compared to SBM alone as replacer of fish meal. This might be due to the elevation of dietary protein levels and inter balancing of deficient EAA by the different ingredients, thereby improving the feed protein utilization.

Researchers of Laboratory of Fish Nutrition of the Kouchi University have conducted feeding experiment of yellowtail to examine the availability of alternate proteins in single moist pellet (SMP). Shimeno *et al.* (1992a) reported that growth and feed efficiency were not adversely affected by including the SBM up to 30% in SMP for young yellowtail; further, SPC contained at 14% (equivalent to 20% SBM) was found to be a better protein source. Moreover, they observed an excellent growth rate for fingerling yellowtail too, when fed a SMP with 20% SBM (Shimeno *et al.* 1992b). Later, Shimeno *et al.* (1993a) investigated the availability of CGM, rapeseed meal, MM, and MBM as partial replacement (10 30%) for fish meal in SMP for young yellowtail. Better growth and feed performances were noted in fish on diets with a 10% replacement involving all the above mentioned protein sources in comparison to the control fish meal diet. The performances further increased only in the case of MM, even up to the maximum replacement of 30%. The trend in performance was just the opposite when plant protein sources were included at 30%. The MPF, which comes from brewer's spent grain, was also evaluated for its potential as an alternate ingredient (0 40%) in SMP to fingerling yellowtail (Shimeno *et al.* 1994). It was reported that this ingredient is useful as an alternative protein source, and can be substituted for fish meal even up to 20% in the diets. Shimeno and co researchers also investigated on the combinational use of alternate proteins in SMP for yellowtail. They reported that juvenile yellowtail fed SMP containing 30% SBM with 10 20% CGM or krill meal hardly reduced the growth and feed performances, indicating that adequate combination of protein sources can spare considerable amounts of fish meal with no problem (Shimeno *et al.* 1993b). Furthermore, Shimeno *et al.* (1993c) concluded that juvenile yellowtail fed SMP containing 20% SBM combined with up to 20% MM or CGM showed comparable growth performance to fish meal diet, suggesting that these combinations can be effectively used to replace large amount of fish meal in yellowtail diets. So far, the above authors suggested that a combination of alternative proteins can be incorporated up to around 40% (substituting about 60% fish meal) in both SDP and SMP for yellowtail without significant difference in growth and feed performances, taking care that their EAA profile are balanced and protein and energy content elevated to a level comparable to the fish meal diet.

In other marine fish, Ukawa *et al.* (1994) reported that it is possible to incorporate 25% SBM in SMP for young red sea bream. Yamamoto *et al.* (1996) also noted that MPF can substitute 30% of white fish meal protein in DP for fingerling red sea bream. With Japanese flounder, a series of studies have been conducted at Central Research Institute of Electric Power Industry. Kikuchi *et al.* (1993, 1994, 1997) conducted feeding experiment with Japanese flounder to examine the utilization of feather meal, SBM, MBM, and MM (Sato and Kikuchi 1997) as substitutional protein sources for fish meal in extruded pellets, and reported that 20 40, about 50, 20, and 60% of fish meal protein can be replaced by the respective ingredient. Yamamoto *et al.* (1995) demonstrated that 20% replacement of white fish meal by MPF in fingerling Japanese flounder diet (DP) resulted in equivalent weight gain and feed efficiency to those of the control diet, and that use in combination with SBM would improve the performances of the fish fed MPF diets. Furthermore, Ukawa *et al.* (1996a, 1997) showed that fingerling tiger puffer can utilize SBM as a partial substitute for fish meal at a maximum level of 20 30% in SMP. They also found MBM as an effective fish meal replacer in SMP for this species (Ukawa *et al.* 1996b).

Thus, these recent investigations on the replacement of fish meal component with alternative protein sources in marine fish feeds have been successful. In marine fish and freshwater fish, however, replacing fish meal either partially or completely with alternative ingredients have varying degrees of success. It seems that the ability of fish to utilize alternate animal or plant meal differs among species. Therefore, the various alternative protein sources should be employed at a pre determined adequate inclusion level according to fish species, genetic

variation, size of fish, culture condition, and water quality.

The future prospects

Aquaculture is expected to play an increasingly important role in fishery production, and demand of available dietary protein ingredients will increase with the increase of formulated feed in the future. A large amount of plant protein feedstuffs has been used in domestic animal feeds, and fish meal inclusion was only 1.2% of the total amount of feed ingredients (1996 data). This suggests the possibility of entirely eliminating the fish meal component from feeds for domestic animals. The final goal for the effective utilization of alternate protein sources for fish is to develop feeds without fish meal, i.e., non fish meal diets. Until date, the high inclusion of alternate proteins in fish feeds have generally resulted in reduced growth and feed performances. The reason for poor utilization of such diets might be due to: 1) improper balance of essential amino acids, 2) adverse effect of anti nutritional factors, 3) presence of high proportions of undigestible fiber and carbohydrate, and 4) decrease of palatability. This necessitates an adequate supplementation of synthetic amino acids in the non fish meal diets to compensate for the EAA deficiency of the selected ingredients, thereby improving the EAA profile of the diets. Moreover, some alternate ingredients should be selectively incorporated in the non fish meal diets to elevate the levels of dietary protein and energy comparable to the ordinary fish meal based diets.

To enhance the utilization of alternate protein sources, supplementation of crystalline amino acids to diets has been attempted for some fish species. The results obtained from several researchers, however, do not show the agreement in the efficacy of supplementing fish feeds with EAA. It has been reported that nutritional quality of diets containing alternate proteins for fish meal could be improved by supplementing EAA mixture for carp (Viola *et al.* 1982, Murai *et al.* 1986, 1989b, Pongmaneerat *et al.* 1993) and rainbow trout (Dabrowska and Wojno 1997). In contrast, Rumsey and Ketola (1975) found no benefit in supplementing SBM with individual EAA for rainbow trout. With yellowtail, Takii *et al.* (1989) demonstrated that supplement of EAA mixture to 20% SPC diet (SMP form) improved growth rate and feed efficiency. However, Shimeno *et al.* (1992a) observed that 20% SBM diet supplemented with methionine and lysine did not enhance performances for this species. Thus, more information on the absorption of individual supplemental EAAs and the effects of such supplements on growth and feed performances is needed for developing non fish meal diets for marine fish.

The situation looks more given from another angle, the decline in capture of meal grade fish has brought about the shortage of not only fish meal but also fish oil which has been used as dietary lipid source. Dietary lipids play an important role as energy and essential fatty acid (EFA) sources. At present, supply of fish oil depends largely on the imports, and the reduction of fish oil component by using alternate lipid sources is necessary for the stable supply of fish feeds. Therefore, effective utilization of alternate lipid source should also be on the priority agenda when re designing the marine fish feeds. The potential of several animal and plant oil as energy source were examined in some fresh water fish (Yu *et al.* 1997a, 1997b, Takeuchi *et al.* 1978, 1979, 1981). It was demonstrated that availability of oil to fish was largely affected by the melting point (mp) which was related with digestibility, but was slightly affected by water temperature (Takeuchi *et al.* 1979). It was reported that animal fat－beef tallow of mp. 38℃－could be effectively utilized as energy source in diets for carp and rainbow trout (Takeuchi *et al.* 1978). Other plant oils like soybean oil and linseed oil also showed high apparent digestibility for rainbow trout. These few pieces of information indicate the possibility of animal and plant oils having a low mp, to serve as a partial fish oil replacer in marine fish feeds, if the dietary EFA level satisfied the requirement of marine fish.

From these viewpoints, the objective of the present study is to obtain basic information to develop a low or perhaps even non fish meal diets for yellowtail and red sea bream. The strategies of the study were:

1) To confirm the effectiveness of alternative proteins in SDP for yellowtail by rearing them on a practical scale for a long period (Chapter 1).
2) To evaluate the availability of alternative protein sources in a newly developed high energy DP for red sea bream (Chapter 1).
3) To investigate the use of alternate protein and lipid sources in practical feeds for yellowtail (Chapter 2).
4) To investigate the effect of fish meal reduction on growth and feed performances in diets for yellowtail and red sea bream (Chapter 3).
5) To evaluate the utilization of non fish meal diets having different ingredient compositions for yellowtail and red sea bream (Chapter 3).
6) To examine the effect of EAA supplementation on the utilization of non fish meal diets for yellowtail, and to evaluate the availability of supplemental EAA in terms of plasma free amino acid concentrations (Chapter 4).

This work will provide valuable information on the practical utilization of alternative protein and lipid sources in diets for yellowtail or red sea bream. The knowledge obtained from this study is intended to contribute significantly to the development of practical least cost, nutritionally balanced formulated diets, circumventing the critical supply of fish meal. Moreover, it may also serve as a guide for the feed manufacture, providing the basal information on utilization of non fish meal diets for marine fish.

General Methodology

Growth and Feed Performances

Growth rate (GR), feed gain ratio (FGR, feed conversion ratio), daily feed consumption (DFC), and protein efficiency ratio (PER) were calculated by the equation given below:

- Growth rate (%) = (Final body wt. (g) − Initial body wt. (g)) / Initial body wt. × 100
- Feed gain ratio = Weight gain (g) / Feed intake (g) in dry matter
- Daily feed consumption (%) = Feed intake (g) / ((Initial body wt. (g) / 2 + Final body wt. (g) / 2 + Sample and dead body wt. (g) / 2) × Feeding day) × 100
- Protein efficiency ratio = Weight gain (g) / Protein intake (g)

Nutrient Composition Analysis

Proximate composition and gross energy content of diets and fish carcass were determined by the following methods/equipments: Crude protein: Kjelchecker (KC 42 Yamato Anritsu, Japan); Crude lipid: extraction by chloroform methyl alcohol (2:1) solution (method of Folch); Crude starch: method of Somogyi Nelson; Crude ash: incineration in a muffle furnace (FA 21, Yamato Co., Japan) at 550℃; Moisture: desiccation at 105℃ to constant weight; Gross energy content: auto calculating bomb calorimeter (CA 4P Shimadzu Co., Kyoto, Japan).

Fatty acid composition was analyzed by gas chromatograph (GC 14A, Shimadzu Co., Kyoto, Japan) with a hydrogen flame ionization detector. Amino acid analysis was done by Japan Food Research Laboratories or Central Research Institute of Nippon Formula Feed Co. Ltd. (Amino acid automatic analyzer or high performance liquid chromatograph methods).

Hematological Characteristics and Hemochemical Constituents

Fish were starved for 24 48 hours prior to blood drawing. The hematocrit value was determined by microhematocrit within 2 hours after the blood samples were collected. A part of blood was centrifuged at 3,000 rpm for 10 15 min to obtain plasma samples. The hemochemical constituents and enzyme activities were analyzed by methods as follows: aspartate aminotransferase (GOT, UK kinetic method of Karmen), alanine aminotransferase (GPT, UV kinetic method of Wroblewski La Du in fresh condition within 24 hours), alkaline phosphatase (ALP, p nitrophenil phosphate method), glucose (GLU, mutarotase glucose oxide method), triglyceride (TG, glycerol 3 phosphate oxidase DAOS method), phospholipid (PL, choline oxidase DAOS method), total cholesterol (TCHO, cholesterol oxidase DAOS method), free cholesterol (FCHO, cholesterol MEHA method), urea nitrogen (BUN, urease GIDH method), total protein (TP, biuret method). All of the items were analyzed by the automatic biochemical analyzer (CL 7100 Shimadzu Co., Kyoto, Japan) using commercial clinical examination kits (Wako Pure Chemical Co., Japan).

Chapter 1: Use of Alternate Protein Sources

1.1 Yellowtail - Long-Term Feeding in Practical Scale Net Cages

Numerous researches have evaluated the potential of various less expensive animal or plant proteins as substitute for fish meal component in fish feeds with varying results. These attempts have been made to deal with the shortage of fish meal, used as a main protein source in formula feeds, due to the quick decline in the meal grade fish in Japan. Our series of trials on the availability of alternate proteins for yellowtail have already demonstrated that SBM, CGM, and MM can be used as excellent protein ingredients in the extruded dry pellet produced by a large twin screw extruder (SDP). We have reported that juvenile and adult yellowtail could use the SBM alone as substitute for fish meal at 30% (substitution of about 55% fish meal) in the SDP (Watanabe *et al.* 1992, Viyakarn *et al.* 1992). Subsequently, we also found that the combination of SBM, CGM, and MM at levels of 42 47% could successfully replace 54 62% fish meal in the SDP for yellowtail (Watanabe *et al.* 1995). The information from these trials will be important for effective formulation of alternate proteins in practical fish feeds. However, these studies were conducted with fish retained in the small size net cages or in the tanks. For development of practical feeds for marine fish, it is necessary to obtain growth and feed performance data in commercial rearing conditions.

In this study, therefore, a long term feeding experiment (until fish grow up to a marketable size) was performed with yellowtail using commercial scale net cages, in order to prove the availability of alternate protein ingredients in practical diets.

Materials and Methods

Experimental Diets

The ingredient composition of the experimental diets and their nutrient contents determined are presented in Table 1 1. A commercial yellowtail SDP with 65% fish meal as a main protein source was used as a control (diet 1). Compositions of alternate protein ingredients of fish meal replaced diets (no. 2 4) were the same as those examined in the earlier study (Watanabe *et al.* 1995). These test diets were designed to contain 20 25% SBM, 5 15% CGM, and 12% MM (42 47% in total), to replace 54 62% of fish meal in the control diet. The diet preparation methods and extrusion conditions using a large size twin screw extruder by Sakamoto Fish Feed Co. were almost the same as described previously (Watanabe *et al.* 1991, Sakamoto *et al.* 1997). Wheat flour (8%) was used as binder, and vitamin and mineral mixtures (2 and 3%, respectively) were supplemented to satisfy the requirements of yellowtail (Takeda 1985). A commercial feed oil was added as lipid source at 15% to elevate the dietary energy levels similar to the control diet. Diet 4 was supplemented with 0.25% crystalline lysine to compensate the deficiency due to the incorporation of 15% CGM.

The levels of crude protein and lipid of the experimental diets ranged 46.0 47.5 and 21.4 22.1%, respectively, and there was no big difference in each

Table 1-1. Composition of the experimental soft dry pellet (SDP) with alternative proteins for yellowtail

Ingredient(%)		Diet no. 1	2	3	4
Local sardine meal	Commercial diet		30	30	25
Soy protein concentrate			0	0	0
Defatted soybean meal			25	20	20
Corn gluten meal			5	10	15
Meat meal			12	12	12
Wheat flour			8	8	8
Mineral mixture			3	3	3
Vitamin mixture			2	2	2
Feed oil			15	15	15
L-Lysine			0	0	0.25
*Analytical composition (%)**					
Crude protein		46.0	46.9	47.5	47.4
Crude lipid		21.5	21.4	21.7	22.1
Crude ash		9.8	8.3	8.0	7.2
Moisture		7.7	6.7	6.9	6.3
Gross energy (kcal/100g)		530	520	535	550
GE/CP (kcal/kg/%CP)		115	109	110	111

* Average of 10 determinations. Each test diet was freshly prepared 10 times according to fish growth during the feeding period of 17 months.

parameter among the treatments. The gross energy content was slightly higher in diet 4. The amino acid profile of the experimental diets determined by Japan Food Research Laboratories is presented in Table 1 2. Although the contents of lysine and methionine of fish meal replaced diets were slightly lower than those of the control diet, all the experimental diets contained sufficient amounts of amino acids to satisfy the requirements for yellowtail*2. The analytical methods for proximate composition of diets were described in the previous paper (Watanabe and Pongmaneerat 1991). Each test diets were prepared 10 times according to fish growth during the feeding period of 17 months, and all of diets were stored at −20℃ until use.

Table 1-2. Composition of essential amino acids of the experimental SDP with alternative proteins sources for yellowtail

Amino acid (g/100g diet)	Diet no.			
	1	2	3	4
Arginine	2.58	2.67	2.64	2.70
Lysine	3.23	2.90	2.87	2.99
Histidine	1.29	1.20	1.20	1.25
Phenylalanine	1.87	1.88	1.99	2.14
Tyrosine	1.40	1.37	1.45	1.56
Leucine	3.49	3.51	3.81	4.23
Isoleucine	1.85	1.75	1.78	1.87
Methionine	1.18	1.06	1.03	1.08
Cystine	0.48	0.51	0.54	0.56
Valine	2.25	2.15	2.19	2.28
Threonine	1.91	1.78	1.79	1.85
Tryptophan	0.49	0.47	0.45	0.48
Total	19.44	21.25	21.74	22.99

Feeding Conditions

The feeding trial was conducted using juvenile yellowtail (*Seriola quinqueradiata*) with an initial body weight of about 170 g at the Seibu Iohjima Sea Ranch. Seventeen thousand fish were acclimated with the commercial SDP for 1 month, and then they weredivided into 4 groups of 3,400 fish each in net cages (10×10×8 m). They were reared for 17 months, from Aug.'92 to Dec.'93, and were fed the respective diets once a day, 6 times per week, each time to near satiation. Water temperature ranged from 14 to 28℃. Average body weight of 30 fish randomly collected from each lot was determined every month, and that of 100 fish was recorded on termination of the experiment. Moreover, 5 fish were randomly sampled at 2 (Oct.'92), 4 (Dec.'92), 7 (Mar.'93), and 11 (Jul.'93) months after the initiation of feeding for determination of moisture, protein, lipid, and ash contents of dorsal muscle by the same analytical methods described in a previous report (Watanabe and Pongmaneerat 1991).

Hemochemical Assessment

At the end of trial, 5 fish from each group were taken for analysis of hemochemical constituents in blood plasma to evaluate the fish health condition. Fish of all groups were starved for 2 days before bloodsampling, and analytical procedures for each item examined were those described in the earlier paper (Watanabe *et al.* 1992). Data from assessment were statistically treated by analysis of variance (ANOVA) and Duncan's multiple range test ($p<0.05$).

Results and Discussion

Feed Performances

The growth and feed performance data are presented in Table 1 3. Palatability and acceptability of the experimental diets were not influenced by inclusion of the alternate proteins during the long feeding period. The initial body weight of 170 g grew up to about 1.0 kg for all the experimental lots after 4 months (Dec.'92), and there was no marked difference in growth between the control and test diet groups. In May (8 months) next year, the growth of fish fed the alternate protein diets was slightly inferior to that of fish on the control diet (the control group: 1.8 kg, the test groups: 1.6 kg). Thereafter, from June to July, the growth of test group fish

*2 T.Watanabe *et al.* : Abst. Metg. Japan. Soc. Fisheries Sci., April, 1995, p. 33 (in Japanese).

retarded because of the shortage of feed supply due to delayed schedule of feed preparation. When the diets were supplied, their growth speed recovered at levels comparable to the control. At the end of feeding, fish fed the control diet showed the highest average body weight and growth rate. Fish on the control diet grew up to 4.7kg on average, while those on the test diets ranged 3.8 4.0 kg. Growth of fish fed the fish meal replaced diets was around 80 85% of that of fish on the control diet. Feed gain ratio varied from 1.75 to 2.40, and the best value was also observed in fish of the control group. Thus, growth and feed utilization in fish fed the fish meal replaced diets were inferior to those of the control. As mentioned above the lower growth of fish on the test diets could be attributed to the lack of feed supply during the summer season. In fact, after test feeds were supplied normally, growth of these test fish returned to normal since August. Therefore, it was assumed that if the test fish groups were fed the normal amount of feeds, their growth and feed performances would be comparable to those of the control. The mortality of fish during the experimental period was mainly caused by bacterial infections such as pseudotuberculosis and streptococcal diseases. The cumulative mortality was 47.5% for the control group and 30.1 52.9% for the test groups, and was higher in fish on diet 2. These results indicated that dietary inclusion of alternate proteins did not exert ill effect on the experimental fish.

Table 1-3. Growth and feed gain ratio of yellowtail fed the experimental SDP with alternative protein sources

Diet no.	Av.body wt. (g)		Growth rate	Feed gain ratio*	Mortality
	Initial	Final	(%)		(%)
1	170.5	4690	2651	1.75	47.5
2	170.5	3780	2117	2.40	52.9
3	170.5	3967	2227	2.28	33.7
4	170.5	3926	2203	2.18	30.1

* g feed / g weight gain.

Previously we reared young yellowtail with test diets having the same ingredient formulation as those used in the present study in small net cages for 146 days, and found that the test diets gave growth and feed performances comparable to the control fish meal diet (Watanabe *et al.* 1995). Also in this experiment, no marked difference was observed in growth performance between the test and control groups during the period of about 10 months, although the growth and feed gain ratio in the test diet groups were inferior to those of the control at the end of feeding due to shortage of diets during the summer season when yellowtail generally show rapid growth. Therefore, it may be concluded that the combination of SBM, CGM, and MM in adequate proportion can be used as substitute of around 50% fish meal in the practical feeds for yellowtail.

Among the alternate protein diets, the lowest growth and feed performance were noted for fish fed diet 2 (25%SBM + 5%CGM + 12%MM). This result was similar to that obtained in our previous work (Watanabe *et al.* 1995), suggesting that this formulation was not suitable to obtain excellent fish growth. The lower feed performances of diet 2 might be due to the lower digestible energy level and/or inadequate amino acid balance in the diet. Therefore, it was suggested that utilization efficiency of diets with alternate proteins could be improved by elevation of dietary energy or supplementation of deficient amino acids.

Proximate Composition of Dorsal Muscle

Results of proximate analysis of dorsal muscle from the experimental fish are presented in Table 1 4. The muscle protein content did not differ largely between the treatments and between the initial (22.9%) and other sampling points (22.2 23.9%). Also the ash content was not greatly affected by dietary inclusion of alternative ingredients. The crude lipid content tended to increase with growth for all the experimental lots until Mar.'93, but there was no remarkable difference among the treatments. However, the lipid value on Jul.'93 was higher in fish fed the control diet (12.9%) than those fed diets containing alternate proteins (6.2 9.4%). Again, this might have resulted from the shortage of feed supply during June and July for the test groups as mentioned above.

Hemochemical Characteristics

The analytical results of hemochemical constituents for the experimental fish are presented in Table 1 5. The values of all the examination items were estimated to be within the normal ranges. ALP (Alkaline phosphatase) activity was higher in the diet 2 group than others. There was no marked difference in glucose value between the treatments. The triglyceride level of fish fed diets 2 and 4 was slightly higher than the rest. Other lipid metabolism parameters such as phospholipid, total cholesterol, an dfree cholesterol were higher in fish on the control and diet 4 than those on diets 2 and 3. This may indicate that the physiological status in fish fed diet 4 was comparable to the control, and was superior to those in fish on other fish meal replaced diets. The blood urea nitrogen, creatinine, and total protein were not significantly different among the groups, suggesting that the protein metabolism was normal for all the experimental lots. Judging from these results, it appeared that there was no marked difference in physiological conditions between the test and control groups, and that health status of fish were not affected by inclusion of alternate proteins in the nutritionally balanced diets.

Table 1-4. Proximate composition (%) of the dorsal muscle of yellowtail fed the experimental SDP in practical scale net cages*1

Diet no.	Moisture	Crude protein	Crude lipid	Crude ash
Initial (Aug. '92)	73.7	22.9 (87.1)*2	3.1 (11.8)	1.5 (5.7)
*Oct. '92 (2-month)*3*				
1	70.6	22.4 (76.2)	6.2 (21.1)	1.9 (6.5)
2	70.8	23.1 (79.1)	5.6 (19.2)	1.8 (6.2)
3	70.8	23.5 (80.5)	5.7 (19.5)	1.8 (6.2)
4	70.2	23.8 (79.9)	5.6 (18.8)	1.7 (5.7)
Dec. '92 (4-month)				
1	65.5	23.1 (67.0)	9.5 (27.5)	1.9 (5.5)
2	65.4	22.6 (65.3)	11.6 (33.5)	1.7 (4.9)
3	67.2	23.0 (70.1)	8.6 (26.2)	1.7 (5.2)
4	69.7	22.8 (75.2)	8.2 (27.1)	1.7 (5.6)
Mar. '93 (7-month)				
1	64.0	23.1 (64.2)	12.5 (34.7)	2.0 (5.6)
2	65.0	23.5 (67.1)	11.1 (31.7)	1.9 (5.4)
3	65.0	23.9 (68.3)	10.6 (30.3)	2.1 (6.0)
4	64.3	22.5 (63.0)	11.9 (33.3)	1.8 (5.0)
Jul. '93 (11-month)				
1	64.2	22.2 (62.0)	12.9 (36.0)	1.9 (5.3)
2	70.2	23.3 (78.2)	6.2 (20.8)	2.0 (6.7)
3	67.4	22.1 (67.8)	9.4 (28.8)	1.8 (5.5)
4	68.2	23.0 (72.3)	6.9 (21.7)	1.9 (6.0)

*1 Samples from 5 fish were pooled for analysis.
*2 Figures in parentheses are values on dry matter basis.
*3 The month of sampling and body weight determination, and feeding period (within parentheses).

Table 1-5. Results of hemochemical assessment for yellowtail fed the experimental SDP with alternative protein sources*

		Diet no. 1	2	3	4
ALP	(IU / l)	98±13	113±12	103±32	101±34
GLU	(mg / 100ml)	89±4	82±2	82±6	85±7
TG	(mg / 100ml)	70±23	157±44	88±72	128±69
PL	(mg / 100ml)	802±117	753±64	683±54	803±116
TCHO	(mg / 100ml)	344±32^{a}	293±23^{b}	265±17^{b}	311±47^{ab}
FCHO	(mg / 100ml)	132±28	125±7	113±3	134±24
EC	(mg / 100ml)	212±9^{a}	167±17^{b}	153±18^{b}	178±29^{b}
Ester ratio	(%)	62.0±4.9	57.1±1.7	57.4±3.2	57.1±3.8
BUN	(mg / 100ml)	9.0±2.1	11.0±2.4	10.3±1.1	11.8±2.0
CRE	(mg / 100ml)	1.2±0.3	1.3±0.4	1.0±0.1	1.2±0.4
TP	(g / 100ml)	3.3±0.2	3.4±0.2	3.4±0.2	3.3±0.2

* Mean ± standard deviation (n=5). Figures in a row with different superscripts are significantly different from each other ($p< 0.05$) when analyzed using Duncan's multiple range test.

From the results of long term feeding together with hemochemical examination, it may be concluded that dietary fish meal could be partially replaced by the combination of SBM, CGM, and MM at the substitution level of around 50% without marked impairment of growth and feed performance, and health of yellowtail, and that the nutritional quality of alternate protein diets was almost comparable to commercial fish meal diet.

1.2 Red Sea Bream

1.2.1 Utilization of Defatted Soybean Meal

To cope with the shortage of fish meal due to the decline in capture of feed grade fish, many feeding studies have been conducted to evaluate substitutive protein sources for fish meal which is the main protein source in formulated fish feed. SBM has been used as a partial replacement protein source for fish meal in many fish feeds, because it is relatively abundant and nutritionally adequate. In the previous studies, we had reported that SBM

alone could be included at about 30% (substituting about 55% fish meal), and SBM combined with CGM and MM could replace up to about 60% of fish meal without any adverse effect in SDP for both juvenile and adult yellowtail (Watanabe *et al.* 1992, 1995, Viyakarn *et al.* 1992).

Red sea bream is one of the most important cultured fish species in Japan, and the annual production was 72,478 t in 1993. Recently, high energy dry pellets (extrusion pellets and/or steam dry pellets) produced by a twin screw extruder or a pellet mill connected with an expander has been newly developed for the red sea bream. It has been reported that growth performance of red sea bream was improved by feeding the high energy pellet in comparison with an ordinary type pellet (Sakamoto 1994). The high energy type pellet may eventually become a preferred culture feed for red sea bream. The present study examined the utilization of a protein source, alternative to fish meal, in the newly developed high energy dry pellets for the fish. Two feeding experiments were conducted to investigate the availability of SBM with or without extrusion processing at a level of 30% in steam dry pellets (DP) and for comparing it with extruded SDP containing 30% SBM (Aoki *et al.* 1996).

Materials and Methods

Experimental Diets

The formulations and proximate compositions of the experimental diets are shown in Table 1 6. The diets were prepared as DP (diets 1 3) and extruded SDP (diet 4) by Sakamoto Fish Feed Co., Japan. Local sardine meal (fish meal) and krill meal were used as protein sources for the control diet (diet 1). Diets 2 and 3 contained SBM and extruded SBM respectively, at a level of 30% (substitution of about 60% fish meal). Diet 4 also contained 30% SBM but was prepared as SDP by a large size twin screw extruder (Buhler Co.) (Watanabe *et al.* 1991). Wheat flour was used as binder and dietary carbohydrate source, and a commercial feed oil as the lipid source. Vitamin and mineral mixtures were supplemented equally to all the experimental diets (Watanabe *et al.* 1991). The diets were kept in the freezer (−20 ℃) until use.

Table 1-6. Composition of the experimental diets for red sea bream

Ingredient(%)	Diet no.* 1	2	3	4
	Protein-energy level			
	HP-HE	MP-HE	MP-HE	MP-HE
Local sardine meal	54	21	21	25
Defatted soybean meal	0	30	0	30
Extruded soybean meal	0	0	30	0
Krill meal	10	10	10	10
Wheat flour	19	19	19	7
Potato starch	0	0	0	3
Wheat gluten	0	0	0	3
Vitamin mixture	2	2	2	2
Mineral mixture	2	2	2	2
Feed oil	11	14	14	18
Binder	2	2	2	0
Nutrient contents determined (% as is basis)				
Expt. I				
Crude protein	47.5	40.5	40.5	42.2
Crude lipid	18.4	17.5	17.7	20.7
Crude starch	15.7	19.7	20.6	15.2
Crude ash	11.1	8.6	8.5	8.5
Moisture	6.2	7.0	5.6	7.7
Expt. II				
Crude protein	47.2	40.3	40.2	42.5
Crude lipid	20.9	20.5	19.7	22.4
Crude starch	15.7	19.7	20.6	15.2
Crude ash	10.8	8.4	8.4	8.5
Moisture	6.7	6.2	6.2	6.7

* Diet 4 was prepared as soft-dry pellet (SDP), and others were all ordinary dry pellets.

The crude protein level was about 47% for the control diet (high protein high energy level: HP HE) and about 40% for the SBM diets (medium protein high energy level; MP HE). The crude lipid level was 17 21% (Expt. I) and 19 23% (Expt. II). Thus the experimental diets were not iso nitrogenous, iso energy diets. Fatty acid compositions of total lipid extracted from the experimental diets are shown in Table 1 7. The proportion of n 3 highly unsaturated fatty acids (n 3 HUFA) which are essential for red sea bream ranged from 22.4 to 24.3%, and there was no marked difference in content among the diets. The analytical methods for proximate composition and fatty acids were described in a previous paper (Watanabe and Pongmaneerat 1991).

Feeding Conditions

Two feeding experiments were conducted with juvenile red sea bream (*Pagrus major*). Expt. I was carried out at the Fisheries Research Institute of Mie in floating net cages set in creeks, and Expt. II at the Nagasaki Prefectural Institute of Fisheries, in aquariums.

Expt. I : Juvenile red sea bream reared up to about 9 g with a commercial diet were used for the experiment. They were divided into four dietary groups of 960 fish each in 3×3×3 m net cages provided with a net cover and fed the different diets for 147 days (from Aug. 3 to Dec. 27, 107 days feeding). The water temperature ranged from 18.2 to 28.3℃ (average 24.0℃). The experimental diets were hand fed, once a day in the morning, 5 or 6 days per week. During the experiment, average body weight of 20 fish that were randomly sampled from each group was recorded every 15 23 feeding days. At the end of experiment, all the fish in net cages were weighed and 10 fish were randomly collected for analysis of proximate composition of dorsal muscle and liver. Analytical techniques followed were as in a previous paper (Watanabe and Pongmaneerat 1991).

Expt. II : Juvenile red sea bream weighing 6 g on average, kept on a commercial diet, were used for the experiment. They were divided into four groups of 25 fish each in 100 *l* aquariums. The fish were fed different experimental diets three times (08:00, 11:30 and 16:00) a day, to satiation (from Aug. 28 to Oct. 8, 42 days feeding). During the experiment, the aquariums were continuously supplied with filtered sea water at an exchange rate of 45 times per day, and water temperature ranged from 23.0 to 29.0℃.

Table 1-7. Fatty acids composition of lipid in the experimental diets

Fatty acid (% area)	Diet no. 1	2	3	4
14:0	5.0	5.1	4.9	5.4
14:1	0.1	0.1	0.1	0.1
15:0	0.3	0.3	0.3	0.3
16:0	16.4	15.7	15.7	16.0
16:1n-7	7.1	7.6	7.4	7.7
17:0	0.6	0.6	0.6	0.6
16:3n-6	0.4	0.4	0.4	0.5
16:3n-3	0.2	0.2	0.2	0.2
18:0	2.8	2.5	2.5	2.3
18:1n-9	13.6	14.1	14.5	13.5
18:1n-7	4.5	4.6	4.4	4.6
18:1n-5	0.2	0.2	0.2	0.2
18:2n-6	4.2	5.6	6.1	3.8
18:3n-6	0.2	0.2	0.2	0.2
18:3n-3	0.9	1.1	1.2	1.1
18:4n-3	1.6	1.7	1.7	2.0
18:4n-1	0.2	0.2	0.2	0.2
20:0	0.2	0.1	0.2	0.2
20:1n-11	3.4	3.8	3.9	4.0
20:1n-9	2.1	2.2	2.2	2.0
20:1n-7	0.2	0.2	0.2	0.2
20:2n-9	0.1	0.1	0.2	0.2
20:2n-6	0.2	-	-	-
20:3n-6	0.1	0.1	0.1	0.1
20:4n-6	0.8	0.7	0.7	0.2
20:3n-3	0.1	0.1	0.1	0.1
20:4n-3	0.5	0.5	0.5	0.5
20:5n-3	13.0	13.2	12.9	13.4
22:1n-13 +22:1n-11	4.2	4.2	4.0	4.1
22:1n-9	0.7	0.4	0.3	0.4
22:1n-7	0.1	0.1	0.1	0.1
22:4n-6	0.1	tr.	tr.	0.1
22:5n-6	0.1	0.1	1.2	0.1
22:5n-3	1.3	1.2	1.2	1.3
22:6n-3	9.4	8.0	7.8	8.2
ΣMonoene	36.3	37.5	37.3	36.9
Σn-6	6.0	7.2	7.6	4.9
Σn-3	27.0	26.0	25.4	26.9
Σn-3 HUFA	24.3	23.0	22.4	23.6

Results

Expt. I

The results of the feed performance in Expt. I are shown in Table 1 8 and Fig. 1 1. Palatability and acceptability in fish were not influenced by inclusion of SBM in both DP and SDP as in the case of yellowtail fed SDP (Watanabe *et al.* 1992, 1995, Viyakarn *et al.* 1992). Growth rate and feed gain ratio of the fish fed diets containing SBM at the 30% level (diets 2 4) were slightly inferior to fish on the control diet without SBM. This might be due to the reduction in dietary protein content in the SBM diets, as shown in Table 1 6. There was no marked difference in both growth rate and feed gain ratio among the dietary groups fed diets containing the different types of SBM. Therefore, the extrusion processing of SBM did not affect feed performance. Protein efficiency ratio ranged from 1.83 to 1.87 and was similarly uninfluenced by the diet treatment. The death of experimental fish on diet 4 during the period Nov. 6 Dec. 9 was accidentally caused by typhoon.

The crude protein and crude lipid contents and moisture of liver and dorsal muscle taken from the experimental fish are shown in Table 1 9. In the fish fed diet 4, the protein content in muscle was slightly lower

Table 1-8. Growth of juvenile red sea bream fed diets containing various types of soybean meal (SBM) in net cages

Diet no.*1		No. of fish	Av.body wt.*2 (g) Initial	Final	Growth rate (%)	Feed gain ratio	Daily feed intake*3	Protein efficiency ratio*4	Mortality (%)
Aug.3 ~ Aug.20 (15 days feeding)									
1	0% SBM	960	8.9	24.6	176.4	0.57	3.55	3.70	0.0
2	30% Un-SBM	960	8.9	23.0	158.4	0.63	3.73	3.90	0.1
3	30% Ex-SBM	960	8.9	22.8	156.2	0.64	3.75	3.85	0.0
4	30% SBM in SDP	960	8.9	23.2	160.7	0.62	3.70	3.80	0.0
Aug.21 ~ Sep.13 (21 days feeding)									
1	0% SBM	940	24.6	39.5	60.6	1.09	2.43	1.92	0.2
2	30% Un-SBM	939	23.0	36.4	58.3	1.22	2.62	2.03	0.0
3	30% Ex-SBM	940	22.8	35.0	53.5	1.34	2.69	1.84	0.4
4	30% SBM in SDP	940	23.2	34.7	49.1	1.38	2.60	1.71	0.2
Sep.14 ~ Oct.10 (16 days feeding)									
1	0% SBM	918	39.5	48.9	23.8	1.60	2.10	1.32	0.3
2	30% Un-SBM	919	36.4	45.9	26.1	1.56	2.26	1.58	0.5
3	30% Ex-SBM	916	35.0	47.2	34.9	1.25	2.27	1.98	3.6
4	30% SBM in SDP	918	34.6	42.5	22.8	1.83	2.31	1.30	6.6
Oct.11 ~ Nov.5 (19 days feeding)									
1	0% SBM	894	48.9	69.3	41.7	1.10	1.99	1.92	0.3
2	30% Un-SBM	894	45.9	61.1	33.1	1.51	2.25	1.63	0.0
3	30% Ex-SBM	863	47.2	57.7	22.2	2.04	2.15	1.21	0.6
4	30% SBM in SDP	837	42.5	65.8	54.8	0.88	1.98	2.70	1.7
*Nov.6 ~ Dec.9 (23 days feeding)*5									
1	0% SBM	871	69.3	101.7	46.8	1.03	1.70	2.04	2.0
2	30% Un-SBM	874	61.1	91.0	48.9	1.19	2.03	2.08	6.4
3	30% Ex-SBM	837	57.7	84.3	46.1	1.26	2.06	1.96	7.9
4	30% SBM in SDP	803	65.8	85.4	29.8	1.39	1.68	1.71	22.0
The entire culture period (Aug.3 ~ Dec.27 : 107 days feeding)									
1	0% SBM	960	8.9	121.1	1261	1.13	1.78	1.86	2.9
2	30% Un-SBM	960	8.9	103.4	1062	1.32	2.04	1.87	6.9
3	30% Ex-SBM	960	8.9	101.1	1036	1.33	2.03	1.85	12.7
4	30% SBM in SDP	960	8.9	101.5	1040	1.29	1.94	1.83	27.4

*1 Un: untreated, Ex: extruded.
*2 Average of 20 fish.
*3 g / 100g body wt.
*4 g gain / g protein intake.
*5 The mortality during this period was mainly caused by typhoon.

than the other groups, although that in liver did not differ markedly from the others. The lipid contents in muscle were highest in fish fed the diet 4. The same tendency was observed for liver lipid too, probably due to the higher amount of dietary lipid.

Expt. II

The results of Expt. II are shown in Table 1 10. Growth rate was highest in fish fed the control diet, and was slightly lower when SBM was included (diets 2 4). The same tendency was observed for feed gain ratio. However, average body weight at the end of the experiment in fish fed diets containing 30% untreated SBM (diet 2) was not significantly different ($p<0.05$) from that infish fed the control diet. Moreover, the feed performance was not significantly different among the groups fed diets containingthe three types of SBM (diets 2 4). In addition, there was no marked difference in protein efficiency ratio among all the dietary groups. The differences in feed performance between the control and test diets might be due

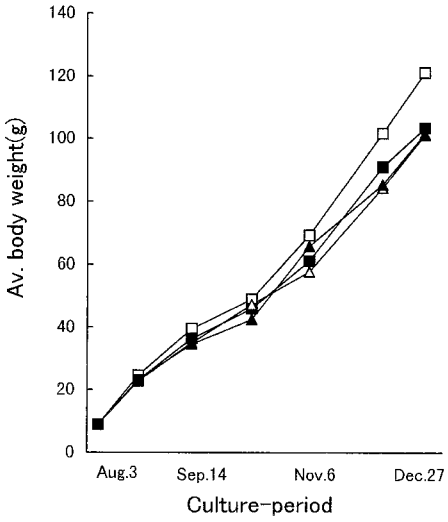

Fig. 1-1. Growth curves of juvenile red sea bream fed diets containing various types of SBM in net cages. □:Diet 1, ■:diet 2, △:diet 3, ▲:diet 4.

to the reduced protein content of SBM diets, as observed in Expt. I.

