ISSN (Print) 1225-9918
ISSN (Online) 2287-3406
Journal of Life Science **2015** Vol. 25. No. 5. 548~556 DOI : http://dx.doi.org/10.5352/JLS.2015.25.5.548

Influence of Dietary Conjugated Linoleic Acid (CLA) and Carotenoids on Growth, Fatty Acid Composition, and 3T3-L1 Cells in Black Seabream (*Acanthopagrus schlegeli*)

Rui Guo[1], Zuliyati Rohmah[1,2], Kwang-Soo Choi[3], Si-Hyang Park[4], Yeong-Lae Ha[5], Seok-Joong Kang[1] and Byeong-Dae Choi[1]*

[1]*Department of Seafood and Aquaculture Science/The Institute of Marine Industry, Gyeongsang National University, Tongyeong 650-160, Korea*
[2]*Faculty of Biology, Universitas Gadjah Mada, Yokyakarta 55281, Indonesia*
[3]*Department of Marine Environmental Engineering, Gyeongsang National University, Tongyeong 650-160, Korea*
[4]*Sunmarine Biotech Co., Tongyeong 650-160, Korea*
[5]*Department of Applied Chemistry, Gyeongsang National University, Jinju 660-701, Korea*

Received January 12, 2015 / Revised May 8, 2015 / Accepted May 8, 2015

Three groups of black seabream (*Acanthopagrus schlegeli*) were fed with treatment diets containing certain concentrations of conjugated linoleic acid (CLA) and carotenoids. The control group feed contained 0% CLA and 0% carotenoids, the CP10 group feed contained 1% CLA and 0.1% carotenoids, and the CP25 group feed contained 2.5% CLA and 0.1% carotenoids. The CP10 and CP25 groups demonstrated the enhanced growth and increased feed conversion efficiency of black seabream. The specific growth rates (SGRs) were 0.74, 0.81, and 0.97, while the feed conversion ratios (FCRs) were 2.65, 2.46, and 2.04 for the control, CP10, and CP25 groups, respectively. The total contents of high unsaturated fatty acid (HUFA) for the control, CP10, and CP25 groups were 41.0%, 41.7%, and 43.5%, respectively. CLA was deposited to the extent of 2.8% and 5.6% in the muscle, and 4.0% and 8.3% in the viscera of the CP10 and CP25 groups, respectively. Meanwhile, treatment with the viscera lipid extract (VLE) from CP25 fish evidently lowered 3T3-L1 adipocytes viability. The lipid extract from the muscle and viscera of black seabream contained ample amounts of beneficial substances, such as CLA, carotenoids, EPA, and DHA. CLA, which enriched black seabream muscle, could be categorized as a functional food and serve as a well-being food. Meanwhile, the fish oil from its viscera could serve as a high function supplement.

Key words : Black seabream, carotenoids, conjugated linoleic acid (CLA), growth performance, 3T3-L1

Introduction

Fish and fishery products are one of the most traded food commodities worldwide. Approximately 148 million tons of fish were produced from wild-captured fisheries and aquaculture in 2010. Total seafood supply was increased by 2.5% more than in 2009 and forecasted would reach 160 million tons in 2013. About 86% of total worldwide fish product was consumed as food [11]. Recently, many studies were made with respect to the role of polyunsaturated fatty acids (PUFAs) contained in fish. The health benefits of omega-3 polyunsaturated fatty acids (n-3 PUFAs), mainly eicosapentaenoic acid (EPA 20:5) and docosahexaenoic acid (DHA, 22:6), have been long known. Conjugated linoleic acid (CLA), one of the PUFAs, is the natural and functional fatty acids in meats and dairy products from ruminant animals. Other significant biological activities such as body fat reduction and modulation of cholesterol content in blood were reported [5, 9, 13, 23]. PUFAs are also major component of cell's membrane. It plays important roles in regulating physiological and biochemical process in cells as well as chemoprotector [12, 39]. Long chain (LC)-PUFAs have vital functions in regulating growth, lipid metabolism, membrane fluidity, immune function, nervous system and vision development [2, 32]. Specific ratio of n-6/n-3 PUFA on diet were proofed influence on aging and degeneration on muscle tissue, brain, as well as suppressed inflammatory and autoimmune diseases [29, 30].

CLA is a group of positional and geometric isomers of

***Corresponding author**
Tel : +82-55-772-9147, Fax : +82-55-648-2038
E-mail : bdchoi@gnu.ac.kr

linoleic acid. Naturally, this compound was found in variety of food product such as dairy product and poultry [7]. CLA is widely used in weight loss management to reduce body fat mass. This fat mass is known as a key factor of the metabolic syndrome like insulin resistance as well as Type 2 diabetes [36]. Dietary CLA has been confirmed to have some beneficial effects on body compositions in mammals by decreasing its body fat mass and increase lean body mass [25]. CLA combined with fish oil was proofed to enhance insulin sensitivity, as well as lower bone marrow adiposity, inflammation, and oxidative stress in aging mice [16]. Higher content of CLA in food products might increase their nutritional and therapeutic value. Several reports have been published for value added of CLA in fish e.g. 0.5 to 2% of CLA were given to rainbow trout [35], Atlantic salmon with 0.5 to 4% of CLA in its feed [3], 0.5 to 1% of dietary CLA given to hybrid striped bass [33]. The results of those studies indicated that the abilities of fish incorporated CLA were vary from species to species. The developmental stage of the fish and the dietary CLA level were shown affecting fish growth and the pattern of lipid metabolism.

Consumers are more aware of the relationship between health and diet. This awareness drives them to choose food which has more nutritional value and benefit to their health. Dietary CLA was one of the ways to amplify its concentration in fish. However, this method has a drawback, since CLA has prominent effect as body fat reducer thus it will lower harvest weight. This study aims are to investigate the influence of dietary conjugated linoleic acid (CLA) in alliance with carotenoids on growth and fatty acid composition in black seabream, and to examine the impact of black seabream lipid extract on 3T3-L1 cells. The addition of carotenoids will feasibly maintain the weight of the fish as well as enhance meat coloring. In the market, CLA mainly sold in form of free fatty acid and derived synthetically from meat and dairy products [24]. The incorporation of CLA into black seabream feed would switch this free fatty acid into triglycerides during its fat metabolism and deposited CLA in its muscle, thus will be safer and more appealing for the consumer.

