Fish Aqua Sci 12(3), 203-208, 2009

KOSFAS FISHERIES AND AQUATIC SCIENCES
The Korean Society of Fisheries and Aquatic Science

Effects of Sources and Levels of Dietary Carbohydrate on Growth and Body Composition of Juvenile Sea Cucumbers, *Apostichopus japonicus*

Jin Choi, Joo-Young Seo[1] and Sang-Min Lee[1]*

Marine Biology Center for Research and Education, Gangneung-Wonju National University, Gangneung 210-853, Korea

[1]Faculty of Marine Bioscience and Technology, Gangneung-Wonju National University, Gangneung 210-702, Korea

A factorial feeding trial of different levels of three carbohydrate sources (wheat flour, α-potato starch, and sodium alginate) was conducted to determine proper sources and levels of dietary carbohydrate for juvenile sea cucumbers. Three replicate groups of juvenile sea cucumbers (770 mg average weight) were fed the experimental diets once a day for 10 weeks. After feeding trial, survival of sea cucumbers was not significantly different between diets. The body weight of sea cucumbers was significantly ($P<0.01$) affected by both source and level of dietary carbohydrate, tending to decrease as dietary carbohydrate level increased. Body weight of sea cucumbers fed a 25% wheat flour diet was the highest but was not significantly different from that of sea cucumbers fed diet containing 10% wheat flour with 15% sodium alginate ($P>0.05$). No significant differences were observed in contents of moisture, crude protein, crude lipid and ash of sea cucumbers among dietary treatments. These results suggest that sea cucumbers utilize wheat flour and sodium alginate more efficiently than they do α-potato starch, and that a formulated diet containing 43-60% carbohydrate may be suitable for juvenile sea cucumber culture.

Key words: *Apostichopus japonicus*, Sea cucumber, Growth, Carbohydrate

Introduction

Appropriate levels and ratios of protein, lipid and carbohydrate are important for the development of practical animal feeds in order to reduce the catabolism of protein for energy (Suarez and Mommsen, 1987; Cowey and Walton, 1989; Wilson, 1994). Feed costs for animal production in aquaculture can be minimized by determining requirements of a given animal for high-cost essential nutrients and by maximizing the use of low-cost energy sources. Carbohydrates are relatively inexpensive and readily available energy sources for animals compared to dietary protein and lipid. However, the ability of fish to utilize carbohydrate varies among species (NRC, 1993). It may be very important to provide an adequate level of carbohydrate in the diet in order to reduce the catabolism of protein for energy which reduces protein retention and increases nitrogen effluent to the environment. However, no quantitative carbohydrate requirements for sea cucumbers have been reported to date.

The sea cucumber, *Apostichopus japonicus*, is the most commercially valuable species of echinoderm in Asia (Huiling et al., 2004). High demand for the species as a human food source in Korea and China makes it a good potential candidate for aquaculture. However, the scarcity of information regarding the dietary nutrient requirements of sea cucumbers has delayed the development of a practical and cost-efficient diet for their aquaculture. Recently, several studies (Yuan et al., 2006; Okorie et al., 2008; Seo et al., 2008; Liu et al., 2009) have been carried out to determine the requirements for dietary protein, lipid, energy and vitamin C, and to develop a practical diet for this species. Feed costs for sea cucumber production could be reduced by maximizing the dietary inclusion of carbohydrate. Thus, the aim of

*Corresponding author: smlee@gwnu.ac.kr

the present study was to investigate the effects of carbohydrate sources and levels in formulated diets on the growth and body composition of juvenile sea cucumbers.

Materials and Methods

Experimental diets

Ingredients and proximate compositions of the experimental diets are shown in Table 1. Six experimental diets were formulated to contain 25% (W25) or 40% (W40) wheat flour and 10% wheat flour with 15% (W10P15) or 30% α-potato starch (W10P30), or with 15% (W10A15) or 30% (W10A30) sodium alginate. Soybean meal was used as the protein source, and squid liver oil and soybean oil were used as lipid sources in the experimental diets. Ingredients of the experimental diets were mechanically mixed with water at a ratio of 100 g of ingredient mixture to 40 g water, pelleted using a laboratory pelleting machine, and dried at room temperature overnight. Pellets were ground using an electric grinder and stored at -30°C until used.

