The Effect of Replacement of Fish Oil (FO) By Canola Oil (CO) On Blood Serum Enzymes of the Rainbow Trout (Oncorhynchusmykiss)

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ABSTRACT

Fatty acids have several effects on immune responses. The present study was performed in order to determine the effect of replacement of fish oil (FO) by canola oil (CO) on blood serum enzymes, i.e. Aspartate aminotransferase (AST), Alanine aminotransferase (ALT), Lactate dehydrogenase (LDH), and Alkaline phosphatase (ALP), of the rainbow trout (Oncorhynchusmykiss). Four experimental diets were formulated with partial or complete (0, 33%, 67%, and 100%) replacement of FO by CO. Fish were fed at 4% of their body weight three times a day (at 8, 12, and 18) for 50 days. Average temperature, dissolved oxygen, and pH were 14°C, 7.5 mg.lit-1, and 7.32, respectively. Measurement of ALT, AST, LDH, and ALP was performed by biochemistry analyzer (Eppendorf, Germany) and laboratory kits. Statistical analysis was performed by one-way ANOVA and Duncan’s multiple-range test (p<0.05). The results derived from the present study revealed that the fish fed by 100% fish oil had the highest level of AST, ALT, LDH, and ALP in their blood serum and it had a significant difference with other treatments (p<005). It is concluded that oils used in fish feeding diets might have profound influence on blood serum enzymes. KEYWORDS: Rainbow trout, canola oil, fish oil, serum enzymes.

1- INTRODUCTION

Cytology is one of the most important branches of clinical studies which can have a high level of contribution in clinical diagnoses. Major focus of cytological studies was on red blood cells whereas other factors such as white blood cells, proteins, and ions are studied in current studies (Kazemi et al., 2010). Extracellular fluid, which covers all cells of a living creature, plays important roles in physicochemical stability of intracellular status, providing energy sources for metabolism, removing metabolic wastes, and molecule shifts, among others (Natochin et al., 1997). Cells contain enzymes which catalyze all biochemical reactions in cells. Enzymes enter interstitial fluids and the, they move to blood serum and cerebrospinal fluid. Therefore, measuring rate and activity of enzymes in biologic fluids can provide valuable information on functions of organs and tissues. Enzyme release into blood circulation might be caused by several reasons such as cellular necrosis and anoxia resulting in disintegration of cell membrane. So, changes in organs can be detected by measuring enzymes in blood serum. International measurement unit for enzymes is International Unit per liter (IU.lit-1) (Mojabi, 1991).

Aspartate aminotransferase (AST) exists in mitochondria and plasma and it is thus known as a nonspecific enzyme. However, along with other enzymes, it can help diagnose liver and muscle disorders. It is also found in kidney tissue and therefore, its increase can not only be indicative of liver disorders but also kidney or even heart disorders. For instance, it increases in nephrocalcinosis in salmonoides (Stoskopf, 1992). Amount of AST does not change in Infectious Salmon Anemia (ISA) while it doubles a day after the infection in cyprinids. It is also revealed that transaminases (e.g. AST and ALT) rise in response to stress. Alanine aminotransferase (ALT) is found in plasma and some cells. The mains source of this enzyme is liver and its activity in blood serum rises considerably in acute hepatic diseases leading to membrane damage and cell necrosis. ALT exists in fish blood serum and it is abundant in fish liver. It rises considerably in acute liver necrotic disorder but decreases in severe infection with Aeromonas; it remain unchanged in noninfectious salmon anemia. Lactate dehydrogenase (LDH) is abundantly found in all tissues although it is 150 times more in red blood cells than in plasma. It is a nonspecific enzyme in liver, muscles, kidney, and heart. It also exists in fish with a similar mechanism found in other animals. Unlike AST, ALT, and ALP, the amount of this enzyme rises in salmon anemia. Alkaline phosphatase (ALP) is one of the first enzymes whose clinical importance has been recognized. Its amount rises in liver and bone disorders. It also increases in hepatic fibrosis, and hepatic lipidosis.

