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Influences of Dietary Lipid Source on the Growth and Fatty Acid Composition of Juvenile Sea Cucumber *Apostichopus japonicus*

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A study was conducted to investigate the effects of dietary lipid sources on the growth and fatty acid composition of juvenile sea cucumber. For 12 weeks, three replicate groups of sea cucumber (average weight 1.4 g) were fed one of three diets, containing squid liver oil (SLO), soybean oil (SO), or linseed oil (LO) as a dietary lipid source, or a control diet (CON) without added lipids. Sea cucumber survival was not significantly different among dietary treatments ($P>0.05$). The highest weight gain was observed in sea cucumber fed the SLO diet, whereas the weight gain of sea cucumber fed the SO diet was the lowest among dietary treatments ($P<0.05$). No significant differences were found in the moisture, crude protein, crude lipid, and ash contents of whole sea cucumber body among dietary treatments ($P>0.05$). Concentrations of 20:5n-3 and 22:6n-3 were significantly higher in sea cucumber fed the SLO diet than in those fed on the other diets. The highest 18:2n-6 and 18:3n-3 contents were observed in sea cucumber fed the SO and LO diets, respectively. The results of this study suggest that squid liver oil could be used as a good lipid source in formulated diets for juvenile sea cucumber.

Key words: *Apostichopus japonicus*, Sea cucumber, Lipid source, Growth, Fatty acid composition

Introduction

Lipid is an important nutrient, providing energy, essential fatty acids (EFA), and fat-soluble nutrients for the normal growth of fish. Providing adequate amounts of EFA is necessary for the normal growth and survival of animals, especially during juvenile stages (Sargent et al., 1999), because it plays important roles in the fluidity, permeability, and enzyme activity of membrane (Stubbs and Smith, 1984). The EFA requirements of aquatic animals are largely affected by species, water temperature, and salinity, and are different from those of terrestrial animals (Castell, 1979; Cowey and Sargent, 1979).

The sea cucumber *Apostichopus japonicus* is an important fishery resource in Korea, China, and Japan (Sloan, 1984). Market demand for the species has increased with increasing awareness of sea cucumber as a healthy food for humans. However, supplies of sea cucumber obtained from the wild have declined due to over-exploitation and pollution (Conand, 2004; Uthicke, 2004). Therefore, it is necessary to increase production and population of the species through aquaculture. Recently, several studies have examined the sea cucumber's requirements for nutrients such as proteins, lipids, carbohydrates, and ascorbic acid (Okorie et al., 2008; Seo et al., 2008; Choi et al., 2009). Feed ingredient studies (Yuan et al., 2006; Liu et al., 2009) have also been carried out to develop a practical diet for efficient sea cucumber culture. However, no information concerning the influence of dietary EFA source on sea cucumber is available. Thus, this study was conducted to evaluate the effects of dietary lipid sources on the growth and fatty acid composition of juvenile sea cucumber.

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Materials and Methods

Diet preparation

Fatty acid composition of the lipid sources used in the experimental diets is shown in Table 1. Table 1. Fatty acid composition (% of total fatty acids) of the dietary lipid sources

Fatty acids	Dietary lipid sources		
	Soybean oil	Linseed oil	Squid liver oil
C14:0			6.4
C15:0			0.6
C15:1			0.2
C16:0	11.4	5.2	22.3
C16:1			0.2
C17:0			0.5
C18:0			1.0
C18:1n-9	30.3	23.0	31.6
C18:2n-6	51.4	17.6	0.3
C18:3n-3	0.8	54.1	1.8
C18:3n-6		0.2	0.2
C20:0	0.4		0.4
C20:1	0.5		0.1
C20:2			2.0
C20:3n-3			3.5
C20:3n-6			0.2
C20:4n-6			0.3
C20:5n-3			14.3
C21:0	5.1		0.1
C22:1			0.4
C22:2			1.1
C22:6n-3			11.9
C23:0			0.8
n-3HUFA[1]			29.7

[1]Highly unsaturated fatty acid (C≥20).

Three diets containing 2% squid liver oil (SLO), soybean oil (SO), or linseed oil (LO) and a control diet (CON) without added lipids were prepared (Table 2). Soybean meal and wheat flour were used as the main protein and carbohydrate sources, respectively. The experimental diets were formulated using a moist pellet machine, and were ground to a powder (180 mesh) after being dried overnight at 60°C. All diets were stored at -30°C until use. The fatty acid composition of the experimental diets is shown in Table 3.

