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EFFECTS OF FISH OIL ETHYL ESTERS ON BODY COMPOSITION AND LIVER OXIDATIVE STRESS IN EUROPEAN SEABASS (*Dicentrarchus labrax*) UNDER THERMAL CHALLENGE

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Introduction
Marine heatwaves driven by climate change threaten aquaculture sustainability in Mediterranean species such as European seabass (*Dicentrarchus labrax*) by disrupting physiological homeostasis, particularly metabolism and oxidative balance. Nutritional strategies to improve thermal resilience are increasingly needed. Fish oil (FO), rich in n-3 long-chain polyunsaturated fatty acids (n-3 LC-PUFA), namely eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), faces sustainability challenges and is increasingly being directly used for human consumption. Ethyl esters (EE), by-products from FO refining for omega-3 supplement production, have recently emerged as a potential alternative lipid source. EE oil is rich in EPA and DHA, which are present as ethyl esters rather than triglycerides, and this may influence bioavailability under heat-stress conditions. Thermal stress can alter membrane fluidity, whose composition is modulated by the dietary lipid source. Previous studies have shown that EE oil has potential as a lipid source under normal temperature conditions, but its impact during thermal challenges has not yet been explored. This study evaluated the effects of dietary EE oil on body composition, fatty acid profile, and liver oxidative stress in seabass under simulated heatwave conditions.

Materials and Methods
Four diets were formulated as isoproteic (48% crude protein) and isolipidic (16% crude lipid), and the lipid sources (% total lipid) were as follows: D1 (72% FO, 28% rapeseed oil, RO; EPA+DHA=12.8% FA), D2 (28% FO, 72% RO; EPA+DHA=4.2% FA), D3 (28% FO, 44% RO, 28% EE; EPA+DHA=4.9% FA), and D4 (28% RO, 72% EE; EPA+DHA=3.6% FA). Firstly, a growth trial was performed at 23 ºC for 88 days with European seabass (*Dicentrarchus* labrax) juveniles with an initial weight of 62g. Then, a thermal challenge was imposed in two phases: 29 ± 0.5 °C for 7 days, followed by 31 ± 0.5 °C for 7 days. At the trial end, three fish per tank were euthanized (overdose of 2-phenoxyethanol) for measurement of body, liver, and viscera weights for hepatosomatic index (HSI) and viscerosomatic index (VSI), and whole-body composition (dry matter, crude protein, lipids, ashes, and energy). Liver and muscle were sampled from 3 other fish per tank to assess fatty acid profiles, and liver oxidative stress biomarkers, including glutathione peroxidase (GPX), glutathione reductase (GR), catalase (CAT), superoxide dismutase (SOD), and lipid peroxidation (LPO). Data was evaluated by one-way ANOVA and Tukey's test ($p < 0.05$) using SPSS 29.0 software for Windows (IBM® SPSS® Statistics, New York, USA).

Results
Under the thermal challenge, dietary treatments had no significant differences in whole-body dry matter, protein, lipids, ash, gross energy content and VSI. The HSI was significantly higher in D4 than in D2. Fatty acid profiles of muscle and liver reflected dietary lipid composition: DHA and total n-3 PUFA levels were significantly reduced in both tissues of fish fed the D4 diet. In muscle, EPA was higher in D4 than in the D2 and D3 diets but remained lower than in the D1. In the liver, EPA, DHA, and total n-3 PUFA were significantly reduced in fish fed the D4 diet compared to the D1 diet. In contrast, saturated fatty acids (SFA) increased significantly in D4, resulting in the highest SFA levels among all dietary treatments. Liver oxidative stress enzymes and LPO levels did not differ significantly among dietary treatments.

Conclusion
In conclusion, dietary EE oil inclusion altered tissue fatty acid composition, particularly reducing n-3 LC-PUFA and increasing SFA levels, without markedly affecting whole-body composition and oxidative stress responses under thermal challenge.

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