Thoughts for the future of aquaculture nutrition: realigning perspectives to reflect contemporary issues related to judicious use of marine resources in aquafeeds

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Running title: Shifting approaches in aquaculture nutrition

Abstract

In recent decades, aquaculture nutrition research has made major strides in identifying alternatives to the use of traditional marine-origin resources. Feed manufacturers worldwide have used this information to replace increasing amounts of fish meal and fish oil in aquafeeds. However, reliance on marine resources remains an ongoing constraint, and the progress yielded by continued monodimensional research into alternative raw materials is becoming increasingly marginal. Feed formulation is not an exercise in identifying “substitutes” or “alternatives”, but a process of identifying different combinations of “complementary” raw materials—including fish meal and oil and others—that collectively meet established nutrient requirements and other criteria for the aquafeed in question. Nutrient-based formulation is the day-to-day reality of formulating industrially compounded aquafeeds, but this approach is less formally and explicitly addressed in aquaculture research and training programs. Here, we (re)introduce these topics and explore the reasons that marine-origin ingredients have long been considered the ‘gold standards’ of aquafeed formulation. We highlight a number of ways in which this approach is inaccurate and constrains innovation before delving into the need to assess raw materials based on their influence on aquafeed manufacturing techniques. We conclude with brief commentary regarding the future funding and research landscape. Incremental progress may continue through the accumulation of small insights, but a more holistic research strategy—aligned with industry needs and focused on nutrient composition and ingredient complementarity—is what will spur future advancement in the aquaculture nutrition domain.

Keywords:

Aquafeed; Fish Nutrition; Fish oil; Fish meal; Research and Development;

Introduction

For many decades, fish nutritionists have endeavored to develop aquaculture feed (aquafeed) formulations that support or enhance growth of cultured fish while controlling costs. Much of this effort has been focused on reducing reliance on limited marine resources. Whereas cultivation of herbivorous and omnivorous species has readily transitioned to feeds containing little-to-no fish meal or oil, such formulations have been more difficult to implement in feeding of carnivorous fish and crustaceans. Despite the various challenges, these efforts have been successful in a broad sense. Fish meal and oil inclusion rates have dropped steadily over the past 20 years (Tacon et al., 2011; Tacon and Metian 2015), and feed prices—while increasing—are not as volatile or high as they would be if the old formulations were sold today. Numerous researchers working largely independently in academia, public agencies, and the private sector have collectively made great strides in addressing the many constraints associated with optimal feeding in aquaculture. Nutritionists, including the authors, celebrate this success. Yet we may wonder what might have be achieved in aquaculture—or what is still possible—with greater emphasis on cohesive, collaborative, long-term partnerships between the public and private sectors, akin to the National Poultry Improvement Plan and associated activities that revolutionized poultry production in the mid-20th century (Boyd 2001).

One might also consider whether there are ways to better leverage limited research and development (R&D) investments to yield the maximum amount of applicable information. Incremental progress can continue through the accumulation of small successes, but transformational change in fish nutrition and the aquaculture industry may require an intentional realignment in approach. Here we (re)introduce a number of fundamental principles in fish meal/oil sparing and their continuing relevance in terms of addressing contemporary issues in aquaculture nutrition. None of these principles are likely to be ‘new’ to anyone who has spent considerable time working in our field—again, we consider them fundamental to the discipline. Perhaps we are sometimes too close to the subject to see it fully; perhaps these fundamentals are sometimes forgotten in the haste to secure funding or the churn of instruction and student mentoring. We also offer a brief commentary on the influence of feed manufacturing techniques, traditional funding mechanisms for aquaculture research, and emerging considerations that are reshaping the ways in which feeds and ingredients are evaluated. Questions of bioavailability, experimental design, statistical analysis, and reporting standards are, of course, intrinsic to any discussion of nutrition research. Rather than belabor those matters here, we refer readers to the well-articulated arguments of others (Shearer 2000; Barrows et al., 2008; Bureau 2011; Salze et al., 2011).

