Effect of Dietary Starch Level and Kind on the Growth and Body Composition of Juvenile Olive Flounder *Paralichthys olivaceus*

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Abstract

A 7-week feeding trial was conducted to investigate the effect of dietary starch level and kind on the growth and body composition of juvenile olive flounder. Triplicate groups of fish (average weight: 1.5 g) were fed iso-nitrogenous (48% crude protein) and isocaloric (4.8 kcal/g diet) diets containing 15-25% α-potato starch and 15% β-potato starch. Survival was not affected by dietary starch level and kind. The weight gain of fish fed the diet containing 20% α-potato starch was significantly higher than that of fish fed the diets containing 15% and 25% α-potato starch levels. The feed efficiency and protein efficiency ratios of fish fed the diets containing 15% β-potato starch were significantly lower than those of the other groups ($P < 0.05$). The protein efficiency ratio tended to increase with increasing α-potato starch. The daily feed intake of fish fed the diet containing 15% β-potato starch was significantly higher than that of the other groups ($P < 0.05$). The hepatosomatic index, condition factor, and proximate composition of the whole body were not affected by the dietary starch level and kind. These results indicate that up to 20% α-potato starch could be incorporated into the juvenile flounder diet for optimum growth.

Key words: Olive flounder, *Paralichthys olivaceus,* Potato starch, Growth, Feed utilization

Introduction

The ability of fish to utilize carbohydrates varies among fish (Wilson, 1994; Hutchins et al., 1998). The carbohydrate utilization of most carnivorous fish is generally lower than that of herbivorous or omnivorous fish. Although no precise carbohydrate requirements of fish have been reported, providing an adequate level of carbohydrate in a fish diet may be very important to generate the least-cost formulation and to reduce catabolism of protein for energy. The maximum dietary dextrin levels that did not reduce growth were 10% for yellowtail, 20% for red sea bream, and 30% for common carp (Millikin, 1982).

Flounder (*Paralichthys olivaceus*) is one of the most important farmed marine fish in Korea. Recently, Lee et al. (2000, 2002a, 2002b) recommended a dietary protein level of 45-

50% for optimum growth and efficient protein utilization in flounder. Similarly, Lee et al. (2002b) suggested that a diet containing 46-51% protein with 6% lipid content is optimal for growth of juvenile flounder. Moreover, the suggestion has been made that dextrin may be more efficiently utilized than lipids as a source of energy by juvenile flounder (Lee et al., 2003). However, limited information exists on the use of different starch kind (raw and gelatinized starch) and levels for juvenile flounder. Thus, determining the ability of juvenile flounder to utilize low-cost dietary sources of energy such as carbohydrates could ultimately reduce the cost of flounder feed production. The present study was conducted to evaluate the effect of starch kind (raw and gelatinized) and gelatinized starch level in diets including the proper protein and lipid lev-

els on the growth and body composition of juvenile flounder.

Materials and Methods

Experimental diets

The ingredients and nutrient contents of the experimental diets are presented in Table 1. The four experimental diets were formulated to contain 15% β-potato starch (B15) and 15 -25% α-potato starch (diets A15, A20, and A25, respectively) with iso-nitrogenous (48% crude protein) and isocaloric (4.8 kcal/g diet) diets according to the result of a previous study (Lee et al., 2002b). To determine the effects of various levels of starch, the amount of gelatinized starch in the diets was increased at the expense of wheat flour. Lipid levels were maintained at 7% (Lee et al., 2002a). For preparing the experimental diets, calculated quantities of air-dried ingredients were thoroughly mixed in a Hobart electric mixer. The experimental diets were formulated using a laboratory pellet machine after 35-40 g water was mixed with a 100-g mixture of ingredients and dried at room temperature overnight. All diets were stored at -30ºC until use.

