

# Nutritional value of Ethyl Ester oils in fish diets

A review on their potential impact on growth and health



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## Report

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## Preface

The Norwegian Seafood Research Fund (FHF) requested a literature review describing the use of ethyl ester oils in Atlantic salmon and Rainbow trout diets. This work is included in the SalmoE2 project (FHF 901847; Nutritional effects of ethyl ester oils in Atlantic salmon and Rainbow trout) and corresponds to work-package 1. The SalmoE2 project aims to investigate the safety and suitability of using ethyl ester oils as ingredients in salmonids' diets and at the same time address one of the major challenges of the Norwegian aquaculture sector, namely the need for locally produced sustainable ingredients for fish feed with low CO<sub>2</sub> footprint.

It is important to note that there is little available research on the nutritional effects of ethyl ester oils in fish, particularly in species like salmon and trout. Therefore, we provide a broader overview of the use of ethyl ester oils, not only in fish but also in humans. Moreover, we discuss the differences between ethyl ester oils and other oil forms and their potential implications for nutrition and metabolism.

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# 1 Summary

## 1.1 Summary in English

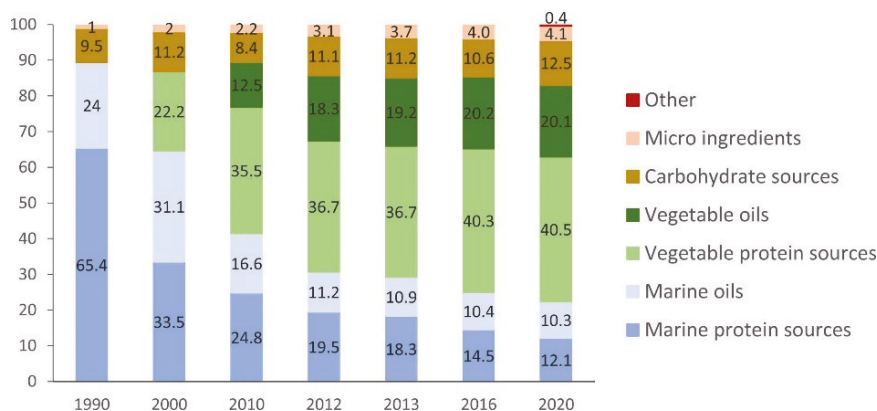
The Norwegian Pelagic sector plays a significant role in producing pelagic fish products for human consumption, including concentrated marine omega-3 fatty acids (FA) used in dietary supplements. To fully utilize fish sources, the sector must explore to use valuable side stream products as ingredients in aquaculture feeds, such as ethyl ester (EE) oils derived from production of concentrated omega-3 FA for dietary supplement. Despite annual production of approximately 10,000 tons of EE oils in Norway, there is limited knowledge about their suitability in aquafeeds. This knowledge gap may stem from the absence of EE in aquatic animals' natural diets, unlike in human dietary supplements. Moreover, varying FA compositions of EE oils used in different fish trials present challenges in drawing definitive conclusions about their effects. Fish metabolism differs from mammals in digesting dietary lipids, lacking the necessary lipase enzymes for monoacylglycerol production during TAG digestion. This may affect the efficiency of lipid transport and metabolism in salmonids, potentially influencing intestinal health and overall lipid metabolism. Furthermore, studies suggest lower digestibility of EE oils compared to free FA or TAG forms, indicating potential limitations in their re-esterification process in enterocytes. Concerns also arise regarding ethanol release during EE hydrolysis in the gut and its potential impact on intestinal health. Additionally, EE oils' susceptibility to oxidation suggests variations in stability in feeds depending on composition and antioxidant content. In SalmoE<sup>2</sup>, we aim to assess the safety and suitability of EE oils in salmonid diets, addressing the challenge of locally sourcing sustainable ingredients with low CO<sub>2</sub> footprints. Understanding the effects of EE oils on salmon and trout performance, health, and fillet quality is crucial for both the pelagic industry and feed producers, optimizing the exploitation of side stream products while promoting sustainable marine product usage. Quantitative data on safety, optimal inclusion, and potential benefits of EE oil use in salmonid diets are essential.

