www.trjfas.org
ISSN 1303-2712
*Turkish Journal of Fisheries and Aquatic Sciences 12: 105-113 (2012)* DOI: 10.4194/1303-2712-v12_1_13

# Digestibility and Liver Fatty Acid Composition of Rainbow Trout (*Oncorhynchus mykiss*) Fed by Graded Levels of Canola Oil

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Received 28 July 2011
Accepted 3 December 2011

**Abstract**

This study was aimed to determined the effects of different concentrations of canola oil (CO) on the digestibility, vicerosomatic index (VSI), hepatosomatic index (HSI) and the contents of fatty acids in the liver of rainbow trout (*Oncorhynchus mykiss*). Rainbow trouts were fed with the experimental diets for 70 days. The diets were given as triplicate groups of rainbow trout (initial weight of 119 g) to apparent satiation twice in a day. There were not significant differences in digestibility of protein and lipid between the experimental diets. VSI and HSI were not significantly different among the all treatment and between the initals and the end of trials ($P>0.05$). Liver fatty acid contents of all groups were different from each other. Liver fatty acid analyses showed that EPA and DHA from n-3 HUFA's in the fish fed by the fish oil diet (K) and the initial liver samples, oleic acid (C18:1n-9, OA) and linoleic acid (C18:2n-6, LA) in the fish fed by the canola oil ($C_{50}$, $C_{75}$, $C_{100}$) were higher than in the fish fed by the control diet. Arachidonic acid (C20:4n-6; ARA) were 2.54, 3.15, 4.15, 4.63 and 5.64% in $C_{75}$, K, $C_{100}$, $C_{50}$ groups and initial, respectively. n3/n6 rate was quite high in all groups.

*Keywords*: Rainbow trout, *Oncorhynchus mykiss,* liver, fatty acids, canola oil, digestibility

**Farklı Oranlarda Kanola Yağıyla Beslenen Gökkuşağı Alabalığı'nın (*Oncorhynchus mykiss*) Karaciğer Yağ Asit Kompozisyonu ve Sindirilebilirliği**

**Özet**

Bu çalışmada, yemlere farklı oranlarda ilave edilen kanola yağının gökkuşağı alabalıklarında karaciğer yağ asitleri kompozisyonu, hepatosomatik indeks, viserosomatik indeks ve sindirilebilirliğe etkisinin belirlenmesi amaçlanmıştır. Gökkuşağı alabalıkları 70 gün süre ile deneme rasyonları ile beslenmişlerdir. Rasyonlar balıklara görülebilir doygunluk sınırına erişinceye kadar günde iki kez verilmiştir. Deneme rasyonları arasında yağ ve protein sindiriminde önemli farklılıklar yoktur. VSI ve HSI'de deneme başlangıcı ve sonunda tüm gruplar arasında farklılığa rastlanmamıştır ($P>0.05$). Bütün grupların karaciğer yağ asitleri içerikleri birbirinden farklı tespit edilmiştir. Karaciğer yağ asidi analizi, deneme başlangıcı karaciğer örneklerinde ve balık yağı ilaveli rasyonla beslenen balıklarda n-3 HUFA'lardan EPA ve DHA, kanola yağı ilaveli rasyonlarla beslenen balıklarda ise oleik (C18:1n-9, OA) ve linoleik (C18:2n-6, LA) asitlerin daha yüksek olduğunu göstermiştir. Araşidonik asit (C20:4n-6; ARA) $C_{75}$, K, $C_{100}$, $C_{50}$ grupları ve başlangıç örneklerinde sırasıyla %2.54, 3.15, 4.15, 4.63 and 5.64 olarak belirlenmiştir. n3/n6 oranları ise tüm gruplarda oldukça yüksek tespit edilmiştir.

*Anahtar Kelimeler*: Gökkuşağı alabalığı, *Oncorhynchus mykiss*, karaciğer, yağ asitleri, kanola yağı, sindirilebilirlik

## Introduction

One of the factors which mostly affects the success of aquaculture in the future is having an advanced feed industry. Food expenses are the major element that restricts the feed production. Beside of the considering cost of food and fish nutrition requirement in the feed production, process techniques of feed raw materials and digestion rate of the fish also play very important role.

Determination of the nutrient digestibility is the first step in evaluation the potential of ingredients for use in the feeding of fish species (Allan *et al.,* 2000; Tibbetts *et al.,* 2006). More nutrients are used for formation of tissues in the body with the diets that is digested easily and less metabolism waste is dischared

out of the body. Therefore, the digestive ability of fish species that are important for aquaculture for various feed materials in different enviromental conditions should be determined (Yiğit and Ustaoğlu, 2003).

