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12 Fish Oil Replacement in Starter, Grow-Out, and Finishing Feeds for Farmed Aquatic Animals

Brett D. Glencross and Giovanni M. Turchini

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ABSTRACT

As aquaculture production continues to grow, there will be an increased use of lipid resources (oils and fats) alternative to fish oil for feed production. The potential for the use of these alternatives varies depending on the feeds in which they are included according to the production phase of the animals to which they are being fed. In starter feeds, where rapid growth, high survival, and normal development are critical priorities, there will remain a need for the use of lipid resources high in omega-3 long-chain polyunsaturated fatty acids (n-3 LC-PUFA). Fish in this starter phase have a critical requirement for the n-3 LC-PUFA docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA), and fish oils remain the only cost-effective source of these nutrients in the volumes required. However, the greatest demand for lipids is in those diets for the
grow-out phase. Most studies on alternative lipid use with animals in this part of the production phase show positive outcomes, in that there are few studies where all the added fish oil cannot be replaced. There are some species, however, where potential replacement levels are suggested to be more conservative, and a general substitution level in this production phase of 75% has been suggested. One of the key effects noted across the grow-out phase is that all alternatives affect the flesh fatty acid characteristics by reducing the level of n-3 LC-PUFA. This issue has provoked the concept of finisher diets, whereby a high n-3 LC-PUFA content diet is fed in order to restore the desired meat fatty acid profiles. Studies examining this concept have found that the tissue triacylglycerol fatty acids were greatly modified and responded in a simple dilution process to the added oil fatty acid composition, whereas the fatty acids of tissue phospholipids were less influenced by dietary fatty acid makeup.

Keywords: animal fat; aquaculture nutrition; crustaceans; fatty acids; finfish; finisher feed; finishing diet; fish oil; vegetable oil; wash-out diet.

12.1 INTRODUCTION

Dietary lipids play a variety of fundamental catabolic and anabolic roles in aquatic organisms, as they serve as sources of energy and of essential nutrients (essential fatty acids [EFA]) (see Chapter 2). Arguably, of all dietary nutrients none has a greater direct impact on the composition of its consumer than lipids (Sargent et al., 2002).

Traditionally aquaculture diets have relied predominantly on the use of fish oil (FO) as the primary dietary lipid source to provide energy (Tacon and Metian, 2008). However, most diets have probably been overspecified for omega-3 long-chain polyunsaturated fatty acids (n-3 LC-PUFA) (Glencross, 2009). Furthermore, essential fatty acids also continue to be one of the least well-understood dietary nutrients in fish nutrition (Watanabe, 1982; Sargent et al., 1995, 2002; Glencross, 2009), as well as in human and mammalian nutrition (Cunnane, 2003).

A recent review on the roles of aquaculture on the global fish and seafood supply (Naylor et al., 2009) has clearly indicated that aquaculture’s current consumption of FO (and not fish meal) is expected to establish the sector’s absolute demand for marine resources. Consequently, in confronting both economical and sustainability concerns, FO is being increasingly substituted by alternative lipid resources in aquaculture feeds (Glencross, 2009; Turchini et al., 2009a). The growing use of alternative lipid sources in starter, grower, and finisher diets for aquaculture species not only has begun to encroach on the fulfillment of EFA requirements for some species, but is also having a notable impact on different aspects of aquaculture production. This in turn demands the question “What is the real purpose of this lipid addition to the diets, and is the present use optimal?” This chapter examines some of the research undertaken to use alternative lipid resources and their application to different aquaculture species, with particular emphasis on the resulting growth performances.

Fish production can be defined as a series of stages that are also normally consistent with different dietary strategies and different aquafeed types. The initial stage of production, from egg until the animal is around 5 g, can be considered the “starter”
stage, although the specific transition size from starter to grower (or grow-out) is somewhat arbitrary. In the present chapter, we make the distinction based on those animals fed a diet <2 mm diameter. The other end of the spectrum is that for finisher diets (also referred to as finishing or wash-out diets). While finisher diets are yet less common in commercial settings, they play a potentially useful role in the FO replacement strategy, and a distinction is made in this regard. A finisher diet is therefore categorized as one that is fed to condition an animal for certain harvest qualities.

Grow-out feeds are primarily formulated to specifications to maximize growth rate, and minimize both feed conversion ratio (FCR) and feed cost. For most aquatic species, this entails a balanced diet that increases in energy density with increasing fish size (crustaceans excepted). To achieve this increase in energy density, the primary method used has been to increase the total lipid density of the diets. Indeed, it is with these grow-out diets that the issue of FO replacement becomes the most pertinent one, as it is this point of production where the vast majority of the resource is used. Accordingly, it is also with this production stage that the majority of research has also been undertaken.

Turchini et al. (2009a) made the generalization that up to 75% of the added oil could be supplied by alternatives to FO for this production phase. Those diets that replaced 100% of the added oil varied in their responses among the lipid resources used and the fish species fed. However, it is highly possible that these high replacement levels are beginning to impinge on the fulfillment of EFA requirements and there is a need to revisit the specification of the requirements for these nutrients in many key species (Glencross, 2009).

12.2 STARTER FEEDS

Most starter feeds have comparatively lower levels of lipids, for both bioenergetic reasons and the practicalities of formulating diets with a higher protein:energy ratio than that usually formulated for grow-out diets (Morais et al., 2005b; Glencross, 2008). This lower level of dietary lipid also occurs because of a poorer ability of larval and young juvenile fish to digest and absorb lipids (Morais et al., 2005a). However, contrasting this poorer ability to deal with high dietary lipid levels, larval and juvenile fish have a proportionally higher demand for EFA for anabolic reasons, particularly in the development of neural tissues (Tocher and Harvie, 1988; Koven et al., 1992; Villalta et al., 2008).

Rinchard et al. (2007) examined the effect of lipid class as a source of fatty acids on the growth of juvenile (0.2 g) rainbow trout (Oncorhynchus mykiss). These authors found that fish fed a diet with the majority of the lipid provided as a phospholipid (soy lecithin) source grew significantly better than those provided with lipids as methyl esters of 18:1n-9 (oleic acid [OA]), or 18:2n-6 (linoleic acid [LA]) and 18:3n-3 (α-linolenic acid [ALA]) provided as triacylglycerols (TAG), or EPA and DHA provided as TAG. It was suggested by these authors that there was a need to further examine the role of lipid class (i.e., neutral or polar lipids) in influencing the utilization and value of different lipid resources and their use in starter diets in general.

In a study with Atlantic salmon (Salmo salar) fry, the fish were fed either a FO or vegetable oil (VO) blend (55% rapeseed oil [RO], 30% palm oil [PO], and 15%
Linseed oil (LOI) diet for a complete production cycle from fish of 0.16 g to around 2.5 kg over a 22-month period (Stubhaug et al., 2007). Interestingly, there were no differences in growth over the first 16 months. Examination of the fatty acid profile of the diets shows that the DHA content of the VO diet was only 1% of total fatty acids, but that total n-3 polyunsaturated fatty acids (PUFA) and LC-PUFA were over 15%.

In a study with the marine flatfish yellowtail flounder (Limanda ferruginea), Copeman et al. (2002) used two different algal-derived oil sources rich in either DHA or arachidonic acid (ARA, 20:4n-6) to examine the nutritional value of enriched live foods. These authors found the best results were observed with fish fed rotifers enriched with the DHA-rich oil, and although such a feeding regime is not a true "starter" diet, the findings consolidate those of others who have identified that there is a critical demand for key EFA in the diets of fish during this part of the production phase.

The nutritional demands for n-3 LC-PUFA, primarily EPA and DHA, by gilthead sea bream (Sparus aurata) have also been extensively studied in larval fish (Koven et al., 1989, 1992; Salhi et al., 1994). It was noted by Koven et al. (1989) that mostly neutral lipids were depleted during starvation with little change in the level of polar lipids. Loss of fatty acids in neutral lipids was in the order of n-6 > n-9 > n-3, but the order reversed to n-3 > n-9 > n-6 in the polar lipids. DHA was retained better than EPA during starvation, and it was concluded that the physiological strategy of the animals at this stage of their life was to conserve the more valuable n-3 fatty acids during starvation. This was taken as an indication of their importance to this species.