## 1.2 Sammendrag på norsk

Norsk pelagisk industrisektor er en av verdens fremste produsenter av pelagisk fisk og fiskeprodukter til både humant konsum og av marine føringredienser til oppdrettsnæringen. For å øke utnyttelsen av villfanget pelagisk fisk i størst mulig grad, må pelagisk industrisektor kontinuerlig videreutvikle prosesser for få øke utnyttelsen av verdifulle sidestrømsprodukter. Spesielt her kan nevnes at det under produksjonen av konsentrerte marine omega-3-fettsyrer (FA) som kosttilskudd til menneske, dannes en sidestrømsfraksjon bestående av en olje på etylester (EE) form. Det er viktig å vurdere om denne EE-oljen kan være egnet som fettkilde i fôr til laksefisk. Til tross for en årlig produksjon på ca. 10.000 tonn EE-oljer i Norge, så er det fortsatt begrenset med kunnskap om hvor egnet denne oljen er som ingrediens i fôr til laksefisk. Mangelen på kunnskap kan skyldes at oljer på EE-form i relativt liten grad tidligere har vært benyttet i fôr til fisk, i motsetning til menneske hvor EPA og DHA på EE-form har vært mye benyttet som kosttilskudd. Videre gir varierende fettsyresammensetning av ulike EE-oljer utfordringer med å trekke endelige konklusjoner om mulige helseeffekter. Det er også utfordrende å trekke konklusjoner fra menneske til fisk. Fettfordøyelsen til laksefisk skiller seg vesentlig fra pattedyrs fordøyelse ved fravær av lipaser for spaltning av monoacylglyserol (MAG) fra triacylglyserol (TAG), noe som kan påvirke effektiviteten av lipidtransport fra tarmen, lipidmetabolisme og tarmhelse. Enkelte studier har vist at inntak av EE-oljer i dietten kan føre til nedsatt fettfordøyelighet. Når EE-oljer hydrolyseres i tarmen fører det til dannelse av frie fettsyrer og en liten mengde etanol, og det er også viktig med ny kunnskap om hvordan dette påvirker tarmhelsen til laksefisk. EE-oljer i fiskefôr kan også være utsatt for lipidperoksidering, noe som igjen kan føre til redusert stabilitet av fôr, men dette vil også være avhengig av antioksidantinnholdet i fôret. I prosjektet SalmoE<sup>2</sup> tar vi sikte på å vurdere sikkerhet og egnethet av EE-oljer i laksefiskfôr. Prosjektet vil bidra med kunnskap om bærekraftig utnyttelse av sidestrømsprodukter fra pelagisk industri og videre øke kunnskapen om effekten av å inkludere EE-oljer i fôr til laksefisk på ytelse, filetkvalitet og helse. Kunnskap som vil være viktig for både pelagisk industri, fôrprodusenter og akvakulturnæringen.

## 2 Background: Ethyl ester oil, a side stream product

In recent years, the aquaculture industry has experienced significant growth, driven by the rising demand for seafood to feed a growing global population (FAO, 2022). Nowadays, aquaculture fulfils nearly 50% of the global seafood needs and the percentage could potentially increase to 70% in the coming years. With this rapid expansion of aquaculture comes an increased need to produce fish diets that can support the nutritional requirements of farmed fish. Feed accounts for 30 to 70% of all the input costs in aquaculture systems (Hodar et al., 2020). Therefore, it's crucial to utilize feed resources efficiently and strategically regarding both economic and sustainability aspects. The composition of fish diets, particularly in salmonids, has dramatically changed since the turn of the millennium. For many years, fish feed (particularly for carnivorous fish species such as Atlantic salmon and Rainbow trout) relied mostly on marine resources, particularly pelagic low-cost fish species like sardines, herring, and anchovies. Nowadays, given limited availability of fish oil (FO) and fish meal (FM), growing costs, and concerns regarding sustainability, plant-derived ingredients have become more desirable substitutes for aquafeed production (Tacon & Metian, 2008). In Norway, in particular, the use of FM and FO in A. salmon diets has decreased from 90% (65% FM, 24% FO) to less than 30% (14% FM, 10% FO) in the last few decades (Figure 1).