Experimental animals and feeding trial

Juvenile sea cucumbers (*Apostichopus japonicus*) were purchased from a local farm (Goseong, Korea). They were acclimated to experimental conditions for 2 weeks while being fed a commercial feed (17% CP, 0.3% CL) before the feeding trial. A factorial (3 carbohydrate sources×2 levels) experimental design with three replicates was employed in this study. Sea cucumbers (mean body weight, 770 mg) were randomly allocated into twenty-one 50-L rectangular plastic tanks (58×38×28 cm) in a flow-through tank system at a density of 70 juveniles per tank. Three replicate groups of sea cucumbers were fed one of the experimental diets and dried *S. thunbergii* once a day (1700 h) at a feeding rate of 5% of body weight per day for 10 weeks. Sand-filtered seawater was supplied at a flow rate of 1 L/min, and supplemental aeration was provided continuously into each tank. Seawater temperature was maintained at 20.6±2.9°C (mean±SD), and the photoperiod followed natural

Table 1. Ingredients and proximate composition of the experimental diets

	Diets						
	W25	W40	W10P15	W10P30	W10A15	W10A30	Algae powder
Ingredients (% of dry matter)							
Soybean meal	48.0	43.5	52.4	52.4	52.4	52.4	
Wheat flour	25.0	40.0	10.0	10.0	10.0	10.0	
α-potato starch	–	–	15.0	30.0	–	–	
Sodium alginate	–	–	–	–	15.0	30.0	*Sargassum thunbergii*
α-Cellulose[1]	19.6	9.3	15.0	–	15.0	–	
Squid liver oil	1.0	1.0	1.0	1.0	1.0	1.0	
Soybean oil	1.2	1.0	1.4	1.4	1.4	1.4	
Vitamin premix[2]	2.0	2.0	2.0	2.0	2.0	2.0	
Mineral premix[3]	3.0	3.0	3.0	3.0	3.0	3.0	
Choline salt	0.2	0.2	0.2	0.2	0.2	0.2	
Nutrient composition (% of dry matter)							
Dry matter	90.8	92.9	92.9	91.3	88.5	84.5	96.6
Crude protein	32.2	31.6	31.0	31.8	30.9	32.2	13.3
Crude lipid	3.1	3.1	3.1	2.9	2.8	3.1	0.1
Crude fiber	22.7	12.5	18.1	3.1	18.1	3.1	6.5
Ash	5.0	4.9	4.9	5.2	8.6	15.5	43.1
Carbohydrate[4]	59.7	60.4	61.0	60.1	57.7	49.2	43.5
Gross energy (kcal/g diet)	5.3	5.7	5.7	5.9	5.0	5.9	2.2
GE/P (kcal/g protein) ratio[5]	16.5	18.0	18.4	18.6	16.2	18.3	16.5

[1]Sigma Chemical, St. Louis, MO, USA.
[2]Vitamin premix, contained the following amount which were diluted in cellulose (g/kg mix): L-ascorbic acid, 200; DL-α-tocopheryl acetate, 20; thiamin hydrochloride, 5; riboflavin, 8; pyridoxine hydrochloride, 2; niacin, 40; Ca-D-pantothenate, 12; myo-inositol, 200; D-biotin, 0.4; folic acid, 1.5; p-aminobenzoic acid, 20; menadione, 4, retinyl acetate, chloecalciferol, 0.003; cyanocobalamin, 0.003.
[3]Mineral premix, contained the following ingredients (g/kg mix): NaCl, 7; $MgSO_4 \cdot 7H_2O$, 105; $NaH_2PO_4 \cdot 2H_2O$, 175; KH_2PO_4, 224; $CaH_4(PO_4)_2 \cdot H_2O$, 140; Ferric citrate, 17.5; Ca-lactate, 21.8; $ZnSO_4 \cdot 7H_2O$, 2.8; CuCl, 0.2; $AlCl_3 \cdot 6H_2O$, 0.11; KIO_3, 0.02; $Na_2Se_2O_3$, 0.007; $MnSO_4 \cdot H_2O$, 1.4; $CoCl_2 \cdot 6H_2O$, 0.07.
[4]Calculated, 100 − (crude protein + crude lipid + ash).
[5]Gross energy/protein ratio.

