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Difference in the health effects of macerated and pressed peanut oils (*Arachis hypogaea*, L.) on stressed rainbow trout (*Oncorhynchus mykiss*)

Diferencia en los efectos sobre la salud de los aceites de maní macerados y prensados (*Arachis hypogaea*, L.) en la trucha arco iris estresada (*Oncorhynchus mykiss*)

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ABSTRACT

Generally, peanut (*Arachis hypogaea*, L.) products have been used as alternative feed additives in trout farming due to their nutritional properties. The maceration process ensures that fat-soluble substances pass into the oil more. It was thought that the application with enriched oil will give different results. Two experimental diets, macerated oil of peanut (MOP) and pressed peanut oil (POP) were used under high stocking density. The trial was studied on antioxidant parameters, hematological parameters, nitro blue tetrazolium (NBT) activity and proximate composition of rainbow trout (*Oncorhynchus mykiss*) for 21 days. It was determined that the highest dry matter content (22.33%) and moisture content (77.79%), it was the lowest ash content (1.17%) and relatively low fat content (5.01%) in POP group. This suggested that the POP group had a higher concentration of dry matter, potentially indicating a higher overall nutrient density. In blood parameters, it was determined that mean corpuscular volume (MCV) (121.54 ± 4.30), platelet (PLT) (25.33 ± 3.68), NBT (1.407 ± 0.382) and lymphocyte (LYM) (93.66 ± 1.17) levels of the MOP group and granulocyte (GRAN) (2.15 ± 0.13) level of the POP group were different compared to the control group (P<0.05). In terms of antioxidant parameters, glutathione peroxidase (GPx) (15.585 ± 2.236), superoxide dismutase (SOD) (17.691 ± 2.250) and catalase (CAT) (12.874 ± 0.620) activities of the POP group and malondialdehyde (MDA) (9.169 ± 0.238) level and glutathione reductase (GR) (12.085 ± 1.034) activity of the MOP group were determined to be different compared to the control group (P<0.05). The results reveals that use of antioxidants (MOP) is an effective way of getting the best result in terms of the lipid peroxidation mechanism and blood production in rainbow trout under high stocking density. It was thought that the amount of oil-soluble antioxidant substances may increase with the maceration method and have a higher effect on the parameters.

Key words: seed oil; fish physiology; alternative herb oils; physiological stress

RESUMEN

Generalmente, el aceite o la pulpa de maní (*Arachis hypogaea*, L.) se han utilizado como un buen solvente y aditivo alimentario alternativo. El proceso de maceración consigue que las sustancias liposolubles pasen más al aceite. Se pensaba que la aplicación con aceite enriquecido daría resultados diferentes. Se utilizaron dos dietas experimentales, aceite de maní macerado (MOP) y aceite de maní prensado (POP), en condiciones de alta densidad de población. El ensayo se estudió sobre parámetros antioxidantes, parámetros hematológicos, actividad del nitro azul tetrazolio (NBT) y composición próximal de la trucha arco iris (*Oncorhynchus mykiss*) durante 21 días. Se determinó que el mayor contenido de materia seca (22,33%) y de humedad (77,79%), y que fue el menor contenido de cenizas (1,17%) y contenido relativamente bajo contenido de grasa (5,01%) fue parael grupo POP. Esto sugirió que el grupo de POP tuvo una mayorconcentración de materia seca, lo que potencialmente indica una mayor densidad general de nutrientes. En los parámetros sanguíneos se determinó que los niveles medios de volumen corpuscular (MCV) (121,54 ± 4,30), plaquetas (PLT) (25,33 ± 3,68), NBT (1,407 ± 0,382), niveles de linfocitos (LYM) (93,66 ± 1,17) del grupo MOP granulocitos (GRAN) (2,15 ± 0,13) del grupo POP fueron diferentes en comparación con el grupo de control (P<0,05). En cuanto a los parámetros antioxidantes, se destacan las actividades glutatión peroxidasa (GPx) (15,585 ± 2,236), superóxido dismutasa (SOD) (17,691 ± 2,250) y catalasa (CAT) (12,874 ± 0,620) del grupo POP y malondialdehído (MDA) (9,169± 0,238) y se determinó que la actividad de glutatión reductasa (GR) (12,085 ± 1,034) del grupo MOP era diferente en comparación con el grupo de control (P<0,05). El resultado revela que el uso de antioxidantes (MOP) es una forma eficaz de obtener el mejor resultado en términos del mecanismo de peroxidación lipídica y la producción de sangre en la trucha arco iris bajo una alta densidad de población. Se pensó que la cantidad de sustancias antioxidantes solubles en aceite podría aumentar con el método de maceración y tener un mayor efecto sobre los parámetros.