*Figure 1 Main sources of feed ingredients (% of feed) in Norwegian salmon feed in 2020 compared to previous years (Aas et al., 2019, Ytrestøyl et al., 2015). Micro ingredients include vitamin- and mineral premixes, phosphorus sources, astaxanthin, crystalline amino acids. 'Other' includes insect meal, single cell protein, fermented products, and microalgae (Aas et al., 2022).*

Lipids play a fundamental role in maintaining overall fish health and contribute to growth, reproduction, and regular bodily functions. They serve as an efficient energy source and are major components of cell membranes, providing essential fatty acids (FAs) that are involved in vitamin absorption, hormone regulation, and enzyme function among others (Hong, 2022; Zhao et al., 2015). FA are important components in fish nutrition, owing to their structural diversity and metabolic significance (Glatz, J. F., 2011). They can be classified into three main groups depending on existence/absence and number of double bonds: saturated FA (SFA), monounsaturated FA and polyunsaturated FA (PUFA) (Hakkinen, P. J., 2005). The Norwegian Pelagic sector are major producers of pelagic fish products for human consumption, including fish oil, rich in essential omega-3 PUFA. The relatively large amounts of such oils that must be ingested in order to reach an appropriate dose of omega-3 PUFA (dietary recommendations between 250 and 500mg/day) have prompted the development of more concentrated compounds (Dyerberg et al., 2010, Offman et al., 2013). Supplements prepared from marine oils are usually concentrated to contain around 30% to 90% of eicosapentaenoic (EPA) and docosahexaenoic acid (DHA) depending on the purification technique used. These supplements come in a variety of forms including ethyl esters (EE), free fatty acids (FFA), phospholipids or re-esterified triglycerides (rTAG) (Minton et al., 2023). To achieve maximum utilization of the fish sources, it is essential for the sector to explore new side stream products of high value. One such product is EE oil, derived from the production

of concentrated omega-3 TAG oils. The synthesis of EE oils involves the reaction of hydrolysed crude fish oil in FFA state with ethanol. Heat distillation under vacuum produces a concentrated omega-3 EE oil. In the final step, an enzymatic reaction process called glycerolysis reattaches the FAs to a glycerol backbone, removing ethanol group and producing rTAG. However, not all fatty acid EE molecules are reattached to the glycerol backbone in this final step, leading to a small fraction of EE remaining (Figure 2). In Norway, approximately 10.000 tons of EE oils are produced annually. There are several alternative usages for EE oils including biodiesel (El-Mashad et al., 2008; Yusoff et al., 2014) and as a potentially important feed ingredient for the aquaculture industry (Grayson et al., 2020). The FA composition of EE oils varies depending on processing methods. Traditionally, side stream marine EE oils contained approximately 8-10% of omega-3 polyunsaturated FAs (PUFAs), such as EPA and DHA, along with 30-40% saturated FAs (SFAs), primarily 16:0 and 18:0, 1-2% n-6 FAs, 20 to 30% monounsaturated FAs (MUFAs), and around 1% cholesterol. However, the pelagic industry is aiming to reduce EPA and DHA levels in marine EE by-products (2-4%) and retain more of these essential FAs in TAG form concentrates for human supplements.

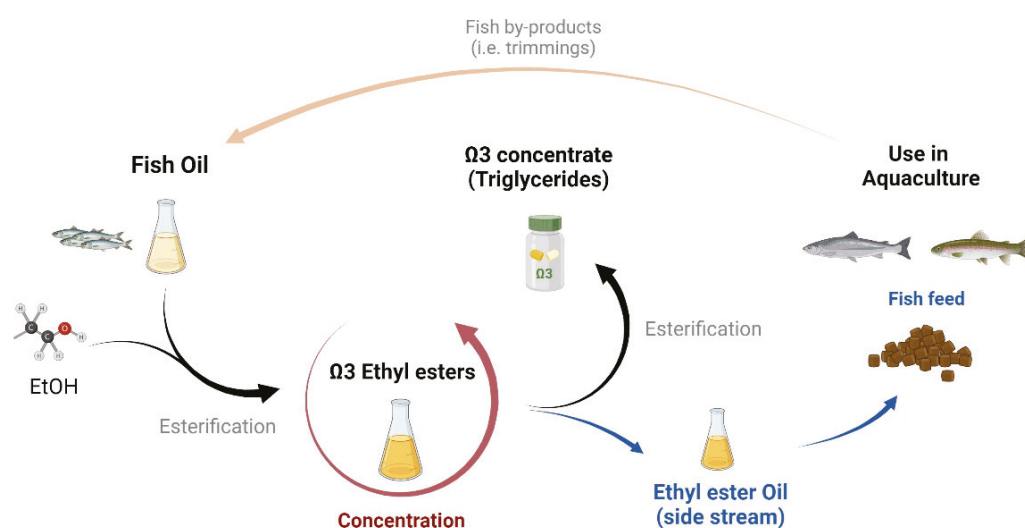


Figure 2 Simplified representation of the production of omega-3 polyunsaturated fatty acid concentrates for human consumption and side stream production of ethyl ester oil for aquaculture feeds