Experimental animals and feeding trials

Juvenile sea cucumber (*Apostichopus japonicus*) were purchased from a local sea cucumber farm (Goseong, Korea). They were acclimated to experimental conditions for 2 weeks before the feeding trial commenced. Sea cucumber (average weight, 1.4± 0.02 g) were randomly distributed into 50 L rectangular plastic tanks, each containing 40 L of water, at a density of 50 sea cucumber per tank. Three replicate groups of sea cucumber were fed one of the four experimental diets at a rate of 5% of sea cucumber body weight once a day (17:00 h) for 12 weeks. Filtered seawater was supplied at a flow rate of 1 L/min, and aeration was provided continuously

Table 2. Ingredients and proximate composition (%) of the experimental diets

	Diets			
	CON	SO	LO	SLO
Ingredients (%)				
Soybean meal	40.0	40.0	40.0	40.0
Wheat flour	44.8	42.8	42.8	42.8
Soybean oil		2.0		
Linseed oil[1]			2.0	
Squid liver oil[2]				2.0
Sargassum thunbergii	10.0	10.0	10.0	10.0
Vitamin premix[3]	2.0	2.0	2.0	2.0
Mineral premix[4]	3.0	3.0	3.0	3.0
Choline salt (50%)	0.2	0.2	0.2	0.2
Proximate composition (%, dry matter basis)				
Crude protein	31.0	29.6	30.1	30.1
Crude lipid	1.6	3.8	3.6	3.6
Ash	9.1	9.6	9.6	9.1
Crude fiber	4.0	4.2	4.1	3.4
N-free extract[5]	54.3	52.8	52.7	53.8
Gross energy (kcal/ g diet)	4.2	4.3	4.3	4.3

[1,2]Provided by E-wha Oil & Fat Ind. Co., Busan, Korea.

[3]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, 1.5; chloecalciferol, 0.003; cyanocobalamin, 0.003.

[4]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.

[5]Calculated by difference (100-crude protein+crude lipid+ash+crude fiber).

into each tank. Water temperature was maintained at 14.7±1.2°C (mean±SD), and the photoperiod followed natural conditions during the feeding trial. Each tank was cleaned by siphoning every two days.

Sample collection and chemical analysis

One hundred sea cucumber at the beginning and all surviving sea cucumber at the end of the feeding trial were sampled and stored at -75°C for chemical analysis. The proximate composition of the sea cucumber was determined using standard methods (AOAC, 1990). Crude protein (N×6.25) was determined by the Kjeldahl method using an Auto Kjeldahl system (Buchi, Flawil, Switzerland). Crude lipid was determined by the ether-extraction method.

Table 3. Fatty acid composition (% of total fatty acids) of the experimental diets

Fatty acid	Diets			
	CON	SO	LO	SLO
C14:0	0.9	0.6	0.3	4.8
C16:0	60.1	42.9	22.4	41.0
C16:1	0.4	0.2	0.5	2.4
C18:0	8.9	9.0	5.6	6.9
C18:1n-9	9.8	19.1	19.4	19.7
C18:2n-6	10.1	18.5	30.9	14.7
C18:3n-3	0.7	1.3	16.8	1.2
C20:1	4.8	3.0	1.3	1.9
C20:2	0.7	1.4	0.7	1.3
C20:4n-6	1.2	1.5	0.3	1.1
C20:5n-3	0.0	0.0	0.0	1.7
C22:0	1.1	0.9	0.4	0.5
C22:2	1.5	1.4	1.4	1.0
C22:6n-3	0.0	0.0	0.0	1.8
n-3 HUFA[1]	0.0	0.0	0.0	3.5

[1]Highly unsaturated fatty acid (C≥20).

Moisture was determined by oven drying at 105°C for 6 hours. Crude fiber was determined using an automatic fiber analyzer (Velp Fiwe6, Milano, Italy) and ash was determined using a furnace muffler at 600°C for 4 hours. The nitrogen-free extract (NFE) was calculated from the difference. Gross energy was analyzed using an adiabatic bomb calorimeter (Parr 1356, Moline, IL, USA). Lipids for fatty acid analysis were extracted by the method of Folch et al. (1957), and fatty acid methylesters were prepared by trans-esterification with 14% BF_3-MeOH (Sigma, St Louis, MO, USA). Fatty acid methyl esters were separated and quantified using a gas chromatograph (HP-6890N, Hewlett-Packard, Palo Alto, CA, USA) with a flame ionization detector, equipped with a SP™-2560 capillary column (100 m × 0.25 mm i.d., film thickness 0.20 μm, Supelco, Bellefonte, PA, USA). Injector and detector temperatures were maintained at 260°C. The column temperature was programmed from 140°C to 240°C at a rate of 4°C min^{-1}. Helium was used as the carrier gas. Fatty acids were identified by comparison with the retention times of standard fatty acid methyl esters (PUFA 37 component FAME Mix, Supelco).

