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Effects of Dietary Lipid Sources on Growth and Body Composition of Snail (*Semisulcospira gottschei*)

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This study was conducted to investigate the effects of dietary lipid sources on survival, growth and body composition of snail (*Semisulcospira gottschei*). Three replicate groups of snail (average weighing 152 mg) were fed the diets containing different lipid sources such as lauric acid (LA), squid liver oil (SO), linseed oil (LO), corn oil (CO), SO+LO, SO+CO, LO+CO and SO+LO+CO for 8 weeks. Survival was not affected by dietary lipid sources ($P>0.05$). Weight gain of snail fed the SO, SO+LO and SO+LO+CO diets was significantly higher than that of snail fed the LA and LO diets ($P<0.05$), and the lowest weight gain was observed in snail fed the LA diet ($P<0.05$). No significant difference was found in crude lipid content of edible portion in snail fed the different diets ($P>0.05$). Contents of 12:0, 18:2n-6, 18:3n-3 and 20:5n-3 from snail fed the LA, CO, LO and SO diets were higher than those from snail fed the other diets, respectively ($P<0.05$). The highest 22:6n-3 content was observed in snail fed the SO+LO but was not significantly different from that of snail fed the SO, SO+CO and SO+LO+CO diets ($P>0.05$). The n-6 highly unsaturated fatty acids such as 20:4n-6 and 22:4n-6 contents of snail were not affected by dietary lipid sources ($P>0.05$). These results suggested that squid liver oil and mixture of squid liver oil and linseed and/or corn oil are good dietary lipid sources for the normal growth of snail. However lauric acid may not be a good lipid source for snail diet.

Key words: Dietary lipid, Fatty acid, Snail, *Semisulcospira gottchei*

Introduction

The freshwater snail genus *Semisulcospira* is widespread in Korea, Japan, Taiwan and China (Davis, 1969). Snail (*Semisulcospira gottschei*) has been used as ingredient of traditional soup and nutritional food in Korean (Kim et al., 1985; Shim et al., 1994). Demand of this species is currently increasing for health food, however snail production from the natural is decreasing. Consequently, snail aquaculture is necessary to increase the production of this species and population resources. In addition, it is essential to develop aquaculture techniques such as artificial larval mass production and feed development for snail aquaculture. Development of nutritionally balanced feed is dependent on the information of essential nutrients requirement for target aquaculture species.

Dietary lipids are important source of energy and essential fatty acids (EFA) for survival and normal growth of fish (NRC, 1993). The EFA affect the fluidity, permeability and enzyme activity of membrane, and are known as the precursors of the prostaglandins and leukotrienes (Broughton et al., 1991; German et al., 1987; Lokesh et al., 1988; Stubbs and Smith, 1984; Swanson et al., 1989). In several studies it has been demonstrated that EFA requirements varied with the species of fish (NRC, 1993). Among the freshwater species, rainbow trout and *Tilapia zillii* require 18:3n-3 and 18:2n-6, respectively (Castell et al., 1972a; 1972b; Kanazawa et al., 1980), however common carp, grass carp and Japanese eel

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require both 18:3n-3 and 18:2n-6 (Takeuchi, 1996; Kanazawa et al., 1980) as EFA. Therefore, it is important to develop feed with satisfying the qualitative and quantitative requirements for EFA of target aquaculture species. However, no information on the EFA nutrition of snail is available. Therefore, this study was conducted to investigate the effects of dietary lipid sources on growth and body composition of snail.

Materials and Methods

Experimental diets

Ingredients and proximate analysis of the experimental diets are presented in Table 1. Eight experimental diets containing different lipid sources such as lauric acid ethyl ester (LA), squid liver oil (SO), linseed oil (LO), corn oil (CO), SO+LO, SO+CO, LO+CO and SO+LO+CO at 7% lipid level were prepared. Casein (Serva, Feinbiochemica GmbH & Co. Heidelberg, Germany) and white fish meal (Produced by steam dry method, Han Chang Fish Meal Co., Busan, Korea) were used as protein sources. White fish meal was defatted by mixture of chloroform and methanol (2:1, v/v) to avoid the influence of fish meal oil. Starch and wheat flour were used as dietary carbohydrate. Dietary protein and lipid levels were designed to be about 29% and 7%, respectively, according to result of Lee et al. (2002)'s study. Fatty acid composition of the experimental diets is shown in Table 2. Experimental diets were formulated by laboratory pellet machine after 35~40 g water was mixed with 100 g mixture of ingredients, and dried at room temperature for overnight. Experimental diets were stored at −30℃ until used.