While fish feeds are optimized according to known nutritional needs, the high inclusion levels of plant sources have led to considerable reductions in the levels of nutritionally important SFA, PUFA such as omega-3 FA, and cholesterol, and an increase in pro-inflammatory n-6 FA and oleic acid (18-1n-9) found in plant oils (Glencross et al 2007). This dietary transition results in an increased omega-6/omega-3 ratio in tissues and organs, impacting their nutritional value and providing an unbalanced supply of these fatty acids for consumers (Katan et al., 2019). The omega-3 PUFA, EPA and DHA, are widely recognized as crucial dietary components in fish (Tocher, 2015) and have garnered a lot of interest due to their numerous physiological roles and potential health benefits (Figure 3). Several studies have evaluated the benefits of increasing dietary concentrations of EPA and DHA in aquafeeds with the aim to minimize the potential negative effects of substituting FO and FM with plant-derived ingredients (Torstensen et al., 2008, Lutfi et al. 2022). In A. salmon, it has become clear that, even if acceptable growth can be achieved, using low levels of these PUFA in the diets may have a negative impact on nutritional value as well as overall fish health and welfare. In this regard, about 16 g/Kg EPA and DHA (1:1 ratio) is regarded as safe and does not reduce growth or fish robustness while increasing levels up to 35 g/Kg appears to further improve growth, robustness, quality, and welfare status of the fish (Lutfi et al., 2022).

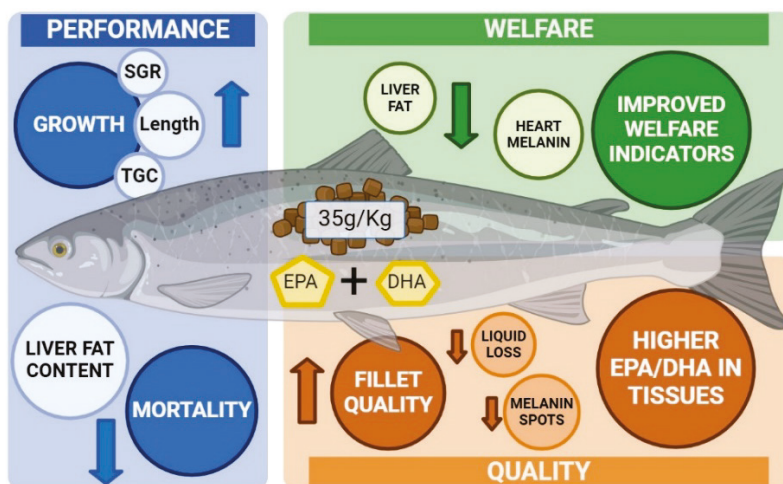


Figure 3 Summary of the main effects of a 35 g/Kg diet inclusion of EPA and DHA on performance, welfare indicators and fillet quality in Atlantic salmon (Lutfi et al. 2022)

Although SFA and cholesterol are not considered essential nutrients for fish, salmonids have evolved to thrive on diets rich in these components, making it unclear how reducing these nutrients in their diet may affect their growth and health. Limited commercial sources of SFA are available in the Norwegian Aquaculture industry, as palm oil, a major source of SFA 16:0, is avoided due to sustainability concerns regarding to deforestation. In addition, the feed industry avoids incorporating fat from terrestrial farmed animals both to address concerns related to animal welfare, potential contamination, and sustainability issues associated with intensive farming practices. Therefore, novel locally produced sources of these SFA are needed. Both feed and fish farming companies operate with their own lower limits of dietary SFA levels due to limited commercial sources, although these limits are based on relatively limited knowledge regarding the need for SFAs and potential consequences for fish health and fillet quality. Recent findings from the FHF project "ERN-samspill" (FHF-901656., Sissener, 2023) suggest that salmon require a minimum of 10% of these FAs in their feed to prevent liquid leakage from their fillets after freezing and thawing, while also positively influencing flesh colour. Excessive fat accumulation in the intestine and liver was observed in salmonids fed high levels of plant oils like rapeseed, flaxseed, or soybean oil, but not in those fed palm or poultry oil, which contain more SFA (Olsen et al 2003). Since most fish species can biosynthesize cholesterol, it is not regarded as an essential component in the diet. Traditional aquafeeds contain FO and FM, which are rich in cholesterol. However, in modern plant-based diets, their inclusion levels are rapidly decreasing and its uncertain how it will impact the cholesterol biosynthesis (Meng et al., 2023). In this regard, studies conducted in different fish species including Japanese flounder, Channel catfish, R. trout and A. salmon indicate that increasing dietary cholesterol levels improves feed intake (Yun et al., 2011), disease resistance against the bacterial pathogen *Aeromonas hydrophila* (Ignatz et al., 2022) and enhances the mobilization of astaxanthin and its retention in fillets (Ignatz et al., 2022).