Lipids are important components of fish diets due to their role in providing energy sources, the essential role of some fatty acids, carriers of fat soluble vitamins and resource of polar lipids and sterols, which are important structural compounds of cell membranes. Fish and fish oils contain omega-3 fatty acids (n-3 polyunsaturated fatty acids) known as eicosapentaenoic acid (EPA, 20:5n-3) and docosahexaenoic acid (DHA, 22:6n-3), whereas plant foods and vegetable oils are devoid of these two important PUFA. However, it is well established that fish require EPA, DHA and arachidonic acid (ARA, 20:4n-6) for normal growth, development, and reproduction (Rodriguez *et al.,* 2004).

The importance of fish as a source of n-3 polyunsaturated fatty acids in human nutrition is widely realized. Among these acids, mainly EPA, DHA as well as its precursor, alpha linolenic acid (LNA, 18:3n-3) stand out. These acids are associated to numberless benefits to human health. DHA plays an important role in the formation, development and working of the brain and retina. EPA has anti-inflammatory properties, and in general contributes to the prevention of heart diseases and to the reduction of biochemical factors associated to cancer (Aguiar *et al.,* 2007).

Lipids that supplies energy are stocked in different tissues in fish, especially in muscle tissue, between the internal organs and liver (Steffens, 1997). Liver is one of the most important organs in fish, which shows the effects of the nutrient material in the diet on growth and development of fish. High amounts of lipids are stored in the liver of fish in case of usage of high amounts of lipids not including the essential fatty acids in the diets. It was reported that this caused liver degeneration (Caballero *et al.,* 1999).

Researchers have recently stated their studies that some parts of fish not consumed as food are suitable for human nutrition may be used in oil extraction. The existence of significant concentrations of polyunsaturated fatty acids in the viscera and liver has been reported (Aguiar *et al.,* 2007). Kozlova (1998) reported that the liver and muscle in fish serve as fat depots, whereby the liver is the main lipid storage organ in the body of many fish species. Rodriguez *et al.* (2004) indicated that the liver plays a critical role in various aspects of lipid metabolism (uptake, oxidation and conversion of fatty acids). However, liver plays a major role in metabolism and has a number of functions in the body, including glycogen storage, decomposition of red blood cells, plasma protein synthesis, hormone production, and detoxification (Anonymus, 2011b).

Therefore, the aim of the present study was to determine the effects of increasing dietary fish oil replacement levels with canola oil on apparent digestibility coefficients of the diets, VSI, HSI and liver fatty acid composition in rainbow trout (*Oncorhynchus mykiss*).

## Materials and Methods

### Fish and Maintenance

The experimental rainbow trout were obtained from a commercial trout farm, Kuzey Su Ürünleri Inc., in Bafra-Samsun and acclimated in Sinop University, Fisheries Faculty in Sinop (Turkey) for ten days before the start of the experiment. During acclimation, the fish were fed with a commercial diet twice a day to satiation. Fish were stocked in centrally drained three 1000-l(birim?) rectangle fiberglass tanks in a flow-through water system in an indoor facility during acclimation. After acclimatization, fish (mean weight of 119±0.17 g) were fasted for a day; batch weighted and randomly distributed among to twelve fiberglasses circular tanks (approximately, water volume 300-L; 60 cm in high; 80 cm in diameter) at a density of 30 fish per tank. The fish that used in the experiment were same size and weight. Water inflow was adjusted to 4 l/min and supplemental aeration was provided via airstone diffusers. The fish were individually weighted at the end and beginning of the experiment with a 1 g sensitive electronic balance. The water quality parameters were monitored on weekly basis and the following parameters were recorded: average temperature of 13.2±0.4°C, dissolved oxygen of 5.46±0.13 mg/l and pH 7.39. At the starting of the experiment, 20 fish were homogenized and analyzed for liver composition and five fish from each tank were analyzed for liver composition at the end of the experiment

### Experimental Diets

Four diets were prepared from ingredients obtained from a local fish feed manufacturer ((Sibal A.Ş. Black Sea Feed, Sinop/Turkey); Table 1). Canola oil was obtained from a commercial feed firm (Çevresel Kimya San.Tic.A.Ş., Edincik, Bandırma/Turkey). In the experiment, canola oil which was the main ingredients in experiment feeds was prepared by chancing with different ratios of fish oil. Diet I, the control diet (K), contained 100% fish oil. Diet II ($C_{50}$), Diet III ($C_{75}$) and Diet IV (C ) contained 50%, 75% and 100% canola oil, respectively. All diets were prepared at Aquaculture Laboratory of Aquaculture and Fisheries Faculty. Chromic oxide was incorporated into the test diets as a marker to assess apparent digestibility of the diets. Ingredients were thoroughly mixed, homogenized, moistened by the addition of 35% water and pelleted (3.0 mm) in a mincer. The pellets were dried at 70°C for 18 h, cut into pieces approximately 5 mm in length and stored at-20°C in plastic bags until need for feeding.