A second study on gilthead sea bream starter diets examined the influence of lipid level on n-3 LC-PUFA requirements in a two-factor factorial study (Salhi et al., 1994). With two levels of n-3 LC-PUFA (0.7% and 2%) and two lipid levels (~13% and ~20%), it was found that increasing the lipid level improved larval growth. The improved growth was unaffected by n-3 LC-PUFA levels in the higher lipid diets. Better growth was observed in the low-lipid diet with high LC-PUFA levels relative to the other low-lipid diet. It was suggested that the n-3 LC-PUFA requirements for juvenile gilthead sea bream were not necessarily related to the lipid content of the diet, but were perhaps influenced by a factor such as the dietary EPA:DHA ratio. This finding provides some support to the notion that partial replacement of FO content in starter diets for this species may have some application.

A study on Senegal sole (Solea senegalensis) examining the manipulation of dietary EFA content in live feeds showed that these nutrients had a significant effect on pigmentation in this species (Villalta et al., 2008). The larvae were fed diets that had been enriched with different amounts of ARA, EPA, or stearidonic acid (SDA, 18:4n-3). The ARA did not have any defined effect on growth but the pigmentation was dramatically affected, which prompted the authors to identify a role for prostaglandins in the regulation of skin pigmentation. The inclusion of either SDA or EPA in the diet also affected the degree of pigmentation. These findings, while showing that there is a clear effect of certain fatty acids on growth, also showed that others play important roles in other physiological functions. As such, the application of alternative oils to starter diets needs to be mindful of the roles that these EFA play in the early development of fish.
In summary, FO replacement in starter feeds seems to be possible, but with due precautions. In fact, the EFA requirements (which commonly correspond to n-3 LC-PUFA) of fish during this early stage of life are significant, and the actual total amount of FO used, and its effect on total production costs, is rather limited. The primary role of nutrition during this phase is that of producing strong and healthy juveniles properly conditioned for the following grow-out phase. Hence, the substitution of FO with cheaper terrestrial alternative oils or fats that lack n-3 LC-PUFA may significantly impact negatively on the primary aim of nutrition during the starter phase and provide only limited economic advantages.

12.3 GROWER FEEDS

Salmonids represent the largest volume usage of FO in aquafeeds of any species. The fish are diadromous (anadromous) and generally farmed in freshwater, estuarine, or marine conditions. Salmonid diets range in lipid content from 15% to 40% (10% to 35% added oil content) (Torstensen et al., 2004; Karolazos et al., 2007; Stubhaug et al., 2007). Not surprisingly, thus far the vast majority of FO replacement research has been done with salmonids (Turchini et al., 2009a). The following sections attempt to compile all principal information available regarding the effects on growth and feed efficiency of FO replacement in diets of farmed aquatic species during the grow-out phase.

12.3.1 SALMONIDS

One of the earliest FO replacement studies was that by Mugrditchian et al. (1981), who compared linseed oil (LO), rendered animal fat, and salmon oil as the added lipid components in a study with Chinook salmon (Oncorhynchus tshawytscha). In each treatment, the level of lipid added was varied to ensure that the n-3 LC-PUFA levels were not lower than 10 g/kg in each test diet. After a 16-week period, there were no differences observed in growth between any of the treatments. Consistent with most studies manipulating the dietary fatty acid composition, the fish in this study had flesh lipid fatty acid profiles that were largely reflective of their diets. However, the authors also suggested that the fish in each treatment maintained a constant level of saturated fatty acids (SFA) irrespective of dietary lipid source. An analysis of the flesh neutral lipid fatty acids showed that these closely resembled those of the diet, while the polar lipid fatty acids were enriched in n-3 LC-PUFA.

A study by Hardy et al. (1987) was another of the early accounts examining the use of alternative lipid sources—herring oil, beef tallow (TAL), or soybean oil (SBO)—in diets fed to Atlantic salmon. The authors substituted up to 37% of the total dietary lipid content with the alternative oil and found that there were no effects of lipid source on growth, feed intake, feed conversion, or total lipid content of the fillets after a 23-week study.

The use of crude palm oil (CPO) was assessed in salmonids by Bell et al. (2002). In this study, CPO was substituted for FO in diets fed to Atlantic salmon. Similar to the SBO studies, the growth and feed utilization of the fish were unaffected by
use of the CPO, but significant changes in both muscle fatty acid composition and hepatic fatty acid metabolism occurred.

Bell et al. (2001) also studied the substitution of refined rapeseed oil (RO) for FO in diets fed to Atlantic salmon. Similar to their study on CPO (Bell et al., 2002), growth and feed utilization of the fish in this experiment were also unaffected by use of the RO, and significant changes also occurred in both muscle fatty acid composition and hepatic fatty acid metabolism.

Dosanjh et al. (1988) also examined the use of RO, pork lard, or herring oil as a replacement for half the dietary lipid content within diets fed to Chinook salmon. These authors found that the source of lipid used did not affect the growth or survival of the fish, but tissue fatty acid compositions largely reflected that of the diets. The exception to this was DHA, which was higher in the tissue than in the dietary lipids in the RO and pork lard dietary treatments.

Thomassen and Rosjo (1989) examined the potential for RO (high- and low-erucic-acid varieties) and SBO in the diets of Atlantic salmon. These authors found that use of either RO did not affect growth or survival, but that there were significant effects of dietary lipid source on the fatty acid composition of both cardiac and muscle lipids. Notably, there were significant increases in muscle lipid LA and decreases in EPA observed in the SBO and both RO treatments. Similar to the findings of Dosanjh et al. (1988), little effect of dietary treatment on the DHA concentration of the muscle was observed. Evaluation of the sensory parameters of the meat from fish from each treatment identified significant effects of lipid source on odor, taste, and color.

Other subsequent studies with Atlantic salmon have also shown that RO can be effectively used as a lipid source (Torstensen et al., 2004). The study by Torstensen et al. (2004) examined the progressive replacement of dietary FO by RO in diets fed to 142 g fish for 42 weeks. At the end of the experiment, no significant effect of RO inclusion on growth was observed, although some effects on hepatic enzyme activities were noted. Huang et al. (2008) also evaluated the influence of FO substitution with RO on the performance of Chinook salmon. The FO content of the diet was progressively replaced with RO at 0%, 33%, 67%, or 100%. After 30 weeks no significant effects on growth, feed utilization, or survival were observed with any of the treatments. Like most other studies, the fatty acid composition of fish also mirrored that of their diets, except the levels of ARA and DHA, which were somewhat conserved irrespective of which treatment the fish were fed. The ionic regulatory capacities of the fish from each treatment were also assessed by a 24-hour seawater challenge, which showed no effect of dietary treatment. However, the whole-body chloride content of the fish was inversely related to the dietary RO level during early development of the fish.

Unfortunately, in many of the published FO replacement studies, little importance has been given to reporting possible differences in growth performances, with presentation of the basic data, such as final fish weight or weight gain, often not reported. Typically, a simple sentence indicating that “no statistically significant differences in fish performance were noted” is reported at the beginning of the “Results” section. However, as addressed by Turchini et al. (2009a), it seems that in many instances the use of relatively short feeding trial periods and/or the use of statistical tests with limited experimental power may have been responsible for type II errors in the interpretation of these results. This is particularly evident for the majority of the
nonsalmonid species, as discussed below, in which many published studies reported that, though not statistically significant, fish fed the control diet containing FO were larger than those fed other treatments. Growth performance of salmonids, however, seems to not be negatively impacted by FO substitution, although there is some evidence suggesting that overall performance may even be improved by substituting FO with some types of oil.