Palabras clave: Aceite de semilla; fisiología de los peces; aceites de hierbas alternativas; estrés fisiológico

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INTRODUCTION

In the production of herbal essential oil, water vapor distillation methods or solvent extraction method in solvents such as alcohol, benzene and hexane are preferred [1, 2, 3]. Pure essential oil obtained by water vapor distillation is both costly and may have side effects for oral use, causing irritation and allergies. Solvent residues in the method using solvent can cause neurological damage [4].

The pressing method is preferred in the production of oils from materials such as almond (*Prunus amygdalus*), peanut (*Arachis hypogaea*), black cumin (*Nigella sativa* L.) and linseed (*Linum usitatissimum*). In the pressing method, the transfer of fat-soluble antioxidant and stimulant substances into the oil occurs in very low amounts [5, 6, 7]. Peanut oil is an important antioxidant with its polyphenols and high vitamin E content [8]. Due to its high oil content, peanuts are easily oxidized during storage and transportation, and this affects the nutritional and agricultural values of peanuts [9]. Macerated oils do not carry these or similar risks and are not treated with any solvents or chemicals. In addition, since it is kept in oil, more oil-soluble substances pass through [10].

Various researches were being carried out on alternative feed additives in international production platforms where the tendency towards alternative herbal resources was increasing. Rising costs due to adverse conditions such as covid and global warming also cause a decrease in the feed and pharmaceutical raw materials used in farming [11]. Due to these properties, peanut oil is preferred as an alternative product in fish feed ration studies [12].

In the literature, moisture content of trout was determined as 65–75%, protein content as 11–25%, ash as 0.6–1.5% and fat as 3–10% [13, 14, 15, 16]. Studies that parallel the proximal composition results of the study were found. There are numerous studies involving the addition of pressed peanut oil to rainbow trout feed. However, no studies using macerate oil have been found [17].

There are feeding studies on trouts prepared by adding peanut oil and pulp to fish feed rations. There are also studies using peanut oil as a carrier in the applications of active ingredients. To give an example of a few of these: Some studies have been reported on the addition of peanut oil to the diets of African catfish (*Clarias gariepinus*) and carp (*Cyprinus carpio*) [18, 19]. In another study, peanut oil was preferred as a carrier oil because it has no side effects and is safe. Pure peanut oil was used as a control in the study of the EROD-inducing β-naphthoflavone (BNF) model or the peroxisome proliferator (PP) 2,4-dichlorophenoxyacetic acid model dissolved in 0.5 or 1 mL peanut oil/100 g fish [20]. In another study conducted to determine the possible roles of arylhydrocarbon receptor agonists and oxidative stress, peanut oil was used as a carrier oil instead of fish oil and applied to rainbow trout [21]. Dernekbasi *et al*. [15] investigated the effects of diets containing different proportions of peanut oil on the growth performance, biochemical and fatty acid compositions of juvenile European sea bass (*Dicentrarchus labrax*) and stated that the use of peanut oil in different proportions in the diets did not have a negative effect on the growth and approximate composition of this fish.