Materials and Methods

Materials

Commercial diet for black seabream was obtained from Kyeongnam Feed Co. (Tongyeong, Korea). The pellets (±6 mm in diameter) were analyzed for its general composition (Table 1) and fatty acid content (Table 2). Feed pellet then coated with 10% (v/w) fish oil contains designated concentration of CLA and carotenoids (Control group: 0% CLA and 0% carotenoids; CP10: 1% CLA and 0.1% carotenoids; CP25: 2.5% CLA and 0.1% carotenoids). CLA (purity ≥ 77%) was supplied by HK Biotech Co. (Jinju, Korea), which consisted: 39% *cis*-9, *trans*-11 CLA; 39% *trans*-10, *cis*-12 CLA; and 2% other CLA isomers (*cis*-9, *cis*-11; *cis*-10, *cis*-12; *trans*-9, *trans*-11, and *trans*-10, *trans*-12 CLA). Commercial carotenoids that being used in this study was Carophyll Pink® (contained 10% astaxanthin) obtained from Vixxol Co. (Anyang, Korea). Fish oil was attained from E-Wha Oil Co. Ltd. (Pusan, Korea). Black seabream (*Acanthopagrus schlegeli*) (weight 130.0±16.1 g) were acquired from Jeil Fisheries Co. (Tongyeong, Korea). Fishes were acclimatized to natural sea conditions and fed with the control diet for two weeks prior to the experiment.

Table 1. Proximate composition of the experimental feed and black seabream after treatment

	Experimental feed[1]			Black seabream[1]					
	Control	CP10	CP25	Control		CP10		CP25	
				Muscle	Viscera	Muscle	Viscera	Muscle	Viscera
Moisture	27.4±0.5[a]	28.3±0.3[a]	28.0±0.3[a]	75.4±0.1[a]		74.5±0.1[a]		74.2±0.3[a]	
Ash	6.9±0.5[a]	6.3±0.3[a]	6.5±0.4[a]	1.6±0.1[a]		1.5±0.2[a]		1.3±0.1[a]	
Crude protein[2]	40.4±0.8[a]	39.8±1.8[a]	40.6±2.9[a]	17.6±0.7[a]		18.6±1.5[a]		19.0±0.7[a]	
Lipids	5.4±0.6[a]	5.8±0.3[a]	5.7±0.5[a]	2.7±0.2[a]	27.8±1.2[a]	2.6±0.1[a]	27.1±0.8[a]	2.4±0.2[a]	25.4±0.5[b]
Carbohydrate[3]	19.9±0.3[a]	19.8±0.6[a]	19.2±0.8[a]						

[1]The values are mean ± SD (n=3), % of dry basis. Different superscript letters within lipids of viscera group represent significant differences between treatments (p<0.05).
[2]Crude protein=N ×6.25.
[3]Total carbohydrates were calculated by subtraction: 100- (% moisture+ % ash + % protein+ % lipid).

Table 2. Biological parameters and growth of black seabream after feeding the experimental diets[1]

Item	Dietary treatments		
	Control	CP10	CP25
Initial mean weight (g)	130.0±6.1	130.0±6.1	130.0±6.1
Final mean weight (g)	197.6±29.8	205.6±21.8	224.6±41.1
Final liver weight (g)	16.8±4.5	17.0±1.6	14.2±4.1
Final viscera weight (g)	2.8±1.5	3.0±1.0	3.2±0.4
SGR[2]	0.74[a]	0.81[b]	0.97[c]
FCR[3]	2.65[a]	2.46[ab]	2.04[c]
VSI[4]	8.50[a]	8.27[ab]	6.32[b]
HSI[5]	1.42[a]	1.46[a]	1.42[a]

[1]Values are means from triplicate groups of fishes (n=15) where the values in each row with different superscripts are significantly different ($p<0.05$).
[2]SGR, Specific growth rate (% day) = 100× (Log_e final weight-Log_e initial weight)/days.
[3]FCR, Feed conversion ratio = [Wet weight gain (g)/dry feed intake (g)] ×100.
[4]VSI, Visceralsomatic index = [Visceral weight (g)/fish weight (g)] ×100.
[5]HIS, Hepatosomatic index = [Liver weight (g)/fish weight (g)] ×100.

Experimental procedures

One thousand and five hundred fishes were randomly distributed into three groups (Control, CP10 and CP25) and allotted to cages of 175 m^3 (5 m × 5 m × 7 m) in size. Treatments were conducted for 8 weeks, feed were given 3% of fish body weight, twice a day at 10:00 am and 17:00 pm until satiation. The experiment was conducted during summer (June-August) in sea area of Tongyeong, Korea. Water quality was monitored twice a week at 08:00-09:00 am. Data on feed intakes and weight gains were acquired every 15 days. Fishes were fasted 24 hr before sampling. Five fishes from each group were captured randomly and exposed to lethal dose of MS-222 (anesthetic, ethyl m-aminobenzoate methanosulphonate).

Fish diets

Commercial fish feed for this study was a mixture between white fish meal 55%, soybean meal 7%, wheat flour 25%, and yeast 1%. These were enriched with vitamins premixes up to 1% and minerals premixes 1%. This commercial feed was later coated with 10% (v/w) fish oil contained CLA and carotenoids. The result of proximate compositions analysis of the feed was shown in Table 1. This composition was within the range that suggested by Hardy and Barrows [18].

