IMPACT OF DIETARY ETHYL ESTERS OIL ON INTESTINE OXIDATIVE STRESS AND IMMUNE PARAMETERS IN EUROPEAN SEABASS (*Dicentrarchus labrax*)

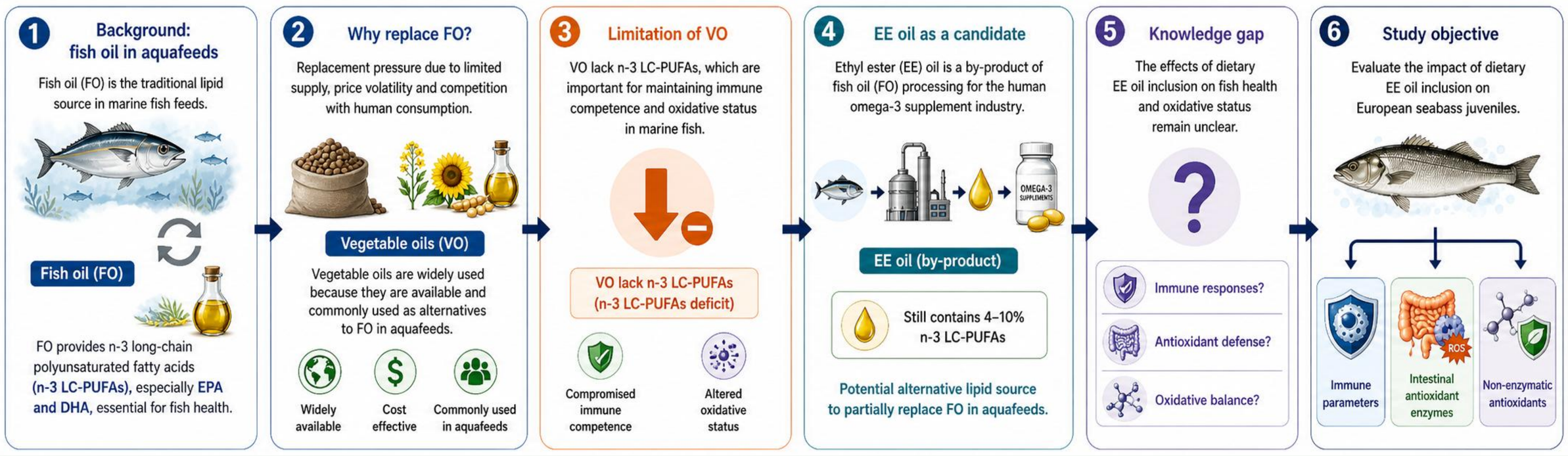
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Introduction

Vegetable oils (VO) are widely used to replace fish oil (FO) in aquafeeds, but they lack n-3 long-chain polyunsaturated fatty acids (n-3 LC-PUFAs), which are critical for maintaining immune competence and oxidative status in marine fish. Ethyl ester (EE) oil, a by-product of the FO processing for the human omega-3 supplement industry, still contains 4–10% n-3 LC-PUFAs and can potentially be used for FO replacement in aquafeeds. However, the effects of dietary EE oil inclusion on fish health and oxidative status remain unclear. This study evaluated the impact of dietary EE oil on immune parameters, intestinal antioxidant enzymes, and non-enzymatic antioxidants in European seabass juveniles.



1 Experimental design

Species	European seabass juveniles
Initial body weight	62 ± 0.5 g
Experimental units	12 tanks; 15 fish tank^{-1}; 3 tanks diet^{-1}
Feeding trial	88-day feeding trial in RAS at 23 ± 1 °C
Feeding management	Hand-fed to apparent satiation, 6 days week^{-1}
Statistics	One-way ANOVA + Tukey's test, $p < 0.05$

2 Diets

Four isoproteic (48%) and isolipidic (16%) diets were formulated using different supplemental oil blends (%).

D1	FO / RO / EE = 78 / 22 / 0	78% FO, 22% RO, 0% EE
D2	FO / RO / EE = 22 / 78 / 0	22% FO, 78% RO, 0% EE
D3	FO / RO / EE = 22 / 56 / 22	22% FO, 56% RO, 22% EE
D4	FO / RO / EE = 0 / 22 / 78	0% FO, 22% RO, 78% EE

FO = Fish oil; RO = Rapeseed oil; EE = Ethyl ester oil.

FO = fish oil; RO = rapeseed oil; EE = ethyl ester oil.
Values represent the proportion of each oil source in the added oil blend, not the percentage of the complete diet.
Analyzed composition was comparable among diets: crude protein 47.5–48.7%, crude lipid 15.9–16.4%, ash 5.77–6.32%, gross energy 24.3–25.4 kJ g^{-1} (dry matter basis), and dry matter 91.5–95.8%.