Hematological studies have been conducted on different macerated oils. One of these is a study investigating the effects of macerated tomato (*Lycopersicon esculentum*) and carrot (*Daucus carota*) oils on the hematological parameters of densely stocked trout; it was observed that there was a significant difference in mean corpuscular hemoglobin (MCH), red blood cell (RBC), Hgb, Hct, LYM and NBT values [22]. In another macerated oil study, the effects of macerated and pressed wheat germ (*Triticum vulgare*) oils added to feed at different rates on the NBT and hematological values of rainbow trout were investigated. Macerated wheat oil group; while a significant difference was obtained in RBC, Hgb, Hct, WBC, PLT values; it was observed that there was no significant difference in NBT levels [23].

Fackjouri *et al*. [24] of soybean oil in *Huso huso* fish; they found that it caused significant changes in MCHC, MCV and RBC levels. They also determined a decrease in the Hct and Hgb values of the POP group in the study. Demir *et al*. [25] observed that there was an increase in the Hct, Hgb, MCV, MCH, MCHC values of tilapia to which they applied press peanut oil.

In this study, the effects of macerated and pressed peanut oils on the proximate composition, hematological parameters and antioxidant parameters of rainbow trout were investigated.

MATERIALS AND METHODS

Fish material and experimental design

This study was applied in the fisheries department of Malatya Turgut Ozal University. Macerated oil of peanut (MOP) and pressed peanut oil (POP) were purchased from a local spice store (Kirkambar Co., Elazig, Turkey). MOP and POP were mixed into trout feed at a rate of 2% of the total weight of the feed (Gumusdoga brand with 45% protein). During the experiment, fish (*Oncorhynchus mykiss*) were fed 2% of its corporal weight twice a day for 21 days (d). The fish in tanks (each tank was filled with 100 liters (L) of water) were subjected to acclimation for two weeks.

In this research, 50 fish were included in each trial group and control group: 10 fish, 110 fish for each repetition, 220 fishes in total wereused. The experiment was executed following a completely randomized design comprising two treatments, each replicated twice control group (low density): 10 fish, MOP group (high density): 50 fish, POP groups (high density): 50 fish. The average weight of rainbow trout in the experiment was weighted as 54.70 ± 2.01 g. with TEM scales, Ns6200, Turkey.

Blood sampling of fish and analysis

At the end of the experiments, blood was collected from the caudal vein of individual fish after anesthetization with benzocaine (25 mg·L^{-1} water) and transferred to the tubes with ethylenediaminetetraacetic acid (EDTA) [22]. Nitro blue tetrazolium (NBT) activity of blood samples was determined spectrophotometrically to detect the total oxidative radical level of neutrophils. The mixture was incubated (ILDAM, ILD-EKH, TÜRKAK 17025, Turkey) at room temperature for 30 min, and 0.05 mL of the NBT–blood cell suspension was removed and added to a glass tube containing 1.0 mL of N,N dimethyl formamide. After centrifugation (Elektromag M 2815 PR, Turkey), the sample absorbance (HACH, DR6000 RFID, Germany) was read at 620 nm in a 1.0 mL cuvette [26]. Hematologic analyze was also performed with Fully Auto Hematology Analyzer (PROCAN PE-6800VET, China). The blood samples were stored (Arcelik, 270482-MI, Turley) one day at 4°C and then for plasma, samples were centrifuged at 1000 G for 15 min. The plasma catalase (CAT), glutathione peroxidase (GPx), glutathione reductase (GR), glutathione peroxidase (GPx) activities and malondialdehyde (MDA) level were measured by commercial kits (SunLong Biotech Co.,

LTD, China). Plasma homogenates were analyzed according to the kit instructions, and the absorbance values were read at 450 nm in a microplate reader (DR–200Bc Microplate Reader. Prokan Electronics, China, Shanghai YL), respectively, and the results were calculated according to the formula provided in the instructions.

