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Influence of Macerated and Cold Press Wheat Germ (*Triticum vulgare*) Oils Added at Different Rates to Trout Feed on The Feed Rate (FCR), Haemotology Parameters and NBT (Nitroblue tetrazolium) Values of Rainbow Trout (*Oncorhynchus mykiss*)

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Abstract

In this study, 2% of macerated and cold press wheat germ oils were added to the feeds of rainbow trout (average weight of 39.8 ± 4 g). At the end of the study, growth rate, blood parameters and NBT (Nitro blue tetrazolium) level of rainbow trout were determined. Feed Conversion Ratios (FCR) of groups were determined as macerated: 1.12, cold press: 1.53, control: 1.11. Statistically significant differences were found in red blood cell (RBC), hemoglobin (HGB), hematocrit (HCT), white blood cell (WBC), platelet (PLT) values ($p<0.05$). There was no statistically significant difference between Nitroblue Tetrazolium (NBT) levels ($p>0.05$).

Keywords: Blood parameters, nitroblue tetrazolium, trout, wheat germ oil

Yemlere Farklı Oranlarda Eklenen Masere ve Soğuk Sıkım Buğday Tohumu (*Triticum vulgare*) Yağlarının, Gökkuşağı Alabalığı (*Oncorhynchu smykiss*)'nın Yem Değerlendirme Oranı (FCR), Hematolojik Parametreler ve NBT (Nitroblue tetrazolium) Değerlerinin Üzerine Etkisi

Öz

Bu çalışmada, ortalama 39,8 ± 4 g ağırlığındaki gökkuşağı alabalıklarının yemlerine % 2 oranında masere ve % 2 oranında soğuk sıkım buğday tohumu yağı ilave edilmiştir. Çalışmanın sonunda, gökkuşağı alabalıklarının büyüme hızı, kan parametreleri ve NBT (Nitroblue tetrazolium) düzeyi belirlenmiştir. Grupların yem çevirme oranları (YÇO): Masere buğday yağı: 1,12, soğuk sıkım buğday yağı: 1,53, kontrol: 1,11 olarak tespit edilmiştir. Eritrosit (RBC), hemoglobin (HGB), hematokrit (HCT), lökosit (WBC), trombosit (PLT) değerlerinde anlamlı bir fark bulunmuştur ($p<0,05$). Nitroblue tetrazolium (NBT) düzeyleri arasında istatistiksel olarak anlamlı farkın olmadığı tespit edilmiştir ($p>0,05$).

Anahtar Kelimeler: Kan parametreleri, nitroblue tetrazolium, alabalık, buğday yağı

INTRODUCTION

In the last two decades, aquaculture has increased dramatically, which has caused a higher demand for fish feed formulas. These feeds may represent as much as 60% or more of the production cost (Özkan et al., 2015). In addition to reducing costs and using them as feed additives, plants are used in fish in various ways as disinfectants or therapeutics in different studies (Chang et al., 2001; Chorianopoulos et al., 2008; Altinterim et al., 2012; Altinterim et al., 2018a-c). The plants are either given directly to the fish in different ways, or they are left in the water and affect the fish by altering the properties of the water (Gulec et al., 2013).

Many vegetable oils have been used as feed additives for fish. However, there are not many studies with wheat oil. Wheat germ oil (WGO) is the best source of tocopherol. Tocopherols are substances that exhibit vitamin E activity. Wheat oil contains alpha, beta, gamma tocopherol. Tocopherols inhibit the synthesis of eicazonoids that activate lipid peroxidation duration (Paranich et al.,

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2000). Vitamin E is an antioxidant and effective against oxidation, known as the first barrier of the defense system both inside and outside the cell. In addition vitamin E has preventive effects on blood clotting, leukocytes and macrophages (Bouts and Gasthuys, 2003). Vitamin E is an oil-soluble vitamin and can be easily oxidized in heat. There are eight forms in nature, four of which are tocopherol (alpha, beta, sigma, and gamma) and four are tocotrienols (alpha, beta, sigma, and gamma). Among them alpha-tocopherol is the most potent antioxidant (Khalifa et al., 2011). WGO contains linoleic, oleic, palmitic, alpha-linolenic, stearic acid, as well as highly unsaturated and poly-saturated fatty acids (Zacchi et al., 2006). Especially alpha-linoleic acid shows anti-inflammatory properties by reducing the amount of super oxide radical (Niu et al., 2011). WGO also contains lutein, zeaxanthin and beta carotene, which are oil-soluble and have an antioxidant effect (Leenhardt et al., 2008). Fatty acids in WGO are particularly important in decreasing the effects of aging, immune system, reproductive system, healthy working of circulatory system. Fatty acids contained in the oil have been found to enhance the stability of the P53 protein which inhibits cell mutation (Omima et al., 2011).

