

RESEARCH ARTICLE

Fatty acid composition and mRNA expression of fatty acid binding protein genes (*fabp3* and *fabp6*) in rainbow trout fed camelina seed oil (*Camelina sativa*)-based diets

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ABSTRACT

Vegetable lipids such as camelina oil (*Camelina sativa*) are used as alternatives oil sources to fish oil in aquafeeds. In this study, we determined fatty acid-binding protein 3 (*fabp3*) and fatty acid-binding protein 6 (*fabp6*) gene expression and fatty acid composition in the liver and muscle tissue of rainbow trout fed different amounts of dietary camelina seed oil [100% (CO100), 67% (CO67), and 37% (CO33)]. Palmitic acid and oleic acid were identified as the most abundant saturated and monounsaturated fatty acids, respectively, in both tissues across all experimental groups. The highest levels of n-6 polyunsaturated fatty acid (Σ n-6 PUFA) were found in the first biopsy (15th day) taken from fish fed a diet of CO100, while the highest Σ n-3 PUFA level was found in the third biopsy (45th day) taken from the same group. The FO100 (fish oil) diet was found to have the highest Σ n-3 / n-6 ratio, as well as the highest levels of eicosapentaenoic acid and docosahexaenoic acid. In general, the fatty acid composition of the fish reflected that of their respective diets. The expression of *fabp3* and *fabp6* genes in the muscle of fish fed camelina seed oil were not significantly different from control group. However, *fabp3* gene expression of liver of FO100 group was found to have significantly higher than CO67 and CO33. A difference in hepatic *fabp6* gene expression was also noted in the FO100 group, but was not found to be statistically significant. Growth parameters and survival rate were not affected after the 45 days feeding trial. These results suggest that camelina seed oil can be used as an alternative to fish oil in rainbow trout diet.

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Introduction

Nutrigenomics is a multidisciplinary science that combines the fields of nutrition, molecular biology, molecular medicine, genomics, epidemiology and bioinformatics. It uses transcriptomics, proteomics, and metabolomics to explore how dietary ingredients can affect the balance between health and disease, and to identify the effects of nutrients on gene expression, transcription activity and the heterogeneous response of gene variants (Mutch et al., 2005). Advances in these technologies have led to a better understanding of the underlying mechanisms of animal nutrition, and could lead to the development of healthier and more economic animal products with reduced risk of disease (Mutch et al., 2005).

Rainbow trout (*Oncorhynchus mykiss*) is the most widely cultured cold freshwater fish in the world, and total production of Rainbow trout (*Oncorhynchus mykiss*) and Atlantic salmon (*Salmo salar*) are 3.284 million tonnes in 2018 (FAO, 2020). α -linolenic acid (ALA), eicosapentaenoic acid (EPA), docosahexaenoic acid (DHA), linoleic acid (LA), and arachidonic acid (ARA) are important for healthy daily life and activity (Baysal, 2004). Aquafeeds containing sufficient essential fatty acids (FAs) are of great importance, particularly during the pre-breeding development of female fish, egg production, and in the early stages of fish development (Çetinkaya, 1995; Bayır, 2011). Deficiencies of these FAs in fish feed can lead to a decrease in hatching rate, and may cause disorders in the embryos. Fatty acid-binding protein FABPs are small (12-15 kDa) intracellular proteins that exist as several distinct types, including liver, intestinal, and adipocyte types. They serve an important role in protecting cells from the repercussions of fatty acids (Glatz & van der Vusse, 1996; Niewold et al., 2004).

Naturally-fed animal meat, poultry eggs, and flaxseed oils are rich in n-3 FAs, while n-6 FAs are found in plants such as sunflower, soybean, and cotton (Aydın, 2004). Fish oil is an important source of the essential FAs EPA and DHA, and is therefore used as a component of fish feed. However, the cost of fish oil has been increasing, due in part to its importance in human health, its use in feed for terrestrial animals, and its stable worldwide production. Studies have shown that cheaper vegetable oil (VO) and meal sources can be used as substitutes for fish oil (FO) and fish meal in the fish feed industry, and different sources of oil and meal have been investigated for this purpose. (Torstensen et al., 2000; Caballero et al., 2002; Bell et al., 2001, 2002; Montero et al., 2003, 2005; Izquierdo et al., 2003, 2005; Ganga et al., 2011). Studies conducted with VOs show

that alternative oil sources can be used partially or completely as a substitute for FO, without adversely affecting the growth performance of fish, and meet the species growth requirement (Bayır, 2011). The availability of VOs that can be used as suitable alternatives to FO will reduce both the fish feed industry's dependence on FO, and the cost of feed (Mourente & Bell, 2006). Camelina oil (CO; *Camelina sativa*) contains less monounsaturated fatty acids (MUFAs) than peanut or rape oil, but more than flax, soybean, and cotton oil. CO contains a similar concentration of PUFAs to that found in soybean and sunflower oil, which is less than in flax oil, but more than in cotton, peanut, and rape oil (Legendre et al., 1995). The composition of saturated fatty acids (SFAs) and PUFAs in CO is also similar to sunflower oil, but CO contains a significantly higher proportion of n-3 PUFA (Kurt & Seyis, 2008). Previous research has investigated changes in gene expression levels in multiple species of fish that were fed different diets using various VOs instead of FOs (Tocher et al., 2003; Ruyter et al., 2000; Jordal et al., 2005; Zheng et al., 2005; Torstensen et al., 2009). Fabps are found in tissues that are highly active in FA metabolism, and are involved in lipid uptake, transport, and homeostasis (Bayır et al., 2015). However, no studies have yet determined how a vegetable oil-based diet may alter mRNA levels of the *fabp3* and *fabp6* genes, which encode proteins involved in intracellular transport in rainbow trout. This study aims to investigate changes in expression of *fabp3* and *fabp6* in rainbow trout fed with CO, which are the FAs in intracellular transport, and to determine differences in the FA composition of liver and muscle tissues in these fish.

Material and Methods

Husbandry of Rainbow Trout

A total of 44 rainbow trout (97.45 ± 15.05 g average weight) were weighed and stocked in 12 experimental tanks. Fish were fed commercial trout feed for 15 days twice daily as 2% of the body. The tank water temperature was 16.0°C, and the dissolved oxygen concentration of the water was 9.3 mg/l.

Four experiments were conducted, the first of which was performed using a FO-fed control group. In each of the other three experiments, fish oil feed was replaced with a feed of 33% CO (CO33), 67% CO (CO67), or 100% CO (CO100) (Table 1). Fish were each randomly assigned to one of the four treatment groups at the beginning of the experiment, and were fed to apparent satiation four times daily for 45 days. Working protocols were approved by Atatürk University Local Ethical Committee for Animal Studies. Liver and muscle samples were

Table 1. 5' → 3' Sequences of primers (Bayır et al., 2015)

Rainbow trout genes	Forward primer (5' → 3')	Reverse primer (5' → 3')	Tm (°C)	qPCR efficiency
<i>fabp3</i>	ATGAAGGCTCTGGGTGTGG	TCCTTGCCATCCCACCTTCTG	54.8	1.04
<i>fabp6</i>	GGGAAAAAGTTCAAGGCCAC	GCTGGTTCCTTTTCAGCACGA	57.4	0.94
β -actin	CTTCTACAACGAGCTGAGGGT	GGTCTCAAACATGATCTGGGT	57.0	0.93

Table 2. Fatty acid composition of rainbow trout diets

Fatty Acid	Cod Liver Oil (100 %)	Camelina Seed Oil (100%)	Camelina Seed Oil (67%)	Camelina Seed Oil (33%)
14:0	6.6	1.6	3.0	4.7
15:0	0.2	0.3	0.5	0.8
16:0	23.7	11.7	14.3	17.6
17:0	0.3	0.4	0.6	0.9
18:0	4.8	6.2	3.5	4.5
20:0	1.6	0.3	0.8	1.2
22:0	0.9	1.4	1.5	1.5
24:0	0.2	0.1	0.2	0.2
ΣSFA	40.3	22.0	24.8	31.4
14:1	0.3	0.1	0.2	0.2
15:1	0.3	0.2	0.5	0.3
16:1	6.5	1.6	3.1	4.6
17:1	0.7	0.2	0.4	0.5
18:1n-9	16.7	14.8	17.1	17.0
20:1n-9	–	–	–	–
22:1	0.5	0.2	0.4	0.5
24:1n-9	0.2	0.1	0.1	0.2
ΣMUFA	25.3	17.2	21.7	23.3
18:2n-6	6.5	18.5	14.5	11.4
18:3n-6	0.2	0.1	0.2	0.3
20:2n-6	1.5	8.6	6.2	4.1
20:3n-6	0.1	0.1	–	0.1
20:4n-6	0.8	0.7	0.6	0.6
22:2n-6	0.2	0.1	0.1	0.2
22:4n-6	0.1	0.2	0.1	0.1
Σn-6 PUFA	9.3	28.2	21.7	16.8
18:3n-3	2.0	24.2	17.3	10.0
18:4n-3	0.1	0.1	0.1	0.1
20:3n-3	0.3	1,1	0.9	0.6
20:4n-3	0.1	0,3	0.5	0.2
22:5n-3	0.3	0.2	0.4	0.5
20:5n-3	8.4	2.3	4.5	6.2
22:6n-3	13.8	4.5	8.3	11.0
Σn-3 PUFA	25.1	32.6	31.8	28.6
EPA+DHA	22.2	6.7	12.7	17.2
Σn-3/Σn-6 PUFA	2.7	1.2	1.5	1.7