Proximate composition

Moisture content, regarding dry matter, fat, total proteins and ash of the muscle samples of fish were carried out. Moisture was measured by using a gravimetric method by drying the sample (3 g) at 105°C until it reached constant weight. Crude protein content was calculated by Micro Kjeldahl method (6.2 × N) (EFLAB, MGD1000X, Turkey) Crude protein content was calculated by Micro Kjeldahl method (6.2 × N) (EFLAB, MGD1000X, Turkey) [27]. The amount of total lipid wasobtained by extracting (Soxhlet system) with light petroleum ether, and the solvent was removed by distillation. Ash was determined from the residue after burning in a muffle furnace at 550°C for around 20 hours [28].

Statistical analysis

The data obtained were statistically tested using the SPSS statistical program at the 0.05 confidence interval one–way analysis of variance (ANOVA) (P<0.05). The mean values were given in the results as mean ± standard error of means. All results were analyzed by the SPSS 24.0 Package Program.

RESULTS AND DISCUSSION

Proximate muscle analysis

When the proximate analysis were examined in the study, it was not found any statistical difference between the control group and the other experimental groups (P>0.05) (FIG. 1).

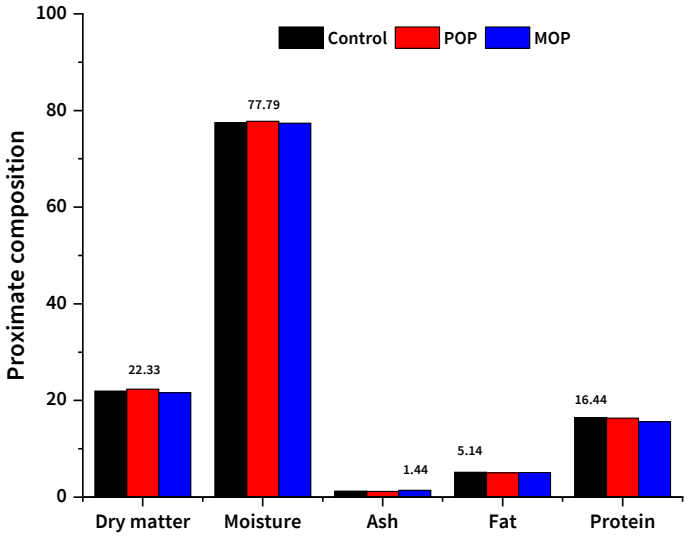

FIGURE 1. Proximate compositions of the muscle in rainbow trout Control and experimental groups. POP: Pressed peanut oil, MOP: macerated oil of peanut

Hematological and serum parameters

It was determined that mean cellular volume (MCV), platelet (PLT), NBT values of bothgroups, lymphocyte (LYM) of the MOP group and granulocyte (GRAN) level of the POP group were found statistically different in the durations (P<0.05) (TABLE I).

TABLE I
Hematological parameters and NBT value (Mean ± Standard Deviation)

