

## Anti-obesity effects of Lipid Extract from Sea-reared of Rainbow Trout (*Oncorhynchus mykiss*) Fed with Sea Squirt (*Halocynthia roretzi*) Tunic's Carotenoids and CLA

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### Abstract

**Purpose:** In order to produce new functional seafood, rainbow trout were acclimation in seawater and fed with inclusion of CLA and carotenoid extract from sea squirt tunic. Lipid extract from the sea-reared rainbow trout muscle (RME) and viscera (RVE) were administered to rats simultaneously with normal or high fat diet for six weeks. We evaluated the effects of the dietary supplementation with CLA, carotenoids, and n-3 HUFA by the beneficial effects of anti-obesity activities.

**Materials and method:** Sea-reared rainbow trout (800 ± 20.5 g; 40.0 ± 1.7 cm) were fed with basal feed which supplemented with 10 mg/kg sea squirt tunic's carotenoids and 3% CLA (w/w) for three months. Lipid was extracted from rainbow trout muscle and viscera using chloroform/methanol (2/1). Forty eight male Sprague Dawley rats (400 ± 2.0 g) were randomly divided into six groups (n=8). Two types of diets, normal types (SN, RVN, RMN) and 45% of high fat research diets (SHF, RVHF, RMHF) were used. The excised liver and epididymal adipose tissues were fixed in 3% formaldehyde solution, embedded in paraffin. Organs sections were stained with hematoxylin-eosin. Histological examination on liver and adipose tissues sections were carried out with a light microscope.

**Results:** Sea farming and inclusion of CLA and carotenoids pigment extracted from the tunic of sea squirt, the content of C18:1n-9 was reduced to 32.5% in the muscle's lipid and 35.8% in the viscera's lipid. Serum lipid and liver enzymes activity in blood serum showed that RME and RVE have the liver protective ability.

**Conclusion:** RME and RVE have potency as weight reducer through fat burner routes as well as liver protective ability. Hence, it could be considered as anti-obesity supplement for the long period of administration.

**Keywords:** CLA; Carotenoids; Anti-obesity; Sea-reared rainbow trout; High fat diets

### Introduction

Rainbow trout (*Oncorhynchus mykiss*, Walbaum 1792) is red muscle fish and known to accumulate carotenoids, especially astaxanthin, in its muscle. The degree of redness will determine the price of it. They are not synthesized in the animal's body, but are ingested from food. While wild rainbow trout naturally take in carotenoids from food, farmed rainbow trout should be supplemented with this substance from feed. The pigment inclusion into feed will increase the costs of the rainbow trout farming industry [1,2]. Sea squirts' tunic, a byproduct of seafood industry in Korea [3], contains many high value substances which one of those is carotenoids. It has 13 types of carotenoids isomers which 6 most abundance of those were alloxanthin, halocynthiaxanthin, diatoxanthin, diadinochrome, mytiloxanthinone, and astaxanthin, as well as minor concentration of lutein, mytiloxanthin, and β-carotene [4]. The sea squirt's tunic is a promising alternative of carotenoid for fish culture [5].

Conjugated linoleic acid (CLA) is one of the geometrical and position isomers of linoleic acid. It has been discovered that this compound naturally exists in diverse foods, such as dairy products or poultry. CLA has powerful anti-adiposity and decrease body fat deposition and changes the route of fat metabolism [6-8]. Body fat is a substance that causes an insulin resistance syndrome, similar to what is observed in type 2 diabetes [9]. Therefore, the effect of CLA intake was shown to reduce body fat while increasing the amount of muscle in mammals. According experiment on mouse models, feeding CLA with fish oil not only increased insulin sensitivity, but it also led to the accumulation of bone marrow fat, caused an inflammatory reaction, and reduced the oxidative stress associated with aging [6].

CLA was also reported to have chemo-protective effects in several tissues during multiple stages of carcinogenesis [10] as well as immune enhancing qualities [6] and possible anti-atherogenic properties [11]. Most of these experiments were used free CLA directly from plant. On the other hand, in this experiment we used CLA that have been incorporated into the muscle or viscera of sea-reared rainbow trout.

This study aimed to develop functional seafood, by combining the beneficial effects of anti-obesity activities of CLA, carotenoids, and n-3 HUFA from the sea-reared rainbow trout's lipid. The rainbow trout was fed with inclusion of sea squirt tunic's carotenoid and CLA, and then the fish oil extracted from sea-reared rainbow trout supplemented to male Sprague Dawley rats. The nutritional aspects were compared with normal and high fat diet group. The bioactivities of accumulated CLA and sea squirt tunic's carotenoid on rainbow trout's expectedly would be conveyed into secondary consumer. Therefore, our goal on tailoring value added fish would be achieved.

## Materials and Method

### Materials

The refined, bleached, deodorized CLA-rich soybean oil was provided by HK Biotech (Jinju, Korea). CLA FAME authentic standards (Sigma-Aldrich Inc., St. Louis, MO, USA) and FAME of menhaden oil standard (Sigma-Aldrich Inc., St. Louis, MO, USA) were used as comparison. All lipid extraction solvents and chemicals for analysis were analytical grade.