Karalazos et al. (2007) published one of the few studies with RO where a significant effect was noted on performance due to the type of lipid resource. The authors added crude RO which made up 0%, 30%, or 60% of the added oil in diets fed to Atlantic salmon. In this study significant increases in growth were observed relative to the addition of the crude RO, but there was no significant effect on FCR (Figure 12.1). This finding implies that the fish ate more of the diets as the proportion of RO increased.

Both Higgs et al. (2006) and Stubhaug et al. (2007) published studies where RO or canola (CO) was blended with other lipid sources (Higgs et al., 2006: 50% CO and 50% poultry oil; Stubhaug et al., 2007: 55% RO, 30% PO, and 15% LO) and fed to Atlantic salmon. The experiment by Higgs et al. (2006) also used three levels (0%, 30%, and 60%) of FO replacement. These authors found that growth rate, feed intake, feed efficiency, and survival were unaffected by dietary treatment, but that

![Graph](image)

**FIGURE 12.1** Effect of replacement of fish oil by rapeseed oil at 0%, 30%, and 60% substitution rates (RO 0%, RO 30%, and RO 60%, respectively) at two different protein:lipid ratios (low and high protein [LP and HP, respectively]) in diets fed to Atlantic salmon. Notable is the improved growth with increasing levels of fish oil replacement (two-way ANOVA, $P = 0.002$), but this effect was more notable at higher dietary protein levels. Minor improvements in FCR with increased replacement of fish oil also occur, but these are concomitant with slight increases in feed intake with the higher rapeseed oil inclusion levels. (Adapted from Karalazos, V. et al. 2007. Aquacult. Nut. 13, 256--265.)


FIGURE 12.2 The effect of replacement of fish oil by a blended vegetable oil in diets fed to Atlantic salmon. Notable is the lack of differences between treatments for a large portion of the study. A significant effect (positive) of replacement is noted at month 22, demonstrating that such fish oil replacement effects may take a long-term approach to identify. (Adapted from Stubhaug, I. et al. 2007. Aquacult. Nut. 13, 145–155.)

the levels of EPA and DHA were significantly reduced with increasing level of FO replacement. The study by Stubhaug et al. (2007) aimed to cover the complete production cycle from fish of 0.16 g to around 2.5 kg over a 22-month period. Initially there were no differences in growth over the first 16 months, consistent with those results reported by Higgs et al. (2006) but, following seawater transfer, the fish fed the VO diet began to perform better. By the end of the experiment, there was a significant difference (~500 g) in weight between fish fed the different diets (Figure 12.2). Notably the fatty acid composition of the VO diet showed that its DHA content was only 1% of the total fatty acids, but that total n-3 PUFA were at over 15% of the total fatty acids, mostly present as ALA.

Sunflower oil (SFO) is another oilseed resource, which, like SBO, is rich in LA (18:2n-6). Bell et al. (1993) evaluated the use of this lipid resource in a study that focused on the phospholipid and eicosanoid chemistry and cardiac health of Atlantic salmon. In that study, fish were fed diets in which the lipid was provided as SFO, LO, or FO. Those fish fed the SFO diet had a notable increase in their cardiac phospholipid LA, 20:2n-6, 20:3n-6, and ARA content, with concomitant decreases in the EPA and DHA levels. Those fish fed the LO diet also had marked increases in their cardiac phospholipid LA, 20:2n-6, and 20:3n-6 content, but in contrast to the SFO-fed fish had a reduced ARA content in their cardiac phospholipids. The production of eicosanoids was also reduced in those fish fed the LO diet compared to those fish fed either the SFO or FO diets. Interestingly, the fish fed the SFO had a significant degree of cardiac histopathologies, which were also present, albeit to a lesser degree, in the FO-fed fish. By contrast, those fish fed the LO diet were virtually free of cardiac histopathologies. SFO was also used by Bransden et al. (2003) in a study where
it was fed in increasing levels to Atlantic salmon as a replacement for FO. These authors found no effects of SFO inclusion on growth or feed efficiency, but like other studies, substantial changes to the tissue fatty acid compositions were noted. Menoyo et al. (2003, 2007) also examined the effect of alternative lipid resources in the diets of Atlantic salmon, with SFO replacing either FO or LO in four levels of replacement (0%, 25%, 50%, and 75%). After 12 weeks, no significant differences in growth were observed among any of the treatments, and the tissue fatty acids reflected those of the diet. These studies consolidated the support for the application of either SFO or LO as lipid resources options for use in Atlantic salmon diets.

In consideration of the well-documented detrimental modifications of FO replacement on the final fillet fatty acid makeup, interest is increasing on the potential utilization of new alternative oils rich in n-3 LC-PUFA. In particular, one of these experimental lipid sources is that derived from thraustochytrids, which are a marine single-cell organism, the oil of which is extremely rich in n-3 LC-PUFA (Lewis et al., 1999). Miller et al. (2007) used this oil source to replace the FO content of diets for Atlantic salmon. Four diets containing 100% thraustochytrid oil, 100% PO, an 80%:20% PO and thraustochytrid oil blend, and FO reference were tested. No significant differences in growth, feed intake, and lipid digestibility between any of the treatments were observed. The fatty acid composition of the fish tissues resembled those of the diets, with fish fed the thraustochytrid oil having significantly elevated DHA levels. The osmolarity of the blood was also examined, as it provides an indication of the ability of the parr to smolt. While both the thraustochytrid oil and FO gave similar results, the PO showed a significant difference to FO, suggesting that Atlantic salmon parr fed solely on PO might have problems at smoltification.

Greene and Selivonchick (1990) examined the use of a wide range of lipid sources including SBO, salmon oil, LO, poultry fat, pork lard, and TAL when fed to rainbow trout. In this study, each lipid source comprised the entire oil content added to each diet. Similar to the majority of studies implemented on salmon, no differences in growth, feed intake, or feed conversion were observed among any of the treatments after a 20-week period. The authors also reported that tissue fatty acids were generally reflective of their diets, excepting the tissue levels of EPA and DHA, which appeared to be regulated at around 10% to 12% of the total fatty acid content.

Turchini et al. (2003) examined the substitution of FO in a commercial extruded pellet by coating the pellets with CO, poultry fat, pork lard, and oleine oil instead of FO when fed to brown trout (Salmo trutta). No differences were recorded in the overall growth performances and feed efficiency. However, some effects of the use of alternative lipid sources were noted on the overall mitochondrial fatty acid β-oxidation activities, and the fish fed the alternative sources had an increased level of fat deposition in the carcass and fillets compared with those fish fed a diet using only FO as its lipid source. Similarly, a study with brown trout using corn oil (CNO) as a replacement for FO had significant effects on the fatty acid profile of muscle and liver lipids, but no effect on fish growth (Arzel et al., 1994).

Another salmonid species, the Arctic charr (Salvelinus alpinus), showed no effect on growth, feed efficiency, or muscle and liver lipid content with the replacement of FO by echium oil (EO) (Tocher et al., 2006b). In addition, the replacement of FO with various blends of LO and coconut oil also had no effect on growth performance
of this species (Olsen and Henderson, 1997). Similarly no major modifications of growth performances were observed in brook charr (Salvelinus fontinalis) fed diets in which FO was replaced by SBO or CO (Guillou et al., 1995).

A recent study by Bureau et al. (2008) with rainbow trout examined the effect of varying the dietary lipid levels from 10% to 18% whilst maintaining a constant digestible protein and energy content. This study also examined the effect of variation in the total n-3 LC-PUFA content of 18% lipid diets by the blending of FO, TAL, and an enriched FO preparation. At the end of the growth study, there were no significant effects on growth or immune response of the fish on any treatment with varying fatty acid composition, but an increase in dietary lipid content reduced growth and feed efficiency.