**Table 1.** Ingredient and proximate compositions of the control and experimental diets

| *Ingredients* | K | $C_{50}$ | $C_{75}$ | $C_{100}$ |
|---|---|---|---|---|
| Fish meal | 44.79 | 44.79 | 44.79 | 44.79 |
| Wheat flour | 10.47 | 10.47 | 10.47 | 10.47 |
| Defatted soybean meal | 23.12 | 23.12 | 23.12 | 23.12 |
| Sunflower Seed Meal | 6.50 | 6.50 | 6.50 | 6.50 |
| Corn protein | 2.00 | 2.00 | 2.00 | 2.00 |
| Fish oil | 12.22 | 6.11 | 3.05 | - |
| Canola oil | - | 6.11 | 9.17 | 12.22 |
| Vitamin premix(*) | 0.20 | 0.20 | 0.20 | 0.20 |
| Mineral premix(*) | 0.20 | 0.20 | 0.20 | 0.20 |
| Chrome oxide ($Cr_2O_3$) | 0.50 | 0.50 | 0.50 | 0.50 |
| *Proximate Composition* | | | | |
| Moisture (%) | 6.87 | 5.38 | 4.75 | 5.20 |
| Protein (%) | 47.34 | 47.54 | 47.28 | 47.30 |
| Lipid (%) | 17.50 | 17.50 | 17.50 | 17.47 |
| Ash (%) | 7.34 | 7.58 | 7.92 | 7.28 |
| Fiber (%) | 2.25 | 2.32 | 2.27 | 2.33 |
| Carbohydrate (%) | 20.95 | 22 | 22.55 | 22.75 |
| NFE | 25.57 | 25.06 | 25.03 | 25.62 |
| Gross energy(kJ/g) | 21.26 | 21.48 | 21.52 | 21.53 |

(*) Vitamin-mineral premix (mg/kg premix): vitamin A, 210000 IU; Vitamin $D_3$, 35000 IU; vitamin E, 7000 mg; vitamim $K_3$, 322 mg; vitamin $B_1$, 588 mg; vitamin $B_2$, 252 mg; vitamin $B_6$, 294 mg; vitamin $B_{12}$, 826 mcg; niacin, 1400 mg; biotin, 7583 mcg; 182 mg folic acid, pantothenic acid, 1722 mg; inositol, 17220 mg; vitamin C, 933.31 mg; Ca, 1414mg.
NFE = 100 - (Protein + Lipid + Ash + Fiber)

**Feeding and Fecal Collection**

The experiment was conducted in triplicates in randomly assigned tanks. During experimental period, fish in all groups were hand fed feed twice a day (at 09:00 am and 16:00 pm) to apparent satiety under a natural light regime for 70 days. All possible care was taken during feeding so that no uneaten feed settled on the tank bottoms. Feed for each tank was weighed daily to a constant amount (100 g) and feed consumption in each tank was determined by subtracting unconsumed feed from the ration. Tanks were thoroughly cleaned after each feeding. Starting on day 7 of the experiment, fecal matter was collected daily between at 11:00 am and 12:00 am and between at 16:00 pm and 17:00 pm by slow siphoning with an 8-mm plastic tube. There were no fecal collections made on weekends. Fecal samples were immediately frozen and stored at -20°C for pending analysis.

**Chemical Analyses**

Chemical composition of dried samples of diets and feces was analyzed by standard methods (AOAC, 1995). Crude protein was analyzed according to the Kjeldahl method (N x 6.25), crude lipid was determined by Soxhlet methods using petroleum ether as a solvent, dry matter by drying at 105°C for 24 h and ash by incineration at 550°C in a muffle furnace for 12 h. Carbohydrate was calculated by difference (100-[moisture+ash+proprotein+lipid]) (Tibbetts *et all.,* 2004). Chromic oxide in the diet and feces was determined with a spectrophotometer according to Bolin *et al.* (1952). Apparent digestibility coefficients (ADC) of nutrients and energy were calculated as: ADC (%)= 100 - [100(% Cr2O3 in diet/% Cr2O3 in feces) x (% nutrient in feces/% nutrient in diet)] as per Degani *et al.* (1997) and Degani (2006); ADC of dry matter (%) = 100 - [100(% Cr2O3 in diet/% Cr2O3 in feces) as per De Silva and Anderson (1995). All chemical analyses were carried out in triplicate and values represented as mean value of triplicate samples.