Use of immune stimulators to increase immune system activity has gained a great deal of attention in aquaculture systems in order to enhance resistance against diseases in cultured fish. Several immune stimulators

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155
have been adopted in vaccines and foods to enhance specific and nonspecific immune responses, respectively. It has been shown that they have important roles in protecting fish against disease (Kumari et al., 2003). Immune stimulators can be prescribed in foods for long time and this can be a benefit over vaccines. In addition, some vaccines cannot be prescribed for many kinds of fish. The present study was formulated in order to evaluate the effect of replacement of fish oil (FO) by canola oil (CO) on blood serum enzymes of the rainbow trout (*Oncorhynchus mykiss*).

### 2- MATERIALS AND METHODS

This study was performed in Educational and Research Center for Aquaculture in Islamic Azad University, Azadshahr Branch, Azadshahr, Iran. Fish were cultured in 12 500-lit fiberglass tanks (105×103×53 cm). Water was stocked from a deep well. The tanks were filled with water until 50 cm. each tank was equipped with cylindrical tubes to close bottom hole of the tanks. Water was discharged through the embedded holes at the end of cylindrical tubes. Fish were purchased from a cold-water aquaculture centers in Firoozkuh City. Three hundred fish (63.5±0.6 g; 70±6.2 cm long) were selected and transferred to the tanks. Twenty five fish were placed in each tank. In order to determine the effect of replacement of fish oil (FO) by canola oil (CO) on blood serum enzymes, four treatments (0, 33%, 67%, and 100% of CO instead of FO) in two iterations were considered. Fish feed (crude protein: 35.31, crude oil: 5.31) was purchased from Mazandaran Cattle and Fish Nutrition Co., Sari, Iran. Then, 150 g canola and/or fish oil was added to 850 g crude feed according to the treatments. The oil was sprayed on dry matter and then, the feed was let to dry so that the oil is absorbed by the feed. Fish were fed at 4% of their body weight three times a day (at 8, 12, and 18) for 50 days. Tank water was reduced to half while feeding to determine fish appearance.

Water and environmental status, i.e. temperature (°C) and dissolved oxygen (mg.lit⁻¹), were recorded daily to make sure environmental status is similar for all the treatments. Average temperature, dissolved oxygen, and pH were 14°C, 7.5 mg.lit⁻¹, and 7.32, respectively.

At the end of the course, 2 fish from each tank (4 fish from each iteration) were selected. The fish were anesthetized by clove concentrate and blood samples were taken from caudal vein. From each sample, 1 cc was poured in tubes without heparin. For serum separation, blood samples were centrifuged at 3000 rpm for 5 min. the serum were kept in freezer until next experiments. Measurement of ALT, AST, LDH, and ALP was performed by biochemistry analyzer (Eppendorf, Germany) and laboratory kits.

Normality test was performed by Shapiro-Wilk test. Statistical analysis was performed by one-way ANOVA and Duncan’s multiple-range test (p<0.05) in SPSS (version 20) and graphs were drawn in Excel (Microsoft Office, 2007).

### 3- RESULTS

#### 3-1- The effect of replacement of fish oil (FO) by canola oil (CO) on blood serum AST of the rainbow trout (*Oncorhynchus mykiss*)

The results showed that the fish fed by 100% fish oil had the highest level of AST in their blood serum and it had a significant difference with other treatments (p<005) (Fig. 1).

![Figure 1: The effect of replacement of fish oil (FO) by canola oil (CO) on blood serum AST of the rainbow trout (*Oncorhynchus mykiss*)](image-url)
3-2- The effect of replacement of fish oil (FO) by canola oil (CO) on blood serum ALP of the rainbow trout (*Oncorhynchusmykiss*)

The results revealed that the fish fed by 100% fish oil had the highest level of ALP in their blood serum and it had a significant difference with other treatments (p<0.05) (Fig. 2).

![Figure 2: The effect of replacement of fish oil (FO) by canola oil (CO) on blood serum ALP of the rainbow trout (*Oncorhynchusmykiss*)](image)

3-3- The effect of replacement of fish oil (FO) by canola oil (CO) on blood serum ALT of the rainbow trout (*Oncorhynchusmykiss*)

According to the results, the fish fed by 100% fish oil had the highest level of ALT in their blood serum and it had a significant difference with other treatments (p<0.05) (Fig. 3).