Table 1-9. Proximate composition (%) of the dorsal muscle and liver from red sea bream fed diets containing various types of SBM in net cages*1

Diet no.		Moisture	Crude protein	Crude lipid
Dorsal muscle				
1	0% SBM	73.8	20.4 (77.9)*2	3.9 (14.9)
2	30% Un-SBM	73.4	19.9 (74.8)	4.9 (18.4)
3	30% Ex-SBM	74.0	19.1 (73.5)	4.6 (17.7)
4	30% SBM in SDP	74.3	17.6 (68.5)	6.0 (23.3)
Liver				
1	0% SBM	65.8	20.8 (60.8)	15.0 (43.9)
2	30% Un-SBM	67.9	20.5 (63.9)	13.4 (41.7)
3	30% Ex-SBM	68.1	19.6 (61.4)	13.5 (42.3)
4	30% SBM in SDP	66.4	20.7 (61.6)	15.6 (46.4)

*1 Samples from 10 fish were pooled for analysis.
*2 Figures in parentheses are values on dry matter basis.

Table 1-10. Growth of juvenile red sea bream fed diets containing various types of SBM in 100 *l* tanks

Diet no.		No. of fish	Av.body wt.* (g) Initial	Final	Growth rate (%)	Feed gain ratio	Daily feed intake	Protein efficiency ratio	Mortality (fish)
1	0% SBM	25	6.0±0.4	36.7±5.1^{a}	512	0.98	3.43	2.17	0
2	30% Un-SBM	25	6.0±0.3	35.0±4.2^{ab}	483	1.10	3.69	2.25	2
3	30% Ex-SBM	25	6.0±0.3	33.5±5.2^{b}	458	1.10	3.69	2.25	1
4	30% SBM in SDP	25	6.0±0.3	33.6±5.3^{b}	460	1.01	3.44	2.33	0

* Mean ± standard deviation. Figures with different superscripts are significantly different from each other ($p < 0.05$) which wewe analyzed using Duncan's multiple range test.

Discussion

There have been many studies on the utilization of SBM as a partial replacement for fish meal in diets of freshwater and marine fish. With regard to the juvenile red sea bream, Ukawa *et al.* (1994) reported that SBM could be included in single moist pellets at a level of 25%. In the present study, not much differences were observed in the feeding activity and feed performances between fish fed the diets containing 30% SBM and the control diet without SBM, except for the slightly reduced growth rate and feed gain ratio, probably induced by the lower protein contents of the diets. Thus these results suggest that the inclusion of 30% SBM in diets did not lead to any adverse effect on palatability of diets and growth in red sea bream.

However, we did not notice any improvement in availability of SBM by extrusion processing for both DP and SDP in the two experiments. The results indicated that there was no marked difference in nutritional quality of SBM as protein source for the three dietary groups (diets 2 4). It has been reported that availability of carbohydrate ingredients in diets was found to be improved by extrusion processing, for rainbow trout and carp, due to the elevation of gelatinized ratio (Takeuchi *et al.* 1990). In addition, Jeong *et al.* (1991) found that extrusion processing enhanced growth of juvenile red sea bream by improving nutritional quality of carbohydrate ingredient in fish meal diets. These studies clearly indicated that the nutritional value of fish diets could be improved by extrusion processing at suitable conditions. On the other hand, Pongmaneerat and Watanabe (1993) reported that nutritional quality of SBM diets (30% level) for rainbow trout was not markedly enhanced by extrusion processing, although the digestibility of starch was elevated. Furthermore, Akimoto (1994) has reported that growth performance in juvenile red sea bream fed dry type diets containing 30% untreated SBM was superior to fish on diets containing extruded SBM prepared at various conditions (screw speed, water injection, material temperature, material press, etc.). A similar result was also observed in yellowtail (Akimoto 1994). Therefore,

detailed experiments are necessary with red sea bream to investigate the effect of extrusion processing on the availability of SBM, at various inclusion levels, for different size of fish.

In conclusion, the results obtained in the present study have suggested that SBM, both processed and unprocessed, can be used as a substitutive protein source at a level of 30% for fish meal, in high energy DP as well as SDP for red sea bream without any adverse effect. However, the protein content and the amino acid balance have to be considered at higher inclusion levels of SBM in diets. It has already been demonstrated that nutritional quality of diet containing an alternate protein source for fish meal could be improved by supplementing essential amino acids mixture (Dabrowska and Wojno 1977, Murai *et al.* 1986, 1989, Takii *et al.* 1989). In another related study we have adopted this approach so as achieve the goal of employing SBM as the sole protein source in fish diets.

1.2.2 Combine Use of Protein Sources

1.2.2-1) Use of Alternative Protein Sources as Substitutes for Fish Meal in Red Sea Bream Diets

A previous study examined the availability to juvenile red sea bream of sole SBM in high energy dry pellets at the level of 30% (Chapter 1.2.1). There were no marked differences in growth performances and feeding activity between fish fed diets containing various types of SBM and those on a control diet without SBM. These results indicated that inclusion of 30% SBM to diet may not adversely affect palatability of diets and growth of red sea bream.

On the other hand, recent investigations on the availability of alternative protein sources for fish meal in diets have shown that combinational use of protein ingredients is superior to the use of a single component, providing better feed performance (Pongmaneerat and Watanabe 1992, Viyakarn *et al.* 1992, Watanabe and Pongmaneerat 1993, Shimeno *et al.* 1993). We have already reported that juvenile yellowtail fed the SDP containing 25% SBM and 15% CGM showed better feed performances than the 40% SBM diets (Viyakarn *et al.* 1992). Furthermore, the combination of SBM, CGM, and MM could replace about 60% of the fish meal in SDP for adult and juvenile yellowtail without any ill effect (Watanabe *et al.* 1995). There was no difference in growth performance and physiological condition of fish between the test groups and control This might be due to the elevation of dietary protein levels and inter balancing of the deficient EAA (essential amino acid) by the different ingredients, thereby enhancing the nutritional value and improving feed utilization. Therefore, this suggests that there would be no difference in nutritional quality between the diets with or without alternative protein sources, provided the proportion of protein ingredients are appropriately incorporated.

From these viewpoints, this study was conducted to examine the availability of combination of SBM, CGM, and MM as partial replacement for fish meal in high energy dry pellets for red sea bream (Aoki *et al.* 1997).

Materials and Methods

Experimental Diets

The ingredient composition and proximate composition of the experimental diets are shown in Table 1 11. Diet 1 was the control diet containing 65% local sardine meal (fish meal) as protein source and was produced by Sakamoto Fish Feed Co. using pellet mill (Watanabe *et al.* 1991). In diets 2 5, 46 62 % of the fish meal was substituted with a combination of SBM, CGM, and MM at levels of 20 30, 5 20 and 3 6%, respectively (total 36 46%). All of the test diets were pelleted to 6mm in diameter for adult fish by a large twin screw extruder (Expt. I). These ellets were also crushed to crumble for juvenile fish (Expt. II). Wheat flour and starch were added

to each of the diets as the binder and feed oil as the lipid source. The supplemental feed oil level was 10% for the control diet and 13 15% for the test diets in order to elevate the dietary energy levels. Mineral mixture and vitamin mixture were supplemented to diet 1 and diets 2 5 at levels of 4 and 5.3% (total), respectively. The crude protein content of diet 1 (47.6%) was slightly higher than that of the other diets (43.5 44.8%). The crude lipid level was proportional to the amount of feed oil included in the diet and was highest in diet 5. Thus the experimental diets in this study were not formulated iso nitrogenously. During the experimental period, all the diets were kept in the freezer (−20℃) until use.

Table 1-11. Composition of the experimental DP with alternative protein sources for red sea bream

	Diet no.*				
Ingredient(%)	1	2	3	4	5
Fish meal	65	35	35	30	25
Defatted soybean meal	0	30	20	20	20
Corn gluten meal	0	5	10	15	20
Meat meal	0	3	6	6	6
Wheat flour	19	4.7	6.7	5.7	4.7
Starch	2	4	4	4	4
Vitamin mixture	2	3.3	3.3	3.3	3.3
Mineral mixture	2	2	2	2	2
Feed oil	10	13	13	14	15
Nutrient contents determined (% as is basis)					
Crude protein	47.6	43.5	44.8	44.5	44.5
Crude lipid	16.3	17.4	17.9	18.6	19.2

* Diet 1: Steam pellet; Diets 2-5: Extruded pellets. All the diets contained 2 mg ethoxyquin (50%) and 3 mg astaxanthin.

Feeding Conditions

The feeding experiments were conducted at the Owase Branch of the Fisheries Research Institute of Mie in floating net cages held within inlets of the bay area (Expt. I) and at the Fishery Research Laboratory of the Kyushu University in aquariums (Expt. II). Red sea bream (*Pagrus major*) of different body sizes were used for experiments to observe the difference in availability of alternative proteins in diets between fish sizes.

Expt. I: Adult red sea bream (one year old) weighing 565 g on average were used. Before starting the experiment, they were fed a commercial diet. They were divided into five groups of 300 fish each in net cages (3×3×3 m) provided with a net cover and reared on the experimental diets for 85 days (from Sep.25 to Dec.18, 65 days feeding). The water temperature ranged from 17.4 to 25.9℃ (average 22.8℃). Fish were hand fed 5 or 6 days a week, once a day in the morning to satiation. All the fish in cages were weighed at the start and finish of the experiment to determine the average body weight. At the end of experiment, 10 fish were randomly taken from each group for analysis of proximate composition of dorsal muscle and liver, and to calculate the relative weights of internal organs. Analytical methods were described in a previous paper (Watanabe and Pongmaneerat 1991).

Expt. II: Juvenile red sea bream weighing 35 g were used for the experiment. They were divided into five groups of 25 fish in 150 *l* aquariums and reared for 56 days (from Oct.16 to Dec.10, 44 days feeding) at water temperature of 23 24℃. Filtered sea water was continuously supplied (540 760 ml/min) to each aquarium with airation. During the experiment, fish were fed the experimental diets to satiation twice a day. All the fish in the aquarium were weighed every 2 weeks and the average body weight was determined.

Examination of Hemochemical Characteristics

In order to evaluate the physiological condition of experimental fish, the level of hemochemical constituents and activities of plasma enzymes were determined at the end of both the experiments.

Expt. I: Five fish were taken from each net cage by a hand net, after fasting them for 48 hours. Whole blood was collected individually from caudal vein or artery with heparinized syringes immediately upon removal from water. The hematocrit value was determined by microhematocrit within 2 hours after the blood samples were collected. Plasma samples were obtained by centrifugation of whole blood at 3,000 rpm for 15 min at the Owase Branch of the Research Institute and then the frozen plasma samples were sent to Tokyo University of Fisheries for analysis. Analytical methods for hemochemical constituents and enzyme activities were described in

the previous paper (Watanabe *et al.* 1992).

Expt. II: Twenty fish were sampled from each group and examined. Fish were anesthetized in 100 ppm tricaine methane sulfonate (MS 222) sea water solution for sampling. Whole blood was collected from cuvierian duct with heparinized syringes. The hematocrit and hemoglobin values were determined within 2 hours after sampling (n=10). The data were compared and their significance was analyzed statistically by T test. The blood samples were also centrifuged at 3,000 rpm for 15 min to obtain plasma. Twenty plasma samples were pooled for analysis of the GOT (aspartate aminotransferase), GPT (alanine aminotransferase), ALP, and total cholesterol levels by the same methods as in Expt. I using commercial clinical examination kits (Chugai Co.). The total protein level was measured with the hand protein refractometer (Atago Co.). In addition, the phosphorus, calcium, and magnesium contents of plasma were analyzed at Kyushu University (Yoshimatsu *et al.* 1992).

Results

Feed Performances

Expt. I: The results of feed performance in Expt. I are shown in Table 1 12. In all dietary groups, fish actively fed the respective experimental diet containing a combination of the alternative protein ingredients. The final average body weight ranged from 744 to 776 g, the differences among the dietary groups being little. Growth rates of the fish fed diets containing 20%SBM + 10%CGM + 6%MM (diet 3) and 30%SBM + 5%CGM + 3%MM (diet 2) were almost at the same level as compared with fish on the control diet, whereas it slightly decreased in fish fed diets 4 and 5. Feed gain ratio values were similar among all the groups, ranging between 1.65 1.81. Diary feed consumption was the highest in fish fed the control diet, and tended to decrease as the proportion of CGM in diet increased. Losses of fish during the experimental period were mainly caused by iridovirus infection, and were unrelated to diets.

Table 1-12. Growth and feed gain ratio of adult red sea bream fed the experimental dry pellets (DP) with alternative protein sources in net cages

Diet no.	Av.body wt. (g)		Growth rate (%)	Feed gain ratio	Daily feed intake	Mortality (%)
	Initial	Final				
Sep.25 ~ Dec.18 (65 days feeding)						
1	561	752	34.0	1.81	0.59	9.7
2	561	744	32.6	1.72	0.56	2.3
3	580	776	33.8	1.70	0.58	0.0
4	565	745	31.9	1.65	0.54	1.0
5	570	744	30.5	1.75	0.53	2.3

Expt. II: The results of feed performance in Expt. II are shown in Table 1 13 and Fig. 1 2. In juvenile fish, acceptability of diet 5 (20%SBM + 20%CGM + 6%MM) was slightly inferior to other diets, resulting in the lowest feed consumption. Fish fed the control diet showed the highest final body weight and growth rate. However, there was no marked difference among fish fed the diets 2, 3 and control for these parameters. The lowest growth was observed for fish fed the diet 5 when the proportion of fish meal was the lowest. Feed gain ratio was high for all the groups considering that the experiments were conducted in aquaria, and it ranged from 1.9 to 3.1, and the highest value was found in fish fed on diet 5. In Expt. II, therefore, feed performance tended to decrease as proportion of CGM in diet increased. Thus the feeding results obtained in Expt. II differ from those in Expt. I.

Table 1-13. Growth and feed gain ratio of juvenile red sea bream fed the experimental DP with alternative protein sources in aquariums

Diet no.	Av.body wt.* (g)		Growth rate (%)		Feed gain ratio	Daily feed intake	Mortality (number)
	Initial	Final	Total	Daily			
Oct.16 ~ Oct.29 (11 days feeding)							
1	35.77	38.74	8.31	0.73	2.86	2.07	0
2	35.89	38.62	7.60	0.67	3.17	2.11	0
3	35.73	40.05	12.09	1.04	2.03	2.10	0
4	35.76	39.45	10.34	0.89	2.34	2.09	0
5	35.78	38.06	6.38	0.56	3.74	2.10	1
Oct.30 ~ Nov.12 (11 days feeding)							
1	38.74	45.24	16.76	1.41	1.80	2.53	0
2	38.62	46.31	19.93	1.65	1.53	2.52	0
3	40.05	44.85	12.00	1.03	2.26	2.33	0
4	39.45	45.48	15.29	1.29	1.85	2.39	0
5	38.06	43.50	14.30	1.21	2.07	2.51	0
Nov.13 ~ Npv.26 (11 days feeding)							
1	45.24	52.82	16.77	1.41	1.75	2.46	0
2	46.31	53.35	15.20	1.28	1.90	2.44	0
3	44.85	50.14	11.78	1.01	2.38	2.41	0
4	45.48	49.94	9.79	0.85	2.94	2.49	0
5	43.50	47.63	9.47	0.82	3.24	2.66	1
Nov.27 ~ Dec.10 (11 days feeding)							
1	52.82	61.47	16.37	1.38	1.85	2.54	0
2	53.35	60.20	12.84	1.10	2.18	2.39	0
3	50.14	57.91	15.51	1.31	1.77	2.32	0
4	49.94	54.35	8.84	0.77	3.09	2.38	0
5	47.63	49.93	4.85	0.43	4.80	2.06	0
Entire duration (44 days feeding)							
1	35.77	61.47	71.84	1.20	1.92	2.31	0
2	35.89	60.20	67.74	1.15	2.00	2.30	0
3	35.73	57.91	62.09	1.08	2.07	2.23	0
4	35.76	54.35	52.00	0.94	2.50	2.35	0
5	35.78	49.93	39.56	0.75	3.08	2.32	2

* Average body weight of fish (n=25).

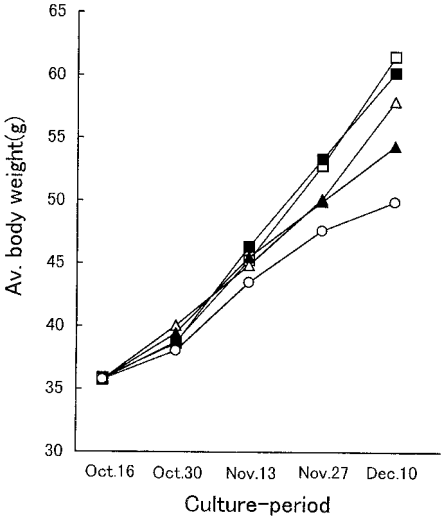

Fig. 1-2. Growth curves of juvenile red sea bream fed the experimental DP with alternative protein sources in aquariums. □:Diet 1, ■:diet 2, △:diet 3, ▲:diet 4, ○:diet 5.

Proximate Composition and Relative Weights of Internal Organs

The protein, lipid, and moisture contents of the liver and dorsal muscle from fish reared in net cages (Expt. I) are shown in Table 1 14. The protein content of the liver did not differ greatly among all the groups, but was slightly lower in the fish fed the diets 3 and 5. The protein content of the dorsal muscle ranged from 22.1 to 22.9% and the levels were similar for all the groups. The lipid content in liver was slightly higher in the control group, but in the case of muscle, the values were slightly above the rest for fish fed diets 3 and 5. On the whole there were no marked differences in protein and lipid contents of both liver and muscle between fish fed the diet containing alternative proteins and control.

Table 1-14. Proximate composition (%) of dorsal muscle and liver from red sea bream fed the experimental DP with alternative protein sources in net cages*1

Diet no.	Moisture	Crude protein	Crude lipid
Dorsal muscle			
1	73.6	22.2 (84.1)*2	3.1 (11.8)
2	73.0	22.5 (83.4)	3.4 (12.6)
3	72.4	22.5 (81.7)	3.9 (14.2)
4	72.8	22.1 (81.4)	3.4 (12.6)
5	72.3	22.9 (82.6)	3.9 (14.0)
Liver			
1	61.6	12.2 (31.8)	18.5 (48.2)
2	67.1	13.1 (39.9)	14.1 (42.9)
3	65.7	11.5 (33.5)	14.7 (43.0)
4	64.4	12.3 (34.5)	16.4 (46.2)
5	63.9	11.8 (32.8)	16.1 (44.6)

*1 Ten samples were pooled for analysis.
*2 Figures in parentheses are values on dry matter basis.

Table 1-15. Relative of internal organs (%) to body weighes in red sea bream fed the experimental DP with alternative protein sources in net cages*1

Diet no.	Viscera	Liver	Digestive organs*2
Initial : *Sep.24*	9.08±0.55	1.32±0.11	1.16±0.15
Final : *Dec.20*			
1	8.53±0.88	1.38±0.35	1.32±0.13
2	9.14±1.07	1.30±0.22	1.46±0.12
3	8.87±1.21	1.49±0.27	1.28±0.17
4	9.00±1.07	1.43±0.26	1.26±0.33
5	10.33±1.17	1.63±0.14	1.23±0.16

*1 Mean ± standard deviation (n=5).
*2 Include stomach, pyloric caeca, and intestine.

The relative weights of viscera, liver, and digestive organs (stomach, pyloric caeca, and intestine) from fish in Expt. I are shown in Table 1 15. Visceral weight was lower in fish fed the control diet than those on the test diets (diets 2 5). Liver weight value was the highest in fish fed diet 5, and generally, those on test diets had a tendency for values higher than the control. Also it was found that the weights of the digestive organs were higher in fish fed the test diets compared with the control. These weight differences between the test and control groups was not great, suggesting that all the fish maintained normal health.

Hemochemical Characteristics

Expt. I : The results of the hemochemical examination in Expt. I are shown in Table 1 16. There was no marked difference in the hematocrit level for fish fed the diets containing fish meal and alternative proteins, although it was slightly lower for fish fed diet 3. The values obtained for GOT, GPT, and ALP activities of fish for all the groups did not indicate any abnormality. This may indicate that all the fish maintained good liver function and physiological condition. In addition, there were no marked differences in the total protein, urea nitrogen, and glucose levels among the groups, suggesting that the fish had normal protein and glucose metabolism. The phospholipid, total cholesterol, and free cholesterol levels in the control fish were slightly higher than those of test groups, and the ester ratio for the control was not abnormal. This may indicate that a greater turn over of lipid metabolites in the control fish, compared to the test groups. There was no distinction in the lipid metabolism among the test group fish which were fed with alternative proteins in some proportion. Summarizing the hemochemical record, it seems that the physiological conditions of adult fish were not influenced largely by feeding the diets containing 36 46% alternative proteins for fish meal.

Table 1-16. Results of hemochemical examination in the adult red sea bream fed the experimental DP with alternative protein sources in net cages*

		Diet no.				
		1	2	3	4	5
Ht	(%)	30.6±4.7	32.1±2.0	27.9±4.4	30.7±2.6	33.0±3.4
ALP	(IU / l)	49±9	29±6	48±28	79±51	36±23
GOT	(IU / l)	8±4	9±4	17±12	15±10	20±10
GPT	(IU / l)	2±1	3±1	3±1	3±1	4±1
CPK	(IU / l)	421±226	482±269	740±724	708±618	718±531
GLU	(mg / 100ml)	55±14	52±2	51±3	47±2	51±5
TG	(mg / 100ml)	864±300	348±256	196±120	385±378	294±134
PL	(mg / 100ml)	741±44	636±27	595±104	648±176	641±60
TCHO	(mg / 100ml)	279±23	198±11	216±29	213±69	210±15
FCHO	(mg / 100ml)	127±17	89±11	85±19	101±40	93±12
Ester ratio	(%)	54.1±8.6	55.0±4.4	61.0±5.1	53.6±3.1	55.5±4.5
BUN	(mg / 100ml)	1.9±0.4	2.7±1.1	2.4±1.1	2.2±0.5	3.2±1.8
CRE	(mg / 100ml)	0.8±0.1	1.1±0.1	0.7±0.0	0.7±0.1	0.7±0.0
TP	(g / 100ml)	3.4±0.1	3.7±0.1	3.5±0.1	3.6±0.4	3.6±0.3
Condition factor		21.8±1.4	22.5±1.4	20.9±1.1	22.7±0.9	22.4±0.5

* Mean ± standard deviation (n=5).

Expt. II: The results from Expt. II are shown in Table 1 17. Hematocrit levels of fish fed the diets 4 and 5 were significantly lower than those of the control. So also were the values of hemoglobin level for fish on diets 4, 5, and 2. The GOT, GPT, and ALP activities were not markedly different among the groups. The total protein levels of fish on diets 4 and 5 were significantly lower than the rest. Both triglyceride and total cholesterol levels were the highest in fish fed the control diet, and the lowest in fish fed diet 5. This result indicates that there maybe some aberration in the lipid metabolism of fish fed the test diets compared to the control. The phosphorus, calcium, and magnesium levels of fish fed the control diet were also higher than other test groups. From these results, the physiological condition of fish fed the control diet was evaluated superior compared to those fed diets containing alternative proteins. Comparing the test groups, fish on diets 2 and 3 were physiologically better compared to those on diets 4 and 5.

Table 1-17. Results of hemochemical examination in the juvenile red sea bream fed the experimental DP with alternative protein sources in tanks*[1]

		Diet no.				
		1	2	3	4	5
Ht	(%)	36.8±2.8	34.2±3.4	36.7±2.3	33.9±2.9[a]	33.9±2.4[a]
Hb	(g / 100ml)	8.5±0.4	7.8±0.7[a]	8.5±0.4	7.9±0.7[a]	7.6±0.6[b]
ALP*[2]		4.6	4	3.6	2.9	3.9
GOT*[3]		52	36	32	70	34
GPT*[3]		19	9	13	18	12
TG	(mg / 100ml)	739	507	377	702	240
TCHO	(mg / 100ml)	410	292	290	280	240
TP	(g / 100ml)	5.9±0.4	5.6±0.5	5.6±0.5	5.1±0.7[b]	5.0±0.7[b]
P	(mg / 100ml)	14.5	12.5	10.4	10.7	10.3
Ca	(mg / 100ml)	19.7	17.3	14.7	16.0	15.4
Mg	(mg / 100ml)	2.45	1.59	2.31	1.46	2.43
Condition factor		37.3±1.5	37.2±3.5	37.6±2.8	35.0±2.2[a]	34.3±2.1[b]
Hepatosomatic index (%)		1.28±0.24	1.20±0.21	1.44±0.23	1.26±0.17	1.17±0.38

*[1] Mean ± standard deviation (n=10). Superscripts a & b indicate significant difference from the control (a: $p<0.05$, b: $p<0.01$).

*[2] King armstrong unit.

*[3] Karmen unit.

Discussion

In the present study, feeding results were different between adult (Expt. I) and juvenile fish (Expt. II). The results in Expt. I showed that there was no marked differences in growth performance and physiological condition between fish fed the diets containing alternative proteins and the control, irrespective of the proportion of protein ingredients in test diets. As for hemochemical constituents and enzyme activities, there were no significant differences between fish fed the test and control diets, and the experimental fish were evaluated as physiologically normal. These results indicated that the adult fish could use the combination of SBM, CGM, and MM as partial substitute for 46 62% of fish meal in diets without any problem. This might be because the levels of protein and lipid in the diets were not greatly different between the control fish meal diet and experimental diets. In juvenile fish, however, lower growth rate and feed gain ratio were found in fish fed the diets 4 and 5 that contained 15 or 20% CGM, although feed performances in fish of diet 2 and 3 groups were approximately equal to those of the control. Furthermore, judging from the results of hemochemical examination, it appeared that physiological condition of fish fed the diets 4 and 5 were inferior to other groups. These results suggested that nutritive value of protein in diets containing high levels of CGM was poor for juvenile fish.

It has been stated that EAA composition of CGM was inferior to SBM, compared to fish meal, although the protein content was high (Pongmaneerat and Watanabe 1991, Shimeno *et al.* 1993). A drop in feed performance due to inclusion of CGM as protein ingredient to diets has been shown in some feeding experiments previously. Pongmaneerat and Watanabe (1991) reported that nutritive value of diets containing CGM to carp in terms of protein quality was the lowest among four feed ingredients (white fish meal, meat meal, meat and bone meal, and corn gluten meal), due to the poor EAA composition. Likewise, Shimeno *et al.* (1993) found that feed performances tended to decrease in juvenile yellowtail fed the diet containing CGM. These previous investigations clearly suggested that the nutritional value of CGM was poor at higher inclusion in diets for carp and yellowtail. Evaluating the present result on the basis of information from other species, the indication is that juvenile red sea bream could not use the CGM as dietary protein source largely, due to the low protein quality in terms of EAA composition. Perhaps there are certain differences in utilization of CGM as protein source in diet for red sea bream based on fish size. This might be due to the difference in protein utilization in terms of EAA requirement linked to fish size. Apart from this, reduction in feed performance observed in Expt. II may be also related to

the palatability of the diet, but a definite reason could not be found. A similar size dependent difference in utilization of another protein source —SBM— was previously reported for rainbow trout (Murai *et al.* 1989) and yellowtail (Takii *et al.* 1989).

In conclusion, the results obtained from this study have confirmed that the combination of SBM, CGM, and MM in some proportions at 36 46% of the diet could help replace about 46 62% of fish meal in diets for adult red sea bream, taking care that the CGM level does not exceed 10% for juvenile. Furthermore, it was also found that the elevation of protein and lipid level in diets containing alternative proteins improved the feed performances of adult fish in agreement with former experiments on yellowtail (Watanabe *et al.* 1995).

1.2.2-2) Feed Protein Ingredients for Red Sea Bream

The quality of an aquaculture diet depends primarily on the protein source and it accounts for two thirds of the feed cost. Fish meal still remains to be the major protein source ranging between 20 60% of the fish feed. The reason for the choice is the premium match of the essential amino acids in relation to the requirements of several fish. Alternative protein sources should preferably contain more than 35% protein and be ideally less expensive and readily available as substitutes for the expensive fish meal component in practical diets. Recent investigations on the use of alternative protein sources in Japanese mariculture have clearly shown that combined use of both plant and animal protein sources, especially the combination of SBM, CGM, and MM, can successfully replace fish meal at levels more than 50% in the formulation (Watanabe *et al.* 1995, Chapter 1.2.2 1). In red sea bream it was also found that inclusion of 30% SBM with or without extrusion processing in ordinary steam pellets and extruded pellets could give feed performances almost comparable to the fish meal diet (Chapter 1.2.1). Whatever drop in feed value of SBM compared to fish meal could be attributed to the undigestible portion of carbohydrate contained in the SBM. Purification of SBM to SPC, which has a high protein content of about 70% on dry matter basis, less carbohydrate and better EAA profile than SBM, may improve the dietary value of the original ingredient.

Based on this background, this study was conducted to investigate the availability of SPC as a protein source and confirm the benefits of several combinations of SBM, CGM, and MM at different levels as fish meal substitute in diets for red sea bream (Aoki *et al.* 1998).

Materials and Methods

Experimental Diets

The composition of the experimental diets and results of proximate analysis are shown in Table 1 18. A commercial high energy steam dry pellet (Sakamoto Fish Feed Co.) containing 65% fish meal as a sole protein source was used as the control diet (diet 1). Diet 2 contained 50% SPC (Danpro A, Aarthus Olie Co.) having about 70% crude protein on dry basis, as a main protein source, thereby reducing the fish meal level to 10% in the diet. Diets 3 5 were formulated to incorporate 20 25% SBM, 5 15% CGM, and 11% MM (a total inclusion of 41 46%), respectively, to substitute 54 62% of the fish meal content in the control diet. Diet 2 was prepared using a large size of twin screw extruder by Sakamoto Fish Feed Co. Diets 3 5 were prepared using a small size of twin screw extruder by Nippon Formula Feed Co. The extruding conditions in both cases were adjusted close to those reported previously*3. Wheat flour or potato starch was contained at levels of 8 9 % as binder. A commercial feed oil was added as the lipid source at 19 % for diet 2 and 15 % for diets 3 5. Diet 5 was supplemented with 1.5% lysine to compensate the deficiency of this amino acid due to the inclusion of 15%

*3 Gyorui Youshoku Taisaku Chousa Gijyou Houkokusho, Suisancho, 1997, p. 16 (in Japanese).

CGM. The mineral and vitamin mixtures were supplemented to diet 2 at levels of 3 and 2%, and to diets 3 5 at 2 and 3%, respectively to satisfy the requirements of yellowtail (Watanabe *et al.* 1991).

The experimental diets were formulated to be close to the control commercial red sea bream diet in proximate composition, but diet 2 with SPC was high in both crude protein (CP) and crude lipid contents, resulting in the highest gross energy (GE) content. This was caused by miscalculation of proximate composition of the SPC. The GE/CP ratio ranged between 109 to 113. The experimental diets were kept at −20℃ during the period of feeding.

Table 1-18. Composition of the experimental diets containing different types and levels of alternative protein sources for red sea bream

Ingredient (%)	Diet no.				
	1	2	3	4	5
Brown fish meal	65	10	30	30	25
Soy protein concentrate	0	50	0	0	0
Defatted soybean meal	0	0	25	20	20
Corn gluten meal	0	6	5	10	15
Meat meal	0	2	11	11	11
Wheat flour	11	8	0	0	0
Potato starch	2	0	9	9	8.5
Lysine	0	0	0	0	0.5
Mineral mixture	6	3	2	2	2
Vitamin mixture		2	3	3	3
Feed oil	16	19	15	15	15
Analytical composition (%)					
Crude protein	46.7	50.7	44.9	45.8	44.6
Crude lipid	22.0	24.2	21.5	20.7	21.6
Crude starch	9.1	12.0	14.8	12.9	13.8
Crude ash	10.4	6.7	8.7	8.3	7.7
Moisture	7.8	3.5	9.8	9.5	10.6
Gross energy (kcal/100g)	530	570	525	530	520
GE/CP (kcal/kg/%CP)*	113	113	109	110	111

* Gross energy / crude protein.

Feeding Conditions

Feeding experiments were conducted with juvenile red sea bream (*Pagrus major*) at the Fisheries Research Institute of Mie (Expt. I) and the Fishery Research Laboratory of Kyushu University (Expt. II).

Expt. I: Juvenile fish weighing about 36 g on average, which had been fed a commercial red sea bream diet (diet 1) during a preliminary feeding, were divided into 5 lots of 960 fish each, stocked into covered net cages of 3×3×3 m in the sea, and fed the experimental diets for 122 days (Aug.27 Dec.16, 83 days feeding). They were fed 6 days a week, once in the morning to satiation. Water temperature ranged from 16.7 to 27.6℃, the mean being 23.1℃. At the end of the feeding experiment, 10 fish were randomly sampled from each group for determination of proximate composition of dorsal muscle and liver. The pooled samples were analyzed for protein, lipid, ash, and moisture contents by the same methods described previously (Watanabe and Pongmaneerat 1991).

Expt. II: Juvenile fish having a mean body weight of about 27 g, which had preliminarily been fed diet 1, were divided into 5 lots of 30 fish each, and kept in 150 *l* glass aquariums. They were fed the experimental diets twice a day, each time to satiation, for 57 days (Oct.6 Dec.1, 44 days feeding) at water temperature of 24 25 ℃. Fish body weight of each group was recorded every two weeks during the experiment. On termination, 10 fish were taken from each group and dorsal muscle and liver were pooled for proximate analysis.

Determination of Hemochemical Characteristics

Five fish from Expt. I and 10 fish from Expt. II were randomly sampled at the end of the experiment for determining the blood biochemical parameters to evaluate the physiological condition. The analytical procedures for blood parameters were the same as those described in previous papers (Chapter 1.2.2 1 Watanabe *et al.* 1992).

Results and Discussion

Feed Performances

Feed performances in terms of growth and feed gain ratio in Expts. I and II are presented in Tables 1 19 and 1 20, respectively.

Expt. I : Palatability and acceptability of the experimental diets were not affected by inclusion of alternate protein sources, except for diet 2 with SPC. Inclusion of 50% SPC slightly reduced its palatability as observed in yellowtail (Watanabe *et al.* 1995). This might be due to the hardness of the diet caused by including the finely powdered ingredient. The best feed performances were obtained for the control fish meal diet (diet 1), but the final body weight and feed gain ratio were not markedly different between the treatments, the former ranged from 112.6 g for diet 2 group to 126.6 g for diet 1 group, and the latter between 1.18 for diet 1 group and 1.28 for diet 3 4 groups. Thus, these results have suggested that inclusion of 41 46% alternate protein sources in different combinations of SBM, CGM, and MM could reduce fish meal levels to 25 30% in diet without marked reduction of feed performances. The use of 50% SPC as a protein source helped to reduce the fish meal level to nearly 10% in diet, indicating that dietary value of SBM was improved by upgrading the ingredient to SPC. In a former experiment, both juvenile and adult yellowtail fed a similar SPC diet showed better growth performances than the control fish meal diet for the initial two months, but thereafter their growth speed and feed gain ratio graduallyreduced, by the end of the four month feeding (Watanabe *et al.* 1995). Although cause of growth reduction was unknown, the results suggested that SPC can not replace fish meal at a high proportion in diets for both the species. Moreover, the inclusion of SPC in fish feed is not practical at present because of its high commercial price in Japan. Further, on comparing the difference in growth and feed gain ratio between fish fed diets 2 4 and diet 5, there was no distinct supplemental effect of lysine. Nevertheless, it should be noted that diet 5 contained a low amount of fish meal and a relatively high proportion of CGM, and this could have caused a drop in performance which has been averted by the lysine supplementation.

Table 1-19. Growth and feed gain ratio of red sea bream fed the experimental diets containing different types and levels of alternative protein sources in net cages

Diet no.	Av.body wt. (g) Initial	Final	Growth rate (%)	Feed gain ratio	Daily feed intake	Mortality (%)
Aug.27 ~ Sep.23 (22 days feeding)						
1	35.9	61.8	71.4	0.96	2.29	1.4
2	35.9	61.3	70.2	0.98	2.30	1.0
3	35.9	63.3	76.0	0.90	2.26	0.8
4	35.9	62.7	74.3	0.92	2.27	0.3
5	35.9	60.7	68.5	1.00	2.32	1.7
Sep.24 ~ Oct.18 (19 days feeding)						
1	61.8	79.8	29.0	1.42	1.90	0.8
2	61.3	77.5	26.8	1.53	1.90	0.6
3	63.3	78.6	24.4	1.65	1.88	1.0
4	62.7	81.7	30.7	1.30	1.82	0.9
5	60.7	79.2	30.4	1.37	1.90	0.4
Oct.19 ~ Nov.15 (20 days feeding)						
1	79.8	109.8	37.4	0.92	1.46	0.8
2	77.5	100.2	29.1	1.07	1.35	0.3
3	78.8	102.7	30.3	1.15	1.51	0.3
4	81.7	105.3	28.8	1.16	1.46	0.1
5	79.2	104.4	31.7	1.10	1.50	0.6
Nov.16 ~ Dec.16 (22 days feeding)						
1	109.8	126.6	15.1	1.73	1.10	0.2
2	100.2	112.6	12.1	1.86	0.96	1.2
3	102.7	118.7	15.2	1.76	1.13	0.5
4	105.3	116.0	10.0	2.44	1.06	0.2
5	104.4	117.9	12.6	2.03	1.10	0.8
Whole period (83 days feeding)						
1	35.9	126.6	246.6	1.18	1.57	3.1
2	35.9	112.6	210.4	1.26	1.56	3.1
3	35.9	118.7	226.6	1.28	1.63	2.6
4	35.9	116.0	221.4	1.28	1.62	1.6
5	35.9	117.9	223.1	1.28	1.62	3.4

Table 1-20. Growth and feed gain ratio of red sea bream fed the experimental diets containing different types and levels of alternative protein sources in aquariums

Diet no.	Av.body wt. (g) Initial	Final	Growth rate (%)	Feed gain ratio	Daily feed intake	Mortality (fish)
Oct.6 ~ Dec.1 (44 days feeding)						
1	27.4	61.2	123.0	1.99	3.48	2
2	27.3	53.5	96.0	2.43	3.57	0
3	27.4	49.0	78.8	2.88	3.72	1
4	27.4	53.3	94.5	2.15	3.10	1
5	27.3	51.3	87.9	2.67	3.69	0

Expt. II : The feed performances in this experiment resembled those in Expt. I , although differences between

the control group and the experimental groups were greater than in Expt. I. The final body weight was 61.2 g for the control and 49.0 53.5 g for the experimental groups, lowest being recorded for the fish fed diet 3. In Expt. I diet 3 had produced growth matching that of diets 4 and 5. Unlike in Expt. I, the lysine supplemented diet could not resemble diet 4 in performance.

In a previous experiment in which extruded dry pellets containing SBM (20 30%), CGM (5 20%), and MM (3 67%) in combination (replacing 42 62% of fish meal) were fed to both adult and juvenile red sea bream, adult fish fed all the combinations showed the same growth rate and feed gain ratio as the control (Chapter 1.2.2 1). In the case of juveniles, however, fish fed the diets containing 15 20% CGM showed inferior performance and hemochemical characteristics. Similar results were also obtained in the present study. The same diet with lysine supplement produced growth performances superior to the control fish meal diet in both juvenile and adult yellowtail in a former study (Watanabe *et al.* 1995), suggesting a slight difference in the effect of this amino acid between red sea bream and yellowtail. The supplemental effect of EAA to diets with alternate proteins has been reported by many researchers and the results hitherto obtained indicate fluctuation largely between EAA, fish species, type of diets, and ingredients (Dabrowska and Wojno 1977, Murai *et al.* 1982a, 1986, 1989a, 1989b, Takii *et al.* 1989, Shimeno 1994, Shimeno *et al.* 1992, Pongmaneerat *et al.* 1993). Further detailed experiments would be necessary to clarify the supplemental effect of EAA to improve protein utilization. However, supplementation of EAA is not cost effective from the practical viewpoint. Combination of various protein ingredients should be tried out to compensate for EAA deficiencies when aiming at least cost formulations.

Proximate Composition of Dorsal Muscle and Liver

Results of proximate analysis for dorsal muscle and liver from fish in Expts. I and II are shown in Tables 1 21 and 1 22, respectively.

Expt. I : There were no marked differences between the treatments in proximate composition of dorsal muscle, although the lipid content increased from the initial value of 1.5% (6.0% on dry basis) before feeding to the final value of 2.4 3.0% (9.4 11.8%) at the end of feeding. The increased lipid levels reflected the slightly reduced moisture contents. A trend similar to that in muscle was also observed in the liver. The lipid content increased markedly at the end of feeding in all the groups, especially in the fish fed the SPC diet, from 10.4% (30.6% on dry basis) to 20.2% (54.0%), reflecting a marked reduction in moisture content. The higher contents of protein and energy in the diet might be responsible for this.