conditions during the feeding period. Each tank was cleaned by siphoning every two days. Sea cucumbers in each tank were collectively weighed every four weeks and at the end of the feeding trial, and the feeding rate was adjusted accordingly.

Sample collection and chemical analyses

One hundred juvenile sea cucumbers at the beginning and all surviving animals in each tank at the end of the feeding trials were sampled and stored at -75°C for chemical analyses. Proximate compositions of the experimental diets and whole body were determined using standard methods (AOAC, 1990). Crude protein was determined following the Kjeldahl method using an Auto Kjeldahl system (Buchi B-324/435/412, Switzerland). Crude lipid was determined by ether extraction using a Soxhlet extractor. Moisture was determined by oven drying at 105°C for 6 h. Crude fiber was determined using an automatic analyzer (Tecator, Hoganas, Sweden), and ash was determined by drying in a muffler furnace at 600°C for 4 h. Carbohydrate was calculated from the remainder. Gross energy was analyzed using an adiabatic bomb calorimeter (Parr 1356, USA).

Statistical analyses

Data were subjected to one-way ANOVA followed by Duncan's multiple range test (Duncan, 1955) at a significance level of $P<0.05$. Two-way analysis of variance (ANOVA) was conducted to test the effects of dietary carbohydrate sources and levels on the growth of sea cucumbers. The data are presented as mean ± SEM of three replications. All statistical analyses were carried out using SPSS version 12.0 (SPSS Inc., Chicago, IL, USA).

Results and Discussion

Survival rate of juvenile sea cucumbers fed on diets containing different carbohydrate sources and levels for 10 weeks is shown in Fig. 1. Sea cucumber survival ranged from 60% to 79% and was not significantly different among dietary treatments. The low survival rate may be due to the small size of the sea cucumbers (770 mg) used in the present study. Mitsunaga and Matsumura (2004) reported that the survival of groups of larger sized sea cucumbers was significantly higher than that of small sized sea cucumbers.

The mean body weight of juvenile sea cucumbers was significantly affected by both the source ($P<0.01$) and the level ($P<0.001$) of dietary carbohydrate (Fig. 2). The body weight of sea cucumbers fed the

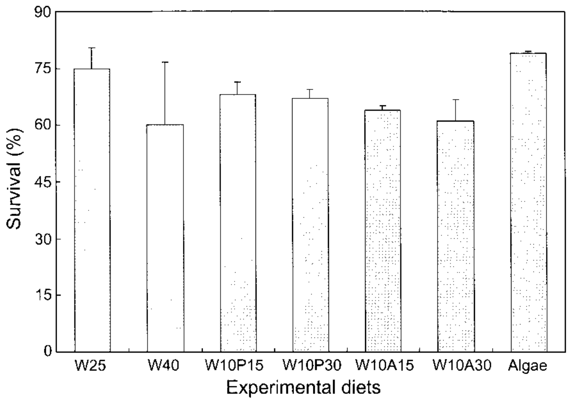

Fig. 1. Survival rate (%) of sea cucumbers fed the experimental diets for 10 weeks.

