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Effects of Macerated and Cold Pressed Sesame (*Sesamum indicum* L.) Oil on Antioxidant Enzyme Activities, Hematological Parameters, Nitro Blue Tetrazolium (Nbt) Activity and Proximate Composition of Rainbow Trout (*Oncorhynchus mykiss*, Walbaum, 1792) at High Stocking Density

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Abstract

The study was aimed to determine the effects of macerated sesame oil (MSO) and cold pressed sesame (*Sesamum indicum* L.) oil (CPSO) on antioxidant enzyme activities, hematological parameters, nitro blue tetrazolium (NBT) activity and proximate composition of rainbow trout (*Oncorhynchus mykiss*, Walbaum, 1792) at high stocking density. Fish (55.89±2.05 g) were fed diets supplemented with same concentration (%2) of MSO and CPSO for 21 days. It was determined that the values of mean corpuscular hemoglobin (MCH), platelet (PLT) values of the CPSO and group and the corpuscular hemoglobin concentration (MCHC), platelet (PLT), mean platelet volume (MPV), platelet-large cell ratio (PLCR), granulocyte (GRAN), nitro blue tetrazolium (NBT) levels of the MSO group were increased. It was found that glutathione peroxidase (GPx) and catalase (CAT) activities of CPSO groups were rised. The results were statistically significant at $P< 0.05$ level. In the study, the dry matter ratio increased in each experimental group compared to the control group, the moisture, ash and protein ratio did not change, the fat ratio decreased compared to the control group. Both macerated sesame oil and cold pressed sesame oil could be recommended to alternative food additives.

Keywords: Blood parameters, fish feeding, immune biomarkers, oxidative stress.

Masere ve Soğuk Pres Susam (*Sesamum indicum* L.) Yağının, Yüksek Yoğunlukta Stoklanmış Gökkuşağı Alabalıkları (*Oncorhynchus mykiss*, Walbaum, 1792)'nın Antioksidan Enzim Aktiviteleri, Hematolojik Parametreleri, Nitro Blue Tetrazolium (NBT) Aktivitesi ve Et Verimi Üzerine Etkileri

Öz

Bu çalışmanın amacı, masere susam yağı (MSO) ve soğuk pres susam (*Sesamum indicum* L.) yağının (CPSO) yüksek yoğunlukta stoklanmış gökkuşağı alabalığı (*Oncorhynchus mykiss*, Walbaum, 1792)'nın antioksidan enzim aktiviteleri, hematolojik parametreler, nitro mavi tetrazolium (NBT) aktivitesi ve yaklaşık kompozisyonu üzerine etkilerinin belirlenmesidir. Balıklar (55.89±2.05 g) 21 gün boyunca aynı konsantrasyonda (%2) MSO ve CPSO içeren yemlerle beslendi. CPSO ve gruba ait ortalama korpüsküler hemoglobin (MCH), trombosit (PLT) değerleri ile korpüsküler hemoglobin konsantrasyonu (MCHC), trombosit (PLT), ortalama trombosit hacmi (MPV), trombosit-büyük hücre oranı değerlerinin olduğu belirlendi. MSO grubunda (PLCR), granülosit (GRAN), nitro blue tetrazolium (NBT) seviyelerinin arttığı, CPSO gruplarının glutatyon peroksidaz (GPx) ve katalaz (CAT) aktivitelerinin arttığı belirlendi. Sonuçlar $P<0.05$ düzeyinde istatistiksel olarak anlamlı bulundu. Araştırmada her deney grubunda kontrol grubuna göre kuru madde oranı artmış, nem, kül ve protein oranı değişmemiş, yağ oranı ise kontrol grubuna göre azaldığı tespit edildi. Alternatif gıda katkı maddeleri olarak hem masere susam yağı hem de soğuk sıkım susam yağı tavsiye edilmektedir.