Overall, the EE oil side stream products have a very interesting fatty acid profile, making them promising ingredients for salmon feed. They are rich in valuable fats that meet the energy-dense dietary needs of carnivorous fish species. Utilizing side stream products like EE oils offers a valuable source of SFA and cholesterol for fish diets, enabling the aquaculture industry to reduce waste and maximize resource utilization. However, there is limited knowledge on how salmonids utilize oil sources in EE forms compared to the natural TAG form.

### 3 Insights from human research: Triglycerides vs. Ethyl Esters

Most studies determining the suitability and nutritional differences of different lipid forms, such as EE, TAG and FFA, have understandably focused on humans. This focus arises from the extensive use of these lipid forms as dietary supplements in human nutrition. During the digestion of neutral lipids in the small intestine in humans, TAG undergo digestion facilitated by bile salts and pancreatic lipase, primarily at positions 1 and 3. Lipases are enzymes that catalyse the breakdown of fats into smaller molecules, making them essential for fat digestion and absorption. Bile salts emulsify fat globules into smaller lipid droplets, thereby increasing the surface area for lipase action. This process releases two FFA and a monoglyceride (a FA attached to glycerol). These components are absorbed by intestinal cells and reassembled into TAG before being transported in lipoproteins to various tissues and organs in the body (Cuenoud et al., 2020). Unlike natural TAG, which consist of FA bound to glycerol, EE oils have replaced the glycerol backbone with ethanol (Figure 4). This absence of glycerol or monoacylglycerol backbone in EE may delay the absorption of FA into the bloodstream, requiring additional carboxyl ester lipases to release single FFA along with ethanol (Offman et al., 2013).

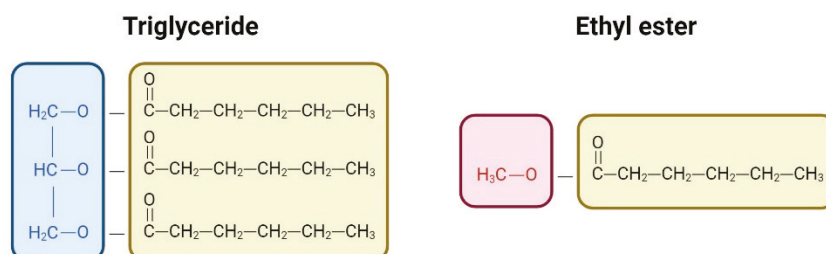


Figure 4 Structural formula of a triglyceride and an ethyl ester. Areas corresponding to a glycerol group (blue) and an alcohol group (red) are highlighted.

A study by Neubronner et al. (2011) compared the efficiency of long-term omega-3 supplementation in TAG vs. EE forms. Results showed that TAG supplementation led to a significantly higher increase in the omega-3 index compared to EE indicating a superior bioavailability of omega-3 in TAG form in humans. Similarly, other studies have demonstrated that FFA exhibit higher bioavailability in blood plasma compared to EE (Ghasemifard et al., 2014, Chevalier et al. 2021), with a consistent trend showing the following order: EE < TAG < FFA (Figure 5). However, conflicting findings exist, with some studies indicating similar absorption rates between natural TAG and EE, regardless of their chemical structure. In a study involving five normolipemic subjects, test meals containing EPA and DHA as either TAG or EEs (with and without added olive oil) demonstrated that both forms were equally well absorbed. Particularly, plasma and chylomicron TAG levels, as well as omega-3 FA responses, were similar between the two forms, indicating equivalent bioavailability of EPA and DHA when provided as either TAG or EE (Nordøy et al., 1991). Another study comparing the enteral absorption of EPA and DHA in EE and TAG form (from natural fish oil) found that both forms were similarly absorbed when given in equivalent doses. Over a 14-day period, EPA and DHA levels in total serum lipids and serum phospholipids were nearly identical between the two ester forms, despite significant differences in *in vitro* lipase hydrolysis rates (Krokan et al., 1993).

