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Research Article

Effects of dietary fish oil replacement by canola oil on some functional and growth parameters in juveniles of *Salmo caspius*

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Abstract

This study aim was demonstrating the effects of total and 50% dietary fish oil replacement by canola oil on growth performance, carcass analysis, blood parameters and serology in juvenile Caspian brown trout (*Salmo caspius*, 4.25±0.68 g) for 60 days. Triplicate groups of 15 fish in nine fiberglass tanks held under identical culture conditions (temperature, aerated, dissolved oxygen and photoperiod) were fed three times a day. Three diets were formulated to replace the dietary fish oil containing the same protein level 43% and fat levels of 14% by canola oil (100% fish oil, 100% canola oil and 50% fish oil 50% canola oil). Fish fed with 50% fish oil and 50% canola oil diets had significantly different growth performance compared with other treatments (p<0.05). Feed conversion ratio was significantly different in all treatments with the highest FCR value in 100% fish oil treatment (p<0.05). There was no significant difference on survival rate, carcass protein, fat and moisture. Levels of liver enzymes (Lactate dehydrogenase and Alanine aminotransferase, alkaline phosphatase, aspartate aminotransferase) were significantly higher in 100% fish oil than other treatments. Canola oil improved the immune system and enzymes including ALP, AST, ALT and LDH. Based on the obtained results it can be inferred that replacing 50% fish oil with 50% canola oil in the diet was a suitable for Caspian brown trout which not only had positive effects on growth indices but also can reduce feed formulation costs.

Keywords: Canola oil, Fish oil, FCR, Liver enzymes, *Salmo caspius*, Survival rate

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Introduction

Nutritional requirements are one of the most important issues that need to be considered in breeding of a species with the aim of economic production and maintenance of reserves. On the other hand, the feed cost includes roughly 60% of the cost of aquatic production (Mohseni *et al.,* 2011). Paying attention to nutritional requirements is of great importance to achieve good and rapid growth and optimum weight at the end of breeding period consequently increasing the economic efficiency. Dietary lipid is a potential source of energy with higher energy efficiency compared to the carbohydrates and proteins, which provide essential fatty acids and fat-soluble vitamins that, are important for physiological functions of the body (Sargent *et al.,* 2002). If non-protein sources, such as carbohydrates and lipids can be used to balance dietary energy, they can prevent protein breakdown in order to produce energy for fish (Demska-Zakeoe *et al.,* 2012) and therefore the dietary protein can be used to exclusively produce tissue and weight gain. As a result protein intake in diet would be increased and final cost of breeding would be reduced. Therefore, determining the lipids requirement is an effective step to produce low-cost and high-efficiency diet in fish growth (Coutinho *et al.,* 2014).

World fish oil used by aquaculture sector has been estimated to be about 75%, 600000 MT (IFFO, 2017). Traditionally, most commercial diets for fish include fish oil which is an important lipid source (Oliva-Teles *et al.,* 2015). Fish oil is considered to be an excellent dietary oil source for fish because it is rich in essential fatty acids (EFAs) such as Eicosapentaenoic acid (EPA, 20:5n-3), Docosahexaenoic acid (DHA, 22:6n-3) and arachidonic acids (ARA, 20:4n-6) that are needed for optimal growth and development. But providing oil from marine fish is limited and the price of these oils has increased due to their direct human usage. Also in recent decades, the production of fish oil has remained steady while the production of vegetable oils has grown considerably in the world (IFFO, 2008).

Vegetable oils which are rich in linoleic (LA, 18:2n-6) and linolenic (ALA, 18:3n-3) acids, has been considered to be a suitable alternatives for fish oil in freshwater fish feeds, because freshwater fish are able to convert dietary LA and ALA to highly unsaturated FAs (HUFA), such as ARA, EPA and DHA (Sargent *et al*., 2002; Tocher 2003, 2010). Fatty acids are essential for fish growth and gonadal evolution.

Canola oil is less expensive and far more abundant than fish oil on a global basis (USDA, 2006; Canola Council of Canada, 2007). Canola oil is a rich source of vitamins E and K and useful herbal fats, which help the heart to be healthy. This oil is resistant to light and its peroxide changes are almost zero compared with other oils. Canola oil has a higher nutritional quality than oil of sunflower, corn and soybeans due to presence of unsaturated fatty acids and lack of cholesterol (Higgs *et al.,* 2006).