Experimental snail and feeding trail

Snail (*Semisulcospira gottschei*) were obtained from a private hatchery (Pyongchang, Korea). They were acclimated to a recirculating system in our

Table 1. Ingredients and proximate analysis of experimental diets

Dietary lipids:	LA	SO	LO	CO	SO+LO	SO+CO	LO+CO	SO+LO+CO
Ingredients (%)								
Casein	5	5	5	5	5	5	5	5
White fish meal[1]	20	20	20	20	20	20	20	20
α-starch	10	10	10	10	10	10	10	10
Wheat flour	52.5	52.5	52.5	52.5	52.5	52.5	52.5	52.5
C12:0[2]	7							
Squid liver oil[3]		7			3.5	3.5		3
Linseed oil[3]			7		3.5		3.5	2
Corn oil				7		3.5	3.5	2
Vitamin premix[4]	2	2	2	2	2	2	2	2
Mineral premix[5]	3	3	3	3	3	3	3	3
Choline salt[2]	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
Proximate analysis (% in dry matter)								
Crude protein	29.8	28.8	28.0	28.5	28.5	28.8	28.6	29.2
Crude lipid	6.5	6.5	6.6	6.6	6.3	6.7	6.3	6.5
n-3 HUFA[6]	−	1.0	−	−	0.7	0.7	−	0.6

[1]Defatted with chloroform-methanol mixture (2:1, v/v).
[2]Sigma Chemical, St. Louis, Missouri, USA.
[3]Provided by E-wha Oil & Fat Ind. Co., Busan, Korea.
[4]Vitamin mix 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; cholecalciferol, 0.003; cyanocobalamin, 0.003.
[5]Mineral mix contained the following ingredients (g/kg mix): NaCl, 10, $MgSO_4 \cdot 7H_2O$, 150; NaH2$PO_4 \cdot 2H_2O$, 250; KH_2PO_4, 320; CaH4 $(PO_4)_2 \cdot H_2O$, 200; Ferric citrate, 25; $ZnSO_4 \cdot 7H_2O$, 4; Ca-lactate, 38.5; CuCl, 0.3; $AlCl_3 \cdot 6H_2O$, 0.15; KIO_3, 0.03; $Na_2Se_2O_3$, 0.01; $MnSO_4 \cdot H_2O$, 2; $CoCl_2 \cdot 6H_2O$, 0.1.
[6]Highly unsaturated fatty acids (C≥20).
LA: lauric acid ethyl ester, SO: squid liver oil, LO: linseed oil, CO: corn oil.

Table 2. Major fatty acids composition (% of total fatty acids) of experimental diets

Dietary lipids	LA	SO	LO	CO	SO+LO	SO+CO	LO+CO	SO+LO+CO
Fatty acids								
12:0	99.0	3.9	5.9	1.5	2.1	5.2	1.7	1.3
16:0	–	12.4	7.5	12.4	11.5	13.8	9.7	11.4
16:1n	–	5.4	–	–	2.4	2.2	–	2.1
18:0	–	1.6	2.1	2.7	2.1	2.5	2.4	2.3
18:1n-9	–	11.9	12.7	16.5	14.1	15.6	15.2	16.4
18:2n-6	–	8.0	22.3	54.2	21.2	35.8	34.9	28.1
18:3n-3	–	1.9	44.1	7.1	24.9	4.2	29.9	21.1
20:5n-3	–	9.9	–	–	5.7	5.6	–	4.7
22:6n-3	–	10.4	–	–	6.5	6.7	–	5.4
n-3 HUFA[1]		27.2			12.2	12.3		10.1

[1]Highly unsaturated fatty acids (C≥20).
–; <0.005.
LA, lauric acid ethyl ester; SO, squid liver oil; LO, linseed oil; CO, corn oil.

laboratory for 1 week by feeding a commercial abalone diet containing 30% protein and 5% lipid. They were then randomly redistributed into 25 L tanks (20 L of water each) at a density of 70 snail (152 mg/snail) per tank. Three replicate groups of snail were fed (ad libitum) once in 2 days for 8 weeks. Before feeding, uneaten diets in each tank was cleaned by siphoning off and 20% of system volume was replaced with freshwater every 2 days. Freshwater was supplied at a flow rate of approximately 0.3 L/min in the recirculating system. Photoperiod was left at the natural condition and water temperature was maintained 23.5 ± 0.47℃ during the feeding trial. Snail in each tank were collectively weighed on the day of initiation and on the day of termination of the experiment after being fasted for 24 h.