Recently, Turchini and Francis (2009) examined the fatty acid metabolism of rainbow trout fed an FO-based diet or an LO-based diet. The authors concluded that the theoretical optimal dietary fatty acid composition for rainbow trout, which provided optimal energy substrate and at the same time minimized anabolic expenditure for in vivo bioconversions, should be characterized by a high content of mono-unsaturated fatty acids (MUFA), including also 20:1 and 22:1 isomers (as these are the favorable energy substrates), and also by high content of DHA and SFA (as these are actively produced by the fish if provided in limited amounts). The authors also suggested that an excessive dietary content of other PUFA, particularly LA, ALA, and/or EPA, seems, from a fish catabolism viewpoint, a relatively wasteful practice.

In almost all feeding trials implemented to assess the suitability of FO replacement with alternative oils, feed intake was unaffected by dietary treatments. However, though commonly implemented feeding trials are based on apparent satiety feeding regimes, they are not specifically designed to evaluate fish preference toward one specific diet compared to another. By implementing a preference test on rainbow trout, Geurden et al. (2005) demonstrated that trout can discriminate between feeds containing different lipid sources, with fish preferring the FO-based diet compared to different VO-based diets. Among the different VO, RO was the most accepted, followed by SFO and LO. These results were subsequently further validated in a second trial based on the measurement of self-feeding activity in the same species (Geurden et al., 2007). The suggestion made by the authors was that, more than the direct modification of the diet palatability, it is likely that nutritional factors could play major roles for this modified feeding behavior. More recently, in a self-selecting feed trial implemented on rainbow trout to assess possible modification of palatability of diets in which the FO component was replaced in 25% increments by RO, similar results were obtained (Pettersson et al., 2009). In particular, it was shown that trout preferred the 100% FO-based diet compared with the diets containing RO, but did not discriminate between different levels of RO inclusion.

It is known that from an olfactory perspective, fish are primarily attracted to low-molecular-weight compounds that are nitrogen containing, nonvolatile, and water soluble; and therefore lipids should play minor roles in determining the palatability of aquafeeds (Turchini et al., 2009a). However, the reported studies discussed in this section are suggesting the existence of a direct effect of dietary lipid source on the actual voluntary feed intake of farmed fish, or at least of rainbow trout. In fact, Geurden et al. (2005, 2007) were unable to evaluate a direct modification of the
pellet palatability in terms of orosensory recognition of lipids in fish. Importantly, Geurden et al. (2005) clearly emphasized the fact that dietary preferences do not necessarily reflect diet acceptance in the absence of choice. This point should be taken into consideration when analyzing the results of FO replacement feeding trials with an eye toward the implementation of efficient FO replacement strategies.

12.3.2 Coldwater Marine Carnivorous Species

Atlantic cod (*Gadus morhua*), a coldwater marine aquaculture species, was studied by Bell et al. (2006) with the use of EO. It was found that this lipid source had no negative effect on growth or feed efficiency of the cod. As in all instances, the fatty acid composition of fish tissues were modified by the dietary lipid source, with the tissue lipids recording elevated levels of SDA, 18:3n-6, and 20:3n-6, and reduced levels of ARA and EPA in those fish fed the EO. It was suggested that EO may have some beneficial effects on some immune parameters, including eicosanoid metabolism, mostly through influencing ARA synthesis in this species.

A study with the coldwater flatfish, Atlantic halibut (*Hippoglossus hippoglossus*), examined the effect of 50% replacement of FO by SBO in diets fed to large fish (initial weight of 1.6 kg) (Haugen et al., 2006). Over a 12-month period, no effect on growth was reported. However, the fatty acid composition of muscle lipids was significantly affected by the dietary fatty acid composition.

Turbot (*Psetta maxima*) are another coldwater marine species of increasing aquacultural importance. This species was used in a study where the fish were fed diets containing FO, SBO, or LO for 13 weeks. This was followed by an additional eight-week finishing period on diets with only FO as the added lipid source. The incorporation of either of the VO into the diet resulted in a slight decrease in growth compared to those fish fed the FO diet (Regost et al., 2003). However, feed and protein efficiency and whole-body composition were not affected by either of the VO-based diets.

12.3.3 Temperate and Warm-Water Freshwater Carnivorous Species

Many freshwater species (as opposed to marine or diadromous species) have been shown to have little need for LC-PUFA as essential dietary nutrients (Takeuchi, 1997). Accordingly it seems to be even less likely that, as for salmonids, the use of alternative lipids will have major detrimental effects on these species. However, the demands of carnivorous fish for the LC-PUFA tend to be greater than that of omnivores and herbivores. In addition, the overall lipid metabolism of temperate and warm-water freshwater carnivorous species has been suggested to be quite different from that of salmonids (Turchini et al., 2009a). Consequently, even though FO substitution is not necessarily responsible for any detrimental effects on the overall growth performance in salmonids species, the situation is quite the opposite for some of the carnivorous freshwater species. In fact, carnivorous freshwater species are characterized by less efficient capabilities in utilizing dietary lipid.

In a series of pioneering works implemented in the 1970s on channel catfish (*Ictalurus punctatus*), it was reported that FO- and animal fat-based diets were responsible for better growth performances when compared to VO-based diets.
Subsequently, it was also shown that a source of n-3 LC-PUFA is required by channel catfish and that, when FO was completely substituted by either VO or animal fats, growth performance and feed efficiency were significantly negatively affected (Satoh et al., 1989; Li et al., 1994; Wilson and Moreau, 1996). Accordingly, significantly reduced growth performances were recorded in channel catfish fed individual terrestrial animal fats or individual VO, when compared to an FO-based diet (Fracalossi and Lovell, 1994). However, catfish fed a blend of these oils recorded overall growth performances similar to the ones of FO-fed fish.

In a study with African catfish (Clarius gariepinus), PO was included in a series of diets as incremental (0%, 25%, 50%, and 100%) replacements of the added FO content (Olurin et al., 2004). Weight gain and feed conversion efficiency of fish from the treatments showed no effect of level of PO inclusion. In another study on African catfish (Clarius gariepinus), PO was used to completely replace the dietary FO content; however, various forms of palm oil, including refined oil, bleached oil, deodorized palm oleine, crude oil, crude palm kernel oil, and combinations of FO with palm fatty acid distillates or residual oil in spent bleaching clay were used (Ng et al., 2003). In this study, the fish fed the FO reference actually produced the poorest growth, and the blending of the FO with the palm oil options improved the growth of the fish. Accordingly, it was reported that African catfish have very low n-3 fatty acid requirements, showing reduced growth performance when fed high amounts of n-3 LC-PUFA (i.e., an FO-based diet) compared to a VO-based diet (Hoffman and Prinsloo, 1995). In a feeding trial, fingerlings of the clarid catfish (Clarias batrachus) fed a 1:1 combination of FO and SFO grew better than fish fed FO or SFO as the sole dietary lipid source (Mukhopadhyay and Mishra, 1998).

In general, it can be suggested that FO replacement seems to be less easily implemented on the channel catfish, while very easily implemented, and likely a suggested strategy, in Asian and African clarid catfishes.

FO replacement with graded levels of CO in diets for the sunshine bass (female white bass Morone chrysops × male striped bass M. saxatilis) resulted in no significant modifications of growth performance and feed efficiency over a 20-week period (Wonnacott et al., 2004). Similarly, no major effects of FO replacement with corn oil were reported in the same hybrid species by Lane et al. (2006).

Growth performance of juvenile pike perch (Sander lucioperca) was not affected by the substitution of FO with LO or SBO (Schultz et al., 2005; Molnár et al., 2006). However, by observing the modifications of the Hepatosomatic Index and fat deposition in the liver, it was suggested that there was a reduced utilization of dietary lipids derived from VO compared with those from FO.

When largemouth bass (Micropterus salmoides) were fed diets containing FO or different vegetable or animal lipid sources, the modification of bodily lipid deposition was recorded, with FO-fed fish recording the higher values, and, although not statistically significant, a trend toward growth reduction in fish fed the alternative lipid sources was observed (Subhadra et al., 2006).

