Japanese Flounder, *Paralichthys olivaceus*

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Introduction

Seedling production has been conducted with various flat-fish species for stock enhancement in the coastal zone of Japan. However, Japanese flounder (*hirame* in Japanese), *Paralichthys olivaceus* (left-eye flounder), is the only flat-fish species that has been commercially produced. Aquaculture of Japanese flounder started in the mid-1970s and commercial production became extensive in the early 1980s with the development of seedling production and farming techniques. The production has increased gradually during the past two decades, was 8583 t in 1997, nearly equal to that of the wild catch, and ranked fourth among marine cultured finfish in Japan next to yellowtail, *Seriola quinqueradiata*, red sea bream, *Pagrus major*, and coho salmon, *Oncorhynchus kisutch* (Fig. 8.1). Flounders are highly valued and their market price is two to three times higher than that of yellowtail and red sea bream. Locally available trash fish, such as sand lance or

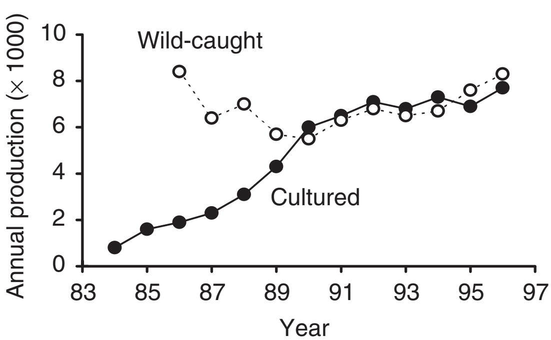

Fig. 8.1. Recent trends in the production of cultured and wild-caught Japanese flounder.

sardines, had been used for grow-out of Japanese flounder. However, the use of dry and moist pellets has increased recently due to the shortage of these trash fish. This chapter provides up-to-date information on the nutritional availability of dietary protein, lipids and carbohydrates, for grow-out of Japanese flounder. Vitamin and mineral requirements are summarized by Takeuchi (1998).

Nutrient Requirements

Protein and amino acids

Similar to other flat-fish and finfish species (Cowey *et al.*, 1970), ten amino acids – arginine, histidine, isoleucine, leucine, lysine, methionine, phenylalanine, threonine, tryptophan and valine – are essential for Japanese flounder (Kanazawa, 1990). Amino acid requirements examined with 3 g fish (Forster and Ogata, 1998) are within the range found for other fresh and marine fish species (Takeuchi, 1998). Growth improvement with dietary taurine, a free amino acid, was demonstrated and research on its biochemical function has been conducted recently (Takeuchi, 1998)

Because of the low availability of dietary carbohydrate and lipid as an energy source, growth of Japanese flounder depends highly on the protein. The optimum ratio of digestible energy to digestible crude protein for juvenile Japanese flounder is considered to be 8.6 kcal g^{-1} (Sato, 1998), which is lower than that for rainbow trout, *Oncorhynchus mykiss* (13.0) (Takeuchi *et al.*, 1978), and carp, *Cyprinus carpio* (9.7–11.6) (Takeuchi *et al.*, 1979).

With declining catches of sardines, the main source of fish-meal for aquaculture diets in Japan, alternative ingredients for dietary fish-meal have been the focus of attention for the last decade. Previous studies indicate that 20–40% of fish-meal protein can be replaced by feather meal (Kikuchi *et al.*, 1994b), 50% by defatted soybean meal (SBM) (Kikuchi *et al.*, 1994b), 20% by meat and bone-meal (MBM) (Kikuchi *et al.*, 1997), 60% by meat meal (Sato and Kikuchi, 1997) and 40% by corn (maize)-gluten meal (CGM) (Fig. 8.2; Kikuchi, 1999a) in the diet of juvenile Japanese flounder, and 20% by malt-protein flour for fingerlings

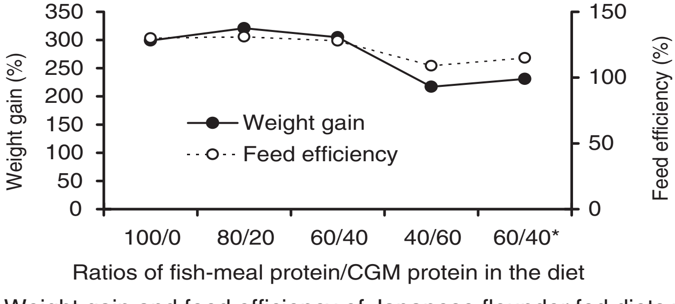

Fig. 8.2. Weight gain and feed efficiency of Japanese flounder fed diets with different ratios of fish-meal and corn (maize)-gluten meal (CGM) for 8 weeks (Kikuchi *et al.*, 1999a). *Crystalline amino acids were not supplied.

