D. 3.1.2. Popular article.

**Manipulation of dietary fatty acids and effects on rainbow trout fry performance**

Ivar Lund\*, Carlo Lazado, Alfred Jokumsen

Technical University of Denmark, DTU Aqua, Section for Aquaculture, The North Sea Research Centre, DK-9850 Hirtshals, Denmark

***Background***

The Organic Regulation of fish farming states strong limitations on the sourcing of protein and fat (oil) ingredients in organic feed, i.e. aiming to phase out fishmeal (FM) and fish oil (FO) obtained from wild fish stocks and this may lead to a higher inclusion of other ingredients, especially of vegetable origin including proteins i.e. soybean; wheat; corn; barley; cottonseed; canola and peas, and oils i.e.: soybean; canola; linseed; sunflower; palm oil a.o. (Gatlin et al., 2007). A complete substitution of (FM) by vegetable protein alternatives may, however, lead to reduced performance in salmonids. Nutritional deficiencies and lack of essential amino acids; high dietary levels of antinutrients/ carbohydrates are typical factors leading to lower feed intake; lower digestibility,- feed utilization and growth and may have additional consequences for survival and robustness.

Several studies have shown that a total substitution of FO by individual or mixtures of vegetable oils do have same negatively effect on growth or feed efficiency provided that essential n-3 (Ω-3) and n-6 (Ω-6) long chain polyunsaturated fatty acids (LC-PUFA) requirements are met by the lipids contained in fish meal (Bell et al., 2003; Richard et al., 2006). Diets free of both FM and FO will contain no LC-PUFAs known to have specific important physiological functions. The three main important LC PUFAs are arachidonic acid (20:4 ARA Ω-6), eicosapentaenoic acid (20:5 EPA n-3) and docosahexaenoic acid (22:6 DHA n-3). While ARA is particularly important in reproduction, EPA and especially DHA are essential for neural development and as part of forming membranes and maintaining their fluidity (Sargent 1995). The addition of LC- PUFAs to vegetable based diets may improve performance and robustness of the fish and act as immune stimulants.

Several studies were performed with rainbow trout fry with supplementation of LC-PUFAs (EPA+ DHA in diets for which FM and /or FO had been partly or fully substituted with alternative ingredients of primarily vegetable origin. Survival growth performance, enzymatic activity; immune response and analytical FA composition were used to evaluate effects.

***Experimental setup***

Two experiments are reported here. I) In a first experiment marine proteins and lipids were partly or fully substituted with alternative mainly vegetable sources (Table 1, Exp. I). Three diets were formulated with low levels of marine derived protein and fat and fabricated in different sizes for broodstock and fry. Rapeseed oil was used as fat source and was gradually substituted/ supplemented by concentrated DHA+EPA oil from Croda. In this way three diets with the same protein content but with increasing levels of LC-PUFA were fabricated. At Lihme trout farm, Vejle, 3 groups of rainbow trout of each 10 females and 5 males were selected from a larger group and kept in three enclosures (Fig. 1) while fed one of the 3 exp. diets for approximately 6 months prior to spawning. A control group from the normal production were reared on a commercial diet as reference. Fertilized eggs were kept at Lihme trout farm, Vejle until eye egg stage and sent to DTU Aqua, Hirtshals where kept in 6 hatchery trays (app. 8 °C) until hatching.

For Exp.II. three diets were formulated for an experiment on fry and diets were formulated with a high level of fish meal protein for which the fish meal was defatted (by supercritical extraction) to contain a low level of marine oils. The three diets were supplemented by gradually increasing levels of concentrated DHA+EPA oil from Croda (Table 1). Fry were also obtained from Lihme dambrug but from a batch of breeders fed on a commercial diet (one year later).