Fish and feeding trial

Juvenile flounder were obtained from a local farm (Uljin, Korea). The fish were acclimated to laboratory conditions for

2 weeks before the start of the feeding trial. Juvenile fish (initial mean weight, 1.5 ± 0.04 g) were allocated randomly into twelve 260-L cylindrical plastic tanks (180 L water volume) with 35 fish per each tank for the feeding trial after being collectively weighed. Three replicate groups of fish were hand-fed to apparent satiation three times a day (08:00, 13:00, and 17:00 for 7 days per week) for 7 weeks. Filtrated seawater was supplied at a flow rate of 4 L/min in each tank and the mean water temperature and salinity were 20.6 ± 1.50ºC and 34 ± 0.1 ppt, respectively. The photoperiod was left under natural conditions during the feeding trail. Records were kept of daily feed consumption, mortalities, and feeding behavior.

Sample collections and analytical methods

At the end of the feeding trial, all of the fish in each tank were collectively weighed after anesthetizing with tricaine methanesulfonate (MS222; Sigma, St. Louis, MO, USA) at a concentration of 100 ppm after starvation for 24 h. Blood was drawn from the caudal vessel with 1 mL heparinized syringes from three fish in each tank and transferred to microcentrifuge tubes. The collected blood was centrifuged at $3,500 \times g$ for 5 min and the plasma was separated and stored in a -75ºC freezer. Before freezing, plasma was divided into separate aliquots for analyses of glucose, total protein, and total cholesterol in the plasma. The remaining fish from each tank were used for proximate analyses. The crude protein content was determined using the Kjeldahl method with the Auto Kjeldahl System (Buchi, Flawil, Switzerland), the crude lipid content was determined by the ether-extraction method, the moisture content was determined with a dry oven (105ºC for 24 h), and the ash content was determined by muffler furnace (550ºC for 6 h). Plasma glucose, total protein, and cholesterol levels were determined using a clinical investigation commercial kit (Asan Pharmaceutical Co., Seoul, Korea).

Statistical analysis

The data were subjected to one-way analysis of variance (ANOVA) using SPSS version 10.0 (SPSS Inc., Chicago, IL, USA). Significant differences ($P < 0.05$) among the means were determined using a Duncan`s multiple range test (Duncan, 1955). The data are presented as the mean ± SE of three replicate groups.

Results and Discussion

The growth performances and feed utilizations of juvenile olive flounder fed the experimental diets for 7 weeks are shown in Table 2. The survival rates of the fish in each dietary group were 90-93% with no difference among treatments. Weight gain was affected by dietary α-starch level ($P < 0.05$). The greatest weight gain (976%) was observed in fish fed the

Table 1. Ingredients and nutrients content of the experimental diets

	Diets			
	B15	**A15**	**A20**	**A25**
Ingredients (%)				
Herring meal	64.0	64.0	64.0	64.0
β-potato starch	15.0			
α-potato starch		15.0	20.0	25.0
Wheat flour	15.0	15.0	10.0	5.0
Squid liver oil	2.0	2.0	2.0	2.0
Vitamin premix[*]	1.5	1.5	1.5	1.5
Mineral premix[*]	2.0	2.0	2.0	2.0
Choline salt	0.5	0.5	0.5	0.5
Nutrient contents (% of dry matter basis)				
Crude protein	48.5	49.9	47.4	45.6
Crude lipid	7.8	7.0	7.2	7.1
Ash	10.0	9.8	10.0	9.7
Crude fiber	1.1	1.1	1.0	0.9
Nitrogen-free extract[†]	32.6	32.2	34.4	36.7
Gross energy (kcal/g)[‡]	4.86	4.84	4.81	4.79

[*]Same as Lee et al. (2000), [†]Calculated by difference (100-crude protein-crude lipid-crude fiber-ash), [‡]Gross energy was calculated based on 5.64 kcal/g protein, 9.44 kcal/g lipid and 4.11 kcal/g nitrogen-free extract.