(Yamamoto *et al.*, 1995). However, most of these protein sources require supplementation of essential amino acids, such as methionine and lysine, to achieve growth comparable to that of the control diet (fish-meal as sole protein source) (Fig. 8.2).

Apparent protein digestibility of meat meal and SBM were comparable to that of fish-meal; however, that of feather meal, MBM, CGM and malt-protein flour was lower (Sato, 1998; Yamamoto *et al.*, 1998). All essential amino acids of CGM were poorly available for Japanese flounder (Yamamoto *et al.*, 1998). A combination of SBM (30% in the diet) and blood meal (10%) successfully replaced 47% of fish-meal protein in the diet without amino acid supplements (Kikuchi, 1999b). Furthermore, inclusion of blue mussel meat (5% dry matter) for an equal weight of SBM in this diet improved the growth of flounder markedly, mostly due to increased feed consumption. Stimulation of feeding with the mussel meat was demonstrated with a diet in which 3% fish-meal protein was replaced by mussel protein (Kikuchi, 1998). Blue mussel meat is an effective protein source that can replace more than 60% of fish-meal protein in the diet with incremental increases in growth and feed utilization and without supplemental amino acids (Kikuchi and Sakaguchi, 1997). Thus, a considerable amount of fish-meal protein can be replaced by several alternative protein sources in the diet of Japanese flounder, as summarized in Table 8.1. However, the results were obtained from a 6–8 week feeding trial with fish of less than 10 g initial body weight. Long-term rearing is required to determine the practical potential of these ingredients.

Lipids and fatty acids

Information on essential fatty acid (EFA) requirements of Japanese flounder is limited to those of the larval stage feeding an *Artemia* (Takeuchi, 1997). Growth

Table 8.1. Replacement levels of alternative protein sources for fish-meal in the diet of juvenile Japanese flounder.

Protein source	Replacement of dietary fish-meal (% protein)	Crude protein of test diets (%)	Amino acid supplements	Reference
Defatted soybean meal	50	49–51	Necessary	Kikuchi *et al.* (1994b)
Maize-gluten meal	40	54–57	Necessary	Kikuchi (1999a)
Silkworm pupa meal	20	45–48	Not necessary	K. Kikuchi (unpublished)
Feather meal	40	53–56	Necessary	Kikuchi *et al.* (1994a)
Meat meal	60	52–56	Not clear	Sato and Kikuchi (1997)
Meat and bone-meal	20	48–50	Not clear	Kikuchi *et al.* (1997)
Blue mussel meat	> 60	44–48	Not necessary	Kikuchi and Sakaguchi (1997)
Cuttlefish meal	100	51	Not necessary	Kikuchi *et al.* (1993)

of larval flounder was improved with an increasing dietary n-3 highly unsaturated fatty acid (HUFA) level in *Artemia* nauplii. However, no difference was found between docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA) (Furuita *et al.*, 1999). Positive effects of DHA enrichment on growth of larvae was also reported with microparticulate diets (Kanazawa *et al.*, 1994). Although arachidonic acid (AA) is considered to be a dietary essential for juvenile turbot (Takeuchi, 1997), the importance of dietary AA for Japanese flounder has not yet been clarified (Kanazawa *et al.*, 1994; Furuita *et al.*, 1998). Takeuchi (1998) recommended that dietary requirements of n-3 HUFA in Japanese flounder are 1.1–1.4% for fish larger than 20 g body weight, and 3% or more with at least 3% of DHA for larvae. Dietary supplement of EPA and DHA is believed to affect brain development of larval Japanese flounder (Furuita *et al.*, 1998).

Dietary supplementation of soybean and hen's-egg lecithin (1% in the diet) also accelerates the growth of 8 g initial body-weight flounder and the effect was superior for soybean lecithin (Kanazawa, 1993). Among phosphatidylcholine (PC), phospatidylethanolamine and phospatidylinositol fractions of soybean lecithin, only PC showed a positive effect on the growth of larval flounder (Kanazawa, 1993). Takeuchi (1998) recommended that the dietary supplement of PC should be more than 1% for larval Japanese flounder.