3 Assessed markers

A. Plasma innate immunity

- Antiprotease activity
- Protease activity
- Peroxidase activity
- Lysozyme activity

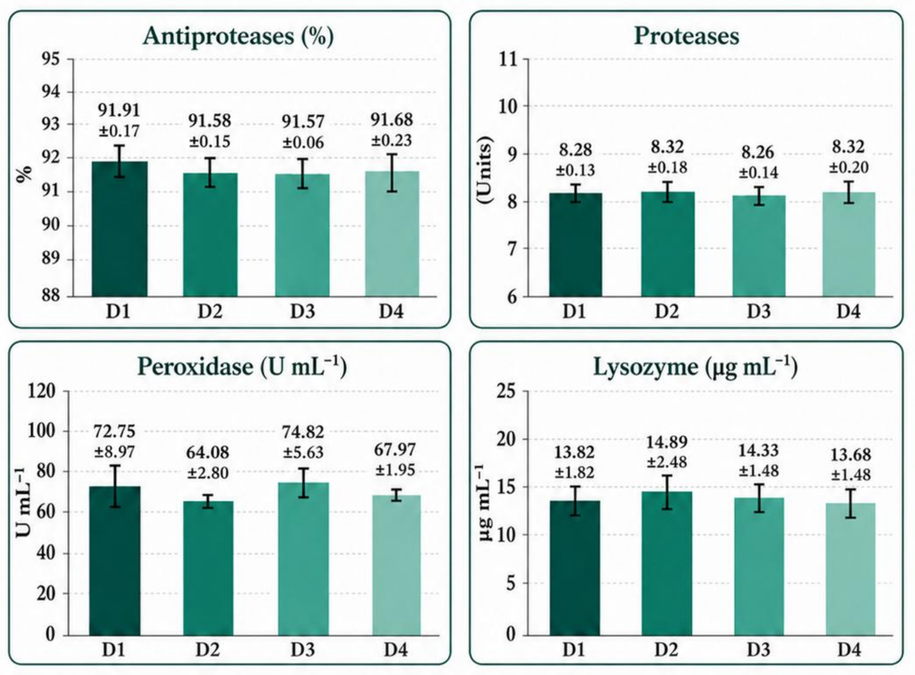
B. Intestinal oxidative status

- CAT
- GR
- SOD
- GPX
- Lipid peroxidation
- Reduced glutathione (GSH)
- Oxidized glutathione (GSSG)

TABLE 1

Plasma innate humoral immune parameters of European seabass fed the experimental diets.

No significant dietary effects were detected for plasma innate humoral immune parameters (Tukey test; $p > 0.05$).



* FO: fish oil; RO: rapeseed oil; EE: Ethyl Esters oil.
Values are presented as means ± SE.

Diet compositions (FO/RO/EE ratio): D1 = 78/22/0 D2 = 22/78/0 D3 = 22/56/22 D4 = 0/22/78

TABLE 2

Intestine antioxidant and oxidative status of European seabass fed the experimental diets

1. Antioxidant enzyme activities (Intestine)

Variable	D1 (78/22/0)	D2 (22/78/0)	D3 (22/56/22)	D4 (0/22/78)	*P-value*	SEM
CAT	146.3	100.5	128.6	136.6	0.39	8.5
GR	14.9	15.3	14.1	15.9	0.90	0.3
SOD	431.4	471.6	439.6	501.4	0.81	13.9
GPX	26.5	34.7	41.0	20.3	0.50	3.9

One-way ANOVA detected no dietary effects for intestine CAT, GR, SOD, or GPX.

2. Glutathione and oxidative stress markers (Intestine)

Variable	D1 (78/22/0)	D2 (22/78/0)	D3 (22/56/22)	D4 (0/22/78)	*P-value*	SEM
tGSH	109.8	112.4	83.0	105.8	0.86	5.83
GSSG	46.6	43.1	44.0	45.1	0.98	0.65
GSH	60.2	69.3	44.7	60.7	0.31	4.44
OSI[1]	100.0	108.1	126.9	99.2	0.50	5.58
LPO	13.63	13.36	13.05	10.56	0.51	0.61

One-way ANOVA detected no dietary effects for intestine tGSH, GSSG, GSH, OSI, or LPO.

FO: fish oil; RO: rapeseed oil; EE: ethyl ester oil.
OSI[1] = 100 × (2 × GSSG / tGSH).
CAT and SOD activities are expressed as U mg^{-1} soluble protein; GPX and GR as mU mg^{-1} soluble protein.
LPO values are expressed as nmol MDA g^{-1} tissue; tGSH, GSSG, and GSH as nmol g^{-1} tissue.

Nine intestines were sampled from each dietary treatment.

4 Conclusion

No adverse effects of dietary EE oil were detected

In European seabass juveniles, dietary ethyl ester (EE) oil inclusion did not significantly affect plasma innate immune parameters or intestinal oxidative stress markers.

- **Plasma innate immunity** remained stable
- **Intestinal oxidative status** remained stable
- **No significant dietary effects among treatments** (one-way ANOVA + Tukey's test; P >0.05)

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