*Exp. 1 - Fig. 1. Rainbow trout breeders separated in three enclosures at Lihme trout farm at same conditions and fed three different diets (photo: Alfred Jokumsen, DTU Aqua)*

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| **Exp. I** | D1 | D2 | D3 | **Exp II** | D1 | D2 | D3 |
| Ingredient (% inclusion) | % | % | % |  |  |  |  |
| Fishmeal CORPESCA Super Prime | 10.0 | 10.0 | 10.0 |  | 48.0 | 48.0 | 48.0 |
| Fish gelatin |  |  |  |  | 2.0 | 2.0 | 2.0 |
| Squid meal | 8.0 | 8.0 | 8.0 |  |  |  |  |
| Porcine blood meal | 7.0 | 7.0 | 7.0 |  | 6.0 | 6.0 | 6.0 |
| Soy protein concentrate (Soycomil) | 14.0 | 14.0 | 14.0 |  | 6.0 | 6.0 | 6.0 |
| Pea protein concentrate | 14.5 | 14.5 | 14.5 |  |  |  |  |
| Potato concentrate | 6.0 | 6.0 | 6.0 |  |  |  |  |
| Wheat Gluten | 10.0 | 10.0 | 10.0 |  | 15.0 | 15.0 | 15.0 |
| Wheat meal | 10.6 | 10.6 | 10.6 |  | 4.6 | 4.6 | 4.6 |
| Incromega DHA 500TG | 0.0 | 1.5 | 5.0 |  | 0.0 | 1.5 | 3.5 |
| Rapeseed oil | 17.0 | 15.5 | 12.0 |  | 17.0 | 15.5 | 13.5 |
| Vit & Min Premix PV01 | 1.0 | 1.0 | 1.0 |  | 1.0 | 1.0 | 1.0 |
| Antioxidant powder (Paramega) | 0.4 | 0.4 | 0.4 |  | 0.4 | 0.4 | 0.4 |
| DCP | 1.5 | 1.5 | 1.5 |  |  |  |  |
| Total | 100.0 | 100.0 | 100.0 |  | 100.0 | 100.0 | 100.0 |
|  |  |  |  |  |  |  |  |
| % (w.w.) | **D1** | **D2** | **D3** |  | **D1** | **D2** | **D3** |
| Crude protein | 54.6 | 55.0 | 55.1 |  | 58.8 | 58.8 | 58.5 |
| Crude fat | 21.8 | 21.6 | 21.2 |  | 20.1 | 19.4 | 19.6 |
| Ash | 5.9 | 6.0 | 6.0 |  | 9.8 | 9.8 | 9.7 |
| DM | 95.3 | 96.2 | 95.4 |  | 93.5 | 93.3 | 93.0 |
| EPA | 0.1 | 0.2 | 0.5 |  | 0.3 | 0.9 | 1.9 |
| DHA | 0.2 | 1.1 | 3.1 |  | 0.1 | 1.0 | 2.7 |
| DHA + EPA | 0.3 | 1.3 | 3.6 |  | 0.4 | 1.9 | 4.6 |
| Marine derived protein | 12.6 | 12.6 | 12.6 |  | 35.6 | 35.6 | 35.6 |
| Marine derived fat (including EPA+ DHA) | 1.5 | 3.2 | 8.2 |  | 1.3 | 2.7 | 5.4 |
|  |  |  |  |  |  |  |  |

Table 1. Dietary ingredients (% total) and proximate composition for the experimental diets in Exp. I and Exp. II

Both experiments were performed at DTU Aqua in a RAS system holding 9 tanks adjusted with the similar water flow and conditions. The experiments were carried out with 3 tanks per diet. For Exp. I half of fry from each dietary origin was divided into two tanks (18 in total) and fed either the same diet as given the broodstock or a commercial feed (Aller Futura) (Fig.2). Temperature was initially kept at 10° C and slowly increased to 13 °C during the first week of acclimation. Oxygen content was always > 70% saturation. Every second day NH4+/NH3 and NO2- (mg L-1) was measured, values were always below detection. At time of up swimming 500 post yolk trout fry were counted into each tank, i.e. app. 1500 per dietary origin.

For Exp. 1 fry were followed from time of start feeding until 45 days old. Growth performance was measured weekly by samplings of 30 fry per tank, similarly muscle growth dynamics were measured by samplings of 20 fry per tank at first feeding and subsequently 3, 10, 15, 25 days after.



**Fig. 2**. Outline of exp. setup in Exp. I. 3 broodstock groups fed 3 exp. diets for 6 months. Eggs divided into two and tested with exp. diets and a commercial control in total 18 tanks.

For Exp. 2 a similar procedure was followed for the fry as for Exp. I, but growth performance of fry was followed until 87 days after start feeding and weighed every 3 weeks. Every week for the first 4 weeks 10 fry per tank were sampled for FA analyses and for digestive enzymatic activity.