![Figure 3: The effect of replacement of fish oil (FO) by canola oil (CO) on blood serum ALT of the rainbow trout (*Oncorhynchusmykiss*)](image)

3-4- The effect of replacement of fish oil (FO) by canola oil (CO) on blood serum LDH of the rainbow trout (*Oncorhynchusmykiss*)

The results showed that the fish fed by 100% fish oil had the highest level of LDH in their blood serum and it had a significant difference with other treatments (p<0.05) (Fig. 4).

![Figure 4: The effect of replacement of fish oil (FO) by canola oil (CO) on blood serum LDH of the rainbow trout (*Oncorhynchusmykiss*)](image)
Effect of oil in feed has not been studied extensively. Recent studies have confirmed the effect of PUFA on fish immune system. The source of fatty acids entering plasma membrane is known as the oil in feed. Therefore, composition of fatty acid of cell membrane denotes oil composition of feed (Clamp et al., 1997). Also, there is a close correlation between fatty acid composition in feed and fatty acid composition in tissue (Sargent et al., 2002). Variations in n-3/n-6 in feed can affect immune cell structure (Thompson et al., 1995; Bell et al., 1993). They are energy source and have role in cell membrane. Manipulation of feed acids can influence on immune system by changing structure of cell membrane (Tacon, 2004).

The results obtained from the present study showed that AST, ALP, ALT, and LDH in the fish fed with a diet containing 100% fish oil were significantly higher than those in other treatment. AST, ALP, and ALT are non-plasma specific enzymes which are found not only in blood plasma but also in liver, heart, gills, kidney, muscles, and other organs. They can also provide valuable information about functions and disorders in the organs. The amount of AST, ALP, and ALT is used as an indicator of liver function and the enzymes are considered important to evaluate health status in fish (Racicot et al., 1975). LDH is often used to evaluate hepatic damages (Yilmaz et al., 2006). Improper management of nutrition might effect on ALT activity (Cech et al., 2000). Activity of serum ALT and AST varies with species. Increase in plasma AST and ALT might be related to stress condition, cell damages in liver, and cell degradation in liver, heart, or muscles caused by heavy metals (Yokoyama et al., 2003).

Menoyo et al. (2004) found that lipid oxidation in bream liver fed by 21% and 11% soybean oil was lower than that in the fish fed by fish oil and linseed oil. This is consistent with the results obtained from the present study. In theory, the diets with high level of unsaturated fatty acids are prone to oxidation resulting in higher level of lipid peroxidation in mitochondria and hepatic cell damage (AbedianKenari et al., 2010). Rehulka and MinaOik (2007) stated that increase in AST activity is a sing of serious liver damage via AST release in mitochondria. Infection rises ALT and LDH. Sakomoto et al. (2001) showed that change in enzyme amount can be due to different blood sampling techniques, analysis methods, age, diet, and habitat.

LDH amount in the fish fed by the diet with 100% fish oil was significantly higher than that in other treatments. High saturation level in the diet might cause shrinkage in red blood cells (Mourente et al., 2006). Khajeh and Peyghan (2007) analyzed some biochemical factors of blood serum of rainbow trout cultured in earth ponds and reported serum ALP, AST, ALT, and LDH to be 722±320, 337±150, 29±24, and 579±353 IU.lit⁻¹. The results acquired from the present study about ALP and ALT were lower than those in the study of Khajeh and Peyghan (2007).

LDH in the present study in all the treatments was higher than that reported by Khajeh and Peyghan (2007). As feeding was performed by hand here and the food was kept at ambient situation, lipid oxidation, especially in fish oil, might have adverse effect on red blood cells and LDH release resulting in higher level of this enzyme in plasma. However, morphological studies on red blood cell is required to prove this.

In conclusion, oils used in fish feeding diets might have profound influence on blood serum enzymes. However, hygienic issues should be considered during oil preservation, especially fish oil, because fatty acid oxidation not only arouses immune system response but also weakens immune system and might have considerable influence on blood serum enzymes.
REFERENCES