### Animals, diets and experimental design

All procedures and experiments with animals in this study were done under animal ethics, supervised and approved by Chosun University Animal Care and Use Committee (CIACU2015-A0045). Forty eight male Sprague Dawley rats ( $400 \pm 2.0$  g) were obtained from Jungang Experimental Animal Co. Ltd. (Seoul, Korea). Animals' treatments were carried out at Animal Research Center Chosun University. Rats were randomly divided into 6 groups ( $n=8$ ). Acclimation were done for 1 week prior of the feeding treatments. Two types of diets, normal type and 45% of high fat research diets were used in this experiment. These were supplemented with three different lipid extracts (soybean oil, rainbow trout viscera's lipid extract (RVE), and muscle lipid extract (RME)) which given orally. Experiment was carried out with randomized block design (Table 1) and done for 6 weeks. The doses of lipid extracts were calculated based on CLA daily dose for human diet (3.0 g/60 kg per day). Water and feed were given ad libitum. The rats' chambers were kept at constant temperature ( $18 \pm 2^\circ\text{C}$ ) and 12 h photoperiods (08:00-20:00). The initial and final body weights of all rats were measured. Weight gain (g/day) was calculated by dividing final weight minus initial weight with days of treatment.

### Rainbow trout lipid extraction and fatty acids analysis

Sea-reared rainbow trout ( $800 \pm 20.5$  g;  $40.0 \pm 1.7$  cm) were fed with basal feed which supplemented with 10 mg/kg sea squirt tunic's carotenoids and 3% CLA (w/w) for 3 months. Lipid was extracted from rainbow trout muscle and viscera using chloroform/methanol (2/1) and total lipid was measured gravimetrically [12]. Fatty acids methyl ester (FAME) analysis was done with method as reported [13]. CLA FAME was analysis by method which briefly as follow: fifty milligrams of lipid extract was added with 1.5 mL 1.0 N NaOH. The mixture was heated at  $100^\circ\text{C}$  for 10 min and cooled to room temperature afterward [14]. Three milliliters of 1.0 N  $\text{H}_2\text{SO}_4$  was added to solution and incubated at  $55^\circ\text{C}$  for 20 min. FAME was partitioned to isooctane by adding 1 mL of it to the mixture. The Isooctane fractions were analyzed with gas chromatography (Clarus 600, Perkin Elmer Co. Ltd., Waltham, MA, USA) and Omegawax-320 column (30 m  $\times$  0.32 mm ID) from Supelco Co. (Bellefonte, PA, USA). Injector and flame-ionization was set at

$250^\circ\text{C}$ . The carrier gas was helium with column-inlet pressure set at constant value,  $1.0 \text{ kg/cm}^2$  and split ratio was 1:100. Oven temperature was set at  $180^\circ\text{C}$  for initial time 8 min to  $230^\circ\text{C}$  with increasing rate  $3^\circ\text{C/min}$ , the final temperature was kept for 15 min. FAME authentic standards of menhaden oil and CLA (Sigma-Aldrich Inc., St. Louis, MO, USA) were used as comparison.

Feed Supplement	Feed	
	Normal	High fat diet
Soybean oil	SN	SHF
Rainbow trout viscera lipid extract (RVE)	RVN	RVHF
Rainbow trout muscle lipid extract (RME)	RMN	RMHF
Fatty acids	RME (%)	RVE (%)
16:0	$17.8 \pm 0.5^a$	$17.4 \pm 0.2^a$
18:0	$6.1 \pm 0.3^a$	$5.6 \pm 0.0^a$
18:1n-9	$32.5 \pm 0.1^a$	$35.8 \pm 0.3^b$
18:2n-6	$12.4 \pm 0.5^a$	$13.3 \pm 0.3^b$
20:5n-3	$1.5 \pm 0.3^a$	$1.5 \pm 0.3^a$
22:6n-3	$5.7 \pm 0.3^a$	$6.5 \pm 0.3^b$
CLA <sup>1</sup>	$9.1 \pm 0.4^a$	$12.5 \pm 0.2^b$
- c9, t11	$8.2 \pm 0.1^a$	$9.1 \pm 0.2^b$
- t10, c12	$0.5 \pm 0.0^a$	$2.1 \pm 0.0^b$
- t8, t10	$0.4 \pm 0.0^a$	$1.3 \pm 0.0^b$
$\Sigma$ saturates	$27.1 \pm 0.2^a$	$25.7 \pm 0.3^b$
$\Sigma$ monoenes	$42.0 \pm 3.0^a$	$35.6 \pm 0.3^b$
$\Sigma$ polyenes	$30.9 \pm 0.1^a$	$38.7 \pm 0.2^b$

**Table 1:** Experimental design of rat feeding regiment and fatty acids composition (%) of RME and RVE [Data are represented as mean  $\pm$  SE ( $n=3$ ). Means at same row that followed with different letters are significantly different at the 5% level of significance. <sup>1</sup>CLA: Conjugated linoleic acid and isomers].