Availability of dietary lipids as an energy source for Japanese flounder has been examined recently. Juvenile flounder of 4–5 g initial body weight were fed 18 experimental diets with six protein levels (40, 45, 50, 55, 60 and 65%) and three lipid levels each (pollack liver oil, 10 to 28%) twice daily to apparent satiation for 8 weeks (Sato, 1998). The growth of fish depended mostly on dietary protein level and increasing level of dietary lipid did not produce positive effects at all protein levels (Fig. 8.3). The protein efficiency ratio (PER) was statistically

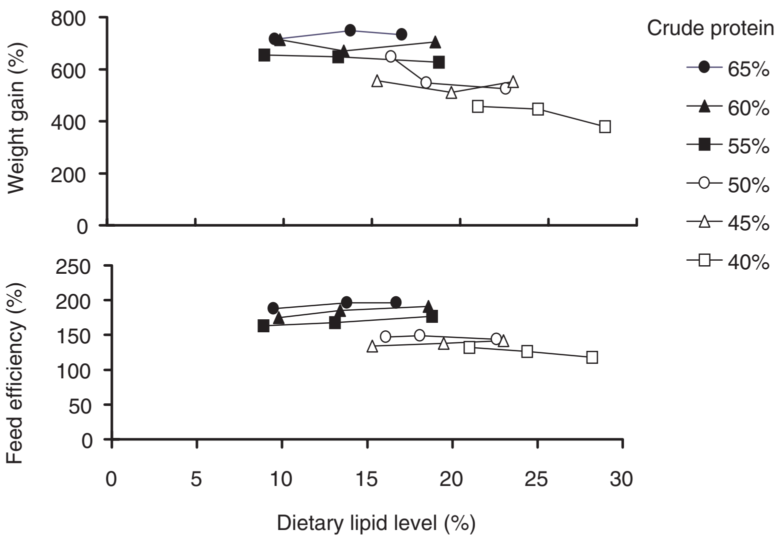

Fig. 8.3. Effect of dietary protein and lipid levels on weight gain and feed efficiency of Japanese flounder (Sato, 1998).

similar among dietary treatments regardless of dietary protein and lipid levels. Different results were obtained from more recent research in our laboratory with fish of 55 g and 245 g initial body weight. The PER of fish fed the diet containing the highest lipid level (20.3%) was significantly higher than that of fish fed the diet with the lowest lipid content (9.8%) in both feeding trials. However, a large quantity of dietary lipids may cause adverse effects on the health condition of the cultured fish by increasing the blood triglyceride level, liver weight and crude lipid content of the liver and muscle (Sato, 1998). Although more study is required to determine the optimum lipid level in the diet, based on the existing experimental findings, it is unlikely that dietary lipids serve as an effective energy source for the growth of Japanese flounder, as was shown in Atlantic salmon, *Salmo salar*, rainbow trout, *O. mykiss*, and yellowtail, *S. quinqueradiata*.

Carbohydrates

Although carbohydrate is one of the most important energy sources for domesticated farm animals, most fish species have a limited ability to utilize carbohydrates. The availability of dietary glucose, maltose, dextrin and potato starch was examined at a 26% inclusion level with juvenile Japanese flounder (Kikuchi *et al.*, 1998). Growth and feed efficiency (FE) were higher in fish fed dextrin and potato-starch diets, and tended to decrease with decreasing molecular weight of dietary carbohydrates. Dietary inclusion of glucose and maltose resulted in a marked increase of blood sugar levels immediately after feeding (Kikuchi *et al.*, 1998), as was shown in other fish species (Furuichi and Yone, 1982). The availability of dietary gelatinized (α) and raw (β) potato starch was compared. However, the difference has not been clarified. Use of carboxymethylcellulose as a binder is not recommended because it inhibited the protease activity of the flounder (Yamamoto and Akiyama, 1995).

The potential utilization of dietary carbohydrates as an energy source was examined with flounder of 3.5 g initial body weight and five diets containing different ratios of fish-meal to potato starch (Fig. 8.4) (Kikuchi *et al.*, 1998). Although the PER was not affected by dietary composition, the FE decreased with increasing potato starch in the diet. Furthermore, a significant growth reduction

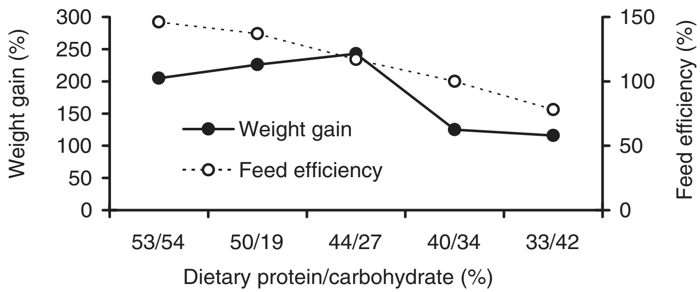

Fig. 8.4. Weight gain and feed efficiency of Japanese flounder fed diets with different ratios of protein to carbohydrate for 6 weeks (Kikuchi *et al.*, 1998).