Expt. II: The dorsal muscle of the fish in the control fish meal group had a slightly higher lipid content, linked to the decrease in moisture content. In the liver, the lipid content in fish fed the alternate protein diets were higher than that

Table 1-21. Proximate composition (%) of dorsal muscle and liver from red sea bream fed the experimental diets containing different types and levels of alternative protein sources in net cages*1

Diet no.	Moisture	Crude protein	Crude lipid	Crude ash
Dorsal muscle				
Initial : Aug. 26	75.9	20.2 (83.9)*2	1.5 (6.0)	1.9 (7.8)
Final : Dec. 18				
1	74.5	21.7 (85.0)	2.4 (9.4)	1.8 (7.0)
2	74.3	21.9 (85.4)	2.6 (10.0)	1.8 (7.0)
3	73.9	20.7 (79.4)	3.0 (11.6)	2.1 (8.0)
4	74.3	20.8 (80.9)	3.0 (11.8)	1.7 (6.5)
5	74.5	21.4 (84.0)	2.7 (10.4)	1.8 (7.0)
Liver				
Initial	66.1	12.6 (37.2)	10.4 (30.6)	1.3 (3.8)
Final				
1	66.1	12.4 (36.6)	14.1 (41.7)	1.4 (4.0)
2	62.6	10.4 (27.7)	20.2 (54.0)	1.1 (3.0)
3	64.4	11.3 (31.7)	16.8 (47.3)	1.4 (4.0)
4	66.6	10.9 (32.7)	15.4 (46.1)	1.7 (5.0)
5	65.8	11.0 (32.3)	14.6 (42.7)	1.7 (5.0)

*1 Samples from 10 fish were pooled for analysis.
*2 Values in parentheses are on a dry matter basis.

of the control group, reflecting the decrease of moisture and protein contents. These differences in the proximate composition could be associated to the differences in growth performances between the control and test groups in terms of utilization efficiency of dietary alternate protein sources.

Table 1-22. Proximate composition (%) of dorsal muscle and liver from red sea bream fed the experimental diets containing different types and levels of alternative protein sources in aquariums

Diet no.	Moisture	Crude protein	Crude lipid	Crude ash
Dorsal muscle				
1	73.9	22.5 (86.2)*	2.2 (8.4)	1.7 (6.5)
2	74.5	22.4 (87.8)	1.7 (6.7)	1.7 (6.7)
3	75.0	22.3 (89.2)	1.6 (6.4)	1.8 (7.2)
4	74.8	22.3 (88.5)	1.4 (5.6)	1.8 (7.1)
5	74.6	22.2 (87.4)	2.0 (7.9)	1.7 (6.7)
Liver				
1	63.2	11.7 (31.8)	10.6 (28.8)	1.1 (3.0)
2	59.0	10.6 (25.9)	16.0 (39.0)	1.4 (3.4)
3	62.3	10.7 (28.4)	14.1 (37.4)	1.5 (4.0)
4	59.9	9.4 (23.4)	15.3 (38.2)	1.4 (3.5)
5	59.1	9.9 (24.2)	15.6 (38.1)	1.6 (3.9)

* See the footnote of Table 1-21.

Hemochemical Characteristics

Blood biochemical parameters including plasma enzyme activities in fish from Expts. I and II are presented in Table 1 23.

Expt. I : There was no marked difference between the treatments in the hematocrit values which ranged from 26 to 30%. The lipid metabolites — triglyceride, phospholipid, total cholesterol, and free cholesterol were all within the normal ranges, but significantly higher in fish fed diets 2 4 than those on the control diet, suggesting that the former fish were maintaining a physiologically stable status. The same tendency was observed for the ALP activity. Both creatinine and total protein levels were also evaluated to be normal for all the groups, although the levels were different between the control and test groups.

Table 1-23. Results of hemochemical assessments in red sea bream fed the experimental diets containing different types and levels of alternative protein sources

		Diet no. 1	2	3	4	5
Expt. I *[1]						
Ht	(%)	27.9±3.4	26.9±1.4	30.0±3.5	28.1±3.4	26.6±3.2
ALP	(IU / l)	87.4±77.3^{ab}	119.6±78.1^{ab}	219.4±166.0^{b}	115.8±61.9^{ab}	78.6±46.4^{a}
GLU	(mg / 100ml)	46.8±9.7^{ab}	46.0±2.9^{ab}	50.0±5.5^{b}	46.6±1.7^{ab}	42.2±2.2^{a}
TG	(mg / 100ml)	162.2±37.4^{a}	448.6±180.8^{b}	336.0±91.1^{ab}	385.4±253.6^{b}	158.4±41.8^{a}
PL	(mg / 100ml)	718.6±89.2^{ab}	799.0±71.8^{bc}	953.2±79.9^{d}	907.6±127.0^{cd}	638.6±83.1^{a}
TCHO	(mg / 100ml)	246.6±25.7^{b}	261.8±18.3^{bc}	302.4±27.7^{d}	288.0±27.3^{cd}	208.8±26.7^{a}
FCHO	(mg / 100ml)	102.8±18.0^{a}	111.0±28.6^{a}	157.6±21.7^{b}	156.2±27.7^{b}	100.0±16.2^{a}
Ester ratio	(%)	58.37±5.42	58.07±8.50	48.02±2.95	45.95±7.36	52.21±3.00
BUN	(mg / 100ml)	5.62±1.28	5.16±1.45	4.98±1.15	4.40±1.00	4.86±1.35
CRE	(mg / 100ml)	0.84±0.05^{b}	0.68±0.08^{a}	0.74±0.09^{a}	0.74±0.05^{a}	0.68±0.04^{a}
TP	(g / 100ml)	2.78±0.16^{ab}	3.32±0.18^{c}	3.16±0.18^{c}	3.02±0.37^{bc}	2.68±0.22^{a}
Expt. II *[2]						
Ht	(%)	39.8±3.1	39.3±4.2	37.1±4.3	38.0±2.3	37.0±2.1^{a}
Hb	(g / 100ml)	8.9±1.2	8.9±0.9	8.1±0.8	9.4±0.6	9.2±0.8
ALP	(IU / l)	29.8	54.0	28.4	27.7	28.4
GOT*[3]		39	70	27	21	22
GPT*[3]		12	33	8	16	12
TG	(mg / 100ml)	744	748	670	885	481
TCHO	(mg / 100ml)	366	388	288	302	278
TP	(g / 100ml)	6.6±0.6	5.9±0.8^{a}	5.4±0.7^{b}	5.8±0.7^{a}	5.6±0.7^{b}

*[1] Mean ± standard deviation (n=5). Figures in a row with different superscripts are significantly different from each other ($p< 0.05$) when analyzed using Duncan's multiple range test.

*[2] Mean ± standard deviation (n=10). Superscripts a & b indicate significant difference from the control (a: $p<0.05$, b: $p<0.01$) when analyzed using t-test.

*[3] Karmen unit.

Expt. II: Hematocrit and hemoglobin levels were not much different between the groups and were within the normal ranges. Both GOT and GPT activities were the highest in the fish fed the SPC diet, although not in abnormal levels, it suggested that the fish were not in a perfect state of health. As for the parameters related to lipid metabolism, the triglyceride level was the highest in fish fed diet 4, and the lowest in those on diet 5. The total cholesterol of fish fed diets 1 and 2 was higher than the rest, though they were within the normal levels. The total protein level was higher for the control than the test groups, suggesting superior protein metabolism in the former fish.

The general trend observed in the hemochemical examination revealed that there was no significant difference in the physiological conditions between fish fed the fish meal diet and those on the alternate proteins.

The results obtained in the present and previous studies indicate that the combination of SBM, CGM, and MM at dietary levels of 36 46% was effective in replacing fish meal in red sea bream diets, when their EAA profiles are balanced and protein and energy contents are elevated to a level comparable to the fish meal diet. The combination seems to be particularly beneficial as a fish meal substitute for adult red sea bream. With regard to SPC, further experiments would be needed to clarify a suitable inclusion level for not only red sea bream but also yellowtail in terms of protein quality and cost efficacy.

Chapter 2: Use of Alternate Protein and Lipid Sources for Yellowtail

Use of Alternate Protein and Lipid Sources in Practical Feeds for Yellowtail

Development of a new type of dry pellet (SDP) for yellowtail and high protein high energy diets for red sea bream has markedly increased the consumption of fish oil in Japan. However, decrease of sardine catch greatly affected not only fish meal but also fish oil production. Both the products are now heavily dependent on the imports.

Therefore, timely efforts are needed to identify substitute sources; thereby reducing the inordinate dependence on fish oil. Combination of fish oil and other lipids would be useful in reducing the dietary unsaturation levels and prolonging the stability of lipids during feed storage and lowering the *in vivo* peroxidation. Lipids play an important role as an energy source and have a protein sparing action in many fish species, but lipids rich in highly unsaturated fatty acids (HUFA) are susceptible to auto oxidation or rancidity. Animal fat like lard and tallow or plant oils are suitable in this respect as energy sources in fish feeds because of their better resistance than fish oil to auto oxidation. They are mainly constituted by saturated and monoethylenic fatty acids, although grease contains a relatively high amount of linoleic acid (18:2n 6).

Their availability to fish as energy source is completely dependent upon their digestibility which is affected by the melting point (mp). Hydrogenated oils with mp. of 53℃ were poorly digested in both carp and rainbow trout, but beef tallow of mp. 38 40℃ was effectively utilized, digestibility being more than 70% regardless of fish size (Takeuchi *et al.* 1978, 1979). These results indicate that these animal fats are available to fish as dietary energy source when they are used with appropriate amounts of marine lipids which can provide the necessary levels of EFA (essential fatty acid) without any adverse effect on fish. In fact, juvenile rainbow trout were cultured with diets containing 7 9% beef tallow for two years until they grew up to broodstock, which produced quality egg (Takeuchi *et al.* 1981).

Recent results on the use of plant oil in SDP for yellowtail have shown that fish oil could be replaced up to 50 60% by palm oil without any ill effects*4. Against this back ground, and on the basis of recent data that the proportion of fish meal can be reduced to 30 40% by using a combination of SBM, CGM, and MM in extruded dry pellets (EP) for yellowtail (Watanabe *et al.* 1995), this experiment was conducted to evaluate nutritional quality for yellowtail of EP formulated to contain both alternative protein and lipids.

Materials and Methods

Experimental Diets

The ingredient composition and their proximate values are shown in Table 2 1. Diets 1 6 were test diets and diet 7 was a control diet which was a commercial yellowtail SDP containing 65% fish meal and 15% fish oil (sardine oil), as main protein and lipid sources (Sakamoto Fish Feed Co.). Test diets were formulated to contain SBM, CGM, MM, and BM in different proportions as alternate proteins at total levels of 28 33%, reducing fish meal levels to 40 30% (substitute 38 54% of fish meal content in the control diet). Diets 1 4 had the same formulation, containing 8% SBM, 10% CGM, and 10% MM, to investigatethe availability of alternate lipid sources. Diet 1 contained 20% fish oil (pollack liver oil) as a main lipid ingredient. Fish oil in diet 1 was diluted by 50% with palm oil in diet 2 and beef tallow (mp. 39 41℃) in diet 3 or a mixture of both lipids in diet 4, respectively. In diets 5 and 6 fish meal component was further decreased to 35 and 30% by inclusion of BM in addition to SBM, CGM, and MM respectively, and fish oil was diluted by 50% with a combination of three oil

*4 T.Watanabe *et al.* : Abst. Metg. Japan. Soc. Fisheries Sci., September, 1997, p. 44 (in Japanese).

ingredients as diet 4. All of the test diets were incorporated with 7.4% gelatinized starch (alpha starch) as the binder. The vitamin and mineral mixtures were supplemented to satisfy therequirement of yellowtail (Takeda 1985). These test diets were all prepared to pellets of 6 and 8 mm diameter using a small size of twin screw extruder (Buhler Co.) by Nippon Formula Feed Co., and the control SDP was produced by a large size twin screw extruder (Buhler Co.). The diets were kept in a freezer (−20℃) during the experimental period.

The crude protein content was 47.4 49.0% for the test diets and 46.2% for the control. The crude lipid level was also lower in the control diet, resulting in the lowest dietary gross energy content. The dietary amino acid contents calculated based on those of each feedstuff are shown in Table 2 2. In all diets, the dietary EAA levels exceeded the requirement of yellowtail determined by Watanabe*et al.*[*2] The fatty acid composition of experimental diets analyzed by the same method described previously is shown in Table 2 3 (Izquierdo *et al.* 1989). The percentage of n 3 HUFA, the EFA for yellowtail, in diet 1 and the control diet was 4.2 and 5.4%, respectively,

Table 2-1. Composition of the experimental diets with alternate protein and lipid sources for yellowtail

Ingredient(%)	Diet no. 1	2	3	4	5	6	7
Fish meal	40	40	40	40	35	30	
Soybean meal	8	8	8	8	8	8	
Corn gluten meal	10	10	10	10	13	15	
Meat meal	10	10	10	10	10	10	
Blood meal	0	0	0	0	2	5	
Pollack liver oil	20	10	10	10	10	10	
Palm oil	0	10	0	5	5	5	
Beef tallow	0	0	10	5	5	5	
Gelatinized starch	7.4	7.4	7.4	7.4	7.4	7.4	
Vitamin mixture	2	2	2	2	2	2	
Mineral mixture	2.6	2.6	2.6	2.6	2.6	2.6	
Nutrient contents determined (% as is basis)							
Crude protein	47.4	47.5	48.1	48.0	49.0	48.4	46.2
Crude lipid	25.3	26.8	27.2	26.0	27.7	28.2	22.5
Crude ash	9.0	9.2	8.9	9.0	8.4	8.2	9.5
Moisture	4.3	3.5	3.1	3.1	3.0	3.3	6.1
Digestible protein	42.7	42.7	43.9	43.8	44.1	43.4	41.6
Digestible energy (kcal / g diet)	4.8	4.8	4.8	4.8	4.9	4.8	4.6

Commercial extruded pellet*

* Fish meal content : 65%

Table 2-2. Amino acid composition of the experimental diets with alternate protein and lipid sources for yellowtail (% of protein ingredients)*

Amino acid	Diet no. 1	2	3	4	5	6
Arginine	3.6	3.6	3.6	3.6	3.5	3.5
Lysine	4.2	4.2	4.2	4.2	4.1	4.0
Histidine	1.7	1.7	1.7	1.7	1.8	1.9
Phenylalanine	2.8	2.8	2.8	2.8	3.0	3.2
Tyrosine	2.1	2.1	2.1	2.1	2.2	2.2
Leucine	5.6	5.6	5.6	5.6	6.0	6.3
Isoleucine	2.6	2.6	2.6	2.6	2.5	2.5
Methionine	1.5	1.5	1.5	1.5	1.5	1.4
Cystine	0.7	0.7	0.7	0.7	0.7	0.7
Valine	3.2	3.2	3.2	3.2	3.3	3.3
Threonine	2.5	2.5	2.5	2.5	2.5	2.5
Tryptophan	0.6	0.6	0.6	0.6	0.6	0.6
Total	31.1	31.1	31.1	31.1	31.7	32.1

* Calculated based on the EAA composition of each ingredient.

Table 2-3. Certain fatty acids in the experimental diets with alternate protein and lipid sources for yellowtail

Fatty acid (% area)	Diet no. 1	2	3	4	5	6	7
14:0	6.2	3.2	4.1	3.8	3.9	3.7	7.4
16:0	16.5	23.3	20.0	22.8	23.4	23.1	18.0
16:1	8.7	4.3	5.7	5.1	5.2	5.1	8.2
18:0	3.2	3.9	9.1	6.4	6.4	6.3	2.2
18:1	22.4	32.9	31.2	32.4	33.4	33.4	10.9
18:2n-6	3.6	7.8	3.6	6.7	6.7	6.9	3.3
20:1	7.1	4.3	4.2	4.2	3.9	3.7	5.9
20:5n-3	9.1	5.2	5.1	4.8	4.6	4.6	11.1
22:1	4.8	3.0	3.0	2.3	2.7	2.5	7.7
22:6n-3	6.0	4.6	4.1	3.8	3.0	3.6	10.5
Σ Saturated	27.9	31.8	35.3	34.7	34.4	33.8	28.4
Σ Monoene	43.0	44.5	44.1	44.0	45.2	44.7	32.7
Σ n-6	4.8	8.6	4.5	7.5	7.5	7.7	4.3
Σ n-3	19.2	12.2	11.6	11.0	9.8	10.4	29.2
Σ n-3 HUFA	16.6	10.8	10.1	9.5	8.3	8.9	23.1
Σ n-3 HUFA in diet (%)	4.2	2.9	2.7	2.5	2.3	2.5	5.2
PI*[1]	127.5	89.6	80.4	79.1	71.7	77.5	191.9
UI*[2]	174.9	141.9	128.4	130.0	123.8	129.3	229.8

*[1] Peroxidizability index = (% of monoene×0.025) + (% of diene×1) + (% of triene×2) + (% of tetraene×4) + (% of pentaene×6) + (% of hexaene×8).

*[2] Unsaturation index = (% of monoene×1) + (% of diene×2) + (% of triene×3) + (% of tetraene×4) + (% of pentaene×6) + (% of hexaene×8).

being higher than the rests (2.3 2.9%) where the fish oil was replaced with alternate lipids. Diets 2 (palm oil) and 3 (beef tallow) were high in 18:2n 6 and 18:0, respectively, reflected by the fatty acids from the corresponding sources. Moreover, dilution of fish oil resulted in reduction of peroxidizability index (PI) and unsaturated index (UI) of diets. The PI of 127.5 in diet 1 was reduced to 71.7 89.6 in the test diet, and similarly the UI of 174.9 in diet 1 was reduced to 123.8 141.9 in the test diets. The difference of PI and UI values between diet 1 and the control diet was due the difference of n 3 HUFA content between feed oil (pollack) and sardine oil. The dietary n 3 HUFA level satisfies the requirement of yellowtail for all the experimental diets (Deshimaru *et al.* 1982).

Feeding Condition

Two feeding experiments were conducted with yellowtail (*Seriola quinqueradiata*) at the Owase Branch, Fisheries Research Institute of Mie (Expt. I) and at the Oita Institute of Marine and Fisheries Science (Expt. II).

Expt. I: Young yellowtail weighing about 172 g on average, which had been preliminarily cultured with commercial extruded pellet for about two months, were divided into seven groups of 350 fish each, maintained in net cages (3×3×3 m) set in an inlet, and reared on the experimental diets for 97 days, from Aug.22 to Nov.26 (67 days feeding). The fish were hand fed once per day in the morning to near satiation level. Water temperature ranged from 18.4 to 27.5℃ with an average of 23.2℃ (2 m in depth) throughout the rearing period.

Expt. II: Young yellowtail with an initial body weight of about 142 145 g, which had been fed a commercial dry pellet for about two months, were stocked in four net cages (3×3×3 m), 300 fish each. They were reared on diets 1 4 for 93 days from Sep.4 to Dec.5 (73 days feeding). Diets were fed to fish once a day in the morning to near satiation. The water temperature ranged from 17.9 to 25.5℃ (average 22.6℃).

In both experiments, all fish in each cage were counted and weighed to determine the average body weight almost every month. At the end of feeding, 10 fish were randomly collected from each group for determination of proximate composition and fatty acid composition of dorsal muscle and liver by the same methods described in the previous papers (Izquierdo *et al.* 1989, Watanabe and Pongmaneerat 1991). In Expt. II, the contents of vitamins A and E in dorsal muscle and liver were also analyzed by the techniques described previously (Verakunpiriya *et al.* 1996).

Examination of Hemochemical Characteristics

In both experiments, 5 fish were taken from each lot on termination of the experiment to determine the hemochemical constituents for evaluation of health status of fish. Blood biochemical parameters were all determined by the same methods described in the previous paper (Watanabe *et al.* 1992). The ANOVA was conducted to determine the significance of treatment effects, and the difference between means were evaluated by Duncan's multiple range test ($p<0.05$).

Results and Discussion

Feed performances

Expt. I: Results of the feeding experiment are shown in Table 2 4. Palatability and acceptability of diets were not influenced by inclusion of both alternate proteins and lipids, and fish in all of the treatments showed good feeding activity as observed in the former experiment*4. Daily feed consumption was not different between diets and ranged from 2.45 to 2.60% for all the treatments. The final body weight was 596 g for the control and 563 607 g for the test groups. The best growth rate was observed in fish fed diet 3 (40%fish meal + 10%fish oil + 10%beef tallow), but there was no marked difference in growth parameters between the fish fed the EP with

40% fish meal, irrespective of the dietary lipid sources, and those on the control diet. On the other hand, growth rate, feed gain ratio, and protein efficiency ratio in fish fed the EP with 35 and 30% fish meal were slightly inferior to those of the control. The decrease of growth and feed performances of fish fed the low fish meal diets might be due to the lower content of methionine resulting from the inclusion of BM. The mortality of fish was generally low for all the treatments, suggesting that all fish were in good health condition during the experimental period.

Expt. II: Feeding results are shown in Table 2 5. As observed in Expt. I, the palatability and acceptability to fish were not affected by inclusion of alternate lipid sources. There was no marked difference in growth performance among the groups for the initial month. The final body weight and growth rate for fish fed diets 2 (10% palm oil) and 3 (10% beef tallow) were slightly lower than those on diets 1 (20% fish oil) and 4 (5%palm oil + 5%beef tallow). Feed gain ratio ranged from 1.54 for the diet 4 group to 1.63 for the diet 2 group, and was also slightly reduced in fish fed diets 2 and 3. The similar tendency was observed for protein efficiency ratio value.

Table 2-4. Growth and feed performances of yellowtail fed the experimental diets with alternate protein and lipid sources

Diet no.	Av.body wt. (g) Initial	Final	Growth rate (%)	Feed gain ratio	Daily feed intake	Protein efficiency ratio	Mortality (%)
Aug.22 ~ Sep.23 (25 days feeding)							
1	171	339	98	1.15	3.00	1.83	0.9
2	171	337	97	1.15	3.02	1.83	0.9
3	171	326	90	1.25	3.08	1.66	1.4
4	171	334	93	1.20	3.04	1.74	2.3
5	171	334	94	1.19	3.04	1.71	0.9
6	171	326	90	1.24	3.09	1.67	0.0
7	172	322	87	1.27	3.07	1.70	1.1
Sep.24 ~ Oct.27 (24 ~ 26 days feeding)							
1	339	491	42	1.77	2.55	1.19	0.3
2	337	479	39	1.90	2.59	1.11	0.0
3	326	489	47	1.69	2.66	1.23	0.9
4	334	484	42	1.85	2.67	1.13	1.2
5	334	463	36	2.10	2.67	0.97	1.5
6	326	467	41	1.91	2.68	1.08	1.4
7	322	459	40	1.99	2.75	1.09	1.5
Oct.28 ~ Nov.26 (18 days feeding)							
1	491	602	20	1.96	1.95	1.08	0.0
2	479	591	19	2.04	1.99	1.03	0.3
3	489	607	21	1.86	1.94	1.12	0.0
4	484	588	19	2.10	2.03	0.99	0.0
5	463	563	18	2.31	2.11	0.88	0.0
6	467	568	18	2.24	2.06	0.92	0.3
7	459	596	25	1.90	2.36	1.14	0.0
Whole period (67 ~ 69 days feeding)							
1	171	602	227	1.54	2.45	1.37	1.1
2	171	591	220	1.60	2.50	1.32	1.1
3	171	607	226	1.55	2.46	1.34	2.3
4	171	588	216	1.62	2.51	1.29	3.4
5	171	563	204	1.72	2.59	1.19	2.3
6	171	568	209	1.69	2.57	1.22	1.7
7	172	596	216	1.68	2.60	1.29	2.6

Table 2-5. Growth and feed performances of yellowtail fed the experimental diets with alternate protein and lipid sources

Diet no.	Av.body wt. (g) Initial	Final	Growth rate (%)	Feed gain ratio	Daily feed intake	Protein efficiency ratio	Mortality (%)
Sep.4 ~ Dec.5 (73 days feeding)							
1	142	704	396	1.56	3.95	1.39	1.5
2	145	674	366	1.63	4.06	1.31	2.5
3	145	676	366	1.61	4.02	1.32	1.0
4	144	715	397	1.54	3.91	1.43	1.0

In an earlier study[*4], growth and feed performances of juvenile yellowtail fed the same test diets with 40% fish meal (diets 2 4) were approximately equal to those of fish on the control 20% fish oil diet, though a 15% beef tallow diet (substitution of 75% fish oil) resulted in slight reduction of these parameters, probably due to deficiency of EFA content in the diet. The performance parameters of fish fed the alternate protein and lipid diets in the present experiment showed almost similar trends as shown in the former experiment[*4]. Thus, it was confirmed that palm oil and beef tallow alone or a combination of both oils in 1:1 can be used as dietary energy source at a level of 10%, replacing 50% of fish oil, in the EP formulated with alternate proteins up to 28% (40% fish meal level) for young yellowtail without ill effects.

Lipid plays an important role as EFA and energy sources in fish feeds. As mentioned above, lipids of any kind, having high digestibility, can be used as dietary energy source, provided they are used along with marine lipids which contribute the necessary level of EFA; n 3 HUFA in the present case. It has been found that some animal and plant lipids combined with marine lipids can be utilized as energy source in diets for rainbow trout (Takeuchi *et al.* 1978, 1981, Yu *et al.* 1977a, 1977b), carp (Takeuchi *et al.* 1978), and channel

catfish (Murray *et al.* 1977) without reduction of growth and feed performances. The present study has also shown that beef tallow with mp. of 39 41℃ could effectively be utilized as energy source in diet for yellowtail.

Proximate Composition

The protein, lipid, ash, moisture, and energy contents and the vitamins A and E contents in dorsal muscle and liver are shown in Tables 2 6 and 2 7, respectively.

Expt. I : The crude protein content in both dorsal muscle and liver did not differ largely between the initial and final fish. There was no marked difference in the protein and ash contents in both tissues among the dietary groups. The final values for the crude lipid content in the tissues recorded a significant rise from the initial values and ranged from 4.6 to 6.6% and from 27.6 to 34.4% in muscle and liver, respectively, and showed no definite relationship linked to dietary treatments. The moisture content in muscle was not markedly different among the treatments, but that in liver appeared to be inversely related to lipid levels.

Expt. II : There were no marked differences in the moisture, protein, lipid, and ash contents of both dorsal muscle and liver from each lot. The lipid content in liver ranged from 39.0 to 45.3%, being generally higher than that obtained in Expt. I. This difference might be due to the difference of fish size and rearing conditions in terms of feeding rate and water temperature. The vitamin A content in both dorsal muscle and liver was slightly high in the fish fed diet 1 with 20% fish oil. This might be due to a higher vitamin A content in fish oil compared to alternate lipids. However, the vitamin E content in both the tissues was higher for the fish fed alternate lipid diets, except the liver of fish on diet 4. The higher vitamin E contents in fish fed diets with lower levels of unsaturation suggested reduced degrees of *in vivo* peroxidation, as already observed in the former

Table 2-6. Proximate composition (%) of dorsal muscle and liver of yellowtail fed the experimental diets with alternate protein and lipid sources*1

Diet no.	Moisture	Crude protein	Crude lipid	Crude ash	Gross energy (kcal / g)
Expt. I					
Dorsal muscle					
Initial : Aug. 22	73.6	23.3 (88.3)*2	1.5 (5.7)	1.7 (6.4)	1.4
Final : Nov.26					
1	68.2	23.6 (74.2)	4.6 (14.5)	1.8 (5.7)	2.1
2	68.9	24.1 (77.5)	5.0 (16.1)	1.9 (6.1)	1.9
3	68.5	24.3 (77.1)	5.4 (17.1)	1.8 (5.7)	1.9
4	68.6	23.8 (75.8)	6.6 (21.0)	1.9 (6.1)	1.9
5	70.4	23.9 (80.7)	5.3 (17.9)	1.8 (6.1)	1.8
6	69.2	24.0 (77.9)	5.7 (18.5)	1.9 (6.2)	1.8
7	68.5	23.7 (75.2)	7.1 (22.5)	2.1 (6.7)	2.0
Liver					
Initial	72.8	13.8 (50.7)	4.8 (17.6)	1.4 (5.1)	1.5
Final					
1	48.6	10.7 (20.8)	33.9 (66.0)	1.0 (1.9)	3.5
2	55.4	11.6 (26.0)	27.6 (61.9)	1.0 (2.2)	4.3
3	54.0	11.2 (24.3)	29.2 (63.5)	1.0 (2.2)	3.8
4	51.7	10.7 (22.2)	34.4 (71.2)	0.9 (1.9)	4.0
5	56.5	12.1 (27.8)	30.2 (69.9)	1.0 (2.3)	3.5
6	54.1	10.8 (23.5)	28.9 (63.0)	0.9 (2.0)	3.8
7	53.5	10.7 (23.0)	29.9 (64.3)	0.9 (1.9)	3.7
Expt. II					
Dorsal muscle					
1	70.1	24.0 (80.3)	4.4 (14.7)	2.6 (8.7)	— *3
2	70.0	25.2 (84.0)	4.6 (15.3)	2.3 (7.7)	—
3	69.8	25.3 (83.8)	4.6 (15.2)	2.6 (8.6)	—
4	69.4	25.0 (81.7)	5.2 (17.0)	2.2 (7.2)	—
Liver					
1	40.6	9.9 (16.7)	45.3 (76.3)	0.5 (0.8)	—
2	44.2	11.2 (20.1)	39.9 (71.5)	0.5 (0.9)	—
3	44.6	10.8 (19.5)	39.0 (70.4)	0.8 (1.4)	—
4	40.9	10.9 (18.4)	40.8 (69.0)	0.8 (1.4)	—

*1 Samples from 10 fish were pooled for analysis.
*2 Figures in parentheses are values on dry matter basis.
*3 Not determined.

Table 2-7. Proximate composition (%) of dorsal muscle and liver of yellowtail fed the experimental diets with alternate protein and lipid sources*1

	Diet no.			
	1	2	3	4
Dorsal muscle				
Vitamin A (IU / g)	0.54	0.43	0.42	0.39
Vitamin E (μg / g)	1.78	2.51	2.79	3.04
Liver				
Vitamin A (IU / g)	1181	945.8	1077	1003
Vitamin E (μg / g)	26.07	34.07	85.03	19.42

* Samples from 10 fish were pooled for analysis.

study[*4].

Fatty Acid Composition

Some fatty acids of total lipids in dorsal muscle and liver from fish in E xpts. I and II are shown in Tables 2 8 and 2 9, respectively.

Expt. I : In both the tissues their fatty acid compositions were clearly reflected by dietary lipids, fish oil and alternate oils. Dilution of fish oil with palm oil or beef tallow increased the percentage of 18:1 and 18:2n 6, and decreased n 3 HUFA such as 20:5n 3 and 22:6n 3. These fatty acids profiles affected the PI and UI values in the muscle and liver. Both PI and UI were reduced in the two tissues compared to the initial data. This might be due to a higher unsaturation level of the diet used in the preliminary feeding. The PI and UI values in the control fish fed fish oil were highest in both muscle and liver, and was lowered by dilution of fish oil with palm oil or beef tallow.

Table 2-8. Fatty acids composition of total lipids in the muscle and liver from yellowtail fed the experimental diets with alternate protein and lipid sources[*1]

Fatty acid (% area)	Initial	Diet no. (Final) 1	2	3	4	5	6	7
Dorsal muscle								
14:0	6.0	4.6	3.0	3.4	3.2	3.4	3.4	6.6
16:0	23.8	16.2	21.7	17.9	19.8	20.4	20.4	19.6
16:1	8.1	7.8	4.7	6.1	5.4	5.3	5.3	9.0
18:0	4.2	3.2	3.6	6.2	5.1	5.2	5.1	2.9
18:1	12.8	25.4	36.5	36.2	36.3	36.2	36.5	13.8
18:2n-6	5.2	3.4	8.4	4.0	6.7	7.0	7.2	4.0
20:1	5.9	9.1	2.0	4.7	4.6	4.5	4.5	7.1
20:5n-3	5.9	7.3	3.7	4.3	4.0	3.6	3.5	7.3
22:1	5.1	6.6	2.9	2.9	2.9	2.7	2.8	7.3
22:6n-3	12.7	6.3	5.1	6.4	5.1	5.3	4.1	8.6
ΣSaturated FA	34.8	24.7	28.8	28.3	28.8	29.6	29.6	29.9
ΣMonoene	31.9	48.9	46.1	49.9	49.2	48.7	49.1	37.2
Σn-6	6.4	4.8	9.1	5.3	7.7	8.0	8.3	5.4
Σn-3	23.4	18.0	11.3	13.4	11.8	11.3	10.0	22.8
Σn-3 HUFA	20.0	15.8	10.0	12.1	10.5	10.0	8.7	17.9
PI[*2]	167	124	87	99	89	87	77	151
UI[*2]	205	177	141	154	145	142	133	194
Liver								
14:0	5.0	2.8	1.8	1.8	2.2	2.2	2.2	3.8
16:0	23.1	17.1	17.6	14.7	17.5	16.8	17.4	18.9
16:1	7.5	6.7	4.0	4.1	4.7	4.0	4.6	8.1
18:0	3.9	4.0	5.2	5.5	5.8	5.8	6.1	3.7
18:1	16.8	35.3	45.8	43.4	45.7	45.6	45.7	24.8
18:2n-6	5.1	3.1	8.3	3.2	6.6	7.1	7.3	3.5
20:1	6.4	8.5	4.8	4.7	4.5	5.0	4.8	7.1
20:5n-3	5.4	4.1	2.1	2.2	2.2	2.1	1.9	5.5
22:1	3.3	3.4	1.6	1.6	1.4	1.7	1.5	3.3
22:6n-3	11.2	4.4	2.5	2.8	2.3	2.5	1.9	7.9
ΣSaturated FA	32.9	24.5	25.0	22.5	26.1	25.3	26.2	26.9
ΣMonoene	33.9	53.9	56.3	53.9	54.3	56.3	56.6	43.3
Σn-6	7.1	4.4	9.5	4.7	7.8	8.3	8.4	4.9
Σn-3	21.7	13.5	7.4	8.1	7.4	7.4	6.4	20.8
Σn-3 HUFA	19.0	12.4	6.7	7.4	6.6	6.6	5.6	18.1
PI[*2]	156	94	59	62	57	58	51	140
UI[*2]	196	151	122	119	120	121	114	188

[*1] Samples from 10 fish were pooled for analysis.
[*2] See the footnote of Table 2-3.

Expt. II : Incorporation of dietary fatty acids into the muscle was almost the same as that observed in Expt. I. Reduction of dietary unsaturation levels by dilution of fish oil with palm oil or beef tallow resulted inlower levels of PI and UI in the muscle. However, no remarkable difference was found in fatty acid composition in liver lipids among the treatments, although the reason for the difference between Expts. I and II was unknown.

In both Expts. I and II, the values for PI and UI were higher for the muscles than liver due to a higher accumulation of n 3 HUFA in the former tissue.

Hemochemical Characteristics

Results of hemochemical examination in Expts. I and II are shown in Table 2 10. In Expt. I, there was no significant difference in all the blood biochemical parameters together with condition factor and hepatosomatic index between the treatments, suggesting that all the fish were in almost the same physiological conditions. Moreover, no abnormal level was observed in blood characteristics in fish of all the groups. Also, in Expt. II, the blood parameters wereallwithin the normal ranges, although the levels of lipid metabolites (phospholipid, total cholesterol, and free cholesterol) from fish fed diets 3 and 4 were higher than those of fish on diets 1 and 2, suggesting that the former fish were probably in a better health condition. From these results, it appeared that fish fed diets with both fish oil and alternate lipid sources were maintaining a good physiological condition. Thus,

feeding the diets containing both alternate protein and lipid sources did not influence the health conditions of yellowtail.

In conclusion, the results of this study clearly confirmed that efficient EP diets for yellowtail may include combinations of SBM, CGM, MM, and BM as dietary protein source up to 38% (substitution of about 54% fish meal), and palm oil and beef tallow alone or in a 1:1 ratio as lipid source (replacing about 50% of fish oil). In general, the results obtained in the present and earlier experiments have clearly shown that adequate alternate protein and lipid sources can successfully replace around 50% of both fish meal and fish oil in the yellowtail EP.

Table 2-9. Fatty acids composition of total lipids in the muscle and liver from yellowtail fed the experimental diets with alternate protein and lipid sources*1

Fatty acid (% area)	Diet no. 1	2	3	4
Dorsal muscle				
18:0	4.1	4.5	6.9	5.6
18:1	23.3	36.0	35.5	36.4
18:2n-6	3.0	7.9	3.9	6.1
20:1	13.0	6.6	6.4	6.3
20:5n-3	5.6	3.2	3.6	3.7
22:5n-3	1.6	1.1	1.2	1.2
22:6n-3	8.6	5.9	6.6	6.3
ΣSaturated FA	22.3	26.1	26.9	26.3
ΣMonoene	52.4	51.2	51.5	51.1
Σn-6	4.5	9.0	5.6	7.5
Σn-3	17.9	11.5	12.9	12.5
Σn-3 HUFA	16.4	10.6	11.8	11.5
PI*2	130	91	98	97
UI*2	161	134	136	136
Liver				
18:0	6.3	5.4	6.3	5.9
18:1	46.5	45.5	46.7	46.5
18:2n-6	4.1	8.7	4.1	6.2
20:1	6.9	6.4	6.9	6.0
20:5n-3	2.4	2.5	2.4	2.6
22:5n-3	2.4	2.1	2.5	2.3
22:6n-3	3.3	3.4	3.4	3.7
ΣSaturated FA	21.1	21.5	21.3	22.2
ΣMonoene	60.9	58.0	60.3	58.0
Σn-6	6.0	10.1	6.0	7.8
Σn-3	9.5	9.3	9.8	10.1
Σn-3 HUFA	8.8	8.7	9.0	9.3
PI*2	72	74	73	77
UI*2	126	132	126	129

*1 Samples from 10 fish were pooled for analysis.
*2 See the footnote of Table 2-3.

Table 2-10. Results of hemochemical examination in yellowtail fed the experimental diets with alternate protein and lipid sources*

		Diet no. 1	2	3	4	5	6	7
Expt. I								
Ht	(%)	50.1±2.3	48.4±4.2	50.3±1.5	50.1±2.4	51.8±1.9	51.9±2.1	50.6±2.8
ALP	(IU / l)	134±18	100±10	108±17	121±35	142±21	125±23	131±23
GLU	(mg / 100ml)	107±17	102±8	108±4	110±6	113±8	110±13	112±12
TG	(mg / 100ml)	247±40	299±34	224±65	265±68	225±27	264±100	227±107
PL	(mg / 100ml)	828±105	766±65	795±79	778±107	762±19	812±95	901±128
TCHO	(mg / 100ml)	303±45	271±30	312±22	291±41	281±27	296±23	329±57
FCHO	(mg / 100ml)	120±17	116±10	120±11	115±15	113±11	115±10	138±13
BUN	(mg / 100ml)	14.6±1.8	10.3±0.6	11.8±2.2	13.3±2.9	12.2±2.0	14.4±2.4	13.0±2.8
CRE	(mg / 100ml)	1.0±0.1	1.0±0.1	1.0±0.2	0.9±0.1	0.9±0.1	1.0±0.1	1.0±0.1
TP	(g / 100ml)	3.7±0.4	3.4±0.3	3.7±0.2	3.6±0.3	3.7±0.3	3.7±0.1	4.0±0.2
Condition factor		16.8±0.4	17.0±1.0	16.4±0.8	16.1±0.4	16.9±1.1	17.2±0.7	17.3±0.6
Hepatosomatic index (%)		1.85±0.17	1.60±0.15	1.62±0.35	1.70±0.25	1.54±0.21	1.83±0.28	1.89±0.05

		Diet no. 1	2	3	4
Expt. II					
ALP	(IU / l)	139±27	154±11	137±14	151±23
GLU	(mg / 100ml)	110±5	116±6	111±5	121±21
TG	(mg / 100ml)	$105±30^{a}$	$82±18^{a}$	$98±44^{a}$	$176±52^{b}$
PL	(mg / 100ml)	802±26	789±54	813±22	863±89
TCHO	(mg / 100ml)	$318±10^{a}$	$328±15^{a}$	$352±8^{b}$	$344±21^{ab}$
FCHO	(mg / 100ml)	116±8	121±9	124±7	141±20
BUN	(mg / 100ml)	$16.1±2.4^{b}$	$12.1±2.0^{a}$	$10.3±2.2^{a}$	$12.4±1.2^{a}$
CRE	(mg / 100ml)	1.0±0.1	1.1±0.2	1.0±0.1	1.1±0.1
TP	(g / 100ml)	$3.4±0.1^{a}$	$3.6±0.2^{ab}$	$3.5±0.2^{a}$	$3.8±0.1^{b}$

* Data are shown as mean ± standard deviation (n=5). Figures in a row with different superscripts are significantly different from each other ($p< 0.05$) when analyzed using Duncan's multiple range test.