**Results Exp. I**

All diets were well accepted by broodstock. Diet D3 with the highest level of DHA+ EPA had a lower production of eggs with a lower hatching rate, however this was not significant. Levels of FA and EPA and DHA LC PUFAs in diets were reflected in eggs, but not in semen (Table 2). After only a few weeks the feed intake was much lower and mortality increased rapidly in fry groups fed the experimental diets D1, D2 and D3 compared with the similar groups fed the commercial diet Aller Futura. The supplementation of LC- PUFAs had no positive effect almost opposite as initial highest mortality was registered in group D3 with highest level of EPA+ DHA. Growth was significantly lowest for D1, D2 and D3 and exp. stopped after 3 weeks as mortality was very high in all tanks fed on exp. diets (data not shown). Results on muscle growth dynamics showed fewer and smaller muscle fibres in fry fed the experimental diets (Fig. 3).

|  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
|  | D1 feed | D2 feed | D3 feed | D1 eggs | D2 eggs | D3 eggs | D1 semen | D2 semen | D3 semen |
| TFA | 92.1±63.1 | 118.8±11.2 | 120.6±10.2 | 137.6±83.6 | 205.9±38.4 | 210.5±45.3 | 115.6±84.5 | 55.4±10.1 | 62.7±7.0 |
| FA |  |  |  |  |  |  |  |  |  |
| 18:1 (n-9) | 53.4±2.6b | 51.9±0.5b | 40.8±0.4a | 35.7±0.1b | 35.4±0.2b | 29.5±0.0a | 12.4±0.6 | 12.2±0.5 | 12.3±0.4 |
| 18:2 (n-6) | 22.2±0.6c | 20.8±0.0b | 17.7±0.2a | 10.2±0.2 | 10.1±0.0 | 10.1±0.0 | 5.8±0.3 | 5.7±0.2 | 5.7±0.1 |
| 20:4 (n-6) ARA | 0.1±0.1a | 0.2±0.0a | 0.6±0.1b | 3.2±0.2c | 2.7±0.1b | 2.0±0.1a | 4.6±0.1a | 4.7±0.2b | 6.1±0.0c |
| 18:3 (n-3) | 8.8±0.0 | 8.3±0.2 | 6.5±0.0 | 2.1±0.0 | 2.2±0.2 | 2.7±0.0 | 0.7±0.1 | 0.7±0.1 | 0.7±0.0 |
| 20:5 (n-3) EPA | 1.0±0.6a | 1.2±0.0a | 2.4±0.2b | 3.7±0.1a | 3.8±0.1a | 4.9±0.3b | 17.6±0.0 | 17.8±0.4 | 17.0±0.1 |
| 22:6 (n-3) DHA | 1.2±0.0a | 5.9±0.3b | 17.6±0.3c | 20.9±0.0a | 23.0±0.0b | 29.3±0.2c | 26.6±0.3 | 26.6±1.3 | 26.7±0.5 |
| DHA/EPA | 1.4±0.8a | 5.0±0.1b | 7.2±0.4c | 5.7±0.1a | 6.1±0.2b | 5.9±0.3ab | 1.5±0.0 | 1.5±0.0 | 1.6±0.0 |
| ARA/DHA | 0.1±0.1 | 0.0±0.0 | 0.0±0.0 | 0.2±0.0 | 0.1±0.0 | 0.1±0.0 | 0.2±0.0 | 0.2±0.0 | 0.2±0.0 |
| ARA/EPA | 0.1±0.0 | 0.2±0.0 | 0.2±0.0 | 0.9±0.0c | 0.7±0.0b | 0.4±0.0a | 0.3±0.0 | 0.3±0.0 | 0.4±0.0 |

Table 2. Exp.I. Main analysed TFA content (mg g-1 d.w.) and FA composition (% of TFA) of broodstock diets, eggs and semen. A different superscript for dietary group denotes as significantly difference (P<0.05).

*Fig. 3. a) Exp. I Number of muscle fibres in fry and b) the size of the muscle fibres (µm)*

In Fig. 4a-c is illustrated the activity of the enzyme alkaline phosphatase, which is an important regulative enzyme in bio-metabolic processes (Ram & Satyyanesan, 1985), and plays a vital role in digestion, absorption, and transition of nutrients (Swarup et al., 1981). Results showed an increasing higher activity in trout fry fed a commercial Aller Futura diet. Several other immune markers such as ceruloplasmin (an acute phase protein), myeloperoxidase ( a pro inflammatory enzyme) and lysosome activity (an innate immune enzyme) revealed some but no consistently differences in activity level until 45 days after start feeding between the tested diets.