**Preparation of Fatty Acid Methylesters and Fatty Acid Analysis**

Total lipid was determined by modified Bligh and Dyer Method (Hanson and Olley, 1963). Fatty acid methyl esters of diet and liver lipids were prepared by sponifying 30-40 mg of lipid with 2 ml of 0.5M methanolic KOH by heating at 100ºC for 7 min. Further 1.5 ml of methanolic $BF_3$ were added and heated for 5 min. The FAMEs were extracted with 2 ml of iso-octane and extracts were decanted into the amber vial. Separation and determination of FAME's were done as described in Öksüz and Özyılmaz (2010).

**Statistical Analyses**

Apparent digestibility coefficients were calculated from the average of three replicate tanks receiving each experimental diet. Statistical analyses were performed using analysis of variance (ANOVA) and in the case of a significant difference, treatment means were differentiated using Tukey's multiple range test. All data reported as a percentage was arcsine transformed prior to ANOVA. Significiancy was determined at a 5% level ($P<0.05$).

## Results

### Diet Composition

The test diet proximate composition was uniform across the canola oil treatments. Protein, lipid and energy ranged from 47.28 to 47.54%, 17.47 to 17.5% and 21.26 to 21.53 kJ $g^{-1}$; respectively and diets were subsequently considered *iso*-nitrogenous, *iso*-lipidic and *iso*-energetic (Table 1). Dietary oils were added at 12.22% of diet, the additional lipid found in the diets originated from the added fish meal and defated soybean meal. The fish oil control treatment (K) was characterized by high levels of saturated fatty acids (SFA), particularly myristic acid, stearic acid and palmitic acid, accounting for 6.31, 20.14 and 4.62% lipid, respectively (Table 2). The K diet had the highest concentrations of palmitoleic acid (16:1n-7, POA), eicosapentaenoic acid (20:5n-3, EPA) and docosahexaenoic acid (22:6n-3, DHA) with values of 6.68, 9.26 and 16.61% lipid, respectively. The fatty acid composition of the $C_{100}$ diet was characterized by high levels of oleic acid (18:1n-9, OA) (45.63% lipid), linoleic acid (18:2n-6, LA) (19.39% lipid) and linolenic acid (18:3n-3, LNA) (59.1% lipid). Levels of the respective fatty acids for canola oil reduced as the level of substitution approached 100% fish oil inclusion. Similarly, the n-3/n-6 ratio decreased progressively relative to the level of substitution, ranging from 3.44-0.72% for the canola oil diets.

### Levels of Hepatosomatic Index and Vicerosomatic Index

Rainbow trout fed the experimental diets increased their mean weight from 119.13 g to 233.08 g after 70 days. According to initial, dietary lipid source not affected statistically hepatosomatic indices (HIS) and vicerosomatic indices (VSI). No significant differences were found for HIS and VSI between the graded canola oil treatments (Table 3).

### Apparent Digestibility of Dry Matter and Nutrients

Apperent dry matter digestibility ($ADC_{dry\ matter}$) and nutrient digestibility values ($ADC_{protein}$, ADClipid and ADCenergy) were given in Table 4. ADCprotein values were similar to in rainbow trout fed by K, C50 and C75 diets, but lower for C100 diet. Statistically no significant differences ($P$<0.05) were apparent between the graded canola oil treatments for lipid digestibility.

### Liver Fatty Acid Composition

The liver fatty acid composition of rainbow trout reared on the graded canola oil diets showed on Table 5. Total saturated fatty acid was the highest in liver of fish in K group and the lowest in fish in $C_{100}$ group. Dominant fatty acids among the saturated fatty acid were palmitic and stearic acids. Although the MUFA content was the highest in fish in the $C_{75}$ group, in general, levels of MUFA were differed among the all groups and oleic acid was the dominant fatty acid in $C_{75}$ group. Total n-6 fatty acids of liver were increased with increasing level of canola oil, compared with beginning of the trial. Total n-3 fatty acids of liver were the highest in initial and K groups and the lowest in $C_{75}$ group. The n3/n6 ratio was the highest in liver of fish in K, the lowest in $C_{100}$ and intermediate in the other groups. Dominant fatty acids among PUFA's were DHA, arachidonic acid, linoleic acid and EPA, respectively. Liver fatty acid compositions were satisfactory for all groups.