Parameters	Groups		
	Control	POP	MOP
WBC (10^3·µL^{-1})	57.48 ± 7.62^a	51.55 ± 6.04^a	54.06 ± 7.02^a
LYM (%)	91.89 ± 0.97^a	93.22 ± 1.11^{ab}	93.66 ± 1.17^b
MID (%)	5.14 ± 0.31^a	4.63 ± 0.12^a	4.45 ± 1.30^a
GRAN (%)	3.13 ± 0.23^a	2.15 ± 0.13^b	2.28 ± 0.33^{ab}
RBC (10^6·µL^{-1})	1.75 ± 0.33^a	1.80 ± 0.34^a	2.06 ± 0.42^a
Hgb (g·dL^{-1})	9.12 ± 1.56^a	8.88 ± 1.28^a	10.44 ± 1.99^a
Hct (%)	22.90 ± 3.69^a	20.97 ± 3.11^a	24.89 ± 4.39^a
MCV (fL)	131.83 ± 6.66^a	118.09 ± 5.18^b	121.54 ± 4.30^b
MCH (pg)	50.51 ± 4.88^a	49.66 ± 2.67^a	50.73 ± 1.59^a
MCHC (g·dL^{-1})	39.72 ± 1.73^a	42.17 ± 1.21^a	41.83 ± 0.90^a
PLT (fL)	11.8 ± 1.46^a	24.4 ± 4.88^b	25.33 ± 3.68^b
MPV (%)	13.15 ± 0.67^a	12.35 ± 0.51^a	13.07 ± 0.63^a
PDW (%)	13.31 ± 6.32^a	21.53 ± 1.40^a	19.23 ± 5.44^a
P–LCR (%)	41.66 ± 3.43^a	36.70 ± 3.63^a	43.05 ± 3.79^a
NBT	0.779 ± 0.072^a	1.256 ± 0.256^b	1.407 ± 0.382^b

POP: Pressed oil of peanut, MOP: macerated oil of peanut, 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

Antioxidant parameters, it was found that glutathione peroxidase (GPx), glutathione reductase (GR), superoxide dismutase (SOD activities of POP group) and, catalase (CAT) and malondialdehyde (MDA) level of MOP group were found statistically different in the durations (P<0.05) (TABLE II).

TABLE II
The values of GPx, CAT, GR, SOD activities and MDA level of experimental groups and control group (Mean ± Standard Deviation)

Parameters	Control	POP	MOP
GPx (units·mg^{-1} protein)	11.418 ± 0.904^a	15.585 ± 2.236^b	13.667 ± 1.692^{ab}
CAT (units·mg^{-1} protein)	12.249 ± 1.301^a	12.874 ± 0.620^a	13.228 ± 1.426^a
GR (units·mg^{-1} protein)	15.251 ± 2.513^a	15.485 ± 2.019^a	12.085 ± 1.034^b
SOD (units·mg^{-1} protein)	12.845 ± 1.879^a	17.691 ± 2.250^b	14.566 ± 0.631^{ab}
MDA (nmol·mg^{-1} protein)	11.287 ± 0.447^a	11.876 ± 0.441^a	9.169 ± 0.238^b

POP: Pressed oil of peanut, MOP: macerated oil of peanut, GPx: Glutathione peroxidase, CAT: Catalase, GR: Glutathione reductase, SOD: Superoxide dismutase, MDA: Malondialdehyde

Antioxidant parameters

In trial, peanut oil administration was shown that GPx, CAT, SOD activities of all groups were increased and MDA level, GR activity of POP group were increased but MDA level and GR activity of MOP group were decreased than control group.

A study was conducted on the evaluation of pressed peanut (*Arachis hypogaea*) oil instead of fish oil in yellow croaker (*Larmichthys crocea*) feed, and it was observed that similar results were obtained to the findings in the trial [17]. In another study, it was stated that all proximal composition values except moisture value were not affected in the application of pressed peanut oil [29]. Sun *et al*. found in their experiments that the proximal composition levels of black carp fry (*Mylopharyngodon piceus*) were not affected by pressed peanut oil added to the diet at different rates [30]. In another study, it was observed that peanut oil applied to juvenile pikeperch fish did not have a significant effect on proximal composition findings [31]. Acar and Turker [32] stated that there was no significant difference in the approximate composition values of trout fed with unrefined peanut oil.

The fact that LYM increased in both the macerated oil and pressed oil groups showed that the acquired immune system was stimulated in both groups. White blood cell (WBC), monocyte (MID) and GRAN values were low in both groups, suggesting that there was no effect on innate immune system markers in general. However, despite the decrease in hemoglobin (Hgb) and hematocrit (Hct) in the POP group, the increase in the MOP group shows that macerated peanut oil stimulates blood production. The data obtained in this study show that both macerated and pressed peanut oils obtained from plants have significant effects on the hematological and NBT levels of trout. However, the NBT value was found to be the highest for the MOP group. Additionally, some studies show different values in hematological results. The reason for these differences is thought to be due to the production technique of the macerated oil. It is obvious that by keeping it in oil for a long time, more oil-soluble substances pass into the oil, causing this effect.