In a 10-week feeding trial implemented on juvenile Eurasian perch (Perca fluviatilis) fed semipurified diets (with minimal fish meal inclusion) containing FO, olive oil (OO), SFO, or LO, the growth performance and feed efficiency parameters were
significantly affected (Xu and Kestemont, 2002). In particular, the highest weight gain (136.8%) was recorded in fish fed the FO, while fish on VO recorded only 32.4%, 43.6%, and 76.8% weight gain for fish fed OO, SFO, and LO, respectively. Similarly, by utilizing a semipurified diet with minimal n-3 LC-PUFA content, a series of experiments on the Australian carnivorous freshwater species, Murray cod (*Maccullochella peeli peeli*), showed that overall growth performance, feed efficiency, and nutrient digestibility were negatively affected by FO substitution with different VO, whether used individually or in blended combination (Francis et al., 2006, 2007a, 2007b, 2007c).

Particularly interesting, from a methodological viewpoint, are the results of one of these studies (Francis et al., 2007c). In this study, FO was replaced in graded increments (25%) with CO or LO. By examining the data using a one-way ANOVA (the most commonly utilized statistical test in FO replacement studies), the only significant difference recorded was for fish fed 100% CO, which had a poorer growth performance. However, by using a linear regression analysis relative to the level of FO substitution, it was shown that the substitution of FO with either of the alternative oils (CO and LO) resulted in significantly reduced growth performance (Figure 12.3). These observations provide a strong indication of the limited power of the ANOVA

![Figure 12.3](image-url)

**Figure 12.3** Example of how the use of different statistical tests (one-way ANOVA vs. linear regression) can generate different data interpretation when analyzing results of a fish oil replacement study. The weight gain percentage of juvenile Murray cod after 16 weeks of feeding with diets with graded substitution level (in 25% increments) of fish oil with linseed oil is reported. Importantly, ANOVA was not able to characterize any significant differences, while linear regression clearly, and significantly, showed that for every 10% of fish oil substitution in the diet, fish were growing 3% less. (Adapted from Francis, D.S. et al. 2007c. Aquacult. Nut. 13, 335–350.)
test in this type of study, highlighting the risk of incurring type II errors in data
test interpretation, as reviewed by Turchini et al. (2009).

12.3.4 Temperate and Warm-Water Marine and Diadromous
Carnivorous Species

Fish, as with all animals, evolved in different environments and adapted to different abundance of n-3 LC-PUFA in the natural food chain. Consequently, with particular regard to the metabolism of ALA to n-3 LC-PUFA, and therefore the nutritional importance of dietary n-3 LC-PUFA, fish can be classified into two main groups: the species with a typical "freshwater" fish pattern (such as herbivorous, omnivorous, and carnivorous freshwater fish which are capable of bioconverting ALA to n-3 LC-PUFA), and the species with a "marine" fish pattern (such as the majority of marine carnivorous species, which seem to have lost the capability to bioconvert ALA and therefore require n-3 LC-PUFA as EFA) (Sargent et al., 2002; Mourente et al., 2005; Tocher et al., 2006a). Thus, as will be discussed in the following sections, FO substitution in this latter group of fish is more challenging.

Sea breams (Sparids) are a temperate marine carnivorous species in which a considerable amount of work has been undertaken on nutritional demands, and lipid requirements in particular. Sparid grow-out diets typically contain 10% to 20% lipid (5% to 15% added oils) (Izquierdo et al., 2003).

Kalogeropoulos et al. (1992) assessed the use of SBO in diets for gilthead sea bream. Diets were made using different blends of SBO and cod liver oil. Growth was strongly influenced by dietary SBO content, and it was suggested that EFA deficiency signs were present in fish fed above 50% inclusion of SBO in the total added lipid content. The EPA and DHA content of the liver phospholipids decreased with increasing SBO content up to the 50% inclusion, after which no further reductions in EPA or DHA were observed. The ARA content of the liver was also found to level out at the 50% SBO inclusion. Similarly, in many instances, it has been shown that the utilization of different alternative oils significantly reduces gilthead sea bream performance (Alexis, 1997; Menoyo et al., 2004; Izquierdo et al., 2005).

Sharpnose sea bream (Diplodus puntazzo) is a sparid fish with more omnivorous feeding habits compared to gilthead sea bream, and no significant effects on sharpnose sea bream performance of FO substitution with LO or SBO have been recorded. However, LO treatment recorded a significantly higher mortality rate (Piedecausa et al., 2007). Nevertheless, Almada-Pagán et al. (2007) reported no effects on survival rate of sharpnose sea bream fed a diet in which FO was substituted by LO.

Glencross et al. (2003a) examined the use of CO in diets fed to red sea bream (Pagrus auratus). The use of crude and refined canola (low-erucic-acid rapeseed) oil and refined SBO was examined as 0%, 25%, 50%, 75%, and 100% replacements for the added FO content. Reduced growth and feed intake were observed with the complete (100%) replacement of added FO by crude rapeseed oil, but no effects on growth were observed with any inclusion level of either the refined rapeseed or SBO. The effect of the alternative lipid resources on the fatty acid composition of the tissues was also considerable, irrespective of plant oil type or processing grade.
There was a notable overall increase in the level of C\textsubscript{18} PUFA (LA and ALA) in the fish fed the VO diets. These trends become more prominent with greater levels of FO replacement by the alternatives examined. There were only nominal reductions in the levels of the n-3 LC-PUFA (EPA and DHA) in the tissues from any of the VO treatments (Glencross et al., 2003a). Similar results were obtained with red sea bream \textit{(Pagrus major)}, in which the substitution of 25\%, 48\%, or 70\% of the dietary FO with CO did not affect fish growth performance, feed efficiency, or percent survival (Huang et al., 2007).

Sea basses include a variety of species, typically diadromous, that are farmed in marine, estuarine, and freshwater systems. Typically the lipid content of manufactured grow-out diets for sea basses ranges from 10\% to 30\% (5\% to 25\% added oil) (Peres and Oliva-Teles, 1999; Izquierdo et al., 2003; Glencross, 2008).

The use of SBO, RO, LO, and a mixture of all three as substitutes for FO in diets fed to European sea bass \textit{(Dicentrarchus labrax)} (as well as in diets fed to gilthead sea bream) was reported by Izquierdo et al. (2003). It was possible to replace up to 60\% of the FO with any of the different alternative options used in this study without compromising growth. The fatty acid composition of the tissues of the fish largely reflected that of their diets. It was also suggested that DHA was selectively retained in the muscle compared to other fatty acids.

A study by Skalli and Robin (2004) examining the n-3 LC-PUFA requirements of European sea bass used six levels of n-3 LC-PUFA ranging from 0.2\% to 1.9\% of the diet by blending an FO with RO. At the end of the experiment, those fish fed the 0.2\% n-3 LC-PUFA diet had significantly poorer growth than those in all other treatments. Progressive improvements in growth were observed up to 0.7\% n-3 LC-PUFA (commensurate with an FO:RO blend of 1.6:7.2), and above this level no further improvements in growth, feed efficiency, or survival were observed. The tissue fatty acid composition of the fish also showed that the neutral lipid fatty acids were reflective of those in the diet, while the polar lipid fatty acids were more conserved.

Richard et al. (2006) examined the potential of two FO–VO blends (40\% FO, 24\% RO, 24\% LO, and 12\% PO; and 40\% FO, 35\% LO, 15\% PO, and 10\% RO) compared to 100\% FO treatment in diets fed to European sea bass. Fish growth was not affected by any of the dietary treatments in this study; however, fish fed the diets with the VO blends had lower plasma very-low-density lipoproteins and low-density lipoprotein cholesterol levels. On the contrary, reduced growth performance in European sea bass fed a blend of VO compared to fish fed the FO diet was reported by Mourente and Bell (2006); and accordingly, Alexis (1997) reported negative effects of FO replacement in European sea bass in a long-term (eight-month) feeding experiment, Montero et al. (2005) reported no differences in growth in European sea bass fed 100\% FO, 60\% SBO, or 60\% or 80\% LO, but fish fed 60\% RO were significantly smaller.

