

Lípidos Alternativos en la Nutrición de Peces Marinos | Alternative Lipids in Nutrition of Marine Finfish

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Abstract

The current paper provides new knowledge regarding LC-PUFA requirements of marine carnivorous fish (White Seabass *Atractoscion nobilis*, California Yellowtail *Seriola dorsalis* and Florida Pompano *Trachinotus carolinus*) in the context of C₁₈ PUFA-rich and SFA- and MUFA-rich alternative lipids. Determine if all LC-PUFAs (ARA, EPA, DHA) are equally important in meeting fatty acids requirements and also determine the effects of dietary SFA, MUFA, and C₁₈ PUFA content in fish oil sparing and tissue deposition of LC-PUFAs. The overall findings highlighted that DHA and ARA appear to be the primary drivers of fatty acid essentiality, whereas EPA is likely required in minor amounts. It was also demonstrated that DHA/EPA ratio had little-to-no effect on fish performance. Additionally, LC-PUFA requirements seem to be more flexible than previously assumed being influenced by dietary fatty acid profile. LC-PUFAs in marine finfish are more bioavailable in the context of SFA-/MUFA-rich alternative lipids, thus, reducing the requirements for these nutrients and allowing the fish's physiological demand to be met with dietary levels below the minimum levels recommended. Finally, these findings suggest that although marine fish accept a variety of alternative lipids, those rich in SFAs and/or MUFAs seem advantageous in terms of limiting the effects of fish oil sparing on tissue fatty acid profiles.

Keywords: Lípidos, Peces marinos, Alimentos

Introduction

Fish oil sparing and replacement is considered one of the biggest challenges faced by the aquaculture industry and aquaculture nutrition because of the unique characteristics of this marine-origin lipid. The high digestible energy and abundance of long-chain polyunsaturated fatty acids (LC-PUFAs), particularly arachidonic (ARA, 20:4n-6), eicosapentaenoic (EPA, 20:5n-3) and docosahexaenoic acids (DHA, 22:6n-3), which are essential nutrients for most carnivorous species, make fish oil an especially valuable ingredient for aqua feed manufacturing (Turchini *et al.* 2009). Consequently, aquaculture consumes most of the fish oil globally available (Tacon & Metian 2015). Growing demand in the context of a limited supply, as well as other factors, has caused the price of fish oil to increase. From 2010 to 2015 the price of fish oil increased from US\$ US\$1,000.00/million ton to US\$2,500.00/million ton (FAO 2015). Increasing cost has encouraged the aqua feed industry to look for alternative lipid sources to spare or replace this expensive, but otherwise fine ingredient (FAO 2015). Vegetable- and terrestrial animal-origin lipids contain little-to-none of the physiologically important LC-PUFAs. Instead, these lipids are rich in other fatty acids: vegetable oils are commonly rich in physiologically inactive C₁₈ polyunsaturated fatty acids (C₁₈ PUFAs; mainly 18:2n-6 and, in few oil types, 18:3n-3), whereas terrestrial animal lipids are rich in saturated fatty acids (SFAs, mainly 16:0) and monounsaturated fatty acids (MUFAs, mainly 18:1n-9) (Turchini *et al.* 2010). Assuming the essential fatty acid requirements of fish are met, fish performance is normally unaffected by dietary lipid source (Turchini *et al.* 2009). However, fish survival rates, growth, and production performance can be impaired as a result fish oil sparing when the diet does not provide adequate levels of essential fatty acids (Turchini *et al.* 2009). These negative effects are most likely to occur in carnivorous fish fed reduced or fish oil-free feeds, due to these species' dietary requirements for LC-PUFAs. In fact, complete fish oil replacement in carnivorous fish without growth impairment is usually only reported for feeds that have been amended with LC-PUFAs supplements or contain considerable residual marine-origin lipid from dietary fish meal. In addition to effects on fish performance, dietary inclusion of alternative lipids also alters the fatty acid profile of fish,

altering the composition and product quality of the edible tissues. It has been extensively reported that the fillet fatty acid profile and associated nutritional value typically reflect the composition of the dietary lipid (Turchini *et al.* 2009). Accordingly, further research on alternative sources of LC-PUFAs, and alternative lipid sources that maintain performance and tissue fatty acid composition are needed in aquaculture nutrition, particularly for carnivorous fish.