was found in fish fed diets with less than 40% crude protein, with a concomitant decrease of protein utilization. Adverse effects of increasing carbohydrates (wheat starch) in the diet on growth and feed utilization are reported in other feeding experiments with fish of 50 g and 310–360 g initial body weight and the effect was more serious in larger fish (Kikuchi *et al.*, 1992). Based on these results, it is considered that Japanese flounder utilize dietary carbohydrates poorly as an energy source and there are no protein-sparing effects of dietary carbohydrate.

Practical Diets and Feeding Practices

Raw fish and moist pellets have been mainly used for grow-out of Japanese flounder. More than five companies produce commercial dry pellets for grow-out, although the total production is not reported. Commercial diets have protein levels ranging from 48 to 56% and lipid levels from 6 to 12% – a high-protein and low-lipid diet (Table 8.2). The protein level tends to increase and the lipid decrease with the growth of fish. Commercial diets contain 75–78% of animal protein, fish and krill meals, 12–13% of wheat and potato starch and maize powder and 10–12% of other constituents. The latter include vitamin and mineral mixtures, pollack liver oil, beer yeast and soybean lecithin. To our knowledge, plant-protein sources as alternatives to fish-meal have not been used for the commercial diet yet. Both pelleted and extruded diets are available for the culture; however, there is a strong tendency to use extruded diets.

Eight to ten pellet sizes ranging from 1 to 18 mm in diameter are used, depending on fish size. Fish are fed diets by hand one to five times a day, each to satiation, at almost all farms. Table 8.3 shows the recommended feeding rate and

Table 8.2. Proximate composition of commercial dry pellets for grow-out of Japanese flounder (%).

Producer	Crude protein*	Crude lipid*	Crude fibre	Ash
A	> 48–52	> 8–10	< 2	< 15
B	> 47–50	> 8–10	< 2.5	< 15
C	> 50–56	> 6–12	< 4	< 17

* Minimum values guaranteed.

Table 8.3. Recommended pellet size, feeding rate and frequency per day of dry pellets for the culture of Japanese flounder.

Producer	February	April	June	August	October	December	February
Temperature (°C)*	15.2	17.7	21.5	27.2	22.7	18.0	14.9
Fish size (g)*	6.4	36.0	94.0	231.0	452.0	686.0	835.0
Pellet size (mm)	2–3	4–5	5–6	8–9	10–11	12–13	16–18
Feeding rate (%)	5.8	1.3	2	1.8	1.3	0.4	0.2
Frequency	5	3	3	3	2	1–2	1–2

* Average temperature and estimated growth.

feeding frequency per day with the growth of fish. The feeding rate increases with the increase of water temperature up to 25°C and decreases thereafter. The fish grows from juvenile to 900 g in 14 months with commercial diets.

References

Cowey, C.B., Adron, J.W. and Blair, A. (1970) Studies on the nutrition of marine flatfish. The essential amino acid requirements of plaice and sole. *Journal of the Marine Biological Association of the United Kingdom* 50, 87–95.

Forster, I. and Ogata, H.Y. (1998) Lysine requirement of juvenile Japanese flounder *Paralichthys olivaceus* and juvenile red sea bream *Pagrus major*. *Aquaculture* 161, 131–142.

Furuichi, M. and Yone, Y. (1982) Availability of carbohydrate in nutrition of carp and red sea bream. *Bulletin of Japanese Society of Scientific Fisheries* 48, 945–948.

Furuita, H., Takeuchi, T. and Uematsu, K. (1998) Effect of eicosapentaenoic and docosahexaenoic acids on growth, survival and brain development of larval Japanese flounder (*Paralichthys olivaceus*). *Aquaculture* 161, 269–279.

Furuita, H., Konishi, K. and Takeuchi, T. (1999) Effect of different levels of eicosapentaenoic and docosahexaenoic acids in *Artemia* nauplii on growth, survival and salinity tolerance of larvae of the Japanese flounder, *Paralichthys olivaceus*. *Aquaculture* 170, 59–69.

Kanazawa, A. (1990) Nutrition and feed in *hirame* culture. *Suisanzoshoku* 38, 398–399 (in Japanese).