### Measurement of TG and TC concentrations in liver and adipose tissues

Lipid from liver, epididymal and mesenteric adipose tissues were extracted according to Folch's method [12]. After gravimetric analysis of the lipid extract, it was then divided into subsamples of 500  $\mu\text{L}$  each. TG and TC contents of liver and adipose tissues were then measured [15, 16].

### Determination of lipoprotein lipase (LPL) activity

Determination of LPL activity was done according to lipoprotein lipase method [17]. Briefly, 50 mg frozen epididymal and mesenteric adipose tissues were minced. These were then defrosted in 0.4 mL of Medium 199 (Gibco BRL, Carlsbad, CA, USA) containing 1% bovine serum albumin and 50 U/mL of heparin. The solutions were incubated in shaking incubator at  $24^\circ\text{C}$  for 45 min. LPL activity in the present of

heparin was measured using the glycerol stabilized <sup>3</sup>H-triolein emulsion as substrate. One unit of LPL activity was defined as the release of 1 μmol of free fatty acid in 1 h.

### Histologic procedure for hepatic tissue lipid accumulation assessment and measurement of adipocyte size

The excised liver and epididymal adipose tissues were fixed in 3% formaldehyde solution, embedded in paraffin. Organ sections were stained with hematoxylin-eosin. Histological examination on liver and adipose tissues sections were carried out with a light microscope (×100 magnifications). The diameters of adipocytes were measured in 10 randomly chosen adipocytes from 10 slides of organs of each independent animals using Olympus microscope system (www.olympus.co.kr) equipped with micrometer. Oil red O staining was used as special staining for hepatic lipid deposition. The fresh frozen liver was sections at 6 μm thick and mounted on slides. The fixation was done with 10% formalin. The slides were then wash with distilled water and let air dry. Absolute propylene glycol was use as dehydration agent. Slides were stained with pre-warmed Oil Red O solution (Sigma-Aldrich Inc.) for 8-10 minutes in 60°C oven. Differentiation was carried out in 85% propylene glycol solution for 2-5 minutes and rinsed with distilled water. Mayer's hematoxylin was used as counterstain. This were quantify by counting the number of lipid droplets (diameter>1 μm) in 5 randomly chosen microscopic area (100 μm<sup>2</sup>) of liver of each independent animals.

### Statistical analysis

The statistical analyses were carried out using Statistical Package for Social Science (SPSS Inc., Chicago, IL, USA, version 12.0) for in vivo assays and Minitab 17.1.0 (Pennsylvania, USA). Each set of data was analyzed with one-way analysis of variance (ANOVA), Tukey's range test was performed as post-hoc test. The difference of the means was considered significant when p-value was less than 0.05.

## Results and Discussion

### Fatty acids composition of rainbow trout muscle extract (RME) and viscera extract (RVE)

The fatty acid composition of RME and RVE is described in Table 1. Although there were no significant differences in the fatty acid compositions of RME and RVE, the content of monoenoic and saturated fatty acid of the second was lower than the previous one. However, the content of polyenoic of RVE was higher than RME (p<0.05). The highest content of C18:1n-9 (oleic acid) and accumulated CLA was found in RVE. Oleic acid is often reported to be the most abundant MUFA in feed and shall be reflected on the fish fatty acid content. The abundance of oleic acid in feed was indirect result of replacement of fish oil with plant oil [18]. To date, researches indicate that up to 80-90% of fish oil can be replaced by vegetable oils and other fat resources in rainbow trout diets without affecting its growth. However, the n-3 PUFA of fish fillet can be affected by this feed alteration. Hence the fillet quality is importance for consumers can be altered and manipulated [19].

In this experiment, as a result of sea farming and inclusion of CLA and carotenoid pigment extracted from the tunic of sea squirt, the

content of C18:1n-9 was reduced to 32.5% in the RME and 35.8% in the RVE. At the same time the content of C22: 6n-3 was increased to 5.7% and 6.5%, for RME and RVE respectively. This suggested that carotenoid extract of sea squirt tunic and dietary CLA would increase the quality of sea-reared rainbow trout lipid content.

### Bioactivities of lipid extract from sea-reared rainbow trout treated with dietary sea squirt tunic's carotenoids and CLA on rats

Feeding RME and RVE to rats would increase it's the food efficiency ratio (FER) especially the one which was fed with high fat diets (Table 2). Table 2 also shows that RME and RVE would significantly (p>0.05) lower the weight gain on high fat groups, though it does not make significantly different for the normal diet groups (p>0.05). The decreasing of the rat body weight could be accounted to the effect of RME and RVE, given that the feed intake among the groups were not significantly different (p>0.05). This result was in agreement with Baraldi et al. [20] who investigated whether dietary supplementation of CLA and extra virgin olive oil (EVOO) would affect the mice metabolism. They reported that there were changes on body metabolism associated with mitochondrial energetics on male C57Bl/6 mice. Even though there were no clear differences for weight gain among the groups.