Analyses of proximate composition and fatty acids profile

Proximate composition of fish feed and black seabream muscle were analyzed. Protein content was determined with Kjeldahl method [1]. Total Lipid content was analyzed with Bligh and Dyer [4] method. The moisture and ash of the samples were determined with methods according to AOAC [1]. For fatty acid methyl ester (FAME) analysis, Park et al. [26] method was employed. In brief, fifty milligrams of lipid extract was added with 1.5 ml 1.0 N NaOH. The mixture was heated at 100°C for 30 min and cooled to room temperature afterward. Three milliliters of 1.0 N H_2SO_4 was added to solution and incubated at 55°C for 20 min. FAME was partitioned to isooctane by adding 1 ml of it to the mixture. Isooctane fraction was analyzed with gas chromatography (Shimadzu GC 17-A, Shimadzu Co., Kyoto, Japan) and Omegawax-320 column (30 m × 0.32 mm ID) from Supelco Co. (Bellefonte, PA, USA). Injector and flame-ionization was set at 250°C. The carrier gas was helium with column-inlet pressure set at constant value, 1.0 kg/cm^2 and split ratio was 1:100. Oven temperature was set at 180°C for initial time 8 min to 230°C with increasing rate 3°C/min, the final temperature was kept for 15 min. FAME authentic standards (Sigma Chemical Co., St. Louis, MO, USA) and an equivalent chain length (ECL) of menhaden oil standard (Sigma Chemical Co.) were used as comparison.

Extraction and analysis of carotenoids content

Carotenoids from black seabream muscle and viscera were extracted with acetone according to Rodriguez-Amaya and Kimura [28]. Extract were concentrated under reduced pressure and partitioned to diethyl ether. Carotenoids were quantified by using UV Spectrophotometer (Shimadzu UV-1700, Shimadzu, Co., Kyoto, Japan), at 460 nm. The total carotenoids content was calculated using equation: Total carotenoids (mg/g) = [O.D. (λmax) × Vol. × 1,000] / [$E^{1\%}_{1cm}$ (2,400) × weight of sample (g)]. The analyses were conducted in triplicate and data presented as mean± standard deviation.

Cell culture

Lipid extract with the highest CLA content among treatment groups (viscera of CP 25) along with control group were analyzed for its effect on 3T3-L1 cells. Mouse 3T3-L1 pre-adipocytes were purchased from American Type Culture Collection (Rockville, MD, USA) and cultured as

described in Kim et al. [20]. Briefly, 3T3-L1 pre-adipocytes were inoculated into ten culture flasks and grown to confluence at 37°C, 5% CO_2 in Dulbecco's modified Eagle's medium (DMEM) containing 10% bovine calf serum (BCS) (Gibco Co., New York, NY, USA) with 1% penicillin/streptomycin. Two days after cell cultures were confluent; cell differentiation was induced with a mixture of 1 μM dexamethasone (Sigma Co. St. Louis, MO, USA), 0.5 mM methyl isobutyl xanthine (Sigma Co.), 1 mM theophylline (Sigma Co.) in DMEM plus 10% BCS and 5 μg/ml insulin. In addition of inducer mixture, 1, 10, 50, 100 μg/ml of black seabream viscera's lipid extract (VLE) from control and CP25 group were added respectively. After 48 hr incubation, the medium was replaced with DMEM plus 10% BCS, 5 μg/ml insulin and VLE of control and CP25 0, 1, 10, 50, 100 μg/ml respectively. Reference group was a culture without addition of VLE. Cultures were incubated for another 96 hr. Cell viability was determined with MTS [3-(4,5-dimethyl-thiazol-2-yl)-5-carboxyethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium] assay. The treatment medium was replaced with 100 μl fresh medium of 10% BCS in DMEM and 20 μl of MTS reagents (Promega Co., Madison, WI, USA). Cells were then incubated at 37°C in 5% CO_2 for 24 hr. The absorbance of cells cultures were read at 490 nm by a microplate reader (Spectramax M^2 Molecular Devices, Sunnyvale, CA, USA).

Calculation and statistical analysis

Growth performance, feed utilization efficiency and body indices were calculated as follows: Specific Growth Rate (SGR) was 100 × ($\log_e$ final weight) − ($\log_e$ initial weight) over number of treatment days. Feed Conversion Ratio (FCR) was dry feed consumed (kg) over wt weight gain (kg). Viscerosomatic index (VI) was viscera weight over body weight times 100, where viscera included liver. Hepatosomatic index (HI) was equal to viscera weight over body weight times 100. Data were analyzed for the degree of variation and significant differences, based on Analysis of Variance (ANOVA), along with the Tukey's pair-wise comparison test between treatment means and Duncan's multiple range test (DMRT) to determine the differences. Analyses were conducted by using the JMP statistical software (SAS Institute Inc., Cary, NC, USA).

Results and Discussion

Environmental parameters and growth performance

Observation on sea water quality during rearing of black seabream revealed that the water temperatures were ranged from 23.7°C to 25.5°C, dissolved oxygen ≥6.8 mg/l, pH 7.9-8.1, total ammonia-nitrogen 0.026-0.038 mg/l, and salinity 31.0 g/l. With high benefit for human health, CLA enriched meat and fishes will have a big market share. Experiments for enhancing CLA level on fish muscle by adding it on its diet have been done. Choi et al. [8] reported that 1% CLA on diets increased weight gains in common carp. However, addition of CLA more than 1% would slow down growth rate in some fishes and lower their final weight as reported by Yasmin and Takeuchi [40] on tilapia. Similar effect also found on yellow catfish [31] and hybrid striped basses [34]. Leaver et al. [22] reported different inclination on Atlantic salmon, its growth rate and FCR were not affected by 1% CLA, but treatment with more than 2% CLA was evidently lowering its final weights. Twibell and Wilson [33] reported similar effect on channel catfish. Experiments to enrich fishes by dietary CLA higher than 1% have shown that it has a tendency to slow down fish growth and reduce its final weight. To overcome the bottleneck, in this study, CLA were given incorporated with carotenoids.