The bioavailability of these FA depends on various factors, including the fat content of diets and the chemical characteristics of the FA, such as chain length and degree of unsaturation. The bioavailability of EPA and DHA in FFA forms appeared to be significantly higher than in EE forms under low-fat dietary conditions, which are typically recommended for patients with cardiovascular disease (Offman et al., 2013). In this scenario, the reduced presence of dietary fat leads to lower secretion and activity of intestinal lipases. Since EE require an additional hydrolysis step by these lipases to release FFA for absorption, the limited lipase activity in patients fed a low-fat diet can hinder the efficient breakdown and

absorption of EE. In contrast, FFA forms do not require this extra hydrolysis step, allowing them to be more readily absorbed even when lipase activity is low. Interestingly, in humans fed a high-fat diet, absorption appears to be similar for both FFA and EE forms of EPA and DHA potentially due to the increased secretion and activity of intestinal lipases, which efficiently hydrolyse both forms, facilitating their uptake (Lawson and Hughes 1988).

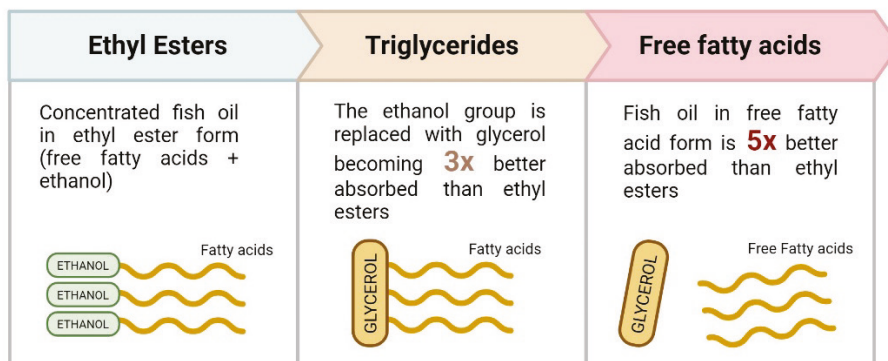


Figure 5 Bioavailability of fish oil in ethyl ester, triglyceride, and free fatty acid forms (Lawson and Hugues, 1988)

The chemical structure of marine oil TAG exhibits certain unique features. For instance, in oils derived from herring, cod, and mackerel, omega-3 fatty acids are predominantly located at the sn-2 position of glycerol, while in the fat composition of polar bears and seals, EPA and DHA may be found at the sn-1 and sn-3 positions (Carlier et al., 1991). Moreover, in rTAG, FA exhibit a distribution across the glycerol backbone that differs from natural TAG, with binding occurring at sn-1, or sn-3 positions and rarely in sn-2, as found in natural TAG. In this regard, studies indicate that binding of omega-3 FA to glycerol in the sn-1 or sn-3 positions enhances lipase hydrolysis of the bond, thereby increasing bioavailability (Schuchardt and Hahn 2013). Furthermore, the presence of monoacylglycerols and diacylglycerols in rTAG mixtures may facilitate micelle formation and increase FA absorption in the intestine (Dyerberg et al., 2010). As previously mentioned, when FA in EE form is consumed, the ethanol is drawn off, and the body must then reassemble the resulting FFA into TAG. Although concerns have been raised about the release of ethanol during the metabolism EE, it is generally regarded as insignificant, and EE are considered safe for human consumption (Bookstaff et al., 2003). However, common side effects include eructation, dyspepsia, taste perversion, constipation, vomiting, and skin rash (Krupa et al., 2024).

## 4 Ethyl ester oils in aquaculture: impacts on metabolism and health

EE oils derived from marine sources have the potential to serve as a valuable lipid source due to their composition, particularly for producing energy-dense diets for carnivorous fish species. However, there is very limited knowledge regarding the efficacy of incorporating EE oils into fish or particularly salmonids' diets. One possible explanation for this knowledge gap is that EEs of FAs, which are common in dietary supplements for humans, are not naturally present in the diets of aquatic animals. Moreover, from the available research in this topic, the FA composition of the EE oil used differs across fish trials, making it challenging to draw definitive conclusions about the potential effects of different oil forms (i.e. EE vs. TAG).