In the Japanese sea bass \textit{(Lateolabrax japonicus)}, the partial substitution of FO with various alternatives resulted in no significant differences in growth performance (Xue et al., 2006), while in red drum \textit{(Sciaenops ocellatus)}, the substitution of FO with coconut oil or tricaprylin was responsible for significantly retarded growth performance; but the inclusion of TAL resulted in improved growth performance even in comparison to FO-fed fish (Craig and Gatlin, 1995). SBO inclusion in diets for red drum produced decreased growth performance and lower feed efficiency (Tucker et al., 1997).
The effect of serial replacement of dietary FO with SBO, RO, or LO in diets fed to Asian sea bass (Lates calcarifer; also known as barramundi) was examined by Raso and Anderson (2003). In this study, growth was significantly reduced by the replacement of FO with either RO or LO, but not with SBO. This observation is significant in that SBO is about 55% LA, and therefore would have substantially altered the n-3 to n-6 ratios of the diets, more so than either RO or LO, both of which have lower levels of LA and higher levels of ALA (n-3 PUFA).

The demands for EFA in Asian sea bass were examined based on a series of blends of anchovy and SBO in a study by Williams et al. (2006). In that study, an optimal (maximal growth and lowest FCR) n-3 to n-6 fatty acid ratio of around 1.7:1 was proposed (equivalent to an SBO:FO blend of 3.5:8.5).

There are a wide variety of other marine fish species that are now being cultivated in various areas throughout the world. Among those different species, there is also a range in the dietary lipid levels used in different formulations. Some species, like the groupers (Epinephelus spp.), are typically raised on a low-lipid diet with levels around 10% being typical (Lin and Shiau, 2007). Other species like cobia (Rachycentron canadum) are being fed diets with lipid levels up to 27% (Romarheim et al., 2008). These two extremes would correspond to about 5% and 20% added oil in each case. Surprisingly, despite the increasing importance of cobia farming and the fact that it requires significantly large amounts of dietary lipid, little information is yet available about possible FO replacement.

A range of alternatives, including SBO, corn oil, SFO, and arachis oil (peanut or groundnut oil), were compared as replacements for FO in diets fed to the tropical marine fish the orange-spot grouper (Epinephelus coioides) (Lin et al., 2007). Among the dietary treatments, no significant differences in the performance of the fish were observed. However, similar to other studies the muscle fatty acid composition of fish fed each treatment reflected that of the dietary lipid sources.

Malabar grouper (Epinephelus malarbaricus), another tropical marine species, has also been used to examine the potential of corn oil as an FO replacement (Lin and Shiau, 2007). Corn oil was progressively (0%, 25%, 50%, 75%, and 100%) used to replace the FO content in diets with a low added lipid content (4%). At the end of the study, the fish fed the 75% corn oil content had a significantly poorer growth rate than those fish fed lower inclusion levels of corn oil. With the 75%:25% corn oil:FO treatment, the levels of n-3 LC-PUFA in the diet had dropped to lower than 5 g/kg, and this might have induced an EFA limitation. Therefore, the results of this study may not necessarily be a reflection of corn oil, but perhaps indicative of the low level of n-3 LC-PUFA that were available in the diet with the high inclusion levels of corn oil.

Shapawi et al. (2008) reported no significant effects on growth and feed efficiency of the humpback grouper (Cromileptes altivelis) when fed FO-, SBO-, PO-, or RO-based diets.

12.3.5 Omnivorous and Herbivorous Species

Several studies have also examined the use of alternative oil resources in diets for omnivorous and herbivorous species. Most of these animals are freshwater species, which have also been shown to have little need for the LC-PUFA as essential dietary
Fish Oil Replacement in Feeds for Farmed Aquatic Animals

nutrients (Stickney and McGeachin, 1985; Takeuchi, 1997). The results of these studies with commonly cultured omnivorous and herbivorous species show some interesting effects.

In general, omnivorous species are reported to require greater amounts of n-6 fatty acids compared to n-3 fatty acids for maximal growth (National Research Council, 1993). However, Stickney and McGeachin (1985) reported that tilapia (*Oreochromis aureus*) did not respond well to high dietary inclusion levels of LA, suggesting that this species would not do well with diets containing high levels of most VO (SBO, SFO, etc.) sources. Contrarily, Takeuchi (1997) found that growth of the *O. niloticus* species was significantly reduced when fed a diet using lipid source high in n-3 LC-PUFA, such as FO, compared to SBO- or corn oil-supplemented diets. Similarly, excessive levels of n-3 PUFA have been reported to negatively affect growth of hybrid tilapia (Huang et al., 1998; Ng et al., 2001). Clearly there is some contention with the fatty acid demands of tilapia and how effective different lipid resource alternatives might be in providing their dietary lipid content.

Indian carp (*Catla catla*) were reported to grow slightly better when fed diets supplemented with SFO compared to FO only, with the best growth rates observed in fish fed equal proportions of the two oils (Mukhopadhyay and Rout, 1996). However, common carp (*Cyprinus carpio*) did not show any significant differences in growth and feed conversion efficiency when fed FO or a variety of different VO (Steffens et al., 1995; Schwarz et al., 1988).

Very little information is currently available on dietary lipid sources in herbivorous fish species. Grass carp (*Ctenopharyngodon idella*) fingerlings fed animal fat (pork lard) or a blend of VO recorded better feed intake and growth performance compared to FO-fed fish (Du et al., 2008). In milkfish fry (*Chanos chanos*), no effects on growth and survival rate of different dietary lipid sources (coconut oil, cod liver oil, or their 1:1 combination) were recorded (Alava, 1998), whilst in striped mullet (*Mugil cephalus*) higher weight gains were recorded in fish fed FO-based diets compared to fish fed diets containing alternative terrestrial sources such as TAL or SBO (Jones and Strawn, 1983).

In summary, the results from these studies on omnivorous and herbivorous species provide limited evidence for the ability of alternative oil sources to replace FO. Indeed, the vagaries in the responses could be an interesting issue, and there is a clear need for some well-designed rigorous experiments with increasingly important omnivorous species such as Pangasius catfish and tilapia. For herbivorous species, though, increased knowledge of the optimal dietary fatty acid composition (hence the most favorable dietary lipid sources) is required.

12.3.6 CRUSTACEANS

Crustaceans, and shrimp in particular, present a contrasting situation to most grow-out fish diets in that these animals have a poor ability to digest lipid levels greater than 10% of the diet (Figure 12.4; Glencross et al., 2002a). In this regard, shrimp diets hold greater similarities with starter diets than grow-out diets for fish. However, contrasting the literature with fish, there are fewer reported studies on FO replacement
Fish Oil Replacement and Alternative Lipid Sources in Aquaculture Feeds

FIGURE 12.4 Three-dimensional model of lipid digestibility by *Penaeus monodon* shrimp with varying essential fatty acids and total neutral lipid content of the diet. The figure shows that above a threshold of dietary lipid content, the ability of shrimp to digest and absorb lipids from the diet dramatically diminishes, and this effect is also influenced by the fatty acid composition of the lipid. (Adapted from Glencross, B.D. et al. 2002a. Aquaculture 205, 157–169.)

in shrimp. Most shrimp diets have total dietary lipid levels at 10% or less, with typically 3% to 6% of the diet as added oil sources.

The substitution of SBO, LO, coconut oil, safflower oil, corn oil, and stearic acid for FO in diets fed to white shrimp (*Penaeus vannamei*) was examined by Lim et al. (1997). These authors noted significant differences in growth of the shrimp among the different treatments. Although FO supported the best growth, good growth was also seen with SBO, LO, and corn oil. Poorer growth was observed with stearic acid, coconut oil, and SFO. The fatty acid composition of the shrimp was also highly consistent with that of their diets.

Zhou et al. (2007) examined a range of alternative lipid sources fed to *Penaeus vannamei*, including pork lard, peanut oil, SBO, RO, an SBO–FO blend, and a straight FO. Each of the diets had similar growth performance, but less than that
of the shrimp fed the FO or the SBO–FO blend. Shrimp fed the pork lard also had poorer survival than of any of the other lipid sources examined. As with other studies, fatty acid composition of the shrimp reflected that of each diet.