Fig. 4. a,b,c Exp. 1. Activity of alkaline phosphatase in fry (U mL-1) until 45 days after start feeding for each of the exp. groups and the commercial control

**Exp 2:** The three diets were well accepted by the fry throughout the experiment and fish grew well on all diets (Fig. 5). Growth rate calculated as specific growth rate: *SGR (% day-1) = 100 x (ln w.w. f − ln w.w. i) t-1*, where ln w.w. f,i = the natural logarithm of the final and initial wet weight, t = time (days) varied from 4.2-4.4 % day -1 over the 87 days duration and was not significantly different (P= 0.052), but tended to be highest for fry fed the commercial Aller Futura. Feed utilization calculated as FCR (i.e. feed conversion ratio; kg feed/kg growth) was not significantly different between treatments (P <0.3) and varied between 0.75 and 0.78.



Fig. 5. Weight of fry (mg ind-1) from first feeding until 87 days after for each exp. group and a commercial Aller Futura control.

The assessment of digestive enzymes activity of gastric (pepsin), pancreatic (trypsin, α-amylase and lipase) and intestinal (alkaline phosphatase) (Fig. 6) revealed that enzymatic activity was present on the first day after hatching. In general the development in activity followed the same pattern with a relatively low activity until 34 days after start feeding apart from amylase, which displayed a higher activity in the early ontogenic phase. For amylase and lipase lower dietary content of EPA and DHA (0.4 (D1)-1.9% (D2) TFA) caused a significantly higher activity than for higher inclusion (D3, 4.5% and Aller F., 12.8 %) at day 34 and 57 days after start feeding .