Result showed that SGR was 0.74, 0.81, and 0.97 and FCR was 2.65, 2.46, and 2.04 for control, CP10, and CP25 respectively (Table 2). VSI of treatment groups was significantly lower from control (p<0.05) but on the other hand the treatments were raised no difference HSI. The SGR value of CP25 > CP10 > control, while FCR value CP25 < CP10 < control and VSI value were CP25 ≤ CP10 ≤ control. This showed that CLA-carotenoid treatments were increased black seabream growth performance and its feed conversion efficiency with dose dependent manner. In the meantime, it also suggested that CP25 was the most effective treatment for promoting growth and muscle development in black seabream.

Proximate compositions and fatty acid profile

The proximate compositions of the fishes muscle (Table 1) shown that there was no significantly (p<0.05) different among the groups. This indicates that the muscle composition of fishes was not changed after treatment for 8 weeks. Fatty acid profile of the diets was shown in Table 3. The

Table 3. Fatty acid compositions[1] of the experimental feed and black seabream after treatment[1]

Fatty acids	Experimental feed			Black seabream					
				Muscle			Viscera		
	Control	CP10	CP25	Control	CP10	CP25	Control	CP10	CP25
14:00	3.9±0.1	3.1±0.0	3.2±0.0	1.7±0.1	1.5±0.1	1.3±0.1	2.3±0.5	2.4±0.1	1.7±0.1
15:00	0.5±0.0	0.3±0.0	0.3±0.0	0.3±0.0	0.3±0.0	0.4±0.0	0.3±0.0	0.2±0.0	0.1±0.0
16:00	16.1±0.3	14.3±0.3	13.7±0.9	21.6±0.4	21.0±1.2	19.3±1.7	13.3±0.4	13.6±1.2	11.9±0.9
17:0+phytane	1.0±0.0	0.9±0.0	0.8±0.0	-	-	-	-	-	-
18:00	4.5±0.1	4.3±0.1	3.9±0.1	5.4±0.0	5.4±0.1	6.1±0.5	2.4±0.4	2.5±0.7	2.8±0.1
20:00	0.3±0.1	0.3±0.2	0.4±0.1	-	-	-	-	-	-
ΣSaturated	26.3^{b}	23.2 ^{a}	22.3 ^{a}	31.8^{a}	30.1^{a}	28.9^{ab}	18.5^{a}	18.9^{a}	16.4^{b}
16:1n-7	5.0±0.5	4.2±0.1	4.2±0.4	5.2±0.0	6.6±0.3	6.7±0.6	9.0±1.4	4.4±0.7	5.7±0.1
16:1n-5	0.2±0.1	0.1±0.1	0.2±0.0	0.3±0.1	0.2±0.0	0.2±0.0	0.3±0.0	0.3±0.0	-
16:1n-9, 7	-	-	-	19.5±1.2	19.0±0.4	18.7±0.6	25.8±1.2	24.1±1.1	23.5±0.9
18:1n-9	18.9±0.8	17.4±0.2	16.5±0.9	-	-	-	-	-	-
18:1n-7	3.0±0.1	2.6±0.1	2.5±0.2	-	-	-	-	-	-
18:1n-5	-	-	-	-	-	-	0.3±0.0	0.5±0.2	0.2±0.0
20:1n-11, 9	1.3±0.2	1.3±0.2	1.5±0.4	1.7±0.2	1.8±0.1	1.6±0.1	2.2±0.2	2.1±0.2	2.4±0.2
22:1n-11, 9	1.7±0.1	1.5±0.1	1.3±0.1	0.4±0.0	0.9±0.0	0.6±0.3	1.1±0.2	0.6±0.1	1.1±0.1
ΣMonounsaturated	30.1^{a}	27.1^{a}	26.2^{a}	27.1^{a}	28.5^{a}	27.8^{a}	38.6^{c}	32.4^{b}	33.0^{b}
16:2n-4	0.5±0.0	0.4±0.0	0.5±0.0	0.5±0.0	0.5±0.1	0.5±0.0	0.2±0.0	0.3±0.0	-
16:3n-4	0.7±0.0	0.6±0.2	0.8±0.0	0.2±0.0	0.2±0.0	0.2±0.0	0.3±0.0	0.3±0.1	-
16:3n-1	0.2±0.0	0.1±0.1	-	2.5±0.0	3.6±0.1	3.8±0.3	-	0.3±0.1	-
18:2n-6	15.2±0.2	13.3±0.2	11.6±0.0	8.1±0.2	6.4±0.7	7.1±0.4	11.2±0.2	10.9±0.7	10.1±0.4
18:3n-3	2.0±0.0	1.7±0.1	1.4±0.0	0.8±0.1	0.4±0.1	0.4±0.1	1.1±0.1	1.6±0.2	1.1±0.1
CLA1	-	3.9±0.4	6.2±0.3	-	1.3±0.0	2.4±0.1	0	2.3±0.2	4.1±0.5
18:4n-3	1.3±0.0	1.2±0.0	1.2±0.0	0.7±0.0	0.1±0.0	0.1±0.0	1.3±0.0	0.5±0.0	0.5±0.0
CLA2	-	4.4±0.2	6.4±0.2	-	1.5±0.2	3.2±0.0	0	1.7±0.2	4.2±0.0
18:4n-1	0.2±0.0	0.2±0.0	0.2±0.0	0.3±0.0	-	-	0.4±0.1	-	-
20:2NMID	0.1±0.0	0.2±0.1	0.2±0.0	-	-	-	-	-	-
20:2n-6	0.3±0.2	0.3±0.1	0.3±0.0	0.3±0.0	0.2±0.0	0.2±0.0	0.2±0.0	0.2±0.0	0.2±0.0
20:4n-6	0.8±0.0	0.5±0.0	0.5±0.0	0.4±0.1	0.2±0.0	0.2±0.0	0.4±0.1	0.3±0.0	0.3±0.0
20:3n-3	0.2±0.0	0.1±0.0	0.1±0.0	1.6±0.3	1.4±0.2	1.6±0.0	1.5±0.3	1.2±0.2	1.6±0.3
20:4n-3	0.5±0.0	0.4±0.1	0.4±0.0	0.5±0.1	0.4±0.1	0.3±0.0	1.0±0.1	1.1±0.1	1.1±0.0
20:5n-3	6.0±0.0	5.5±0.0	5.6±0.0	6.2±0.1	5.6±0.1	5.5±0.8	10.2±1.2	11.7±1.3	10.8±0.8
22:4n-6	-	0.1±0.0	0.1±0.0	0.3±0.0	0.4±0.0	0.3±0.0	0.3±0.0	0.2±0.0	0.3±0.0
22:5n-6	0.2±0.0	0.2±0.0	0.2±0.0	0.4±0.0	0.5±0.0	0.3±0.0	0.4±0.0	0.3±0.0	0.4±0.0
22:5n-3	1.6±0.1	1.3±0.0	1.3±0.0	2.1±0.0	2.1±0.0	1.8±0.1	2.9±0.3	3.0±0.3	3.0±0.1
22:6n-3	7.7±0.6	7.1±0.1	5.6±0.0	15.4±0.3	16.2±0.4	15.8±1.4	10.4±1.1	11.8±1.3	12.7±0.3
ΣPolyunsaturated	37.5^{a}	42.5^{b}	43.8^{c}	41.0^{b}	41.7^{b}	43.5^{c}	42.9^{c}	48.4^{d}	50.5^{e}
Total CLA	0^{a}	9.3^{c}	13.8^{d}	0.0^{a}	2.8^{b}	5.6^{b}	0.0^{a}	4.0^{b}	8.3^{c}
Σn-6	16.5	14.4	12.7	11.3^{c}	12.4^{b}	15.0^{a}	13.9^{c}	17.2^{b}	21.2^{a}
Σn-3	21.3	19.3	15.6	25.7^{a}	24.8^{a}	23.8^{ab}	27.6^{b}	30.0^{a}	29.0^{a}
Σn-6/Σn-3	0.77	0.75	0.81	0.44	0.50	0.63	0.50	0.57	0.73