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The amount of saturated fatty acids in canola oil is very low (7%), while its unsaturated fatty acids (especially omega-3 Alpha-Linolenic acid) are high (11%). Canola oil is more suitable for consumption due to a pleasant fit of 2:1 between linolenic and linoleic acids (Przybylski and Eskin, 2011). The effects of fish oil replacement by vegetable oils and its consequent effects on growth, survival, immunity, nutrition, carcass analysis and carcass fatty acid composition have been evaluated in several studies such as Atlantic salmon (Bell *et al.*, 2002; Jacobs *et al.*, 2002), rainbow trout (Drew *et al.*, 2007; Masiha *et al.*, 2015), *Huso huso* (Nikzad *et al.*, 2013), Caspian brown trout (Abedian Kenari *et al.*, 2010).

Caspian brown trout *(Salmo caspius*), is an endangered anadromous species distributed in southern region of Caspian Sea which migrates to rivers connected to the Caspian Sea, like Sefid-Rud, Gorgan-Rud, and Cheshme Kile rivers for breeding (Armantrout 1980, Kiabi *et al.*, 1999; Coad, 2000). As a result of river pollution, destruction of natural spawning regions, poaching and over-fishing, natural populations of Caspian brown trout have decreased dramatically and is considered critically endangered in southern parts of Caspian Sea according to IUCN criteria (Abdoli, 2000; Coad, 2000; Barannik *et al.*, 2004; Niksirat and Abdoli, 2009). Since fish stock and natural resources protection is one the Iranian fisheries research objectives, the most activity on Caspian trout rehabilitation was focused on releasing thousands of smolts to the rivers discharge to the Caspian Sea. Thus, study on quality of provided brood stocks and producing fries with suitable quality can help the rehabilitation and rearing of this valuable species. Nowadays, aquaculture is being considered as the fastest growing sectors of food production in recent years. Of course, aquaculture is always accompanied by some problems such as nutritional problems. Hence, the present study was designed to investigate the combined effects of fish oil and canola oil in diet on growth performance, survival rate, carcass analysis, some blood parameters and serology in juvenile Caspian brown trout.

Materials and methods

Experimental diets and feeding regime

To prepare the diets, the required ingredients were transferred to Food Analysis Laboratory of the National Research and Livestock Science Center, Tonekabon and based on chemical composition of raw materials, the diets were adjusted. Three experimental diets based on lipid source were formulated on a gross basis to be most isoenergetic and aftermath fish were fed for 60 days (Table 1). The lipid sources used in diets were fish and canola oils. The lipid requirement for the first diet (control or T1) was totally formulated by fish oil, while the fish oil was totally replaced by canola oils in the second diet (T2) and finally for the third diet, 50% of the fish oil was replaced by canola oil. The standard method was used to perform approximate analysis of chemical

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compounds (AOAC, 2005). Difference in diets was only in the type of oil. The amount of energy was calculated according to the standard physiological fuel value (Pike *et al*., 1967) and the protein 4, carbohydrate 4, and fat 9 $kcalg^{-1}$ and diets set using the Lindo Formula Program (Lindo Inc., 1994). The pellets were 2 mm in diameter. The pellets were dried, packed and kept in freezer (18°C) until consumption. An hour before food distribution, diets were removed from freezer and after being equilibrated to room temperature were weighed with a digital scale.

Table 1: Chemical composition of the experimental diets (g kg^{-1} as is basis).

Compositions	Control (100% fish oil)	T2 (100% canola oil)	T3 (50% fish oil and 50% canola oil)
dry matter	878	883	874
Protein	401	395	411
Fat	141	142	145
Ash	56.2	55.8	55.2
Carbohydrate	16	17	16
Fiber	2.5	2.8	3.1
Gross energy (kcal/kg Food)	3807.2	3804.2	3800.9

Sample preparation and proximate composition analysis

Fish specimens (n=135) with a mean initial body weight of 4.6±0.23g (mean ±SD) were cultured in a covered place under a complete randomized design. Groups of 15 fishes were randomly distributed among 9 fiberglass tanks. These tanks were equipped with aeration system, central water drainage, tune water valves (fountain) and were filled with freshwater (1700L per each tank) at a flow rate of 5.89 L min^{-1}.