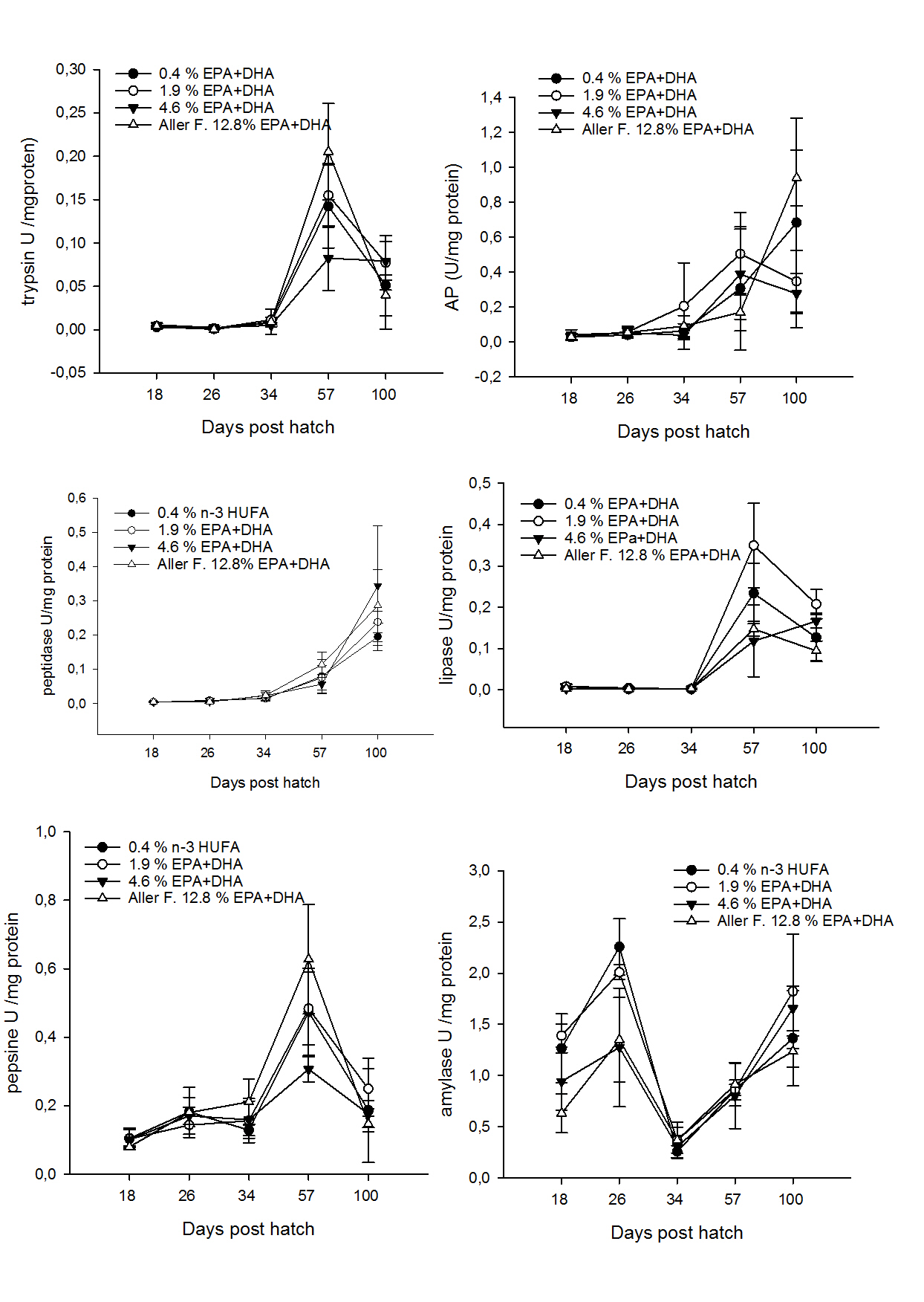


Fig. 6. Digestive enzymatic activity in trout fry digestive tract from18 -100 days after start feeding.

The tissue level of FA revealed a significant correlation between EPA and DHA in diet and in fry (data not shown) and a relative depletion for fry fed the lowest dietary level, however, results though also indicated a certain desaturation and elongase activity, as some intermediary LC-PUFAs displayed higher values, however this capacity was not enough to avoid a depletion of EPA and DHA in tissues.

**Discussion**

Partial substitution of marine protein mainly vegetable sources indicated a negative effect on growth and survival and caused lower muscle fibre growth and a change in activity of some immune related enzymes in first feeding fry. The supplementation of various levels of EPA+DHA seemed to have a limited effect. The reason for the poor growth on all experimental diets may be explained by the high inclusion of vegetable proteins that may have caused a lower acceptance of the feeds due to palatability issues or a nutritional imbalance in amino acid availability (Pratoomyot et al. 2010). However, only highly concentrated vegetables alternatives were used with a known composition and that previously had been used in other experiments for larger fish without negative effects.

In diets for which fish meal was not substituted but for which fish meal had been depleted of oils (down to 1.6 %) by super critical extraction, results revealed only minor effects on growth and enzymatic activity until 100 days after start feeding. The low dietary content of LC PUFAs (> 0.5 %) and marine derived fat (1.3 % of 20.1 %) however, caused a gradual depletion of LC- PUFAs in fish tissues. Rainbow trout fry seem to be able to mobilize sufficient LC- PUFAs for their physiological requirements by an enzymatic activation of enzymes related with desaturation and elongation of shorter chain PUFAs (Veron et al., 2016).

Overall results indicate that a partial substitution of marine proteins with mainly vegetable concentrated proteins have negative consequences for performance and survival of first feeding rainbow trout. An almost complete substitution of fish oil with vegetable oils without LC- PUFA, however, seem to have minor effects on performance of fish with no beneficial effects by increased supply of EPA and DHA.

*Dansk resume*

Det blev undersøgt hvorvidt foderets indhold af HUFA i foder til avlsfisk hhv. yngel påvirkede ynglens robusthed og performance.

Tre grupper af avlsfisk blev fodret med tre forskellige typer forsøgsfoder med reduceret indhold af marine ingredienser (protein and olie) og erstattet med vegetabilske produkter. Forskellige mængder omega-3 fedtsyrer (EPA og DHA) blev tilsat de tre diæter. Efter ca. 6 måneders fodring blev æg fra de respektive moderfiske grupper befrugtet og flyttet til DTU Aqua klækkeri som øjenæg og klækkede kort efter.

Analyser af total indhold af fedtsyrer og indhold af EPA og DHA i forsøgsdiæterne viste lineær stigning parallelt med inklusions niveauerne, som også afspejledes i analyserne af de tre grupper æg, dvs. en ”carry over effect af EPA og DHA” fra foder til æg. Tilsvarende var dog ikke tilfældet ved analyser af hannernes sæd.