taken from the fish four times (15th, 30th, and 45th days of the experiment) throughout the trial. At the end of the trial the fish were euthanized, and their liver and muscle weights recorded. The muscle and liver samples intended for study of mRNA expression were stored in RNAlater at -80°C until RNA

isolation could be performed. The other samples were frozen immediately in liquid nitrogen and stored at -80°C until their FA composition could be analyzed. At the end of the study, growth parameters of rainbow trout were calculated as follow:

$$SGR(\%day^{-1}) = 100 \times \frac{\ln(Final\ weight) - \ln(Initial\ weight)}{Feeding\ days} \quad (1)$$

$$WG(\%) = 100 \times \frac{Final\ weight - Initial\ weight}{Initial\ weight} \quad (2)$$

$$SR(\%) = 100 \times \frac{Final\ number\ of\ fish}{Initial\ number\ of\ fish} \quad (3)$$

where SGR is the specific growth rate, WG is the weight gain, SR is the survival rate.

Lipid and Fatty Acid Analysis

Lipid analysis was performed using the Folch et al. (1957) method for lipid extraction. Accordingly, the liver and muscle tissue samples of fish from each tank were homogenized with a ratio of 2:1 v/v chloroform/methanol containing 0.01% (w/v) butylated hydroxytoluene (Sigma, ≥99.0% gas chromatography). The organic solvent was allowed to evaporate under a nitrogen stream before the lipid content was determined gravimetrically.

Table 3. Fatty acid composition of 100% fish oil and 100% camelina seed in muscle tissue (mean±SD)

Fatty acids	Initial	100% Fish Oil			100% Camelina Seed Oil		
		First sample (15th day)	Second sample (30th day)	Third sample (45th day)	First sample (15th day)	Second sample (30th day)	Third sample (45th day)
14:0	2.0 ± 0.2	3.0 ± 0.1 ^a	2.8 ± 0.0 ^a	3.0 ± 0.2 ^a	2.0 ± 0.0 ^b	1.9 ± 0.0 ^b	1.7 ± 0.0 ^b
15:0	0.3 ± 0.0	0.4 ± 0.0 ^a	0.5 ± 0.0 ^a	0.5 ± 0.0 ^a	0.2 ± 0.0 ^b	0.4 ± 0.0 ^b	0.3 ± 0.0 ^b
16:0	15.6 ± 0.3	17.6 ± 0.2 ^a	21.0 ± 0.3 ^a	21.4 ± 0.1 ^a	16.7 ± 0.1 ^a	17.9 ± 0.5 ^b	19.1 ± 0.1 ^b
17:0	0.5 ± 0.0	0.4 ± 0.0 ^a	0.5 ± 0.0 ^a	0.5 ± 0.0 ^a	0.4 ± 0.0 ^a	0.5 ± 0.0 ^a	0.4 ± 0.0 ^b
18:0	4.7 ± 0.3	4.7 ± 0.1 ^a	5.0 ± 0.1 ^a	4.8 ± 0.3 ^a	4.5 ± 0.0 ^a	5.2 ± 0.1 ^a	4.9 ± 0.1 ^a
20:0	0.6 ± 0.1	0.5 ± 0.0 ^b	0.5 ± 0.0 ^b	0.6 ± 0.0 ^b	0.8 ± 0.0 ^a	0.7 ± 0.0 ^a	0.7 ± 0.0 ^a
22:0	1.0 ± 0.0	0.9 ± 0.1 ^b	0.6 ± 0.1 ^a	0.5 ± 0.0 ^b	1.0 ± 0.0 ^a	0.6 ± 0.1 ^a	0.7 ± 0.1 ^a
24:0	0.4 ± 0.1	0.7 ± 0.0 ^a	0.4 ± 0.0 ^b	0.3 ± 0.0 ^b	0.3 ± 0.0 ^b	0.5 ± 0.1 ^a	0.4 ± 0.2 ^a
ΣSFA	25.0 ± 0.5	28.3 ± 0.2^a	31.4 ± 0.2^a	31.5 ± 0.2^a	25.9 ± 0.2^b	27.7 ± 0.6^b	28.2 ± 0.3^b
14:1	0.1 ± 0.0	0.1 ± 0.0 ^a	0.1 ± 0.0 ^a	0.1 ± 0.0 ^a	0.1 ± 0.0 ^a	0.2 ± 0.1 ^a	0.1 ± 0.0 ^a
15:1	0.3 ± 0.1	0.3 ± 0.0 ^a	0.2 ± 0.1 ^b	0.2 ± 0.0 ^b	0.3 ± 0.1 ^a	0.3 ± 0.1 ^a	0.3 ± 0.1 ^a
16:1	2.9 ± 0.3	3.9 ± 0.1 ^a	5.9 ± 0.3 ^a	5.3 ± 0.2 ^a	3.2 ± 0.1 ^b	3.3 ± 0.2 ^b	3.1 ± 0.1 ^b
17:1	0.3 ± 0.0	0.4 ± 0.0 ^a	0.4 ± 0.0 ^a	0.4 ± 0.0 ^a	0.3 ± 0.0 ^b	0.3 ± 0.0 ^b	0.3 ± 0.0 ^b
18:1n-9	26.2 ± 0.2	22.7 ± 0.1 ^b	24.0 ± 0.1 ^a	20.7 ± 0.5 ^a	24.8 ± 0.2 ^a	20.6 ± 0.4 ^b	19.9 ± 0.3 ^a
20:1n-9	0.4 ± 0.0	0.2 ± 0.0 ^a	0.2 ± 0.0 ^a	0.1 ± 0.0 ^a	0.2 ± 0.0 ^a	0.2 ± 0.0 ^a	—
22:1	0.4 ± 0.0	0.6 ± 0.0 ^a	0.5 ± 0.0 ^a	0.5 ± 0.0 ^a	0.4 ± 0.0 ^b	0.2 ± 0.0 ^b	0.5 ± 0.0 ^a
24:1n-9	0.4 ± 0.1	0.6 ± 0.1 ^a	0.5 ± 0.1 ^b	0.1 ± 0.0 ^a	0.3 ± 0.0 ^b	1.1 ± 0.2 ^a	0.2 ± 0.0 ^a
ΣMUFA	30.9 ± 0.4	28.8 ± 0.1^b	31.8 ± 0.3^a	27.5 ± 0.4^a	29.5 ± 0.2^a	26.2 ± 0.4^b	24.4 ± 0.3^b
18:2n-6	12.9 ± 0.4	10.6 ± 0.1 ^b	8.0 ± 0.1 ^b	8.1 ± 0.2 ^b	12.4 ± 0.1 ^a	11.1 ± 0.4 ^a	11.4 ± 0.1 ^a
18:3n-6	0.2 ± 0.0	0.3 ± 0.1 ^a	0.2 ± 0.0 ^a	0.1 ± 0.0 ^b	0.2 ± 0.0 ^a	0.2 ± 0.0 ^a	0.3 ± 0.1 ^a
20:2n-6	2.0 ± 0.2	1.7 ± 0.0 ^b	1.8 ± 0.0 ^b	1.5 ± 0.1 ^b	3.3 ± 0.1 ^a	2.8 ± 0.2 ^a	3.5 ± 0.1 ^a
20:3n-6	0.2 ± 0.1	0.3 ± 0.0 ^a	0.3 ± 0.0 ^a	0.2 ± 0.0 ^b	0.1 ± 0.0 ^b	0.1 ± 0.0 ^b	0.3 ± 0.0 ^a
20:4n-6	1.1 ± 0.0	1.1 ± 0.0 ^a	1.1 ± 0.0 ^a	1.0 ± 0.0 ^a	0.9 ± 0.0 ^a	1.0 ± 0.0 ^a	1.0 ± 0.0 ^a
22:2n-6	0.2 ± 0.0	0.3 ± 0.0 ^a	0.1 ± 0.0 ^a	0.1 ± 0.0 ^a	0.2 ± 0.0 ^b	0.1 ± 0.0 ^a	0.1 ± 0.0 ^a
22:4n-6	0.1 ± 0.0	0.2 ± 0.0 ^a	0.1 ± 0.0 ^b	0.1 ± 0.0 ^b	0.1 ± 0.0 ^b	0.4 ± 0.1 ^a	0.1 ± 0.0 ^b
Σn-6 PUFA	16.7 ± 0.4	14.4 ± 0.1^b	11.6 ± 0.2^b	11.0 ± 0.1^b	17.3 ± 0.1^a	15.8 ± 0.6^a	16.5 ± 0.1^a
18:3n-3	2.9 ± 0.3	2.4 ± 0.0 ^b	2.4 ± 0.0 ^b	3.1 ± 0.1 ^b	6.0 ± 0.1 ^a	7.3 ± 0.1 ^a	7.4 ± 0.3 ^a
18:4n-3	0.3 ± 0.0	0.2 ± 0.0 ^a	0.2 ± 0.0 ^a	0.1 ± 0.0 ^b	0.2 ± 0.0 ^a	0.2 ± 0.0 ^a	0.2 ± 0.0 ^a
20:3n-3	0.8 ± 0.1	0.9 ± 0.0 ^b	0.7 ± 0.0 ^b	0.6 ± 0.0 ^b	1.1 ± 0.0 ^a	1.0 ± 0.0 ^a	1.2 ± 0.0 ^a
20:4n-3	0.6 ± 0.0	0.6 ± 0.0 ^a	0.5 ± 0.0 ^b	0.4 ± 0.0 ^b	0.6 ± 0.0 ^a	0.6 ± 0.0 ^a	0.6 ± 0.0 ^a
20:5n-3	4.3 ± 0.2	1.4 ± 0.0 ^a	3.7 ± 0.0 ^a	4.3 ± 0.1 ^a	0.9 ± 0.0 ^b	3.6 ± 0.1 ^a	3.3 ± 0.0 ^b
22:5n-3	1.1 ± 0.1	4.4 ± 0.2 ^a	0.9 ± 0.1 ^a	1.0 ± 0.0 ^a	3.4 ± 0.1 ^b	0.8 ± 0.1 ^a	0.8 ± 0.0 ^a
22:6n-3	17.8 ± 0.3	18.7 ± 0.3 ^a	16.7 ± 0.1 ^a	20.3 ± 0.2 ^a	15.3 ± 0.2 ^b	16.7 ± 0.7 ^a	17.4 ± 0.5 ^b
Σn-3 PUFA	27.7 ± 0.4	28.6 ± 0.4^a	25.2 ± 0.2^b	29.8 ± 0.4^b	27.4 ± 0.2^b	30.3 ± 0.7^a	30.8 ± 0.4^a
EPA+DHA	22.0 ± 0.3	23.1 ± 0.4^a	20.5 ± 0.1^a	24.6 ± 0.3^a	18.7 ± 0.2^b	20.3 ± 0.8^a	20.7 ± 0.5^b
Σn-3/Σn-6 PUFA	1.7 ± 0.0	2.0 ± 0.0^a	2.2 ± 0.0^a	2.7 ± 0.1^a	1.6 ± 0.0^b	1.9 ± 0.1^b	1.9 ± 0.0^b