Each diet was given to three fiberglass tanks three times a day (8h, 12h and 16h). The amount of daily food was calculated based on the feeding table (Hardy, 1998) and according to the average water temperature, weight and number of fish in the tanks. To reduce stress, fish feeding was stopped 12 hours before and after bioassay. For bioassay, fish were anaesthetized with clove powder at the concentration of 300 ppm. The length and weight of each fish was measured and the amount of diet was adjusted for the next 14 days. During the experimental period, mean water temperature was 14.7±1.5°C and dissolved oxygen was 7.6±0.86 mg/L.

Proximate analysis

Feed ingredients, experimental diets, and fish samples were analyzed for proximate compositions (protein, lipid, ash, fiber and moisture) according to the standard methodology of AOAC (1995). At the end of the period, to ensure that gastrointestinal contents were evacuated 12 hours starvation was given to the fishes. Then fish were anaesthetized with clove powder with the concentration of 300 ppm and three specimens were randomly taken from each replicate. The

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carcasses were sent to the laboratory for carcass decomposition (protein, fat, moisture and ash). To measure moisture, samples of diets and fish were dried at 105°C until a constant weight (Khater *et al.*, 2014). Protein was extracted by using Kjeldahl method with estimated total nitrogen (N×6.25). The fat content was extracted using Soxhlet method through chloroform solvent with boiling point 50 to 60°C for 4 to 6 hours. The amount of energy in the food composition was measured by the calorimeter bomb and ash was measured by burning in an electric furnace at 550°C for 9 hours.

Growth performance

Measurements of all fish specimens were conducted and statistical analyses of growth factors such as weight gain (WG), specific growth rate (SGR) and feed conversion ratio (FCR) were calculated per each group (Mohseni *et al.*, 2018):

Body weight increase (BWI) = (final weight–initial weight) / (initial weight) × 100
Specific growth rate (SGR) = [(ln final Weight-ln initial weight) / days] × 100
Feed conversion ratio (FCR) = [feed intake (g)] / [weight gain (g)]
Protein efficiency ratio (PER) = wet weight gain (g) / protein intake (g)

Eighteen hours after starvation, 2 mL blood samples from each fish was taken from the caudal vein, to evaluate blood parameters (CBC), biochemical indices (total protein and albumin) and hepatic enzymes including alanine aminotransferase (ALT) and aspartate aminotransferase (AST).

Hematological assessments

For hematology indices, 0.5 mL of blood was poured into Eppendorf tubes coated with an anticoagulant (heparin). The remaining 1.5 mL of blood was transferred into non-heparinized Eppendorf tubes for serology and blood studies. The number of red blood cells (RBCs), white blood cells (WBC), hematocrit (PCV) and hemoglobin (Hb) were measured using common laboratory methods (Borges *et al.*, 2004). For serological studies, blood samples in the Eppendorf tubes without antagonistic heparin, were centrifuged (3000 rpm, 10 min, Labofuge model, Heraeus sepatch, Germany). The serum was extracted and poured in a new tube and stored at -80°C. These indices were measured using laboratory kits (Pars Azmun, Karaj, Iran) and auto analyzer machine (Eurolyser, Belgium). Albumin was measured by Bromocresol green method and total protein was measured by biuret method.

Enzymological assessment

Alanine aminotransferase (ALT), aspartate aminotransferase (AST), lactate dehydrogenase (LDH), and alkaline phosphatase (ALP) were quantified using the biochemical analyzer apparatus (Ependorf, Germany). Measurements were performed according to the manufacturer's instructions using enzyme-linked laboratory kit. AST and

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ALT enzymes were detected based on Reitman and Frankel's method (1957) using Pars-Azmoon kit (Tehran, Iran). ALP was measured based on Belfield and Goldberg (1971) and LDH activity was assessed according to Tietz' protocol (1976).

Statistical analysis

One-way analysis of variance (ANOVA) was conducted to analyse the row data using SPSS 20.0 software (SPSS Inc., Chicago IL, USA). Comparison of differences among treatment values was performed by Duncan's multiple range test (p<0.05).