Anahtar Kelimeler: Balık besleme, immün biyobelirteçler, kan parametreleri, oksidatif stres.

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INTRODUCTION

Fish obtained from sea, fresh waters and aquaculture facilities have an important and nutritious place in our diet. Many aquatic products are an excellent source of vitamins and minerals for human nutrition. Among the protein sources, seafood with a high degree of digestibility is quite low in terms of fat when compared to other high protein foods. In addition, seafood is the only source of ω-3 series polyunsaturated fatty acids with proven health benefits (Turan et al., 2006). The nutritional content of cultured fish meat depends on several factors such as species, season, diet, habitat and age (Drazen 2007; Tuna Kelestemur and Ozdemir, 2013). Food scientist is interested in making fish foods high protein foods while providing the best quality taste, color, smell, texture and safety that can be obtained with maximum nutritional value (Mohamed et al., 2010). For these reasons, knowledge on the chemical composition of freshwater fish in general is valuable to nutritionists interested in food sources that are readily available, such as most low-fat and high-protein freshwater fish (Mozaffarian et al., 2003; Foran et al., 2005).

Feed additives are indispensable components of fish diets. In the fish diet, feed additives play an important role for the aquaculture and immunity of animals. Feed additives are substances that are added to the fish diet in small amounts and act as preservatives. Some authors found that the feed additive is antimicrobial, anti-oxidant, growth-promoting, and immune-enhancing (Tuna Kelestemur, 2011; Yadav et al., 2021).

Plants and herbal products are used as feed additives in fish farming. These additives are added to improve blood and antioxidant values. These substances are made by adding oil, hydrosol (Altınterim et al., 2012; Küçükgül et al., 2013; Altınterim et al., 2018a; Tuna Kelestemur et al., 2021), herbal extracts (Kılıç et al., 2007) or plant ingredients obtained in powder form to the feed. Cold pressing and heat treatment applications are generally used to obtain herbal oils. Maceration is an another technique which has been used in many fields including alternative medicine in Southeast Asia since ancient times (Hsu et al., 2013; Kantawong et al., 2017), although it has not been used much in aquaculture studies. In recent studies, it has been observed that there are positive improvements in the health of fish fed by macerated oil added into feed. (Altınterim et al., 2018b; Altınterim et al., 2018c).

Sesame (*Sesamum indicum*, L.) has been cultivated in various ecological regions of Turkey. Sesame seed oil has anti-inflammatory, anti-bacterial, hypolipidemic and antitumor effects (Anilakumar et al., 2010; Özdemir et al., 2018). It includes mono and polyunsaturated fatty acids, proteins, minerals, many compounds such as phytosterols, tocopherols, mainly oleic and linoleic, sesamol, sesaminol, sesamolinol, pinoresinol, matairesinol, lariciresinol and episesamine (Yamashita et al., 1995). Sesame seed oil is highly strong to oxidation (Budowski 1964) and its biochemical mixture also makes this oil one of the most resistant seed oils against oxidation. Sesamol, an antioxidant, was detected only in cold pressed sesame oil (CPSO). Sesamin and sesaminol have anti-mutagenic, anti-oxidant and anti-inflammatory effects and free radical scavengers (Shakoori et al., 1990; Osawa et al., 1995). These antioxidants were determined to have inhibitory effects on lipid peroxidation (Kang et al., 1998). Sesame oil inhibits the formation of reactive oxygen and free radicals. Natural antioxidants in sesame oil are aforethought useful for the prevention of oxidative damage (Das 2000). Non-enzymatic antioxidants in sesame oil could act to overcome the oxidative stress (Maree et al., 2009). Sesame oil was experimented on the various hematologic and oxidative stress parameters in animals and humans (Sankar et al., 2006; Namiki, 2007; Saleem et al., 2012). The role of the antioxidant defense system, which includes glutathione peroxidase (GPx), glutathione reductase (GR), catalase (CAT), superoxide dismutase (SOD) and malondialdehid (MDA) in protection against oxidative insults is well characterized (Huang et al., 1994).