Chapter 3: Trial to Culture Yellowtail and Red Sea Bream with Non-Fish Meal Diets

3.1 Effect of Dietary Fish Meal Levels on Growth Performances

Over the last several years, there has been a slump in the domestic production of fish meal for fish feeds because of the quick drop in the catch of sardine, and the soaring cost for feed production. In this context, many studies are being conducted to investigate the availability of alternate protein sources as substitute for fish meal in order to maintain a constant feed supply. Our attempts to replace fish meal component have proved that SBM can be included up to 30% in combination with fish meal in dry pellets for yellowtail (Watanabe *et al.* 1992, Viyakarn *et al.* 1992) and red sea bream (Chapter 1.2.1). Moreover, it was also demonstrated that a combination of SBM with CGM and MM can replace around 60% of fish meal in diets for both two species (Watanabe *et al.* 1995, Chapter 1.2.2 1,2). These results showed that SBM, CGM, and MM have the potential of becoming excellent feed protein ingredients for marine fish. The knowledge obtained from these studies would contribute to develop practical feeds formulated with alternate ingredients.

On the other hand, the availability of several animal by products, such as MBM, BM, and PFM as fish meal replacer have also been examined in fish feeds. The protein content of these materials is generally high (around 50 85%), but their amino acid profiles are inferior to fish meal in terms of quality and quantity of EAA. Therefore, it was reported that nutritive value of MBM was lower than that of fish meal for rainbow trout (Watanabe and Pongmaneerat 1991) and common carp (Pongmaneerat and Watanabe 1991). However, some studies indicated that the proportion of fish meal in the diets could be partially replaced by MBM and/or PFM without marked decrease of growth and feed performances in yellowtail (Terada 1994) and Japanese flounder (Kikuchi *et al.* 1994, 1997).

Thus, serious efforts are being made to reduce fish meal component in practical diets using other animal and plant protein sources. The final goal is to entirely eliminate fish meal from fish feeds and develop non fish meal diets as in the case of animal feeds. In the present study, therefore, three feeding experiments were conducted to obtain basic information on the availability to yellowtail and red sea bream of low or non fish meal diets formulated with SBM, CGM, and MM including some animal by products.

Materials and Methods

Experimental Diets

The ingredient composition and proximate analysis of the experimental diets are shown in Table 3 1. The experimental diets were prepared as extruded pellets (6 and 8 mm in diameter) using a large size of twin screw extruder by Sakamoto Fish Feed Co. Diet 1 was the control diet containing 65% fish meal as a main protein source. In diet 2, the combination of 8% SBM, 10% CGM, and 10% MM was used to replace 31% of fish meal in the control diet (fish meal 40%). Besides these three alternate materials, 10% MBM was added to diet 3 to substitute 54% of fish meal in the control diet (fish meal 30%). Diets 4 and 5 contained the combination of these four materials (each 10%) together with PFM (4 and 8%) and BM (5 and 8%) to replace 69 and 85% of fish meal in the control diet, respectively (fish meal 20 and 10%). Diet 6 was a non fish meal diet prepared by replacing 10% fish meal in diet 5 with 10% krill meal. Control and test diets were supplemented with vitamin and mineral mixtures, at 2 4 and 5%, respectively, to satisfy the requirements of yellowtail (Watanabe *et al.* 1991). Fish oil was added at 16 20% to diets to adjust the lipid level to approximately 20%. The diets were kept in freezer (−20℃) until use.

The crude protein content was about 47% for all the diets, except a slightly lower value of 45% for diet 6. The crude lipid level of the control diet was 23.7%, and was higher than that of test diets (18.4 20.5%). Gross energy content of the control diet (5.6 kcal/g) was slightly higher than that of test diets (5.0 5.2 kcal/g). The crude ash content of control diet was slightly lower than that of the rest. The amino acid contents of the experimental diets analyzed by Japan Food Research Laboratories are given in Table 3 2. Among the EAA for fish, the content of lysine, methionine, threonine, and isoleucine decreased linearly with decrease in dietary level of fish meal. Especially the lysine and methionine contents in diets 5 and 6 were only about 60 70% of the control diet. In contrast, the leucine and phenylalanine contents increased as fish meal decreased in the diets. Other EAA contents in test diets were generally lower than those of the control. The total amount of EAA was highest in the control diet. Thus, the EAA levels of test diets were lower than the control diet, but EAA were not supplemented to the test diets to adjust their EAA levels to be equivalent as the control diet, in order to observe the effect of EAA deficiency on feed performance in yellowtail and red sea bream.

Table 3-1. Composition of the experimental low or non fish meal diets for yellowtail and red sea bream

Ingredient(%)	Diet no. 1	2	3	4	5	6
Fish meal	65	40	30	20	10	0
Krill meal	0	0	0	0	0	10
Defatted soybean meal	5	8	9	10	10	10
Corn gluten meal	0	10	10	10	10	10
Meat meal	0	10	10	10	10	10
Meat and bone meal	0	0	10	8	10	9
Poultry feather meal	0	0	0	4	8	8
Blood meal	0	0	0	5	8	8
Wheat flour	10	9	8	8	8	8
Mineral mixture	5	5	5	5	5	5
Vitamin mixture	4	2	2	2	2	2
Feed oil	11	16	16	18	19	20
Nutrient contents determined : As is basis(%)						
Crude protein	47.2	47.4	47.9	46.8	46.7	45.1
Crude lipid	23.7	19.5	19.8	19.1	18.4	20.5
Crude ash	9.0	11.3	11.6	11.0	11.1	10.7
Moisture	5.1	8.2	7.0	7.0	5.5	6.5
Gross energy (kcal / g)	5.6	5.0	5.1	5.1	5.1	5.2
Dry matter basis(%)						
Crude protein	49.7	51.6	51.5	50.3	49.4	48.2
Crude lipid	25.0	21.2	21.3	20.5	19.5	21.9
Crude ash	9.5	12.3	12.5	11.8	11.7	11.4

Table 3-2. Amino acid composition of the experimental low or non fish meal diets for yellowtail and red sea bream

Amino acid (g/100g diet)	Diet no. 1	2	3	4	5	6
Arginine	2.61	2.42	2.42	2.41	2.42	2.38
Lysine	3.42	2.74	2.55	2.53	2.36	2.37
Histidine	1.43	1.14	1.06	1.21	1.36	1.25
Phenylalanine	1.89	1.90	1.87	1.99	2.19	2.21
Tyrosine	1.47	1.45	1.43	1.43	1.49	1.66
Leucine	3.31	3.65	3.54	3.76	4.00	3.91
Isoleucine	2.06	1.87	1.80	1.67	1.61	1.62
Methionine	1.32	1.10	1.05	0.92	0.78	0.75
Valine	2.30	2.18	2.12	2.30	2.49	2.45
Alanine	2.64	2.84	2.77	2.79	2.83	2.65
Glycine	2.59	2.92	3.07	3.00	3.01	2.73
Proline	2.20	2.58	2.77	2.85	3.10	2.94
Glutamic acid	6.64	6.61	6.48	6.33	6.28	6.10
Serine	1.93	1.91	1.97	2.13	2.42	2.31
Threonine	1.92	1.72	1.68	1.70	1.71	1.63
Aspartic acid	4.16	3.74	3.60	3.72	3.72	3.69
Tryptophan	0.51	0.43	0.42	0.43	0.43	0.43
Cystine	0.48	0.51	0.54	0.61	0.70	0.69
Total	42.88	41.71	41.14	41.78	42.90	41.77
Total EAA*	22.72	21.11	20.48	20.96	21.54	21.35

* Total essential amino acids (including tyrosine and cystine).

Feeding Conditions

In this study, two experiments were conducted with yellowtail (*Seriola quinqueradiata*) (Expts. I and II) and one with red sea bream (*Pagrus major*) (Expt. III). Yellowtail experiments were performed at Fisheries Research Institute of Mie (Expt. I) and Oita Institute of Marine and Fisheries Science (Expt. II). Expt. III was carried out at Fishery Research Laboratory of Kyushu University.

Expt. I : Young yellowtail with an average body weight of about 140 g, which were fed a commercial dry pellet for about one and a half months before starting the experiment, were divided into six groups of 350 fish each in net cages (3×3×3 m) located in Owase Bay. They were cultured with each experimental diet for 101 days, from Aug.10 to Nov.18. During this time span they were fed for 67 days, once a day in the morning to near satiation. The water temperature ranged from 22.4 to 28.5℃ (average 24.5℃). All fish in each net cage were weighed at the start, after 24 and 58 days, and at the end of the experiment. On termination of the feeding experiment, 5 fish were collected from each lot to analyze the proximate composition of dorsal muscle and

liver.

Expt. II: Young yellowtail weighing 102 107 g on average were used. They were divided into six groups of 300 fish each, stocked in net cages (3×3×3 m), and reared for 97 days from Aug 22 to Nov. 26, at water temperature of 19.7 26.5℃ (average 23.3℃). During the period of experiment, fish were fed respective diets to near satiation, once a day in the morning, for 77 days. The average body weight of each group was determined every month by weighing all the fish in the net cages. At the end of feeding, 10 fish were taken from each lot for proximate analysis of dorsal muscle and liver. Analysis of proximate composition in both Expts. I and II was conducted at Tokyo University of Fisheries, and analytical methods were all described in a previous paper (Watanabe and Pongmaneerat 1991).

Expt. III: Thirty juvenile red sea bream having a mean body weight of about 30 g were selected and stocked in each of six rectangular aquariums (150 *l* capacity). Fish were reared for 70 days (10 weeks), from Sep.19 to Nov.28, in aquaria supplied with sand filtered sea water at a temperature of 21.5 25.5℃. Each experimental diet was given twice daily, each time to satiation for 60 days. During the rearing period, all fish in each lot were weighed every two weeks in order to record the mean body weight. At the end of experiment, 10 fish were collected from each aquarium for analyzing the composition of dorsal muscle and liver at Kyushu University.

Determination of Hematological Characteristics

At the final of both yellowtail experiments, 5 fish were taken from each lot for determination of hemochemical constituents and plasma enzyme activities by the same methods described previously (Watanabe *et al.* 1992, Chapter 1.2.2 1), to evaluate the health condition of the fish. The hematocrit, hemoglobin and plasma protein concentrations of red sea bream (n=10) were measured at the Kyushu University (Chapter 1.2.2 1). The data were statistically analyzed by ANOVA and Duncan's multiple range test, or Fisher's PLSD test, at a significance level of $p<0.05$.

Determination of Postprandial Concentration of Free Amino Acid in Blood Plasma

To evaluate the feed protein utilization efficiency, post feeding changes of free amino acid (FAA) concentration in plasma of fish from each group was determined in Expt. I. Fish were fed on each diet to satiation, at a feeding rate of 5.8 7.1% (on dry matter basis), after they were starved for 72 hours. Then five fish were collected from each net cage at pre feeding and at 3, 6, 12, 18, 24, 30, and 48 hours post feeding, to draw whole blood. Plasma was obtained by centrifugation of whole blood at 3,000 rpm for 15 min, and five plasma samples from each lot were pooled for analysis. Sampling was conducted after 39 days after initiation of feeding. Water temperature ranged from 24.5 25.8℃ during the examination. Analytical methods of FAA concentration were the same as described in an earlier paper (Watanabe *et al.* 1998).

Results

1. Yellowtail Experiments

Feed Performances

The results of growth and feed performances from Expts. I and II are shown in Table 3 3, and the growth curves are presented in Figs. 3 1 and 3 2.

Expt. I: Palatability and acceptability of the experimental diets were good for all groups, not affected by inclusion of alternate proteins and dietary fish meal levels and daily feeding rate was 2.7% for the control and 3.0 3.3% for the test groups. Final body weight and growth rate were highest in fish fed the control fish meal diet (599 g, 319%), and tended to decrease with decrease of fish meal component in alternate diets.

(355 547 g, 150 283%). The feed gain ratio and protein efficiency ratio were also best in fish fed the control diet and tended to decrease as fish meal substitution levels increased. The lowest growth and feed performance were noted for fish fed diets 5 (fish meal 10%) and 6 (fish meal 0%). However, there were no marked differences in these parameters between fish fed the control diet and those on diets 2 and 3 (fish meal 40 and 30%). In the anatomical test at the end of the experiment, the so called green liver symptom was observed for fish on diets 5 and 6 at the occurrence rate of 20 and 70%, respectively. The mortality mainly caused by bacterial disease (pseudotuberculosis and streptococcal bacterial disease) and/or parasitic disease (gill disease) ranged from 2.3 to 10.3% for all treatments, and the rates for fish on diets 2 and 3 were lower than others.

Expt. II: Growth and feed performances showed almost the same tendency as those observed in Expt. I. Fish fed the control diet had the maximum final body weight and growth rate (622 g, 509%), and these parameters decreased in fish fed the test diets with decreasing levels of fish meal component (336 590 g, 226 453%). A similar trend was observed for feed gain ratio and protein efficiency ratio, as shown in Expt. I. However, these parameters of fish on diets 2 and 3 (40 and 30% fish meal, respectively) were approximately equal to those of the control. On screening for the state of liver at the end, the occurrence of green liver was found at a high rate (50 90%) for fish fed the diets with fish meal at levels below 20%. The cumulative mortality was high for

Table 3-3. Growth and feed performances of yellowtail fed the experimental low or non fish meal diets

Diet no.	Av.body wt. (g)		Growth rate (%)	Feed gain ratio	Daily feed intake	Protein efficiency ratio	Mortality (%)
	Initial	Final					
Expt. I (Mie prefecture)							
1 FM diet	142.9	598.6	318.9	1.59	2.71	1.33	8.0
2 40% FM	142.9	546.8	282.6	1.81	2.98	1.17	2.3
3 30% FM	141.4	513.2	262.9	1.94	3.06	1.08	4.0
4 20% FM	142.6	464.0	225.4	2.20	3.23	0.97	8.0
5 10% FM	142.0	355.0	150.0	2.72	3.27	0.79	7.7
6 0% FM	141.4	402.6	184.7	2.34	3.09	0.95	10.3
Expt. II (Oita prefecture)							
1 FM diet	102.0	621.6	509.4	1.64	2.36	1.29	6.0
2 40% FM	106.5	589.1	453.1	2.07	2.60	1.02	5.4
3 30% FM	107.0	590.2	451.6	1.95	2.69	1.07	16.7
4 20% FM	106.6	527.9	395.2	2.21	2.90	0.97	20.7
5 10% FM	104.9	342.6	226.6	3.10	3.17	0.69	66.0
6 0% FM	103.2	336.3	225.9	3.49	3.42	0.64	79.3

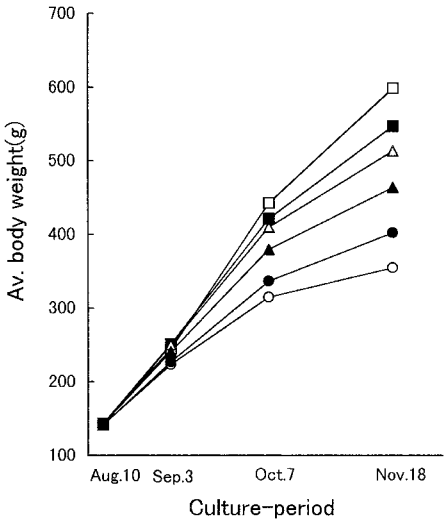

Fig. 3-1. Growth curves of young yellowtail fed the low or non fish meal experimental diets. □:Diet 1, ■:diet 2, △:diet 3, ▲:diet 4, ○:diet 5, ●:diet 6.

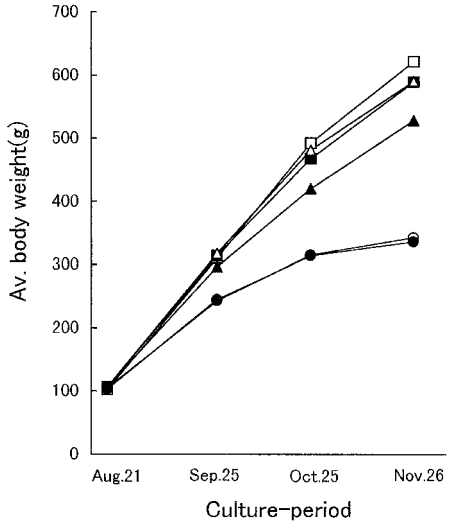

Fig. 3-2. Growth curves of young yellowtail fed the low or non fish meal experimental diets. □:Diet 1, ■:diet 2, △:diet 3, ▲:diet 4, ○:diet 5, ●:diet 6.

fish on diets 5 and 6, and was mainly due to parasitic disease (skin fluke disease) in the final phase of experiment (Oct.25 Nov.26). Moreover, the anatomical examination showed that most of dead fish had the green liver symptom (Maita *et al.* 1997), suggesting the abnormal health condition for fish fed diets 5 and 6.

Proximate Composition

The proximate compositions of the dorsal muscle and liver from the experimental fish in Expts. I and II are shown in Table 3 4. In Expt. I, no great difference was found between the treatments in the protein content of the dorsal muscle (around 24%). Also, the protein content in liver did not differ among the groups. The lipid and energy contents were highest in both muscle and liver of fish fed the control diet, and the values tended to reduce with decreased dietary fish meal levels, although the lipid level was approximately same for all experimental diets except for the control. In Expt. II, the results of proximate composition in dorsal muscle and liver were similar to those in Expt. I. The protein content was slightly lower in muscle of fish fed the control diet. The lipid and energy contents of muscle and liver decreased as the proportion of fish meal in diet decreased.

Table 3-4. Proximate composition (%) of dorsal muscle and liver of yellowtail fed the experimental low or non fish meal diets*1

Diet no.	Moisture	Crude protein	Crude lipid	Crude ash	Energy (kcal/g)
Expt. I (Mie prefecture)					
Dorsal muscle					
Initial	73.6	23.7 (89.8)*2	2.6 (9.8)	1.8 (6.8)	1.5
Final					
1 FM diet	69.4	23.4 (76.5)	6.5 (21.2)	2.0 (6.5)	1.9
2 40% FM	71.5	24.6 (86.3)	4.0 (14.0)	1.9 (3.2)	1.6
3 30% FM	72.2	24.5 (88.1)	4.3 (15.5)	1.7 (6.1)	1.7
4 20% FM	72.4	24.2 (87.7)	3.8 (13.8)	1.8 (6.5)	1.6
5 10% FM	72.7	23.4 (85.7)	3.1 (11.4)	1.7 (6.2)	1.4
6 0% FM	74.5	22.7 (89.0)	2.3 (9.0)	1.6 (6.3)	1.4
Liver					
Initial	69.4	15.9 (52.0)	11.4 (37.3)	1.4 (4.6)	2.1
Final					
1 FM diet	60.3	13.5 (34.0)	21.9 (55.2)	1.2 (3.0)	3.0
2 40% FM	65.2	13.6 (39.1)	16.6 (47.7)	1.1 (3.2)	2.5
3 30% FM	64.6	13.9 (39.3)	16.5 (46.6)	1.3 (3.7)	2.6
4 20% FM	66.6	13.3 (39.8)	14.5 (43.4)	1.2 (3.6)	2.5
5 10% FM	70.9	13.2 (45.4)	11.6 (39.9)	1.2 (4.1)	2.0
6 0% FM	74.3	13.8 (53.7)	8.2 (31.9)	1.2 (4.7)	1.7
Expt. II (Oita prefecture)					
Dorsal muscle					
1 FM diet	69.0	25.0 (80.6)	6.4 (20.6)	2.1 (6.8)	1.9
2 40% FM	69.8	25.4 (84.1)	5.5 (18.2)	2.3 (7.6)	1.8
3 30% FM	70.3	24.8 (83.5)	5.0 (16.8)	2.1 (7.1)	1.7
4 20% FM	71.9	25.3 (90.0)	3.1 (11.0)	2.0 (7.1)	1.5
5 10% FM	73.8	24.0 (91.6)	2.0 (7.6)	1.6 (6.1)	1.4
6 0% FM	74.7	23.2 (91.7)	1.6 (6.3)	1.5 (5.9)	1.3
Liver					
1 FM diet	56.7	12.5 (28.9)	26.8 (61.9)	1.2 (2.8)	3.4
2 40% FM	59.2	14.3 (35.0)	22.5 (55.1)	1.3 (3.2)	3.1
3 30% FM	61.8	15.4 (40.3)	19.9 (52.5)	1.4 (3.7)	2.8
4 20% FM	63.4	15.1 (41.3)	19.0 (51.9)	1.3 (3.6)	2.7
5 10% FM	72.7	16.5 (60.4)	9.5 (34.8)	1.5 (5.5)	1.8
6 0% FM	76.3	16.9 (71.3)	5.1 (21.5)	1.5 (6.3)	1.4

*1 Samples from 10 fish were pooled for analysis.

*2 Figures in parentheses are values on dry matter basis.

Hemochemical Characteristics

The results of hemochemical examination in Expts. I and II are shown in Table 3 5. In Expt. I, the hematocrit value of fish fed diets 5 and 6 was significantly lower than that of others, being about 33%, indicating an anemic state. There were no significant differences in the phospholipid, total cholesterol and free cholesterol levels between fish on the control diet and those on diets 2 and 3. However, these lipid metabolites of fish from diets 4 6 groups were significantly lower than those of fish on diets 1 3. Thus, plasma levels of the lipid metabolites tended to decrease with reduction of fish meal component in the diets. There were no marked differences in the protein and glucose metabolites among treatments. Moreover, the condition factor was highest for fish fed the control diet, and tended to decline as the fish meal content in diets decreased. There was no significant difference in hepatosomatic index among the treatments. In Expt. II, the values for blood parameters generally showed the same tendency as those obtained in Expt. I. The common observations for the two experiments were 1) the anemic state of fish on diets 5 and 6, 2) significantly lower lipid metabolites in fish on diets 4 6.

Table 3-5. Results of hemochemical examination in yellowtail fed the experimental low or non fish meal diets*

		Diet no.					
		1	2	3	4	5	6
Expt. I							
Ht	(%)	41.0±4.9^{a}	40.1±1.6^{a}	44.4±2.8^{a}	39.1±2.5^{ab}	32.7±4.4^{b}	33.7±4.8^{b}
ALP	(IU / l)	153±22^{b}	137±12^{b}	172±15^{ab}	162±36^{b}	212±37^{a}	143±19^{b}
GLU	(mg / 100ml)	120±5	116±12	123±19	117±14	126±26	117±23
TG	(mg / 100ml)	196±54	126±26	182±24	146±26	167±57	150±19
PL	(mg / 100ml)	847±45^{a}	751±23^{b}	859±34^{a}	695±64^{bc}	621±81^{c}	575±91^{c}
TCHO	(mg / 100ml)	292±22^{a}	285±6^{a}	308±11^{a}	251±24^{b}	202±23^{c}	202±32^{c}
FCHO	(mg / 100ml)	123±6^{ab}	111±4^{b}	127±3^{a}	101±10^{bc}	92±147^{c}	85±11^{c}
Ester ratio	(%)	57.7±3.0^{a}	61.2±2.1^{a}	58.9±1.0^{ab}	59.8±0.9^{ab}	54.6±2.1^{b}	57.4±2.3^{b}
BUN	(mg / 100ml)	18.2±2.0^{ab}	20.9±3.1^{a}	20.4±1.8^{ab}	19.9±2.8^{ab}	16.2±2.6^{b}	12.2±4.2^{b}
CRE	(mg / 100ml)	1.3±0.3^{ab}	1.1±0.1^{ab}	1.0±0.1^{b}	1.3±0.3^{a}	1.0±0.1^{b}	0.9±0.1^{b}
TP	(g / 100ml)	3.5±0.2	3.6±0.1	3.8±0.1	3.5±0.3	3.6±0.4	3.3±0.4
Condition factor		16.9±1.3^{a}	16.2±0.4^{a}	16.1±0.6^{a}	15.3±0.5^{b}	13.9±0.3^{c}	13.7±0.6^{c}
Hepatosomatic index (%)		1.48±0.24	1.39±0.07	1.41±0.15	1.48±0.15	1.39±0.22	1.42±0.22
Expt. II							
Ht	(%)	41.9±1.8^{a}	42.3±2.0^{a}	41.4±1.9^{a}	39.9±2.5^{a}	30.1±5.8^{b}	24.0±5.2^{c}
ALP	(IU / l)	152±24	153 ±26	134 ±23	144±25	141±16	141±36
GLU	(mg / 100ml)	87±5^{b}	88 ±4^{b}	99±6^{ab}	92±8^{b}	102±10^{a}	97±5^{ab}
TG	(mg / 100ml)	200±36^{a}	184±62^{ab}	139±58^{bc}	138±35^{bc}	111±31^{c}	111±21^{c}
PL	(mg / 100ml)	769±64^{a}	713±51^{ab}	711±60^{ab}	631±102^{b}	502±77^{c}	513±83^{c}
TCHO	(mg / 100ml)	301±25^{a}	271±20^{ab}	306±74^{a}	202±41^{b}	171±19^{c}	165±15^{c}
FCHO	(mg / 100ml)	124±17^{a}	106±9^{b}	112±11^{ab}	98±24^{bc}	75±10^{c}	81±5^{c}
Ester ratio	(%)	59.1±2.2^{a}	61.0±2.4^{a}	61.4±9.2^{a}	55.9±3.7^{ab}	56.2±1.7^{ab}	50.8±2.1^{b}
BUN	(mg / 100ml)	11.6±1.8^{b}	13.3±0.7^{b}	15.4±2.8^{ab}	16.1±2.3^{a}	15.5±1.9^{ab}	16.2±1.6^{a}
CRE	(mg / 100ml)	1.2±0.4^{a}	0.8±0.1^{b}	0.8±0.0^{b}	0.9±0.1^{b}	0.8±0.0^{b}	0.9±0.1^{b}
TP	(g / 100ml)	3.1±0.1^{ab}	3.3±0.1^{a}	3.2±0.2^{a}	3.3±0.3^{a}	3.1±0.2^{a}	2.8±0.1^{b}

* Mean ± standard deviation (n=5). Figures in a row with different superscripts are significantly different from each other ($p<0.05$) when analyzed using Duncan's multiple range test.

Concentration of Plasma FAA

The periodical changes of FAA contents in blood plasma from fish fed the experimental diets are shown in Table 3 6. Remarkable differences in concentration of some FAA in plasma were observed among dietary treatments with different levels of fish meal. Plasma levels of methionine, lysine, threonine, isoleucine, arginine and total EAA showed a tendency to decrease with decrease of fish meal and/or EAA content in the diets. Especially, the lysine and methionine contents of fish fed the diets 5 and 6 (10 and 0% fish meal) were reflected by lower levels of these EAA in the diets and remarkably lower than those on the control diet (cf. Fig. 3 3). On the other hand, these amino acid concentrations in fish on diets 2 and 3 (40 and 30% fish meal) were approximately in a same level as the control. These FAA increased gradually after feed intake, reaching a plateau at 18 24 hours post feeding, although there were similar periodical changes in these EAA irrespective of the dietary treatments. With regard to other EAA, tryptophan and histidine levels for fish fed the alternate protein diets were lower than those on the control, but no distinct relation between these EAA levels and dietary fish meal contents was observed. Moreover, plasma concentrations of leucine and valine were similar for all the treatments, and no definite relation was found between the level of plasma FAA and these EAA contents in the diets.

Table 3-6. Periodical changes of free amino acid contents in blood plasma of yellowtail fed the experimental low or non fish meal diets (μmol/100ml plasma)

Diet no.	Hours after feeding (h) 0	3	6	12	18	24	30	48
Total EAA *[1]								
1 FM diet	108.8	195.8	179.7	235.6	282.4	206.9	166.5	92.4
2 40% FM	117.9	147.4	153.7	256.7	270.9	240.6	180.1	118.6
3 30% FM	148.0	177.4	177.7	248.1	279.0	186.1	149.5	115.8
4 20% FM	153.6	161.8	166.5	225.1	248.8	209.1	193.7	93.2
5 10% FM	141.3	143.5	163.3	202.7	239.8	198.3	182.5	104.2
6 0% FM	152.9	134.4	156.1	211.6	250.3	206.2	203.1	110.4
Total NEAA *[2]								
1 FM diet	190.6	209.2	124.5	129.3	171.9	183.6	178.4	146.1
2 40% FM	207.6	153.7	148.7	146.6	173.8	225.7	205.9	182.7
3 30% FM	250.1	175.9	167.7	186.3	198.7	192.9	199.5	184.1
4 20% FM	227.6	169.3	212.8	188.0	224.8	234.6	246.2	150.6
5 10% FM	251.1	168.1	178.5	201.6	217.8	217.7	212.0	191.2
6 0% FM	259.4	159.8	175.6	207.9	222.6	219.8	231.1	190.0
Total FAA *[3]								
1 FM diet	299.3	405.0	304.2	364.8	454.3	390.4	344.8	238.5
2 40% FM	325.4	301.1	302.5	403.4	444.7	466.3	386.0	301.3
3 30% FM	398.1	353.4	345.4	434.3	477.7	379.0	348.9	300.0
4 20% FM	381.2	331.0	379.2	413.1	473.6	443.7	440.0	243.8
5 10% FM	392.3	311.7	341.8	404.3	457.5	416.0	394.5	295.3
6 0% FM	412.3	294.2	331.8	419.5	472.9	425.9	434.3	300.4

*[1] Total essential amino acids: arginine, lysine, histidine, phenylalanine, tyrosine, leucine, isoleucine, methionine, valine, threo nine, tryptophan, and cystine.

*[2] Total non essential amino acids: alanine, glycine, glutamic acid, serine, taurine, and aspartic acid.

*[3] Total free amino acids.

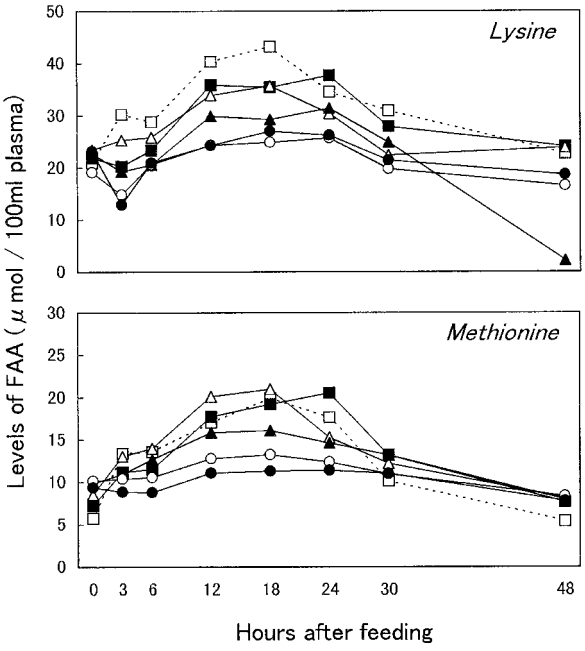

Fig. 3-3. Periodical changes of plasma lysine and methionine levels in yellowtail after feeding the experimental low or non fish meal diets.
□:Diet 1, ■:diet 2, △:diet 3, ▲:diet 4, ○:diet 5, ●:diet 6.

2. Red Sea Bream Experiment

Feed Performances

The results of growth and feed performances in Expt. Ⅲ are shown in Table 3 7. Palatability and acceptability of diets was not influenced by replacing fish meal with alternate protein ingredients. The average body weight of fish and feed gain ratio at the end of feeding was not much different between the treatments (50 55 g, 1.6 1.8), although these values were slightly higher for fish fed diet 3 (fish meal 30%). A similar trend was also found for the protein efficiency ratio value (1.19 1.35). These parameters in fish on diet 5 were slightly lower than those of other groups. Diet 6 (non fish meal diet), which produced the lowest feed performance in yellowtail, was comparable to the control diet in dietary value. The reason for this observation was unknown. Thus, unlike yellowtail, growth and feed performances in red sea bream were found to be independent of the fish meal content in diets.

Table 3-7. Growth and feed performances of red sea bream fed the experimental low or non fish meal diets

Diet no.	Av.body wt. (g)		Growth rate	Feed gain ratio	Daily feed intake	Protein efficiency ratio	Mortality (numbers)
	Initial	Final	(%)				
1 FM diet	29.7	53.2	79.1	1.65	1.56	1.29	2
2 40% FM	29.6	51.3	73.3	1.77	1.59	1.21	0
3 30% FM	29.6	55.3	86.8	1.59	1.61	1.35	2
4 20% FM	29.7	51.5	73.4	1.70	1.51	1.27	1
5 10% FM	29.6	50.3	69.9	1.79	1.55	1.19	0
6 0% FM	29.6	53.4	80.4	1.59	1.53	1.34	0

Proximate Composition and Hematological Characteristics

The results of proximate analysis are shown in Table 3 8. The moisture, protein, lipid, and ash contents of the muscle were almost the same among the groups. In the liver, however, the lipid content of fish fed diets 1 and 2 was slightly higher than that of fish on other diets, and was the lowest in the diet 6 group. The fish on diets 1 and 2 showed slightly low moisture contents in the liver, reflecting the higher lipid contents. Hepatosomatic index value of fish on diet 5 was significantly lower than that of the rest.

The results of hematological test are presented in Table 3 9. Both hematocrit and hemoglobin were not markedly different among the groups and evaluated to be within the normal range for red sea bream. The plasma protein varied from 5.3 7.1 g/100ml, and showed a tendency to decline with decreased fish meal levels in diets.

Table 3-8. Proximate composition (%) of dorsal muscle and liver of red sea bream fed the experimental low or non fish meal diets*1

Diet no.	Moisture	Crude protein	Crude lipid	Crude ash
Dorsal muscle				
1 FM diet	74.2	21.0 (81.4)*2	2.7 (10.5)	1.7 (6.6)
2 40% FM	73.9	21.1 (80.8)	2.7 (10.3)	1.7 (6.5)
3 30% FM	74.5	21.2 (83.1)	2.5 (9.8)	1.7 (6.7)
4 20% FM	73.4	21.6 (81.2)	2.8 (10.5)	1.8 (6.8)
5 10% FM	74.1	21.7 (83.8)	2.2 (8.5)	1.8 (6.9)
6 0% FM	73.3	21.7 (81.3)	2.5 (9.4)	1.9 (7.1)
Liver				
1 FM diet	63.3	12.0 (32.7)	12.9 (35.1)	1.5 (4.1)
2 40% FM	62.0	11.0 (28.9)	14.0 (36.8)	1.3 (3.4)
3 30% FM	64.8	10.6 (30.1)	10.4 (29.5)	1.5 (4.3)
4 20% FM	65.6	11.6 (33.7)	9.1 (26.5)	1.5 (4.4)
5 10% FM	65.1	11.5 (33.0)	10.3 (29.5)	1.5 (4.3)
6 0% FM	65.3	11.3 (32.6)	9.0 (25.9)	1.6 (4.6)

*1 Samples from 10 fish were pooled for analysis.

*2 Figures in parentheses are values on dry matter basis.

Table 3-9. Results of blood characteristics of red sea bream fed the experimental low or non fish meal diets*

		Diet no.					
		1	2	3	4	5	6
Hematocrit	(%)	36.8±2.0^{b}	36.4±1.7^{b}	37.9±4.1^{b}	37.5±3.0^{b}	38.7±2.7^{ab}	40.8±3.6^{a}
Hemoglobin	(g / 100ml)	9.4±0.8^{b}	10.7±0.7^{a}	10.9±1.1^{a}	10.2±1.3^{ab}	10.1±0.8^{ab}	10.3±1.0^{a}
Plasma protein	(g / 100ml)	7.1±1.2^{a}	6.7±1.7^{ab}	6.2±0.9^{bc}	6.2±0.7^{bc}	5.3±0.3^{c}	5.9±0.4^{bc}
Hepatosomatic index	(%)	1.91±0.36^{ab}	2.09±0.34^{a}	1.74±0.27^{b}	1.96±0.47^{ab}	1.33±0.16^{c}	1.75±0.33^{b}

* Mean ± standard deviation (n=10). Values in the same row bearing different superscripts letters are significantly different from each other ($p < 0.05$) when analyzed using Fisher's PLSD test.

Discussion

In yellowtail, the growth and feed performances were clearly influenced by fish meal levels in the diets. The performance parameters of fish tended to reduce with decreasing content of fish meal in the diets. This could be attributed to the deficiency of some EAA in the diets. Especially the contents of methionine and lysine of diets 5 and 6, which produced poor feed performances, seemed to be below the requirement level of yellowtail[*2]. An EAA deficiency state might have resulted and this idea was supported by the fact that plasma levels of several EAA decreased as fish meal contents in the diet decreased.

Among the diets with alternate proteins, diets 2 and 3 could produce feed performances close to those on the fish meal control diet. Moreover, the health condition of these alternate protein diet groups was evaluated to be in a good status, comparable to the control. These facts indicated that the combination of SBM, CGM, MM, and MBM was nutritionally efficient as fish meal replacer to reduce the fish meal level to 30% in the feeds for yellowtail. This replaceable level is consistent with the result obtained in our previous studies on the use of alternate proteins for yellowtail (Watanabe *et al.* 1992, 1995, Viyakarn *et al.* 1992). On the other hand, decreasing fish meal levels below 20% (diets 4 6) resulted in marked reduction of growth rate and feed gain ratio, and high mortality. Besides, the plasma chemical parameters related to lipid metabolism which is a special indicator of health status for yellowtail (Maita *et al.* 1998a, 1998b) were significantly lower in fish fed these low or non fish meal diets than those on the control diet, suggesting poor health condition. In addition, occurrence of green liver symptom and the low lipid content in fish of these groups might indicate the abnormality in liver function and lipid metabolism. The plasma FAA concentration of the diets 5 and 6 groups was especially low compared to the control, suggesting the insufficiency of FAA level in plasma to synthesize the body protein for normal growth. Thus, these facts suggested that the diets with alternate ingredients at higher inclusion levels were deficient in some EAA such as lysine and methionine.

Several studies have demonstrated that high inclusion of alternate ingredients as a partial substitution for fish meal resulted in the reduction of feed utilization in terms of growth and feed performances in both marine and fresh water fish. As for yellowtail, the reduction of weight gain due to replacement of fish meal with either SBM, CGM, MBM, rapeseed meal or malt protein flour at high inclusion levels in extruded dry pellets and/or moist pellets has been observed (Watanabe *et al.* 1992, Viyakarn *et al.* 1992, Shimeno *et al.* 1992b, 1993a, 1994). This might be due to the impairment of amino acid balance, decrease of protein and energy contents in diets and/or influence of anti nutritional factors in feedstuffs. The present results have shown that the poor growth and feed performances obtained in fish on diets with alternate ingredients at higher inclusion levels might be attributed to a deficiency in some EAA judging from the plasma FAA concentrations. For improvement of feed utilization, supplementation of crystalline amino acids may be effective as observed in rainbow trout (Dabrowska and Wojno 1977), carp (Murai *et al.* 1986, 1989) and yellowtail (Takii *et al.* 1989). Therefore, further studies will be necessary to investigate the effect of EAA supplementation to alternate protein diets for yellowtail.

With regard to red sea bream, no marked difference was observed in growth and feed performances among dietary treatments. This suggests that the nutritive value of alternate protein diets was comparable to the fish meal based diet for red sea bream, unlike yellowtail. Moreover, the health condition of fish fed the alternate protein diets seemed to be in good status judging from the hematological characteristics. Thus, the availability of alternate protein ingredients was different between red sea bream and yellowtail, and this might partly be due to difference in protein utilization and EAA requirement. Juvenile red sea bream can utilize alternate animal and plant protein ingredients more efficiently than yellowtail, indicating that formulation of low or non fish meal diets is relatively easy for red sea bream. In fact Takagi *et al.* (1999) succeeded to culture red sea bream with low or non fish meal diets with alternative protein ingredients for a long period. Nutritional quality of low or non fish meal

diets of course depends on combination of alternate ingredients in terms of EAA balance. In our previous experiment, inclusion of CGM at levels exceeding 15% in extruded pellets caused a reduction in growth performance of juvenile red sea bream (Chapter 1.2.2 1). In another experiment, we found that the diet containing SPC at a high level (50%) also led to a decrease in growth rate and feed gain ratio for the juveniles (Chapter 1.2.2 2). Accordingly, further work is needed to clarify an adequate combination of some useful alternative protein sources in diets without fish meal component.

In conclusion, the results of present study have shown that fish meal can be replaced partially with several plant and animal protein ingredients at a substitution level of around 50% in yellowtail diets. However, improvement of feed quality is necessary when alternate proteins are included to replace fish meal above this level. Supplementation of certain amino acids would be effective for improvement of utilization efficiency of alternate ingredients. As for red sea bream, it was found that fish meal component can be completely substituted by combination of various protein ingredients without ill effects.

3.2 Growth Performances on Non-Fish Meal Diets

3.2.1 Yellowtail

3.2.1-1) A Trial to Culture Yellowtail with Non-Fish Meal Diets

Though the present total production of animal feeds in Japan is estimated to be around 25 million t, the quantum of fish meal being employed in formulations stands at a meager 1.2%. In sharp contrast, out of the 500,000 t of fish feed produced during 1996 in Japan, the proportion of fish meal was about 56%, a manifold higher than that of other animal feeds.