By understanding fatty acid essentiality of intended fish species, fish nutritionists will be able to further address the fish oil bottleneck in aquafeed manufacturing. However, determining fatty acids requirements is complex because they commonly vary among species according to trophic level and the environment in which they evolved, as well as within species according to the developmental stage and physiological condition (Sargent *et al.* 2002). Additionally, other external and internal factors, including basal dietary composition, feeding strategy, and rearing conditions are also known to influence fatty acid requirements (Sargent *et al.* 2002). There is growing evidence, for example, that C₁₈ PUFAs—long considered essential nutrients for all vertebrates—are physiologically inactive and serve only as precursors to the LC-PUFAs which possess true biochemical or physiological functionality (Sargent *et al.* 2002). Preliminary results suggest that not all LC-PUFAs (ARA, EPA, and DHA) may be equally required (Trushenski *et al.* 2012). DHA seems to be important, whereas EPA appears to be expendable in some species; ARA, apparently required in comparatively minor amounts, has simply not been investigated to the same extent as the n-3 LC-PUFAs (Trushenski *et al.* 2012). Additionally, it is becoming clearer that the fatty acid composition of dietary alternative lipid influences fatty acid bioavailability and the degree of tissue fatty acid distortion (Turchini *et al.* 2009). More specifically, alternative lipids rich in SFAs and/or MUFAs appear to influence LC-PUFA bioavailability and, in some instances, seem to enhance fillet LC-PUFA deposition. Clearly, the questions of essential fatty acid requirements of fish, especially marine carnivores, are multifold, complex, and interrelated. In this context, the current paper sought to provide new knowledge regarding LC-PUFA requirements of marine carnivorous fish in the context of C₁₈ PUFA-rich, SFA-, and MUFA-rich alternative lipids. Further, it sought to determine if ARA, EPA, and DHA are equally important drivers of fatty acid

essentiality in representative species. Finally, it sought to determine the effects of dietary SFA, MUFA, and C₁₈ PUFA content on tissue deposition of LC-PUFAs in marine carnivorous fish.

Results and Discussion

Marine carnivorous fish exhibit physiological demand for ARA, EPA, and DHA, but may not require each of them to be provided intact in the diet. Trushenski *et al.* (2012) demonstrated that DHA is essential, whereas EPA is largely expendable or required only in trace amounts in Cobia (a marine carnivore, trophic level = 4.0) feeds. These results contradicted the previous reported joint requirements for EPA and DHA, but similar results were repeatedly demonstrated, adding further credibility to these findings. I found similar results regarding the relevance of DHA in White Seabass (trophic level = 4.3) fed fish-oil free feeds. My study with California Yellowtail (trophic level = 4.2) provided further supporting information, emphasizing the greater relevance of DHA versus EPA, as well as demonstrating the essentiality of ARA. Furthermore, DHA appears to be more influential than EPA in the context of promoting growth of Florida Pompano (trophic level = 3.5). Based on these findings and previous results, I suggest that DHA and ARA appear to be the primary drivers of fatty acid essentiality in marine carnivorous fish with trophic level equal or higher than 3.5; EPA, if required, appears necessary only in trace amounts.

These findings draw a clear contrast with the reported requirements of lower trophic level species and, to a lesser extent, the requirements previously reported for marine carnivores. With respect to omnivorous and herbivorous species, it has been reported that they require 18:2n-6 and/or 18:3n-3 as essential fatty acids (NRC 2011). For example, Tilapia spp. require 18:2n-6 between 0.5-1.0% of the diet, Channel Catfish *Ictalurus punctatus* require 18:3n-3 between 1.0-2.0% of dry diet, and Carp spp. require both C₁₈ precursors between 0.5-1.0% of the diet (NRC 2011). For marine carnivores it has been highlighted the importance of n-3 LC-PUFAs (i.e. EPA + DHA) but not of n-6 LC-PUFAs (i.e. ARA) to fatty acid essentiality (NRC 2011). The reported EPA + DHA requirement of representative marine carnivorous species, including Cobia, *Seriola* spp., Turbot

Scophthalmus maximus, Grouper *Epinephelus* spp., Red Drum *Sciaenops ocellatus*, Red Sea Bream *Pagellus bogaraveo* and Gilthead Sea Bream *Sparus aurata* consist in a range between 0.5-3.9% of the the diet (NRC, 2011; Trushenski *et al.* 2012).