Note: SD is the standard deviation, n=6. Different letters indicate groups that differ from each other (p<0.05)

Table 4. Fatty acid composition of 67% and 33% camelina seed in muscle tissue (mean±SD)

Fatty acids	67% Camelina Oil			33% Camelina Seed Oil			
	Initial	First sample (15th day)	Second sample (30th day)	Fatty acids	Initial	First sample (15th day)	Second sample (30th day)
14:0	2.0 ± 0.2	2.4 ± 0.1 ^b	2.2 ± 0.1 ^b	2.3 ± 0.2 ^b	2.7 ± 0.1 ^b	2.6 ± 0.0 ^b	2.6 ± 0.1 ^b
15:0	0.3 ± 0.0	0.4 ± 0.0 ^a	0.4 ± 0.0 ^b	0.4 ± 0.0 ^b	0.4 ± 0.0 ^a	0.4 ± 0.0 ^b	0.4 ± 0.0 ^b
16:0	15.6 ± 0.3	16.1 ± 0.1 ^b	19.3 ± 0.4 ^b	18.9 ± 0.4 ^b	16.5 ± 0.1 ^b	19.2 ± 0.1 ^b	20.7 ± 0.1 ^b
17:0	0.5 ± 0.0	0.5 ± 0.0 ^a	0.5 ± 0.0 ^a	0.4 ± 0.0 ^b	0.5 ± 0.1 ^a	0.5 ± 0.0 ^a	0.5 ± 0.0 ^a
18:0	4.7 ± 0.3	4.6 ± 0.0 ^a	5.3 ± 0.2 ^a	4.4 ± 1.0 ^a	4.4 ± 0.2 ^c	5.0 ± 0.1 ^b	5.0 ± 0.1 ^a
20:0	0.6 ± 0.1	0.9 ± 0.0 ^a	0.7 ± 0.0 ^a	0.6 ± 0.0 ^a	0.7 ± 0.1 ^b	0.6 ± 0.0 ^b	0.5 ± 0.0 ^b
22:0	1.0 ± 0.0	1.3 ± 0.2 ^a	0.7 ± 0.1 ^a	0.7 ± 0.0 ^a	1.2 ± 0.0 ^{ab}	0.7 ± 0.1 ^a	0.6 ± 0.0 ^b
24:0	0.4 ± 0.1	0.4 ± 0.1 ^b	0.3 ± 0.1 ^b	0.4 ± 0.2 ^a	0.3 ± 0.0 ^c	0.2 ± 0.0 ^c	0.4 ± 0.1 ^a
ΣSFA	25.0 ± 0.5	26.5 ± 0.5^b	29.4 ± 0.9^b	27.9 ± 1.0^b	26.7 ± 0.2^b	29.2 ± 0.1^b	30.7 ± 0.1^a
14:1	0.1 ± 0.0	0.1 ± 0.0 ^a	0.1 ± 0.0 ^a	0.1 ± 0.0 ^a	0.1 ± 0.0 ^a	0.1 ± 0.0 ^a	0.1 ± 0.0 ^a
15:1	0.3 ± 0.1	0.3 ± 0.0 ^a	0.1 ± 0.0 ^b	0.3 ± 0.1 ^a	0.3 ± 0.0 ^a	0.1 ± 0.0 ^b	0.5 ± 0.1 ^a
16:1	2.9 ± 0.3	3.3 ± 0.2 ^b	3.9 ± 0.2 ^b	4.5 ± 1.0 ^b	4.0 ± 0.5 ^a	4.6 ± 0.1 ^b	4.0 ± 0.1 ^c
17:1	0.3 ± 0.0	0.4 ± 0.0 ^a	0.4 ± 0.0 ^a	0.4 ± 0.1 ^a	0.4 ± 0.0 ^a	0.4 ± 0.0 ^a	0.4 ± 0.0 ^a
18:1n-9	26.2 ± 0.2	25.0 ± 0.4 ^a	22.2 ± 0.6 ^b	23.2 ± 0.5 ^a	27.1 ± 0.3 ^a	23.5 ± 0.3 ^b	20.8 ± 0.1 ^b
20:1n-9	0.4 ± 0.0	0.2 ± 0.0 ^a	0.1 ± 0.0 ^b	0.1 ± 0.0 ^a	0.2 ± 0.0 ^a	0.2 ± 0.0 ^a	0.1 ± 0.0 ^a
22:1	0.4 ± 0.0	0.4 ± 0.0 ^b	0.5 ± 0.0 ^a	0.4 ± 0.0 ^b	0.4 ± 0.0 ^b	0.5 ± 0.0 ^a	0.5 ± 0.0 ^a
24:1n-9	0.4 ± 0.1	0.4 ± 0.2 ^b	0.4 ± 0.0 ^b	0.6 ± 0.5 ^a	0.3 ± 0.0 ^c	0.2 ± 0.1 ^c	0.4 ± 0.1 ^b
ΣMUFA	30.9 ± 0.4	30.2 ± 0.4^a	27.7 ± 0.8^b	29.3 ± 0.6^a	32.8 ± 0.8^a	29.8 ± 0.3^b	26.8 ± 0.2^c
18:2n-6	12.9 ± 0.4	11.6 ± 0.3 ^a	10.9 ± 0.1 ^a	12.9 ± 0.8 ^a	12.4 ± 0.1 ^a	9.5 ± 0.1 ^b	10.6 ± 0.1 ^b
18:3n-6	0.2 ± 0.0	0.2 ± 0.0 ^b	0.2 ± 0.0 ^a	0.2 ± 0.0 ^a	0.1 ± 0.0 ^c	0.2 ± 0.0 ^a	0.1 ± 0.0 ^b
20:2n-6	2.0 ± 0.2	3.5 ± 0.3 ^a	2.7 ± 0.0 ^a	2.6 ± 0.0 ^a	2.8 ± 0.1 ^b	2.3 ± 0.0 ^b	2.0 ± 0.1 ^b
20:3n-6	0.2 ± 0.1	0.2 ± 0.0 ^b	0.2 ± 0.0 ^b	0.1 ± 0.0 ^b	0.3 ± 0.1 ^a	0.3 ± 0.1 ^a	0.2 ± 0.0 ^a
20:4n-6	1.1 ± 0.0	0.9 ± 0.0 ^b	1.0 ± 0.1 ^a	0.8 ± 0.1 ^b	0.8 ± 0.0 ^c	1.1 ± 0.0 ^a	1.0 ± 0.0 ^a
22:2n-6	0.2 ± 0.0	0.2 ± 0.0 ^b	0.1 ± 0.0 ^a	0.1 ± 0.0 ^a	0.2 ± 0.0 ^b	0.1 ± 0.0 ^a	0.1 ± 0.0 ^a
22:4n-6	0.1 ± 0.0	0.1 ± 0.0 ^b	0.1 ± 0.0 ^a	0.1 ± 0.0 ^a	0.1 ± 0.0 ^b	0.1 ± 0.0 ^a	0.1 ± 0.0 ^a
Σn-6 PUFA	16.7 ± 0.4	16.6 ± 0.1^a	15.2 ± 0.1^a	16.9 ± 0.8^a	16.8 ± 0.2^a	13.5 ± 0.1^c	14.0 ± 0.1^b
18:3n-3	2.9 ± 0.3	5.7 ± 0.3 ^a	5.4 ± 0.2 ^a	5.6 ± 0.4 ^a	4.1 ± 0.1 ^b	4.0 ± 0.0 ^b	4.4 ± 0.1 ^b
18:4n-3	0.3 ± 0.0	0.3 ± 0.0 ^a	0.1 ± 0.0 ^b	0.1 ± 0.0 ^a	0.2 ± 0.0 ^b	0.2 ± 0.0 ^a	0.1 ± 0.0 ^a
20:3n-3	0.8 ± 0.1	1.0 ± 0.0 ^b	1.0 ± 0.0 ^b	1.0 ± 0.1 ^b	1.0 ± 0.0 ^a	0.8 ± 0.0 ^a	0.9 ± 0.0 ^b
20:4n-3	0.6 ± 0.0	0.5 ± 0.0 ^b	0.7 ± 0.0 ^a	0.5 ± 0.0 ^a	0.4 ± 0.0 ^c	0.5 ± 0.0 ^b	0.5 ± 0.0 ^a
20:5n-3	4.3 ± 0.2	1.0 ± 0.1 ^b	3.4 ± 0.0 ^a	3.2 ± 0.2 ^b	0.8 ± 0.0 ^c	4.0 ± 0.1 ^a	3.8 ± 0.1 ^b
22:5n-3	1.1 ± 0.1	3.8 ± 0.2 ^b	0.9 ± 0.0 ^a	0.9 ± 0.2 ^a	3.4 ± 0.2 ^c	1.0 ± 0.1 ^a	0.9 ± 0.1 ^b
22:6n-3	17.8 ± 0.3	14.6 ± 0.3 ^b	16.0 ± 0.3 ^a	14.5 ± 0.3 ^b	13.7 ± 0.7 ^c	16.7 ± 0.3 ^a	18.0 ± 0.3 ^b
Σn-3 PUFA	27.7 ± 0.4	26.7 ± 0.4^b	27.6 ± 0.2^a	25.8 ± 0.4^b	23.6 ± 0.8^c	27.4 ± 0.5^a	28.6 ± 0.3^a
EPA+DHA	22.0 ± 0.3	18.4 ± 0.2^b	19.4 ± 0.3^b	17.6 ± 0.2^b	17.0 ± 0.9^b	20.7 ± 0.4^a	21.8 ± 0.3^b
Σn-3/Σn-6 PUFA	1.7 ± 0.0	1.6 ± 0.0^b	1.8 ± 0.0^b	1.5 ± 0.1^b	1.4 ± 0.1^c	2.0 ± 0.0^a	2.0 ± 0.0^b

