D. 3.1.1. Technical note:

**Fatty acid manipulation of rainbow trout fry**

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**Background**

The EU regulation (EC 710/2009) on Organic Aquaculture states that the principles of organic rearing shall include the whole trout production cycle, i.e. the fish production shall be based on organic fry. The Organic Regulation of fish farming states strong limitations on the sourcing of protein and fat ingredients in organic feed, i.e. aiming to phase out fish oil obtained from wild fish stocks and this may lead to a higher inclusion of other oils, especially vegetable oils as these are widely used.

The main difference between vegetable oils (VO) and fish oils (FO) is related to the content of so called long chain essential n-3 (previously denoted Ω-3) and n-6 (previously denoted Ω-6) polyunsaturated fatty acids (LC- PUFAs). These are main constituents of primarily marine oils (i.e. fish oil) but are non-existing in vegetable oils. LC– PUFAs are considered important in early fish larval and fry ontogenesis for optimal physiological development. The dietary content and requirement of specific essential n-3 and n-6 fatty acids is however generally regarded as less important in freshwater fishes than in marine fish species, as some freshwater fishes such as salmonids i.e. rainbow trout to a certain degree are capable to synthesize some of these essential fatty acids for their physiological requirements.

Thus, substitution of marine oils with vegetable oils in feeds for organic aquaculture causes a reduced content of LC- PUFAs. Studies on salmonids (salmon, trout) have examined consequences for growth and physiological influences on juvenile and ongrowing salmonid fish, but little is known regarding short and long term effects on start feeding fry and their immunecompetance and ability to cope with stress and robustness towards inflectional diseases.

**What are fats, oils and fatty acids in fish feeds?**

We use different terminology when considering fats and oils. While fats are normally solid at room temperature oils are liquid. Oils are substances that belong to a lipid subgroup called glycerides, specifically triglycerides, but can also contain other important groups such as phospholipids. The oils used in organic or conventional aquaculture, whether of marine – or vegetable origin are mostly composed of triglycerides (Fig. 1), which means that they have a glycerol backbone upon predominantly one- three **fatty acids** are attached (this make them liquid at room temperature).



Glycerol + 3 fatty acids attached

**Fig. 1** Example of triglyceride with a glycerol backbone (left) and 3 fatty acids attached

**Fatty acids** are chains of hydrocarbons – chains of carbon atoms (or various length), with each of the carbon atoms (at each connection point in the chain) connected to one or two hydrogen atoms (Fig.2).



**Fig. 2** Example of a long chain essential fatty acid, eicosapentaenoic acid, 20:5n-3

As mentioned fatty acids can be saturated or unsaturated, but predominantly they are unsaturated. Unsaturation means that the carbon atom chain in the fatty acid has one or more double bonds (Illustrated Fig. 2, this fatty acids have 5 double bonds). Fatty acids are divided into different groups according to the number of carbon atoms. In oils they are mostly long-chain fatty acids: between 14 and 20 carbon atoms or very-long chain fatty acids: > 20 carbon atoms. The very long chain fatty acids are often polyunsaturated meaning they have two or more double bonds. Among these are the so called essential LC- PUFAs, that are required in diets for many organisms, as they can not synthesize these themselves.

**Oil types used in fish feeds for (organic) aquaculture**

Oils used in conventional aquaculture feeds have in many years been mainly industrial fish oil obtained from a variety of wild caught species (sprat, sandeel, herring, sardines etc.). In both conventional and organic fish farming in many parts of the world, there is a wish to phase out fish oil obtained from wild fish stocks, which is the majority of the industrial production today. Either because supplies are becoming limited or as this fishery is not considered sustainable (organic farming). Marine fish oils are now gradually substituted to a certain extent with a variety of other (cheaper) oils animal by-product fats including vegetable seed oils like, rapeseed oil, sunflower oil, soybean oil. All these oils have a different FA profile, which determines their quality and use, but none of the vegetable oils contain LC-PUFAs, but only FA up to 18 carbon atoms. A complete substitution with oils containing no LC- PUFAs may have some negative consequences on growth and on for instance stress coping ability of the fish and may compromise stress resilience and immune-competence. This may result in substantial losses and become a bottle neck for further growth of organic (salmonid/trout) farming.

**Requirement of oils and FA in feeds for organic rainbow trout**

Most fish species have specific requirements of nutrition for protein, oil, carbohydrate, vitamins and minerals. Rainbow trout is a carnivorous species with a high requirement for protein and energy rich diets, but with a relatively poorer ability to digest and uptake carbohydrates. As oil is an energy rich nutrient, this is widely used in dietary formulations of salmonid fish feeds to obtain a fast and good growth performance.