Nutrient-based aquafeed formulation

Modern compounded aquafeeds are a sophisticated, engineered mix of ingredients (raw materials) used for their nutritional and physical properties. These include commodity meals, oils, and concentrates intended to satisfy demand for macronutrients and premixes and specialty products included as sources of minerals, vitamins, pigments, binding agents, etc. The nutritionist's task is to identify a mixture of ingredients that satisfy the intended species' dietary requirements and tolerances and can be manufactured to the desired pellet specifications. As discussed below, fish meal and oil can greatly simplify formulation because they possess so many uniquely desirable properties. That said, fish meal and oil are not requisite ingredients in any aquafeed, and feed formulation is not an exercise in identifying "needed levels" of any specific ingredients, "substitutes", or "alternatives". Rather, formulation is the process of identifying different combinations of "complementary" raw materials—including fish meal and oil and others—that collectively meet established criteria for the aquafeed in question.

Several key datasets are needed to support nutrient-based formulation. Complete compositional profiles are essential, but the most informative raw material 'dossiers' also include digestibility, palatability, utilization, and functionality data in at least one representative cultured species. Ideally, these datasets are generated using more than a single raw material batch or source so that product variability is also captured. Such information takes time and resources to generate, but the ultimate value of a prospective raw material cannot be accurately judged without it.

As most experienced aquaculture nutritionists are well aware, nutrient-based formulation is the day-to-day reality of formulating industrially compounded aquafeeds. That said, the nutrient-based approach is less formally and explicitly addressed in aquaculture research and training programs. We encourage students and early-career aquaculture nutritionists to be particularly mindful of the nuanced difference between the search for fish meal/oil alternatives and the development of more broadly applicable informative datasets that facilitate incorporation of novel ingredients or optimize use of existing ingredients in aquafeeds. Similarly, we advise researchers working in the raw materials sector to recognize their products aren't solely judged in terms of their similarity to marine-derived ingredients, but also how they compare to and complement other raw materials.

Fish meal and fish oil: the 'gold standards' in aquafeed formulation

Fish meal (hereafter abbreviated as FM; a dry, high-protein powder derived from the rendering of whole fish, frames, or offal) and fish oil (hereafter abbreviated as FO, an oil extracted during the rendering of fish meal, typically rich in long chain polyunsaturated fatty acids [LC-PUFAs] of the omega-3 [n-3] series) are principally derived directly or indirectly (e.g., from seafood processing wastes or discards) from capture fisheries. Both ingredients have long been used in various types of animal feeds, but have proven uniquely valuable in aquafeed formulation (Gatlin et al., 2007; Hardy 2010; Tacon and Metian 2008; Turchini et al., 2009).

FM and FO were originally used because they were, at the time, inexpensive and palatable sources of protein and lipid. Today, they are used most often because they are the most economical means of formulating nutrient-dense feeds containing nutrients not usually found in abundance outside of the marine environment. FM contains a considerable amount of highly digestible, well-balanced protein matching the amino acid requirements of aquatic livestock, an oil fraction rich in phospholipids and LC-PUFAs, and a purported “unknown growth factor” (most likely a cocktail of naturally-occurring amines and steroids; Hardy, 2010). FM is also highly palatable to cultured species, contains no antinutritional factors if properly produced and stored, and has limited carbohydrate and fiber content (Gatlin et al., 2007; Glencross et al., 2007; Hardy 2010). FO is a triglyceride-rich oil with a unique fatty acid composition, typically comprising roughly equal amounts of saturated fatty acids (SFAs), monounsaturated fatty acids (MUFAs), and LC-PUFAs, particularly those in the n-3 series (Tocher 2015; Turchini et al., 2009). Because of their distinctive composition and other attributes, few if any raw materials match the feeding value of FM and FO in aquafeeds.

Despite the utility of FM and FO in aquafeed formulation, the incorporation of wild-caught fish in aquafeeds has attracted considerable criticism from scientists and the public, consumers and markets (Naylor et al., 2000; Cao et al., 2013; Jones et al., 2015). These criticisms are largely based on the seemingly illogical use of one type of fish to produce another. The accusation that the aquaculture industry consumes more fish (in the form of FM and FO) than it produces is incorrect (Byelashov and Griffin 2014) and nutritionists had been addressing the issue of over-reliance on marine-origin raw materials well before publication of the article that triggered the contemporary debate (Kaushik and Troell 2010). Nonetheless, use of FM and FO in aquafeeds continues to be a source of concern to many, and growing demand for FM and FO as raw materials has been identified as a possible contributor to over-exploitation of capture fisheries and a global fisheries crisis (Naylor et al., 2000, 2009).