In this study, it was aimed to investigate the effect of adding sesame oils obtained by maceration and cold pressing methods to rainbow trout feed on some blood parameters, nitro blue tetrazolium (NBT) and antioxidant values and proximate composition.

MATERIAL AND METHODS

Fish material and experimental design

This study was carried out in the Fisheries Department of Malatya Turgut Özal University. Average weight of trouts was 55.89±2.05 g. prior to the starting of experiments, fish were subjected to acclimation for two weeks. Sesame seeds were waited in sunflower oil (1/10) for 15 days to obtain

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macerated sesame oil (MSO). CPSO was purchased from a local store (Kırkambar Co., Elazığ, Turkey) and these pressed oils were added to trout feed at %2 rate. In intensive stocking trial groups, 50 fish were stocked in 250 L tanks. 300 rainbow trout were divided into 3 groups (CPSO, MSO and control groups) and each group consisted of two tanks (two replicates). Fish were fed 2% of the weight of fish twice a day (morning and night) for 21 days.

Blood sampling of fish and analysis

The fish were anesthetized with an anaesthetic matter [Benzocaine (ethyl 4-aminobenzoate 99%, Sigma Aldrich Co, USA) 30 mg/L] before blood samples collection (Altınterim and Aksu 2020). Blood samples were taken from tail veins of the anaesthetized fish and transferred to ethylenediaminetetraacetic acid (EDTA) tubes. The NBT activity (total oxidative radical production of neutrophils) was determined by spectrophotometric method from blood samples with EDTA. Hematologic analyze was also performed with Fully Auto Hematology Analyzer PROCAN PE-6800VET (Shenzhen Prokan Electronics, Guangdong, China). The blood samples were waited one day at 4 °C and then centrifuged at 1000 g for 15 min to obtain plasma. Antioxidant enzymes activities including CAT, GPx, GR and MDA levels were measured with detection kits (Shanghai YL Biotech Co., Ltd., China) by using DR-200Bc Microplate Reader (Shenzhen Prokan Electronics, China).

Proximate composition

The proximate composition of fish samples in terms of moisture, fat, total proteins and ash were carried out according to AOAC (1990). Moisture was measured by using a gravimetric method by drying the sample at 105 °C until it reached constant weight. Crude protein content was calculated by Micro Kjeldahl method (6.2 x N). The amount of total lipid was obtained by extracting (Soxhlet system) with light petroleum ether, and the solvent was removed by distillation. The ash was determined from the residue after burning in a muffle furnace at 550 °C for about 20 hours.

Statistical analysis

In this study, ANOVA multivariate Duncan test was applied using SPSS Statistics 25.0 package program. The Kruskal Wallis test was used for the values of normal spreads and non-homogeneous variances. The results were expressed with the letters “a, b, c” (Çimen 2015).