Most of these studies using EE oils in aquaculture investigate the impact of incorporating marine omega-3 EE oils into fish diets as alternative sources of EPA and DHA. A study using A. salmon post-smolts fed four different diets including two natural TAG oils (herring oil and canola oil) and two blends of EE oils (EPA+DHA concentrate from fish oil and arachidonic acid concentrate from hen eggs) revealed that diets containing EPA/DHA concentrates in EE form led to reduced body fat content, potentially attributed to less efficient EE utilization. However, no significant differences between TAG and EE preparations were observed in terms of health, growth, or lipolytic enzyme activity in frozen fillets (Polvi et al 1992). Similarly, Lochmann and Gatlin (1993) examined the effects of different types and levels of TAG, both alone and in combination with omega-3 EEs, in the diets of juvenile red drum (*Sciaenop ocellatus*). The EE preparations were a mixture of approximately 60% EPA and DHA and were incorporated into the four diets at a level of 2.5% (2 diets) or 5% (2 diets) complemented with linseed oil to produce diets containing 7 and 14% of total lipid. The results indicated no significant effects in survival, feed efficiency, growth, or appearance among fish fed diets containing EE forms although quantitative EPA and DHA requirements increased with dietary lipid. In a more recent study using R. trout, varying dietary levels of EE inclusion substituting FO (25, 50, 75, and 100%) demonstrated an increase in voluntary feed intake and a reduction in lipid vacuole size in the intestine and liver in diets with 100% EE oil, potentially due to diminished FA absorption (Grayson and Dabrowski 2020). Nowadays, given the relatively low levels of EPA and DHA in the EE oil by-products used in aquaculture, the focus of the research has shifted to providing an alternative source of SFAs which are also crucial for maintaining optimal growth and health in fish.

Fish metabolize dietary lipids differently from mammals due to their lack of the necessary lipase enzyme to produce monoacylglycerol during the digestion of TAG (Denstadli et al., 2004, Bøgevik et al., 2008a). Instead, fish possess a non-specific lipase that releases FFAs from all esterified lipids in the gut. Bile salt-dependent lipase (BSDL) is considered the primary lipase in fish, as opposed to the colipase-dependent pancreatic lipase that predominates in mammals (Lie & Lambertsen 1985). Consequently, salmon, and similar species must undergo additional enzymatic steps to reassemble TAG within intestinal cells for further lipid transport via lipoproteins. In the case of EE oils, the absence of the glycerol backbone in TAG requires that FFAs taken up by enterocytes be reattached to glycerol and converted back into TAG before being transported in the bloodstream via lipoproteins. Previous research has demonstrated salmon's ability to produce glycerol from glucose via the tricarboxylic acid cycle (Bou et al., 2026). However, it remains unclear whether salmonids can efficiently synthesize sufficient backbone glycerol to produce TAG when fed high-fat diets. Limitations in this process may lead to the accumulation of lipid in the intestine, potentially impairing intestinal function, and overall health. Additionally, it may impact the synthesis of very low-density lipoproteins (VLDL) in the liver, affecting the proper transport of lipids to peripheral tissues and organs. A study investigating the digestibility of different forms of FAs from fish oil in farmed salmon found that EEs exhibited significantly lower digestibility compared to FFAs or TAG forms. Despite the dietary fat type, only FFAs were detected in faeces, indicating complete hydrolysis of both TAG and EE to free FFAs in the intestine (Sigurgisladottir et al., 1992). This suggests that re-esterification of FFAs to TAG in enterocytes and their incorporation into lipoproteins for transport

to the bloodstream may be lower in the EE group. Furthermore, growth and feed conversion ratio were also significantly lower when diets contained more than 50% of EE inclusion, which is probably linked to the reduced digestibility. Other studies also reported reduced growth efficiency on EE diets in different fish species, however, no specific mechanistic interpretations for these results were provided (Castell et al., 1972; Lochmann and Gatlin, 1993).