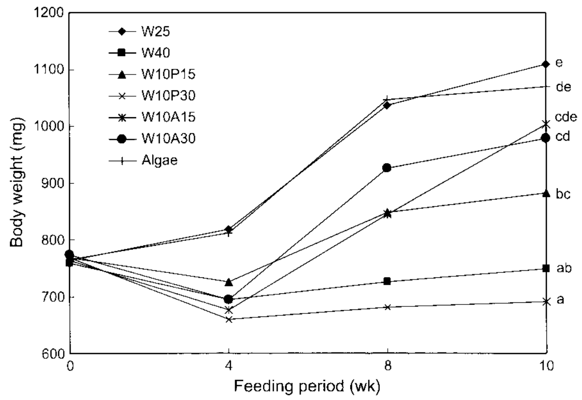

Fig. 2. Mean body weight (mg) of sea cucumber fed the experimental diets for 10 weeks. Lines having different superscripts are significantly different ($P<0.05$).

W25 diet containing 25% wheat flour was highest among the dietary treatments, but it was not significantly different from the body weight of sea cucumbers fed the W10A15 diet containing 10% wheat flour with 15% sodium alginate and dried *S. thunbergii* powder. It is notable that sea cucumbers fed on the W10A15 diet grew rapidly after 4 weeks of feeding and may have caught up with the growth of sea cucumbers fed on the algae powder if the course of feeding were prolonged. This suggests that sea cucumbers may require some time to become accustomed to the formulated W10A15 diet. Several interpretations of the differences in body weight of sea cucumbers fed on different levels and sources of dietary carbohydrate are possible. First, this might be due to the ability of sea cucumbers to digest different dietary carbohydrate sources. Wilson (1994) reported that the growth and feed utilization of fish could be affected by dietary carbohydrate sources. Differences in growth and feed utilization with differing dietary carbohydrate sources have been observed in flounder (Lee et al., 2003), channel catfish (Wilson and Poe, 1987), and tilapia (Shiau and Chuang, 1995). However, no differences in weight gain were found for abalone and snails fed on diets containing different carbohydrates (Lee et al., 1998; Lim and Lee, 2003). Species differ in their ability to utilize dietary carbohydrate, depending on their digestive and metabolic systems' adaptations to different aquatic environments (Walton and Cowey, 1982) and on the sources and levels of dietary carbohydrate (Bergot, 1979; Hutchins et al., 1998). Carbohydrate utilization in most carnivorous fish is lower than that in herbivorous or omnivorous fish (Wilson, 1994; Ruohonen et al., 2003). Second, the excessive inclusion of carbohydrate could inhibit the digestion and absorption of other nutrients from the diet and consequently poor growth in sea cucumbers. Rosenlund et al. (2004) reported that increased levels of dietary carbohydrate adversely affected feed efficiency in cod because of its lower utilization as an energy source. Studies (Hillestad and Johnsen, 1994; Aksnes, 1995; Hemre et al., 1995) have reported that the inclusion of high levels of carbohydrate in diets can reduce the digestibility of other nutrients in fish. The growth values observed in this study suggest that an appropriate carbohydrate level in formulated diets for juvenile sea cucumbers could be 43-60%. This value was higher than the optimum dietary carbohydrate level reported for abalone (Lee et al., 1998). Interestingly, in the present study, sea cucumbers fed on the W25 and W10A15 diets, containing 18-23% fiber, showed higher body weights compared to those fed on other diets with lower fiber content (3-18%), except for those fed on algae powder. This result suggests that sea cucumber may have cellulase activity or cellulolytic microflora in the gut. Obrietan et al. (1991) reported that echinoderm species have cellulase activity in gut tissues. In contrast, dietary fiber is not hydrolyzed by fish (Hung et al., 1989), and high fiber levels may reduce the utilization of other nutrients (Anderson et al., 1984). Other studies have shown a negative correlation between dietary cellulose level and growth and/or nutrient digestibility in fish (Hilton et al., 1983; Fynn-Aikins et al., 1992; Lee, 2002). Finally, the final body weight of sea cucumbers in the present study was significantly correlated with the energy/protein ratio of the experimental diet ($y=-116.6x+2966.5$, $r=0.71$) (Fig. 3). The energy/protein ratios (16.2-16.5 kcal/g protein) of the W25 and W10A15 diets and the algae powder were relatively low compared to those (18.0-18.6 kcal/g protein) of