Note: SD is the standard deviation, n=6. Different letters indicate groups that differ from each other (p<0.05)

Preparation of Fatty Acid Methyl Esters (FAMES)

The crude lipids obtained from the samples were weighed and transferred to clean tubes, to which 1.5 ml of 2 M NaOH was added. The saponification process was performed by filling the tubes with nitrogen gas and subjecting them to a temperature of 80°C for 1 hour. After cooling the samples, 2 ml of BF₃ (25% of brontrifluoride methanol) was then added, and the tubes were again filled with nitrogen and kept at 80°C for 30 minutes. After the incubation period, the samples were allowed

to cool again. 1 ml of hexane was added and the samples were vortexed; following this, 1 ml of ultrapure water was added and the samples were vortexed again. The hexane layer in each tube was then taken and transferred to a new tube containing sodium sulphate. After adding another 1 ml of hexane and vortexed again, the upper hexane layer of each tube was transferred to 2 ml GC vials, which were then filled with nitrogen (Metcalf & Schmitz, 1961). Finally, the vials were placed on the Hewlett Packard Agilent 6890 N model gas chromatography (GC) for analysis of FAME (Bayır, 2011).

Table 5. Fatty acid composition of 100% fish oil and 100% camelina seed in liver tissue(mean±SD)

Fatty Acids	Initial	100% Fish Oil			100% Camelina Seed Oil		
		First sample (15th day)	Second sample (30th day)	Third sample (45th day)	First sample (15th day)	Second sample (30th day)	Third sample (45th day)
14:0	1.0 ± 0.0	2.8 ± 0.2 ^a	2.8 ± 0.0 ^a	3.0 ± 0.2 ^a	1.1 ± 0.0 ^b	1.0 ± 0.0 ^b	1.0 ± 0.0 ^b
15:0	0.2 ± 0.0	0.4 ± 0.0 ^a	0.5 ± 0.0 ^a	0.5 ± 0.0 ^a	0.2 ± 0.0 ^{ab}	0.2 ± 0.0 ^b	0.2 ± 0.0 ^b
16:0	16.8 ± 0.1	17.7 ± 0.1 ^a	21.0 ± 0.3 ^a	21.4 ± 0.1 ^a	15.4 ± 0.2 ^c	16.4 ± 0.4 ^a	14.1 ± 0.4 ^a
17:0	0.4 ± 0.0	0.4 ± 0.0 ^a	0.5 ± 0.0 ^a	0.5 ± 0.0 ^a	0.3 ± 0.0 ^a	0.5 ± 0.0 ^a	0.3 ± 0.0 ^b
18:0	7.2 ± 0.2	4.7 ± 0.1 ^a	5.0 ± 0.1 ^b	4.8 ± 0.3 ^b	5.6 ± 0.3 ^{ab}	7.8 ± 0.1 ^a	6.0 ± 0.0 ^a
20:0	0.2 ± 0.0	0.5 ± 0.0 ^a	0.5 ± 0.0 ^a	0.6 ± 0.0 ^a	0.4 ± 0.0 ^a	0.3 ± 0.0 ^a	0.3 ± 0.0 ^a
22:0	0.3 ± 0.0	0.9 ± 0.1 ^a	0.6 ± 0.1 ^a	0.5 ± 0.0 ^a	0.5 ± 0.0 ^a	0.3 ± 0.0 ^a	0.3 ± 0.0 ^a
24:0	0.4 ± 0.1	0.6 ± 0.0 ^a	0.4 ± 0.0 ^b	0.3 ± 0.0 ^b	0.5 ± 0.1 ^b	0.3 ± 0.0 ^d	0.5 ± 0.1 ^a
ΣSFA	26.5 ± 0.2	28.2 ± 0.2^a	31.4 ± 0.2^a	31.5 ± 0.2^a	24.0 ± 0.3^b	26.8 ± 0.5^b	22.7 ± 0.5^b
14:1	0.1 ± 0.0	0.1 ± 0.0 ^a	0.1 ± 0.0 ^a	0.1 ± 0.0 ^a	0.1 ± 0.0 ^a	—	0.1 ± 0.0 ^a
15:1	0.2 ± 0.0	0.3 ± 0.0 ^a	0.2 ± 0.1 ^a	0.2 ± 0.0 ^a	0.2 ± 0.0 ^a	0.1 ± 0.0 ^a	0.1 ± 0.0 ^b
16:1	2.5 ± 0.1	3.9 ± 0.1 ^b	5.9 ± 0.3 ^a	5.3 ± 0.2 ^b	3.4 ± 0.1 ^d	2.4 ± 0.4 ^b	3.0 ± 0.2 ^c
17:1	0.2 ± 0.0	0.4 ± 0.0 ^a	0.4 ± 0.0 ^a	0.4 ± 0.0 ^b	0.3 ± 0.0 ^{ab}	0.4 ± 0.0 ^a	0.3 ± 0.0 ^c
18:1n-9	21.2 ± 0.3	22.8 ± 0.0 ^b	24.0 ± 0.1 ^a	20.7 ± 0.5 ^b	21.6 ± 0.1 ^d	17.2 ± 0.1 ^c	19.9 ± 1.1 ^d
20:1n-9	0.1 ± 0.0	0.2 ± 0.0 ^a	0.2 ± 0.0 ^a	0.1 ± 0.0 ^a	0.2 ± 0.1 ^a	0.1 ± 0.0 ^a	—
22:1	0.5 ± 0.0	0.6 ± 0.0 ^b	0.5 ± 0.0 ^b	0.5 ± 0.0 ^b	0.6 ± 0.0 ^c	0.7 ± 0.0 ^b	0.7 ± 0.0 ^{ab}
24:1n-9	0.2 ± 0.1	0.6 ± 0.1 ^a	0.5 ± 0.1 ^a	0.3 ± 0.3 ^a	0.2 ± 0.0 ^{bc}	0.2 ± 0.1 ^b	0.5 ± 0.1 ^a
ΣMUFA	25.0 ± 0.4	28.9 ± 0.2^a	31.8 ± 0.3^a	27.5 ± 0.4^a	26.7 ± 0.2^b	21.2 ± 0.5^b	24.7 ± 1.1^b
18:2n-6	9.2 ± 0.2	10.6 ± 0.1 ^a	8.0 ± 0.1 ^a	8.1 ± 0.2 ^a	8.1 ± 0.3 ^a	6.8 ± 0.1 ^c	6.2 ± 0.5 ^b
18:3n-6	0.2 ± 0.0	0.3 ± 0.1 ^a	0.2 ± 0.0 ^a	0.1 ± 0.0 ^a	0.2 ± 0.0 ^c	0.1 ± 0.0 ^b	0.1 ± 0.0 ^b
20:2n-6	1.6 ± 0.1	1.6 ± 0.1 ^b	1.8 ± 0.0 ^b	1.5 ± 0.1 ^b	4.3 ± 0.1 ^a	1.8 ± 0.1 ^b	2.8 ± 0.2 ^a
20:3n-6	0.1 ± 0.1	0.3 ± 0.0 ^a	0.3 ± 0.0 ^a	0.2 ± 0.0 ^a	0.2 ± 0.0 ^c	0.1 ± 0.0 ^c	0.2 ± 0.0 ^c
20:4n-6	2.9 ± 0.1	1.1 ± 0.0 ^b	1.1 ± 0.0 ^b	1.0 ± 0.0 ^b	2.0 ± 0.1 ^b	4.1 ± 0.1 ^a	3.8 ± 0.1 ^a