RESULTS AND DISCUSSION

The values proximate compositions of control and experimental groups were not statistically found different in the durations ($P>0.05$) (Table 1). The MCH, PLT values of the CPSO group and the MCHC, PLT, MPV, PLCR, GRAN, NBT levels of the MSO group were statistically found different in the durations ($P<0.05$) (Table 2). In the present study, it was found that GPx and CAT activities of CPSO group were statistically found different in the durations ($P<0.05$) (Table 3). Proximate compositions in current study are in agreement with Özpolat (2020). There are many studies involving the addition of various forms of sesame plants to rainbow trout feed. However, studies using MSO could not be found. A study was conducted on the evaluation of sesame (*Sesamum indicum*, L.) seed instead of soybean in rainbow trout (*Oncorhynchus mykiss*, Walbaum, 1792) feed (Dernekbaşı et al., 2017). According to this study, moisture and ash were reported as 76.07% and 1.49%, respectively which is consistent with the data obtained in our study. On the other hand, Dernekbaşı et al. (2017) stated that fat and crude protein were 3.39% and 10.52%, respectively. The reason for the inconsistency of fat and protein values with the present study (Table 1) may be due to different brands of feed and the different content of these feeds. The difference in the size of the fish used in the studies may also have an effect on this. Sesame cake and meal were used in other studies, while MSO was added into fish feed in this study and it was pruposed to investigate the effect of this oil mostly on blood and antioxidant parameters. Another study (Nang et al., 2011) was conducted on rainbow trout fry with a body weight of 1.42 g. In the experiment, the effect of adding sesame oil cake (SOC) to the feeding ration of the juveniles at different rates was investigated. When SOC protein was added to 0%, 13% and 26% feed gradually, it was observed that it had a significant contribution on the enlargement and development of rainbow trout fry. This oil supplement also increased feed requests and increased SOC nitrogen holding capacity. The data obtained showed that there is no need for extra amino acid supplementation in rainbow trout fry, which are carnivorous.

In another study, sesame oil was added to diets instead of fish oil (Olude et al., 2019). Native male tilapia fish were fed with these diets and their effects on growth, food utilization and muscle fatty acid

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composition of these fish were considered. At the end of the experiment, positive results were obtained and it was stated that sesame seed oil should be used instead of fish oil in feeds. This study shows that the use of sesame oil as a feed additive has beneficial effects on fish with different diets.

In another study, it was determined that a decrease in the RBC, HGB and HCT levels, but an increase in the WBC, lymphocyte (LYM) and monocyte (MID) counts for European sea bass (*Dicentrarchus labrax*) (Saleh, 2020). Iqbal et al. (2022) showed that sesame oil had beneficial influence on hemoglobin level in rabbit. Moradi et al. (2013) reported that experimental diets (sesame oil cake and corn gluten) caused a reduce significant difference in hematocrit and hemoglobin levels of treatments respect with control group, no significant differences were found in WBCs, RBCs of groups in carp (*Cyprinus carpio*). Contrary to our study, these experiments show that sesame oil contains substances that stimulate blood production, but its sesame cake and meal do not.

Although macerated oils are used in many production areas, but they are not widely used as fish feed additives. Apart from MSO, there are studies on other macerated plants. In these studies, blood parameters and NBT were examined as in our study.

Altınterim and Aksu (2020) studied the addition of macerated oils of Tunceli garlic (*Allium tuncelianum*, Koll.) and garlic (*Allium sativum* L.) to the feeds of high stocked rainbow trout (*Oncorhynchus mykiss*, W.). It was observed that the values of MCH, platelet large cell ratio (P-LCR), plateletcrit (PCT), platelet count (PLT) and mean platelet volume (MPV) were significantly better in the groups given macerated garlic than control group. No significant difference was found in WBC, MID, LYM, HCT, RBC, red cell distribution width-standard deviation (RDW-SD), HGB, MCV, MCHC, platelet distribution width (PDW) and red cell distribution width-coefficient of variation (RDW-CV) parameters between groups. It was observed that the NBT levels of trout fed with macerated garlic oil were significantly reduced. This shows that the active ingredients of garlic reduce oxidative stress in cells (Altınterim and Aksu 2020). The effect of macerated fenugreek (*Trigonella foenum graecum* L.) oil mixed to trout feed at different rates was investigate on blood parameters and NBT values of rainbow trout. All blood parameters except MCHC increased numerically in the experimental groups. Although this increase was a little bit, a statistically significant difference was observed only in MID values. At the end of the study, there were an increase in NBT values compared to pre-experimental values, which are thought to increase NBT activity due to water-soluble phenolic compounds in fenugreek and are not important (Altınterim 2019a). In another study, the effects of macerated and cold pressed wheat germ (*Triticum vulgare*, L.) oils added to feeds at different rates on NBT and hematological values of rainbow trout were investigated. Another study showed that a significant difference was found in PLT, RBC, HCT, HGB and WBC values. It was observed that there was no difference between NBT levels (Altınterim and Aksu, 2019b). In the study with rainbow trout stocked at high density, the effect of macerated tomato (*Lycopersicon esculentum*, L.) and carrot (*Daucus carota*, L.) oils was investigate on the hematological parameters of the fish. Significant difference was found in MCH, RBC, HGB, HCT, LYM and NBT values. High levels of both macerated carrot and macerated tomato oils have been found to stimulate the non-specific immune system. In particular, it has been determined that macerated carrot oil is more effective than macerated tomato oil (Altınterim and Aksu, 2019c). This affects the blood parameters of biological differences in the life stages of other living things. This effect was also seen in rainbow trout fish (Altınterim et al., 2018d).