Results

Growth performance

Based on the obtained results from the analysis, no significant difference was observed between fish fed with 100% fish oil (treatment 1) and 100% canola oil (treatment 2), considering mean weight, body weight increase and specific growth rate (p>0.05). While the treatment T3 (50% fish oil with 50% canola oil) showed significant differences when it's compared to the other two treatments (p<0.05). So that treatment 3 had the highest average of final weight, weight increase and specific growth rate. No significant difference was observed among different treatments in terms of food conversion ratio and protein efficiency ratio (p>0.05). All treatments had a significant difference in mean fish length, so that the maximum length of fish was related to treatment 3 and the lowest amount was related to treatment 1 (Table 2).

Table 2: Growth performance and feed efficiency indices in juveniles of *S. caspius* fed with diets containing different lipids after 60 days (n=3, Mean ± SD).

Growth factors	T1 (100% fish oil)	T2 (100% canola oil)	T3 (50% fish oil and 50% canola oil)
Initial weight	4.0±7.21	4.0±8.26	4.0±4.15
Final weight(g)	22.0±7.86^{a}	23.0±6.96^{a}	26.0±5.7^{b}
Length(cm)	12.0±5.15^{a}	12.0±9.2^{b}	13±5.25^{c}
BWI (%)	386.8±5.7^{a}	391.32±9.3^{a}	497.35±8.9^{b}
FCR (%)	1.62± 0.17 ^{a}	1.63± 0.11 ^{a}	1.41± 0.01 ^{a}
SGR (%)	2.63± 0.11 ^{a}	2.65±0.11^{a}	2.97± 0.10 ^{b}
PER (%)	1.0±26.2	1.0±29.3	1.0±36.2

Different letters in each row indicate significant differences (p<0.05).

Whole body proximate composition

Whole body proximate composition of juveniles fed with diets containing different oils are shown in Table 3. There was no significant difference between treatments (p>0.05). The amount of carcass ash in treatment 3 (fish oil and canola oil) was significantly higher than that in treatment 1 (fish oil, p<0.05), while there was no significant difference between T2, canola oil and T3, fish oil with canola oil treatments (p>0.05).

Blood indices

As it can be seen, results of the blood indices evaluation in juveniles of *S.*

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caspius are shown in Table 4. There were no significant difference in numbers of white blood cells, hemoglobin and hematocrit concentrations among treatments (p>0.05), while number of red blood cells showed a remarkable increase in treatment T3 (50% fish oil with 50% canola oil) compared to treatment T1 (100% fish oil, $p<0.05$). There were no significant difference between treatments T2 (100% canola oil) and the other treatments ($p>0.05$).

Table 3: Body composition of juveniles of *Salmo caspius* fed with diets containing different lipids after 60 days (n=3, Mean±SD).

Index	T1 (100% fish oil)	T2 (100% canola oil)	T3 (50% fish oil and 50% canola oil)
Protein	16.0±3.17	16.0±1.47	16.0±3.32
Fat	4.0±5.34	4.0±3.23	4.0±9.22
Ash	2.0±62.12^{a}	2.0±93.14^{ab}	3.0±22.2^{b}
Moisture	75.6±7.69	76.4±5.76	76.1±1.78

Different letters in each row indicate significant differences ($p<0.05$).

Table 4: Blood indices of juveniles of *Salmo caspius*, fed with diets containing different lipids after 60 days (n=3, Mean ± SD)

Index	T1 (100% fish oil)	T2 (100% canola oil)	T3 (50% fish oil and 50% canola oil)
Red blood cell ($\times 10^6$ /mm^3)	1.53±0.08^{c}	1.59±0.06^{bc}	1.67±0.03^{ab}
White blood cell ($\times 10^6$ /mm^3)	8.70±0.81	9.30±0.17	8.76±0.50
Hemoglobin (g / dl)	9.16±0.55	9.7±0.50	9.7±0.69
hematocrit (%)	40.66±1.52	41.33±1.15	41.33±2.08

Different letters in each row indicate significant differences ($p<0.05$).

Liver enzymes

Obtained results from the liver enzymes of *S. caspius* juveniles fed with different diets based on lipid sources are shown in Table 5. LDH, ALP, ALT and AST enzymes values in treatment T1 (100% fish oil) showed significant difference with treatments T2 (100% canola oil) and T3 (50% fish oil with 50% canola oil, $p<0.05$), although no significant difference was observed between treatments T2 and T3 ($p>0.05$).