22:2n-6	0.5 ± 0.1	0.3 ± 0.0 ^b	0.1 ± 0.0 ^b	0.1 ± 0.0 ^b	0.3 ± 0.0 ^b	0.9 ± 0.0 ^a	0.8 ± 0.0 ^a
22:4n-6	0.2 ± 0.1	0.2 ± 0.0 ^b	0.1 ± 0.0 ^a	0.1 ± 0.0 ^a	0.1 ± 0.0 ^b	0.1 ± 0.0 ^c	0.1 ± 0.0 ^a
Σn-6 PUFA	14.7 ± 0.2	14.4 ± 0.1^b	11.6 ± 0.2^a	11.0 ± 0.1^b	15.2 ± 0.3^a	14.1 ± 0.0^b	14.0 ± 0.3^a
18:3n-3	1.2 ± 0.2	2.4 ± 0.0 ^a	2.4 ± 0.0 ^a	3.1 ± 0.1 ^a	4.0 ± 0.0 ^a	2.2 ± 0.1 ^a	3.3 ± 0.2 ^a
18:4n-3	0.2 ± 0.0	0.2 ± 0.0 ^a	0.2 ± 0.0 ^a	0.1 ± 0.0 ^b	0.1 ± 0.0 ^a	0.2 ± 0.0 ^b	0.2 ± 0.0 ^a
20:3n-3	1.4 ± 0.0	0.6 ± 0.0 ^a	0.7 ± 0.0 ^b	0.6 ± 0.0 ^b	1.9 ± 0.0 ^a	1.4 ± 0.0 ^b	1.8 ± 0.1 ^a
20:4n-3	1.0 ± 0.0	0.8 ± 0.4 ^a	0.5 ± 0.0 ^b	0.4 ± 0.0 ^b	1.4 ± 0.0 ^b	1.4 ± 0.1 ^b	1.1 ± 0.0 ^b
20:5n-3	3.6 ± 0.1	1.4 ± 0.1 ^a	3.7 ± 0.0 ^b	4.3 ± 0.1 ^a	0.8 ± 0.0 ^b	5.5 ± 0.1 ^a	4.8 ± 0.2 ^a
22:5n-3	1.1 ± 0.1	4.4 ± 0.2 ^a	0.9 ± 0.1 ^a	1.0 ± 0.0 ^b	3.1 ± 0.1 ^a	1.1 ± 0.0 ^b	1.1 ± 0.0 ^b
22:6n-3	25.3 ± 0.2	18.7 ± 0.3 ^b	16.7 ± 0.1 ^b	20.3 ± 0.2 ^b	22.8 ± 0.2 ^b	26.0 ± 0.5 ^{bc}	26.5 ± 0.6 ^a
Σn-3 PUFA	33.9 ± 0.4	28.5 ± 0.3^b	25.2 ± 0.2^b	29.8 ± 0.4^b	34.2 ± 0.2^a	37.8 ± 0.7^a	38.7 ± 0.7^a
EPA+DHA	28.9 ± 0.3	23.1 ± 0.4^b	20.5 ± 0.1^b	24.6 ± 0.3^b	25.9 ± 0.2^a	31.5 ± 0.7^a	31.4 ± 0.5^a
Σn-3/Σn-6 PUFA	2.3 ± 0.1	2.0 ± 0.0^b	2.2 ± 0.0^b	2.7 ± 0.1^a	2.2 ± 0.1^a	2.7 ± 0.0^a	2.8 ± 0.1^a

Note: SD is the standard deviation, n=6. Different letters indicate groups that differ from each other (p<0.05)

Reverse Transcriptase and Quantitative Polymerase

Chain Reaction (RT-qPCR) Analysis

For this study, six fish were anesthetized with 0.2% tricaine methanesulfonate (Finquel MS-222, Argent Chemical Laboratories, Redmond, WA, USA), and liver and muscle samples of approximately 0.5 g were taken. These samples were stored in 2 ml RNAlater and transferred to the Molecular Biology Laboratory of Department of Agricultural

Biotechnology, Atatürk University, Erzurum, Turkey, where they were stored at -80°C until analysis could be performed. After the isolation of total RNA (TRIzol method, Life Technologies), agarose gel electrophoresis and nanodrop were used to determine the quality of total RNA and the total RNA concentration, respectively. Before cDNA synthesis, RNA was treated with DNase (DNase I Deoxiribonuclease I, Amplification Grade Cat No: 18068-015) to eliminate genomic DNA contamination. Omniscript Reverse Transcription kit (Qiagen, Düsseldorf, Germany) was used for cDNA synthesis

from a total of 2 µg of RNA from each tissue (Vélez-Calabria et al., 2021). The steady-state levels of rainbow trout *fabp6* and *fabp3* mRNA transcripts for liver and muscle tissues were assayed using RT-qPCR as outlined by Bustin et al. (2005). A Rotor-Gene 6000 thermal cycler system (Qiagen GmbH, Düsseldorf, Germany) and a QuantiTect SYBR Green PCR kit (Qiagen) were used for RT-qPCR analyses according to the manufacturers' instructions. For RT-qPCR, initial denaturation occurred at 95.0°C for 15 min, and was followed by 40 cycles consisting of denaturation (at 95.0°C for 20 s), primer annealing (for 30 s) and elongation (at 72.0°C for 30 s). To calculate normalized steady-state levels of *fabp* mRNA transcripts in rainbow trout liver and muscle tissues, the mean copy number of *fabp* mRNA transcripts was divided by the mean copy number of the reference gene (β -actin, which is constitutively expressed at approximately the same steady-state levels in all tissues) (Torstensen et al., 2009; Anderson & Elizur, 2012). SPSS (Version 10.0) was used for statistical analysis. Differences in gene expression between the study groups seemed significant as a result of the two-way ANOVA test were subjected to Duncan's multiple comparison test (SPSS, 2011).