## Discussion

In the past, high-quality marine fish oils have been used almost exclusively as dietary lipid sources in the formulation of commercial fish feeds. However, vegetable oils used as an alternative to fish oil consisted lately an important part of the research on fish nutrition. This study was aimed to determined the

**Table 2**. Apparent digestibility coefficients (%) of the diets

| Parameters | K | $C_{50}$ | $C_{75}$ | $C_{100}$ |
|---|---|---|---|---|
| Dry matter | 86.44±0.42$^{c}$ | 83.35±1.86$^{b}$ | 81.02±1.89$^{a}$ | 86.36±0.26$^{c}$ |
| Crude protein | 84.34±0.32$^{b}$ | 84.84±0.47$^{b}$ | 84.90±0.42$^{b}$ | 82.84±0.49$^{a}$ |
| Crude lipid | 95.29±0.33$^{a}$ | 94.84±0.26$^{a}$ | 95.31±0.25$^{a}$ | 95.16±0.23$^{a}$ |
| NfE | 69.93±0.21$^{a}$ | 70.13±0.16$^{a}$ | 68.94±0.38$^{a}$ | 69.76±0.22$^{a}$ |
| Gross energy | 84.41±0.26$^{c}$ | 82.63±0.13$^{b}$ | 80.84±0.33$^{a}$ | 85.22±0.15$^{d}$ |

Different superscripts within the row denote significant differences.

**Table 3.** Levels of hepatosomatic index (HSI) and vicerosomatic index (VSI)

| | Initial | K | $C_{50}$ | $C_{75}$ | $C_{100}$ |
|---|---|---|---|---|---|
| HSI (%) | 1.04±0.04$^{a}$ | 0.84±0.02$^{a}$ | 0.91±0.13$^{a}$ | 0.80±0.01$^{a}$ | 0.88±0.08$^{a}$ |
| VSI (%) | 11.99±0.49$^{a}$ | 10.03±0.69$^{a}$ | 10.38±0.22$^{a}$ | 10.17±0.68$^{a}$ | 10.48±0.34$^{a}$ |

Different superscripts within the row denote significant differences. HSI (%) = (liver weight / body weight) x 100 VSI (%) = (viscera weight/body weight) x 100

**Table 4.** Fatty acid composition of the experimental diets (% of total fatty acids)

| Fatty Acids | Diet Groups | | | |
|---|---|---|---|---|
| | K | $C_{50}$ | $C_{75}$ | $C_{100}$ |
| C14:0 | 6.31[a] | 3.83[b] | 2.86[c] | 1.45[d] |
| C16:0 | 20.14[a] | 14.79[b] | 13.29[c] | 11.03[d] |
| C17:0 | 0.82 | 0.55 | 0.34 | nd |
| C18:0 | 4.62[a] | 3.78[b] | 3.57[b] | 3.05[c] |
| C20:0 | 1.35[a] | 1.05[b] | 0.95[b] | 0.71[c] |
| C16:1n-7 | 6.68[a] | 4.16[b] | 3.18[c] | 1.71[d] |
| C18:1 n-9 | 17.93[a] | 30.44[b] | 35.89[c] | 45.63[d] |
| C20:1 | 0.56[a] | 1.19[b] | 1.37[b] | 1.46[b] |
| C18:2 n-6 | 7.31[a] | 12.67[b] | 15.22[c] | 19.39[d] |
| C18:3 n-3 | 1.29[a] | 3.73[b] | 4.76[c] | 5.91[d] |
| C20:2 | nd | 0.14 | 0.18 | nd |
| C20:3 n-6 | nd | 0.20 | nd | nd |
| C20:4 n-6 | 0.83 | 0.36 | 0.50 | 0.26 |
| C20:5n-3 | 9.26[a] | 5.49[b] | 4.18[c] | 2.32[d] |
| C22:4 | 0.19 | 0.37 | 0.24 | nd |
| C22:5 n-3 | 0.81 | 0.57 | 0.38 | nd |
| C22:6 n-3 | 16.61[a] | 10.97[b] | 8.85[c] | 5.91[d] |
| ΣSFA | 33.24[a] | 26.00[b] | 21.01[c] | 16.24[d] |
| ΣMUFA | 24.98[a] | 35.79[b] | 40.44[c] | 48.80[d] |
| ΣPUFA | 36.30[a] | 34.50[ab] | 34.31[ab] | 33.79[b] |
| Total n-3 PUFA | 27.97[a] | 20.76[b] | 18.17[c] | 14.14[d] |
| Total n-6 PUFA | 8.14[a] | 13.23[b] | 15.72[c] | 19.65[d] |
| n-3/n-6 | 3.44[a] | 1.57[b] | 1.16[c] | 0.72[d] |

Different superscripts within the row denote significant differences. nd (not detected)