In reality, past claims that increasing demand from the aquafeed sector would result in greater exploitation of reduction fisheries have not borne out: global production of FM and FO has remained fundamentally static at about 5.5 and 1 million tons per year, respectively, over the last 30 years (FAO 2015). Reduction fisheries are some of the most carefully and aggressively managed in the world and may actually support modest growth in the future despite continued growth of the aquaculture industry (FAO 2014). What's more, by 2022, half of the FM and FO that is used is expected to come from improved capture and processing of seafood waste, and not purpose-driven reduction fisheries (FAO 2014). Nonetheless, use of FM and FO in aquafeeds is considered a 'black mark' in terms of ecological sustainability assessments and certifications. Although experts quickly recognized early applications of the "fish in, fish out" concept (Tacon and Metian, 2008) as deceptive and fundamentally flawed (Jackson 2009; Kaushik and Troell 2010), the simplicity of 'FIFO' scoring is appealing to lay audiences and FIFO-based criticism of aquaculture remains pervasive in the blogosphere and op-ed journalism (Byelashov and Griffin 2014). In response, fish farmers and feed producers are increasingly using reduced FM and FO feed formulations for marketing and public relations purposes. The unfortunate consequence of this strategy is that it reinforces a misinformed public perception. The 'feeding fish to fish' quandary is further complicated by concern over the socioeconomic prudence of transforming low-cost, potentially edible fish into highly priced seafood products intended for premium food markets (Tacon and Metian 2013, 2015).

Though the environmental and socio-political aspects are important parts of the debate over FM and FO use in aquafeeds, the most significant factor influencing FM and FO usage patterns is the rising cost of these raw materials. Strong and growing demand for FM and FO, coupled with a relatively static supply and consistent growth in intensive aquaculture, have resulted in variable, but generally increasing prices (FAO 2014). There is considerable economic incentive to reduce utilization and dependence on FM and FO, and the combination of these and other incentives related to notions of sustainability, marketing, and consumers' expectations is a powerful one. After examining various factors related to the role of seafood in maintaining global food security through to 2050, Bene et al. (2015) argued that fisheries and aquaculture will continue to contribute positively to global food security, but only if some conditions are met, including reductions in FM and FO dependency.

Moving beyond the gold standards

The attributes of FM and FO make them immensely valuable feed resources, but they are not required, per se, in any aquafeed. Moreover, recent research has revealed that FM and FO are not the 'be-all, end-all' of raw materials for the aquafeed sector. Prior to the discovery of the importance of taurine in nutrition of marine carnivorous finfish (reviewed by Salze and Davis 2015), replacing FM with plant proteins seemed hopeless. Once this key constraint was identified, FM sparing was no longer an impossibility for these species and, in some cases, growth on reduced FM feeds has surpassed that associated with traditional formulations. Similarly, some combinations of lipids may be even better than FO in terms of n-3 LC-PUFA bioavailability and efficiency in different finfish species. Dubbed the "omega-3 sparing effect", lipid sources rich in SFAs and/or MUFAs appear to improve utilization of n-3 LC-PUFAs and, in effect, reduce dietary requirements for these nutrients (Rombenso et al., 2015; Bowzer et al., 2016; Emery et al., 2016). Likewise, providing crustaceans with the correct balance of n-3 and n-6 C_{18} PUFA, eicosapentaenoic acid (EPA, 20:5n-3) and docosahexaenoic acid (DHA, 22:6n-3), reduces fatty acid requirements, improves utilization of n-3 LC-PUFA, and can yield growth beyond that normally achieved when FO is the primary or only dietary lipid source (Glencross et al., 2002a, 2002b).