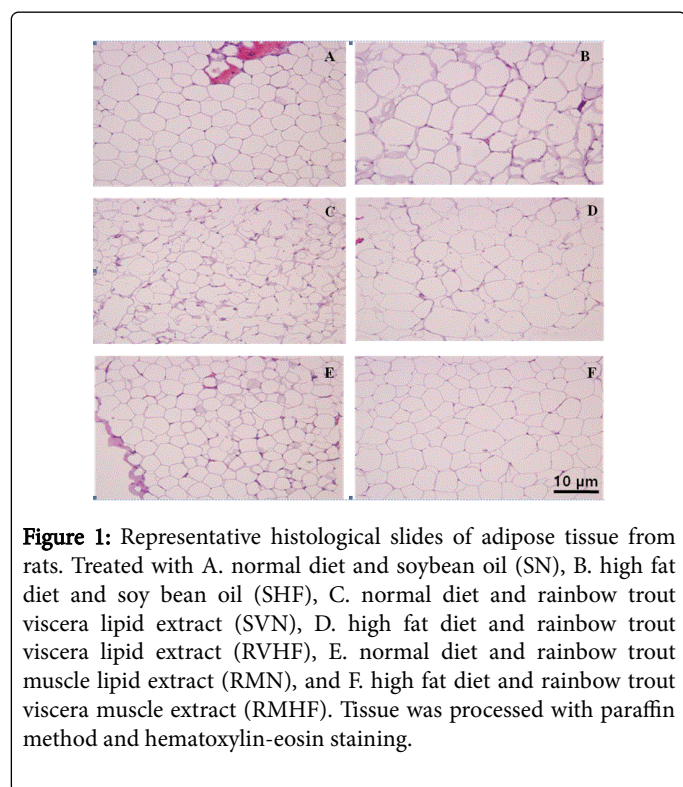
The treatment groups (RVN, RMN, RVHE, and RMHF) had lower total lipid weight compare to high fat diet control group (SHF) (Table 2). The CLA content of RME and RVE was most prominent to affect the retroperitoneal adipose tissue mass. On the other hand, the treatment showed no effect on other adipose tissues in the body (p>0.05). Even though, some of the adipose tissues (perirenal and mesenteric) started to be decreased. This result was similar to those reported [20, 21]. Mice fed CLA- and CLA+EVOO-supplemented diet had less white adipose tissue weight compared to that of control mice [20].

The peritoneal adipose tissue was the most reactive toward CLA treatments [22]. In contrast of this finding that the epididymal adipose tissue is the less responsive, in this study epididymal adipose tissue is more responsive to the treatment than mesenteric and perirenal ones. Recent study suggested that supplementation CLA decreases fat deposition by inducing adipocyte apoptosis [23]. The anti-obesity effect of CLA has been ascribed to reduced adipocyte size, reduced adipocyte proliferation, increased adipocyte lipolysis and apoptosis, as well as to enhanced fatty acid oxidation and energy expenditure, which is particularly found to be higher in t10-CLA-fed mice [24].

Since the accumulation of neutral fat in fat cells due to the intake of a high fat diet is a result of increased fat cell diameter, measuring the size of fat cells is an effective way to prove anti-obesity effects [25]. Therefore, this study monitored the size of epididymal fat cells, and the results are shown in Figure 1 and Table 2. The size of the epididymal fat cells in the group that was orally administered the RME and RVE in the normal or high fat diet tended to decrease when compared to the SN and SHF groups, which were administered the soybean oil. According to the measurements (Table 2), the size of the fat cells was in the order of SHF≥RVHF≥RMHF≥SN>RVN>RMN (p<0.05). It appears that the size of the fat cells of the white mice that orally administered RME and RVE were decreased as a result of controlling fat accumulation (Figures 1C-1E).