Studies using different aquatic animals or other FA forms such as methyl esters (ME) or wax esters (WE) have also been done. Fatty acid MEs are a type of FA ester that are derived by transesterification of fats with methanol. Although ethanol is now preferable as it is easier to handle and poses a potentially lower toxicity risk compared to methanol, MEs offered an advantage in extraction from reaction mixtures, as EE tend to form stable emulsions that might complicate the extraction process. In a direct comparative study of different chemical forms of FAs, the shrimp *Panaeus monodon*, were fed purified diets with different neutral lipid sources: TAG, FFA, EE, and ME (Glencross & Smith, 1997). After 50 days, shrimp on the TAG diet exhibited the highest weight gain (94%) compared to those on FFA (84%), ME (73%), and EE (54%) diets, with poor growth on the EE and ME diets attributed to ineffective digestion and metabolism of these esters. In particular, the apparent digestibility of the EE diet was significantly lower (90.9%) than the other diets (96.5 ± 0.9%: TAG, 98.1 ± 0.6%: FFA, 96.1 ± 0.9%: ME). In contrast, Ibeas et al. (2000) assessed the utilization of dietary omega-3 PUFAs provided as ME, TAG, or a mixture of both in gilthead seabream diets. Fish were fed 3 experimental diets containing 2% of omega-3 PUFAs as ME, TAG, or a mixture, and a fourth diet including 5% of these FAs in TAG form. After 8 weeks, no significant differences were observed in growth, hepatosomatic index, total lipid content, or fatty acid composition in different tissues between the experimental groups. WE are esters from marine animal origin that consists of a long-chain FA and a monohydric long-chain fatty alcohol (Cowey & Sargent 1977). Although some studies indicate that many fish species can utilize dietary wax esters, their rate of intestinal hydrolysis is generally lower than that of TAG (Patton, Nevenzel & Benson 1975; Tocher & Sargent 1984; Bøgevik 2011). Early studies using dietary WE in fish showed poor growth of R. trout fed frozen-thawed zooplankton over 3 months compared to fish fed commercial diets, potentially due to loss of vitamin and protein during thawing (Sargent et al. 1979). Olsen et al., (2004) evaluated the use of a WE-rich oil from the copepod *Calanus finmarchicus* in A. salmon diets. Results showed that after 140 days of feeding, there were no significant differences in weight gain, specific growth rates, feed conversion ratios, or apparent digestibility between the fish fed a FO diet and the ones fed a Calanus-oil diet (up to 37.5% of inclusion). However, a study conducted in A. salmon and R. trout revealed that BSDL are more efficient at hydrolysing TAG than other lipid forms, such as wax and sterol esters (Bøgevik et al 2008b) indicating that, although depending on the inclusion level, WE might also have lower bioavailability when compared to TAG as seen for ME and EE.

From a feed production perspective, lipids are known to influence the pellet quality, feed dustiness, palatability and improve digestibility, all of which might affect feed utilization as well as the growth rate of the fish (Kerr et al., 2015). In most fish species there is a negative correlation between dietary fat and feed conversion ratios (Hillestad et al., 1998; Rodehutsord and Pfeffer, 1999) and in some cases also with growth parameters (Samuelsen et al., 2021). Extensive research indicates that oxidation of lipid ingredients in fish diets can compromise the nutritional quality of the feed, consequently affecting fish health (Martins et al., 2007; Zhong et al., 2007), growth (Peng et al., 2009) or even reducing PUFA content in the muscle (Zhong et al., 2008). However, there is a lack of data on the potential effects of lipid ingredients in different FA forms on feed quality and oxidative stability. Despite their high nutritional value, PUFA such as EPA and DHA, are highly prone to oxidation, leading to deterioration and quality loss (De Leonardis et al., 2008). Consequently, the production and handling of omega-3 concentrates, fish oils, or omega-3 enriched food products require the use of antioxidants for maintaining the integrity and efficacy of these products during processing and storage prior to consumption. In this context, oxidative stability refers to the resistance to oxidation, a chemical reaction that involves the loss of electrons, leading to the formation of free radicals and reactive oxygen species. This process can

degrade omega-3 FA, causing rancidity and a loss of nutritional and sensory quality. While most research on oxidative stability has focused on fish oils with low to medium omega-3 levels, data on concentrated omega-3 oils is limited. Although contradictory findings exist regarding the oxidation of EE oils compared to TAG oils, most studies suggest that the TAG structure offers greater stability, emphasizing the need for antioxidants such as tocopherols for protecting EE oils from oxidation (Ahonen et al., 2022). As previously mentioned, the current EE oil by-products contain relatively small amounts of EPA and DHA as the industry seeks to retain these fatty acids for TAG concentrates used in human supplements, thereby minimizing potential oxidation issues associated with PUFAs. Moreover, we anticipate that the EE oil in the current composition will exhibit high oxidative stability, as it is rich in SFA, which lack double bonds and are therefore less susceptible to oxidation. However, further studies are needed to confirm these statements in the context of present EE oil side stream production.

In summary, the chemical form of dietary lipids impacts digestibility and absorption in fish, as reported in humans. TAG show higher bioavailability compared to EE, potentially due to the presence of a glycerol backbone, which simplifies metabolic processing. In general, fish fed TAG-based diets generally exhibit better growth performance and health than those fed EEs. However, contradictory have been reported, indicating that FA composition, lipid levels, and species may influence lipid utilization, fish performance, and health outcomes.

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