Despite these promising findings, the steady decline in FM and FO inclusion rates (Tacon and Metian 2008), and more than 60 years of other landmark achievements in aquaculture nutrition and aquafeed manufacturing (see Halver 1957; Gatlin et al., 2007; Glencross et al., 2007; Turchini et al., 2009; Hardy, 2010; Tocher 2015; Jobling 2016), the reality is that feeds containing little or no marine inputs do not routinely yield the same growth performance as traditional feeds in carnivorous species. Of those high-performing FM/FO-free formulations, not all are considered economically viable as they rely on specialized raw materials or costly supplements to replace the nutrients found in marine-origin resources and ensure feed attractability/palatability. Given that most of the 'low hanging fruit' in FM/FO sparing has already been picked, how can nutritionists and feed manufacturers continue to drive down the use of marine-derived resources and still produce feeds that are economical and yield acceptable growth?

To answer this question, it is instructive to examine how we have gotten to where we are at present. Although some researchers have investigated simultaneous sparing of FM and FO, most have focused exclusively on FM replacement/alternative protein sources <u>or</u> FO replacement/alternative lipid sources. Even though the protein and lipid 'divisions' of aquaculture nutrition have, generally speaking, worked independently from each other (likely because of the different knowledge, skills, and analytical approaches involved in these two fields of study), both shared the same conceptual and experimental approach. Nutritionists have intensively sought alternatives to FM and FO, testing various raw materials as direct substitutes to the marine-origin resources and using FM/FO-feeds as gold standards for the purposes of comparison. Nutritionists have been prolific in their use of approach: a search of the existing scientific literature using the search terms "alternative AND aquafeeds" reveals 7,390 articles/documents dealing with alternative protein and/or alternative lipid sources in aquaculture feeds; using the search terms "alternative AND aquaculture AND nutrition" returns more than 80,300 results (from Google Scholar database, retrieved on 9 January

2018). It is almost impossible to summarize this vast scientific literature; instead, in Table 1, a succinct summary of reviews dealing with different aspects of FM and/or FO replacement in aquafeeds is provided.

Although much of this work lacked the nutrient-based approach discussed herein, testing a wide range of potential alternatives has greatly expanded the portfolio of possible aquafeed ingredients and allowed FM/FO sparing to progress to its current place. That said, one could argue that this approach has reached (or will soon reach) the point of diminishing returns. Most raw materials that could feasibly serve as protein or lipid sources in aquafeeds have now been tested in at least one, if not more cultured aquatic species. The search for alternatives yielded substantial insight when so many raw materials had yet to be evaluated in aquafeeds. As the number of truly novel resources dwindles, testing raw materials as direct substitutes for FM/FO is less likely to yield advances beyond marginal, incremental progress. The staggering diversity of species, rearing systems, and culture conditions involved in aquaculture will always strain the resources available for R&D and force researchers to thinly spread investments and effort across a broad array of data gaps. Instead of 'doubling down' on the search for alternative raw materials, limited R&D resources may yield greater dividends if redirected to research questions more likely to 'move the needle'. New raw materials will periodically emerge and should be assessed, but focusing on alternative raw materials as direct substitutes for FM and FO is perhaps no longer the most strategic approach.

In some ways, direct comparison between various protein and lipid sources and the marine-origin gold standards FM and FO has always been flawed. Other than the marine-origin raw materials themselves, no single feedstuff has the precise composition, nutrient availability, and other characteristics of FM or FO. For example, some of the nutrients present in FM are also present in soybean meal, but the nutritional characteristics of these raw materials are not equivalent. Rather than seeking alternatives that might directly replace FM or FO, researchers are much more likely to find greater success in identifying essential or beneficial attributes of aquafeeds and developing complementary raw materials accordingly. The concept of raw material complementation is not new. Rather, it is central to human evolution and history: the traditional food habits of many cultures with limited/no animal food consumption regularly pair the nutrients found in legumes and cereals to achieve nutritional balance that reflects nutrient requirements and energy demand (Young and Pellett, 1994). Evaluating raw materials in terms of their ability to complement rather than replace other raw materials is not just a semantic distinction, but a realignment that changes how the problem is understood, how potential solutions are conceived, and how both are addressed through research intended to help aquaculture use marine-origin resources more efficiently and judiciously. By expanding our thinking beyond alternatives and substitution values to include the concept of complementarity of raw materials, we are shifting our focus from ingredients to nutrients and making room for more promising research directions:

- What nutrients are truly essential vs. nonessential, and how do we resolve questions of whether a nutrient is conditionally essential or merely beneficial?
- How does modified consumption of essential and nonessential nutrients affect the performance of cultured fish and shellfish?
- How can different energy sources be used to satisfy independent demands for bioenergetic 'fuel' vs. essential nutrients?
- How do different raw materials complement each other and how can their properties be leveraged to maximize the value of limited FM/FO inclusion?
- How can the attributes of raw materials (including compositional and physical characteristics) be used strategically, processed and/or blended, to optimize nutrient availability, utilization, palatability, etc., to satisfy nutrient requirements and optimize performance?

- How do the physical and nutritional qualities of raw materials affect feed manufacturing and pellet quality?
- What are the tolerances for nutrient density and variation in raw materials and do trade-offs between product refinement and processing costs offer opportunity for cost savings?
- How can innovation in feed and husbandry (e.g., feed management, breeding) be integrated as nutritional strategies better suited to resolve modern challenges in aquaculture?

Refocusing on nutrients and the way ingredients can complement each other will likely open numerous and as-yet untapped possibilities for improving the next generation of aquafeeds. Those who have adopted this approach have already proven the merits of doing so, as described in the sections below.

Lessons learned from nutrient-based research in FM sparing

Typically, FM replacement/alternative protein studies have primarily focused on protein digestibility and amino acid composition, particularly essential amino acid (EAA) content. However, a recent and important review on amino acid nutrition in animals (Wu et al., 2014) highlights the limitations of focusing only on EAA and the importance of considering other aspects of protein sources. Nutritionally nonessential amino acids (NEAA) and conditionally essential amino acids (CEAA) are now known to contribute significantly to the health, growth and overall performance of cultured animals. All dietary amino acids, whether considered EAA, NEAA or CEAA have physiological importance, serving not only as building blocks for protein synthesis, but as precursors to various metabolites and as factors contributing to the regulation of gene expression, cell signaling, and overall metabolism (Wu et al., 2014). Similarly, in their review of recent developments in amino acid nutrition of fish, Li et al. (2009) concluded that continuing advances in amino acid nutrition technologies, including EAA, NEAA, and CEAA, will play a defining role in shaping the viability and sustainability of aquafeed formulation and manufacturing. The need to take a broader view of aquaculture nutrition and expand our focus on essential nutrients was recently summarized by one of the field's pioneering scientists with the following elegant, if ironic statement: "non-essential dietary nutrients may in fact be so essential that the cell/body actually produces them" (Albert Tacon, pers. comm.).

Beyond questions of essentiality or nonessentiality, there is the matter of energetic costs: *de novo* synthesis of any nonessential nutrient uses energy that, in the context of aquaculture, would be better used to support somatic growth. As such, experts are beginning to question the assumption that NEAA are not relevant in terms of feed formulation or supporting maximal growth and optimal health (Kaushik and Seiliez 2010; Wu et al., 2014). Numerous discoveries that taurine, glutamine, glycine, proline and hydroxyproline promote growth and health of cultured aquatic species further underscore the importance of considering all dietary AA during feed formulation (Li et al., 2009). Table 2 provides a summary of some selected studies in which the substitution of dietary FM with different raw materials (in isolation and/or in combination) was tested in different commercially important aquaculture species. In most cases, it was shown that better results could be achieved by blends of raw materials, and/or balancing all AA, not just the first few limiting EAA. Clearly, all dietary AA are important to some extent (Wu 2014) and diets for aquatic animals must contain the proper balance of all AA (NEAA, CEAA and NEAA) to optimize growth, health and reproduction. This more holistic approach takes the "ideal protein concept" a step forward (Rollin et al., 2003). Balancing dietary levels of EAA, NEAA, and CEAA can be achieved through specific amino acid fortification or—better yet—by carefully blending raw materials according to their complementary characteristics and composition.