	Groups					
	SN	RVN	RMN	SHF	RVHF	RMHF
Initial weight (g)	192.8 ± 4.8 <sup>NS</sup>	192.8 ± 4.2	192.8 ± 6.4	192.7 ± 4.9	193.0 ± 3.1	193.7 ± 4.5
Final weight (g)	460.0 ± 18.8 <sup>NS</sup>	456.3 ± 16.7	454.8 ± 14.8	495.9 ± 12.5	468.7 ± 11.3	433.0 ± 14.6
Weight gain (g/day)	6.4 ± 0.4 <sup>ab</sup>	6.3 ± 0.3 <sup>ab</sup>	6.2 ± 0.4 <sup>ab</sup>	7.2 ± 0.3 <sup>a</sup>	6.6 ± 0.2 <sup>ab</sup>	5.7 ± 0.4 <sup>b</sup>
Feed intake (g/day)	25.2 ± 1.1 <sup>NS</sup>	24.7 ± 1.0	24.8 ± 1.5	21.4 ± 1.6	21.3 ± 1.1	20.2 ± 0.9
FER <sup>1</sup>	0.25 ± 0.00 <sup>c</sup>	0.25 ± 0.01 <sup>bc</sup>	0.25 ± 0.01 <sup>c</sup>	0.34 ± 0.02 <sup>a</sup>	0.31 ± 0.01 <sup>ab</sup>	0.28 ± 0.01 <sup>bc</sup>
Liver (g)	14.0 ± 0.5 <sup>NS</sup>	13.9 ± 0.5	13.3 ± 0.4	15.2 ± 0.4	14.1 ± 0.6	13.7 ± 0.5
Weight of adipose tissue (g)						
- Epididymal	6.8 ± 0.8 <sup>NS</sup>	7.0 ± 0.6	6.8 ± 0.6	9.2 ± 0.5	8.8 ± 0.6	9.0 ± 0.5
- Mesenteric	4.6 ± 0.39 <sup>NS</sup>	4.4 ± 0.5	4.5 ± 0.6	5.6 ± 0.5	4.6 ± 0.5	4.6 ± 0.4
- Retroperitoneal	9.3 ± 0.6 <sup>ab</sup>	7.1 ± 0.47 <sup>b</sup>	8.3 ± 1.26 <sup>ab</sup>	11.6 ± 0.80 <sup>a</sup>	9.5 ± 0.84 <sup>ab</sup>	9.8 ± 0.53 <sup>ab</sup>
- Perirenal	2.4 ± 0.3 <sup>NS</sup>	2.4 ± 0.3	2.2 ± 0.4	3.5 ± 0.4	2.6 ± 0.4	2.3 ± 0.2
- Total	23.2 ± 1.7 <sup>ab</sup>	20.9 ± 1.6 <sup>b</sup>	21.8 ± 2.8 <sup>ab</sup>	29.9 ± 2.1 <sup>a</sup>	25.6 ± 2.1 <sup>ab</sup>	25.6 ± 1.1 <sup>ab</sup>
Adipocytes diameter (µm)	4.7 ± 0.6 <sup>bc</sup>	3.8 ± 1.0 <sup>c</sup>	4.0 ± 0.9 <sup>c</sup>	8.6 ± 1.4 <sup>a</sup>	5.5 ± 0.9 <sup>b</sup>	4.0 ± 0.6 <sup>c</sup>
Number of lipid droplet in liver	21.26.0 <sup>b</sup>	13.84.8 <sup>cd</sup>	12.83.0 <sup>d</sup>	28.82.6 <sup>a</sup>	21.42.4 <sup>b</sup>	20.61.7 <sup>bc</sup>

**Table 2:** Weight gain, feed intake, feed efficiency ratio (FER), liver, adipose tissue weights, and Adipocytes diameter of rats treated with RME and RVE. Data are represented as mean ± SE (n=6). Values with different superscripts in the same row are significantly different (p<0.05) from each other. NS: not significant. <sup>1</sup>FER (feed efficiency ratio): weight gain (g/day)/feed intake (g/day).



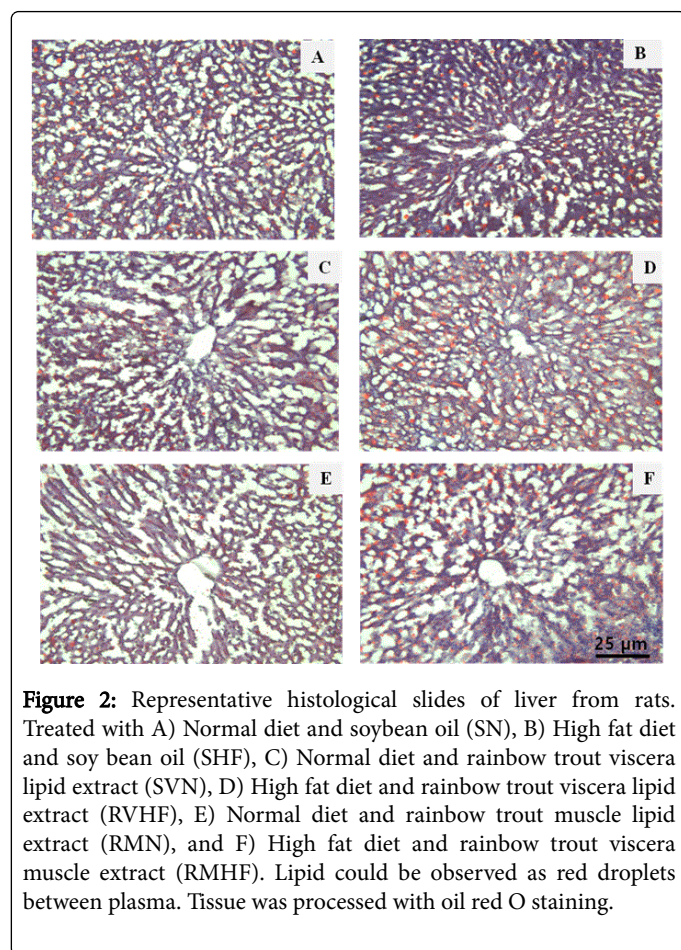
**Figure 1:** Representative histological slides of adipose tissue from rats. Treated with A. normal diet and soybean oil (SN), B. high fat diet and soy bean oil (SHF), C. normal diet and rainbow trout viscera lipid extract (SVN), D. high fat diet and rainbow trout viscera lipid extract (RVHF), E. normal diet and rainbow trout muscle lipid extract (RMN), and F. high fat diet and rainbow trout viscera muscle extract (RMHF). Tissue was processed with paraffin method and hematoxylin-eosin staining.