A recent rapid decline of the sardine catch in Japan has resulted in a shortage of fish meal supply for the feed industry, prompting reliance on imported fish meal. To overcome this shortfall, efforts are now being directed to finding alternative protein sources of good nutritional value as a substitute for the expensive fish meal component in practical diets. The development of a new type of dry pellet, classified as "SDP" for yellowtail (Watanabe *et al.* 1991), has opened up the possibility of utilizing alternative protein sources as substitutes for fish meal in practical diets for marine finfish. Therefore, availability of various plant and animal protein ingredients as a protein source has already been tested using SDP in red sea bream and yellowtail (Watanabe *et al.* 1992, 1994, 1995, Viyakarn *et al.* 1992, Chapter 1.2.1, 1.2.2 1). The results hitherto obtained have shown that SBM can be included as a protein source up to 30% in place of fish meal without any adverse effects (Watanabe *et al.* 1992, Viyakarn *et al.* 1992), and even up to 50% SBM without influencing palatability and acceptability (Viyakarn *et al.* 1992). In addition, a diet with 25% SBM and 15% CGM showed better feed performance than the 40% SBM diet, suggesting the availability of CGM as a protein source in combination with SBM (Viyakarn *et al.* 1992). Later experiments conducted on a practical scale in net cages for two years have demonstrated that a combination of SBM, CGM, and MM at different levels could replace 50 60% of fish meal in diets for yellowtail and red sea bream (Watanabe 1994). Thus, the recent experience in the replacement of fish meal with alternatives in aquafeeds has been successful, and the expensive fish meal component is still being gradually relieved by substitution with various protein meals. Further efforts are now needed for aquaculture to develop a new type of fish feed formula based on alternative protein sources.

The purpose of this series of studies is to develop a non fish meal diet for yellowtail by replacing fish meal with plant and animal protein sources. Thus, non fish meal diets were formulated using SPC, SBM, CGM, and MM as protein sources for this trial with yellowtail (Watanabe *et al.* 1998).

Materials and Methods

Experimental Diets

Feeding experiments were conducted with juvenile (Expt. I) and young (Expt. II) yellowtail.

The composition of the experimental non-fish meal diets and their proximate values analyzed by the same methods described previously are shown in Table 3-10 (Watanabe and Pongmaneerat 1991). All the diets were formulated to have around 45% crude protein (CP) and 21% crude lipid (CL) as those hitherto used in yellowtail experiments. A commercial yellowtail SDP with 67% fish meal (CP: 68%) as a main protein source was used as a control diet. The fish meal component was entirely eliminated from the experimental non-fish meal diets and replaced by a combination of alternative protein sources such as SPC (Protao, a product of Aarhus Olie; CP: 70%), SBM (CP: 46%), CGM (CP: 65%), and MM (CP: 80%) at different levels. TheSPC was used as a main protein source, ranging from 40% in diet 2 to 20% in d iets 5 and 6, CGM being correspondingly included at a level of 3% in diet 2 and 23% in diets 5 and 6. Diets 3 and 4 contained 30 and 25% SPC and 13 and 18% CGM, respectively. All the diets except the control diet were incorporated with 2% krill meal in order to enhance palatability to juveniles, although its non-inclusion did not impair palatability in a preliminary study with 900 g yellowtail. The wheat flour as a carbohydrate source, which also served as a binder, was included only at 8%, a provision for elevating the protein level. The mineral and vitamin mixtures were prepared to satisfy the requirements of yellowtail (Watanabe *et al.* 1991). A mixture of 1.5% L-lysine, 0.5% DL-methionine, 0.5% L-threonine, and 0.2% L-tryptophan was supplemented in diets 2-5 to match the amino acid profile with that of the control fish meal diet. The same amino acid mixture, coated with alpha-starch was added to diet 6 which had the same dietary composition as diet 5, in order to compare availability between crystalline amino acids with and without coating. The test diets were prepared as dry pellets by a small twin screw extruder (Suehiro Iron Works Co.), while the control fish meal diet was made as SDP using a large twin screw extruder (Buhler Co.); the extruding conditions of both the machines were adjusted close to those reported previously. The prepared diets were kept in a cold storage room (5℃) during the period of feeding.

The amino acid composition of the test diets analyzed by Japan Food Research Laboratories is shown in Table 3-11. The amino acid composition of all

Table 3-10. Composition of the fish meal and non-fish meal diets for yellowtail

Ingredient(%)	Diet no. 1	2	3	4	5	6
Soy protein concentrate	Commercial yellowtail SDP	40	30	25	20	20
Defatted soybean meal		10	10	10	10	10
Corn gluten meal		3	13	18	23	23
Meat meal		12	13	15	14	14
Krill meal		2	2	2	2	2
Wheat flour		8	8	8	8	8
Mineral mixture		1.35	1.35	1.35	1.35	1.35
Calcium phosphate*[1]		1	1	1	1	1
Fe (peptide)		0.1	0.1	0.1	0.1	0.1
Vitamin mixture		1.5	1.5	1.5	1.5	1.5
Choline chloride (50%)		0.98	0.98	0.98	0.98	0.98
Vitamin E (50%)		0.02	0.02	0.02	0.02	0.02
AMP*[2]		0.05	0.05	0.05	0.05	0.05
Feed oil		20	19	17	18	18
Amino acid mixture						
Free*[3]	—	2.7	2.7	2.7	2.7	—
coated (AP-7)*[4]	—	—	—	—	—	5.0
Total		102.7	102.7	102.7	102.7	105.0
Nutrient contents determined (% as is basis)						
Crude protein	47.1	46.5	47.4	47.7	47.7	46.7
Crude lipid	23.0	23.9	23.7	23.2	23.5	23.5
Crude ash	9.9	5.7	5.3	5.1	4.8	4.8
Moisture	6.5	4.3	4.2	6.2	5.4	5.5
Calcium	ND*[5]	0.57	0.56	0.58	0.56	0.54
Phosphorus	ND	0.89	0.85	0.85	0.82	0.80

*[1] Monobasic.
*[2] Mg-L-ascorbyl-2-phosphate.
*[3] Lysine 1.5, methionine 0.5, threonine 0.5, and tryptophan 0.2.
*[4] Lysine 1.5, methionine 0.5, threonine 0.5, tryptophan 0.2, and other 2.3.
*[5] Not determined.

test diets was comparable to that of the control diet, containing a sufficient amount of EAA to satisfy the requirement of yellowtail[*2]. The fatty acid composition of the experimental diets analyzed by the method described previously is shown in Table 3 12 (Watanabe and Pongmaneerat 1991). The percentage of n 3 HUFA, the essential fatty acids for fish, did not differ between the test diets, but was quite high in the control diet which included both supplemental fish oil and that derived from fish meal. The content of n 3 HUFA in all diets satisfied the EFA requirement of yellowtail (Deshimaru *et al.* 1982, Watanabe *et al.* 1991).

Table 3-11. Amino acid composition of the fish meal and non fish meal diets for yellowtail

Amino acid (g / 100g diet)	Diet no.					
	1	2	3	4	5	6
Arginine	2.66	3.03	2.84	2.74	2.63	2.58
Lysine	3.42	3.71	3.52	3.36	3.27	3.00
Histidine	1.32	1.19	1.13	1.15	1.13	1.07
Phenylalanine	1.89	2.18	2.26	2.32	2.42	2.38
Tyrosine	1.51	1.47	1.58	1.65	1.73	1.71
Leucine	3.38	3.54	4.16	4.51	4.92	4.81
Isoleucine	1.95	1.89	1.88	1.88	1.91	1.88
Methionine	1.24	1.06	1.15	1.18	1.26	1.11
Cystine	0.49	0.60	0.64	0.66	0.67	0.64
Valine	2.34	2.15	2.17	2.20	2.22	2.19
Alanine	2.78	2.28	2.63	2.85	3.05	2.95
Glycine	2.94	2.57	2.61	2.67	2.62	2.58
Proline	2.19	2.85	3.15	3.30	3.55	3.55
Glutamic acid	6.13	8.11	8.38	8.58	8.83	8.88
Serine	1.89	2.25	2.31	2.32	2.36	2.32
Threonine	1.95	2.15	2.16	2.17	2.16	2.07
Aspartic acid	4.29	4.59	4.32	4.16	4.08	4.00
Tryptophan	0.56	0.72	0.68	0.67	0.64	0.61
Total	42.93	46.34	47.57	48.37	49.45	48.33
Total EAA*	22.71	23.69	24.17	24.49	24.96	24.05

* Total essential amino acids (including tyrosine and cystine).

Table 3-12. Certain fatty acids of the fish meal and non fish meal diets for yellowtail

Fatty acid (% area)	Diet no.					
	1	2	3	4	5	6
14:0	6.3	4.8	4.9	4.8	4.6	4.7
16:0	16.9	13.0	13.3	13.5	13.1	13.3
16:1	7.2	8.2	8.1	7.8	7.6	7.6
18:0	2.7	2.8	2.8	3.1	2.9	2.9
18:1	16.2	20.7	20.5	20.7	20.4	20.4
18:2n-6	2.1	3.9	4.8	5.7	6.0	6.4
18:4n-3	2.5	1.8	1.8	1.8	1.8	1.8
20:1	6.0	12.6	12.2	11.8	12.0	11.7
20:5n-3	13.0	9.0	9.0	8.7	8.9	9.0
22:1	6.0	10.6	10.2	9.8	10.3	9.9
22:5n-3	1.7	0.8	0.8	0.7	0.8	0.8
22:6n-3	10.0	4.5	4.5	4.4	4.7	4.7
Σ Saturated	27.3	21.5	21.9	22.4	21.6	21.8
Σ Monoene	35.4	52.1	51.0	50.1	50.3	49.6
Σ n-6	3.9	4.9	5.7	6.6	6.9	7.3
Σ n-3	29.0	17.4	17.6	17.0	17.6	17.6
Σ n-3 HUFA	25.5	14.9	14.9	14.3	14.9	15.0
n-3 / n-6	7.4	3.6	3.1	2.6	2.6	2.4
DHA / EPA	0.8	0.5	0.5	0.5	0.5	0.5

Feeding Conditions

Expt. I : Juvenile yellowtail (*Seriola quinqueradiata*) with an average body weight of about 13.1 g were randomly sampled from stock which had previously been fed a commercial SDP (Sakamoto Fish Feed Co.) for two weeks. They were divided into six polycarbonate tanks (500 *l* capacity, 400 *l* water volume), 30 fish in each, provided a continuous water supply, and aerated at a water temperature of 23.9±2.0℃. Fish were fed the test diets for 45 days, twice a day (08:00 and 17:00), each time to satiation. At the beginning and end of the experiment, fish were starved for 24 hours and anesthetized with aminobenzoate (100 ppm) before being weighed individually (initial and final body weight). The ten fish were collected from each lot at the initial and final phases for proximate analysis.

Expt. II : Young yellowtail weighing around 130 g were used for the feeding trial. Initially these fish were collected as wild caught fry, and were fed with a commercial SDP (Sakamoto Fish Feed Co.) for two months. They were then divided into six groups of 350 fish each, kept in the covered net cages (3×3×3 m), and fed the

experimental non fish meal diets during the August October period (50 days of feeding). Fish were fed six days a week, once in the morning to satiation. Corresponding to the growth of the fish, the number of fish per cage was gauged every month during the determination of the total body weight. The initial water temperature of 27.3 ℃ dropped to 21.8℃ in the final phase. At the end of feeding trial, five fish were taken from each lot after 24 hour starvation for proximate analysis of muscle and liver. The analytical procedures for proximate composition were those described previously (Watanabe and Pongmaneerat 1991).

Determination of Hematological Characteristics

Blood was sampled from each fish in both Expts. I and II, after fish were starved for 24 hours. Details of blood collection, treatments, and methods of determining each parameter were all as described in a previous paper (Watanabe *et al.* 1992).

Periodical Changes of Free Amino Acids in Blood Plasma and Digesta Contents

In order to compare the post feeding changes in plasma amino acid and digesta content in fish fed the non fish meal diets containing crystalline amino acids with and without coating (diets 5 and 6, respectively), and the control diet, the free amino acid content in plasma and digesta contents in the stomach and intestine were determined in Expt. II. After a three day fasting, five fish were taken from each net cage for collecting whole blood to determine the fasting level of plasma amino acid. Plasma sample was obtained by centrifugation of blood at 3,000 rpm for 15 min. Then the experimental fish were fed the respective diets to satiation. Five fish were sampled from each group at 3, 6, 12, 18, and 24 hours after feeding, at water temperature of 21.3 24.5℃. The average body weight of fish samples was 269 273 g for the non fish meal diet group and 347 g for the control. After withdrawing blood, the digesta in the stomach and intestine were collected from fish to calculate relative weights. Analysis of plasma for free amino acid constituents was conducted by the Central Research Institute of Nippon Formula Feed Co. Ltd.

Results and Discussion

Feed Performances

Expt. I : Growth rate and feed gain ratio in fish fed the non fish meal diets are shown in Table 3 13. Palatability of the test diets was found to be generally poor among juveniles throughout the feeding period, unlike young yellowtail which showed active feeding, indicating a size related difference. Therefore, growth of fish fed the non fish meal diets (average body weight 62 78 g) was quite inferior to that of the control fish which grew up to 158 g from 13 g during 45 days of feeding. The same pattern was obtained for feed gain ratio, 0.89 for the control SDP as against 1.55 1.83 for the non fish meal diets. The poor feed performance might be partly due to low palatability and quality of non fish meal diets. However, anatomical observation at the end of the feeding experiment revealed that almost all the fish fed the non fish meal diets had the so called "green liver" status, unlike the control fish. This symptom was found to be caused by occlusion of the bile duct due to parasitic mucosporozoa.

Table 3-13. Growth and feed gain ratio in juvenile yellowtail fed the fish meal and non fish meal diets in tanks

Diet no.	Av.body wt.* (g)		Growth rate (%)	Feed gain ratio	Daily feed intake	Total feed consumption (g)
	Initial	Final				
1	12.9±1.4	158.3±19.9	1127.1	0.89	3.25	3093.6
2	13.4±1.4	62.9±8.2	369.4	1.89	4.26	1942.2
3	13.5±1.4	78.6±12.8	482.2	1.66	4.86	2601.7
4	12.8±1.5	76.2±13.0	495.3	1.55	4.49	2240.8
5	13.3±1.3	69.4±11.1	421.8	1.70	4.71	2239.4
6	13.3±1.6	72.4±10.2	444.4	1.59	4.65	2422.1

* Mean ± standard deviation.

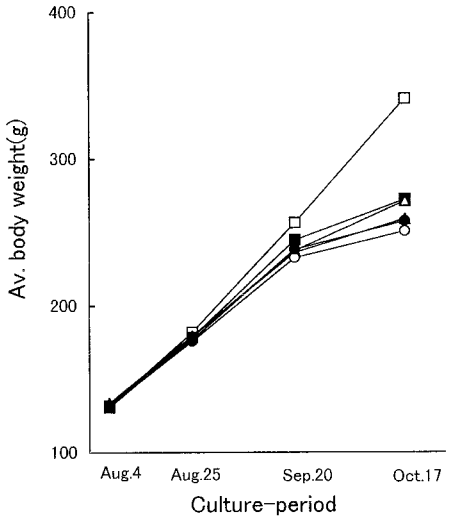

Fig. 3-4. Growth curves of young yellowtail fed the low or non fish meal experimental diets. □:Diet 1, ■:diet 2, △:diet 3, ▲:diet 4, ○:diet 5, ●:diet 6.

Table 3-14. Growth and feed gain ratio of young yellowtail fed the fish meal and non fish meal diets in net cages

Diet no.	Av.body wt. (g) Initial	Final	Growth rate (%)	Feed gain ratio	Daily feed intake	Mortality (%)
Aug.4 ~ Aug.24 (16 days feeding)						
1	131.9	181.8	37.8	0.98	2.11	0.0
2	130.9	178.5	36.4	1.02	2.13	0.0
3	133.4	179.0	34.2	1.06	2.11	0.3
4	133.8	179.6	34.2	1.06	2.11	0.0
5	132.2	175.9	33.1	1.12	2.14	0.9
6	132.2	176.9	33.8	1.09	2.14	1.3
Aug.25 ~ Sep.19 (15 days feeding)						
1	181.8	256.4	41.0	1.19	2.91	0.9
2	178.5	244.8	37.1	1.37	3.03	4.1
3	179.0	237.4	32.6	1.52	3.06	1.3
4	179.6	236.2	31.5	1.56	3.06	0.9
5	175.2	232.6	32.2	1.58	3.14	0.9
6	176.9	238.3	34.7	1.49	3.10	4.4
Sep.20 ~ Oct.17 (17 days feeding)						
1	256.4	340.8	32.9	1.89	3.20	1.0
2	244.8	272.3	11.2	4.23	2.59	8.4
3	237.4	271.2	14.2	3.68	2.80	9.0
4	236.2	259.1	9.7	5.41	2.80	11.9
5	232.6	250.7	7.8	6.60	2.82	10.6
6	238.3	257.6	8.1	5.85	2.47	28.8
Total feeding period (50 days feeding)						
1	131.9	340.8	158.4	1.41	2.75	1.9
2	130.9	272.3	108.0	1.76	2.67	11.9
3	133.4	271.2	103.3	1.86	2.76	10.3
4	133.8	259.1	93.7	2.02	2.82	12.5
5	132.2	250.7	89.6	2.13	2.87	12.2
6	132.2	257.6	94.9	1.89	2.65	32.2

Expt. II: The results of growth and feed utilization in fish fed the test diets in the net cages are shown in Table 3 14 and Fig. 3 4. The average body weight of fish fed the non fish meal diets was almost comparable to that of the control group until the 46th day (the initial phase of 31 days of feeding) after the initiation of feeding, being 233 245 g for the non fish meal groups and 256 g for the control. However, feed gain ratio of the non fish meal diets (1.37 1.58) were all inferior to those of the control diet (1.19). The daily feed consumption of the test diets was comparable to that of the control diet until this time (46 days), thereafter feeding activity of these groups gradually reduced, resulting in retarded growth. At the end of feeding, fish on the control SDP grew up to 341 g, while those on the non fish meal diets averaged only between 250 and 272 g. During the final phase of 19 days of feeding, the control fish gained 33% in growth rate, while it was only 8 14% for the test groups. The average feed gain ratio for the entire feeding period was 1.41 for the control and 1.76 2.13 for the non fish meal diets. During the final phase of feeding, mortality was frequently observed in the non fish meal groups (10 32% in total). Dissection of the experimental fish revealed that most of the fish fed the non fish meal diets were suffering from green liver, but not so in the case of the control fish, tallying with the earlier observation (Expt. I). The mortality linked to the green liver condition might have again resulted from inhibition of bile juice transport due to the reason mentioned above. Since both juvenile and young yellowtail exhibited the same abnormality only in the non fish meal diet groups, it may be possible to postulate that the growth retardation in fish fed the non fish meal diets was due to the occurrence of the green liver condition, although it is too early to classify the condition as a nutritional pathogenesis. It remains unknown whether poor feed performance of the non fish meal diets was due to the inferior quality of the diets in terms of availability of supplemental crystalline amino acids or due to the appearance of green liver. Further experiments are necessary to examine these aspects.

Proximate Composition of Muscle and Liver

Proximate values of dorsal muscle and liver in both juvenile and young yellowtail are presented in Table 3 15.

Expt. I : There was no marked difference in the proximate values of dorsal muscle among the non fish meal diet groups which had lower lipid levels and markedly higher moisture than the control group. A similar trend was observed in the liver, with the exception of a slightly high protein content in the control. Hepatosomatic index ranged from 1.0 to 1.3% in the non fish meal groups and was the lowest (0.9%) in the control.

Expt. II : Proximate composition values of young yellowtail followed the trend similar to those in juvenile fish. However, slightly higher values were observed for muscle lipid of young fish and liver protein of juveniles.

In both Expts. I and II, the non fish meal groups revealed the high moisture and low lipid contents which are usually observed in fish with retarded growth caused in part by low quality of diets.

Table 3-15. Proximate composition (%) of dorsal muscle and liver from yellowtail fed the fish meal and non fish meal diets in tanks and in net cages

Diet no.	Moisture	Crude protein	Crude lipid	Crude ash
Expt. I (Juvenile fish, n=10)				
Dorsal muscle				
1	74.3	22.5 (87.5)*	3.3 (12.8)	1.7 (6.6)
2	77.6	21.5 (96.0)	1.4 (6.3)	1.6 (7.1)
3	77.0	22.0 (95.7)	1.5 (6.5)	1.7 (7.4)
4	77.1	22.0 (96.1)	1.5 (6.6)	1.6 (7.0)
5	76.5	22.1 (94.0)	1.7 (7.2)	1.8 (7.7)
6	77.2	22.4 (98.2)	1.6 (7.0)	1.6 (7.0)
Liver				
1	71.4	18.3 (64.0)	8.1 (28.3)	1.4 (4.9)
2	79.7	17.7 (87.2)	3.9 (19.2)	1.6 (7.9)
3	77.3	17.7 (78.0)	4.6 (20.3)	1.7 (7.5)
4	76.5	17.1 (72.8)	5.4 (23.0)	1.6 (6.8)
5	76.0	17.2 (71.7)	6.2 (25.8)	1.5 (6.3)
6	74.9	17.3 (68.9)	5.0 (19.9)	1.6 (6.4)
Expt. II (Young fish, n=5)				
Dorsal muscle				
1	74.0	23.1 (88.8)	3.0 (11.5)	1.7 (6.5)
2	76.1	21.4 (89.5)	2.6 (10.9)	1.5 (6.3)
3	75.3	21.9 (88.7)	2.8 (11.3)	1.6 (6.5)
4	75.5	22.2 (90.6)	2.3 (9.4)	1.5 (6.1)
5	75.4	21.8 (88.6)	2.8 (11.4)	1.5 (6.1)
6	75.6	21.7 (88.9)	2.4 (9.8)	1.6 (6.6)
Liver				
1	72.8	15.7 (57.7)	8.3 (30.5)	1.3 (4.8)
2	79.7	14.9 (73.4)	4.6 (22.7)	1.3 (6.4)
3	79.0	15.4 (73.3)	4.8 (22.9)	1.3 (6.2)
4	79.1	15.3 (73.2)	4.5 (21.5)	1.3 (6.2)
5	77.2	15.3 (67.1)	5.7 (25.0)	1.3 (5.7)
6	76.4	15.8 (66.9)	6.6 (28.0)	1.4 (5.9)

* Figures in parentheses are values on dry matter basis.

Hematological Characteristics

Results of hematological measurements in both juvenile and young yellowtail are shown in Tables 3 16 and 3 17, respectively.

Expt. I : Blood biochemical parameters could not be determined for the diet 5 group due to sampling failure. The hematocrit value of fish fed the non fish meal diets ranged from 18.0 to 38.2% on average, and was lower than that of the control (42.6%), indicating that the test groups were in an anemic state. ALP activity and the glucose content were higher in the test groups. Among the blood lipid metabolites, fish on

Table 3-16. Results of hemochemical assessments in juvenile yellowtail fed the fish meal and non fish meal diets in tanks*

		Diet no. 1	2	3	4	6
Ht	(%)	42.6 ± 3.2^{a}	18.0 ± 5.5^{c}	27.0 ± 3.7^{b}	21.6 ± 2.4^{c}	38.2 ± 4.6^{a}
ALP	(IU / l)	103 ± 7^{b}	137 ± 28^{ab}	173 ± 36^{a}	155 ± 34^{a}	191 ± 37^{a}
GLU	(mg / 100ml)	112±10	178±20	151±19	216±124	177±36
TG	(mg / 100ml)	101 ± 33^{ab}	68 ± 25^{b}	67 ± 25^{b}	88 ± 20^{b}	126 ± 32^{a}
PL	(mg / 100ml)	570 ± 84^{a}	281 ± 63^{c}	304 ± 82^{bc}	345 ± 54^{b}	438 ± 69^{b}
TCHO	(mg / 100ml)	243 ± 34^{a}	104 ± 22^{c}	116 ± 23^{bc}	128 ± 29^{bc}	149 ± 19^{b}
FCHO	(mg / 100ml)	95 ± 10^{a}	45 ± 11^{c}	52 ± 10^{c}	57 ± 10^{bc}	67 ± 8^{b}
Ester ratio	(%)	60.7 ± 4.1^{a}	56.9 ± 1.4^{b}	54.6 ± 2.1^{b}	55.4 ± 3.2^{b}	55.0 ± 0.5^{b}
BUN	(mg / 100ml)	17.1 ± 0.8^{b}	32.5 ± 4.0^{a}	33.1 ± 6.5^{a}	29.5 ± 4.6^{a}	28.2 ± 3.0^{a}
CRE	(mg / 100ml)	1.28 ± 0.13^{a}	0.82 ± 0.04^{b}	0.92 ± 0.23^{b}	0.80 ± 0.07^{b}	1.22 ± 0.16^{a}
TP	(g / 100ml)	2.94 ± 0.11^{a}	2.04 ± 0.21^{c}	2.36 ± 0.32^{b}	2.20 ± 0.23^{bc}	2.88 ± 0.22^{a}

* Mean ± standard deviation (n=5). Data from diet 5 group could not be included due to sampling failure. Figures in a row with different superscripts indicate significant different from each other ($p<0.05$) when analyzed using Dancan's multiple range test.

Table 3-17. Results of hemochemical assessments in young yellowtail fed the fish meal and non fish meal diets in net cages*

		Diet no.					
		1	2	3	4	5	6
Ht	(%)	41.2±3.0^{a}	23.3±4.5^{b}	22.1±4.1^{b}	18.1±3.9^{bc}	20.6±2.6^{b}	19.8±3.6^{b}
ALP	(IU / l)	105±10	91±12	97±34	99±16	107±24	108±16
GLU	(mg / 100ml)	84±7^{b}	106±15^{a}	109±13^{a}	105±17^{a}	98±8^{ab}	117±8^{a}
TG	(mg / 100ml)	93±72	172±85	124±50	113±36	174±56	134±30
PL	(mg / 100ml)	641±76^{a}	373±26^{b}	354±51^{b}	354±38^{b}	371±69^{b}	395±21^{b}
TCHO	(mg / 100ml)	246±20^{a}	120±8^{b}	116±9^{b}	113±14^{b}	115±14^{b}	128±7^{b}
FCHO	(mg / 100ml)	98±12^{a}	60±5^{b}	56±5^{b}	59±8^{b}	61±9^{b}	63±4^{b}
Ester ratio	(%)	60.3±4.0^{a}	49.8±3.8^{b}	51.8±2.0^{b}	47.6±1.1^{bc}	47.5±2.4^{bc}	50.5±3.6^{b}
BUN	(mg / 100ml)	15.0±1.5^{a}	14.6±2.2^{a}	13.9±1.1^{a}	12.1±1.7^{ab}	10.8±1.7^{b}	13.6±2.8^{a}
CRE	(mg / 100ml)	0.86±0.45	0.64±0.17	0.76±0.15	0.82±0.29	0.80±0.30	0.82±0.39
TP	(g / 100ml)	2.90±0.16^{a}	2.28±0.08^{bc}	2.46±0.25^{b}	2.58±0.19^{b}	2.34±0.25^{b}	2.56±0.27^{b}
Condition factor		13.7±0.9a	11.9±0.3^{b}	12.1±0.4^{b}	12.6±0.7^{b}	12.2±0.5^{b}	12.5±0.5^{b}
Hepatosomatic index (%)		1.17±0.05	1.38±0.28	1.26±0.10	1.18±0.06	1.27±0.07	1.26±0.16

* Mean ± standard deviation (n=5). Figures in a row with different superscripts indicate significant different from each other ($p<0.05$) when analyzed using Dancan's multiple range test.

the non fish meal diets had significantly lower phospholipid content, total cholesterol, and free cholesterol levels and cholesterol ester ratio, suggesting abnormal liver functioning. The urea nitrogen level was higher for the non fish meal groups, and in the case of the control group it was creatinine.

Expt. II : The trend similar to those in juvenile fish was observed in young fish, although the protein metabolites of the control group were slightly higher than all the test groups. The anemic conditions and abnormal lipid metabolism observed in both the experiments were thought to be directly linked to the green liver pathogenesis. All the hematological indicators examined were in favor of the control diet.

Periodical Changes of Free Amino Acids and Digesta

Free amino acid levels in plasma in Expt. II : The periodical changes of methionine, lysine, and threonine, and the total free essential amino acids in the blood plasma of fish from Expt. I are shown in Table 3 18 and Fig. 3 5. The levels of the three supplemental amino acids for fish fed the non fish meal diets increased immediately after feeding. The maximum values of these amino acids for the test groups were extremely high compared with those of the control. These results suggested that supplemental crystalline amino acids, both coated and uncoated, were effectively absorbed. These amino acids were fortified in the test diets to give the same levels as those of the control fish meal diet, but all their plasma levels, especially methionine, were markedly higher than those of the control, and might result in amino acid imbalance, which may have caused lower feed performance in the test groups. Therefore, the supplemental amounts of these amino acids should be reduced to maintain the balance of plasma free amino acids after feed intake. Both lysine and threonine exhibited peaks at the third hour, and in the case of methionine overtly high levels were attained in the early hours after digestion, but unlike the former amino acids a distinct reduction was not observed with respect to the control values, even by 24 hours. The supplemental methionine, therefore, seems to have upset the normal physiological levels of the amino acid in the blood plasma. The combined plasma level of the three supplemented amino acids in fish on the test diets peaked at 3 hours post feeding, and yet another peak was observed at 18 hours when the diet contained coated amino acid mixture. The total essential amino acid levels for fish on the non fish meal diets also increased after feeding and peaked at the third hour, regardless of the coating of amino acid mixture. Later, it gradually decreased to the fasting level after 24 hours, however fish fed the diet with coated amino acids had a second peak at 18 hours. Conversely, the amino acid level in the control did not show a rapid increase after feeding, but gradually peaked at 12 hours. Thus, there were remarkable differences in the postprandial pattern of

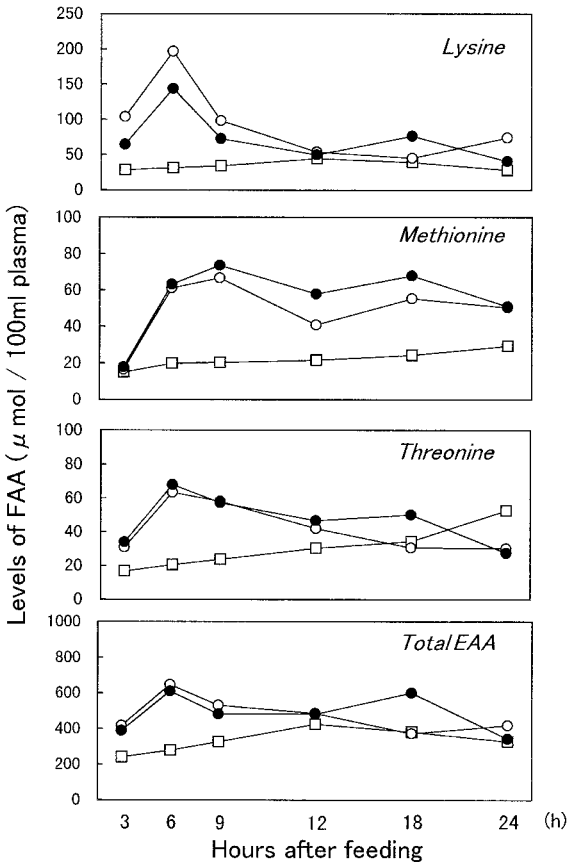

Fig. 3-5. Periodical changes of the free amino acids in blood plasma of yellowtail fed the control SDP and EP with coated (cAA) or uncoated (ucAA) amino acids. □:Diet 1 (control SDP), ○:diet 5 (EP+ucAA), ●:diet 6 (EP+cAA).

Table 3-18. Periodical changes of free amino acid contents in blood plasma of yellowtail fed the control SDP and EP with coated (cAA) or uncoated (ucAA) amino acids (μmol/100ml plasma)

total free amino acids between fish fed the control and test diets, and also between those fed diets containing an amino acid mixture with or without coating. However, the effect of coating the amino acids remained ambiguous because some of the uncoated amino acids (leucine, phenylalanine, and arginine) also appeared to peak at 18 hours. These variations could be attributed to the differences in the evacuation time of individual diets in the stomach and intestine, based on their physical properties. The non fish meal diets prepared by the small twin screw extruder was digested much faster than the control diet processed by the large twin screw extruder, the former diets releasing amino acids into blood plasma faster than the latter diets. For effective utilization, absorption of the supplemental crystalline EAA must be synchronized with those derived from dietary protein; therefore, the SDP type diet is desirable to enhance EAA utilization efficiency. In this context, the coated EAA seemed more effective than those without coating, although the coating effect was not clearly observed in this experiment. Further precise experiments are necessary to obtain information on utilization of supplemental amino acids.

Digesta content: Changes in the stomach digesta content (wet % of body weight) after feed intake are shown in Fig. 3 6. It was higher in the fish on the control diet than those on the non fish meal diets. The stomach content in fish on the non fish meal diets decreased to the fasting level within 18 24 hours, although the level for the control group was 3% at 24 hours after feeding. There was no marked difference in the postprandial change of stomach and intestine contents between the non fish meal diets with or without coating. Thus the digesta of the non fish meal diets passed rapidly through the stomach compared to the fish meal diet. This resulted in the

difference in plasma amino acid patterns between the two types of diets as mentioned earlier.

Results of this trial have shown that the non fish meal diets formulated with SPC, SBM, CGM, and MM as protein sources could sustain normal growth of young yellowtail for 46 days after starting feeding, but thereafter the growth gradually retarded. Most fish of these groups revealed the green liver condition, which might be the cause of growth retardation and the subsequent high mortality. If not for the green liver condition, fish could have grown normally on the non fish meal diets. In fact, subsequent trials have shown that yellowtail could grow normally on non fish meal diets for about two months until the green liver appeared in the fish (Chapter 3.2.1 2). The green liver, which was observed only in the fish fed the non fish meal diets, is thought to be induced by the occlusion of bile duct by parasitic mucosporozoa. The same non fish meal diets used in this trial with yellowtail were also fed to rainbow trout fingerlings of about 10 g for 20 weeks (Watanabe *et al.* 1997). These diets produced excellent growth and feed performances for rainbow trout without any abnormalities. The fish could also grow well on the non fish meal diets without EAA supplement. This has shown that the content of EAA in the non fish meal diets was sufficient to satisfy the EAA requirement of rainbow trout. Thus, the extruded diets without fish meal which could not sustain normal growth of yellowtail and caused the green liver, seemed not only to be harmless to rainbow trout, but also efficient in growth promotion and performance. The results on rainbow trout proved that the diets are not solely responsible for the abnormality. The link between the green liver status and dietary components needs to be examined closely in order to effectively develop non fish meal diets for yellowtail.

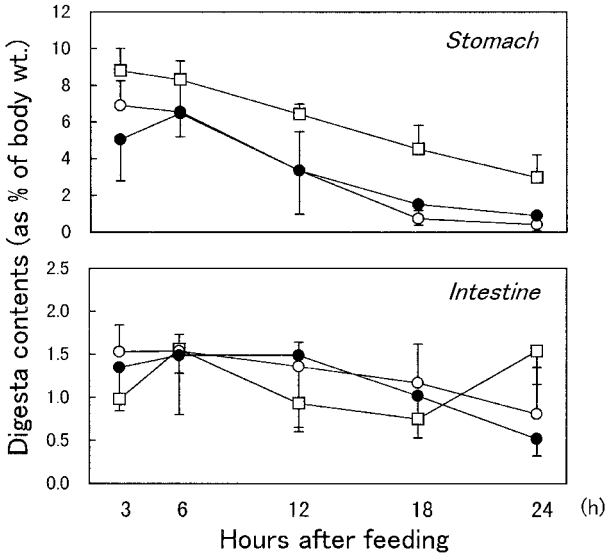

Fig. 3-6. Periodical changes of the stomach and intestine digesta contents of yellowtail fed the control SDP and EP with coated (cAA) or uncoated (ucAA) amino acids. □:Diet 1 (control SDP), ○:diet 5 (EP+ucAA), ●:diet 6 (EP+cAA). Results are expressed as the mean with standard deviation (n=5).

3.2.1-2 Use of Non-Fish Meal Diets for Yellowtail: Second Trial

The first trial to culture juvenile (13 g on average) and young (130 g on average) yellowtail with extruded dry pellets (EP) formulated without fish meal has shown that the experimental non fish meal diets were of poor palatability to juvenile fish, but were actively accepted by young yellowtail and sustained normal growth for the first 46 days of feeding, but thereafter resulted in stagnant growth, poor feed gain ratio, and high mortality irrespective of the dietary treatments (Chapter 3.2.1 1). Moreover, at the end of the experiments, both juvenile and young fish fed the non fish meal diets revealed the green liver symptom and poor blood characteristics, indicative of the abnormal physiological status.

These non fish meal diets were formulated to include SPC, SBM, CGM, MM as alternate protein sources, and crystalline amino acid mixture was supplemented in the diets to match the amino acid profile with that of the control fish meal diet. Therefore, the reduced growth performances observed in fish fed the non fish meal diets might partly be due to the poor utilization efficiency of supplemental amino acids, and partly be linked with the appearance of green liver, although its mechanism remains unknown.

Thus, the present study was conducted to confirm the results obtained in the first trial whether the

appearance of green liver in yellowtail is always closely linked with feeding non-fish meal diets, and to investigate supplemental effect of crystalline amino acids on feed performances of non-fish meal diets.

Materials and Methods

Experimental Diets

The composition of the experimental non-fish meal diets and their proximate values analyzed by the same methods described previously are shown in Table 3-19 (Watanabe and Pongmaneerat 1991). All the diets were formulated to have around 45% crude protein and 20% crude lipid those hitherto used in yellowtail experiments. A commercial yellowtail dry diet containing 65% fish meal (CP:68%) as a main protein source was used as a control diet (diet 1) and was produced by Sakamoto Fish Feed Co. using large size twin screw extruder. The fish meal component was entirely eliminated from the experimental non-fish meal diets and replaced by a combination of alternate protein sources such as SPC (Protao, a product of Aarhus Olie; CP:70%), SBM (CP:46%), CGM (CP:65%), MM (CP:82%), and PFM (CP:82%) at different levels. The combination and proportion of these protein ingredients are shown in Table 3-19. All the diets except the control diet were incorporated with 10% krill meal to enhance palatability to juveniles, although its non-inclusion diet not impair palatability to young yellowtail. The wheat flour or starch (only the control diet) as a carbohydrate source and binder was included at 8 or 5%, respectively. The vitamin and mineral mixtures were added to match the requirements of yellowtail (Watanabe *et al.* 1991). The supplemental feed oil level was 13% for the control and 17% for the test diets to elevate the dietary energy levels. Moreover, diets 3-6 contained 2.7% amino acid mixtures (1.5% L-lysine, 0.5% DL-methionine, 0.5% L-threonine, and 0.2% L-tryptophan) to match the amino acid profile with that of the control diet. The experimental diets were prepared to dry pellets using small size twin screw extruder by Nippon Formula Feed Co. There were no large differences in crude protein and lipid levels among the experimental diets. The gross energy content of the control diet was slightly lower than that of other

Table 3-19. Composition of the experimental non-fish meal diets for yellowtail

Ingredient (%)	Diet no.					
	1	2	3	4	5	6
Local sardine meal	67	0	0	0	0	0
Soy protein concentrate	0	30	30	20	13	10
Defatted soybean meal	0	10	10	10	10	5
Corn gluten meal	0	8	8	10	15	15
Meat meal	0	10	10	15	15	15
Poultry feather meal	0	0	0	3	5	10
Krill meal	0	10	10	10	10	10
Wheat flour	8	8	8	8	8	8
α-Starch	5	0	0	0	0	0
Vitamin and mineral mixtures	7	7	7	7	7	7
Feed oil	13	17	17	17	17	17
Amino acid mixture*	0	0	2.7	2.7	2.7	2.7
Total	100	100	102.7	102.7	102.7	102.7
Proximate composition: As is basis (%)						
Crude protein	45.0	45.2	43.5	43.8	45.9	44.9
Crude lipid	20.5	20.5	19.5	20.4	20.3	20.7
Crude ash	9.0	8.3	8.0	7.7	7.7	7.1
Moisture	11.5	4.9	7.2	7.5	5.7	6.8
Total phosphorus			1.6-1.9			
Dry matter basis (%)						
Crude protein	50.9	47.5	46.9	47.4	48.7	48.1
Crude lipid	23.2	21.5	21.0	22.1	21.6	22.2
Crude ash	10.2	8.7	8.6	8.3	8.2	7.6
Gross energy (kcal / g)	4.9	5.4	5.2	5.3	5.4	5.3

* Lysine 1.5, methionine 0.5, threonine 0.5, and tryptophan 0.2.

diets. The EAA contents of the experimental diets analyzed by Japan Food Research Laboratories are presented in Table 3 20. The non fish meal diets except for diet 2 seemed to contain enough EAA to satisfy the requirement level for yellowtail[*2]. The experimental diets were kept in freezer (−20℃) during the feeding period.