Glencross et al. (2007) commented on the importance and technical complexity of assessing interference in nutrient utilization resulting from incorporation of different raw materials. These authors also highlighted the existence of clear needs to improve the understanding, and the possible quantification, of the nutritional and functional interactions among raw materials. "As the adoption of alternatives to fish meal increases, there will probably be increasingly complex interactions among feed ingredients. The nature of such ingredient interactions may also have important implications for the study of ingredient functionality" (Glencross et al., 2007). Given these observations and other lessons learned from nutrient-based research, it is unsurprising that Gatlin et al. (2007) stated that a combination of (plant-derived) feed ingredients, not a single alternative ingredient, will be required to successfully replace FM.

Lessons learned from nutrient-based research in FO sparing

Regarding lipids, there are also a series of recent studies that illustrate the value of focusing on nutrients, rather than raw materials (Table 3). Though none of these trials explicitly invoked the concept of complementarity, they suggest there is considerable potential for this approach. Research evaluating how different lipid sources and fatty acids interact and how they can influence the efficiency of n-3 LC-PUFA utilization has proven more informative than studies in which FO is directly substituted with one alternative lipids or another. The discovery of the omega-3 sparing effect is a particularly compelling example (Trushenski 2009; Turchini et al. 2011; Codabaccus et al., 2012; Eroldogan et al., 2013; Salini et al., 2017).

Similar to EAA-driven research in FM replacement, much of the attention in FO replacement studies has focused on essential (or conditionally essential) fatty acids, particularly the n-3 LC-PUFA and n-6 LC-PUFAs found almost exclusively in marine-origin ingredients. DHA, EPA, and arachidonic acid (ARA, 20:4n-6) are inarguably important in the feeding of most if not all carnivorous fish (Bell and Sargent 2003; Tocher 2015), but, non-essential lipids also have nutritional importance. Turchini and

Francis (2009) suggested that the optimal dietary fatty acid composition for a growing fish would be a fatty acid composition that would minimize *in vivo* bio-conversion processes (to reduce unnecessary energetic costs), while simultaneously providing an efficient substrate for energy production. Their findings in Rainbow Trout support this 'ideal lipid concept', indicating that higher dietary inclusion of saturated fatty acids, monounsaturated fatty acids, and DHA improved performance, whereas excessive amounts of dietary polyunsaturated fatty acids, including EPA, were wasted (Turchini and Francis 2009).

Likewise, other nonessential lipids have been shown to play important nutritional roles. For example, cholesterol is well known as nonessential for teleosts; given its many physiological roles, cholesterol is highly regulated and biosynthesized efficiently if not provided in sufficient amounts with the diet. However, this happens at a significant metabolic cost (18 acetyl-CoA, 18 ATP, 16 NADPH and 4 O_2 molecules per molecule of cholesterol) and it has been suggested that aquafeeds not providing sufficient quantities of cholesterol (e.g., plant-based formulations) should be fortified with additional cholesterol to improve overall fish performance (Norambuena et al., 2013). Accordingly, cholesterol is garnering additional interest from fish nutritionists (Leaver et al., 2008; Yun et al., 2012; Zhu et al., 2014; Guerra-Olvera and Viana 2015).

Individual dietary fatty acids, essential or otherwise, may trigger differential responses in regulation of gene transcription (Coccia et al., 2014; Kjaer et al., 2016). For example, in Rainbow Trout, fatty acid catabolism for energy production appears to be stimulated by stearic acid (18:0), oleic acid (18:1n-9), α-linolenic acid (18:3n-3), ARA and DHA and inhibited by palmitic acid (16:0), linoleic acid (18:2n-6) and EPA (Coccia et al., 2014). Consequently, catabolic processes and, in turn, retention and tissue deposition of n-3 LC-PUFA can be modulated by manipulating intake of these fatty acids in a species-specific manner (Turchini et al. 2011; Eroldogan et al., 2013; Gause and Trushenski 2013;

Trushenski et al., 2013; Emery et al., 2014; Francis et al., 2014). This research has encouraged investigation of previously underappreciated lipid sources, such as rendered animal fats (Trushenski and Lochmann 2009), in aquafeed formulation.