Sample collection and chemical analysis

Snail were sampled at the initiation (200 snail) of the feeding trial and all surviving snail at the termination were sacrificed for chemical analysis after they were starved for 24 h and stored at −70℃ as separate aliquots for analysis lipid and fatty acids. After measuring of body weight, edible portion of snail was separated. Crude protein content was determined by Kjeldahl method using Auto Kjeldahl System (Buchi B-324/435/412, Switzerland), and crude lipid content was determined by ether-extraction method. Lipid of diets and snail was extracted by the method of Folch et al. (1957) and fatty acid methyl esters were prepared by transesterification with 14% BF_3-MeOH (Sigma, USA). Fatty acid methyl esters were analyzed by using a gas chromatography (HP 5890, Hewlett-Packard, USA) with flame ionization detector, equipped with HP-INNOWax capillary column (30 m×0.32 mm i. d., film thickness 0.5 μm, Hewlett-Packard, USA). Injector and detector temperatures were 250℃ and 270℃, respectively. The column temperature was programmed from 170℃ to 225℃ at a rate of 1℃/min. Helium was used as the carrier gas. Fatty acids were identified by comparison with retention times of the standard fatty acid methyl esters (Sigma Chemical Co., USA). Fatty acid contents on a dry matter basis were calculated from the following equation (Yoshimatsu et al., 1997):

$$\text{Fatty acid content of dry weight \%} = \text{total lipid of dry weight \%} \times \text{area \%} \times 0.892.$$

Statistical analysis

The data were subjected to one-way analysis of variance (ANOVA) and if significant ($P<0.05$) differences were found, Duncan's multiple range test (Duncan, 1955) was used to rank the groups using the SPSS (SPSS Inc., Michigan Avenue, Chicago, Illinois, USA).

Results and Discussion

Survival and weight gain of snail fed the diets containing different lipid sources for 8 weeks are presented in Table 3. Survival of snail was not

Table 3. Growth performance of snail fed the diets containing different lipid sources for 8 weeks[1]

Dietary lipids	Survival (%)	Weight gain (mg/snail)
LA	76.7 ± 5.85	14 ± 3.4[a]
SO	65.7 ± 6.22	64 ± 10.8[d]
LO	69.5 ± 2.09	37 ± 8.8[b]
CO	76.6 ± 5.37	50 ± 4.9[bcd]
SO+LO	71.9 ± 0.50	66 ± 5.7[d]
SO+CO	73.8 ± 3.45	70 ± 7.1[d]
LO+CO	69.5 ± 0.47	40 ± 5.8[bc]
SO+LO+CO	69.1 ± 2.53	60 ± 3.5[cd]

[1]Values (mean ± SE of three replications) in each column not sharing a common superscript are significantly different ($P<0.05$).
LA, lauric acid ethyl ester; SO, squid liver oil; LO, linseed oil; CO, corn oil.

affected by dietary lipid sources ($P>0.05$). However, weight gain of snail was significantly influenced by dietary lipid sources ($P<0.05$). Weight gain of snail fed the diets containing squid liver oil (SO) such as SO, SO+linseed oil (LO) and SO+LO+corn oil (CO) diets was significantly higher than that of snail fed the lauric acid (LA), LO and LO+CO diets ($P<0.05$). The lowest weight gain was observed in snail fed the LA diet ($P<0.05$), and this may be due to the accelerating side effect of 12:0 on EFA deficiency as also observed in carp and *Tilapia* (Watanabe et al., 1975; Kanazawa et al., 1980). The lipid kind and level in the diet are important for growth and body composition of animal. Major fatty acids composition varied in the different dietary lipid sources (Lee, 1994). Since marine fish oils such as squid liver oil showing good growth in this study contain high proportion of n-3 HUFA and adequate ratio of EPA/DHA (Kalogeropoulos et al., 1992) for some fish species, especially marine fish such as rockfish *Sebastes schlegeli* (Lee, 2001) and abalone *Haliotis discus hannai* (Lee and Park, 1998), these could be used as good dietary lipid sources to satisfy EPA and DHA requirements. Good growth of snail in the diets containing SO indicates that n-3 HUFA could be EFA of snail.