Table 2. Growth performance of juvenile flounder fed diets containing different starch level for 7 weeks

	Diets			
	B15	**A15**	**A20**	**A25**
Initial mean weight (g)	1.5 ± 0.02	1.5 ± 0.18	1.4 ± 0.14	1.5 ± 0.03
Survival (%)	93 ± 2.3	93 ± 5.2	90 ± 2.3	93 ± 3.4
Weight gain (%)[*]	934 ± 18.6[ab]	844 ± 50.0[a]	976 ± 34.0[b]	847 ± 37.5[a]
Feed efficiency (%)[†]	85 ± 0.5[a]	93 ± 1.7[b]	97 ± 1.3[b]	97 ± 2.1[b]
Daily feed intake (%)[‡]	3.9 ± 0.03[b]	3.5 ± 0.04[a]	3.4 ± 0.04[a]	3.4 ± 0.06[a]
Daily protein intake (%)[‡]	1.9 ± 0.01[d]	1.7 ± 0.02[c]	1.6 ± 0.02[b]	1.5 ± 0.03[a]
Protein efficiency ratio (%)[§]	1.7 ± 0.01[a]	1.9 ± 0.03[b]	2.0 ± 0.02[c]	2.1 ± 0.05[c]
Hepatosomatic index[¶]	0.9 ± 0.03	0.9 ± 0.02	1.0 ± 0.05	0.9 ± 0.06
Condition factor[**]	1.1 ± 0.05	1.1 ± 0.02	1.1 ± 0.08	1.1 ± 0.05

Values (mean ± SE, $n = 3$) in the same row not sharing a common superscript are significantly different ($P < 0.05$).
[*](Final body weight − initial body weight) × 100/initial body weight, [†]Fish wet weight gain × 100/feed intake (dry matter), [‡]Feed (or protein) intake × 100/ [(initial fish wt.+ final fish wt.+ dead fish wt.)/2 × days reared], [§]Fish wet weight gain/protein intake, [¶]Liver weight × 100/body weight, [**]Body weight × 100/ total body length[3].

diets containing 20% α-starch. Feed efficiency was affected by the dietary starch kind at the same level ($P < 0.05$). The feed efficiency and protein efficiency ratios of fish fed the diet containing 15% β-starch were lower than those of fish fed the diet containing 15-25% α-starch. The daily feed intake and protein intake of fish fed the diets containing 15% β-starch were significantly higher than those of the other groups. The hepatosomatic index and condition factor were not affected by the dietary starch level and kind. The proximate compositions of the whole bodies of juvenile olive flounder fed the experimental diets are shown in Table 3. Dietary starch level and kind had no significant effect on the moisture, crude protein, crude lipid, and ash contents of the whole body. Plasma glucose, total protein, and total cholesterol of juvenile olive flounder (Table 4) were not affected by the dietary starch level and kind ($P > 0.05$).

Minimizing feed costs for fish production in aquaculture can be achieved by determining the requirements of high-cost essential nutrients such as protein while maximizing the use of low-cost energy sources. Carbohydrates are the most cost-effective energy source for fish, although the abilities of fish to utilize carbohydrates vary among species. The use of dietary carbohydrates by fish appears to be related to their digestive systems and metabolic pathways adapted to a variety of aquatic environments (Walton and Cowey, 1982) as well as dietary carbohydrate level and complexity (Bergot, 1979; Hutchins et al., 1998; Small and Soares, 1999). Generally, herbivorous or omnivorous fish use much higher levels of carbohydrates than carnivorous coldwater fish such as marine fish and salmon (Wilson, 1994). We found that the weight gain of olive

Table 3. Proximate composition (%) of whole body of juvenile flounder at the end of feeding trial

	Diets			
	B15	**A15**	**A20**	**A25**
Moisture	75.9 ± 0.40	75.2 ± 0.26	76.0 ± 0.38	76.0 ± 0.89
Crude protein	17.5 ± 0.40	17.7 ± 0.44	17.2 ± 0.76	17.6 ± 0.83
Crude lipid	1.3 ± 0.45	2.4 ± 0.32	2.0 ± 0.43	1.9 ± 0.42
Ash	3.5 ± 0.08	3.5 ± 0.04	3.5 ± 0.08	3.7 ± 0.25

Values are mean ± SE, $n = 3$.