The result of the oil red O staining on liver is shown in Figure 2. Lipid was identified as red color droplet among hepatocytes. There was clear evidence of excessive of fat accumulation on the liver which can cause to fatty lipid [24]. The number and size of lipid droplet varied among treatment groups (Table 2 and Figure 2). Result revealed that SHF has the highest number of lipid droplets compare to other groups (p<0.05).

### Biochemicals change of serum

Table 3 shows the analysis of the serum of the rat fed RME and RVE. Compared to the normal diet groups, the serum activity of alanine transaminase (ALT), aspartate transaminase (AST), alkaline phosphatase (ALP), and lactate dehydrogenase (LDH) all increased in the high fat diet groups (SHF, RVHF, and RMHF). It has been reported that the present and activities of ALT and AST in the serum corresponded to the liver function. The release of these enzymes from liver will be accelerated on as high-fat diets, high-cholesterol diets, and alcohol consumption. These diets and consumption caused fatty liver or the accumulation of liver toxic substances which damaged the liver tissues [26]. In this experiment, RVE significantly affected the concentration of ALT and AST compare to RME (p<0.05) in both normal diet and high fat diets. This result showed that RVE is good for health compare to RME.





**Figure 2:** Representative histological slides of liver from rats. Treated with A) Normal diet and soybean oil (SN), B) High fat diet and soybean oil (SHF), C) Normal diet and rainbow trout viscera lipid extract (SVN), D) High fat diet and rainbow trout viscera lipid extract (RVHF), E) Normal diet and rainbow trout muscle lipid extract (RMN), and F) High fat diet and rainbow trout viscera muscle extract (RMHF). Lipid could be observed as red droplets between plasma. Tissue was processed with oil red O staining.

The present of ALP in the blood serum was sign of biliary duct obstruction or hepatocytes membrane damage [27]. This could be repaired by increasing the concentration of cholesterol and elimination disorders of bile acid in the liver. In addition, LDH is an enzyme that is involved in both oxidation and reduction reactions at the final stage of

the internal anaerobic process; the level of this enzyme is known to significantly increase in acute hepatitis, early hepatitis, myocardial infarction, pernicious anemia, and leukemia [28]. In this research however, there was no significant difference in the activity of AST and LDH among experimental groups (Table 3). The activity of ALT and ALP in the SHF were the highest compared to the other groups. This demonstrated that high fat diets could cause obstruction on liver function. Whilst, group fed the RME and RVE showed lower concentration of those enzymes. This revealed that CLA and carotenoid pigment extracted from sea squirt tunic exhibited liver-protective actions even though it has been accumulated in rainbow trout.

Table 4 shows the results of neutral lipid levels (TG), total cholesterol (TC), low-density lipoprotein (LDL) cholesterol and high-density lipoprotein (HDL) cholesterol in the serum, as well as the arteriosclerosis index and the cardiovascular index calculation. According to the analysis, the groups that fed the high fat diet had higher levels of TG, TC, and LDL-cholesterol, as well as high values for the arteriosclerosis index and cardiovascular index, but a low level of HDL-cholesterol when compared to those groups administered the regular diet. There was no significant ( $p > 0.05$ ) difference between the experimental groups in terms of TC, HDL-cholesterol levels, arteriosclerosis index values, and cardiovascular index values in the serum. On the other hand, the level of TG and the content of LDL-cholesterol in serum was significantly different ( $p < 0.05$ ).

In mammals, the effect of CLA on blood lipid levels was ever ambiguous. In mice, TG and TC levels and the (TC-HDL)/HDL ratio decreased, but HDL and lipoprotein lipase (LPL) levels increased with increasing CLA addition [29]. In the comparison among the groups that fed with high fat diet, SHF had the highest levels of TG and LDL-cholesterol compared to the others ( $p < 0.05$ ). In contrast, RMN had the significantly lowest levels of TG and LDL-cholesterol in serum compare to other groups. Addition of dietary CLA in the feed of obese juvenile *A. schrenckii* resulted in a lower growth rate [30]. Dietary CLA reduced body and liver lipid accumulation as well as lower lipid level in the blood. An inclusion of 2.0% CLA was found adequate to achieve the maximum effect.