Kanazawa, A. (1993) Essential phospholipids of fish and crustaceans. In: Kaushik, S.J. and Luquet, P. (eds) *Fish Nutrition in Practice*. INRA, Paris, pp. 519–530.

Kanazawa, A., Matsumoto, H., Teshima, S., Koshio, S. and Osada, T. (1994) *Abstracts for the Meeting of the Japanese Society of Fisheries Science*, 1–4 October, Mie. 74 pp. (in Japanese).

Kikuchi, K. (1998) Blue mussels in the diet of juvenile Japanese flounder. *UJNR Technical Report* 26, 269–274.

Kikuchi, K. (1999a) Partial replacement of fish meal with corn gluten meal in diets for Japanese flounder *Paralichthys olivaceus*. *Journal of the World Aquaculture Society* 30, 357–363.

Kikuchi, K. (1999b) Use of defatted soybean meal as a substitute for fish meal in diets of Japanese flounder (*Paralichthys olivaceus*). *Aquaculture* 179, 3–11.

Kikuchi, K. and Sakaguchi, I. (1997) Blue mussel as an ingredient in the diet of juvenile Japanese flounder. *Fisheries Science* 63, 837–838.

Kikuchi, K., Honda, H. and Kiyono, M. (1992) Effect of dietary protein level on growth and body composition of Japanese flounder, *Paralichthys olivaceus*. *Suisanzoshoku* 40, 335–340 (in Japanese, with English abstract).

Kikuchi, K., Honda, H., Kiyono, M. and Miyazono, I. (1993) Total replacement of fish meal with other protein sources in the diet of Japanese flounder, *Paralichthys olivaceus*. *Suisanzoshoku* 41, 345–351.

Kikuchi, K., Furuta, T. and Honda, H. (1994a) Utilization of feather meal as a protein source in the diet of juvenile Japanese flounder. *Fisheries Science* 60, 203–306.

Kikuchi, K., Furuta, T. and Honda, H. (1994b) Utilization of soybean meal as a protein source in the diet of juvenile Japanese flounder, *Paralichthys olivaceus*. *Suisanzoshoku* 42, 601–604.

Kikuchi, K., Sato, T., Furuta, T., Sakaguchi, I. and Deguchi, Y. (1997) Utilization of meat and bone meal as a protein source in the diet of juvenile Japanese flounder. *Fisheries Science* 63, 29–32.

Kikuchi, K., Sato, T. and Deguchi, Y. (1998) Effect of dietary carbohydrates on the growth of juvenile Japanese flounder. *Suisanzoshoku* 46, 541–546 (in Japanese, with English abstract).

Sato, T. (1998) Development of formulated feeds for juvenile Japanese flounder. PhD thesis, Tokyo University of Fisheries, Tokyo, Japan (in Japanese).

Sato, T. and Kikuchi, K. (1997) Meat meal as a protein source in the diet of juvenile Japanese flounder. *Fisheries Science* 63, 877–880.

Takeuchi, T. (1997) Essential fatty acids requirements of aquatic animals with emphasis on fish larvae and fingerlings. *Reviews in Fisheries Science* 5, 1–25.

Takeuchi, T. (1998) Nutritional requirements of larval and juvenile Japanese flounder. In: *Text Book of Basic and Theoretical Course in Sea Farming – XII*. Fisheries Agency and Japan Sea Farming Association, Tokyo, pp. 1–23 (in Japanese).

Takeuchi, T., Yokoyama, M., Watanabe, T. and Ogino, C. (1978) Optimum ratio of dietary energy to protein for rainbow trout. *Bulletin of Japanese Society of Scientific Fisheries* 44, 729–732 (in Japanese, with English abstract).

Takeuchi, T., Watanabe, T. and Ogino, C. (1979) Optimum ratio of dietary energy to protein for carp. *Bulletin of Japanese Society of Scientific Fisheries* 45, 983–987 (in Japanese, with English abstract).

Yamamoto, T. and Akiyama, T. (1995) Effect of carboxymethylcellulose, α-starch and wheat gluten incorporated in diets as binders on growth, feed efficiency, and digestive energy activity of fingerling Japanese flounder. *Fisheries Science* 61, 309–313.

Yamamoto, T., Unuma, T. and Akiyama, T. (1995) Utilization of malt protein flour in fingerling Japanese flounder diets. *Bulletin of National Research Institute of Aquaculture* 24, 33–42.

Yamamoto, T., Unuma, T. and Akiyama, T. (1998) Apparent availabilities of amino acids from several protein sources for fingerling Japanese flounder. *Bulletin of National Research Institute of Aquaculture* 27, 27–35.