Table 5: Liver indices of *S. caspius* juveniles, fed with diets containing different lipids after 60 days (n=3, Mean ± SD)

	T1 (100% fish oil)	T2 (100% canola oil)	T3 (50% fish oil with 50% canola oil)
LDH	2716.125±3.7^{b}	1717.51±3.7^{a}	1618.72±3.9^{a}
ALP	676.32±3.8^{b}	365.36±7.9^{a}	351.39±7.5^{a}
ALT	11.1±5.79^{b}	7.1±4.94^{a}	7.1±1.21^{a}
AST	519.18±4.9^{b}	326.16±7.1^{a}	331.20±7.3^{a}

Different letters in each row indicate significant differences ($p<0.05$)

Discussion

In this study, part of fish oil replacement with canola oil had a positive effect on growth factors and fish fed with this diet had the highest final weight. The reason for this observation can be expressed in

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the high amount of unsaturated fatty acids in canola oil (Shafaeipour *et al.*, 2008). Bell *et al.* (2002) showed that replacing fish oil with canola oil in salmon diet resulted in similar GR and FCR and no negative effect on growth and efficiency of fish food. Webster and Lim (2002) also found that canola meal has the potential to be used as a substitute for fish meal in blue catfish (*Ictalurus furcatus*) diet, which reduces the cost of food. Considering growth performance indices, the obtained results through this study support the performed study on juvenile yellowtail *Seriola quinqueradiata* in which the best results obtained by 50% replacement of fish oil with Canola oil (Fukada *et al.*, 2017).

A study performed by Drew *et al.* (2007) showed that replacement of fish oil and fish meal with canola concentrate Protein and canola and flax seed oil combination, had no negative effect on rainbow trout (*Oncorhynchus mykiss*) growth performance and has the potential of replacing expensive sources of diet, which is similar to the results of the present study.

Kazemi *et al.* (2016) expressed that replacement of fish oil with vegetable oils, especially canola and sunflower oil is possible for enrichment of *Artemia naupli* to feed rainbow trout larvae. It provides better growth and higher survival rate in fish that those fed with vegetable oils in the first stage of growth.

Results about the effect of using vegetable oils on growth and nutritional factors in fish are sometimes contradictory. Nikzad *et al.* (2013) used the following treatments to replace vegetable oils with fish oil in juvenile beluga sturgeon (*Huso huso*) diet: first treatment (50% fish oil+25% soybean oil+25% sunflower oil), second treatment (50% fish oil+25% sunflower oil and 25% canola oil) and control treatment (100% fish oil). Their results indicated that the mean final weight in control treatment was significantly lower than that of other treatments. However, there was no significant difference in other growth parameters including WI, CF, SGR, FCR and PER among different treatments.

Bayraktar and Bayır (2012) also investigated the effect of complete replacement of cod liver oil with animal fat (Goose meat, sheep tail fat and cow fat) on growth performance, survival rate and fatty acid profile of rainbow trout. It was concluded that the final fish weight did not differ significantly among treatments, which is consistent with the results of this study.

Mohammadi Ashnani *et al.* (2008) investigated the effect of replacing different levels of linseed oil (25, 50, 75 and 100%) with fish oil in rainbow trout diet. They reported that there was no significant difference in final weight and growth rate of fishes ($p>0.05$) but the percentage of carcass fat, protein and total fatty acids3-n increased significantly with increase in linseed oil content in the diet ($p<0.05$).

The results of Güler and Yildiz (2011) also showed that partial and total substitution of fish oil with linseed oil in trout diet did not affect growth factors

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and food conversion ratio. Research by Bell *et al.* (2002), Şener and Yildiz (2003) and Torstensen *et al.* (2004) in replacement of vegetable oils with fish oil in rainbow trout and Atlantic salmon (*Salmo salar*) diets showed no significant difference in growth factors and food conversion ratio ($p>0.05$). However, the use of vegetable oils in some fish such as Surubim, *Pseudoplatystoma coruscans* (Martino *et al.*, 2002) and sea bass, *Sparus auratus* (Menoyo *et al.*, 2004) caused significant change in growth. It seems that the reason for these contradictory results is the amount of fish meal of diets and ability of fish to consume fat as energy and thus have more protein storage. For example, Atlantic salmon has the ability to store protein and use fat as an energy source, while in fish like Murray cod this ability does not exist and any change in the composition of dietary fatty acids affect the growth of the fish due to the role of these fatty acids in cell structure (Francis *et al.*, 2007).