Dietary DHA/EPA ratio has been used to investigate n-3 LC-PUFAs in several marine species. This approach is seemingly based on the biosynthetic relationship between these two fatty acids and emphasizes the importance of the relative proportions of these two nutrients in the diet. However, DHA and EPA have distinct physiological roles and different degrees of importance in terms of fatty acid essentiality, undermining the relevance of examining these two nutrients in proportion to one another. Thus, perhaps understandably, it has been reported that DHA/EPA ratio had little-to-no effect on juvenile Cobia performance (Trushenski *et al.* 2012). Likewise, my results with juvenile California Yellowtail and Florida Pompano provide further evidence that DHA/EPA ratio is not a critical benchmark of proper feed formulation. In the California Yellowtail study, fish oil-free feeds supplemented with EPA and all LC-PUFAs (ARA, EPA, and DHA) at the 100% level had equivalent DHA/EPA ratios of 1.0. However, fish fed the former feed exhibited reduced growth performance; whereas those fed the latter feed exhibited improved performance. In the Florida Pompano trial, dietary treatments had DHA/EPA ratios ranging from 0.4 – 2.5, but none of these yielded significantly different fish performance. Dietary ratios of DHA to EPA seem substantially less important than the absolute dietary concentrations of both nutrients, at least in the context of fish with limited ability to bioconvert EPA to DHA. Further research regarding the subject of DHA/EPA ratios may be warranted, but the collective results of this dissertation suggest that a focus on absolute, independent requirements for these nutrients is likely to be a more successful strategy to formulate marine finfish feeds and use fish oil as judiciously as possible.

Requirements for LC-PUFAs seem to be more flexible than previously presumed. Specifically, it is increasingly apparent that such requirements being influenced by dietary fatty acid composition and the effect of overall dietary composition on the availability of LC-PUFAs. The present results demonstrated that fish oil sparing with C₁₈ PUFA-rich lipids is largely constrained by dietary availability of LC-PUFAs (mainly DHA and ARA).

In California Yellowtail, adequate dietary DHA and ARA supplementation in soybean oil-based feeds (high C₁₈ PUFA content) was necessary to completely replace fish oil without impairing production performance and reducing LC-PUFA content of fish tissues. Such supplements were also necessary in White Seabass feeds containing C₁₈ PUFA-rich soybean oil, but not those containing SFA-rich hydrogenated soybean oil. It was previously reported that alternative SFA-rich lipids are advantageous in respect of meeting LC-PUFA requirements of marine carnivorous fish fed reduced or fish oil-free feeds. These authors demonstrated that LC-PUFAs requirements of White Seabass were effectively reduced in the context of SFA-rich feeds. In my study with the same species, it was demonstrated that the level of DHA needed to satisfy the dietary requirement of White Seabass was lower in the context of SFA-rich feeds compared to C₁₈ PUFA-rich feeds. Similarly, I demonstrated that n-3 LC-PUFAs were more bioavailable and the presumptive LC-PUFAs requirements of Florida Pompano for EPA and/or DHA were reduced in the context of SFA- and MUFA-rich feeds. Recent research with California Yellowtail and Cobia fed SFA- and/or MUFA-rich feeds provide further evidence in support of the hypothesis that dietary LC-PUFAs are more bioavailable in the context of SFA- and/or MUFA-rich lipids, thus, reducing the requirements for these nutrients and allowing the fish's physiological demand to be met with dietary levels below the minimum levels typically recommended.