[1]The values (in % of total fatty acids) are mean ± S.D. (n=5). Different superscript letters on the same rows of enclosed column represent significant differences between treatments (p<0.05). Σn-6: incorporated total CLA; Total CLA: sum of isomers *cis*-9, *trans*-11 and *trans*-10, *cis*-12.

total CLA detected in Control, CP10, and CP25 feed were 0%, 9.3%, and 13.8% from total fatty acid respectively. The EPA and DHA level for Control, CP10, and CP25 feed were 6.0±0.0% and 7.7±0.6%, 5.5±0.0% and 7.1±0.1%, 5.6±0.0% and 5.6±0.0% respectively. The fatty acid profile of black seabream muscle showed decreasing concentration of saturated fatty acid (SFA) in treatment groups, whereas the monounsaturated fatty acid (MUFA) concentration showed no differences among the groups. Dietary inclusion of 2.5% CLA was proofed to boost polyunsaturated fatty acids

(PUFA) concentration on seabream muscle, which was 41.0%, 41.7%, and 43.5% for control, CP10, and CP25 feed group compare to those of hybrid striped bass, respectively [34]. Previous studies [8, 40] suggested that final weight, growth performance, EPA, and DHA contents of fish would be declined with the increasing dietary CLA levels. These were one of the immense problems with dietary CLA for fish. This study showed that CLA incorporated with carotenoids on diets could augment PUFA level in black seabream. The percentages of CLA deposited in muscle of CP10 and CP25 feed group were 2.8% and 5.6%, while their levels in viscera were 4.0% and 8.3% respectively. These exhibited significant differences (p<0.05) between the two (Table 3). The level of CLA deposited in muscle and viscera of black seabream were linearly affected by its concentration in the diet.

Total PUFA in viscera of control, CP10, and CP25 feed group was 42.9%, 48.4%, and 50.5% correspondingly. Increasing n-6 and decreasing of n-3 will be resulted on increasing of n-6/n-3 ratio. N-6 and n-3 concentrations on back seabream viscera were 13.9%, 17.2%, 21.2% and 27.6%, 30.0%, 29.0% for control, CP10, and CP25 feed group respectively. The increase of n-6/n-3 ratio (Table 3) could be attributed to increase of CLA. Some studies suggested that high ratio of n-6/n-3 pose greater risk of cancer [6, 38] and cardiovascular disease [37]. However in this study that risk was outweighed by the merit of CLA. There is a positive relationship between plasma n-6/n-3 ratio with inflammatory marker such as IL-6 and TNF-α in healthy adult as was reported by Ferrucci et al. [10]. An observational study of dietary n-3 and n-6 fatty acids intake in relation to plasma inflammatory markers in healthy men and women suggested that the combination of n-6 and n-3 fatty acids was associated with the declining levels of inflammation and might inhibit inflammatory cytokines [27].

Carotenoids content of black seabream muscle and viscera

Addition of 0.1% carotenoid in the diet resulted higher carotenoids content on CP10 and CP25 muscle and viscera compare to those in control (Table 4). The additions of same concentration of carotenoid incorporated with different level of CLA were yielded different deposition of carotenoid in muscle and viscera. The carotenoids level in muscle of control, CP10, and CP25 were 0.02±0.01, 0.04±0.0, and 0.05±0.01 mg/g, while in viscera were 0.38±0.00, 0.62±0.08, and 0.56±0.03 mg/g respectively. There is an extensive attention to incorporation of dietary carotenoids in consumption and ornamental fishes. The effects of carotenoids on growth performance are consistent with the acknowledged relationship between carotenoids signals to growth and health [14]. This study demonstrated that even though the CLA has fat reducing activity, addition of 0.1% carotenoids would diminish that effect and promoted growth on black seabream.

Table 4. Carotenoids content of muscle and viscera (mg/g) in black seabream fed with different diets

	Control	CP10	CP25
Muscle	0.02±0.01[a]	0.04±0.01[b]	0.05±0.01[b]
Viscera	0.38±0.00[a]	0.62±0.08[b]	0.56±0.03[b]

The values are mean ± S.D. (n=3). Different superscript letters within the same rows represent significant differences between treatments (p<0.05).