Many routine haematological methods already exist in human medicine to assist in providing evidence, and possible identification, of an abnormality or disease process. Some of the methods could readily be adapted for use with fish blood as has indeed been done by some workers (Blaxhall, 1972). It was therefore decided to establish some routine haematological screening methods with healthy fish which may assist in assessing fish health (Blaxhall and Daisley, 1973).

Blood consists of three different cells and a fluid that leads to their functions. Red cells are the cells in the bloodstream that are larger in quantity, nucleated in fish and carry oxygen and carbon gas. Leukocytes are defense cells, and platelets can be used to assess the immune system because they are related to blood clotting with organic defense and have phagocytic function (Cicero et al., 2014).

In this study, we purposed to determine the feed conversion ratio (FCR), some blood parameters and NBT values of the rainbow trout (*O. mykiss*) of macerated and cold pressed wheat germ oil added to rasion at 2% ratio.

MATERIAL AND METHODS

Rainbow trout (*Oncorhynchus mykiss*, Walbaum, 1792) with an initial average weight of 39.8 ± 4 g supplied from Sürgü, Malatya, Turkey. Fish were adapted to the experimental system for two weeks. Then, the experiment was started. The fish were kept in 1000 l fiberglass tanks supplied with aerated spring water. A total 90 rainbow trout were randomly divided into 3 groups each group consisted of two tanks and each tank contained 15 fish. Fish were anesthetized (Benzocaine 30 mg/L, Stehly et al. (2000) before the blood collection. Experiments were conducted in accordance with ethical rules (Inonu University, Faculty of Medicine, Experimental Animal Ethics Committee, Protocol No:2017/A-24). Blood samples were taken from the tail veins of fishes by vacutainer and transferred to tubes containing EDTA. On the same day nitroblue tetrazolium (NBT) activity (total oxidative radical production of neutrophils) from EDTA blood samples was determined spectrophotometrically. Full blood count was also performed with PROCAN PE-6800VET brand fully automated hematology analyze.

Wheat germ (*Triticum vulgare*) (crushed) was kept in 1/10 sunflower oil for 15 days and macerated oil was obtained. The pelleted fat was added to Pınar commercial trout diet at a rate of 2%. The study groups were formed as control (no wheat germ oil added to the diet), experiment group 1 (D1: 2% macerated wheat germ oil added to the diet) and experiment group 2 (D2: 2% cold pressed wheat germ oil added to the diet (it was purchased from a local store). Ten fish were studied in each group and three were repeated. Fish were fed daily with feed at a rate of 2% of their body. Feeding was done twice a day for 21 days, morning and evening. In order to determine the feed conversion ratio (FCR) of the fish, the weight of the fish at the beginning and end of the experiment and the amount of feed consumed were determined by Hoşsu et al. (2001) was calculated with the formula developed.

$$\text{FCR} = \frac{\text{Amount of Feed Consumed (g)}}{\text{Weight Gain (g)}}$$

White blood cells (WBC), lymphocyte (LYM), monocytes (MID), granulocyte (GRAN), red blood cells (RBC), hemoglobin concentration (HGB), hematocrit (HCT), mean erythrocyte volume

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(MCV), cell hemoglobin average (MCH), cell hemoglobin percentage (MCHC), platelet (PLT), were measured using a blood count device.

The data obtained were statistically tested using the SPSS statistical program at the 0.05 confidence interval by Tukey test.

RESULTS AND DISCUSSION

Statistically significant differences were determined in the WBC, RBC, HGB, HCT, PLT parameters ($p<0.05$) between groups and no statistically significant difference was determined in other blood parameters ($p>0.05$). The blood parameters measured at the end of the 21-day trial and blood parameters are shown in Table 1.

In this study, the best FCR value was obtained in the control group. Although there were numerical differences between the control and thetwo experimental groups, no statistically significant difference was observed ($p>0.05$). Table 2 shows FCR, mean weight change values.

The mean NBT values were 0.457 in the control group, 0.474 in the D1 group and 0.455 in the D2 group. NBT activity was not significant in the case of macerated and cold pressed oil application.It has been stated that wheat oil significantly reduces the negative effects of toxic substances by changing hepatic enzyme activities and can be used for hepatoprotective purposes. In particular, this effect is thought to be effective in wheat germ oil (Khalifa et al., 2011). In one study, WGO has been found to be successful in preventing oxidative stress against harmful effects of toxic substances (Aboubakr et al., 2014).

Hematocrit values are an indicator of physiological stress and oxidative stress in fish (Kiron et al., 2004).