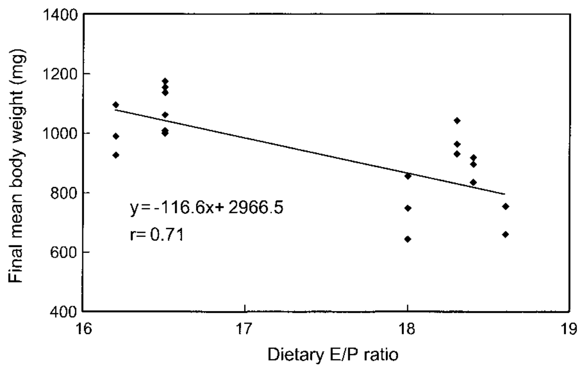

Fig. 3. Relationship between dietary E/P ratio and final body weight of sea cucumber fed the experimental diets for 10 weeks.

the other diets. Yuan et al. (2006) and Liu et al. (2009) reported that energy deposition in sea cucumbers was low compared to that of other echinoderms and that energy loss through feces and respiration accounted for the majority of ingested energy. Lovell (1989) reported that feeding animals on diets containing excessive energy relative to protein levels led to a reduction of feed consumption and poor growth due to the lack of necessary nutrients for growth. The better growth of sea cucumbers fed on algae powder and on the W25 and W10A15 diets may be due to the balance of nutrients in *S. thunbergi* and in the diets. Considering these results, it is suggested that diets containing 25% wheat flour or 10% wheat flour with 15% sodium alginate could be suitable for juvenile sea cucumbers.

The proximate compositions of the whole body of sea cucumbers fed the experimental diets are shown in Table 2. No significant differences were observed in moisture (91.2-91.8%), crude protein (3.2-3.5%), lipid (0.13-0.29%), or ash (3.4-3.8%) contents.

Many factors, including dietary nutrients and energy (Yuan et al., 2006; Okorie et al., 2008; Seo et al., 2008) and water temperature (Dong et al., 2006; An et al., 2007), could affect the growth of sea cucumbers. However, under the conditions of this study, it is suggested that juvenile sea cucumbers utilized wheat flour and sodium alginate more efficiently than they did α-potato starch, and a formulated diet containing 43-60% carbohydrate is recommended for juvenile sea cucumbers.

Acknowledgements

This work was supported by the funds of the Ministry for Food, Agriculture, Forestry and Fisheries in Korea.

Table 2. Proximate compositions of whole body in juvenile sea cucumber fed the diets containing different carbohydrate source and level for 10 weeks. Values (means ± SEM of three replications) in the same column not sharing a common superscript are significantly different (P<0.05)

Diets	Moisture (%)	Crude protein (%)	Crude lipid (%)	Ash (%)
Initial	92.3	3.1	0.3	3.6
W25	91.4 ± 0.09	3.4 ± 0.06	0.27 ± 0.023	3.5 ± 0.07[ab]
W40	91.2 ± 0.18	3.5 ± 0.07	0.23 ± 0.128	3.7 ± 0.09[ab]
W10P15	91.5 ± 0.15	3.5 ± 0.14	0.29 ± 0.119	3.5 ± 0.01[ab]
W10P30	91.5 ± 0.16	3.4 ± 0.01	0.20 ± 0.128	3.8 ± 0.04[b]
W10A15	91.2 ± 0.05	3.3 ± 0.21	0.20 ± 0.068	3.4 ± 0.07[a]
W10A30	91.7 ± 0.12	3.2 ± 0.04	0.13 ± 0.062	3.4 ± 0.11[a]
Algae	91.8 ± 0.07	3.5 ± 0.16	0.20 ± 0.052	3.6 ± 0.15[ab]
Two-way ANOVA				
CHO source	P < 0.3	P < 0.2	P < 0.7	P < 0.02
CHO level	P < 0.5	P < 0.9	P < 0.5	P < 0.08
Interaction	P < 0.1	P < 0.4	P < 0.9	P < 0.2

References

Aksnes, A. 1995. Growth feed efficiency and quality of salmon (*Salmo salar*) given feeds with different ratios of carbohydrate and protein. Aquacult. Nutr., 1, 241-248.