Groups	Serum activity (IU/L)			
	Alanine amino transferase (ALT)	Aspartate amino transferase (AST)	Alkaline phosphatase (ALP)	Lactate dehydrogenase(LDH)
SN	18.3 ± 1.4 <sup>b</sup>	95.8 ± 5.7 <sup>NS</sup>	432.3 ± 32.0 <sup>b</sup>	493.8 ± 68.5 <sup>NS</sup>
RVN	18.8 ± 1.1 <sup>b</sup>	98.7 ± 4.5	514.7 ± 26.6 <sup>ab</sup>	453.8 ± 46.7
RMN	21.2 ± 0.9 <sup>ab</sup>	118.2 ± 9.0	421.0 ± 35.3 <sup>b</sup>	683.2 ± 79.5
SHF	27.8 ± 1.8 <sup>a</sup>	125.3 ± 5.14	678.2 ± 64.0 <sup>a</sup>	603.3 ± 87.4
RVHF	24.8 ± 1.7 <sup>ab</sup>	109.5 ± 12.8	654.5 ± 36.5 <sup>a</sup>	535.8 ± 87.1
RMHF	26.7 ± 2.7 <sup>a</sup>	124.7 ± 12.1	542.0 ± 42.2 <sup>ab</sup>	680.0 ± 113.0

**Table 3:** Serum activities of AST, ALT, ALP and LDH of rats treated with RME and RVE. Data are represented as mean ± SE (n=8). Values with different superscripts in the same column are significantly different ( $p < 0.05$ ). NS: Not significant.

Groups	Concentration (mg/dL)				AI <sup>1</sup>	CRF <sup>2</sup>
	Triglyceride	Total Cholesterol	LDL-cholesterol	HDL-cholesterol		
SN	78.7 ± 6.4 <sup>b</sup>	83.3 ± 3.0 <sup>NS</sup>	52.2 ± 3.4 <sup>bc</sup>	46.8 ± 1.7 <sup>NS</sup>	0.79 ± 0.08 <sup>NS</sup>	1.79 ± 0.08 <sup>NS</sup>
RVN	81.0 ± 7.3 <sup>b</sup>	84.0 ± 5.7	52.0 ± 7.8 <sup>bc</sup>	48.2 ± 5.3	0.88 ± 0.27	1.88 ± 0.27
RMN	75.3 ± 10.1 <sup>b</sup>	81.7 ± 3.0	46.7 ± 6.9 <sup>c</sup>	50.0 ± 5.8	0.78 ± 0.28	1.78 ± 0.28
SHF	135.2 ± 13.9 <sup>a</sup>	101.8 ± 5.8	89.0 ± 6.9 <sup>a</sup>	39.8 ± 2.0	1.59 ± 0.17	2.59 ± 0.17
RVHF	118.2 ± 3.8 <sup>a</sup>	88.2 ± 4.7	68.5 ± 3.8 <sup>abc</sup>	43.3 ± 3.5	1.07 ± 0.11	2.07 ± 0.11
RMHF	104.7 ± 6.6 <sup>ab</sup>	98.2 ± 6.9	75.1 ± 8.3 <sup>ab</sup>	44.0 ± 1.9	1.27 ± 0.22	2.27 ± 0.22

**Table 4:** Serum concentrations of triglyceride, total cholesterol LDL-cholesterol, HDL-cholesterol, AI and CRF of rats treated with RME and RVE. Data are represented as mean ±SE (n=8). Values with different superscripts in the same column are significantly different (p<0.05). NS: Not significant. <sup>1</sup>AI (atherosclerotic index)=(total cholesterol-HDL-cholesterol)/HDL-cholesterol. <sup>2</sup>CRF (cardiac risk factor)=total cholesterol/HDL-cholesterol.

## Conclusions

New functional seafood based on sea-reared rainbow trout inclusion with CLA, carotenoids, and n-3 HUFA was developed. RME and RVE have potency as weight reducer through fat burner routes as well as liver protective ability. Hence, it could be considered as anti-obesity supplement of seafood for the long period of administration.