Table 3-20. Amino acid content of the non fish meal diets for yellowtail

Amino acid (g / 100g diet)	Diet no.					
	1	2	3	4	5	6
Arginine	2.79	3.02	2.82	2.71	2.68	2.61
Lysine	3.58	2.75	3.82	3.59	3.41	3.27
Histidine	1.53	1.11	1.07	1.00	0.96	0.89
Phenylalanine	1.95	2.23	2.14	2.10	2.21	2.14
Tyrosine	1.46	1.68	1.60	1.57	1.67	1.58
Leucine	3.62	3.99	3.82	3.89	4.32	4.30
Isoleucine	1.99	1.96	1.87	1.79	1.81	1.82
Methionine	1.30	0.85	1.18	1.15	1.19	1.15
Cystine	0.51	0.61	0.59	0.66	0.76	0.92
Valine	2.38	2.15	2.06	2.08	2.17	2.26
Threonine	2.04	1.82	2.20	2.16	2.23	2.17
Tryptophan	0.55	0.49	0.63	0.59	0.58	0.56
Total	23.70	22.66	23.80	23.29	23.99	23.67

Feeding Conditions

Two feeding experiments were conducted with yellowtail (*Seriola quinqueradiata*) at the Owase Branch, Fisheries Research Institute of Mie in floating net cages set within the coastal bay area (Expt. I) and at the Nagasaki Prefectural Institute of Fisheries in polycarbonate tanks (Expt. II).

Expt. I : Young yellowtail weighing about 210 g on average, fed with the commercial dry pellet (the control diet) for about two months before starting the experiment, were used. They were divided into six groups of 300 fish each in net cages (3×3×3 m) with a net cover and reared on each diet for 113 days (from Aug.8 to Nov.28, 70 days feeding). The water temperature ranged from 21.4 to 29.6℃ (average 25.3℃). Fish were hand fed 5 or 6 times per week, once a day in the morning to near satiation. All the fish were counted and weighed every 26 31 days, to calculate the average body weight. On termination, 5 fish were randomly collected from each lot for analysis of proximate composition of dorsal muscle and liver, by the same method described in an earlier paper (Watanabe and Pongmaneerat 1991).

Expt. II : Juvenile yellowtail with an average weight of 8.4 g, which had previously been fed a commercial EP (Sakamoto Fish Feed Co.) for 2 weeks, were used for the experiment. They were divided into six groups of 30 fish each in 500 *l* polycarbonate tanks (400 *l* water volume) and reared for 41 days (from Jun.30 to Aug.9, 37 days feeding) at water temperature of 23.0 31.0℃. Filtered seawater was continuously supplied (8 22 *l* /min) to each tank with airation. Fish were fed the respective experimental diets to near satiation twice a day (08:00 and 17:00). All the fish in each tank were weighed at the initial and final of the experiment to determine the mean body weight after starved for 24 hours and anesthetized with aminobenzoate (100 ppm). At the end of feeding experiment, proximate analysis of whole body from each lot was conducted (n=10).

Determination of Hematological Characteristics

At the final of the both two experiments, 5 fish were randomly sampled from each treatment and blood biochemical characteristics were determined to evaluate the physiological status. The analytical procedures for blood parameters and statistical analysis method of data were the same as those described in previous papers (Watanabe *et al.* 1992, 1995).

Results and Discussion

Feed Performances

The results of growth and feed performances in Expts. I and II are presented in Tables 3 21 and 3 22, respectively. The growth curves of fish in Expt. I are shown in Fig. 3 7.

Expt. I : The experimental non fish meal diets showed good palatability to the fish. The best final body weight and growth rate were obtained for fish on the control fish meal diet. There was no large difference in average body weight between fish on the non fish meal diets and the control diet until the 59th day (Oct.6, 39

Table 3-21. Growth and feed gain ratio of young yellowtail fed the non fish meal diets in net cages

Diet no.	Av.body wt. (g) Initial	Final	Growth rate (%)	Feed gain ratio	Daily feed intake	Mortality (%)
Aug.8 ~ Sep.4 (21 days feeding)						
1	213.0	322.7	50.9	1.33	2.57	0.0
2	208.5	300.7	43.8	1.58	2.71	0.0
3	210.3	317.2	47.5	1.46	2.66	0.0
4	203.5	295.6	44.2	1.61	2.78	0.0
5	207.3	300.0	44.1	1.61	2.76	0.0
6	207.3	310.8	49.4	1.45	2.73	0.0
Sep.5 ~ Oct.5 (18 days feeding)						
1	322.7	448.5	33.4	1.65	2.62	0.8
2	300.7	392.7	26.2	2.25	2.90	4.5
3	317.2	419.7	28.7	1.95	2.72	2.9
4	295.6	390.4	28.7	2.07	2.89	4.1
5	300.0	389.3	26.5	2.22	2.88	4.1
6	310.8	402.8	25.7	2.18	2.75	4.5
Oct.6 ~ Oct.31 (16 days feeding)						
1	448.5	620.1	35.7	1.82	3.15	0.6
2	392.7	445.1	12.4	3.58	2.58	3.3
3	419.7	447.0	6.2	5.49	2.32	17.8
4	390.4	446.5	13.2	3.32	2.58	6.7
5	389.3	436.0	11.1	3.71	2.52	7.8
6	402.8	416.2	3.1	10.27	2.12	11.1
Nov.1 ~ Nov.28 (15 days feeding)						
1	620.1	692.1	13.4	3.55	3.00	0.0
2	445.1	432.4	-	-	2.01	26.2
3	447.0	375.0	-	-	1.69	48.5
4	446.5	432.7	-	-	1.64	47.8
5	436.0	432.8	-	-	1.46	47.3
6	416.2	401.6	-	-	1.46	43.0

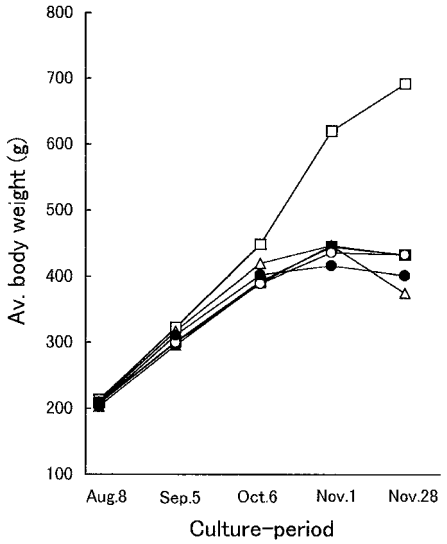

Fig. 3-7. Growth curves of young yellowtail fed the experimental non fish meal diets for yellowtail in net cages. □:Diet 1, ■:diet 2, △:diet 3, ▲:diet 4, ○:diet 5, ●:diet 6.

Table 3-22. Growth and feed gain ratio of juvenile yellowtail fed the non fish meal diets in polycarbonate tanks

Diet no.	Av.body wt.* (g) Initial	Final	Growth rate (%)	Feed gain ratio	Daily feed intake	Protein efficiency ratio	Nitrogen retention (%)	Mortality (fish)
Jun.30 ~ Aug.9 (37 days feeding)								
1	8.4±0.7	124.4±19.8	1080	0.95	4.34	2.5	44.2	2
2	8.3±0.8	43.0±8.6	298	1.89	6.13	1.4	20.0	5
3	8.4±0.8	72.6±15.9	536	1.16	4.57	2.4	41.7	3
4	8.3±0.8	56.9±11.9	392	1.36	4.85	2.1	35.3	4
5	8.4±0.9	67.2±12.8	539	1.25	4.95	1.9	33.8	1
6	8.5±0.9	51.4±12.3	326	1.40	4.68	2.1	35.1	4

* Mean ± standard deviation (n=25 ~29).

days feeding) from the start of feeding (being 390 420 g for the non fish meal diet groups and 450 g for the control). But, thereafter the feeding activity of non fish meal diet groups became gradually poor, and growth rate of these diet groups was extremely reduced at the end of the experiment without regard to dietary components. The final body weight of fish fed the control diet was 692 g, and was remarkably different from that of the fish on the non fish meal diets (375 433 g). Thus the non fish meal diets used in this study could not sustain normal growth of yellowtail for more than 2 months. The same tendency was also obtained for feed gain ratio, showing that this value of non fish meal diet groups worsened with growth retarded. Both growth rate and feed gain ratio were not different among the non fish meal diet groups. On comparing the performance parameters between fish on diets 2 and 3, namely the diets with or without crystalline EAA, there seemed to be no supplemental effect on improvement of the feed quality.

High mortalities (26 49%) were observed for fish of the non fish meal diet groups during the final feeding period of Nov.1 28, while no mortality in the control group. Dissection of dead fish revealed that most of fish had green liver status as observed in the former trial (Watanabe *et al.* 1998), indicating the poor health condition

for fish on the non fish meal diets irrespective of the dietary components. The anatomical assessment conducted about every 2 weeks from the start of feeding showed that green liver symptom peculiarly appeared in fish on the non fish meal diet after the end of October (Maita *et al.* 1997). Thereafter, the occurrence rate of this symptom increased, and almost all the fish on the non fish meal diets suffered at the end of the experiment. Contrary, there were few green liver fish in the control groups. The histological examination conducted at the Laboratory of Fish Physiology, Tokyo University of Fisheries revealed that this symptom was caused by occlusion of bile duct due to parasitic mucosporozoa, as same in the first trial (Watanabe *et al.* 1998).

Expt. Ⅱ: Feeding activity in fish fed the non fish meal diets was generally poor for all the diet treatments during the whole feeding period, unlike young fish in Expt. Ⅰ, suggesting the difference in palatability to test diets between fish sizes. Growth and feed performances showed almost the same tendency as those obtained in Expt. Ⅰ. The final average body weight and feed gain ratio were 124 g and 0.95 for the control and 43 73 g and 1.16 1.89 for the test groups, respectively. The best protein efficiency ratio value was also observed for the control diet. Mortality was generally low, ranged between 1 5 fish (n=30) for all the treatments. But the anatomical examination at the final of feeding showed that all of fish on the non fish meal diets had green liver status due to the same reason mentioned earlier. On the other hand, when comparing the performance parameters between fish on diets 2 and 3, growth rate, feed gain ratio, and protein efficiency ratio of fish fed diet 2 (without EAA) were inferior to fish on diet 3 (with EAA), unlike in the case of Expt. Ⅰ. This result indicates the effectiveness of EAA supplementation to the non fish meal diets to improve to some extent the feed utilization. Therefore, more detailed study must be carried out to investigate the availability of supplemental amino acids to diets with alternate ingredients.

In the first trial on the use of non fish meal diets to yellowtail (Watanabe *et al.* 1998), normal growth and feed performances were obtained until one and a half months after the initiation of the feeding (net pen experiment), but thereafter growth gradually retarded and subsequently high mortality was observed. Moreover, the anatomical examination at the end of the experiment revealed that almost all the fish fed the non fish meal diets were suffering from green liver, while few fish on the control fish meal diet. These results agreed well with those obtained in the present study. Therefore, although the cause of the reduction of growth and feed performances was unknown, the results from the present and former studies suggested that there was some nutritional inadequacies in the non fish meal diets to yellowtail, regardless of the dietary components. The inferior feed performances and high mortality observed in fish fed the non fish meal diets might partly be due to poor utilization efficiency of supplemental amino acids and partly be related to the appearance of green liver symptom. The reason for green liver appearance induced by feeding the non fish meal diets was still unknown. Therefore, further studies are need to clarify the outbreak mechanism of this phenomenon to develop a non fish meal diet.

Proximate Composition

The results of proximate analysis of the dorsal muscle, liver (Expt. Ⅰ), and whole body (Expt. Ⅱ) of fish are presented in Table 3 23. In Expt. Ⅰ, the protein content of the dorsal muscle did not differ largely among the treatments, but this value of the liver in fish on the non fish meal diets slightly higher than that in fish on the control diet. The lipid content of muscle in fish on the non fish meal diets ranged from 2.0 to 3.7%, and was remarkably lower than that on the control diet (8.2%). A similar trend was shown in the liver lipid too, 21.2% for the control and 6.0 13.0% for the non fish meal diet groups. The moisture content was inversely lower in both muscle and liver of fish fed the control diet reflected by higher lipid content. In Expt. Ⅱ, the patterns of proximate composition were similar to Expt. Ⅰ. The lipid content in the whole body was the highest in fish on the control diet. The lower lipid levels were reflected by lower growth of fish of the non fish meal diet groups. The results of proximate analysis from this study showed the same tendency as those obtained in the previous

experiment (Watanabe *et al.* 1998).

Hemochemical Characteristics

The results of hemochemical assessments of fish in Expts. I and II are presented in Table 3 24. In E xpt. I , the hematocrit value of fish fed the non fish meal diets was significantly lower than that on the control diet, 40.7% for the control and 15.6 23.8% for the test groups, showing the anemic state in fish of the latter groups. Also, fish fed the non fish meal diets had greatly lower blood lipid metabolism items, namely phospholipid, total cholesterol, free cholesterol, and cholesterol ester ratio. This indicates that an abnormal liver function in the fish of thetest groups, compared to the control group. The total protein and creatinine levels of test groups were also lower than those of the control, suggesting the poor nutrient condition. On the contrary, ALP activity and glucose content were higher in fish fed the non fish meal diets. The results in Expt. II showed almost the same trend as those obtained in Expt. I . Fish of the non fish meal diet groups were in an anemic state and had significantly lower lipid metabolites, regardless of dietary treatments. From these results, the

Table 3-23. Proximate composition (%) of dorsal muscle, liver, and whole body from yellowtail fed the non fish meal diets

Diet no.	Moisture	Crude protein	Crude lipid	Crude ash
Expt. I (n=5)[*1]				
Dorsal muscle				
1	66.2	23.1 (68.3)[*2]	8.2 (24.3)	2.1 (6.2)
3	75.0	21.1 (84.4)	2.0 (8.0)	1.6 (6.4)
4	75.7	20.6 (84.8)	2.1 (8.6)	2.3 (9.5)
5	74.0	20.9 (80.4)	3.7 (14.2)	2.5 (9.6)
6	76.0	20.6 (85.8)	2.0 (8.3)	2.1 (8.8)
Liver				
1	56.7	11.7 (27.0)	21.2 (49.0)	1.0 (2.3)
3	69.3	12.8 (41.7)	13.0 (42.3)	1.3 (4.2)
4	77.8	13.7 (61.7)	6.0 (27.0)	1.8 (8.1)
5	78.4	11.1 (51.4)	8.7 (40.3)	1.5 (6.9)
6	69.1	16.8 (54.4)	10.8 (35.0)	2.6 (8.4)
Expt. II (n=10)				
Whole body				
1	71.1	17.5 (60.6)	7.1 (24.6)	2.6 (9.0)
2	79.3	15.2 (73.4)	1.9 (9.2)	3.8 (18.4)
3	74.7	17.6 (69.6)	4.2 (16.6)	3.0 (11.9)
4	75.6	16.8 (68.9)	4.7 (19.3)	3.0 (12.3)
5	74.0	17.4 (66.9)	4.1 (15.8)	2.9 (11.2)
6	75.9	17.3 (71.8)	2.9 (12.0)	3.1 (12.9)

*[1] No data for the diet 2 group due to failure of sample preparation.
*[2] Figures in parentheses are values on dry matter basis.

Table 3-24. Results of hemochemical examination in yellowtail fed the experimental non fish meal diets*

		Diet no. 1	2	3	4	5	6
Expt. I							
Ht	(%)	40.7±3.2[a]	18.0±5.5[b]	15.5±3.0[b]	21.3±7.9[b]	23.5±5.3[b]	23.8±9.8[b]
ALP	(IU / l)	136±36	185±50	189±55	203±31	208±18	177±19
GLU	(mg / 100ml)	85±12[b]	152±39[a]	149±47[a]	166±26[a]	139±31[a]	127±21[ab]
TG	(mg / 100ml)	261±67[a]	127±83[b]	158±82[b]	165±50[a]	257±70[a]	188±49[ab]
PL	(mg / 100ml)	851±75[a]	480±122[b]	517±102[b]	558±90[b]	558±109[b]	637±189[b]
TCHO	(mg / 100ml)	297±35[a]	163±30[b]	176±20[b]	188±28[b]	173±23[b]	189±16[b]
FCHO	(mg / 100ml)	119±11[a]	70±12[b]	78±10[b]	80±11[b]	76±12[b]	77±7[b]
EC	(mg / 100ml)	178±24[a]	93±18[b]	98±11[b]	108±17[b]	97±12[b]	112±10[b]
Ester ratio	(%)	59.9±1.4[a]	57.0±1.4[b]	55.7±2.0[b]	57.5±1.3[b]	56.3±1.7[b]	59.3±1.9[ab]
BUN	(mg / 100ml)	11.3±1.1	11.4±3.5	9.1±3.0	11.3±2.3	9.8±0.6	12.7±2.5
CRE	(mg / 100ml)	1.14±0.05	0.94±0.11	0.94±0.05	1.00±0.20	0.90±0.19	0.90±0.07
TP	(g / 100ml)	3.34±0.25	2.62±0.64	2.90±0.35	3.08±0.53	3.00±0.21	3.04±0.25
Condition factor		16.1±0.3[a]	13.4±1.5[b]	12.8±0.8[b]	13.5±0.7[b]	12.8±0.9[b]	13.5±1.3[b]
Hepatosomatic index	(%)	1.56±0.09	1.41±0.29	1.56±0.26	1.29±0.19	1.26±0.19	1.25±0.20
Expt. II							
Ht	(%)	38.4±3.6[a]	25.9±4.4[b]	23.8±5.3[bc]	22.0±3.5[bc]	29.4±3.0[b]	26.7±3.5[b]
ALP	(IU / l)	120±21	197±53	226±115	198±45	181±49	169±45
GLU	(mg / 100ml)	108±12[b]	163±19[b]	220±98[a]	158±27[b]	170±42[ab]	165±22[a]
TG	(mg / 100ml)	156±57[a]	77±43[b]	107±33[b]	112±40[b]	147±39[ab]	134±46[b]
PL	(mg / 100ml)	485±63[a]	288±119[b]	335±114[b]	292±66[b]	411±103[a]	398±100[ab]
TCHO	(mg / 100ml)	187±35[a]	107±33[b]	125±36[b]	117±23[b]	132±47[b]	129±36[b]
FCHO	(mg / 100ml)	77±10[a]	49±18[b]	57±15[b]	55±9[b]	62±16[ab]	63±13[ab]
EC	(mg / 100ml)	110±27[a]	59±16[b]	68±21[b]	62±15[b]	70±32[b]	66±24[b]
Ester ratio	(%)	58.0±5.0	55.6±4.5	54.1±1.4	52.7±4.2	50.6±9.9	50.6±3.5
BUN	(mg / 100ml)	8.8±1.6[c]	15.1±2.3[ab]	15.0±3.9[ab]	12.5±2.6[bc]	14.8±4.3[b]	18.4±1.7[a]
TP	(g / 100ml)	3.1±0.6[a]	1.8±0.4[b]	2.0±0.2[b]	1.9±0.3[b]	2.2±0.3[b]	2.0±0.3[b]

* Data are shown as mean ± standard deviation (n=5). Figures in a row with different superscripts are significantly different from each other ($p < 0.05$) when analyzed using Duncan's multiple range test.

physiological condition of fish fed the non fish meal diets was evaluated as being quite inferior to those on the control fish meal diet.

In conclusion, the feed performances together with hemochemical examination have shown that the non fish meal diets could not produce normal growth and health condition, and cause green liver symptom for yellowtail, irrespective of the ingredient composition, as already observed in the previous trial (Watanabe *et al.* 1998). Additional works are necessary to clear the nutritional defect of the non fish meal diets, especially from the viewpoint of availability of the supplemental amino acids.

3.2.2 Red Sea Bream

The series of our study has investigated the availability to red sea bream of various plant and animal protein ingredients as partial replacements for fish meal in the feeds, in order to deal with the shortage of fish meal, a main protein source, due to the quick decrease in the catch of feed grade fish. Consequently, it was demonstrated that SBM, both processed and unprocessed by extruder, could be employed as fish meal replacer at around 30% in the diets for juvenile fish (Chapter 1.2.1). Further, it was showed that SBM combined with CGM and MM could substitute about 60% of fish meal component without any ill effects on the growth and feed parameters in the diets for both juvenile and adult fish (Chapter 1.2.2 1,2). Thus, these trials on the alternate proteins to red sea bream have been successful, and new type fish meal replaced diets would be practically developed in the near future.

On the other hand, at present, marine fish culture industry is expected to play an important role as provider of high value fish, and the demand of available protein feedstuffs will increase with the increase of formula feed production in Japan. Accordingly, the further efforts for reduction of fish meal component using effective alternate proteins are needed to supply the least cost diets stably. The goal of studying on the employ of alternate proteins is to develop a diet without fish meal component, namely the non fish meal diet. In yellowtail and rainbow trout, availability of the non fish meal diets was already examined, and it was shown that there was a certain difference in its feed utilization between both the species (Watanabe *et al.* 1997, Chapter 3.2.1 1, 2).

The present study was, therefore, conducted with red sea bream to examine availability of low or non fish meal diets formulated with SPC, SBM, CGM, MM, and PFM as protein sources.

Materials and Methods

Experimental Diets

In this study, two feeding experiments were performed using different test diets. The ingredient composition of the experimental diets and their proximate composition in Expts. I and II are shown in Table 3 25.

Expt. I (low or non fish meal diets): Diet 1 was a commercial diet for red sea bream containing fish meal as a main protein source, and was used as the control. Diet 2 was a non fish meal diet containing 30% SPC, 10% SBM, 8% CGM, and 10% MM as dietary proteins, and was supplemented with a crystalline amino acid mixture (1.5% L lysine, 0.5% DL methionine, 0.5% L threonine, and 0.2% L tryptophan) to match the EAA profile with that of the control diet for red sea bream. Diets 3 5 contained 10% fish meal with 20 and 10% SPC, 10% SBM, 8 and 10% CGM, 10 and 15% MM, and 5 8% PFM. The proportion of protein ingredients in diets 3 and 4 was the same, and EAA mixture was added to diet 4 only to investigate the supplemental effect. There was no marked difference in dietary protein content among the treatments, while slightly lower in diet 2. The crude lipid content was high in diet 2, reflecting the amount of feed oil added in the diet.

Expt. II (non fish meal diet): The experimental diets were the same as those used in the previous trial with

Table 3-25. Composition of the low or non-fish meal diets for red sea bream

Ingredient(%)	Experiment I					Experiment II				
	Diet no.					Diet no.				
	1	2	3	4	5	1	2	3	4	5
Fish meal	Commercial diet	0	10	10	10	Commercial diet	0	0	0	0
Soy protein concentrate		30	20	20	10		40	30	25	20
Defatted soybean meal		10	10	10	10		10	10	10	10
Corn gluten meal		8	8	8	10		3	13	18	23
Meat meal		10	10	10	15		12	13	15	14
Poultry feather meal		0	7	5	8		0	0	0	0
Krill meal		10	5	5	5		2	2	2	2
α-starch		0	8	10	10		0	0	0	0
Wheat flour		8	0	0	0		8	8	8	8
Vitamin mixture		2	3	3	3		2.55	2.55	2.55	2.55
Mineral mixture		5	5	5	5		2.45	2.45	2.45	2.45
Feed oil		17	14	14	14		20	19	17	18
Amino acid mixture*		2.7	0	2.7	2.7		2.7	2.7	2.7	2.7
Total		102.7	100	102.7	102.7		102.7	102.7	102.7	102.7
Nutrient contents determined (% as is basis)										
Crude protein	44.4	42.4	44.6	44.5	45.6	47.1	46.5	47.4	47.7	47.7
Crude lipid	15.1	21.0	17.5	16.9	13.3	23.0	23.9	23.7	23.2	23.5
Crude ash	9.4	8.3	8.3	8.2	8.3	9.9	5.7	5.3	5.1	4.8
Moisture	8.3	3.8	7.2	7.4	7.9	6.5	4.3	4.2	6.2	5.4
Dry matter basis(%)										
Crude protein	48.4	44.1	48.1	48.1	49.5	50.4	48.6	49.5	50.9	50.4
Crude lipid	16.5	21.8	18.9	18.3	14.4	24.6	25.0	24.7	24.7	24.8
Crude ash	10.3	8.6	8.9	8.9	9.0	10.6	6.0	5.5	5.4	5.1

* Lysine 1.5, methionine 0.5, threonine 0.5, and tryptophan 0.2.

yellowtail (Chapter 3.2.1-1). Commercial pellets prepared using a twin screw extruder was used as the control diet (diet 1). In diets 2-5, 20-30% SPC, 10% SBM, 3-23% CGM, and 12-15% MM were incorporated in combination, and all the diets contained 2.7% EAA mixture with the same composition as that used in Expt. I. All of the non-fish meal diets were produced using small size twin screw extruder (Chapter 1.2.2-2). The crude protein and lipid contents were almost the same for all the experimental diets. The amino acid composition of the experimental diets analyzed by the Japan Food Research Laboratories is shown in Table 3-26. Each EAA content of the non-fish meal diets was comparable to that of the control fish meal diet, and was satisfying the requirement of yellowtail[*2].

The control and test diets in both experiments were added with the vitamin and mineral mixtures to satisfy the requirements of yellowtail (Watanabe *et al.* 1991). All the experimental diets were kept at −20℃ during the rearing period.

Table 3-26. Amino acid composition of the fish meal and non-fish meal diets for red sea bream (Expt. II)

Amino acid (g / 100g diet)	Diet no.				
	1	2	3	4	5
Arginine	2.66	3.03	2.84	2.74	2.63
Lysine	3.42	3.71	3.52	3.36	3.27
Histidine	1.32	1.19	1.13	1.15	1.13
Phenylalanine	1.89	2.18	2.26	2.32	2.42
Tyrosine	1.51	1.47	1.58	1.65	1.73
Leucine	3.38	3.54	4.16	4.51	4.92
Isoleucine	1.95	1.89	1.88	1.88	1.91
Methionine	1.24	1.06	1.15	1.18	1.26
Cystine	0.49	0.60	0.64	0.66	0.67
Valine	2.34	2.15	2.17	2.20	2.22
Alanine	2.78	2.28	2.63	2.85	3.05
Glycine	2.94	2.57	2.61	2.67	2.62
Proline	2.19	2.85	3.15	3.30	3.55
Glutamic acid	6.13	8.11	8.38	8.58	8.83
Serine	1.89	2.25	2.31	2.32	2.36
Threonine	1.95	2.15	2.16	2.17	2.16
Aspartic acid	4.29	4.59	4.32	4.16	4.08
Tryptophan	0.56	0.72	0.68	0.67	0.64
Total	42.93	46.34	47.57	48.37	49.45
Total EAA*	22.71	23.69	24.17	24.49	24.96

* Total essential amino acids (including tyrosine and cystine).

Feeding Conditions

The feeding experiments were conducted at the Fishery Research Laboratory of Kyushu University in aquariums (Expt. I), and at the Owase Branch of Fisheries Research Institute of Mie in net cages (Expt. II).

Expt. I: Juvenile red sea bream (*Pagrus major*) with a mean body weight of about 27 g were divided into five groups of 30 fish each in 150 *l* rectangular aquariums. They were reared on each experimental diet for 56 days (8 weeks, 50 days feeding), from Jan.12 to Mar.10. Fish were fed the respective diets to near satiation

twice a day. The aquariums were continuously supplied with filtered sea water, and water temperature was kept at 22.0 ± 0.5℃. During the experimental period, all of fish in each lot were weighed every two weeks to determine the average body weight. At the final, 5 fish were taken from each aquarium for proximate analysis of dorsal muscle and liver at Kyushu University. Analytical techniques were described in a previous paper (Watanabe and Pongmaneerat 1991).

Expt. II: Adult red sea bream (one year old) weighing 538 579 g on average were used. They were reared on a commercial dry pellet for about 10 months before the initiation of the experiment. Then they were divided into five groups of 240 fish each and stocked in net cages (3×3×3 m), and reared for 183 days (from Nov.11 to May 12 of the next year, 87 days feeding). The water temperature at farming site (2 m in depth) ranged from 14.5 20.6℃ (average 16.9℃). The respective diets were fed to each fish, once a day in the morning to near satiation, 3 4 times per week. At the start and end of the experiment, all of fish in net cages were counted and weighed, and average body weight of 60 fish, which were randomly collected from each lot, was recorded on Dec.26 and Feb.13.

Hematological and Hemochemical Assessments

In both the experiments, 5 fish were randomly taken from each lot on termination and hematological characteristics and hemochemical constituents were determined to evaluate the health condition, by the same methods described previously (Watanabe *et al.* 1992, Viyakarn *et al.* 1992). Statistical analysis of the data was carried out using ANOVA. Fisher's PLSD test or Duncan's multiple range test was used to compare the differences of treatment mean value at a significant level of 0.05.

Results and Discussion

Feed Performances

The results of growth and feed performances in Expts. I and II are shown in Tables 3 27 and 3 28, respectively.

Expt. I: Both final average body weight and growth rate were highest in fish fed the control fish meal diet, and significant difference ($p<0.05$) was found in body weight between the control (71.5 g) and test groups (47.5 64.1 g). Among the low or non fish meal diets, the average body weight of fish fed diet 2 (non fish meal diet) was significantly higher than that of fish on diets 3 5. The worst growth performance was obtained for the diet 5 group. The same tendency was observed for feed performances in terms of feed gain ratio and protein efficiency ratio, though these values were almost similar between the control and diet 2 groups. Thus, the feed quality of diet 2 seemed to be superior to other test diets with alternate proteins. The growth performance tended to decrease with reduction of proportion of SPC in the test diets. We have already examined the availability of SPC to juvenile red sea bream, and have shown that SPC could utilize as an alternative protein source for fish meal in the diet. These facts suggest that the nutritional value of SPC was superior to other alternate ingredients. However, the lipid content of diet 5 was

Table 3-27. Growth and feed gain ratio of red sea bream fed the low or non fish meal diets in aquariums

Diet no.	Av.body wt.* (g)		Growth rate (%)	Feed gain ratio	Daily feed intake	Protein efficiency ratio	Mortality (fish)
	Initial	Final					
Jan.12 ~ Mar.10 (50 days feeding)							
1	27.0±1.6	71.5±7.3^{a}	164.8	1.13	1.98	2.00	0
2	27.1±1.7	64.1±11.9^{b}	136.5	1.11	1.56	2.12	2
3	27.1±1.6	57.4±8.2^{c}	111.8	1.34	1.84	1.67	0
4	27.0±1.6	55.3±9.2^{c}	104.8	1.24	1.64	1.81	0
5	27.0±1.6	47.5±7.7^{d}	75.9	1.68	1.78	1.31	0

* Mean ± standard deviation (n=28 ~30). Values in the same row bearing different letters are significantly different ($p < 0.05$, Fisher's PLSD test).

the lowest among the experimental diets, indicating that poor growth and feed performance in fish on this diet were due to the insufficiency of energy content. Therefore, the distinct cause of reduction of growth performances in fish on the low-fish meal diets was unknown in the present study. On the other hand, on comparing the feeding results between fish fed diets 3 and 4, there was no clear supplemental effect of the crystalline amino acid mixture. Similarly in a previous study, we did not observe the supplemental effect of crystalline lysine to a fish meal replaced diet for juvenile red sea bream (Chapter 1.2.2-2), although the same diet produced growth and feed performance parameters comparable to the commercial fish meal diet for yellowtail (Watanabe *et al.* 1995). Further study should be done to investigate the availability of supplemental amino acids, to improve the feed protein utilization.

Table 3-28. Growth and feed gain ratio of red sea bream fed the non-fish meal diets in net cages

Diet no.	Av.body wt. (g) Initial	Final	Growth rate (%)	Feed gain ratio	Daily feed intake	Protein efficiency ratio	Mortality (%)
Nov.11 ~ Dec.26 '93 (30 days feeding)							
1	560	650	16.1	1.60	0.79	1.33	0.0
2	579	657	13.4	1.67	0.70	1.29	0.0
3	551	625	13.5	1.67	0.70	1.26	0.4
4	544	615	13.1	1.76	0.72	1.19	0.8
5	538	610	13.4	1.67	0.70	1.25	0.0
Dec.27 ~ Feb.13 '94 (19 days feeding)							
1	650	690	6.2	1.44	0.54	1.48	0.0
2	657	700	6.5	1.39	0.52	1.55	0.0
3	625	705	12.8	0.77	0.51	2.74	0.4
4	615	637	3.6	2.04	0.51	1.03	0.4
5	610	618	1.3	3.27	0.52	0.64	0.0
Feb.14 ~ May 12 '94 (38 days feeding)							
1	690	714	3.5	6.73	0.62	0.32	0.6
2	700	736	5.1	4.70	0.61	0.89	0.6
3	705	675	–	–	0.56	–	1.1
4	637	655	2.9	8.24	0.63	0.25	0.6
5	618	641	3.7	6.04	0.64	0.35	0.0
Total feeding period (87 days feeding)							
1	560	714	27.5	2.32	0.61	0.92	0.4
2	579	736	27.0	2.27	0.57	0.95	0.4
3	551	675	22.6	3.09	0.57	0.68	1.7
4	544	655	20.5	3.20	0.59	0.66	1.7
5	538	641	19.1	3.06	0.58	0.69	0.0

Expt. II: Palatability and acceptability of the experimental diets were not affected by the entire elimination of fish meal component, irrespective of the combination of alternate proteins. The growth rate ranged from 19.1% for the diet 5 group to 27.5% for the control group, and tended to decrease with decrease of SPC content in the diets, as shown in Expt. I. This value was generally low for all the treatments due to low water temperature during the winter season. The growth performance of fish fed diet 2 (40%SPC + 10%SBM + 3%CGM + 12%MM) was comparable to that of the control group, and was superior to other test diets, though nutrient contents were almost in the same levels among the non-fish meal diets. Feed gain ratio values of fish on the control diet and diet 2 were better than those on diets 3-5, 2.32 and 2.27 for the former groups and 3.06-3.20 for the latter groups. The same tendency was observed for protein efficiency ratio. These results indicated that there was no difference in nutritional quality between the fish meal diet and the non-fish meal diet containing 40% SPC. It was also suggested that SPC had excellent quality to adult red sea bream as alternate dietary proteins, compared to other feed ingredients. The cumulative mortality of fish during the experimental period was low for all the treatments. However, the dissection of the fish at the end of feeding revealed that fish had green liver status for both the control and test groups. The occurrence rate of this symptom was 60% for the control group and 60-100% for the test groups (n=5). These results indicated that physiological condition of the experimental fish was generally poor for both the control and test groups, although the reason remained unknown.

In a previous trial, the same non-fish meal diets were shown to be efficient to sustain normal growth and health of young yellowtail for about one and a half months after initiation of the feeding, but thereafter the growth retarded and high mortality was observed, irrespective of the dietary composition (Chapter 3.2.1-1). Moreover, most of fish on the non-fish meal diets developed green liver symptom, which was caused by occlusion of the bile duct due to parasitic mucosporozoa, for all the treatments. On the contrary, both retardation of the growth and mass mortality were not found in fish of all groups in this experiment, though the growth

performance of test groups was inferior to the control. Therefore, it was suggested that there was difference in availability of the non-fish meal diets between red sea bream and yellowtail. In the present experiment, the good growth and feed performances comparable to the control fish were obtained in the fish on the non-fish meal diet formulated with 40% SPC as a main protein source. These findings demonstrated that there would be no difference in nutritive value between diets with and without fish meal component for both juvenile and adult red sea bream when non-fish meal diets are formulated with alternate protein ingredients in an adequate balance. Further researches should be carried out to clarify suitable ingredient and proximate compositions of the non-fish meal diets for red sea bream.

Proximate Composition

Results of proximate analysis for dorsal muscle and liver from fish in Expt. I are shown in Table 3-29. The protein content of dorsal muscle was not greatly affected by the dietary compositions. The lipid content of this tissue in fish fed diets 1-3 was higher than those of fish fed diets 4 and 5. The lipid value in the diet 5 group was lowest among the treatments, reflected by a lower dietary lipid level. There was an inverse relationship between lipid and moisture contents, and the highest moisture value was obtained for fish on diet 5. The muscle ash content was slightly higher in fish fed the control diet than those fed the test diets, reflected by the ash content in the diet. In the liver, the protein content in fish fed the control diet was not different from fish fed diets 2 and 3, and was slightly higher than that in fish on diets 4 and 5. The lipid, ash, and moisture contents of liver showed the same tendencies with those observed in dorsal muscle.

Table 3-29. Proximate composition (%) of dorsal muscle and liver from juvenile red sea bream fed the low or non-fish meal diets in aquariums*1

Diet no.	Moisture	Crude protein	Crude lipid	Crude ash
Dorsal muscle				
1	75.3	20.6 (83.4)*2	2.1 (8.5)	1.7 (6.9)
2	75.2	21.5 (86.7)	2.3 (9.3)	1.6 (6.5)
3	74.5	22.3 (87.5)	2.2 (8.6)	1.6 (6.3)
4	75.9	21.9 (90.9)	1.6 (6.6)	1.6 (6.6)
5	76.1	20.5 (85.8)	1.6 (6.7)	1.6 (6.7)
Liver				
1	61.0	13.1 (33.6)	19.8 (50.8)	2.0 (5.1)
2	58.2	13.2 (31.6)	21.3 (51.0)	1.3 (3.1)
3	59.7	13.2 (32.8)	20.8 (51.6)	1.8 (4.5)
4	64.5	11.2 (31.5)	16.5 (46.5)	1.7 (4.8)
5	67.2	11.4 (34.8)	13.2 (40.2)	1.6 (4.9)

*1 Samples from 5 fish were pooled for analysis.
*2 Data in the parentheses are in dry basis.

Hematological and Hemochemical Characteristics

Results of hematological and hemochemical examinations in Expts. I and II were shown in Table 3-30.

Expt. I : The hematocrit value varied from 31.4 to 36.4% and was within the normal range for all the groups. There was no significant difference in hemoglobin concentration between fish fed the control diet and test diets. The triglyceride content in plasma was the highest for the diet 2 group and lowest for the diet 5 group, probably reflected by dietary lipid levels. Total plasma protein value indicated the normality irrespective of the dietary treatments, although this level in fish fed diets 4 and 5 were significantly lower than those on diets 1-3. The almost similar levels were found in both phosphorus and calcium in plasma, suggesting the normal health status of fish. From these data, the indication was that the physiological condition was not markedly different between fish fed the fish meal diet and low or non-fish meal diets, and that all of fish were kept in good health status.

Expt. II : The Ht values were not markedly different among the treatments and were within the normal levels (around 30%). Some of fish in all the groups, however, showed very high ALP activity, suggesting a slightly poor health status. The glucose level of fish fed the non-fish meal diets was significantly lower than that of the control diet, though its definite reason was unknown. The items related to lipid metabolism, phospholipid, total cholesterol, free cholesterol, and cholesterol ester ratio, were not significantly different among the treatments, though these levels in fish on the control diet were slightly higher than those on the test diets. These results indicated better lipid metabolism or liver function in the control group than in the test groups. There were no

Table 3-30. Results of hemochemical examination in red sea bream fed the low or non fish meal diets

		Diet no.				
		1	2	3	4	5
Expt. I *[1]						
Ht	(%)	32.1±1.6^{bc}	34.5±1.7^{ab}	36.4±4.7^{a}	31.8±2.9^{c}	31.4±2.4^{c}
Hb	(g / 100ml)	8.1±0.7^{a}	8.4±0.8^{a}	8.4±1.3^{a}	8.4±1.3^{a}	8.1±0.5^{a}
TG	(mg / 100ml)	591	699	543	490	339
TP	(g / 100ml)	6.6±0.4^{bc}	7.2±0.6^{a}	6.8±0.6^{ab}	6.1±0.7^{d}	6.2±0.4^{cd}
P	(mg / 100ml)	9.5	10.6	10.5	10.4	9.7
Ca	(mg / 100ml)	14.1	15.6	15.5	13.5	14.5
Expt. II *[2]						
Ht	(%)	32.2±3.1	28.2±2.5	28.4±5.0	30.8±3.6	29.0±1.9
ALP	(IU / l)	64±73	75±71	47±23	104±114	97±105
GLU	(mg / 100ml)	113±75^{a}	46±14^{b}	44±13^{b}	45±5^{b}	51±12^{b}
TG	(mg / 100ml)	190±135	276±165	138±88	74±57	184±130
PL	(mg / 100ml)	704±197	676±95	638±328	623±124	518±120
TCHO	(mg / 100ml)	260±61	217±36	175±56	183±61	182±39
FCHO	(mg / 100ml)	113±34	114±23	84±37	99±25	81±23
Ester ratio	(%)	56.7±6.5	47.5±6.0	53.6±8.9	44.2±9.9	56.1±5.3
BUN	(mg / 100ml)	3.7±0.6	4.9±1.4	3.7±0.8	5.1±1.9	3.8±0.3
TP	(g / 100ml)	3.2±0.4	3.0±0.5	2.6±0.4	2.8±0.3	2.7±0.2
Condition factor		21.3±1.1^{a}	21.9±1.4^{a}	18.7±1.4^{b}	22.8±2.2^{a}	19.9±1.7^{ab}
Hepatosomatic index	(%)	2.25±0.45^{a}	1.85±0.44^{a}	1.26±0.50^{b}	1.80±0.36^{a}	1.65±0.53^{a}

*[1] Mean ± standard deviation (n=5). Values in the same row bearing different letters are significantly different ($p<0.05$, Fisher's PLSD test).