Effect of black seabream viscera's lipid extract (VLE) on 3T3-L1 cells viability

3T3-L1 preadipocytes were treated with black seabream VLE at various concentrations (1, 10, 50 and 100 μg/ml) for 24 hr during differentiation steps. Cells viability was determined with MTS assay. Fig. 1 showed that adipocytes viability was alleviated after treatment with 1 μg/ml VLE of

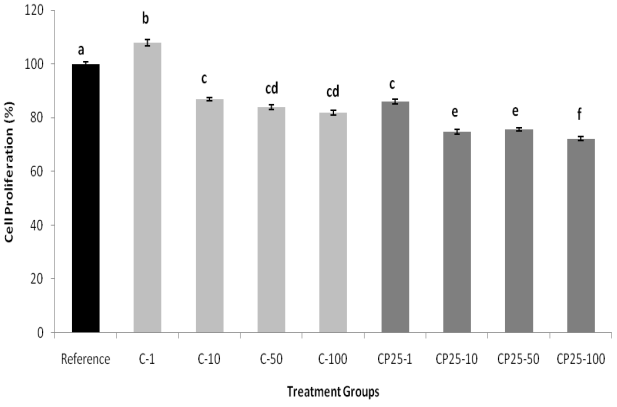

Fig. 1. Effect of black seabream viscera lipid extract (VLE) on 3T3-L1 preadipocytes. Cells viability was determined with MTS assay. All values are mean ± S.D. of triplicate. Different letters above the bars indicated significant differences between the treatments (p<0.05). C-1, C-10, C-50, and C-100 contain 1, 10, 50, and 100 μg/ml respectively of VLE from control group fishes; CP25-1, CP25-10, CP25-50, and CP25-100 contain 1, 10, 50, and 100 μg/ml respectively of VLE from CP25 fish which is contain sum of isomers *cis*-9, *trans*-11 and *trans*-10, *cis*-12 of CLA.

control fish group but it was lower on the other concentrations. Cell viability after treatment with 1, 10, 50 and 100 μg/ml VLE from control fish group were 107.9±1.2, 86.8±0.5, 83.9±0.8, and 81.9±0.9% respectively. Meanwhile, treatment with VLE from CP25 fish group were proofed lower adipocytes viability compare to those of reference and VLE of control group. They were 85.9±0.8, 74.8±0.9, 75.5±0.6, 72.1±0.7% for 1, 10, 50 and 100 μg/ml of CP25 feed group's VLE respectively. The effect of the treatment was dose dependent.

The declining viability of 3T3-L1 after treated with VLE during differentiation could be attributed to the present of PUFA on the VLE from control group and PUFA plus carotenoids on VLE CP25. This was aligned with study from Kim et al. [21] that suggested DHA inhibits adipocytes differentiation and induces apoptosis in 3T3-L1 preadipocytes. Han et al. [17] recommended that most of PUFA reduced triglyceride level and DHA inhibited palmitate-induced expression of SAA3 and MCP-1 which resulted smaller lipid droplet in adipocytes. Furthermore Inoue et al. [19] suggested that carotenoids especially astaxanthin would inhibit lipid accumulation trough modulation of PPARɣ target genes in 3T3-L1 adipocytes.

In conclusion, the present research demonstrated dietary CLA in corporate with carotenoids will increase CLA deposit in black seabream without hinder its growth. This diet regime generated higher final weight on black seabream, which are 205.6±21.8 and 224.6±41.1 g for CP10 and CP25 feed group, respectively and lower the VSI, therefore CLA-carotenoids enriched feed could be recommended as seabream growth enhancer. Dietary CLA 1% and 2.5% were resulted its deposit to the extent of 2.8% and 5.6% on muscle, and 4.0% and 8.3% in viscera of black seabream, respectively. The lipids extract from muscle and viscera of black seabream contain ample amount of beneficial substance such as CLA, carotenoids, EPA and DHA. These substances are known to have positive effect on human health. CLA enriched black seabream muscle could be categorized as functional food and serve as well-being food. Meanwhile, the fish oil from its viscera which declined the viability of 3T3-L1 cells could be utilized as high function supplement.

Acknowledgement

This study was funded by Technology Development Program in Yeongnam Seagrant (grant no. R&D/YSG-RB0801), Ministry of Land Transport and Maritime, The Republic of Korea.