EFAs and their requirements of fish differ among species (NRC, 1993). Freshwater fish require 18:3n-3 and/or 18:2n-6, whereas marine fish require n-3 HUFA (0.5~2.0% in diet) such as EPA and DHA. Abalone (*H. discus hannai*) requires n-3 and n-6 HUFA as EFAs, and the n-3 HUFA requirement was estimated to be about 0.5~1.0% in the diet (Lee and Park, 1998; Uki et al., 1986). Weight gain of snail fed the SO+LO+CO diet containing 0.6% n-3 HUFA was not significantly different to that of snail fed the SO, SO+LO and SO+CO diets containing 0.7~1.0% n-3 HUFA in the present study. Lee (1994) and Lee et al. (2002) found that no side effect of soybean oil substitution with squid liver oil in diet was observed when n-3 HUFA requirement for the optimal growth of rockfish was met. In considering these results and negative effect of other fish fed the excess amounts of n-3 HUFA (Takeuchi and Watanabe, 1979), it is not necessary to adding excessive n-3 HUFA in the diet for normal growth and healthy condition of fish. Growth of snail in this study also indicates that 0.6% n-3 HUFA may be recommended for practical snail diets, and plant oil could be used as a energy source when n-3 HUFA requirement is satisfied.

Crude lipid content and fatty acids composition of edible portion in snail are reported Table 4. The crude lipid content of edible portion in snail fed LA diet was the lowest among the groups, however no significant difference was found in snail fed the different experimental diets ($P>0.05$). This is an agreement with other studies showing that body lipid content of rainbow trout fed the diets containing only lauric acid is lower compared to that of fish fed the diets containing mixture of lauric acid and linolenic acid, or soybean oil and cod liver oil as lipid sources (Castell et al., 1972b). The reduced lipid levels of gilthead bream fed n-3 HUFA-deficient diets have also been reported (Kalogeropoulos et al., 1992).

It is well established that the fatty acid pattern of fish lipid reflects the fatty acid composition of dietary lipid (Bell et al., 1994; Sargent et al., 1995). The highest 12:0 content was observed in snail fed the LA diet and was significantly different from that of snail fed the other diets ($P<0.05$). Snail fed the LO diets contained high content of 18:3n-3 was showed the highest contents of 18:3n-3 and 20:3n-3 ($P<0.05$). Significantly higher contents of 20:5n-3 were observed in snail fed the SO diet than that of fish fed the other diets ($P<0.05$). The highest 22:6n-3 content was observed in snail fed the SO+LO diet but not significantly different from that of

Table 4. Lipid and fatty acids composition (% of dry matter) of edible portion in snail fed the diets containing different lipid sources for 8 weeks[1]

Dietary lipids	LA	SO	LO	CO	SO+LO	SO+CO	LO+CO	SO+LO+CO	SEM[2]
Crude lipid	1.1	2.6	3.1	3.3	2.6	2.4	2.7	3.0	0.73
Fatty acids									
12:0	0.04^{b}	0.01^{a}	—^{a}	—^{a}	—^{a}	—^{a}	—^{a}	—^{a}	0.005
14:0	0.02^{a}	0.06^{b}	0.04^{ab}	0.04^{ab}	0.04^{ab}	0.04^{ab}	0.03^{ab}	0.04^{ab}	0.012
14:1n	0.03	0.05	0.06	0.04	0.03	0.04	0.04	0.05	0.015
16:0	0.08	0.23	0.21	0.22	0.18	0.19	0.20	0.18	0.053
16:1n	0.08	0.14	0.13	0.12	0.10	0.11	0.12	0.13	0.033
17:0	0.04	0.04	0.06	0.05	0.03	0.04	0.04	0.05	0.014
18:0	0.05	0.09	0.12	0.12	0.08	0.09	0.10	0.10	0.027
18:1n-9	0.06^{a}	0.30^{abc}	0.35^{abc}	0.43^{c}	0.30^{abc}	0.31^{abc}	0.33^{abc}	0.38^{bc}	0.087
18:2n-6	0.07^{a}	0.14^{ab}	0.31^{ab}	0.79^{c}	0.24^{ab}	0.39^{ab}	0.44^{abc}	0.49^{bc}	0.118
18:3n-3	0.01^{a}	0.03^{a}	0.45^{d}	0.08^{ab}	0.26^{c}	0.04^{a}	0.22^{bc}	0.24^{bc}	0.053
20:0	0.08^{a}	0.27^{b}	0.22^{ab}	0.21^{ab}	0.19^{ab}	0.19^{ab}	0.19^{ab}	0.21^{ab}	0.054
20:1n-9	-	0.05	0.04	0.04	0.04	0.04	0.04	0.04	0.014
20:2n-6	0.05	0.06	0.11	0.13	0.05	0.08	0.10	0.10	0.026
20:4n-6	0.10	0.13	0.17	0.19	0.10	0.11	0.16	0.16	0.044
20:3n-3	—^{a}	—^{a}	0.04^{b}	—^{a}	0.02^{a}	—^{a}	0.02^{a}	0.01^{a}	0.004
20:5n-3	0.02^{a}	0.18^{b}	0.05^{a}	0.06^{a}	0.09^{a}	0.08^{a}	0.06^{a}	0.08^{a}	0.028
22:0	0.02^{ab}	0.04^{b}	0.01^{a}	0.01^{ab}	0.02^{ab}	0.03^{ab}	0.01^{ab}	0.02^{ab}	0.009
22:1n-9	0.14	0.16	0.22	0.17	0.13	0.14	0.15	0.16	0.045
22:2n-6	0.02	0.03	0.05	0.09	0.05	0.02	0.07	0.05	0.027
22:4n-6	0.02	0.02	0.03	0.02	0.02	0.01	0.02	0.01	0.008
22:5n-3	0.03	0.07	0.05	0.04	0.04	0.04	0.03	0.04	0.014
22:6n-3	0.03^{a}	0.20^{ab}	0.08^{a}	0.07^{a}	0.30^{b}	0.13^{ab}	0.06^{a}	0.11^{ab}	0.057
n-3 HUFA[3]	0.09^{a}	0.46^{b}	0.22^{ab}	0.17^{ab}	0.45^{b}	0.25^{ab}	0.18^{ab}	0.24^{ab}	0.091
n-6 HUFA[3]	0.19	0.25	0.36	0.44	0.21	0.23	0.35	0.32	0.099