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## References

- Bjerkeng B (2008) Carotenoids in aquaculture: fish and crustaceans. Carotenoids. Britton G, Liaaen-Jensen S, Pfander HH (eds.), Springer Co, NY, USA.
- Jobling M, Tveiten H, Hatlen B (1998) Cultivation of Arctic char: An update. Aqua Interna 6: 181-196.
- KMI (Korea Maritime Institute), Fisheries outlook.
- Choi BD, Kang SJ, Choi YJ, Youm MG, Lee KH (1994) Utilization of ascidian (*Halocynthia roretzi*) tunic: Carotenoid composition of ascidian tunic. Korean J Fish Aqua Sci 27: 344-350.
- Rohmah Z, Jeong UC, Ticar BF, Kim JS, Lee JJ, et al. (2016) Effect of ascidian (*Halocynthia roretzi*, Drasche 1884) tunics carotenoids on enhancing growth and muscle coloring of sea-reared rainbow trout (*Oncorhynchus mykiss*, Walbaum 1792). Aquaculture Reports 4: 22-29.
- Cook ME, Miller CC, Park Y, Pariza MW (1993) Immune modulation by altered nutrient metabolism: Nutritional control of immune-induced growth depression. Poultry Sci 72: 1301-1305.
- Poirier H, Shapiro JS, Kim RJ, Lazar MA (2006) Nutritional supplementation with trans-10, cis-12-conjugated linoleic acid induces inflammation of white adipose tissue. Diabetes 55: 1634-1641.
- Terpstra AM, Javadi M, Beynen AC, Kocsis S, Lankhorst AE, et al. (2003) Dietary conjugated linoleic acids as free fatty acids and triacylglycerols similarly affect body composition and energy balance in mice. J Nutr 133: 3181-3186.
- Boden G, Laakso M (2004) Lipids and glucose in type 2 diabetes: What is the cause and effect? Diabetes Care 27: 2253-2259.
- Belury MA (1995) Conjugated dienoic linoleate: a polyunsaturated fatty acid with unique chemoprotective properties. Nutr Rev 53: 83-89.
- Lee KN, Kritchevsky D, Pariza MW (1994) Conjugated linoleic acid and atherosclerosis in rabbits. Atherosclerosis 108: 19-25.
- Folch J, Lees M, Sloane-Stanley G (1957) A simple method for the isolation and purification of total lipids from animal tissues. J Biol Chem 226: 497-509.
- Sabetian M, Delshad ST, Moini S, Islami HR, Motalebi A (2012) Identification of fatty acid content, amino acid profile and proximate composition in rainbow trout (*Oncorhynchus mykiss*). J Am Sci 8: 670-677.
- Park SJ, Park CW, Kim SJ, Kim JK, Kim YR, et al. (2002) Methylation methods for the quantitative analysis of conjugated linoleic acid (CLA) isomers in various lipid samples. J Agric Food Chem 50: 989-996.
- Biggs HG, Erikson TM, Moorehead WR (1975) A manual colorimetric assay of triglyceride in serum. Clin Chem 21: 437-441.
- Zlatkis A, Zak B (1969) Study of a new cholesterol reagent. Anal Biochem 29: 143-148.
- Nilsson-Ehle P, Schotz MA (1976) A stable, radioactive substrate emulsion for assay of lipoprotein lipase. J Lipid Res 17: 536-541.
- Baron CP, Svendsen GH, Lund I, Jokumsen A, Nielsen HH, et al. (2013) Organic plant ingredients in the diet of rainbow trout (*Oncorhynchus mykiss*): impact on fish muscle composition and oxidative stability. Eur J Lipid Sci Technol 115: 1367-1377.
- Turchini GM, Hermon K, Cleveland BJ, Emery JA, Rankin T, et al. (2013) Seven fish oil substitutes over a rainbow trout grow-out cycle: I) Effects on performance and fatty acid metabolism. Aquac Nutr 19: 82-94.
- Baraldi FG, Vicentini TM, Teodoro BG, Dalalio FM, Dechandt CRP, et al. (2016) The combination of conjugated linoleic acid (CLA) and extra virgin olive oil increases mitochondrial and body metabolism and prevents CLA-associated insulin resistance and liver hypertrophy in C57Bl/6 mice. J Nutr Biochem 28: 147-154.
- Goonewardene LA, Mir PS, Wang Z, Okine EK, Mir Z, et al. (2004) Study of carcass, organ, muscle, fat tissue weight, and concentration in rats fed CLA or its precursors by principal component analysis. Can J Animal Sci 84: 537-543.
- Azain MJ, Hausman DB, Sisk MB, Flatt WP, Jewell DE (2000) Dietary conjugated linoleic acid reduces rat adipose tissue cell size rather than cell number. J Nutr 130: 1548-1554.
- Qi R, Yang F, Huang J, Peng H, Liu Y, Liu Z (2014) Supplementation with conjugated linoleic acid decreases pig back fat deposition by inducing adipocyte apoptosis. BMC Vet Res 10: 141-147.

24. Halade GV, Rahman MM, Fernandes G (2010) Differential effects of conjugated linoleic acid isomers in insulin-resistant female C57Bl/6J mice. J Nutr Biochem 21: 332-337.
25. Kim HS, Kim TW, Kim DJ, Hwang HJ, Lee HJ, et al. (2007) Effects of natural plants supplementation on adipocyte size of the epididymal fat pads in rats. J Korean Soc Food Sci Nutr 36: 419-423.
26. Sollof LA, Futenberg HL, Lacko AG (1973) Serum cholesterol esterification in patients with coronary artery disease. Am Heart J 85: 153-161.
27. Lim SS, Kim MH, Lee JH (1997) Effect of *Artemisia princeps* var *orientalis* and *Cirsium japonicas* var *ussuriense* on liver functions body lipid and bile acid hyperlipidemic rat. Korean J Nutr 30: 797-802.
28. Ki HY, Song SW, Ha CS, Han SS (1993) Effects of the population density on growth and various physiological values of Sprague-Dawley rats. J Animal Sci Technol 9: 71-82.
29. Xiuheng X, Zhigeng W, Juhua W, Lin M, Mi L (2006) Effect of fortified conjugated linoleic acid milk on body fat and plasma lipids in mice. Acta Nutrimenta Sini 28: 120-123.
30. Yang CG, Wen H, Jiang M, Liu W, Wu F, et al. (2014) Effect of dietary conjugated linoleic acid supplementation on the growth, lipid metabolism, and plasma lipid levels of juvenile Amur sturgeon (*Acipenser schrenckii* Brandt, 1869). J Appl Ichthyol 30: 1626-1631.