Table 4. Hematological changes of the plasma of juvenile flounder at the end of feeding trial

	Diets			
	B15	**A15**	**A20**	**A25**
Total protein (g/100 mL)	3.8 ± 0.23	3.5 ± 0.27	3.5 ± 0.11	3.2 ± 0.55
Glucose (mg/100 mL)	49 ± 6.2	48 ± 12.3	46 ± 4.1	47 ± 4.9
Total cholesterol (mg/100 mL)	197 ± 8.0	187 ± 7.2	176 ± 9.8	192 ± 14.5

Values are mean ± SE, $n = 3$.

flounder was not affected by carbohydrate kind at the same level, but that the feed intake of flounder fed a diet containing β-starch increased compared to that of fish fed a diet containing α-starch. This result was likely due to the low digestibility of β-starch. The feed efficiency and protein efficiency ratio of flounder fed the diet containing α-starch improved compared

to that of fish fed the diet containing β-starch. These results are similar to those reported for other fish (Peres and Oliva-Teles, 2002). The relative efficiency of the utilization of dietary carbohydrates by fish has been associated with the technological treatments applied (Bergot and Brèque, 1983) such as the gelatinized ratio of carbohydrates. For example, Jeong et al. (1992) found that rainbow trout fed a diet containing 30% starch exhibited the best overall growth performances when the gelatinized ratio reached 40%. Furthermore, Hilton and Atkinson (1982) observed that the growth performance of rainbow trout decreased when levels of starch comprised more than 14% of the diet. Conversely, other authors have reported that higher levels of dietary carbohydrates led to a significant increase in protein and energy retention (Kaushik and de Oliva-Teles, 1985; Kaushik et al., 1989; Takeuchi et al., 1990). For example, Pieper and Pfeffer (1980) concluded that gelatinized starch was an effective energy source for rainbow trout. Finally, when compared to raw starch, the incorporation of gelatinized starch has been demonstrated to have beneficial effects on the growth and feed efficiency of various fish species (Furuichi et al., 1987; Takeuchi et al., 1992; Kaushik and Médale, 1994). Thus, the technological treatment of starch deserves particular attention.

Although the carbohydrate requirements of fish have not been reported, it is the least expensive nutrient in feed formulating and can improve the physical properties of extruded and steam pelleted feeds. Therefore, many studies have been focused on optimizing formulation to determine the maximum level of dietary carbohydrates that fish can utilize efficiently. The growth, feed efficiency, and protein efficiency ratios of juvenile olive flounder fed the diet containing 20% α-starch in the present study were higher than those of fish fed the diet containing 15% α-starch. However, the growth of flounder fed the diet containing 25% α-starch decreased compared to that of fish fed the diet containing 20% α-starch, indicating that a dietary inclusion of 25% α-starch would be excessive for the growth of olive flounder. These results suggest that the inclusion of 20% α-starch is an optimal level for the growth and feed utilization of flounder. For marine fish species, reports indicate that the dietary incorporation of digestible carbohydrates should not exceed 20% (Kaushik and de Oliva-Teles, 1985; Beamish and Medland, 1986; Kaushik et al., 1989; Takeuchi et al., 1990; Hemre et al., 1995; Lanari et al., 1999). Conversely, Gouveia et al. (1995) reported that digestible carbohydrates could account for 25% of sea bass diets with no significant effects on growth performance and feed utilization efficiency. Additionally, Peres and Oliva-Teles (2002) observed that a dietary incorporation of 25% starch had no negative effects on growth or feed efficiency for European sea bass. In addition, better growth and feed utilization have been reported for rainbow trout fed a diet containing 30% dextrin compared to one that included 8% dextrin (Yamamoto et al., 2000). Finally, Lee et al. (2003) reported that a diet containing 15-25% dextrin was superior to one containing 5% dextrin for

flounder.

Providing an adequate dietary carbohydrate level is important to reduce catabolism of proteins for energy and for the synthesis of glucose, which decreases protein retention and increases nitrogen release to the environment (Suarez and Mommsen, 1987; Cowey and Walton, 1989; Wilson, 1994). The results reported herein indicate that α-starch should account for up to 20% of the diet of juvenile flounder to achieve optimal growth.

Acknowledgments

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