An, Z., Y. Dong and S. Dong. 2007. Temperature effects on growth-ration relationships of juvenile sea cucumber *Apostichopus japonicus* (Selenka). Aquaculture, 272, 644-648.

Anderson, J.S., A.J. Jackson, A.K. Matty and B.S. Capper. 1984. Effects of dietary carbohydrate and fiber on the tilapia, (*Oreochromis niloticus*). Aquaculture, 37, 303-314.

AOAC (Association of Official Analytical Chemists). 1990. Official Methods of Analysis. 15th ed. Association of Official Analytical Chemists, Arlingon, Virginia, 1298.

Bergot, F. 1979. Carbohydrate in rainbow trout diets: effects of the level and source of carbohydrate and the number of meals on growth and body composition. Aquaculture, 18, 157-167.

Cowey, C.B. and M.J. Walton. 1989. Intermediary metabolism. In: Fish Nutrition. 2nd. ed. Halver, J.E., ed. Academic Press, New York, U.S.A, 259-329.

Dong, Y., S. Dong, X. Tian, F. Wang and M. Zhang. 2006. Effects of diel temperature fluctuation and proximate body composition in the sea cucumber *Apostichopus japonicus* Selenka. Aquaculture, 514, 514-521.

Duncan, D.B. 1955. Multiple-range and multiple *F tests*. Biometrics, 11, 1-42.

Fyun-Aikins, K., S.S.O. Hung, W. Liu and H. Li. 1992. Growth, lipogenesis and liver composition of juvenile white sturgeon fed different levels of D-glucose. Aquaculture, 105, 61-72.

Hemre, G.I., K.Ø. Lie, O. Torrissen and R. Waagbo. 1995. Carbohydrate nutrition in Atlantic salmon, I. Growth and feed utilization. Aquacult. Res., 26, 149-154.

Hillestad, M. and F.T. Johnsen. 1994. High-energy/low-protein diets for Atlantic salmon, effects on growth nutrient retention and slaughter quality. Aquaculture, 124, 109-116.

Hilton, J.W., J.I. Atkinson and S.J. Slinger. 1983. Effect of increased dietary fiber on the growth of rainbow trout (*Salmo gairdneri*). Can. J. Fish. Aquat. Sci., 40, 81-85.

Huiling, S., L. Mengqing, Y. Jingping and C. Bijuan. 2004. Nutrient requirements and growth of the sea cucumber, *Apostichopus japonicus*. In: Advances in Sea Cucumber Aquaculture and Management. Lovatelli, A., C. Conand, S. Purcell, S. Uthicke, J.F. Hamel and A. Mercier, eds. FAO, Rome, Italy, 327-331.

Hung, S.S.O., K.F. Ftun-Alikins, P.B. Lutes and R.P. Xu. 1989. Ability of juvenile white sturgeon (*Acipenser transmontanus*) to utilize different carbohydrate source. J. Nutr., 119, 727-733.

Hutchins, C.G., S.D. Rawles and D.M. Gatlin III. 1998. Effects of dietary carbohydrate kind and level on growth, body composition and glycemic response of juvenile sunshine bass (*Moronechrysops* ♀ × *M. saxatilis* ♂). Aquaculture, 161, 187-199.

Lee, S.M. 2002. Apparent digestibility coefficients of various feed ingredients for juvenile and grower rockfish (*Sebastes schlegeli*). Aquaculture, 207, 79-95.

Lee, S.M., K.D. Kim and S.P. Lall. 2003. Utilization of glucose, maltose, dextrin and cellulose by juvenile flounder (*Paralichthys olivaceus*). Aquaculture, 221, 427-438.