Results and Discussion

Nutrigenomics of Rainbow Trout *fabp3* and *fabp6* Genes

The aim of this study is to investigate the effect of camelina oil as a replacement of fish oil in rainbow trout diets for to evaluate fatty acid composition, gene expression of fatty acids binding protein genes (*fabp3* and *fabp6*) of liver and muscle tissues and investigate the growth parameters.

Results showed that hepatic *fabp6* gene expression levels were significantly higher ($p < 0.05$) in the CO67 (25.15±0.46) experimental group than in the CO100 (22.76±0.87) and CO33 (23.19±0.66) groups, but were not significantly higher than expression levels in the FO (23.76 ± 0.52) group. Statistical analysis showed that differences in hepatic expression of *fabp6* between the CO33, CO100 and FO groups were not significant. Similarly, differences in expression of the *fabp6* gene in muscle tissue were not found to be significant across the experimental groups [FO (23.76±0.52), CO100 (22.52±0.46), CO67 (23.27±0.09), CO33 (22.48±0.58)].

Hepatic *fabp3* gene expression levels were found to be significantly higher in the FO group (24.46±0.52) than in the CO100 group (22.71±0.87) ($p < 0.05$). Hepatic expression was lower in the CO67 group (23.44±0.52) than in the CO33 group

(22.76±0.46), but this difference was found to be statistically insignificant. Muscle *fabp3* gene expression levels were found to be higher in the FO group (22.78±0.52) than in all three of the CO-fed groups [CO67 (22.55±0.52), CO100 (23.76±0.87) and CO33 (23.76±0.46)], but the difference was very slight, and was found to be insignificant as a result of statistical analysis.

The mRNA expression levels of *fabp3* and *fabp6* genes in liver and muscle tissues, and indication of effects of replacing dietary FO with 33%, 67%, and 100% CO mix is presented here in rainbow trout. It was observed that muscle and liver tissues fatty acid composition analysed for gene expression analysis reflected the dietary fatty acid composition in this study.

Intracellular lipid chaperones, known as FABPs, are a group of molecules that regulate lipid response in cells, and are strongly associated with metabolic and inflammatory pathways (Furuhashi & Hotamisligil, 2008). Free FAs are transported into the cell by protein transporters and *fabps* from the plasma membrane. Tissues such as liver and muscle have rapid fat metabolism, and have high *fabp* levels in proportion to their FA intake and use (Storch & Corsico, 2008). Fish convert ALA (18:3 n-3) to DHA (22:6 n-3) with EPA and LA (18:2 n-6) to ARA (20:4 n-6) cannot be synthesized by vertebrates, and are therefore referred to as "essential" FAs (Kanazawa et al., 1979; Sargent et al., 2002). They can be converted to n-3 and n-6 HUFA by desaturation, elongation, and alternative chains (Sargent et al., 2002; Nakamura & Nara, 2004). In fish, it has been shown that an increase in 18:2n-6 and 18:3n-3 FAs increases desaturation activity for the production of HUFAs. In this study, however, the low levels of *fabp3* gene expression and high levels of *fabp6* gene expression were found in the muscle of fish fed a FO-rich diet (Tocher et al., 2002; Ling et al., 2006).

Bayır et al. (2015) used nucleotide sequence databases (*fabp1*, 2, 3, 6, 7, 10 and 11) and phylogenetic analysis to determine the mRNA transcripts encoded by 14 different *fabp* genes in rainbow trout, and reported that most of these genes are duplications resulting from teleost-specific whole genome duplication (WGD) events. However, the *fabp3* and *fabp6* genes were found as single copies in the rainbow trout genome. Steady-state transcript levels differ between *fabp3* and *fabp6* genes. While some *fabp* transcripts such as *fabp3* are found large quantities in many tissues, others (such as *fabp6*) are limited to a few tissues. In the study, levels of *fabp3* and *fabp6* mRNA expression in fish fed CO-based diets were very similar to those found in fish fed FO-based diets. No adverse effects were found on FA metabolism in the muscle and liver.

Table 6. Fatty acid composition of 67% and 33% camelina seed in liver tissue(mean±SD)