Table 1. Mean values of blood parameters measured at the end of 21 days feeding according to experimental groups ($p<0.05$)

Blood parameters	Control	Group D1	Group D2
WBC (10^3/µL)	57.34±4.26^{b}	50.15±8.03^{a}	55.23±4.43^{ab}
RBC (10^6/µL)	1.64±0.13^{ab}	1.40±0.28^{a}	1.78±0.39^{b}
HGB (g/dl)	8.61±0.74^{ab}	7.36±1.43^{a}	9.74±1.70^{b}
HCT (%)	21.40±1.94^{ab}	17.58±3.33^{a}	23.65±4.81^{b}
PLT (10^3/µL)	33.40±16.60^{b}	23.00±5.70^{ab}	12.00±3.43^{a}
LYM (10^3/µL)	92.37±1.31	92.65±1.13	93.4±1.25
MID (10^3/µL)	4.78±0.54	4.57±0.67	4.54±0.76
GRAN (%)	2.83±0.81	2.97±0.52	2.53±0.70
MCV (fL)	131.15±5.66	128.91±4.90	129.66±3.24
MCH (pg)	52.58±2.05	52.66±2.38	53.06±1.11
MCHC (g/dl)	40.2±0.61	40.97±1.17	40.19±1.06
RDW-SD (fL)	77.72±12.09	81.2±6.53	69.86±5.97
RDW-CD (%)	12.54±1.61	14.18±1.56	11.46±1.14
MPV (fL)	12.22±1.39	12.65±0.81	14.58±0.97
PDW (%)	13.71±6.53	14.46±3.80	14.9±5.26
PCTY (%)	0.034±0.02	0.021±0.01	0.014±0.01
PLCR (%)	36.63±8.64	38.84±5.03	50.16±6.10
NBT (Neutrophil counts)	1.826±0.18	1.89±0.15	1.82±0.16

Table 2. FCR, mean weight change values

	Control	D 1	D 2
Average weight 0 day	39.8±2.1	39.4±2.7	40.2±3.2
Average weight 21 day	61.4±2.6	59.0±3.5	49.2±3.3
FCR	1.11	1.12	1.53

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Because red blood cells (RBC) are one of the main production sites of free radicals. Hematocrit and hemoglobin values are needed when blood needs to increase oxygen carrying capacity or meet increasing energy needs change. This gives good clues about the environment, nutrients and fish physiology (Caruso et al., 2005). In this study, RBC values decreased in macerated oil group and increased in cold press group compared to control group. This shows that antioxidant effect of cold-pressed oil is greater.

Since there is no study on the blood parameters of fish related to wheat oil, studies on different animals have been examined. In ourstudy, RBC, HGB and HCT values decreased in the table and increased in the cold press oil. 3% ground wheat was carried out on the offspring, RBC, HCT, were found to be at the highest level (Gāliņa and Valdoska, 2017).

In another study performed on mice, it was determined that RBC, HGB, HCT values were increased according to the group in which heavy metal was applied. In subjects who were exposed to heavy metal, blood production was suppressed, and it was found that the group administered wheat oil increased the stimulation of erythropoietin in the adrenal gland (Abdou et al., 2017).

In this study, WBC rates decreased significantly in both D1 and D2. Especially in D1, this decrease was higher. This may mean that WGO has a curative effect, as in the study of rats.

The cold press wheat oil group, which had decreased PLT value compared to the control group of both groups, was observed at the lowest level. This decrease is in parallel with the suppression of immunity.

Phytoestrogens in wheat have been found to show estrogenic effects in fish, causing vitellogenesis in fish and causing low growth rate (Pelissero et al., 1991; Kaushik et al., 1995). Tusche et al. (2012), In an FCR study on wheat gluten and potato-fed rainbow trout, no significant difference was found between the experimental groups and the control group. In this study, the best FCR value was obtained in the control group. Although there were numerical differences between the control and the two experimental groups, no statistically significant difference was observed ($p>0.05$).

NBT activity was not significant in the case of macerated and cold pressed oil application ($p>0.05$). In similar study, it was not stimulated respiratory burst in sea bream (Sitjà-Bobadilla et al., 2005). Ferreira et al. (2015), in the study conducted on tilapia NBT value, which is the determinant of the non-specific immune system, decreased in macerated olive oil and riviera and extra virgin olive oil groups statistically significant increase. Similar differences have emerged in this study. NBT values in the purer cold press group were similar to control group and increased in macerated oil group.

CONCLUSION

As a result, when the RBC and NBT values were examined, it was seen that the use of wheat oil as a feed additive in rainbow trout had antioxidant effects. On the other hand, wheat oil has no significant effect on growth.

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