Lee, S.M., S.J. Yun, K.S. Min and S.K. Yoo. 1998. Evaluation of dietary carbohydrate sources for juvenile abalone (*Haliotis discus hannai*). J. Aquacult., 11, 133-140.

Lim, T.J. and S.M. Lee. 2003. Effect of different dietary carbohydrate sources on growth and body composition of juvenile snail (*Semisulcospira gottschei*). J. Aquacult., 16, 187-189.

Liu, Y., S. Dong, X. Tian, F. Wang and Q. Gao. 2009. Effects of dietary sea mud and yellow soil on growth and energy budget of the sea cucumber *Apostichopus japonicus* (Selenka). Aquaculture, 286, 266-270.

Lovell, R.T. 1989. Nutrition and Feeding of Fish. Van Nostrand Reinhold, New York, USA.

Mitsunaga, N. and Y. Matsumura. 2004. Growth and survival of hatchery juveniles of sea cucumber *Apostichopus japonicus* in different release size. Bull. Nagasaki Prefect. Inst. Fish., 30, 7-13.

NRC (National Research Council). 1993. Nutrient Requirements of Fish. National Academy Press, Washington, U.S.A.

Obrietan, K., M. Drinkwine and D.C. Williams. 1991. Amylase, cellulase and protease activities in surface and gut tissues of *Dendraster excentricus*, *Pisaster ochraceus* and *Strongylocentrotus droebachiensis* (Echinodermata). Mar. Bio., 109, 53-57.

Okorie, O.E., S.H. Ko, S. Go, S.H. Lee, J.Y. Bae, K.M. Han and S.C. Bai. 2008. Preliminary study of the optimum dietary ascorbic acid level in sea cucumber, *Apostichopus japonicus* (Selenka). J. World Aquacult. Soc., 39, 758-765.

Rosenlund, G., Ø. Karlsen, K. Tveit, A. Mangor-Jensen and G.I. Hemre. 2004. Effect of feed composition and feeding frequency on growth feed utilization and nutrient retention in juvenile Atlantic cod *Gadus morhua* L. Aquacult. Nutr., 10, 371-378.

Ruohonen, K., J. Koskela, J. Vielma and J. Kettunen. 2003. Optimal diet composition for European whitefish (*Coregonus lavaretus*), analysis of growth and nutrient utilization in mixture model trials. Aquaculture, 225, 27-39.

Seo, J.Y., J. Choi, G.U, Kim, S.S. Cho, H.G. Park and S.M. Lee. 2008. Effects of dietary protein and lipid levels on growth and body composition of juvenile sea cucumber *Stichopus japonicus*. J. Aquacult., 21, 19-25.

Shiau, S.Y. and J.C. Chuang. 1995. Utilization of disaccharides by juvenile tilapia (*Oreochromis niloticus* × *O. aureus*). Aquaculture, 133, 249-256.

Suarez, P.K. and T.P. Mommsen. 1987. Gluconeogenesis in teleost fishes. Can. J. Zool., 65, 1869-1882.

Walton, M.J. and C.B. Cowey. 1982. Aspects of intermediary metabolism in fish. Comp. Biochem. Physiol., 73B, 59-79.

Wilson, R.P. 1994. Utilization of dietary carbohydrate by fish. Aquaculture, 124, 67-80.

Wilson, R.P. and W.E. Poe. 1987. Apparent inability of channel catfish to utilize dietary mono- and disaccharides as energy source. J. Nutr., 117, 280-285.

Yuan, X., H. Yang, Y. Zhou, Y. Mao, T. Zhang and Y. Liu. 2006. The influence of diets containing dried bivalve feces and/or powdered algae on growth and energy distribution in sea cucumber *Apostichopus japonicus* (Selenka) (Echinodermata: Holothuroidea). Aquaculture, 256, 457-467.

(Received 17 June 2009; Revised 1 September 2009; Accepted 16 September 2009)