Patniak et al. (2006) substituted the FO component of a shrimp (Penaeus vannamei) diet with either VO or oil derived from algal-microbial fermentation products rich in ARA and DHA. The growth, survival, and feed conversion were unaffected by the substitution of oil source in this study. A study by Browdy et al. (2006) also examined the replacement of FO by DHA-enriched algal oil products in organic plant-based feeds for P. vannamei, and found no significant effects between the feeds using the DHA-enriched algal oils and the control diets. Both studies provided preliminary support that algal oils provide a viable nutritional option for the replacement of FO sources in shrimp feeds.

The effects of a range of lipid sources, including LO, on the growth of black tiger shrimp (Penaeus monodon) were examined by Deering et al. (1997). A variety of different oil sources was evaluated, including LO, RO, pork lard, cod liver oil, SBO, and medium-chain triglycerides plus or minus cod liver oil. At the end of the study, the growth of shrimp was significantly better in those treatments fed the diets with LO, RO, SBO, and the medium-chain triglycerides and cod liver oil blend than those shrimp fed the cod liver oil and pork lard. The tissue fatty acids of the shrimp reflected those of the respective diets. The high growth performance of the shrimp fed the LO was consistent with the findings of Glencross et al. (2002b, 2002c), who showed that balancing LA, ALA, EPA, and DHA in diets for this species could promote growth better than that seen with FO alone.

Kumaraaguru vasagam et al. (2005) examined the nutritional value of a series of different VO sources fed to black tiger shrimp. PO, SFO, and peanut oil were compared against an FO reference. Each of the lipid sources was also evaluated with or without the inclusion of phospholipid (soy lecithin). No effect of lipid sources on growth was observed, though the inclusion of phospholipid significantly improved growth.

Although lipid inclusion levels in shrimp diets are low, the sheer volume of feed used by this sector means that they are one of the larger users of FO resources. The use of alternative oils in shrimp diets, however, presents some critical issues that are not as prevalent with many of the finfish diets. The poor ability of shrimp to emulsify dietary lipids and the dramatic effect that EFA optimization can have on their growth mean that substituting oils cannot simply be done without careful consideration of those EFA requirements.

12.4 Finisher Feeds

Most studies examining the use of alternative oils to FO have shown no, or minimal, reduction in growth, but have shown significant alterations in fatty acid composition of the fish's tissues (Turchini et al., 2009a). Despite these dramatic changes in fatty acid profile, the possible subsequent deterioration of the sensory parameters of the meat from the fish fed the alternative lipid diets is more subtle, but still present (Thomasen and Rosjo, 1989; Glencross et al., 2003a; Turchini et al., 2003, 2004, 2007a, 2009a). However, a further and important consideration is the effect of dietary lipids on the nutritional-quality characteristics of the meat from the fish.
to which they are fed (Steffens, 1997; Hunter and Roberts, 2000). A high level of importance has been placed on the presence of n-3 LC-PUFA in fish meat as an important quality factor. Even species such as catfish, which reputedly do not have a marked n-3 LC-PUFA requirement of their own, will face increasing pressure to include n-3 LC-PUFA in their diets in order to satisfy market demands (Morris et al., 1995). This fatty acid composition shift and the associated reduction in LC-PUFA content have prompted some researchers to suggest the need for "finisher" diets as a means of restoring the tissue fatty acid profile back to what was present when the fish were fed FO-based diets (Glencross et al., 2003b; Robin et al., 2003; Jobling, 2004; Turchini et al., 2007b).

The potential for manipulating the fatty acid composition of juvenile red sea bream that had previously been fed either SBO or RO was examined by Glencross et al. (2003b). In this study, fish were fed FO, SBO, or RO diets for three months before being changed to treatments in which they were either fed an FO diet or kept on their original conditioning diets for another five weeks. At the end of the experiment, fish fed an FO diet after previously being fed an SBO diet showed a slight growth enhancement, although most treatments showed no differences in growth performance. No significant changes in the relative fatty acid composition were observed in those treatments where fish were maintained on their original lipid source. In contrast, those fish that were changed to an FO diet had significant changes in both the relative (% total fatty acids) and absolute (g/kg live-weight) fatty acid content. Glencross et al. (2003b) showed that the rates of absolute (g/kg live-weight) change and accumulation of these fatty acids followed an exponential equation that differed for each fatty acid in each treatment.

Robin et al. (2003) also examined the potential of finisher diets used to restore fatty acid profiles of brown trout and turbot (Scophthalmus maximus). These authors hypothesized that the fatty acids would change based on a simple dilution model, with the fatty acids in the initial tissues simply being "washed out" by those coming in from the new diet. It was proposed that this could be modeled using the following equation:

\[ P_i(t) = P_i + \frac{P_i f}{Q(t)/Q_o} \]

where \( P_i(t) \) is the percentage of a fatty acid in the tissue of the fish fed a test diet at a certain time \( t \), \( P_i \) is the initial fatty acid concentration in the tissues, \( P_i f \) is the fatty acid concentration in the "reference" fish tissues at time \( t \), \( Q_o \) is the initial amount of fatty acids and total fat in the fish, and \( Q(t) \) is the amount of fatty acid and total fat in the fish at time \( t \) in the fish fed the test diet. It was observed that the fatty acids of the neutral lipids in the muscle followed this model of dilution, but in contrast the fatty acids of the polar lipid of the muscle underwent a much faster rate of change.

This concept of a dilution model of fatty acid composition, total body lipid content, and time was also developed and tested against that of actual data from Atlantic salmon by Jobling (2004). In this study the fish were initially fed a VO-based diet (an RO and LO blend) for a period of time. After this, the fish were then fed an FO-based diet, and
the changes in fatty acids monitored over a 98-day period. It was argued by Jobling (2004) that the changes in fatty acids were largely following a dilution response.

The use of a finishing strategy following a grow-out phase on a VO-based diet on Murray cod was examined by Turchini et al. (2006, 2007b). In the latter experiment, fish were initially fed for eight weeks one of three diets that differed in the lipid source: FO, a low-PUFA VO blend (PO:OO, 50%:50%), and a high-PUFA VO blend (LO:SFO, 50%:50%). At the end of the initial eight-week period, the fatty acid composition of tissues was markedly different and reflective of the respective diets. During a subsequent eight-week finishing period in which the fish were fed the FO diet, those differences in fatty acid composition of the tissues decreased. The change in fatty acid profile of the tissues was more rapid in those fish that were previously fed the low-PUFA VO diet. Importantly, though, during the final finishing period, those fish that were previously fed the VO-based diets grew significantly faster than fish that had been continuously fed the FO-based diet. It was suggested that the reduced levels of LC-PUFA in the fish previously fed the VO-based diets positively stimulated lipid metabolism, which led to a growth enhancement when the fish reverted back to an FO-based diet. Turchini et al. (2007b) proposed this effect as “lipo-compensatory” growth. Accordingly, a similar effect was noted by Glencross et al. (2003b) with the marine fish, red sea bream, when they were initially fed an SBO-based diet and then finished on an FO-based diet.

A study was undertaken by Palmeri et al. (2009) of change in flesh fatty acid profiles. Juvenile Murray cod were initially fed either FO- or RO-based diets for 90 days before both were fed an FO diet for a further 90 days. Prior to the introduction of the finisher diet, the fish were starved for 0, 5, 10, or 15 days to reduce the initial lipid content of the animal. However, in this experiment, the fish did not show a significant decline in lipid content with starvation period, nor were there any effects of starvation on improving the uptake of EPA or DHA into the fillet. Despite this result, it should be noticed that, in consideration of the fact that the dilution model seems to describe accurately the fatty acid modification during the finishing period, at least in semifat or fat fish species, the potential reduction of total lipid content of the fillet before the finishing period should speed up the restoration of FO-like fatty acid makeup. The problem is how to efficiently reduce fillet lipid content in growing fish, without excessively negatively impacting growth performance and/or fish health and well-being. A prefinishing lean diet could be a possible solution, but this needs to be properly assessed.