*[2] Mean ± standard deviation (n=5). Figures in a row with different superscripts are significantly different from each other ($p<0.05$, Duncan's multiple range test).

marked differences in the blood urea nitrogen and total protein levels among the groups, suggesting that all fish had normal protein metabolism.

Summarizing the hematological and hemochemical examinations in both the experiments, the physiological condition of fish fed the low or non fish meal diets was comparable to those fed the control diet. Therefore, it appears that health status of red sea bream was not affected largely by rearing them on diets without fish meal.

In conclusion, present experiments have shown that feed utilization of low or non fish meal diets was somewhat lower than that of the fish meal diet. However, it seemed that there were not adverse effects on growth and health status of red sea bream due to feeding the low or non fish meal diets. Therefore, red sea bream could use the low or non fish meal diets unlike yellowtail if dietary protein ingredients were appropriately incorporated to balance EAA profiles.

Chapter 4: Improvement of Quality of Non-Fish Meal Diets for Yellowtail by Amino Acid Supplementation

4.1 Plasma Free Amino Acid Patterns of Yellowtail Fed Different Types of Diets with Supplemental Amino Acids

Rapid expansion of world aquaculture (9.6% av. compound growth rate per annum since 1984) (Tacon 1998) and improvements in fish culture techniques have compounded the demand for fish feeds which depend inordinately on fish meal and fish oil as the major dietary components because of their ideal nutritional quality. However, there has to be a sea change from feeding fish to fish by finding alternatives to fish meal and fish oil since the raw materials are becoming scarce. Therefore, there is an increasing trend to substitute the fish meal and fish oil with alternate plant and/or animal protein and lipid components in aquatic feeds. The results from our studies have shown that SBM, CGM, and MM have excellent potential as substitute protein sources for fish meal in diets for rainbow trout (Pongmaneerat and Watanabe 1992, 1993a, 1993b, Watanabe and Pongmaneerat 1993, Watanabe *et al.* 1993, 1997), yellowtail (Watanabe *et al.* 1992, 1994, 1995, Viyakarn *et al.* 1992), and red sea bream (Chapter 1.2.1,2). However, incorporation of large amount of these feedstuffs to diets resulted in reduced growth and feed performances of fish, which might partly be due to the lack of some EAA such as methionine and/or lysine in the diets. The nutritional quality of diets with substitutive protein ingredients has been reported to be improved when such diets are supplemented with deficient EAA for rainbow trout (Dabrowska and Wojno 1977) and carp (Murai *et al.* 1986, 1989). These reports suggest that supplementation of EAA may also improve feed utilization in yellowtail. In a previous feeding experiment with yellowtail, we found remarkable differences in the periodical changes of both feed digesta contents in digestive tracts and plasma FAA profiles between fish fed a fish meal diet produced by a large twin screw extruder and a non fish meal diet prepared by a small twin screw extruder (Chapter 3.2.1 1). Incorporation of supplemental EAA into plasma was faster in the latter diet suggesting that its utilization may be affected by physical properties of the diets such as hardness and elasticity. Thus the absorption of supplemental EAA along with those derived from dietary protein could be closely monitored from the plasma levels and the evacuation time of stomach, which in turn depends on the digestion of the diets based on their physical properties of diets.

The present study was, therefore, conducted to clarify the effect of physical properties of diets on their retention time in digestive tracts and plasma FAA patterns, by feeding yellowtail with extruded pellets or single moist pellets, both having a similar ingredient composition and supplemented with three crystalline amino acids, to gather basic data needed for enhancing the nutritional value of diets with alternative proteins through efficient supplementation of EAA.

Materials and Methods

Experimental Diets

The ingredient composition and proximate composition of experimental diets are shown in Table 4 l. Diets 1 3 were formulated to be almost similar in ingredient composition. These diets contained 64 65% fish meal (Jack mackerel), and 15% SBM as dietary protein sources. Diet 1 (abbreviated as SDP) was prepared by Sakamoto Fish Feed Co. using a large size of twin screw extruder (Watanabe *et al.* 1991). Diet 2 (abbreviated as EP) was produced by Nippon Formula Feed Co. using a small size of twin screw extruder*3. The operating conditions for both the extruders were described previously*3. Diet 3 was a single moist pellet (SMP) prepared by mixing formulated mash with water (6:4) using pelleting machine at the Owase Branch, Fisheries Research Institute of

Mie. A commercial SDP for yellowtail (Sakamoto Fish Feed Co.) was used as the control (diet 4). Diets 1 3 were supplemented with L lysine, DL methionine, and L tryptophan at levels of 3.3, 1.2, and 0.5% (total 5.0%), respectively to compare plasma free amino acid patterns between the type of diets. Vitamin and mineral mixtures were added to diets 1 3 at levels of 3.1 and 1.9%, respectively, to satisfy the requirements of yellowtail (Takeda 1985, Watanabe *et al.* 1991). These three diets were formulated to be isoproteic (50% CP) and isoenergetic (digestible energy: DE, 3.9 kcal/g diet).

Table 4-1. Composition of the experimental diets

Ingredient(%)	Diet no. 1	2	3	4
	Diet type*[1] SDP+AA	EP+AA	SMP+AA	SDP
Fish meal (Jack mackerel)	65	64	64	Commercial yellowtail SDP
Defatted soybean meal	15	15	15	
Potato starch	10	8	0	
α-Potato starch	0	0	8	
Vitamin mixture	3.1	3.1	3.1	
Mineral mixture	1.9	1.9	1.9	
Guar gum	0	0.5	0.5	
CMC-Na	0	2.5	2.5	
Soy lecithin	0	2	2	
Feed oil	10	8	8	
L-Lysine	3.3	3.3	3.3	
DL-Methionine	1.2	1.2	1.2	
Tryptophan	0.5	0.5	0.5	
Total	110	110	110	–
Calculated proximate composition (%)				
Crude protein	49.6	49.3	49.3	46.9
Crude lipid	14.4	14.4	14.4	22.3
Digestible energy*[2] (kcal / kg diet)	3882	3857	3857	4224

*[1] SDP; Soft dry pellet produced by a large size twin screw extruder, EP; Extruded pellet produced by a small size twin screw extruder, SMP; Single moist pellet, AA; Amino acid.

*[2] Calculated based on the following numerical values: crude protein; 4.5kcal/g, crude lipid; 8.0kcal/g, crude carbohydrate; 2.8kcal/g.

Experimental Procedure

Feeding experiment was conducted at the aquaculture facility of the Owase Branch, Fisheries Research Institute of Mie. Yellowtail (*Seriola quinqueradiata*) which had been fed a commercial SDP and/or moist pellet (mash:minced sardine=5:5) for about 10 months, was used for the experiment (about 690 g on average). Three hundred fish were divided into 4 groups of 75 fish each and stocked in floating net cages (3×3×3 m) set in the Owase bay. Before initiation of the experiment, fish on diets 1, 2, and 4 were fed the commercial SDP (diet 4), and those on diet 3 the experimental SMP (diet 3), at the daily feeding rate of 1.9 (diets 1, 2, 4) and 2.9% (diet 3) of body weight for 11 days, respectively. Then the fish of all groups were starved for 48 hours, and fed the respective diets to near satiation at 09:00. Four fish from each cage were randomly sampled before feeding (only the control group) and at 3, 6, 12, 18, 24, 30, and 48 hours after feeding. Water temperature at 1 m depth of the culture site ranged from 21.4 to 24.0℃. Blood samples were taken from the heart with heparinized syringe for the determination of free amino acid constituents in blood plasma (Chapter 3.2.1 1). The whole blood was centrifuged at 3,000 rpm for 10 min and in all the sampling points, four plasma samples from each group were pooled for analysis. The analytical procedure for plasma free amino acids was described in the previous paper (Chapter 3.2.1 1). After blood sample was taken, the experimental fish of each group were dissected and the feed digesta of stomach and intestine were sampled to calculate their relative weights to body weight.

Results and Discussion

Periodical Changes of Digesta Contents

Figure 4 1 shows the periodical changes in the contents of stomach and intestine digesta on dry matter percentage of body weight for diets 1 4 groups. The feeding rate (dry matter basis) for diets 1 4 groups was estimated to be 4.0, 4.3, 3.3, and 4.4%, respectively, slightly lower in diet 3. Stomach digesta content of fish on the test SDP (diet 1) was the highest among the groups at all the sampling points, and remained so until 30 hours after feeding. The periodical changes in the mass of stomach digesta from fish on the commercial SDP (diet 4) was similar to that of the test SDP. On comparing SDP with EP, both of which were extruded, SDP remained longer in the stomach than EP. On the other hand, when SMP was fed, the stomach digesta content was the least during the experimental period of 30 hours, almost disappearing from the stomach by about 18

hours after feeding.

Digesta contents of intestine were dependent on periodical changes in the stomach. Intestine digesta contents of fish on the test SDP, which passed the stomach at a relatively slow pace, reached a maximum level at the 24th hour after feeding. On the other hand, in the fish on the SMP, the intestine digesta rapidly increased and attained the highest level at 6 12 hours, and thereafter graduallydecreased until 24 hours. The fish on either the commercial SDP or the EP attained a peak between 12 18 hours.

Thus, the results from this study clearly show that there was remarkable difference in the periodical changes of feed digesta contents between the diet types. Gastric evacuation time seemed to be related to the collapse of diet shape in the stomach. SDP was able to retain its shape in the stomach until 12 hours after feeding, while EP and SMP had lost its shape as early as 3 hours. This characteristic of SDP may have caused the prolonged stomach digesta emptying time, indicating that it is probably the physical properties that affect the collapse of diet. Therefore, it is concluded that the diet preparation conditions determine the physical properties like shape/form, hardness, stability etc; and this in turn is responsible for the influence the evacuation time of stomach and intestine feed digesta.

Periodical Changes of Plasma Free Amino Acids

The periodical changes of three supplemental amino acids, methionine, lysine, and tryptophan, and the total free amino acids in blood plasma from fish fed diets 1 4 are shown in Table 4 2 and Fig. 4 2.

The levels of the three amino acids for fish fed diets 1 3 increased immediately after feeding, and the maximum values for the experimental groups were extremely high compared with those of the control. These results indicated that crystalline amino acids were effectively absorbed when supplemented to diets, without regard to their types, SDP, EP, and SMP. The plasma levels of these amino acids, especially methionine, were markedly higher than those of the control at all the sampling points, as already observed in the previous study (Chapter 3.2.1 1). This high

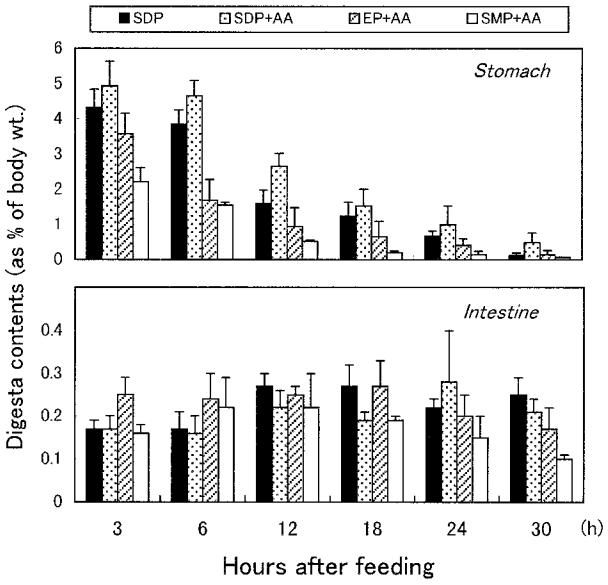

Fig. 4-1. Periodical changes of feed digesta contents in yellowtail fed the experimental diets. Data are shown as mean with standard deviation (n=4).

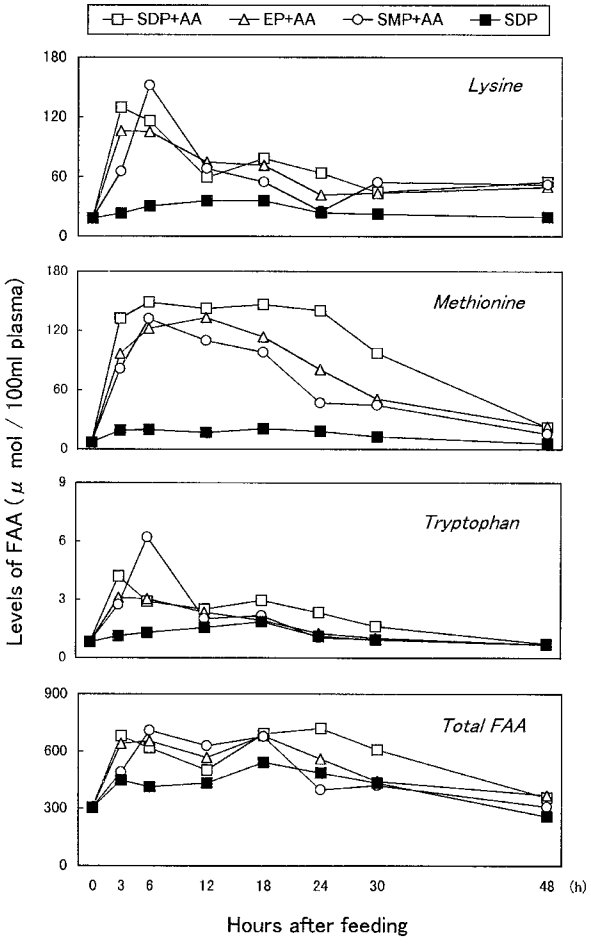

Fig. 4-2. Periodical changes of free amino acid contents of yellowtail fed the experimental diets.

Table 4-2. Periodical changes of free amino acid contents in blood plasma of yellowtail fed the experimental diets (μmol/100ml plasma)

Diet no.	Hours after feeding (h) 0	3	6	12	18	24	30	48
Lysine								
1 SDP+AA	18.3	129.9	116.3	59.5	78.8	64.0	44.6	55.0
2 EP+AA	18.3	106.3	105.4	74.9	71.7	41.7	43.4	49.6
3 SMP+AA	18.3	65.5	151.9	68.5	54.7	25.3	54.4	52.2
4 SDP	18.3	23.1	30.3	35.9	35.7	23.7	22.4	19.2
Methionine								
1 SDP+AA	7.1	132.4	148.9	142.5	146.5	140.4	97.3	22.3
2 EP+AA	7.1	96.8	122.4	133.1	113.8	80.6	50.6	23.0
3 SMP+AA	7.1	81.4	132.0	109.9	97.9	46.8	44.4	15.6
4 SDP	7.1	19.3	19.7	16.9	20.6	18.2	12.7	5.7
Tryptophan								
1 SDP+AA	0.8	4.2	2.9	2.5	3.0	2.3	1.6	0.7
2 EP+AA	0.8	3.1	3.0	2.4	1.9	1.3	1.0	0.7
3 SMP+AA	0.8	2.7	6.2	2.0	2.2	1.0	0.9	0.7
4 SDP	0.8	1.1	1.3	1.6	1.9	1.1	0.9	0.7
*Total EAA**1								
1 SDP+AA	156.2	521.8	522.0	415.2	538.4	520.8	400.6	225.6
2 EP+AA	156.2	513.0	543.6	456.8	492.1	347.7	266.8	226.6
3 SMP+AA	156.2	378.4	578.4	447.8	427.4	225.4	256.5	191.7
4 SDP	156.2	250.2	275.6	294.0	330.8	263.3	222.8	131.2
*Total NEAA**2								
1 SDP+AA	148.2	158.4	96.0	86.8	153.7	200.1	208.3	132.7
2 EP+AA	148.2	127.8	110.4	110.1	189.9	213.2	176.0	143.9
3 SMP+AA	148.2	112.3	130.7	181.2	250.5	173.6	164.9	118.1
4 SDP	148.2	197.2	138.1	138.7	211.0	223.2	211.3	127.8
*Total FAA**3								
1 SDP+AA	304.4	680.3	618.0	502.0	692.0	720.9	608.9	358.2
2 EP+AA	304.4	640.8	654.0	566.9	682.0	560.9	442.8	370.5
3 SMP+AA	304.4	490.7	709.1	629.0	677.9	399.0	421.4	309.8
4 SDP	304.4	447.4	413.6	432.7	541.8	486.5	434.1	259.1

*1 Total essential amino acids: arginine, lysine, histidine, phenylalanine, tyrosine, leucine, isoleucine, methionine, valine, threo nine, and tryptophan.

*2 Total non essential amino acids: alanine, glycine, serine, and aspartic acid.

*3 Total free amino acids.

methionine levels of the test groups may result in amino acid imbalance, leading to lower feed performances. Therefore, the supplemental amounts of these amino acids should be reduced to maintain the balance of plasma FAA after feed intake. The supplemental amino acids exhibited peaks at 3 6 hours, except for the 12th hour peak of methionine in the fish fed diet 3 (SMP); and both lysine and tryptophan showed a distinct reduction at 12 hours with respect to the control values, while the methionine levels remained high until 18 hours (until 24 hours for the fish fed diet 1). The supplemental methionine therefore seems to have upset the normal physiological levels of the amino acid in the blood plasma as already observed in the previous experiment (Chapter 3.2.1 1).

The total FAA levels of all the groups also increased after feeding and peaked at 3 6 hours, while second any peaks appeared at 18 24 hours; those of the test groups being reflected by supplemental amino acids. The total FAA levels of the test groups were higher than that of the control until 18 hours after feeding, and that of fish fed diet 1 (SDP) even by 48 hours. Thus, there were remarkable differences in the postprandial pattern of total FAA between fish fed the control and test diets, and also slightly between those fed different types of diets. Supplemental amino acids were quickly absorbed without regard to types of diets and methionine level of fish fed diet 1 was maintained high until 24 hours, even by 30 hours after feeding 70% of the maximum level was detected. Plasma total FAA together with supplemental EAA, especially methionine, in the fish fed diet 1 (SDP) were kept high through the experimental period, and showed a pattern similar to that of fish fed the commercial SDP (diet 4), suggesting that supplemental amino acids absorbed would be in a manner matching those derived from dietary proteins. Control diets without supplemental EAA were not prepared for EP and SMP, therefore it remained unknown whether EAA supplemented to EP and SMP would be efficiently utilized at rates equivalent to

those observed in SDP, by combining with those derived from dietary protein sources. The variation in the plasma FAA pattern could partly be attributed to the difference in the retention timings of individual diets in the stomach and intestine, based on their physical properties. For effective utilization, absorption of the supplemental crystalline EAA must be synchronized with those derived from dietary protein; therefore the SDP type of diet may be desirable to promote EAA utilization efficiency. Further precise experiments using various types of diets with or without supplemental EAA are necessary to obtain more precise information on utilization of supplemental amino acids.

4.2 Effect of Supplemental Amino Acids in Different Types of Non-Fish Meal Diets on Growth Performances

In a previous experiment growth and feed efficiency in yellowtail fed non fish meal diets were inferior to those of the control fish meal diet, although there were no great differences between non fish meal and fish meal diets in the protein and energy levels, and essential amino acid profiles (Chapter 3.2.1 1,2). Feed performances of yellowtail fed the non fish meal diets were gradually reduced after about one and a half months feeding, corresponding to the occurrence of green liver irrespective of the ingredient composition of non fish meal diets. These results suggest a relation between nutritional quality of the non fish meal diets and appearance of green liver.

Our recent work with yellowtail indicated that postprandial changes of the plasma level of amino acids supplemented to diets and feed digesta transit time were affected by the physical properties of diet in terms of stability of diets in digestive tracts, which in turn was related to the feed manufacturing conditions. In fact, several researchers have demonstrated that nutritive value to fish of soybean meal was improved to some extent by feed extrusion and/or preparation conditions (Sandholm *et al.* 1976, Smith *et al.* 1980, Viola *et al.* 1983, Abel *et al.* 1984, Wilson and Poe 1985, Wee and Shu 1989, Shimeno *et al.* 1992c). These results suggested that both nutritive value of the non fish meal diets containing various protein ingredients and availability of supplemental crystalline amino acids would be different, depending on feed preparation methods in terms of physical properties of diets.

In the present study, therefore, three types of non fish meal diets were prepared by different processing conditions to clarify the effect of physical properties of diets on the utilization efficiency of non fish meal diets and supplemental amino acids in yellowtail.

Materials and Methods

Experimental Diets

The type of diets and their ingredient composition and proximate compositions are presented in Table 4 3. The experimental non fish meal diets were prepared to three different forms: SDP, EP, and SMP. SDP was prepared using a large size twin screw extruder (Buhler Co.) by Sakamoto Fish Feed Co. according to the following extrusion conditions: —material temperature: 96℃; material press:40 kg/cm^2; water injection: 330 kg/h; feed production rate: 2000 kg/h. EP was prepared using a small size of twin screw extruder (Buhler Co.) by Nippon Formula Feed Co. according to following extrusion conditions: —material temperature: 105℃; material press: 49 kg/cm^2; water injection: 30 kg/h; feed production rate: 120 kg/h. SMP was prepared by mixing formulated mash and fresh water at the ratio of 6:4 and mechanically pelleting it at the Owase Branch, Fisheries Research Institute of Mie, every two weeks during the experimental period. Diet 1 was the control diet containing 67% fish meal as the sole protein source, and was prepared as SDP by Sakamoto Fish Feed Co. (Watanabe *et al.* 1991) Non fish meal diets 2 7 contained SPC, SBM, CGM, MM, and krill meal as protein

Table 4-3. Composition of the different types of the experimental non-fish meal diets with or without supplemental essential amino acids for yellowtail

Ingredient(%)	Type of diet*[1]						
	SDP			EP		SMP(Mash)	
	Diet 1*[2]	Diet 2	Diet 3	Diet 4	Diet 5	Diet 6	Diet 7
Fish meal (Jack mackerel)	67	0					
Soy protein concentrate	0	30					
Defatted soybean meal	0	10					
Corn gluten meal	0	8					
Meat meal	0	10					
Krill meal	0	10					
Wheat flour	8	8		2		0.5	
α-Starch	5	0		8		5.5	
Guar gum	0	0		0		2	
Carboxymethylcellulose	0	0		0		2	
Mineral mixture	5	5		5		5	
Vitamin mixture	2	2		2		2	
Feed oil	13	17		15		15	
Amino acid mixture*[3]	0	0	2.7	0	2.7	0	2.7
Total	100	100	102.7	0	102.7	0	102.7
Proximate composition: As is basis (%)							
Crude protein	41.4	43.0	45.0	41.0	42.3	25.7	26.6
Crude lipid	19.5	19.0	18.3	19.1	18.7	11.9	11.3
Crude ash	9.1	8.9	8.9	7.7	7.8	5.2	4.9
Moisture	13.7	7.3	5.4	10.6	9.8	45.4	44.5
Dry matter basis (%)							
Crude protein	48.0	46.3	47.5	45.8	46.8	47.1	47.9
Crude lipid	22.6	20.5	19.3	21.4	20.7	21.9	20.4
Crude ash	10.6	9.5	9.4	8.6	8.7	9.5	8.8

*[1] SDP; Soft dry pellet, EP; Extruded pellet, SMP; Single moist pellet.

*[2] Control fish meal diet.

*[3] Lysine 1.5, methionine 0.5, threonine 0.5, and tryptophan 0.2.

sources at levels of 30, 10, 8, 10, and 10%, respectively, and were prepared as the three forms mentioned earlier (diets 2 and 3: SDP, diets 4 and 5: EP, diets 6 and 7: SMP). The combination of these protein ingredients as protein source was already evaluated to be high in availability to young yellowtail in a preliminary study (Chapter 3.2.1-2). Diets 3, 5, and 7 were supplemented with 1.5% L-lysine, 0.5% DL-methionine, 0.5% L-threonine, and 0.2% L-tryptophan (total 2.7%) to match the amino acid profile with that of the control fish meal diet (Chapter 3.2.1-1), in order to investigate the supplemental effect on the dietary value of the respective non-fish meal diets. The amino acid composition of the non-fish meal diets with and without the four supplemental EAA, and the control diet containing 67% fish meal used in the preliminary experiment mentioned earlier is presented in Table 4-4 for comparison (Chapter 3.2.1-2). The amino acid composition of all test diets with supplemental EAA was comparable to that of the control diet, containing a sufficient amount of EAA to satisfy the requirement of yellowtail*[2]. Vitamin and mineral mixtures were added to meet the levels recommended for yellowtail (Takeda 1985, Watanabe *et al.* 1991). Wheat flour and alpha-starch were used as main binder in diets 1-3 and diets 4-7, respectively. Guar gum and carboxymethylcellulose (CMC) were also used as binder in SMP (diets 6 and 7). On dry matter basis, the crude protein and lipid contents of the experimental diets were 45.8-48.0% and 19.3-22.6%, respectively. Moisture content of SMP was approximately

Table 4-4. Amino acid content of the experimental non-fish meal diets for yellowtail

Amino acid (g / 100g diet)	Fish meal diet	Non-fish meal diet	
		without EAA*	with EAA*
Arginine	2.79	3.02	2.82
Lysine	3.58	2.75	3.82
Histidine	1.53	1.11	1.07
Phenylalanine	1.95	2.23	2.14
Tyrosine	1.46	1.68	1.60
Leucine	3.62	3.99	3.82
Isoleucine	1.99	1.96	1.87
Methionine	1.30	0.85	1.18
Cystine	0.51	0.61	0.59
Valine	2.38	2.15	2.06
Threonine	2.04	1.82	2.20
Tryptophan	0.55	0.49	0.63
Total	23.70	22.66	23.80

* Essential amino acid mixture. See Table 4-3.

45%, while those of the dry type diets ranged from 5.4 to 13.7%. All the diets were stored at −20℃ during the experimental period.

Feeding Conditions

In this study, two feeding experiments were conducted with young yellowtail (*Seriola quinqueradiata*) in floating net cages at the Owase Branch of the Fisheries Research Institute of Mie (Expt. I) and in aquariums at the Nagasaki Prefectural Institute of Fisheries (Expt. II).

Expt. I: Young yellowtail weighing 134 g on average, which had previously been fed a commercial dry pellet for one and half months, were divided into 7 groups of 350 fish each in net cages (3×3×3 m). Fish were fed the respective diets for 93 days (from Aug.8 to Nov.8, 58 days feeding), once a day in the morning to near satiety. Water temperature ranged from 21.9 to 28.1℃ (average 24.6℃, 2 m in depth). All of the fish in each group were weighed and counted periodically (every 16 28 rearing days). At the end of experiment, sixteen fish collected randomly from each group were pooled for analysis of proximate composition of whole body, dorsal muscle, and liver.

Expt. II: Five groups of 15 young yellowtail having a mean initial weight of 237 g were placed in individual aquarium (500 *l*) with aeration and reared at a water temperature of 18 24.5℃. The aquariums were supplied with filtered seawater at the rate of 7 20 *l*/min. Fish were fed diets 1 5, twice a day (9:00 and 14:00), each time to near satiety. The experimental SMP (diets 6 and 7) were not used in Expt. II. The experiment lasted for 44 days (from Sep.27 to Nov.9, 39 days feeding) and fish weights were recorded at the start and end. On termination, five fish were pooled for analysis of whole body composition. Analytical methods of proximate composition of fish from Expts. I and II were the same as those described in a previous paper (Watanabe and Pongmaneerat 1991).

Examination of Hemochemical Characteristics

At the end of Expt. I, six fish were randomly collected from each group for analyzing hemochemical indicators of the physiological condition, as well as screening them for green liver pathology. The levels of hemochemical constituents and activities of enzymes in blood plasma were determined by the same analytical procedures described in previous papers (Watanabe *et al.* 1992, Viyakarn *et al.* 1992). Differences in blood parameters among the groups were compared statistically by using Duncan's multiple range test at significance level of $p<0.05$, if ANOVA indicated significant changes.

Results

Feed Performances

The results of feeding in Expts. I and II are presented in Tables 4 5 and 4 6, respectively, and the growth curves in Expt. I are shown in Fig. 4 3.

Expt. I: The control diet as well as the different types of non fish meal diets were found to be palatable to the fish. Daily feeding rate on dry matter basis did not differ much among the groups, ranging between 2.69 2.91%. The final body weight was 535 g for the control and 317 446 g for the non fish meal diet groups, being lowest for the fish fed the SMP without supplemental EAA (diet 6). The best growth rate was obtained for fish fed the control fish meal diet. Similar trend was found in feed gain ratio on dry matter basis, 1.39 for the control and 1.70 2.04 for others. Moreover, the highest protein efficiency ratio was obtained for the control diet. Among the non fish meal diet groups, fish fed the SDP supplemented with EAA (diet 3) showed the best growth rate and feed gain ratio, and fish on the EP supplemented with EAA (diet 5) were the second best. The growth rate of fish

Table 4-5. Growth and feed performances of yellowtail fed the experimental non fish meal diets in net cages

Diet no.	Av.body wt. (g) Initial	Final	Growth rate (%)	Feed gain ratio	Daily feed intake	Protein efficiency ratio	Mortality (%)
Aug.8 ~ Aug.28 (16 days feeding)							
1 Control	134.3	203.2	51.1	1.40	3.57	1.73	0.6
2 SDP	136.6	194.8	42.5	1.55	3.40	1.50	0.3
3 SDP+EAA	134.3	200.9	48.9	1.34	3.29	1.66	1.4
4 EP	130.9	195.1	49.3	1.45	3.59	1.68	0.9
5 EP+EAA	134.9	201.4	49.6	1.41	3.51	1.68	0.9
6 SMP	132.9	179.0	35.3	2.88	5.39	1.35	1.1
7 SMP+EAA	134.3	186.3	37.9	2.59	5.15	1.45	4.0
Aug.29 ~ Sep.25 (17 days feeding)							
1 Control	203.2	358.1	70.8	1.19	3.68	2.03	0.0
2 SDP	194.8	321.9	59.9	1.35	3.66	1.72	0.6
3 SDP+EAA	200.9	342.8	66.2	1.22	3.58	1.82	0.6
4 EP	195.1	319.0	59.1	1.38	3.70	1.77	0.3
5 EP+EAA	201.4	337.6	62.3	1.27	3.54	1.86	1.7
6 SMP	179.0	261.7	42.2	2.90	5.94	1.34	1.4
7 SMP+EAA	186.3	284.5	47.9	2.54	5.78	1.48	4.8
Sep.26 ~ Oct.23 (17 days feeding)							
1 Control	358.1	485.4	34.6	1.96	3.41	1.23	0.3
2 SDP	321.9	390.7	20.9	2.72	3.03	0.85	1.4
3 SDP+EAA	342.8	418.2	21.5	2.45	2.79	0.91	3.0
4 EP	319.0	357.3	8.7	5.10	2.49	0.48	38.0
5 EP+EAA	337.6	404.4	19.2	2.83	2.92	0.84	4.0
6 SMP	261.7	309.3	17.7	4.75	4.54	0.82	7.4
7 SMP+EAA	284.5	330.9	16.7	4.96	4.50	0.76	8.6
Oct.24 ~ Nov.8 (5 ~ 8 days feeding)							
1 Control	485.4	534.8	10.0	2.46	2.94	0.98	0.0
2 SDP	390.7	415.6	6.2	3.82	2.88	0.61	4.3
3 SDP+EAA	418.2	446.2	6.1	3.66	2.69	0.61	2.1
4 EP	357.3	385.5	6.8	2.29	3.00	1.07	15.3
5 EP+EAA	404.4	422.9	4.1	3.51	2.80	0.67	9.5
6 SMP	309.3	316.5	1.8	18.67	4.78	0.21	31.3
7 SMP+EAA	330.9	351.1	5.5	6.33	4.81	0.59	16.5
Entire duration (55 ~ 58 days feeding)							
1 Control	134.3	534.8	266.2	1.61	3.16	1.50	0.9
2 SDP	136.6	415.6	183.7	1.90	3.14	1.22	5.4
3 SDP+EAA	134.3	446.2	207.7	1.70	2.99	1.31	6.3
4 EP	130.9	385.5	163.3	1.86	3.04	1.31	40.6
5 EP+EAA	134.9	422.9	192.6	1.75	3.12	1.35	13.4
6 SMP	132.9	316.5	124.7	3.74	5.13	1.04	32.9
7 SMP+EAA	134.3	351.1	139.1	3.30	4.84	1.14	27.1

Table 4-6. Growth and feed performances of yellowtail fed the experimental non fish meal diets in aquariums

Diet no.	Av.body wt. (g) Initial	Final	Growth rate (%)	Feed gain ratio	Daily feed intake	Protein efficiency ratio	Mortality (numbers)
Oct.6 ~ Dec.1 (39 days feeding)							
1 Control	235.3	584.8	148.5	1.4	3.4	1.73	0
2 SDP	236.5	340.6	44.0	3.0	1.3	0.78	1
3 SDP+EAA	236.6	437.1	84.7	1.5	2.2	1.48	0
4 EP	241.1	285.3	18.3	2.5	1.1	0.98	2
5 EP+EAA	232.9	390.2	67.5	1.6	1.9	1.48	1

on SDP supplemented with EAA was 207.7%, and was about 80% of the control group (266.2%). Fish on the SMP were lowest in both growth rate and feed gain ratio. The growth and feed performances were improved by supplementation of the EAA mixture irrespective of the types of non fish meal diets and were in the order of SDP, EP, and SMP. Nevertheless, losses of fish were observed due to bacterial and/or parasitic diseases during the experimental period. Cumulative mortality reached 27 41% in fish on diets 4 (EP without EAA), 6, and 7 (SMP with and without EAA), whereas the mortality was low in groups fed the control (1%) and non fish meal diets produced as SDP (5 7%). Mass mortalities in the groups 4, 6, and 7 during October seemed to be caused by skin fluke disease (parasitism of *Benedenia seriolae*) but interestingly almost all dead fish had the green liver

abnormality.

Expt. II: Growth and feed performances in Expt. II showed the trend similar to those in Expt. I in general. However, palatability and acceptability of each non fish meal diet in Expt. II were slightly inferior to those in Expt. I. Growth rate, feed gain ratio, and protein efficiency ratio of fish fed the control diet were superior to those of fish on the non fish meal diets as observed in Expt. I. Fish fed the SDP supplemented with EAA showed the best growth rate and feed gain ratio, and those fed the EP without supplementation were the worst among the non fish meal diet groups. However, growth and feed performances of fish fed the non fish meal diets were improved when the EAA mixture was supplemented in both SDP and EP diets.

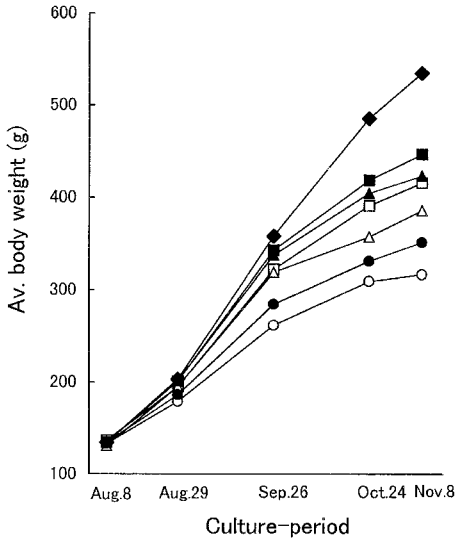

Fig. 4-3. Growth curves of yellowtail fed the experimental non fish meal diets and control fish meal diet in net cages. ◆:Diet 1, □:diet 2, ■:diet 3, △:diet 4, ▲:diet 5, ○:diet 6, ●:diet 7.

Proximate Composition

The results of proximate analysis for whole body, dorsal muscle, and liver in fish from Expts. I and II are presented in Table 4 7. In Expt. I, the final group of fish fed each diet showed almost the same protein content (19.3 20.3%) of whole body as compared with the initial fish (20.2%), and levels were similar for all the groups. On the other hand, the final lipid content in whole body of fish fed the control diet (10.5%) was slightly higher than those fed the non fish meal diets (5.3 9.5%). Comparing within the non fish meal diet groups, the lipid value of fish on diets 6 and 7 (SMP) was lower than the rest, although dietary lipid content was almost same for all treatments. Moisture content in whole body of fish fed diet 6 was the highest among the groups, and there was an inverse relationship between moisture and lipid content. In dorsal muscle, the same tendency was observed in protein, lipid and moisture contents. The protein content in liver of fish on the control diet was slightly lower than the rest, and lipid content of the control was the highest among the groups as observed in whole body and muscle. The low lipid and high moisture contents in both muscle and liver were also observed in a previous

Table 4-7. Proximate composition (%) of whole body, dorsal muscle, and liver of yellowtail fed the experimental non fish meal diets

Diet no.	Moisture	Crude protein	Crude lipid	Crude ash
Expt. I				
Whole body (n=6)				
Initial	71.6	20.2 (71.7)*	5.6 (19.7)	3.5 (12.3)
Final				
1 Control	67.4	20.1 (61.7)	10.5 (32.2)	2.7 (8.3)
2 SDP	69.8	19.7 (65.2)	8.1 (26.8)	2.7 (8.9)
3 SDP+EAA	69.9	19.8 (65.8)	8.3 (27.6)	2.8 (9.3)
4 EP	69.3	19.3 (62.9)	9.5 (30.9)	2.8 (9.1)
5 EP+EAA	69.0	19.7 (63.5)	8.8 (28.4)	2.9 (9.4)
6 SMP	71.8	20.3 (72.0)	5.3 (18.8)	3.5 (12.4)
7 SMP+EAA	71.3	20.0 (69.7)	6.5 (22.6)	2.8 (9.8)
Dorsal muscle (n=10)				
Initial	74.6	23.3 (91.7)	1.3 (5.1)	1.9 (7.5)
Final				
1 Control	71.8	24.6 (87.2)	3.5 (12.4)	2.5 (8.9)
2 SDP	74.3	24.0 (93.4)	1.7 (6.6)	2.0 (7.8)
3 SDP+EAA	74.5	24.2 (94.9)	2.0 (7.8)	2.0 (7.8)
4 EP	74.1	23.6 (91.1)	2.6 (10.0)	1.7 (6.6)
5 EP+EAA	73.7	24.8 (94.3)	1.9 (7.2)	2.1 (8.0)
6 SMP	75.5	23.6 (96.3)	1.5 (6.1)	1.7 (6.9)
7 SMP+EAA	74.5	23.9 (93.7)	1.3 (5.1)	2.0 (7.8)
Liver (n=10)				
Initial	74.7	15.7 (62.1)	4.7 (18.6)	1.4 (5.5)
Final				
1 Control	62.0	12.4 (32.6)	19.3 (50.8)	0.9 (2.4)
2 SDP	74.3	15.8 (61.5)	9.2 (35.8)	1.4 (5.4)
3 SDP+EAA	71.8	15.5 (55.0)	10.3 (36.5)	1.3 (4.6)
4 EP	71.0	14.2 (49.0)	12.9 (44.5)	1.2 (4.1)
5 EP+EAA	65.7	14.1 (41.1)	17.7 (51.6)	1.1 (3.2)
6 SMP	76.1	16.0 (66.9)	6.7 (28.0)	1.3 (5.4)
7 SMP+EAA	75.8	16.3 (67.4)	6.8 (28.1)	1.3 (5.4)
Expt. II (whole body, n=5)				
Initial	68.1	20.3 (63.6)	9.4 (29.5)	2.6 (8.2)
Final				
1 Control	62.6	20.7 (55.3)	15.2 (40.6)	2.4 (6.4)
2 SDP	68.9	20.7 (66.6)	9.3 (29.9)	2.7 (8.7)
3 SDP+EAA	66.3	20.2 (59.9)	12.1 (35.9)	2.3 (6.8)
4 EP	68.0	20.2 (63.1)	9.6 (30.0)	2.9 (9.1)
5 EP+EAA	63.9	20.4 (56.5)	13.8 (38.2)	2.7 (7.5)

* Figures in parentheses are values on dry matter basis.

experiment with yellowtail fed the non-fish meal diets (Chapter 3.2.1-1, 2). In Expt. II, the highest body lipid content was in the fish fed the control diet, as shown in Expt. I. The lipid content of fish fed the non-fish meal diets was high when EAA was supplemented in both SDP and EP diets. There was no marked difference in the protein content of whole body among the groups.