**Table 5.** Levels of saturated (SFA), mono-unsaturated (MUFA), and poly-unsaturated (PUFA) fatty acids (% of total fatty acids) in liver of rainbow trout fed the experimental diets

| Fatty Acids | Diet Groups | | | | |
|---|---|---|---|---|---|
| | Initial | K | $C_{50}$ | $C_{75}$ | $C_{100}$ |
| C14:0 | 0.93 | 1.02 | 0.83 | 0.85 | 0.53 |
| C16:0 | 19.83 | 22.18 | 19.26 | 18.28 | 17.67 |
| C17:0 | 0.38 | 0.44 | nd | 0.3 | 0.2 |
| C18:0 | 5.8 | 7.04 | 7.02 | 6.77 | 6.41 |
| C20:0 | 0.14 | 0.27 | nd | 0.22 | 0.27 |
| C16:1 | 1.95 | 1.99 | 1.92 | 2.25 | 1.75 |
| C18:1 n-9 | 12.66 | 14.35 | 22.91 | 29.43 | 26.29 |
| C20:1 | 0.61 | 1.28 | 2.26 | 2.16 | 2.53 |
| C18:2 n-6 | 3.92 | 2.33 | 4.17 | 5.97 | 5.59 |
| C18:3 n-3 | 0.94 | 0.5 | nd | 0.68 | 0.58 |
| C20:2 | 0.67 | 0.62 | 1.02 | 1.22 | 1.47 |
| C20:3 n-6 | 0.7 | 0.56 | nd | 1.31 | 1.31 |
| C20:4 n-6 | 5.64 | 3.15 | 4.63 | 2.54 | 4.15 |
| C20:4 n-3 | 0.58 | 0.43 | nd | 0.27 | 0.5 |
| C20:5n-3 | 3.63 | 3.93 | 2.81 | 1.79 | 1.8 |
| C22:4n-4 | 2.1 | 1.64 | 1.18 | 0.52 | 0.73 |
| C22:5 n-6 | 0.33 | 0.42 | nd | 0.42 | 0.52 |
| C22:5 n-3 | 1.07 | 1.11 | nd | 0.6 | 0.43 |
| C22:6 n-3 | 34.67 | 34.74 | 32 | 24.22 | 26.01 |
| ΣSFA | 27.08 | 30.95 | 27.11 | 26.42 | 25.08 |
| ΣMUFA | 15.22 | 17.62 | 27.09 | 33.84 | 30.57 |
| ΣPUFA | 54.25 | 49.43 | 45.81 | 39.54 | 43.09 |
| Total n-3 PUFA | 40.89 | 40.71 | 32 | 27.56 | 29.32 |
| Total n-6 PUFA | 10.59 | 6.46 | 8.80 | 10.24 | 11.57 |
| n-3/n-6 | 3.86 | 6.30 | 3.64 | 2.69 | 2.53 |

nd (not detected)

effects of different concentrations of canola oil (CO) on the digestibility, vicerosomatic index (VSI), hepatosomatic index (HSI) and the contents of fatty acids in the liver of rainbow trout (*Oncorhynchus mykiss*).

Essentially, dry matter, protein and energy digestibility values were usually high and significantly affected by the dietary lipid source.

Small differences were detected for $ADC_{dry\ matter}$, energy and protein digestibility among the all groups. However, Martins *et al.* (2006) reported that lipid digestibility was quite similar and high in diets containing canola oil and fish oil. Nevertheless, lipid digestibility values were high across all treatments, comparable to values reported by Martins *et al.* (2009) and Francis *et al.* (2006), but lower when compared to the values reported by Martins *et al.* (2006) and in general conformity with the statement that lipid well digested by fish (Olsen and Ringø, 1997) and ADC values are in general accordance with ADC for carnivorous species (NRC, 1993). Similarly, previous studies have reported a metabolic acceptance in various fish species for diets containing several vegetable oil types, including canola oil (Olsen and Ringø, 1997; Caballero *et al.,* 2002). Nevertheless, lipid digestibility was higher in diets containing vegetable oil than with animal lipid in Atlantic salmon fed diets based on flaxseed oil (Menoyo *et al.,* 2007) and in Atlantic halibut fed diets based on vegetable oils (Martins *et al.,* 2009).

As fish stores energy in muscle tissues, they collect the extra energy in liver as glycogen. Therefore, the proportional size of the liver is accepted as an index of growth speed with nutritional status of fish. HSI is an index that is used to investigate the effects of feeding on the liver which is a key organ for metabolism. If the hepatosomatic index is higher than the standard values (between 1-2% for *osteichthyes*), it shows that feeding or the feed causes some troubles in fish especially in the carbohydrate and fat metabolism, the existence of oxidized feed in the diet, extra carbohydrate and vitamin deficiency (Munshi and Dutta**,** 1996; Anonymus, 2011a). In this study, HSI ranged from 0.80 to 1.04; there were no differences among all the fish fed canola and fish oil (Panserat *et al.,* 2009). Relative liver weights did not differ among dietary groups, indicating that this variable was not affected by the dietary lipid composition (Lin *et al.,* 2007).