Statistical analysis

Data were subjected to one-way ANOVA to test the effects of dietary lipid source. When significant ($P<0.05$) differences were detected, Duncan's multiple range test was used to rank the groups. The data are presented as mean ± SE of the three replicate groups. All statistical analyses were carried out using SPSS version 12.0 (SPSS, Chicago, IL, USA).

Results and Discussion

The growth performance of juvenile sea cucumber fed for 12 weeks on the experimental diets containing various lipid sources is shown in Table 4. Sea cucumber survival ranged from 74% to 83% with no significant differences among dietary treatments ($P>0.05$). The weight gain of sea cucumber was significantly affected by dietary lipid source ($P<0.05$). The highest weight gain was observed in sea cucumber fed the SLO diet, whereas the weight gain of sea cucumber fed the SO diet was the lowest ($P<0.05$). The growth results indicate that juvenile sea cucumber requires n-3 highly unsaturated fatty acids (n-3 HUFA) such as 20:5n-3 and 22:6n-3 for normal growth. Interestingly, sea cucumber fed the SO and LO diets exhibited lower weight gain than did those fed the CON diet without added lipids. This was thought to be associated with the low availability of plant lipids to sea cucumber and may indicate that it is not necessary to add 18:2n-6 and 18:3n-3 to sea cucumber feed.

Several studies have reported that dietary n-3 HUFA is essential for the normal growth of invertebrates (Lee et al., 2002) and marine fish species such as the flounder *Paralichthys olivaceus* (Kim et al., 2002). Type and level of essential fatty acids required for normal growth differ among fish species. Whereas freshwater species require 18:2n-6 and/or 18:3n-3 (Castell et al., 1972a, b; Kanazawa et al., 1980; Takeuchi, 1996), marine species generally require n-3 HUFA such as 20:5n-3 and 22:6n-3 for normal growth and development (Van Ballaer et al., 1985; Izquierdo et al., 1989; Kim et al., 2002). Because marine oils, such as the squid liver oil, which was found to increase growth rates in this study, contain a high proportion of n-3 HUFA and an adequate 20:5n-3/22:6n-3 ratio (Kalogero-poulos et al., 1992), this could be used as a good dietary lipid source to satisfy the 20:5n-3 and 22:6n-3 requirements of juvenile sea cucumber.

Weight gains recorded in this study were lower than those reported from other nutritional studies concerning sea cucumber (Yang et al., 2005; Dong et al., 2006; Zhou et al., 2006; An et al., 2007; Okorie et al., 2008). This may be related to the presence of anti-nutritional factors and/or deficiency in essential amino acid such as methionine in dietary soybean meal (Rumsey et al., 1994; Anderson and Wolf, 1995). In addition, remarkable size variations among individuals in each tank were observed after the feeding trial. This phenomenon may be another possible reason for the low mean growth rate of the

Table 4. Growth performance and survival of juvenile sea cucumber fed the diets containing different lipid

sources for 12 weeks[1]

	Diets			
	CON	SO	LO	SLO
Initial mean weight (g/ sea cucumber)	1.4 ± 0.01	1.4 ± 0.01	1.4 ± 0.01	1.4 ± 0.01
Survival (%)	74 ± 5.0	77 ± 1.8	83 ± 1.8	81 ± 2.4
Weight gain (%)[2]	23.6 ± 6.5^{b}	3.8 ± 1.4^{a}	14.1 ± 1.1^{ab}	45.2 ± 5.0^{c}

[1]Values (mean ± SE of triplicate groups) in each row with different superscripts are significantly different ($P<0.05$).
[2]Weight gain (%) = (final body weight – initial body weight) × 100 / initial body weight.

Table 5. Proximate compositions (%) of whole body in sea cucumber fed the diets containing different lipid sources for 12 weeks

	Initial	Diets			
		CON	SO	LO	SLO
Moisture	92.3	90.7 ± 0.28	90.1 ± 0.19	91.0 ± 0.75	91.1 ± 0.14
Crude protein	3.1	4.0 ± 0.18	3.8 ± 0.14	3.7 ± 0.07	3.7 ± 0.06
Crude lipid	0.3	0.6 ± 0.50	0.2 ± 0.13	0.4 ± 0.03	0.2 ± 0.10
Ash	3.6	3.5 ± 0.12	3.5 ± 0.03	3.5 ± 0.03	3.4 ± 0.04

Table 6. Fatty acid composition (% of total fatty acids) of whole body in sea cucumber fed the diets containing different lipid sources for 12 weeks[1]