Benedito-Palos et al. (2009) examined both an FO replacement and also whether changes in the tissue fatty acid profile of gilthead sea bream followed a simple test dilution model after changing the lipid resources used in their diets. The fish were initially fed diets with either an FO or a VO blend (RO:LO:PO; 17%:58%:25%) replacing 33% and 66% of the added FO. After a 14-month period on the test diets, a further three-month phase examined the feeding of an FO “finishing” diet to monitor the changes in the fatty acid profile. There were no significant effects of any of the treatments on growth of the fish; however, the partial replacement of FO increased the OA, LA, and ALA, consistent with the fatty acid composition of those diets. As for the FO finishing diet, the simple dilution process of the tissue fatty
acids was a reasonable model of the changes observed, consistent with the findings of others (Glencross et al., 2003b; Robin et al., 2003; Jobling, 2004; Turchini et al., 2006).

In a further study with gilthead sea bream, a reference diet using FO as the sole added lipid resource was compared against other diets where SBO, PO, or RO were used as alternatives (replacing 69% of the added oil) (Fountoulaki et al., 2009). After a six-month study period, each of the treatments was then fed an FO finishing diet, and the progressive change in the fillet fatty acid profiles examined. No significant differences on growth or feed utilization were observed among any of the treatments. The use of the FO finishing diet (over a 120-day period) was shown to not be adequate for restoration of DHA, ARA, and EPA. In addition, sensory analysis did not identify any differences in the organoleptic characteristics of the treatments.

12.5 CHALLENGES ASSOCIATED WITH FO REPLACEMENT

Most studies examining the use of alternative lipid sources have shown a sound ability to replace FO, with limited effects on growth and feed utilization, but most have had a notable effect on the fatty acid composition of the fish (reviewed by Turchini et al., 2009a). Generally, it could be regarded that any of the main plant-derived oils (SBO, PO, RO, etc.) or terrestrial animal fats (TAL, poultry by-product fat, pork lard, etc.) can be viably used to replace roughly up to 75% of the added dietary FO for most species (Turchini et al., 2009a). However, there are several issues that still need to be addressed to make such replacement technology cost-effective and sustainable and to minimize possible detrimental effects on fish performances, health and well-being, and final product quality.

More work is required on the grade of such alternative oils needed in the diets and how refined they have to be, or how much contaminants can be tolerated by the fish, for them to still be viable replacements for FO. This aspect of the FO replacement "issue" has received limited research in studies with RO and PO (Glencross et al., 2003a; Ng et al., 2003). There is still a need for further nutritional research on the effects of oil processing grades and oil-based contaminants (non-TAG content) on fish from oil resources such as SBO and LO. This issue of lipid source purity and quality also requires some evaluation for rendered oils from bovine-, ovine-, porcine-, and poultry-processing streams.

Most of the major VO have been assessed to some degree in the key aquaculture species. However, there is still a need for some dose-response (FO dilution) studies with some of these oils in rapidly emerging key species (e.g., Pangasius catfish), simply to provide confidence to the feed sector that particular oil types do not have tolerance thresholds, or if they do, then to provide information as to what those tolerances are. While a significant amount of work has been spent on examining many of the primary VO options, further work is still required on rendered (poultry, ovine, bovine, and porcine) oils with many aquaculture species.

The potential of transgenic or genetically modified (GM) oilseed products is also another emerging prospect. In particular the potential of a DHA-producing rape-seed variety is already being intensively pursued and offers significant potential for
alleviating pressure on fishery resources (Lopez Alonso and Garcia Maroto, 2000; Scarth and Tang, 2006). As with other genetically modified agricultural resources, there may be concern and skepticism associated with the release of such oils. Like other GM raw materials, an assessment of benefits and risks associated with their use will provide significant clarity on their value as a feed ingredient.

Increasing levels of both fish meal and FO replacement in aquaculture feeds will also reduce the n-3 LC-PUFA content of aquaculture diets. There are examples where this has already begun to impinge on EFA requirements of some species (e.g., studies by Skalli and Robin, 2004), and therefore there needs to be regard given to the issue of coreplacement and how potential EFA limitations may be avoided. This will also increase the need for more accurate information on the actual EFA requirements (for both maintenance of health and maximization of growth performances) for each species, so that precise specifications can be made with regard to what EFA needs to be added and how much needs to be included in any specific formulation (reviewed by Glencross, 2009).

A further aspect that should be considered in FO replacement studies is that, though the available data are still limited, there is evidence that, in fish, the modification of dietary fatty acid composition can affect leptin metabolism (Turchini and Paolucci, in press). Leptin is a hormone with several metabolic actions, including the regulation of voluntary feed intake. In two recent feeding trials implemented on Murray cod and rainbow trout, it was noted that VO-based diets were responsible for increased preprandial plasma concentration of leptin, when compared to fish receiving an FO-based diet, resulting in decreased voluntary feed intake (Turchini et al., 2009b). This could potentially explain the differences recorded in voluntary feed intake in fish fed alternative oils by Geurden et al. (2005, 2007) and Pettersson et al. (2009). Moreover, Turchini et al. (2009b) speculated that, when FO replacement is protracted for sufficient time, a form of leptin resistance occurs; this is responsible for triggering an increased appetite which, in turn, can explain the increased growth performance recorded, for example, by Karalazos et al. (2007) and Stubhaug et al. (2007) in Atlantic salmon (Figures 12.1 and 12.2). These salmon were fed for a prolonged period of time with a VO-based diet, with the increased performances recorded in some instances during the finishing period (Glencross et al., 2003b; Turchini et al., 2007b), and the increased lipid deposition sometimes reported in fish fed an FO-replaced diet (Arzel et al., 1994; Turchini et al., 2003). However, these hypothesized metabolic modifications are responsible for relatively minor and subtle alterations of feed intake, performance, or tissue chemical composition, and hence have been difficult to evaluate; but it seems evident this area of fish oil research needs the increased attention of fish nutritionists.

Without exception, all studies examining the use of alternatives have also noted changes in the fatty acid composition of the tissues of the fish. This therefore raises another issue—the effect of substitution on the nutritional value of fish to the consumer. Fish consumption is promoted as one of the best sources of n-3 LC-PUFA in the human diet (Steffens, 1997; Horrocks and Yeo, 1999; Hunter and Roberts, 2000), and if there is to be increasing substitution of n-3 LC-PUFA-rich lipid sources by alternatives with high levels of n-3 and n-6 PUFA, or MUFA, or even SFA, then
inevitably this has to impact the nutritional qualities of the meat product being produced. To address this problem, there are essentially three options which allow us to also sustain a growing demand for aquaculture feeds.

One strategy is to improve the overall efficiency of feed utilization in aquaculture so that greater outputs are achieved from the same inputs. Achieving this outcome is not limited solely to nutritional strategies, as genetic selection for more efficient use of lipids and improved growth efficiency in general will also increase the outputs from the same level of resource use.

A second option is to identify lipid resources that will allow the fatty acid profile of the fish to be maintained within certain tolerances of the desired profile. This may require further developing genetically modified variants of rapeseed or other oilseeds that produce oil containing EPA and DHA (Heinz, 2002; Scarth and Tang, 2006; Bimbo, 2007), and/or upscaling the technology to allow the commercial production of single-cell oils (such as thraustochytrid oil) which have high DHA levels (Lewis et al., 1999; Harel et al., 2002).

A third option is based on improving the understanding of the potential for restoration of fatty acid profiles by using “finisher” diets in which fish are fed a diet enriched in n-3 LC-PUFA for a short period before harvest. So far, this concept has primarily focused on the issue of fatty acid turnover and several studies have already examined its potential, but further work is needed to develop commercially applicable protocols. In particular, the benefits of using DHA- and EPA-enriched oils, higher or lower lipid density diets, and partial feed restriction prior to feeding the “finisher” diets are all further possible considerations.

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