VSI are used to determine the rate of fat accumulated all body of the fish. At the end of the experiment, the value of VSI did not affected by the source of vegetable oil used in the study ($P > 0.05$). At the beginning of the experiment VSI was higher than at the end of the experiment. In this situation, canola oil used in feeds for rainbow trout trial can be used effectively without accumulation in the body. In present study, VSI determined between 10.03-11.99; similar conclusion reported for rainbow trout (Şener and Yıldız, 2003; Figueiredo-Silva *et al.,* 2005; Caballero *et al.,* 2002; Panserat *et al.,* 2009) and extensively for many different fish species (Yıldız and Şener, 2003; Menoyo *et al.,* 2005).

Liver fatty acid compositions of the rainbow trout were significantly influenced by the dietary lipid source. Despite each trial diets were *iso*-nitrogenous, *iso*-lipidic and *iso*-energetic, differences among liver fatty acid compositions were in all likelihood a result of the differing fatty acid compositions of the trial diets (Tocher *et al.,* 2003; Francis *et al.,* 2007). Increased levels of 18:2n-6 and 18:1n-9 were observed in the liver of rainbow trout fed canola oil-based diets. Similarly, high levels of 18:2n-6 and 18:1n-9 were observed in farmed rainbow trout muscle due to they were fed with artificial diet (Oksuz, 2000). In contrast, low level of 18:2 n-6 fatty acid was reported in wild brown trout muscle with a considerable amount of 18:3 n3 fatty acid (Kayım *et al.,* 2011). Replacement of fish oil with canola oil resulted in reduced levels of total n-3 PUFA and SFA, and an increased level of total n-6 PUFA and MUFA in liver tissues. High level of linoleic acid (n-6) in the liver may be considerd as an indicator of vegetable sourced lipid containing feed. Generally, there was a direct dietary influence in terms of PUFA levels found in fish tissues. In contrast, liver total saturated fatty acid levels were minimally affected by dietary treatments from 25.08% in the $C_{100}$ diet to 30.95% in the K diet. The minimal impact of diets on saturated fatty acids in fish liver tissues was also observed by other researchers (Greene and Selivonchick, 1990; Shapawi *et al.,* 2008). Similar to the results of the present study, replacement of fish oil with vegetable oils had been reported to result in significant changes in liver fatty acid composition in many other marine fish species such as the European sea bass (Mourente *et al.,* 2005), red sea bream (Glencross *et al.,* 2003) and Atlantic salmon, *Salmo salar* L. (Ng *et al.,* 2007). In general, replacement of dietary fish oil with vegetable oil had resulted in a lower level of n-3 PUFA (especially EPA and DHA) in fish tissues.

Liver EPA and DHA levels of rainbow trout were strongly influenced by the dietary levels of EPA and DHA. Feeding rainbow trout canola oil-based diets markedly decreased the concentrations of these essential fatty acids in the tissue. In fish the main PUFA to be considered are 20:4n-6 (Arachidonic acid, ARA) and its metabolic precursor 18:2n-6 (linoleic acid, LA), together with 20:5n-3 and 22:6n-3 and their metabolic precursor 18:3n-3 (linolenic acid, ALA). Arachidonic acid is known as the primary eicosanoid precursor in fish. Eicosanoids have been found in a large range of freshwater and marine fish (Tocher, 2003). They observed that DHA and EPA levels in liver tissue of rainbow trout were reflected the dietary levels of DHA and EPA. Linear regression analysis revealed that there was a strong correlations between the OA, LA, EPA and DHA fatty acid composition of each trial diets and the OA, LA, EPA and DHA composition of the liver (Figure 1); a trend reported for rainbow trout (Caballero *et al.,* 2002; Fonseca-Madrigal *et al.,* 2005) and extensively for many different fish species (Izquierdo *et al.,* 2003; Francis *et al.,* 2007; Lin *et al.,* 2007). Naturally, the concentration of OA and LA increased as the canola oil inclusion approached 100%. However, levels of each fatty acid were found in lower concentration across each of the canola oil treatments in the liver for