The data obtained in other studies and this study show that macerated oils obtained from plants have significant effects on the blood values of trout. In addition, some studies show different values in the NBT results, while NBT levels are found similar in some studies, they may be different in others. The reason for the differences in blood values may be due to the different effects of the substances in the contents of different plants. The reason why MSO in NBT values give different results than oils obtained by control and other methods is due to the fact that different substances in the plant's content pass into the oil during the maceration stage. Also in some studies mentioned above, it has been determined that the effects of MSO are more than CPSO.

In the present study, the increase in GPx and CAT levels of CPSO group showed that these oils increased the antioxidant capacity of the trout. The antioxidant values in our experiment coincided with the values in similar studies (Richardson et al., 1975;

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Epp et al., 1983). Shenoy et. al. (2011) reported that sesamolin preventing the oxidative degradation. These findings indicated that sesame ingredients exhibit antioxidant and anti-inflammatory activities (Köse and Yıldız, 2013). Mitsiopoulou et al. (2021) found that the SOD and CAT activities were increased, but MDA content declined in blood plasma of goats. Cao et al. (2020) determined that sesamolin treatment attenuated the production of reactive oxygen species (ROS) and oxidative stress and the increase of CAT and SOD activities in zebra fish exposed to fluoride.

Table 1. *Proximate compositions of control and experimental groups*

Groups	Dry matter (%)	Moisture (%)	Ash (%)	Fat (%)	Protein (%)
Control	21.97±0.02^{a}	77.51±0.60^{a}	1.26±0.34^{a}	5.14±1.54^{a}	16.44±0.22^{a}
CPSO	22.72±0.46^{b}	77.02±0.54^{a}	1.20±0.83^{a}	4.57±0.86^{b}	16.18±0.84^{a}
MSO	22.31±0.03^{b}	77.18±0.74^{a}	1.23±0.62^{a}	4.64±1.32^{b}	16.06±0.49^{a}

*CPSO: Cold pressed sesame oil, MSO: macerated sesame oil).

Table 2. *Hematological parameters and NBT value*

Groups	Control	CPSO	MSO
WBC (10^3/µL)	57.48±3.40^{a}	51.83±2.22^{a}	53.80±1.43^{a}
RBC (10^6/µL)	1.74±0.14^{a}	1.95±0.15^{a}	1.90±0.04^{a}
HGB (g/dl)	9.11±0.69^{a}	9.73±0.73^{a}	9.90±0.27^{a}
HCT (%)	22.90±1.65^{a}	22.80±1.74^{a}	21.83±0.68^{a}
MCV (fL)	133.36±2.65^{a}	117.46±2.48^{a}	114.93±0.93^{a}
MCH (pg)	52.38±0.81^{a}	49.03±0.80^{b}	51.90±0.36^{a}
MCHC (g/dl)	39.71±0.77^{a}	42.98±0.42^{ab}	45.30±0.36^{b}
PLT (10^3 /µL)	11.83±0.60^{a}	17.16±1.10^{b}	34.33±0.42^{c}
MPV (fL)	13.10±0.32^{a}	13.10±0.27^{a}	11.60±0.29^{b}
PDW (%)	14.51±2.70^{a}	13.11±2.14^{a}	9.30±012^{a}
PLCR (%)	41.66±1.53^{a}	43.70±2.22^{a}	32.23±1.79^{b}
LYM (%)	91.80±0.45^{a}	92.75±0.35^{a}	93.03±0.08^{a}
MID (%)	5.16±0.21^{a}	4.73±0.19^{a}	4.63±0.06^{a}
GRAN (%)	3.13±0.30^{a}	2.68±0.09^{a}	2.23±0.04^{b}
NBT	0.78±0.03^{a}	0.77±0.04^{a}	1.00±0.04^{b}