Similar to the effects of dietary fatty acid composition on LC-PUFA availability, it is becoming more evident that dietary fatty acid also influences tissue deposition of LC-PUFAs. In most cases, C₁₈ PUFA-rich feeds appear to reduce the availability and deposition of LC-PUFAs in tissues. Conversely, SFA-, and MUFA-rich feeds exhibit a less overt effect on tissue fatty acid composition and attenuate the loss of beneficial LC-PUFAs typically associated with fish oil sparing. I observed this so-called LC-PUFA sparing effect, in my research with White Seabass and Florida Pompano. Although the same magnitude of SFA- and MUFA-induced sparing of LC-PUFA is not observed in all circumstances and seems to vary according to fish species (e.g., White Seabass were able to maintain tissue fatty acid profile equivalent to control, but Florida Pompano were not able to maintain tissue profile), diets rich in SFAs and/or MUFAs still outperformed the other

experimental diets (rich in C₁₈ PUFAs) in terms of reducing diet-related losses of fillet LC-PUFA levels and preserving the overall fatty acid profile.

In recent years, n-3 LC-PUFAs have been highlighted as human health-promoting fatty acids mainly due to the anti-inflammatory actions of the signaling molecules (i.e., eicosanoids) derived from EPA and the various physiological and physical attributes of DHA. The value of n-3 fatty acids is most often described in comparison to the less beneficial, pro-inflammatory n-6 fatty acids. The Western diet is widely considered to contain far too much n-6 fatty acid content and too little n-3 fatty acid content. Consumers are urged to increase their consumption of n-3 fatty acid-rich foods (specifically seafood) in an attempt to balance out consumption of foods with high n-6 fatty acid levels. Thus, to maintain product quality and value, it is important that farmed seafood be as good a source of valuable n-3 fatty acids as wild fish, while containing as little n-6 fatty acid content as possible. In this context, SFA- and MUFA-rich alternative lipids offer dual advantage over C₁₈ PUFA-rich lipids: SFA- and MUFA-rich lipids minimize the loss of n-3 LC-PUFAs from farmed fish fillets and contain few n-6 fatty acids that would otherwise become enriched in the fillets. Although marine finfish accept a variety of alternative lipids, those rich in SFAs and MUFAs seems advantageous for feed formulations in terms of limiting tissue fatty acid profile distortion, enhancing LC-PUFA bioavailability, and in some cases, reducing LC-PUFAs requirements.

In terms of tissue total lipid content, it appears that effect of dietary lipid source on tissue lipid content may be species-specific and a function of the fatty acid composition of alternative lipids used. For example, although no statistically significant differences in total lipid content of fillet and liver tissues were observed in Florida Pompano, numeric differences appeared to suggest somewhat elevated lipid content in those tissues of fish fed the beef tallow-based feeds (low C₁₈ PUFA, high SFA/MUFA content), and reduced lipid content in tissues of those fed SFA-rich soybean oil (low C₁₈ PUFA/MUFA, high SFA content) or poultry fat (similar C₁₈ PUFA/SFA/MUFA content). Further, total lipid content of fillet and liver tissues of White Seabass was significantly reduced in fish fed SFA-rich soybean oil-based feeds (low C₁₈ PUFA/MUFA, high SFA content) compared to those fed fish oil (high LC-PUFA content) or C₁₈ PUFA-rich soybean oil-based feeds (high C₁₈

PUFA, low SFA/MUFA content). The value of farmed fish fillets is based on both fatty acid profile and total lipid content, thus further research to investigate the effects of SFA- and MUFA-rich feeds on tissue lipid content is warranted.

Conclusion

In summary, the present results provide further insights regarding fatty acid essentiality in marine carnivorous finfish, fatty acid composition of the alternative lipid source, degree of tissue fatty acid profile distortion, and also highlight the importance of assessing and providing LC-PUFAs on the basis of individual fatty acids. Not all LC-PUFAs are required in marine carnivorous fish feeds, with DHA and ARA being the primary drivers of fatty acid essentiality, whereas EPA is likely required in minor amounts. Further, DHA/EPA ratio has little-to-no effect on fish performance. The fatty acid composition of alternative lipid sources influences bioavailability of and requirements for LC-PUFAs. The requirements themselves seem to be more flexible than previously presumed. Finally, alternative lipids rich in SFAs and/or MUFAs are likely advantageous ingredients in aqua feeds in the context of preserving tissue fatty acid profile, and enhancing bioavailability of LC-PUFAs.

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