References

1. AOAC International. 2003. Official methods of analysis of AOAC International. 17th edition, 2nd revision. Gaithersburg, MD, USA, Association of Analytical Communities.
2. Benitez-Santana, T., Masuda, R., Carrillo, J., Ganuza, E., Valencia, A., Hernandez-Cruz, C. M. and Izquierdo, M. S. 2007. Dietary n-3 HUFA deficiency induces a reduced visual response in gilthead seabream *Sparusaurata* larvae. *Aquaculture* **264**, 408-417.
3. Berge, G. M., Ruyter, B. and Asgard, T. 2004. Conjugated linoleic acid in diets for juvenile Atlantic salmon (*Salmo salar*); effects on fish performance, proximate composition, fatty acid and mineral content. *Aquaculture* **237**, 365-380.
4. Bligh, E. G. and Dyer, W. J. 1959. A rapid method of total lipid extraction and purification. *Can. J. Biochem. Physiol.* **37,** 911-917.
5. Breslow, J. L. 2006. Omega-3 fatty acids and cardiovascular disease. *Am. J. Clin. Nutr.* **83** S1477-S1482.
6. Brown, I., Wahle, K. W., Cascio, M. G., Smoum-Jaouni, R., Mechoulam, R., Pertwee, R. G. and Heys, S. D. 2011. Omega-3 N-acylethanolamines are endogenously synthesised from omega-3 fatty acids in different human prostate and breast cancer cell lines. *Prostaglandins Leukot. Essent. Fatty Acids* **85**, 305-310.
7. Chin, S. F., Liu, W., Storkson, J. M., Ha, Y. L. and Pariza, M. W. 1992. Dietary sources of conjugated dienoic isomers of linoleic acid, a newly recognized class of anticarcinogens. *J. Food Composit. Anal.* **5**, 185-197.
8. Choi, B. D., Kang, S. J., Ha, Y. L. and Ackman, R. G. 1999. Accumulation of conjugated linoleic acid (CLA) in tissues of fish fed diets containing various levels of CLA, pp. 61-71. In: Xiong, Y. L., Ho, C. T. and Shahidi, F. (eds.), *Quality Attributes of Muscle Foods.* Kluwer Academic/ Plenum Publishers, New York, USA.
9. Cockbain, A. J., Toogood, G. J. and Hull, M. A. 2012. Omega-3 polyunsaturated fatty acids for the treatment and prevention of colorectal cancer. *Gut* **61**, 135-149.
10. Ferrucci, L., Cherubini, A., Bandinelli, S., Bartali, B., Corsi, A., Lauretani, F., Martin, A., Andres-Lacueva, C., Senin, U., Jack, M. and Guralnik, J. M. 2006. Relationship of plasma polyunsaturatedfatty acids to circulating inflammatory markers. *J. Clin. Endocrinol. Metab.* **91**, 439-446.
11. Food and Agriculture Organization (FAO). 2010. World review of fisheries and aquaculture, pp. 3-89. In: FAO Fisheries and Aquaculture Department, (eds.), *The State of World Fisheries and Aquaculture*. FAO Sales and Marketing Group, Rome, Italy.
12. Graham, I. A., Larson, T. and Napier, J. A. 2007. Rational metabolic engineering of transgenic plants for biosynthesis of omega-3 polyunsaturates. *Curr. Opin. Biotechnol.* **18**, 142-147.

13. Gu, Z., Suburu, J., Chen, H. and Chen, Y. Q. 2013. Mechanisms of omega-3 polyunsaturated fatty acids in prostate cancer prevention. *Biomed. Res. Int.* **2013**, 824563.
14. Güroy, B., Sahin, I., Mantoglu, S. and Kayali, S. 2012. Spirulina as a natural carotenoid source on growth, pigmentation and reproductive performance of yellow tail cichlid *Pseudotropheusacei. Aquacult. Int.* **20**, 869-878.
15. Ha, Y. L., Grimm, N. K. and Pariza, M. W. 1987. Anticarcinogens from fried ground beef: heat-altered derivatives of linoleic acid. *Carcinogenesis* **8**, 1881-1887.
16. Halade, G. V., Rahman, M. M., Williams, P. J. and Fernandes, G. 2010. High fat diet-induced animal model of age-associated obesity and osteoporosis. *J. Nutr. Biochem.* **21**, 1162-1169.
17. Han, C. Y., Kargi, A. Y., Omer, M., Chan, C. K, Wabitsch, M., O'Brien, K. D., Wight, T. N. and Chait, A. 2010. Differential effect of saturated and unsaturated free fatty acids on the generation of monocyte adhesion and chemotactic factors by adipocytes. *Diabetes* **59**, 386-396.
18. Hardy, R. W. and Barrows, F. T. 2002. Diet formulation and manufacturing, pp. 505-600. In: Halver, J. E. and Hardy, R. W. (eds.), *Fish Nutrition*. Academic Press Inc., New York, USA.
19. Inoue, M., Tanabe, H., Matsumoto, A., Takagi, M., Umegaki, K., Amagaya, S. and Takahashi, J. 2012. Astaxanthin functions differently as a selective peroxisome proliferator-activated receptor g modulator in adipocytes and macrophages. *Biochem. Pharm.* **84**, 692-700.
20. Kim, M. J., Chang, U. J. and Lee, J. S. 2009. Inhibitory effects of fucoidan in 3T3-L1 adipocyte differentiation. *Mar. Biotechnol.* **11**, 557-562.
21. Kim, H. K., Della-Fera, M. A., Lin, J. and Baile, C. A. 2006. Docosahexaenoic acid inhibits adipocyte differentiation and induces apoptosisin 3T3-L1 preadipocytes. *J. Nutr.* **136**, 2965-2969.
22. Leaver, M. J., Tocher, D. R., Obach, A., Jensen, L., Henderson, R. J., Porter, A. R. and Krey, G. 2006. Effect of dietary conjugated linoleic acid (CLA) on lipid composition, metabolism and gene expression in Atlantic salmon (*Salmo salar*) tissues. *Comp. Biochem. Physiol.* **145A**, 258-267.
23. Lee, K. N., Kritchevsky, D. and Pariza, M. W. 1994. Conjugated linoleic acid and atherosclerosis in rabbits. *Atherosclerosis* **108**, 19-25.
24. Lowery, L. M., Appicelli, P. A. and Lemon, P. W. R. 1998. Conjugated linoleic acid enhances muscle size and strength gains in novice body builders. *Med. Sci. Sports Exerc.* **30**, S182.
25. Ostrowska, E., Muralitharan, M., Cross, R. F., Bauman, D. E. and Dunshea, F. R. 1999. Dietary conjugated linoleic acids increase lean tissue and decrease fat deposition in growing pigs. *J. Nutr.* **129**, 2037-2042.
26. Park, S. J., Park, C. W., Kim, S. J., Kim, J. K., Kim, Y. R., Park, K. A., Kim, J. O. and Ha, Y. L. 2002. Methylation methods for the quantitative analysis of conjugated linoleic acid (CLA) isomers in various lipid samples. *J. Agric. Food Chem.* **50**, 989-996.
27. Pischon, T., Hankinson, S. E., Hotamisligil, G. S., Rifai, N., Willett, W. C. and Timm, E. B. 2003. Habitual dietary intake of n-3 and n-6 fatty acids in relation to inflammatory markers among US men and women. *Circulation* **108**, 155-160.
28. Rodriguez-Amaya, D. B. and Kimura, M. 2004. Harvest Plus Handbook for Carotenoid Analysis. Washington DC, WD, USA.
29. Sapieha, P., Stah, A., Chen, J., Seaward, M. R., Willett, K. L., Krah, N. M., Dennison, R. J., Connor, K. M., Aderman, C. M., Liclican, E., Carughi, A., Perelman, D., Kanaoka, Y., San Giovanni, J. P., Gronert, K. and Smith, L. E. H. 2011. 5-Lipoxygenase metabolite 4-HDHA is a mediator of the antiangiogenic effect of n-3 polyunsaturated fatty acids. *Sci. Transl. Med.* **3**, 69 ra12.
30. Simopoulos, A. P. 2008. The omega-6/omega-3 fatty acid ratio, genetic variation, and cardiovascular disease. *Asia Pac. J. Clin. Nutr.* **17**, 131-134.
31. Tan, X. Y., Luo, Z., Xie, P., Li, X. D., Liu, X. J. and Xi, W. Q. 2010. Effect of dietary conjugated linoleic acid (CLA) on growth performance, body composition and hepatic intermediary metabolism in juvenile yellow catfish *Pelteobagrus fulvidraco. Aquaculture* **310**, 186-191.
32. Trichet, V. V. 2010. Nutrition and immunity: an update. *Aquacult. Res.* **41**, 356-372.
33. Twibell, R. G. and Wilson, R. P. 2003. Effects of dietary conjugated linoleic acids and total dietary lipid concentrations on growth responses of juvenile channel catfish, *Ictaluruspuncatus. Aquaculture* **221**, 621-628.
34. Twibell, R. G., Watkins, B. A., Rogers, L. and Brown, P. B. 2000. Effects of dietary conjugated linoleic acids on hepatic and muscle lipids in hybrid striped bass. *Lipids* **35**, 155-161.
35. Valente, L. M. P., Bandarra, N. M., Figueiredo-Silva, A. C, Rema, P., Vaz-Pires, P., Martins, S., Prates, J. A. M. and Nunes, M. L. 2007. Conjugated linoleic acid in diets for large-size rainbow trout (*Oncorhynchus mykiss*): effects on growth, chemical composition and sensory attributes. *Br. J. Nutr.* **97**, 289-297.
36. Watras, A. C., Buchholz, A. C., Close, R. N., Zhang, Z. and Schoeller, D. A. 2007. The role of conjugated linoleic acid in reducing body fat and preventing holiday weight gain. *Int. J. Obes.* **31**, 481-487.
37. Wijendran, V. and Hayes, K. C. 2004. Dietary n-6 and n-3 fatty acid balance and cardiovascular health. *Annu. Rev. Nutr.* **24**, 597-615.
38. Williams, C. D., Whitley, B. M., Hoyo, C., Grant, D. J., Iraggi, J. D., Newman, K. A., Gerber, L., Taylor, L. A., McKeever, M. G. and Freedland, S. J. 2011. A high ratio of dietary n-6/n-3 polyunsaturated fatty acids is associated with increased risk of prostate cancer. *Nutr. Res.* **31**, 1-8.
39. Yalpani, M. 2002. New technologies for health foods and nutraceuticals. *J. Food Sci. Nutr.* **7**, 222-229.
40. Yasmin, A. and Takeuchi, T. 2002. Influence of dietary levels of conjugated linoleic acid (CLA) on juvenile tilapia *Oreochromis niloticus. J. Zhejang Univ. Sci.* **9B**, 691-700.