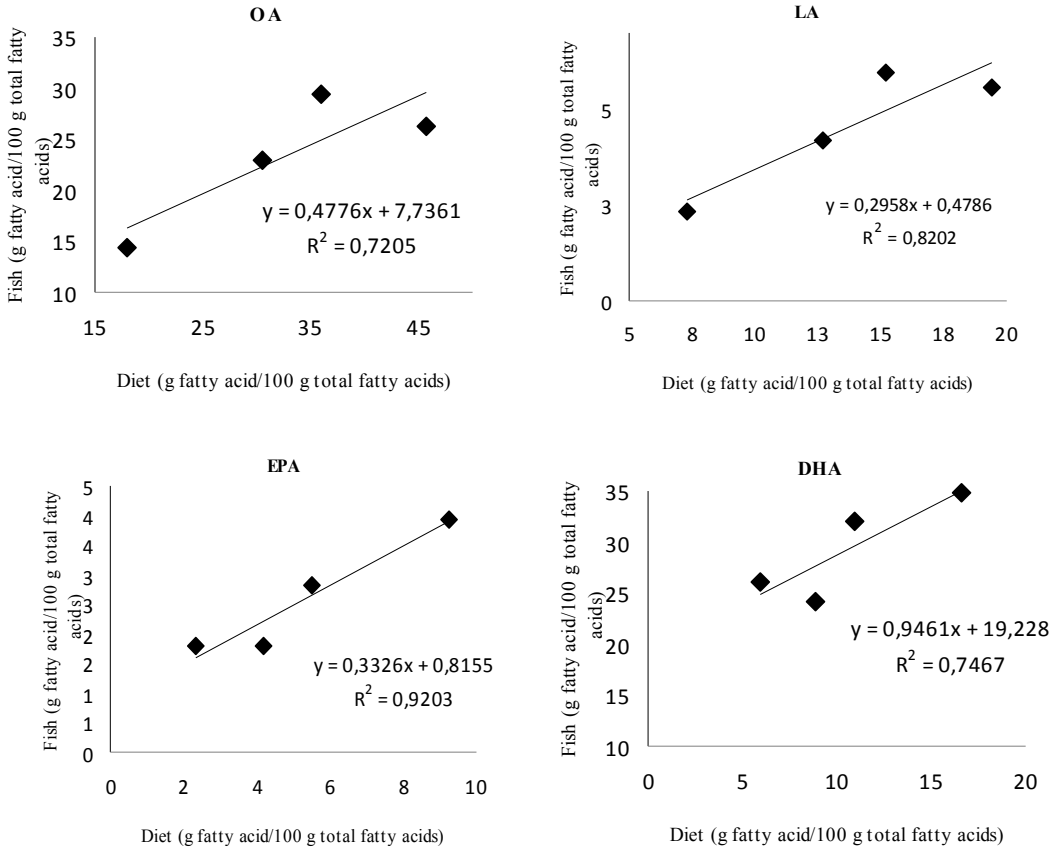


**Figure 1.** Relationships between concentrations of (a) oleic acid (18:1n-9; OA), (b) linoleic acid (18:2n-6; LA), (c) eicosapentaenoic acid (20:5n-3; EPA) and (d) docosahexaenoic acid (22:6n-3; DHA) in diets containing different amounts of canola oil and in liver of the rainbow trout fed by the experimental diets.

the $C_{75}$ and $C_{100}$ treatments in comparison with the concentrations detected in the diet. These results tend to indicate that these fatty acids (OA, LA and LNA), when present in suitable quantities in the diet are selectively utilized in the liver for β-oxidation, as suggested by Bell *et al.* (2003) and Torstensen *et al.* (2004). It is shown that the inclusion of vegetable oils in fish diets stimulates desaturation and elongation activity in the liver (Tocher *et al.,* 2000; Bell *et al.,* 2003; Zheng *et al.,* 2005). In addition, despite the increasing addition of canola oil, n3/n6 rates in the liver were quite high (Caballero *et al.,* 2002; Tocher *et al.,* 2003; Fonseca-Madrigal *et al.,* 2005; Francis *et al.,* 2007).

In summarising the results obtained, lipid digestibility, HSI, VSI and liver fatty acid composition of the rainbow trout was not negatively affected by the graded levels canola oil diets used in the present study. Our results showed that the liver fatty acid composition was higher in DHA and EPA, respectively, and thus, the liver contents more n-3 PUFA. Consequently, accumulation of unsaturated fatty acid in the liver tissue of the fish fed by canola oil showed that liver could be an effective fatty acid source.

## Acknowledgements

This paper was carried out in conjunction with Seval DERNEKBAŞI's PhD thesis. We extend our gratefulness to Osman PARLAK, the Owner of the Kuzey Su Ürünleri fish farm, for providing us the fish.

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