Moisture, fat, ash and protein ratios in fish may vary depending on seasons, gender, species and age. And in the same application, while there was no difference in RBC, Hct and MCV values as a result of hematological analysis, an increase similar to the MOP group data was obtained in Hgb, MCH and mean corpuscular hemoglobin concentration (MCHC) values [32].

A decrease in GRAN and MID values of WBC was detected in fish fed with MOP and POP at high stocking density. This decrease indicates that the fish's innate immune system is suppressed due to stress. In the MOP group, an increase was observed in lymphocyte and NBT values, which are indicators of acquired immunity. The increase in RBC, Hgb, Hct, MCV, MCH, PLT, MPV values in the MOP group is remarkable. This is an indication that macerated peanut oil affects erythropoiesis and thrombocytosis. Although this group appears to be a microcytic hyperchromic picture, high Hgb and Hct values indicate that hematopoietic organs, especially the thymus and pronephros, are stimulated by lymphocytosis to compensate for anemia. Increasing NBT values despite granulopenia in both groups indicate that neutrophils with phagocytosis ability are stimulated.

Studies have been conducted to investigate the effects of peanut products on the antioxidant parameters of fish. Zhu *et al.* [33] found in their experiment that peanut cake increased the MDA level of juvenile hybrid grouper, and a similar increase was seen in the POP group in the study [25]. In this study, the stimulating effects of MOP on MDA and GR inhibitory and antioxidant defense system parameters (SOD, CAT, GR) were parallel to the results of another study conducted with vitamin E [9].

In this study, a significant decrease in MDA level was detected in the fish group fed with MOP. It is thought that this decrease is due to the possible increase in the concentration of n-3, which has oil-soluble properties in MOP. Higher n-3 content in diets causes higher SOD activity in rainbow trout [34].

In another similar study, it was stated that peanut supplemented feeds increased the SOD and CAT activities of Pompano fish [35]. In another study conducted on a sea bass species, it was determined that palm oil and coconut oil increased the SOD and GPx activities on the antioxidant parameters of fish [36]. Darsini *et al*. determined that elephant-apple (*Limonia acidissima*) fruit increased the SOD, GPx, and GST activities in carp, as in the POP group, and decreased the MDA level [37]. In another study, it was determined that fish fed with vitamin C supplemented feed increased SOD, GPx, CAT activities similar to the MOP group [38].

CONCLUSIONS

When the results of the experiment were examined, it was seen that peanut oil applications did not have a different effect on the proximate composition values of the fish.

POP successfully coped with oxidative stress caused by high stocking density. The use of MOP and POP at high stocking density reduced their negative effects on most of the antioxidant parameters, which could be attributed to the antioxidant role of peanut oil as an antioxidant. POP is a good source of antioxidants, but MOP prepared by soaking in oil showed a stronger MDA suppression effect than POP. It is thought that this effect occurs when peanuts are kept in oil, and more fat-soluble components such as vitamin E, resveratrol and coenzyme Q10 pass into the oil. The fact that the GPx, GR and SOD levels of the POP group and the CAT level of the MOP group were higher than the control group and the other experimental group showed that antioxidant enzyme activities may change under stress. Minimizing this sensitivity is important for the continuity of fish health. In the study, the superiority of the MOP group was clearly revealed.

In conclusion, incorporating diets rich in macerated peanut oil into the feeding regimen of fish may offer significant benefits by mitigating the adverse effects associated with high-density stress levels, thereby promoting better overall health and well-being in aquatic species.

Conflicts of interest

The author declares that I have no conflict of interest.

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