Resultaterne viste også, at erstatning af marint protein med vegetabilsk kan have negativ effekt på foder indtag, vækst og fordøjelighed og dødelighed hos regnbue ørred yngel.

Endelig tydede resultater på, at regnbueørred er i stand til at danne HUFA ud fra kortere kædede fedtsyrer.

**References**

Bell, J.G., McGheea, F., Campbell P.J. Sargent J.R. 2003. Rapeseed oil as an alternative to marine fish oil in diets of post-smolt Atlantic salmon (Salmo salar): changes in flesh fatty acid composition and effectiveness of subsequent fish oil ‘‘wash out’’ Aquaculture 218, 515 – 528.

Gatlin D.M. et al. 2007. Expanding the utilization of sustainable plant products in aquafeeds: A review. Aquacult Res 38, 551–579.

[Pratoomyot, J.](http://findit.dtu.dk/en/catalog?l%5Bauthor%5D=Pratoomyot%2C+J.), [Bendiksen, E. A.](http://findit.dtu.dk/en/catalog?l%5Bauthor%5D=Bendiksen%2C+E.+A.); [Bell, J. G.](http://findit.dtu.dk/en/catalog?l%5Bauthor%5D=Bell%2C+J.+G.)[Tocher, D. R.](http://findit.dtu.dk/en/catalog?l%5Bauthor%5D=Tocher%2C+D.+R.), 2010. Effects of increasing replacement of dietary fishmeal with plant protein sources on growth performance and body lipid composition of Atlantic salmon (Salmo salar L.) [Aquaculture](http://findit.dtu.dk/en/journal?ignore_search=%E2%9C%93&issn%5B%5D=18735622&issn%5B%5D=00448486&key=00448486%7C000089%7C000305%7C000001%7C000000)305, 124-132.

Ram RN, Satyyanesan AG. 1985. Mercurie chloride, cythion and ammonium sulfate induced changes in the brain, liver, and ovarian alkaline phosphatase content in the fish [J]. Channo Puntactus Emir Ecol, (3): 263-268.

Richard, N., Kaushik, S., Larroquet, L., Panserat, S., Corraze, G., 2006. Replacing dietary 603 fish oil by vegetable oils has little effects on lipogenesis, lipid transport and tissue lipid 604 uptake in rainbow trout (Oncorhynchus mykiss). Br. J. Nutr. 96, 299-309.

Sargent, J.R., 1995. Origin and Functions of Egg Lipids: Nutritional Implications. In: Broodstock Management and Eggs and Larval Quality, Bromage, N.R. and R.J. Roberts (Eds.). Blackwell Science, London

Swarup G. 1981. Selective dephosphorylation of proteins containing phosphotyrosine by alkaline phosphatasess [J]. Biol Chem, 256: 8197-8201.

[Véron, V](https://www.ncbi.nlm.nih.gov/pubmed/?term=V%C3%A9ron%20V%5BAuthor%5D&cauthor=true&cauthor_uid=26746847)., [Panserat,S](https://www.ncbi.nlm.nih.gov/pubmed/?term=Panserat%20S%5BAuthor%5D&cauthor=true&cauthor_uid=26746847)., [Le Boucher,R](https://www.ncbi.nlm.nih.gov/pubmed/?term=Le%20Boucher%20R%5BAuthor%5D&cauthor=true&cauthor_uid=26746847)., [Labbé, L](https://www.ncbi.nlm.nih.gov/pubmed/?term=Labb%C3%A9%20L%5BAuthor%5D&cauthor=true&cauthor_uid=26746847)., [Quillet, E](https://www.ncbi.nlm.nih.gov/pubmed/?term=Quillet%20E%5BAuthor%5D&cauthor=true&cauthor_uid=26746847)., [Dupont-Nivet, M](https://www.ncbi.nlm.nih.gov/pubmed/?term=Dupont-Nivet%20M%5BAuthor%5D&cauthor=true&cauthor_uid=26746847)., [Médale, F](https://www.ncbi.nlm.nih.gov/pubmed/?term=M%C3%A9dale%20F%5BAuthor%5D&cauthor=true&cauthor_uid=26746847). 2016. Long-term feeding a plant-based diet devoid of marine ingredients strongly affects certain key metabolic enzymes in the rainbow trout liver. [Fish Physiol Biochem.](https://www.ncbi.nlm.nih.gov/pubmed/26746847) 42, 771-85.