Hemochemical Characteristics

The results of hemochemical examination in Expt. I are presented in Table 4-8. Hematocrit level of fish fed diets 6 and 7 (SMP) was under 40%, and was significantly lower than that of the control, indicative of slight anemia. There were no significant differences in the ALP activity and glucose, urea nitrogen, and total protein levels among the groups, when the data was statistically analyzed using ANOVA. In addition, triglyceride level was not significantly different between the test and control groups. With regard to the lipid metabolites, the values obtained for phospholipid, total cholesterol, free cholesterol, and ester ratio of fish from the non-fish meal diet groups were significantly lower than those of the control, the exception being triglyceride. A similar result was also observed in the previous study with yellowtail fed the experimental non-fish meal diets having various alternate proteins at different levels (Chapter 3.2.1-1,2). Among the non-fish meal diet groups, the plasma levels of these lipid metabolites in fish fed the SMP diets were lower than those on SDP and/or EP diets. Moreover, these lipid values were higher in fish on the diets with supplemental EAA than those on diets without EAA, irrespective of the type of non-fish meal diet. The creatinine level in the control fish was significantly higher than those of the test groups, while no marked differences were found among the test groups.

The anatomical examination showed that 1-5 out of 6 fish for each non-fish meal diet group, except the diet 7 group (SMP with EAA), had the green liver. The occurrence rate of green liver symptom was the highest for fish on diet 4, and significantly this symptom was not observed for fish on the control fish meal diet.

Table 4-8. Results of hemochemical examination in yellowtail fed the experimental non-fish meal diets in net cages*[1]

		Diet no.						
		1	2	3	4	5	6	7
Ht	(%)	48.3 ± 1.4^{ab}	49.0 ± 2.9^{a}	45.3 ± 5.8^{ab}	42.8 ± 4.1^{b}	44.8 ± 7.7^{ab}	37.4 ± 2.7^{b}	39.2 ± 3.3^{b}
ALP	(IU / l)	143±27	140±22	149±21	156±16	144±29	159±17	137±8
GLU	(mg / 100ml)	134±15	141±19	158±23	154±14	154±19	163±18	140±17
TG	(mg / 100ml)	152 ± 29^{ab}	110 ± 31^{b}	161 ± 42^{ab}	160 ± 46^{ab}	185 ± 54^{a}	117 ± 31^{b}	99 ± 20^{b}
PL	(mg / 100ml)	761 ± 59^{a}	632 ± 39^{b}	674 ± 88^{ab}	627 ± 95^{b}	651 ± 120^{b}	506 ± 56^{c}	517 ± 35^{c}
TCHO	(mg / 100ml)	319 ± 16^{a}	239 ± 23^{bc}	248 ± 35^{b}	208 ± 25^{c}	218 ± 47^{bc}	176 ± 23^{c}	198 ± 7^{c}
FCHO	(mg / 100ml)	112 ± 8^{a}	86 ± 3^{bc}	97 ± 11^{b}	81 ± 10^{bc}	95 ± 14^{b}	74 ± 7^{c}	80 ± 6^{c}
Ester ratio	(%)	65.0 ± 2.4^{a}	63.9 ± 2.7^{ab}	60.4 ± 3.8^{b}	56.2 ± 0.9^{bc}	55.2 ± 5.4^{bc}	57.5 ± 3.5^{bc}	59.6 ± 2.7^{b}
BUN	(mg / 100ml)	21.4±3.2	20.2±1.4	19.8±6.1	21.4±2.8	18.4±5.2	18.3±2.3	17.1±2.8
CRE	(mg / 100ml)	1.8 ± 0.4^{a}	1.2 ± 0.0^{b}	1.2 ± 0.1^{b}	1.1 ± 0.1^{b}	1.2 ± 0.1^{b}	1.1 ± 0.2^{b}	1.1 ± 0.1^{b}
TP	(g / 100ml)	3.7±0.1	3.6±0.3	3.9±0.2	3.5±0.2	3.7±0.5	3.3±0.2	3.6±0.2
Hepatosomatic index	(%)	1.79	1.16	1.15	1.38	1.30	1.28	1.10
Appearance of green liver*[2]		0 / 6	1 / 6	3 / 6	5 / 6	1 / 6	2 / 6	0 / 6

*[1] Mean ± standard deviation (n=6). Figures in a row with different superscripts are significantly different from each other ($p<0.05$) when analyzed using Duncan's multiple range test.

*[2] Ratio of fish with green liver / total number examined.

Discussion

In this study, growth and feed performances of non fish meal diet groups were inferior to the control. This suggests that nutritive value of the non fish meal diets prepared as different diet forms used in this study was lower than the control fish meal diet. Among the three types of non fish meal diets, SMP diet produced inferior growth in yellowtail compared to SDP and EP diets, though the ingredient composition and proximate composition were almost similar for all of the diets. Moreover, there was no big difference in the daily feeding rate value (dry matter basis) among the test diet groups. Nutritional quality of SMP diet was, therefore, considered to be the lowest among the three types of diet. This might be attributed to the low availability of carbohydrate ingredient (about 20% each on dry matter basis) in diets. It has been demonstrated that availability of this component to some fish could be improved by extrusion processing which elevates the gelatinized ratio (Takeuchi *et al.* 1990, 1994, Jeong *et al.* 1991, 1992a, 1992b, Hernadez *et al.* 1994). With regard to the yellowtail, Takeuchi *et al.* (1992). reported that juvenile yellowtail utilize gelatinized starch more effectively than raw starch, and that gelatinized ratio of diet for yellowtail should be above 50% for optimal growth performances. On the basis of these observations, we assume that the availability of carbohydrate to yellowtail in both SDP and EP diets used in this study also seemed to be elevated by extrusion processing. This may have produced the excellent feed performances for fish fed the SDP and EP diets compared to those fed the SMP diet, although we cannot definitely conclude because gelatinized ratio of diets was not determined in this study. Further, feeding results in this study suggested that SDP diet was superior to EP diet in the nutritional quality. This difference could be attributed to the extrusion conditions maintained during pellet production. The results from feeding experiments with rainbow trout, yellowtail, and red sea bream performed by Akimoto (1994) have indicated that nutritional qualities of diets (containing 30% SBM) were influenced by extrusion conditions, especially the material temperature. However the present data is insufficient to pin point which of the extrusion conditions affected the feed quality of the non fish meal diets. Further experiments will be necessary to investigate the effect of extrusion conditions on the optimum utilization of non fish meal diets, and to determine the suitable manufacturing conditions.

The results obtained in this study have clearly shown that EAA supplementation to the non fish meal diets improved growth and feed performances of yellowtail, irrespective of the diet type. This might be due to the enhanced feed protein utilization, promoted by the compensation for deficient EAA in diets. Moreover, our observations indicate that yellowtail can efficiently utilize crystalline amino acids supplemented to the individual non fish meal diets. However, feed performances of fish fed the non fish meal diet supplemented with EAA were inferior to those fed control diet, though there were no marked difference in the EAA composition between the test and control diets, as shown in Table 4 4. This suggests that EAA supplementation to the non fish meal diets could not produce protein utilization matching that obtained with the control fish meal diet. Therefore, more information is needed on the availability of EAA supplemented to diets for yellowtail. We determined the postprandial changes in plasma free amino acids of yellowtail after feeding the respective non fish meal diets with or without supplemental EAA during the experimental period, in order to investigate the absorption of supplemental EAA and their availability. The results of the investigation will be described in a subsequent paper.

As for physiological condition of fish, the control group is considered to have maintained a healthy status during the experimental period. Among the hemochemical constituents, significantly lower levels of lipid metabolites were observed for fish on the non fish meal diets, indicating abnormal liver function. The blood parameters related to lipid metabolism —total cholesterol, free cholesterol, and phospholipid— are thought to be special indices of health condition for yellowtail, because it was stated that the fish having low levels of these lipid items was susceptible to the pathogens (Maita *et al.* 1998a, 1998b). Accordingly, it appeared that the non

fish meal diet groups were physiologically inferior to the control. Comparing the non fish meal diet groups, the lipid metabolites and hematocrit values of fish fed the SMP diets were lower than those of fish on the SDP and/or EP diets, suggesting poorer status of health. Body composition analysis also revealed that fish on the SMP diets were malnourished compared to other non fish meal diet groups. Moreover, fish fed diets 6, 7 (SMP), and 4 (EP without EAA) showed a high mortality together with poor growth rate and feed gain ratio. Judging from these facts, SMP diets seem to be nutritionally inadequate both quality wise as well as health wise, among the three diet forms tested. This also brings to light that the physiological condition of experimental fish has a close relation to feed performances, and that feed quality affects the state of fish health.

In the anatomical test at the end of experiment, green liver symptom as a pathological feature was observed for fish on the non fish meal diets. This followed our earlier description in juvenile and young yellowtail (Chapter 3.2.1 1,2). The reason why green liver occurs only in fish fed the non fish meal diets remained unknown. However, the results from this study have indicated that outbreak of green liver was controlled by nutritional quality of the non fish meal diets. Perhaps, the appearance of green liver was related to the level of biodefense and/or physiological function in fish. We are minutely investigating the link between occurrence of green liver and changes in the physiological condition of yellowtail during experimental rearing in another feeding trial.

In conclusion, the feeding results from this study have proved that quality of the non fish meal diets was influenced by diet type (preparation method), and also probably by the conditions during extrusion processing. Further, it was demonstrated that supplementation of EAA to the non fish meal diets to compensate the deficient amino acids resulted in the improvement of growth and feed performances for yellowtail, irrespective of diet types. Consequently, feed quality of the non fish meal diet produced as SDP, supplemented with EAA, was evaluated to be superior to those of other types, with the exception of the control diet. More work has to be done to identify the reasons behind the fall in performances of the non fish meal diets, so as to establish them as fish meal alternates.

4.3 Comparison of Plasma Free Amino Acid Patterns between Three Types of Non-Fish Meal Diets with Amino Acid Supplement

In a previous experiment, in which non fish meal diets of three different types－SDP, EP, and SMP－ all having almost same formulation with or without supplemental EAA were fed to young yellowtail, it was demonstrated that the feed performances of non fish meal diets were improved by supplementation of EAA, irrespective of diet types (Chapter 4.2). However, the growth and feed gain ratio of fish fed the non fish meal diets with EAA were inferior to those of fish on the control fish meal diet, in spite of the fact that EAA were fortified in the non fish meal diets to give the same levels as those of the control fish meal diet. This inferior feed performances may be attributed to the same observation made in another experiment, that supplemental EAA were effectively absorbed and their plasma levels, especially of methionine, were markedly higher than those of the control, but probably resulting in an amino acid imbalance that may have caused lower feed performances in the test groups fed diets with high levels of alternate proteins (Chapter 4.1). For effective utilization, absorption of supplemental crystalline EAA into plasma must be synchronized with those derived from dietary protein, and this would be closely related to evacuation timing of diet from stomach. Thus, the utilization efficiency would differ between types of diets in terms of their physical properties such as hardness.

This study was, therefore, conducted to compare the supplemental effect of EAA from the viewpoint of the periodical changes of feed digesta in stomach and intestine, and plasma free amino acid patterns between fish fed different diet types such as SDP, EP or SMP.

Materials and Methods

Experimental Diets

The diet type, ingredient composition, and proximate composition of experimental diets are presented in Table 4 9. All the experimental diets were the same as those used in the feeding trial with yellowtail reported previously (Chapter 4.2). Diet 1 was the control fish meal diet and diets 2 7 were non fish meal diets containing 30% SPC as a main protein source. Non fish meal diets were prepared to three types of diets (SDP, EP, and SMP), each supplemented with or without EAA mixture. The EAA mixture contained 1.5% L lysine, 0.5% DL methionine, 0.5% L threonine, and 0.2% L tryptophan. SDP and EP were prepared by the large and small size twin screw extruders, respectively. The preparation conditions for the different diets were presented in a former paper (Chapter 4.2).

Experimental Procedure

The experiment was conducted at the Owase Branch, Fisheries Research Institute of Mie using floating net cages (3×3×3 m). Young yellowtail (*Seriola quinqueradiata*) were maintained on each experimental diet for 35 days. The average fish weight was about 280 g for the control and 220 270 g for the test groups, and total

Table 4-9. Composition of the different types of the experimental non fish meal diets with or without supplemental essential amino acids for yellowtail

Ingredient(%)	Type of diet*1						
	SDP			EP		SMP(Mash)	
	Diet 1*2	Diet 2	Diet 3	Diet 4	Diet 5	Diet 6	Diet 7
Fish meal (Jack mackerel)	67			0			
Soy protein concentrate	0			30			
Defatted soybean meal	0			10			
Corn gluten meal	0			8			
Meat meal	0			10			
Krill meal	0			10			
Wheat flour	8	8		2		0.5	
α-Starch	5	0		8		5.5	
Guar gum	0	0		0		2	
Carboxymethylcellulose	0	0		0		2	
Mineral mixture	5	5		5		5	
Vitamin mixture	2	2		2		2	
Feed oil	13	17		15		15	
Amino acid mixture*3	0	0	2.7	0	2.7	0	2.7
Total	100	100	102.7	0	102.7	0	102.7
Proximate composition: As is basis (%)							
Crude protein	41.4	43.0	45.0	41.0	42.3	25.7	26.6
Crude lipid	19.5	19.0	18.3	19.1	18.7	11.9	11.3
Crude ash	9.1	8.9	8.9	7.7	7.8	5.2	4.9
Moisture	13.7	7.3	5.4	10.6	9.8	45.4	44.5
Dry matter basis (%)							
Crude protein	48.0	46.3	47.5	45.8	46.8	47.1	47.9
Crude lipid	22.6	20.5	19.3	21.4	20.7	21.9	20.4
Crude ash	10.6	9.5	9.4	8.6	8.7	9.5	8.8

*1 SDP; Soft dry pellet, EP; Extruded pellet, SMP; Single moist pellet.

*2 Control fish meal diet.

*3 Lysine 1.5, methionine 0.5, threonine 0.5, and tryptophan 0.2.

fish number of each group was 325 341. Five fish were collected from each treatment after fasting them for 2 days, and whole blood was taken from heart with heparinized syringe to determine the pre feeding level of plasma FAA. Then fish were fed each experimental diet to near satiation at 09:00, and subsequently five fish were collected from each group to measure the feed digesta in both stomach and intestine for determination of the relative weights immediately after feeding. Thereafter, whole blood and feed digesta were taken from five fish of

each group at 3, 6, 9, 12, 18, and 24 hours post feeding. Water temperature ranged from 24.5 to 26.8℃ during the experimental period (2 m in depth). The whole blood was centrifuged at 3,000 rpm for 10 min to obtain plasma, and five plasma samples were pooled for FAA analysis of each group. Analytical methods of plasma FAA were the same as described in a previous paper (Chapter 3.2.1 1).

Results and Discussion

Plasma Levels of FAA

The results of postprandial changes of the four supplemental EAA and other EAA, together with total EAA concentrations in the plasma for fish fed the non fish meal diets with or without supplemental EAA and those on the control diet are presented in Table 4 10 and Fig. 4 4.

Comparing the plasma levels of four supplemental amino acids between fish fed the non fish meal diets without EAA supplement and the control fish meal diet, lysine, methionine, and threonine except tryptophan were clearly lower in the former groups than in the control group, during the whole experimental period, regardless of diet types, suggesting a shortage of these EAA in the test diets. The level of tryptophan was not much different between the treatments, suggesting that the test diets were sufficient in this amino acid. In fish fed the test diets with supplemental EAA, the plasma levels of these EAA increased immediately afterfeeding, and their maximum values were quite higher than those of the control. These results agreed well with those obtained in the former experiment that supplemental crystalline amino acids were effectively absorbed irrespective of the diet type

Table 4-10. Periodical changes of free amino acid contents in blood plasma of yellowtail fed the experimental non fish meal diets (μmol/100ml plasma)

Diet no.	Hours after feeding (h)						
	0	3	6	9	12	18	24
Total EAA[*1]							
1 Control	182.7	299.4	326.5	411.5	387.9	466.9	347.1
2 SDP	193.3	281.0	288.3	362.9	413.4	456.1	272.4
3 SDP+EAA	213.5	464.7	492.5	452.8	448.9	496.3	332.4
4 EP	196.0	240.7	273.9	380.8	436.7	494.2	266.1
5 EP+EAA	240.6	404.2	475.9	488.4	562.3	601.0	340.4
6 SMP	210.0	226.1	303.3	356.1	385.3	471.9	311.2
7 SMP+EAA	210.3	374.6	423.1	437.2	488.0	491.1	349.4
Total NEAA[*2]							
1 Control	300.1	336.5	272.7	261.9	214.4	275.0	337.7
2 SDP	313.9	310.6	302.2	239.4	244.1	305.9	348.5
3 SDP+EAA	325.2	334.1	391.6	267.2	238.5	274.0	311.1
4 EP	303.8	244.7	290.8	241.2	259.3	366.5	362.2
5 EP+EAA	320.7	282.7	355.1	238.8	260.1	303.3	301.5
6 SMP	247.1	195.1	270.1	225.4	259.7	362.4	346.2
7 SMP+EAA	238.6	242.8	278.0	253.6	248.6	325.2	333.1
Total FAA[*3]							
1 Control	482.8	635.9	599.2	673.5	602.2	741.9	684.8
2 SDP	507.2	591.5	590.5	602.2	657.5	762.0	620.9
3 SDP+EAA	538.6	798.9	884.1	720.0	687.4	770.4	643.4
4 EP	499.8	485.4	564.7	622.0	696.0	860.7	628.4
5 EP+EAA	561.3	686.9	831.0	727.2	822.3	904.3	642.0
6 SMP	457.1	421.2	573.4	581.5	645.1	834.3	657.4
7 SMP+EAA	448.9	617.5	701.1	690.8	736.6	816.3	682.4

*[1] Total essential amino acids: arginine, lysine, histidine, phenylalanine, tyrosine, leucine, isoleucine, methionine, valine, threo nine, tryptophan, and cystine.

*[2] Total non essential amino acids: alanine, glycine, glutamic acid, serine, taurine, and aspartic acid.

*[3] Total free amino acids.

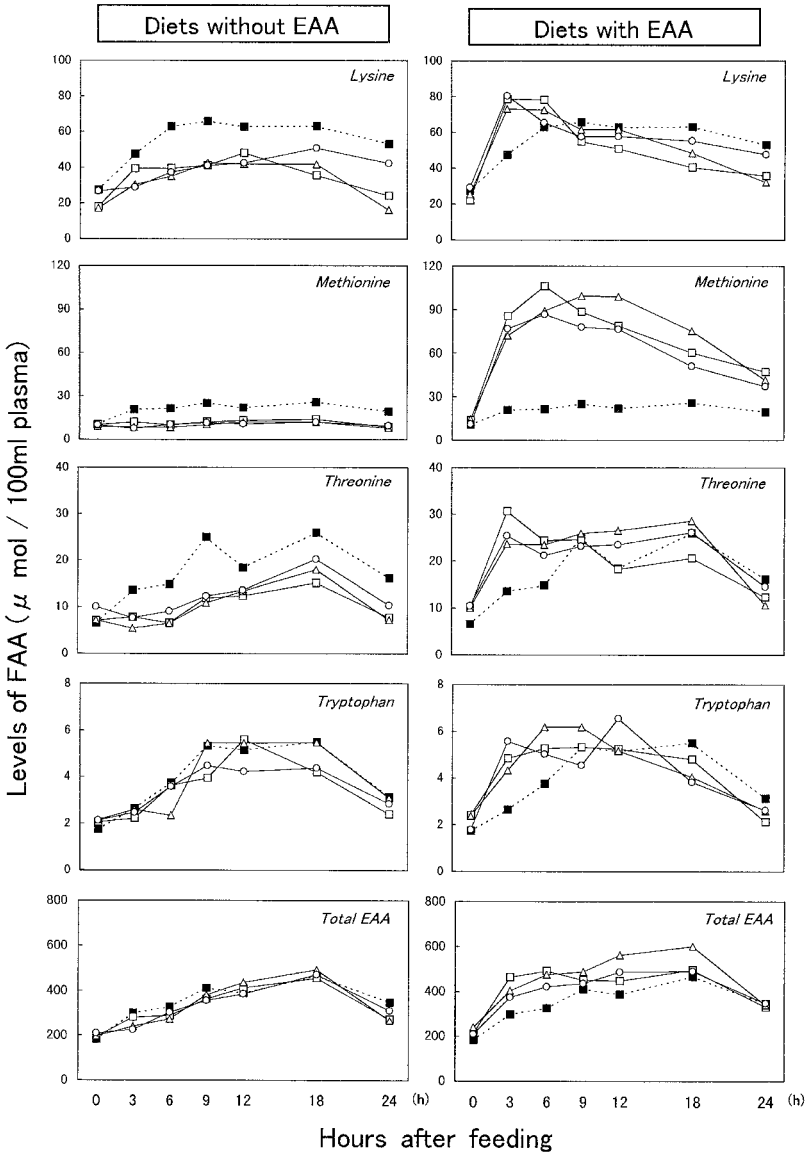

Fig. 4-4. Periodical changes of free amino acid levels in plasma of yellowtail fed the experimental non fish meal diets with or without supplemental amino acids. ■:Control SDP, □:SDP, △:EP, ○:SMP.

(Chapter 4.1). Both lysine and threonine exhibited peaks at the third hour as observed previously and there was no marked difference between the treatments in the pattern of periodical changes. In case of methionine, too high levels were attained in the early hours after digestion, and continued to be so for 24 hours in fish fed the test diets with EAA, irrespective of the type of diet. The supplemental methionine seemed to have upset the normal physiological levels of the amino acid in the plasma. Methionine was fortified in the test diets to provide the same level as that of the control fish meal diet, but its plasma level for the test groups was significantly higher than that of the control, resulting an amino acid imbalance that may have lowered the feed performances in the test groups. Therefore, the amounts of supplemental amino acids should be adjusted to maintain the balance of plasma free amino acids after feed intake.

A remarkable difference was found in postprandial patterns of plasma lysine, methionine, and threonine

between fish fed the control diet and non-fish meal diets with EAA. It was indicated that these amino acids derived from supplement were absorbed much earlier than those from feed protein ingredients. In other experiments with rainbow trout (Yamada *et al.* 1981, Murai *et al.* 1987, Schuhmacher *et al.* 1997) and/or carp (Plakas *et al.* 1980, Murai *et al.* 1982b), it has been revealed that the time required for appearance of peak level of plasma FAA concentration after feeding was earlier for supplemental amino acids than those derived from the protein ingredient. For enhancement of the availability of supplemental amino acids, plasma FAA levels due to supplemental EAA must be synchronized with those derived from dietary protein. Therefore, further experiments will be necessary to determine suitable supplemental levels of individual crystalline amino acids in feeds based on the absorption rate of each amino acid. Moreover, Murai *et al.* (1982c) have reported that coating amino acids with casein improved plasma amino acid balance due to the alteration of absorption and retention times of some amino acids. A similar trial using coated amino acids will be useful in improving the dietary value of non-fish meal diets for yellowtail.

With regard to other non-supplemental EAA, there were no marked differences in their postprandial plasma concentrations, as well as in the periodical changes between fish fed the control and test diets, and also between the different types of diets. The plasma levels of the total EAA in fish fed the non-fish meal diets both with and without supplemental EAA peaked at 18 hours post feeding and quickly decreased thereafter, but in fish of SDP with supplemental EAA group had another peak at 6 hours post feeding. In the former experiment each supplemental EAA and their total levels peaked at 3 hours post feeding with non-fish meal diets supplemented with the same four EAA, the appearance rate being faster than the present experiment (Watanabe *et al.* 1998). The reason for this may be the difference in physical properties of the diets in terms of their evacuation timing from stomach and intestine.

Feed Digesta Contents

The results of postprandial changes of feed digesta contents (dry % of body weight) for fish fed the non-fish meal diets with or without EAA and the control diet are presented in Fig.4-5. Feed digesta content in stomach for fish fed the control fish meal diet was slightly higher than those on other non-fish meal diets, irrespective of EAA supplement during the period of 24 hours. There was no marked difference in decreasing pattern of stomach content among non-fish meal diet groups and the fasting level was attained within 24 hours. With regard to the shape of feed digesta, all the types of non-fish meal diets with or without EAA collapsed in the stomach even at the 3rd hour post feeding, while the control fish meal diet keep its shape until 24 hours. Moreover, no remarkable difference was found in postprandial pattern of feed digesta content in intestine among all the experimental groups. The intestine contents were the maximum either at 6 or 9 hours post feeding.

In our previous study, it was shown that feed digesta of the fish meal diets prepared as SMP forms passed rapidly through the stomach compared to those as SDP forms (Chapter 4.1). Therefore, the SMP used in the present experiment seemed different from those used in the earlier experiment in characteristics such as stability in the digestive tract. The stability of the diet basically would depend on feed ingredient, the kind of dietary binder and its content. In addition, present results indicated that the non-fish meal diets prepared as SDP collapsed much earlier than control fish meal SDP during the digestion process, suggesting that feed ingredients may also have an influence on this.

In conclusion, the results from this study have demonstrated that crystalline lysine, methionine, threonine, and tryptophan supplemented to the non-fish meal diets are utilized by yellowtail irrespective of diet forms, and this may result in improvement of nutritional quality of non-fish meal diets as reported in feeding trial earlier (Chapter

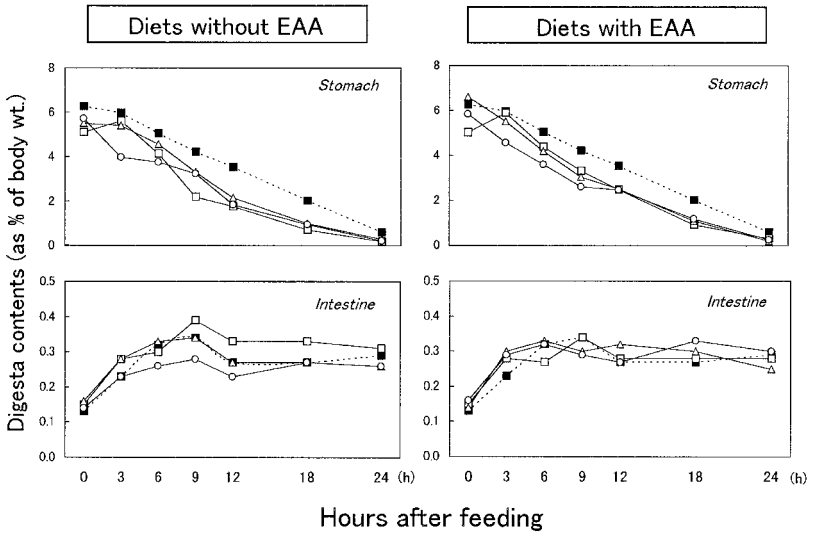

Fig. 4-5. Periodical changes of feed digesta contents in yellowtail fed the experimental non fish meal diets with or without supplemental amino acids. ■:Control SDP, □:SDP, △:EP, ○:SMP.

4.2). However, enhanced utilization could be achieved only if there is a synchronization in the absorption rates, and in turn on the plasma levels of amino acids derived from the supplemented EAA and from the dietary protein sources.

Concluding Remarks

The role of fisheries as provider of precious animal protein for humanity has become more important of late. Aquaculture industry is, especially, projected to produce high value fish, and the recent growth in world aquaculture indicates this trend. This has led to an increased demand for aquatic formula feeds that depend on fish meal and fish oil as main protein and lipid sources, respectively. In Japan this demand is greater compared to other countries, because of the popular high quality extruded dry type pellet available for marine fish culture. However, recent rapid decline in the landings of sardine, a prominent raw material for fish meal and fish oil, has necessitated the exploration of efficient alternate feed ingredients.

This thesis highlights a series of experiments conducted to obtain information on the availability of various plant and animal ingredients as alternative protein source for fish meal in diets for yellowtail and red sea bream. Practical inclusion levels of SBM, CGM, MM, and others were tried out in diets for the two species. The results from these trials have clearly confirmed that a combination of SBM, CGM, and MM can replace more than 50% of fish meal in dry pellets for both yellowtail and red sea bream without any adverse effect. Moreover, it was also demonstrated for yellowtail that fish oil can be substituted up to 50% by beef tallow or palm oil. However, in some of the experiments, the results were not very encouraging because the non fish meal diets produced certain adverse effects on growth and health of yellowtail. The poor growth and feed performances are thought to be related to the appearance of green liver. The etiology of this liver condition and preventive measures must be clarified through future studies. This study also revealed that supplementation of crystalline amino acids was effective in enhancing availability of the non fish meal diets for yellowtail. Unfortunately, we could not define an adequate level of amino acid supplementation based on their absorption efficiencies, and this needs to be worked out. Further research on suitable formulation of alternative protein sources in diets for red sea bream is also required.

The final goal in these selection trials with alternative protein sources is to totally replace fish meal in aquatic feeds, namely the development of the non fish meal diets. The knowledge obtained from this thesis will contribute greatly to the feed preparation technologies for developing practical diets containing alternate ingredients, and meet the challenges of the fish culture industry in the 21st century.

Summary

Fish meal and fish oil, primarily sourced from sardine which has become a scarce commodity, are major ingredients for aquatic feed formulation in Japan. The fish feed industry in Japan is now almost entirely dependent on the meal and oil imported from South America and Europe, because of the quick decline in domestic catch of feed grade fish. This situation will lead to instability in farm management. Thus, serious efforts are being made to reduce fish meal and fish oil components in practical diets using other animal and plant ingredients. The aim is to optimally utilize alternate ingredients, thereby developing feeds without fish meal, as succeeded in the case of some other animal feeds. The present study was, therefore, conducted to examine availability of alternate ingredients in diets for yellowtail and red sea bream, in order to obtain basic information necessary for the development of low or perhaps even non fish meal diets for both two species. The results of the study are summarized below:

Chapter 1

Feeding experiments were performed to investigate the availability of various plant and animal protein sources as partial replacement for fish meal in diets for yellowtail and red sea bream.

1) Dietary fish meal can be replaced up to 50 60% by combining defatted soybean meal (SBM), corn gluten meal (CGM), and meat meal (MM) in practical extruded pellets (soft dry pellet; SDP) for yellowtail.
2) SBM, both unprocessed and processed by extruder, can be used as sole substitutive protein source at a level of 30% for fish meal (substitution of 55% fish meal) in high energy steam dry pellet (DP) as well as in SDP for juvenile red sea bream.
3) The combination of SBM, CGM, and MM in some proportion can replace up to about 60% of fish meal in the DP for both adult and juvenile red sea bream.
4) Soy protein concentrate, purified from SBM, has excellent potential as substitutive protein source for fish meal in diets for yellowtail and red sea bream. However, this ingredient cannot be used at high proportion in diets because it slightly reduces the palatability for both the species.

Chapter 2

The dietary value of extruded pellets formulated to contain both alternate protein and lipid sources was evaluated for yellowtail.

5) The combined use of alternate proteins such as SBM, CGM, MM, and blood meal could contribute to dietary protein at around 40%, while palm oil and beef tallow (melting point 39 41℃) either individually or in 1:1 combination could replace 50% of fish oil in the extruded pellets for yellowtail.

Chapter 3

The purpose of feeding trials in this part was to obtain information on the use of non fish meal diets for yellowtail and red sea bream.

6) Growth and feed gain ratio of young yellowtail tend to decrease with reduction of dietary level of fish meal, probably due to deficiency of essential amino acids such as lysine and methionine in the diets.
7) Feed performances in yellowtail fed the diets containing alternate proteins, substituting over 50% of the fish meal, are remarkably poor compared to fish fed on the fish meal diet.
8) Young yellowtail actively fed the non fish meal diets and sustained normal growth for the initial 1 2 months, but thereafter growth became stagnant, feed gain ratio was poor eventually resulting in high mortality irrespective of the dietary treatments.
9) The green liver symptom is singularly observed for yellowtail fed the non fish meal diets irrespective of the ingredient composition. This symptom is caused by occlusion of the bile duct due to parasitic mucosporozoa, the parasitism succeeding only when the diets are devoid fish meal.

10) The inferior performance in yellowtail fed the non fish meal diets is thought to be related to the appearance of green liver symptom but its mechanism is still unknown.

11) Fish meal component in diets can be completely replaced by a suitable combination of substitutive protein sources, for both juvenile and adult red sea bream.

Chapter 4

The availability and effect of crystalline amino acids supplemented to three types of non fish meal diets were studied with yellowtail.

12) Gastric evacuation time seemed to be related to the collapse of diet shape in the stomach.

13) Crystalline lysine, methionine, threonine, and tryptophan supplemented to diets seemed to be absorbed immediately after feed intake into blood plasma irrespective of the diet types.

14) Post feeding change of free amino acid concentrations in blood plasma seemed to be linked to emptying time of stomach digesta.

15) Postprandial plasma methionine concentration of yellowtail fed the diets with supplemental crystalline methionine is remarkably higher than that of fish on diets without supplementation (3 24 hours post feeding). This may imbalance the plasma free amino acid levels, and cause lower feed performances of fish fed the non fish meal diets with supplemental amino acids compared to the control fish meal diet.

16) Supplementation of crystalline amino acids can improve the quality of non fish meal diets irrespective of the diet types.

17) Nutritional quality of non fish meal diet is affected by the diet preparation method (extrusion condition).

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Appendix - Japanese Summary

代替原料の有効利用による新養魚飼料の開発に関する研究

現在，海面魚類養殖業は高級魚の安定的な生産を担う産業として，我が国の沿岸漁業の中で重要な位置を占めている。海面養殖の対象魚種はブリ，マダイなど肉食性傾向の強い魚種であり，その餌料としては大量に漁獲されてきた安価なマイワシがほぼ 100％の割合で用いられてきた。ところが，近年，マイワシの漁獲量が急激に減少し，餌料としての供給が著しく困難な状況となったため，生餌に代わって配合飼料の使用量が急速に増加しつつある。一方，配合飼料の主原料である魚粉・魚油についても，その国内生産はマイワシ漁獲量の推移と連動して大幅に減少している。そのため，現在，魚粉・魚油の供給はほとんどを海外からの輸入に頼らざるを得ないという不安定な状態に陥っている。

このような背景のもとに，本研究は魚粉や魚油のみに依存しない新しいタイプの海水魚用ドライペレットの開発に必要な知見を集積することを目的に，代替タンパク質や代替油脂を配合した低魚粉飼料の実用飼料としての有効性を調べ，さらに魚粉を全く配合しない飼料の利用性に関して，特に結晶アミノ酸の補足効果の観点から検討を加えたものである。

先ず，第１章では海水魚における各種タンパク質原料の利用性に関する今までの研究成果に基づき，代替タンパク質を配合したブリおよびマダイ用低魚粉飼料の利用性について検討した。代替タンパク質として大豆油粕(**SBM**)，コーングルテンミール(**CGM**)およびミートミール(**MM**)を併用して魚粉含量を50-60％置き換えた二軸エクストルーダー製ドライペレット(**EP**)を用いてブリを大型生簀で２年間にわたって飼育した。その結果，飼育成績は代替タンパク質を配合したことによる悪影響はみられず，ブリに対する低魚粉飼料の有効性を実証することができた。マダイでは，通常のスチームペレット(**DP**)を用いて**SBM**を単独で30％(魚粉代替率55％)，また上述した３種類の原料を併用することによりタンパク質源として46％(代替率62％)まで配合できることがわかった。さらにマダイ稚魚における濃縮大豆タンパク質(**SPC**)の利用性についても明らかにした。

第２章では，第１章の結果に基づき代替タンパク質配合の低魚粉**EP**を用いて代替油脂の利用性についてブリで検討した。その結果，**SBM**，**CGM**，**MM**および血粉を併用して魚粉含量を30-40％としたEPでは，ブリの必須脂肪酸要求を満足する量の魚油と併用すれば，パーム油および牛脂をそれぞれ単独で，あるいは両者を併用して10％前後配合することが可能であることがわかった。このことから，ブリ用EPでは魚粉および魚油の両者とも配合割合の50％程度を他の原料で有効に代替できることが明らかとなった。

以上のように，ブリおよびマダイでは，魚粉配合率の半分以上を代替タンパク質で置換した低魚粉飼料が十分に利用可能であること，またブリでは魚粉および魚油の配合割合を大幅に削減したEPの有効性を明らかにすることができた。しかし，マイワシ資源の早期の回復が望めない現状では，飼料の安定供給を将来にわたって確保するためには，さらに代替原料の積極的な利用を図り，タンパク質源に魚粉を全く使用しない養魚飼料の開発が必要であると考えられる。そこで，第３章および第４章ではブリおよびマダイを用いて無魚粉飼料開発の可能性について検討した。

第３章では，先ず飼料中の魚粉含量がブリの飼育成績に及ぼす影響を調べるため、魚粉配合率を0-65％としてEPを作製して飼育試験を行ったところ，飼育成績は魚粉含量と正の相関を示し，特に魚粉20％以下の区では65％区に比べて著しく劣った。これは，低魚粉あるいは無魚粉EPではリジンやメチオニンなどの必須アミノ酸(**EAA**)含量がブリの要求量以下のレベルとなり，飼料タンパク質の栄養価が低下したためであると推察された。一方，マダイでは飼料中の魚粉含量にかかわらずほぼ同じ飼育成績が得られた。次に，**SPC**を中心に**SBM**，**CGM**等をタンパク質源とし，**EAA**含量が魚粉飼料と同程度となるように結晶**EAA**を補足添加した無魚粉飼料の利用性を２回調べた。その結果，成長は飼育開始後１ヶ月(第１回試験)から２ヶ月(第２回試験)程度までは順調であったが，その後いずれも徐々に停滞し，終了時には魚粉区に比べて著しく劣った。これは，飼料に添加した**EAA**が有効に利用されなかったことや，無魚粉区の魚に特異的に発症した緑肝が原因ではないかと考えられた。この緑肝症は，胆管中に大量に寄生した粘液胞子虫によって引き起こされたもので，胆汁の流通阻害によって脂肪の脂肪の消化吸収が影響を受けたことも成長低下の一因と推察された。なお，無魚粉飼料給餌による粘液胞子虫寄生のメカニズムは不明である。一方，マダイでは無魚粉飼料給餌による成長阻害などは観察されず，無魚粉飼料の利用性が両魚種で大きく異なることがわかった。また，マダイに対する無魚粉飼料の適正配合組成を検討した試

験では，SPC配合率の高い飼料区で飼育成績が優れる傾向がみられ，SPCの利用性もブリと異なることが示唆された。

このように第３章の結果から，ブリにおける無魚粉飼料の利用性はかなり劣ることが明らかとなった。そこで第４章ではブリ用無魚粉飼料の性能の改善について，結晶アミノ酸の補足添加の点から検討した。無魚粉飼料に添加したEAAが有効に利用されるためには，原料タンパク質由来のEAAと添加したEAAの吸収における同時性が求められる。また添加EAAの吸収速度は飼料の物性（硬度や胃内における崩壊性など）の影響を受けると考えられる。そこで先ず，添加EAAの吸収速度および飼料の消化管内滞留時間に及ぼす飼料形態の影響を調べた結果，大型の二軸エクストルーダー（Ex）で製造したドライペレット（SDP）では小型Ex製ペレット（EP）およびシングルモイストペレット（SMP）に比べて胃内における崩壊が緩やかに進み，原料由来のアミノ酸と結晶EAAの吸収にはほぼ同時性がみられ，飼料の消化管内滞留時間と血漿FAAの消長に相関があることが明らかとなった。また，SMPの場合にも，飼料の粘着性を高めて消化管内における崩壊を遅らせるとSDPの場合と同じ効果がみられることがわかった。これらの結果に基づき，ほぼ同一の配合組成で無魚粉のSDP，EPおよびSMPを製造し，EAAの添加効果を比較したところ，いずれの場合にも成長・増肉係数に対する添加効果が認められた。無魚粉区の飼育成績は魚粉区には及ばなかったものの，３飼料の中ではSDP区が他区よりも優れた成績を示した。このことから，無魚粉飼料の性能は製造方法や条件によって異なることが示唆された。ブリの給餌後の血漿遊離アミノ酸（FAA）濃度をみると，無魚粉飼料に添加したEAAは形態にかかわらずいずれも効率よく吸収されることがわかった。無魚粉飼料には魚粉飼料のEAA組成に合わせて結晶EAAを補足添加したが，無魚粉区の血漿FAA濃度は魚粉区よりもかなり高くなっており，特にメチオニンの濃度は著しく高く，アミノ酸インバランスを引き起こしている可能性が示唆された。したがって，不足するアミノ酸を補足する場合は，個々のアミノ酸の吸収率や血漿中のFAAバランスを考慮した添加が必要であることがわかった。

以上，本研究では，最近の逼迫した魚粉・魚油の需給状況に鑑み，代替タンパク質・代替油脂配合の新しいタイプの養魚飼料の開発を試みるとともに，養魚飼料の安定供給，品質保持，価格低減化を計るためには魚粉に依存しない飼料の開発が不可欠であると考え，そのために必要な基礎資料の集積を行った。本研究で得られた成果は，21世紀の魚類養殖における飼料開発の方向を示す有益な情報を提供するものである。