Fatty Acids	Initial	33 % Camelina Seed Oil			67% Camelina Seed Oil		
		First sample (15th day)	Second sample (30th day)	Third sample (45th day)	First sample (15th day)	Second sample (30th day)	Third sample (45th day)
14:0	2.0 ± 0.2	2.7 ± 0.1 ^b	2.6 ± 0.0 ^b	2.6 ± 0.1 ^b	2.4 ± 0.1 ^b	2.2 ± 0.1 ^b	2.3 ± 0.2 ^b
15:0	0.3 ± 0.0	0.4 ± 0.0 ^a	0.4 ± 0.0 ^b	0.4 ± 0.0 ^b	0.4 ± 0.0 ^a	0.4 ± 0.0 ^b	0.4 ± 0.0 ^b
16:0	15.6 ± 0.3	16.5 ± 0.1 ^b	19.2 ± 0.1 ^b	20.7 ± 0.1 ^b	16.1 ± 0.1 ^b	19.3 ± 0.4 ^b	18.9 ± 0.4 ^b
17:0	0.5 ± 0.0	0.5 ± 0.1 ^a	0.5 ± 0.0 ^a	0.5 ± 0.0 ^a	0.5 ± 0.0 ^a	0.5 ± 0.0 ^a	0.4 ± 0.0 ^b
18:0	4.7 ± 0.3	4.4 ± 0.2 ^c	5.0 ± 0.1 ^b	5.0 ± 0.1 ^a	4.6 ± 0.0 ^a	5.3 ± 0.2 ^a	4.4 ± 1.0 ^a
20:0	0.6 ± 0.1	0.7 ± 0.1 ^b	0.6 ± 0.0 ^b	0.5 ± 0.0 ^b	0.9 ± 0.0 ^a	0.7 ± 0.0 ^a	0.6 ± 0.0 ^a
22:0	1.0 ± 0.0	1.2 ± 0.0 ^{ab}	0.7 ± 0.1 ^a	0.6 ± 0.0 ^b	1.3 ± 0.2 ^a	0.7 ± 0.1 ^a	0.7 ± 0.0 ^a
24:0	0.4 ± 0.1	0.3 ± 0.0 ^c	0.2 ± 0.0 ^c	0.4 ± 0.1 ^a	0.4 ± 0.1 ^b	0.3 ± 0.1 ^b	0.4 ± 0.2 ^a
ΣSFA	25.0 ± 0.5	26.7 ± 0.2^b	29.2 ± 0.1^b	30.7 ± 0.1^a	26.5 ± 0.5^b	29.4 ± 0.9^b	27.9 ± 1.0^b
14:1	0.1 ± 0.0	0.1 ± 0.0 ^a	0.1 ± 0.0 ^a	0.1 ± 0.0 ^a	0.1 ± 0.0 ^a	0.1 ± 0.0 ^a	0.1 ± 0.0 ^a
15:1	0.3 ± 0.1	0.3 ± 0.0 ^a	0.1 ± 0.0 ^b	0.5 ± 0.1 ^a	0.3 ± 0.0 ^a	0.1 ± 0.0 ^b	0.3 ± 0.1 ^a
16:1	2.9 ± 0.3	4.0 ± 0.5 ^a	4.6 ± 0.1 ^b	4.0 ± 0.1 ^c	3.3 ± 0.2 ^a	3.9 ± 0.2 ^b	4.5 ± 1.0 ^b
17:1	0.3 ± 0.0	0.4 ± 0.0 ^a	0.4 ± 0.0 ^a	0.4 ± 0.0 ^a	0.4 ± 0.0 ^a	0.4 ± 0.0 ^a	0.4 ± 0.1 ^a
18:1n-9	26.2 ± 0.2	27.1 ± 0.3 ^a	23.5 ± 0.3 ^b	20.8 ± 0.1 ^b	25.0 ± 0.4 ^a	22.2 ± 0.6 ^b	23.2 ± 0.5 ^a
20:1n-9	0.4 ± 0.0	0.2 ± 0.0 ^a	0.2 ± 0.0 ^a	0.1 ± 0.0 ^a	0.2 ± 0.0 ^a	0.1 ± 0.0 ^b	0.1 ± 0.0 ^a
22:1	0.4 ± 0.0	0.4 ± 0.0 ^b	0.5 ± 0.0 ^a	0.5 ± 0.0 ^a	0.4 ± 0.0 ^b	0.5 ± 0.0 ^a	0.4 ± 0.0 ^b
24:1n-9	0.4 ± 0.1	0.3 ± 0.0 ^c	0.2 ± 0.1 ^c	0.4 ± 0.1 ^b	0.4 ± 0.2 ^b	0.4 ± 0.0 ^b	0.6 ± 0.5 ^a
ΣMUFA	30.9 ± 0.4	32.8 ± 0.8^a	29.8 ± 0.3^b	26.8 ± 0.2^c	30.2 ± 0.4^a	27.7 ± 0.8^b	29.3 ± 0.6^a
18:2n-6	12.9 ± 0.4	12.4 ± 0.1 ^a	9.5 ± 0.1 ^b	10.6 ± 0.1 ^b	11.6 ± 0.3 ^a	10.9 ± 0.1 ^a	12.9 ± 0.8 ^a
18:3n-6	0.2 ± 0.0	0.1 ± 0.0 ^c	0.2 ± 0.0 ^a	0.1 ± 0.0 ^b	0.2 ± 0.0 ^b	0.2 ± 0.0 ^a	0.2 ± 0.0 ^a
20:2n-6	2.0 ± 0.2	2.8 ± 0.1 ^b	2.3 ± 0.0 ^b	2.0 ± 0.1 ^b	3.5 ± 0.3 ^a	2.7 ± 0.0 ^a	2.6 ± 0.0 ^a
20:3n-6	0.2 ± 0.1	0.3 ± 0.1 ^a	0.3 ± 0.1 ^a	0.2 ± 0.0 ^a	0.2 ± 0.0 ^b	0.2 ± 0.0 ^b	0.1 ± 0.0 ^a
20:4n-6	1.1 ± 0.0	0.8 ± 0.0 ^c	1.1 ± 0.0 ^a	1.0 ± 0.0 ^a	0.9 ± 0.0 ^b	1.0 ± 0.1 ^a	0.8 ± 0.1 ^b
22:2n-6	0.2 ± 0.0	0.2 ± 0.0 ^b	0.1 ± 0.0 ^a	0.1 ± 0.0 ^a	0.2 ± 0.0 ^b	0.1 ± 0.0 ^a	0.1 ± 0.0 ^a
22:4n-6	0.1 ± 0.0	0.1 ± 0.0 ^b	0.1 ± 0.0 ^a	0.1 ± 0.0 ^a	0.1 ± 0.0 ^b	0.1 ± 0.0 ^a	0.1 ± 0.0 ^a
Σn-6 PUFA	16.7 ± 0.4	16.8 ± 0.2^a	13.5 ± 0.1^c	14.0 ± 0.1^b	16.6 ± 0.1^a	15.2 ± 0.1^a	16.9 ± 0.8^a
18:3n-3	2.9 ± 0.3	4.1 ± 0.1 ^b	4.0 ± 0.0 ^b	4.4 ± 0.1 ^b	5.7 ± 0.3 ^a	5.4 ± 0.2 ^a	5.6 ± 0.4 ^a
18:4n-3	0.3 ± 0.0	0.2 ± 0.0 ^b	0.2 ± 0.0 ^a	0.1 ± 0.0 ^a	0.3 ± 0.0 ^a	0.1 ± 0.0 ^b	0.1 ± 0.0 ^a
20:3n-3	0.8 ± 0.1	1.0 ± 0.0 ^a	0.8 ± 0.0 ^a	0.9 ± 0.0 ^b	1.0 ± 0.0 ^b	1.0 ± 0.0 ^b	1.0 ± 0.1 ^b
20:4n-3	0.6 ± 0.0	0.4 ± 0.0 ^c	0.5 ± 0.0 ^b	0.5 ± 0.0 ^a	0.5 ± 0.0 ^b	0.7 ± 0.0 ^a	0.5 ± 0.0 ^a
20:5n-3	4.3 ± 0.2	0.8 ± 0.0 ^c	4.0 ± 0.1 ^a	3.8 ± 0.1 ^b	1.0 ± 0.1 ^b	3.4 ± 0.0 ^a	3.2 ± 0.2 ^b
22:5n-3	1.1 ± 0.1	3.4 ± 0.2 ^c	1.0 ± 0.1 ^a	0.9 ± 0.1 ^b	3.8 ± 0.2 ^b	0.9 ± 0.0 ^a	0.9 ± 0.2 ^a
22:6n-3	17.8 ± 0.3	13.7 ± 0.7 ^c	16.7 ± 0.3 ^a	18.0 ± 0.3 ^b	14.6 ± 0.3 ^b	16.0 ± 0.3 ^a	14.5 ± 0.3 ^b
Σn-3 PUFA	27.7 ± 0.4	23.6 ± 0.8^c	27.4 ± 0.5^a	28.6 ± 0.3^a	26.7 ± 0.4^b	27.6 ± 0.2^a	25.8 ± 0.4^b
EPA+DHA	22.0 ± 0.3	17.0 ± 0.9^b	20.7 ± 0.4^a	21.8 ± 0.3^b	18.4 ± 0.2^b	19.4 ± 0.3^b	17.6 ± 0.2^b
Σn-3/Σn-6 PUFA	1.7 ± 0.0	1.4 ± 0.1^c	2.0 ± 0.0^a	2.0 ± 0.0^b	1.6 ± 0.0^b	1.8 ± 0.0^b	1.5 ± 0.1^b

Note: SD is the standard deviation, n=6. Different letters indicate groups that differ from each other (p<0.05)

Table 7. Growth parameters of rainbow trout fed camelina seed oil (*Camelina sativa*)-based diet(mean±SD)

Diets	Weight Gain Rate (%)	Specific Growth Rate (%)	Feed Conversion Rate (%)	Survival Rate (%)
FO100	261.80 ± 11.25 ^a	2.16 ± 0.56 ^a	0.98±0.08 ^a	100 ^a
CO100	254.65 ± 23.82 ^a	2.06 ± 0.15 ^a	0.97±0.03 ^a	100 ^a
CO67	250.27 ± 23.70 ^a	1.99±0.13 ^a	0.97±0.01 ^a	100 ^a
CO33	252.42 ± 24.91 ^a	2.19±0.15 ^a	0.96±0.07 ^a	100 ^a

Note: SD is the standard deviation, n=6. Different letters indicate groups that differ from each other (p<0.05)

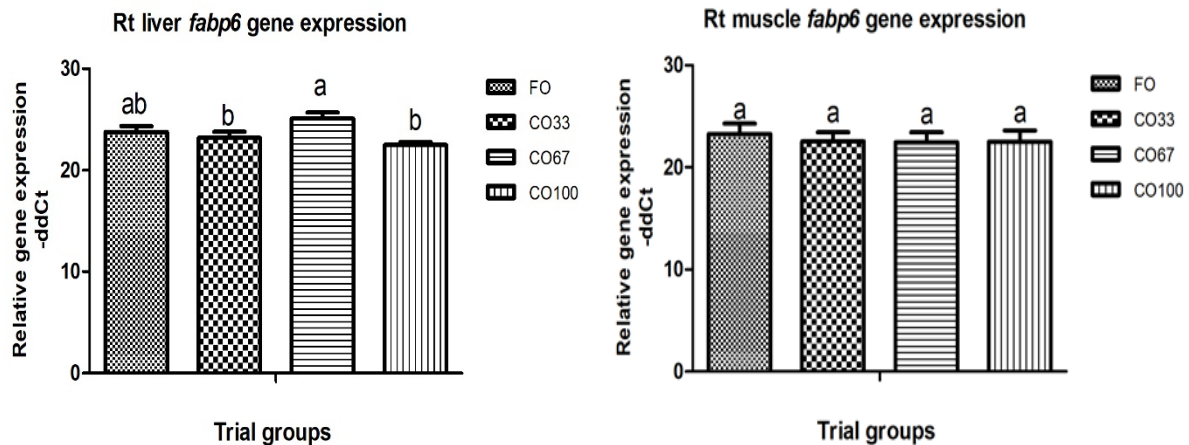


Figure 1. Relative gene expression ($-\Delta\Delta C_t$) for *fabp6* gene expression in liver and muscle of fish fed different lipid contain diets. Bars represent the difference in least square mean relative expression (ΔC_t camelina oil (different amount) $-\Delta C_t$ fish oil) \pm SE.