Suitable feed conversion ratio (FCR) reduces the cost and amount of food and as a result, reduces water pollution and secondary infections. The results of this study showed that the combination of fish oil and canola oil significantly reduced the conversion rate of food compared to the other treatments. These results are similar to the results of Nikzad *et al.* (2013) on juvenile cultured beluga sturgeon.

Requirement of lipids is different in fishes. Types of dietary fat affect fish's appetite and physical properties of food, such as fragility of food grains due to physical pressures, thus this may change the amount of food intake.

The results of this study showed that different levels of fish oil and canola oil did not have a significant effect on protein, fat and moisture content of Caspian brown trout in different treatments. Similar results are observed in the study of Masiha *et al.* (2015) in replacing linseed oil with fish oil (ratio 1 to 1) in fingerling trout diet. Also, in the study of Nikzad *et al.* (2013), like the results of this study, addition of canola and soybeans vegetable oils did not show a significant effect on chemical composition of carcass in juvenile beluga sturgeon (*Huso huso*). Badpa Roodsari *et al.* (2013) showed that replacing 5% soybean oil with 5% fish oil increased the amount of carcass fat and moisture in fingerling trout significantly but did not have a significant effect on other variables such as ash and crude protein.

The results of this study showed that in treatments fed with 100% fish oil, the levels of ALP, AST, LDH and ALT were significantly higher than other treatments. AST, ALT and ALP are nonspecific enzymes. These enzymes not only are found in blood plasma but also in the liver, heart, gills, kidneys, muscles and other organs. They can also provide specific information on function and failure of these organs. AST, ALT, and ALP are known as indicators of liver function and are important enzymes for checking health status of fish (Racicot *et al.*, 1975) and liver cells are rich in these enzymes. LDH is often measured to

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evaluate liver tissue damage (Yilmaz *et al.*, 2006).

Incorrect nutrition management may affect the activity of ALT (Cech *et al.*, 2006). ALT and AST serum activity is significantly different depending on fish species. Increased plasma levels of ALT and AST may be caused by stress, cellular damage, or cellular degradation due to heavy metals in liver, heart, or muscle (Yokoyama *et al.*, 2003). Menoyo *et al.* (2004) stated that fat oxidation was lower in liver of Bream fed with 11% and 21% soybean oil compared to fish fed with fish oil and linseed oil. The results of these researchers were in accordance with the results of the recent research. Theoretically, diets that contain high unsaturated fatty acids in their composition are susceptible to fat oxidation, which increase the level of peroxidation of mitochondrial fat and damage liver cells (Abedian Kenari *et al.*, 2010). Amount of LDH enzyme in fish fed with 100% fish oil was significantly higher than that in other treatments. High levels of saturation in diet may result in wrinkling red blood cells (Mourente and Bell, 2006). This enzyme is high in all tissues but its amount in red blood cells is 150 times higher than plasma. Even a very small amount of red blood cell hemolysis will make a significant difference in the enzyme. Therefore, the probable cause of high levels of this enzyme in the above treatment is the damage to red blood cells and the entry of this enzyme into plasma. According to the results, oils used in the diet have a significant effect on fish immune system. On the other hand, care should be taken during the storage of various oils, especially fish oil and suitable packaging after preparing food is needed. Oxidation of fatty acids not only increases immune response, but also weakens the immune system. According to the results, it is recommended to use canola oil in combination with fish oil in cultured brown trout due to the presence of significant amounts of essential fatty acids, alpha linolenic (C18: 3 n-3) and linoleic acid (C18: 2 n-6) in canola oil.

The present study has shown the possibility of replacing fish oil by canola oil in *Salmo caspius* dietary without a negative effect on growth and feed utilization. So our finding indicates that canola oil is a potentially trustable dietary lipid source for *S. caspius* and totally can be used instead of fish oil with the best results being partial substitution (50% FO and 50% CO). As bearing in mind economic importance of cost efficiency, this substitution can reduce the related feed preparation costs through rearing period of *S. caspius*.

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