Fatty acids	Initial	Diets			
		CON	SO	LO	SLO
C14:0	1.1	1.3 ± 0.02^{b}	1.0 ± 0.03^{a}	1.0 ± 0.03^{a}	1.0 ± 0.06^{a}
C14:1	0.3	0.3 ± 0.14	0.2 ± 0.02	0.2 ± 0.02	0.2 ± 0.12
C16:0	4.5	5.2 ± 0.35^{c}	3.3 ± 0.05^{a}	3.7 ± 0.21^{ab}	4.8 ± 0.57^{bc}
C16:1	3.0	3.0 ± 0.19^{b}	2.6 ± 0.19^{a}	3.2 ± 0.05^{bc}	3.6 ± 0.05^{c}
C18:0	5.5	4.1 ± 0.20^{b}	3.5 ± 0.06^{a}	3.6 ± 0.04^{a}	4.4 ± 0.09^{b}
C18:1n-9	16.2	14.5 ± 0.22^{a}	15.7 ± 0.26^{b}	16.6 ± 0.35^{b}	20.2 ± 0.36^{c}
C18:2n-6	7.3	28.8 ± 0.98^{c}	30.2 ± 0.52^{c}	25.5 ± 0.62^{b}	22.2 ± 0.23^{a}
C18:3n-3	-	1.1 ± 0.08^{a}	1.3 ± 0.05^{a}	6.0 ± 0.66^{b}	0.7 ± 0.35^{a}
C20:0	11.2	9.9 ± 0.49	9.7 ± 0.35	9.2 ± 0.54	9.4 ± 0.15
C20:2	3.8	9.6 ± 0.27^{c}	9.4 ± 0.15^{c}	8.4 ± 0.33^{b}	7.6 ± 0.14^{a}
C20:4n-6	24.2	15.9 ± 1.14^{b}	14.4 ± 0.81^{b}	13.7 ± 0.23^{b}	9.9 ± 0.43^{a}
C20:5n-3	15.4	6.5 ± 0.09^{a}	7.0 ± 0.36^{a}	6.8 ± 0.09^{a}	11.6 ± 0.19^{b}
C22:6n-3	7.4	2.8 ± 0.91^{a}	1.8 ± 0.21^{a}	2.1 ± 0.51^{a}	4.5 ± 0.02^{b}
n-3 HUFA[2]	22.9	9.3 ± 0.98^{a}	8.7 ± 0.15^{a}	8.9 ± 0.44^{a}	16.1 ± 0.17^{b}

[1]Values (mean ± SE of triplicate groups) in each row with different superscripts are significantly different ($P<0.05$).
[2]Highly unsaturated fatty acid (C≥20).

sea cucumber. Similar phenomena have been observed in sea cucumber aquaculture facilities and in our previous studies (Seo et al., 2008; 2009). Although the exact reasons for the observed size variations are not known, they are thought to be related to natural characteristics of the sea cucumber, variations in environmental conditions, etc.

Proximate and fatty acid compositions of the sea cucumber whole body are presented in Tables 5 and 6, respectively. No significant differences were observed in the contents of moisture, crude protein, crude lipid, and ash ($P>0.05$). It is well established that the fatty acid composition of animals reflects the fatty acid composition of their dietary lipid sources (Bell et al., 1994; Sargent et al., 1995; Lee et al., 2002; Seo et al., 2008). In the present study, 18:1n-9, 18:2n-6, and 20:4n-6 were found to be abundant fatty acids in sea cucumber, and the highest 18:2n-6, 18:3n-3, and n-3 HUFA contents were observed in juvenile sea cucumber that were fed the SO, LO, and SLO diets, respectively. In addition, the 18:1n-9 contents of sea cucumber fed the SO, LO, and SLO diets, the 18:3n-3 contents of those fed the LO treatment, and the n-3 HUFA contents of those fed the SLO treatment were significantly higher than those of sea cucumber fed the control diet ($P<0.05$).

These findings indicate that fatty acid com-positions of sea cucumber varied with variations in fatty acid compositions of their dietary lipid sources, which is in accordance with previous studies of sea cucumber (Seo et al., 2008), snail (Lee et al., 2002), and flounder (Kim et al., 2002). The lower n-3 HUFA contents of sea cucumber fed on the LO diet compared with the SLO diet may be due to the very limited capacity of sea cucumber to convert 18:3n-3 to 20:5n-3 or 22:6n-3. Similar findings were reported for marine fish such as turbot (Owen et al., 1975). Although, all diets had low 20:4n-6 levels (0.3% to 1.5%), the relatively high 20:4n-6 levels (9.9% to 15.9%) in sea cucumber at the end of the feeding trial indicate that sea cucumber may have the capacity to elongate or desaturate 20:4n-6 from shorter poly-unsaturated fatty acids.

Based on the results of this study, it is concluded that squid liver oil could be used as a good source of lipid in formulated diets for juvenile sea cucumber.

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