Fish have a higher need for specific nutrients during embryogenesis and early ontogeny before and after first feeding than during later juvenile or ongrowing stages. Immediate and long term effects of dietary fatty acid composition have been demonstrated in other freshwater fishes, e.g. pike perch (*Sander lucioperca*). Thus, larvae of this species fed low levels of docosahexaenoic acid (DHA), one of the major essential LC- PUFAs in fish oil, showed higher vulnerability to salinity stress and caused neurophysiological changes (Lund et al., 2014). While studies have shown, that rainbow trout can reproduce and produce viable offspring for which fish oils have been completely replaced by vegetable oils (Lazzarotto et al., 2015), there is strikingly little information about how and if dietary fatty acid composition affects performance, stress resilience and immunocompetence in rainbow trout. This is needed for securing the production of robust organic trout fry based on sustainable lipid sources.

In contrast to pike perch several anadromous fishes such as rainbow trout has a certain ability to synthesize some of the shorter chain C-18, n-3 FAs to LC- n-3 PUFAs (n-3 is a group of FAs that may be desaturated (addition of double bond) and elongated (extension of fatty acid carbon chain) to EPA and DHA LC- PUFAs) and the need for dietary inclusion of LC-PUFA is therefore considered lower. However, despite this, studies have demonstrated that rainbow trout fed a vegetable oil-based diet was insufficient in preserving the LC-PUFA EPA, 20:5n-3 and DHA 22: 6n-3 contents of the whole body, which were, respectively, 5·0- and 3·0-fold lower than the fish fed the FO-based diet (Turchini & Francis 2009). The study demonstrated that much of the shorter chain FA are either deposited or used for energy production. Therefore diets high in DHA and with a high content of shorter chain monounsaturated fatty acids, as substrate for energy production should be considered.

**FA manipulation**

Nutritional changes during critical periods in early development can permanently induce changes in animal metabolism and physiology, as a result of adaptive changes at the cellular, molecular and biochemical levels (Geurden et al. 1997) and can have impacts on PUFA metabolism as well as carbohydrate utilization.

The FA composition of fish fry can be manipulated in several ways. Broodstock nutrition is known to play a major role in quality of eggs and survival of fry and fry quality, and lipid and fatty acid composition has been identified as a main factor, that determines successful reproduction and survival of offspring (Rennie et al., 2005). Lipids derived directly from the dietary intake of broodstock in the period preceding gonadogenesis determine the essential fatty acids vital for early survival and development of newly hatched progeny. Different essential FA are stored in the ova and ARA from the 18:2 n-6 precursor (LA) is particular important, since ARA plays a major role in the reproductive process.

In order to manipulate the offspring a broodstock is typically fed different dietary formulations 6-12 months prior to ovulation / spawning and prior to gonadogenesis (development of gonads) in this respect the reproductive system i.e. gametes - eggs or sperm often reflect the dietary composition. Another method is to start feed the fry offspring with different experimental diets for a certain period of time after hatching. There is also the possibility to feed one type of experimental feeds to broodstock and either the same or another type to the first feeding fry which can give further information on combinations of feeds with different dietary oils and or FA composition). In this respect various effects and importance of dietary formulation can be examined.

Some oils are formulated and manufactured from various organisms with considerable high contents of LC-PUFAs containing up to 80-90 % of the total oils. Such oils can be supplemented in low amounts to vegetable seed oils and thereby create oils with designed levels of LC-PUFA contents of essential FA such as EPA and DHA or arachidonic acid (20:4n-6). – This gives the opportunity to statistically compare various inclusion levels and FA ratios and effects of these on the fish fry physiology.

*Dansk resume*

Ifølge EU regelsættet for økologisk akvakultur skal den økologiske fiskeproduktion udelukkende være baseret på økologisk yngel. Da medicinbehandling af økologisk ørred er uønsket og kun er tilladt i stærkt begrænset omfang, er ynglens robusthed overfor bl.a. den alvorligste yngelsygdom i dansk ørredopdræt, YDS (”yngeldødeligheds-syndrom”), særdeles vigtig. Ynglens robusthed kan muligvis relateres til ynglens swim-up adfærd (first feeding) og performance under opvæksten. Indholdet af specifikke essentielle Ω-3 fedtsyrer i foderet til ynglen er særlig vigtig for fiskens vækst, sundhed og velfærd, herunder robusthed overfor stress.

Der mangler imidlertid viden om evt. effekt af erstatning af fiskeolie med plantebaserede olier og deraf følgende ændringer i fedtsyre sammensætning og evt. afledede effekter på vækst og overlevelse, samt fysiologiske markører som stress resilience og immunkompetence. Dettegælder i såvel moderfiskefoder som yngelfoderet.

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