[1]Values (mean of three replications) in each column not sharing a common superscript are significantly different ($P<0.05$).
[2]Standard error of the treatment mean calculated from the residual mean square in the analysis of variance
[3]Highly unsaturated fatty acids (C≥20).
—; <0.005.
LA: lauric acid ethyl ester, SO: squid liver oil, LO: linseed oil, CO: corn oil.

snail fed the SO, SO+CO and SO+LO+CO diets ($P>0.05$). These results suggest that some of 20:3n-3 might be elongated from 18:3n-3 supplied with linseed oil but no or very low converting from 18:3 n-3 to 20:5n-3 and 20:6n-3 are occurred in snail. However, freshwater fish such as rainbow trout, ayu and eel have capacity to convert from 18:3n-3 to 20 :5n-3 and 20:6n-3 by elongation and desaturation (Kanazawa et al., 1979). This difference could be resulted from the difference of converting capacity of 20:5n-3 and 22:6n-3 from 18:3n-3 in fish (Owen et al., 1975; Yamada, 1980; Kissil, 1987).

Although there was no significant difference in n-6 HUFA contents (% of dry matter) of snail among all diets ($P>0.05$), the relative proportion (% of total fatty acids) of n-6 HUFA such as 20:4 n-6 and 22:4n-6 contents of total fatty acids in snail fed the LA diets were significantly higher than those of snail fed the other diets (data of % total fatty acid were not shown in the Table) ($P<0.05$). This phenomenon indicates that this snail may has capacity to elongate or desaturate n-6 HUFA from shorter chain polyunsaturated fatty acids. However, more detailed studies on fatty acids synthesis of snail are needed.

Various deficiency signs in aquaculture species are apparent in response to the EFA-deficient diets, and fish fed the EFA-deficient diets exhibited lower growth, higher liver lipid content and increase of n-9 fatty acids (NRC, 1993). It has been proposed that the ratio of 18:1n-9 or 20:3n-9 to n-3 HUFA can serve as an index of EFA adequacy for other

fish (Castell et al., 1972c; Kalogeropoulos et al., 1992). However, values for 18:1n-9/n-3 HUFA ratio and 18:1n-9 content of snail fed the LA diet in this study were not increased compare to those of snail fed n-3 HUFA-sufficient diets. This result is not in accordance with data obtained from the several studies showed that 18:1n-9 and 20:3n-9 contents of freshwater fish increased in the EFA insufficient diets (Kanazawa et al., 1980; Takeuchi et al., 1980; Takeuchi, 1996; Satoh et al., 1989). This different response to fatty acids composition of snail as compared to other fish is not clear, but it may be due to differences in species and feeding habitat.

Based on the results of this study, it is concluded that squid liver oil or mixture of squid liver oil and linseed and/or corn oil are good dietary lipid sources, and lauric acid is an undesirable source for the normal growth of snail.

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