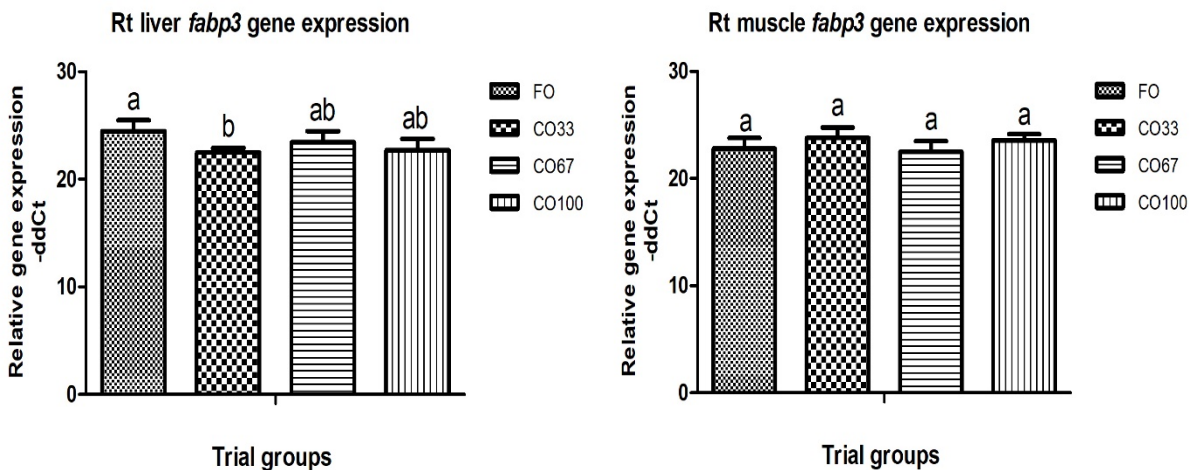


Figure 2. Relative gene expression ($-\Delta\Delta C_t$) for *fabp3* gene expression in liver and muscle of fish fed different lipid contain diets. Bars represent the difference in least square mean relative expression (ΔC_t camelina oil (different amount) $-\Delta C_t$ fish oil) \pm SE.

In general, as the energy requirements of a fish increase, its use of lipids also increases (Tocher et al., 2003). For example, the same literature reported that the quantities of Σ SFA, ARA, and DHA increased during the larval development period of many fish species. In this study, it was observed that the levels of Σ SFA increased in all trial groups compared to the initial value. As SFA is a carcass tissue element (Wiegant et al., 1996), levels will increase with the growth of the fish. 16:0 is used as a source of metabolic energy during fish growth, and was found to be the dominant FA of the Σ SFAs, and it also overlaps with many studies such as Mohamed & Al-Sabahi, (2011), Henderson & Tocher (1987), Vázquez et al. (1994), Sargent et al. (1999a), Luo et al. (2009), and Bayır et al. (2010).

Of the MUFAs, oleic acid (OA; C18:1) and palmitoleic acid (C16:1) are the predominant FAs that account for approximately 67.69% and 22.85% of Σ MUFA, respectively (Yanar et al., 2006). In this study, C18:1 was found to be the

most abundant FA overall, while palmitoleic acid (C16:1) was the most abundant in liver and muscle tissues in the experimental groups, followed closely by oleic acid (C18:1). At the first sampling, the CO100, CO67, and CO33 groups were found to have significantly higher Σ MUFA content than the fish fed the control diet; while at the end of the trial, the CO67 group had more Σ MUFA content than any of the other groups. As CO is rich in C18:1, these findings are likely to reflect the FA composition of the diets.

The lipid content of fish meat mainly consists of triglycerides and long-chain polyunsaturated fatty acids (PUFA). Like all vertebrates, fish require three FAs from PUFAs for normal growth, development, and reproduction (Piedicausa et al., 2007). It has been shown that the essential FA composition changes depending on the molecular size of the lipids present in the fish's diet (Watanabe, 1982; Ensminger et al., 1990; Greene & Selivonchick, 1990; Tucker, 1998).

The levels of n-6 HUFA in fish muscle and liver tissue were found to be directly correlated to the quantity of LA in their diet, with the highest amount of n-6 HUFA found in the CO100 group and the lowest in the FO100 group.

Finally, the levels of EPA and DHA were determined across the experimental groups, with the highest quantities found in fish in the FO100 group. It is well recognized that marine and freshwater fish have different requirements for essential FAs; while marine fish require high quantities of HUFAs in the n-3 series, freshwater fish have an additional requirement of FAs in the n-6 series. These FAs enable effective growth and feed utilization rate, which reduces the levels of protein required in the fish feed (Sowizral et al., 1990; Sargent et al., 1999b; Rinchart et al., 2007).

From the results of this study, it can therefore be concluded that CO can be used as an alternative oil source to FO in rainbow trout diets. This is supported by the lack of significant difference in expression of the *fabp3* and *fabp6* genes in rainbow trout muscle tissue between fish fed CO-based diets, compared to those fed FO-based diets. This suggests that the fundamental molecular lipid metabolism was not negatively impacted by the change in diet. However, rainbow trout should be fed a finishing diet prepared with FO for the last months before being prepared for consumption (Bordignon et al., 2020), which would restore the majority of the FA content of the fish.

The effects of feeding camelina seed oil on the weight gain, specific growth rate, feed conversion rate, and survival rate are presented in Table 7. Weight gain rate of rainbow trout were 261.80 ± 11.25 , 254.65 ± 23.82 , 250.27 ± 23.70 , and 252.42 ± 24.91 in FO100, CO100, CO67, and CO33, respectively. Feed conversion rate of rainbow trout were 0.98 ± 0.08 , 0.97 ± 0.03 , 0.97 ± 0.01 , and 0.96 ± 0.07 in FO100, CO100, CO67, and CO33, respectively. Weight gain, specific growth rate, feed conversion rate, and survival rate decreased in the fish fed camelina seed oil-based diets compared to control group, but the differences was not significantly important among groups. The survival rates were 100% in all groups. In this study, it was observed that the use of camelina seed oil completely or partially replace of fish oil did not have a negative effect on rainbow trout growth rates and survival rate. Many studies have been carried out in which various vegetable oils have been tested in trout and these oils have provided good growth in fish (Torstensen et al., 2000; Bell et al., 2001, 2002; Grisdale-Helland et al., 2002). Fish meal and fish oil, which are the main ingredients of feed in aquaculture, may be insufficient due to the rapid development of fish farming, and this causes the search for alternative oil

sources (Olsen et al., 2003). Studies with vegetable oils show that alternative oil sources can be used to partially or completely replace fish oil and meet the fatty acid needs of the species to be grown without adversely affecting the growth performance of the fish (Dernekbaşı & Karayücel, 2010). The availability of vegetable oils that can replace with fish oil will both eliminate the dependence on fish oil and reduce the cost of feed (Mourente & Bell, 2006). Camelina oil has high levels of PUFA compared to other plant oil (Ni Eidhin et al., 2003). N-3 PUFA and LA are essential dietary components for ensuring optimum fish growth (Chou & Shiau, 1999). The growth performance of salmonids, most notably the offspring of anadrome salmon and trout, is increased when they are fed a diet containing PUFAs, particularly ALA (Sargent et al., 1989). ALA, DHA, and EPA are therefore essential for the growth and development of rainbow trout juveniles. Vegetable oils are poor in n-3 HUFA, but rich in C18 PUFAs, particularly LA, ALA, and OA (Ganga et al., 2011). This was confirmed by the high levels of C18 FA in the liver and muscle tissues of fish fed CO-based diets in this study. Camelina seed oil has potential as a lipid source in diets for rainbow trout replace FO.

Conclusion

In the study, levels of *fabp3* and *fabp6* mRNA expression in fish fed camelina seed oil-based diets were very similar to found in fish fed fish oil -based diets. When the fish meat was evaluated in terms of fatty acid composition, it was observed that the n-3/n-6 ratio was lower in the group fed with camelina seed oil as the oil source. However, it was observed that feeding with camelina seed oil did not have a negative effect on growth parameters and survival rate. For this reason, it is thought that camelina seed oil can be replace with fish oil in rainbow trout feeds, but it would be much more appropriate to apply a fish oil-based final feeding regimen, especially prepared with a high amount of long chain PUFA.

Compliance With Ethical Standards

Authors' Contributions

This manuscript is produced from Sinem Keşan's master thesis. Author SK took part in the literature review and writing of manuscript as well as performing the trial phase and laboratory studies of the study. MB designed the study and wrote the first draft of the manuscript, GA performed and managed statistical analyses. All authors read and approved the final manuscript.

Conflict of Interest

The authors declare that there is no conflict of interest.

Ethical Approval

Ethics committee approval was not required at the time this study was conducted. Working protocols were approved by Atatürk University Local Ethical Committee for Animal Studies. The studies have been approved by Atatürk University research ethics committee and have been performed in accordance with the ethical standards of Atatürk University and its later amendments or comparable ethical standards.

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