*CPSO: Cold pressed macerated oil, MSO: Macerated sesame oil, WBC: White blood cell, LYM: Lymphocyte, RBC: Red blood cell, HGB: hemoglobin, HCT: hematocrit, MCV: Mean corpuscular volume, MCH: Mean corpuscular hemoglobin, MCHC: Mean corpuscular hemoglobin concentration, PLT: Platelet, MPV: Mean platelet volume, PDW: Platelet distribution width, PLCR: Platelet-large cell ratio, LYM: lymphocyte, MID: Monocyte, GRAN: Granulocyte, NBT: Nitro blue tetrazolium.

Table 3. *The values of GPX, CAT, GR, SOD and MDA levels of experimental groups*

Antioxidant Parameters and MDA levels	Control	CPSO	MSO
GPx (units/mg protein)	11.41±0.45^{a}	15.60±1.80^{b}	13.03±0.56^{ab}
CAT (units/mg protein)	12.24±0.58^{a}	15.46±0.65^{b}	13.78±0.48^{ab}
GR (units/mg protein)	15.83±1.17^{a}	17.34±0.96^{a}	15.72±0.88^{a}
SOD (units/mg protein)	11.28±0.22^{a}	12.16±0.67^{a}	11.18±0.38^{a}
MDA (nmol/mg protein)	12.95±1.01^{a}	17.42±1.22^{a}	14.07±0.98^{a}

*CPSO: Cold pressed macerated oil, MSO: Macerated sesame oil, GPx: Glutathione peroxidase, CAT: Catalase, GR: Glutathione reductase, SOD: Superoxide dismutase, MDA: Malondialdehyde.

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CONCLUSION

It can be concluded that CPSO could be used to improve the oxidative stress caused by high stocking density. Using CPSO on high stocking density reduced its negative effects on most of the antioxidant parameters and this may be referred to the therapeutic role of sesame oil as antioxidant. The sesamolin and sesamin in sesame oil have been the high oxidative stability. CPSO is a good source of anti-oxidants, omega-3 and omega-6 fatty acids. CPSO prepared by soaking in oil showed a stronger effect than MSO. This effect is thought to occur due to the fact that sesame contains more oil-soluble components. In our study proved the beneficial effect of CPSO that significantly recovered the reverse free radical generative influence of stress than control and MSO. Especially, it was determined that MSO decreased lipid peroxidation level. Interestingly, significant increase was found in the MDA levels for treatment with CPSO compared to the control. It is thought that this difference was due to the more intense transition of substances such as sesamol and sesamin to CPSO. MSO was significantly increased blood profile: MCHC, PLT, MPV, PLCR, GRAN and immunity NBT level of rainbow trouts. On the other hand, CPSO and MSO treatments did not cause any different effect on the nutritional values of the fish. Macerated oils were found to be more effective than press oils on hematological values and NBT activity. This effect was thought to occur due to the transition of oil-soluble substances of plants and the blood production mechanism of fish were stimulated by these substances.

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COFLICT OF INTEREST

The Author reports no conflict of interest relevant to this article.

RESEARCH AND PUBLICATION ETHICS STATEMENT

The author declares that this study complies with research and publication ethics.

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