초록 : CLA 첨가사료가 감성돔(*Acanthopagrus schlegeli*) 성장과 지방산 조성 및 내장 추출지방이 지방세포 3T3-L1에 미치는 영향

곽 뢰[1] · 쥴리야티 로마[1,2] · 최광수[3] · 박시향[4] · 하영래[5] · 강석중[1] · 최병대[1]*

([1]경상대학교 해양식품생명의학과, [2]가자마다대학교 생물학과, [3]경상대학교 해양환경공학과, [4]선마린바이오, [5]경상대학교 환경생명화학과)

공액리놀레산(CLA)과 카로테노이드 일정량을 함유하는 두 가지 사료구와 대조구를 감성돔(*Acanthopagrus schlegeli*)에 급여하였다. 대조구에는 CLA와 카로테노이드가 포함되지 않았고, CP10구에는 CLA 1%와 카로테노이드 0.1%가 포함되었고, CP25구에는 CLA 2.5%와 카로테노이드 0.1%가 포함되었다. 대조구, CP10 및 CP25구의 일일성장률(SGR)은 0.74, 0.81, 0.97이었고, 사료요구율은 2.65, 2.46 및 2.04이었다. 사육 후 근육 내에 축적된 CLA 함량은 CP10 및 CP25구에서 각각 2.8% 및 5.6%였으며, 내장에 축적된 CLA 함량은 4.0% 및 8.3%였다. 한편, CP25구의 내장에서 추출된 지용성 물질(VLE)을 첨가한 3T3-L1 지방세포 생존율은 대조구와 비교하였을 때 처리량에 따라 영향을 받는 것으로 나타났다. 따라서 CP25구의 VLE 추출물 농도를 1, 10, 50 및 100 μg/ml으로 처리하였을 때 각각 85.9±0.8%, 74.8±0.9%, 75.5±0.6% 및 72.1±0.7% 감소시켰다. 이와 같은 사료로 감성돔을 사육하는 경우 CP10 및 CP25구의 내장에서 추출한 지질함량 중 CLA함량은 각각 4.0% 및 8.5%, 카로테노이드는 각각 0.62 mg/g 및 0.56 mg/g, EPA 및 DHA는 각각 11.7%와 10.8% 및 11.8%와 12.7%로 증가하였고, 총 PUFA 함량도 각각 48.4% 및 50.5%로 증가하여 인체에 긍정적인 영향을 미치는 성분을 동시에 섭취할 수 있어 기능성 어류의 생산이 가능한 것으로 나타났다.