Future research horizons in aquaculture nutrition

The challenge of FM/FO replacement is more likely to be addressed with a strategy, not a single raw material. These alternative strategies will comprise a combination of technological and nutritional strategies (e.g., dietary supplementation with amino acids, palatants/attractants, exogenous enzymes; pre- and probiotics; further development of mechanical and biological raw material processing technologies, feed manufacturing technologies; genetic modification of crops; [Gatlin et al., 2007]) and innovation in selective breeding (Quinton et al., 2007; Gjedrem et al., 2012; Overturf et al., 2013), rearing systems, and so forth. For example, replacement of FM was achieved in Tiger Shrimp *Penaeus mondon* not by using an alternative raw material, but an alternative nutritional strategy, via the utilization of microbial biomass, complementing terrestrial protein sources (Glencross et al., 2014). In this case, the growth-stimulating properties of the microbial biomass combined with the blending of land animal proteins with vegetable proteins to balance the amino acid profile allowed all of the dietary FM and FO to be replaced without affecting production performance; in some cases, shrimp performed better on the FM/FO-free feeds.

A variety of oils containing the health-promoting and highly sought n-3 LC-PUFA (namely, EPA and DHA) have proven able to directly and completely replace FO in aquafeeds. Some are also derived from wild-caught marine organisms, such as krill, amphipods, copepods and mesopelagic species (Olsen et al., 2011). Of course, the promise of these raw materials is constrained by the same factors that incentivize reduced reliance on FO, so it is perhaps best to think of these ingredients as supplements to the available FO supply. Other marine/aquatic derived alternative oils containing n-3 LC-PUFA are those derived from fisheries byproducts (i.e., seafood processing wastes or bycatch). Production of these raw materials is expanding (Rustad et al., 2011; Shepherd and Jackson 2013), and evaluations in aquafeeds show good potential (Fernandez Palacios et al., 1997; Turchini et al., 2003; Goncalves et al., 2012; Sevgili et al., 2012). These products also have the advantage of competitive pricing and, since they are mostly considered unacceptable or undesirable for direct human consumption, are not seen as aggravating the emerging issue of food vs. feed (Tacon and Metian 2009).

A series of novel non-marine oils containing n-3 LC-PUFA have been developed and are at different levels of commercialization and availability (Miller et al., 2011). The most promising of these novel n-3 LC-PUFA-containing oils are derived from microalgae/single-cell organisms (Miller et al. 2007; Ganuza et al., 2008; Hemaiswarya et al., 2011; Eryalcin et al., 2015; Sprague et al., 2015 ; Sarker et al., 2016) and genetically modified oilseed crops (Kitessa et al., 2014; Betancor et al., 2015, 2016). Although the overall content of n-3 LC-PUFA of these oils is comparable to or higher than that of FO, they typically contain more DHA and less EPA than traditional FO. These products are the focus of considerable, promising research (Vizcaino-Ochoa et al. 2010; Codabaccus et al., 2012; Trushenski et al. 2012; Betiku et al. 2016; Emery et al., 2016). These oils present a series of exciting opportunities for the sustainable expansion of the aquaculture sector, but also highlight a partial knowledge gap: the dearth of research addressing individual fatty acid requirements. Previous lipid nutrition research, relying primarily on traditional terrestrial and marine oils, assessed essential fatty acid requirements in terms of total n-3 or n-6 fatty acids. Now, evidence is mounting to suggest that the different n-3 LC-PUFA vary substantially in their nutritional value, n-6 LC-PUFA are also nutritionally important, and the functional differences between C_{18} PUFA and LC-PUFA have not been adequately communicated (Glencross and Smith 2001; Koven et al.; Bell and Sargent 2003; Van Anholt et al., 2004; Lund et al., 2007; Norambuena et al., 2015; Ding et al., 2018). Accordingly, a much greater effort into basic research to define individual requirements for key fatty acids—nutrients, rather than raw materials—and elucidate their specific roles in aquatic animal health and optimal performance is needed.

More than nutrients and ingredients: the influence and constraints of manufacturing techniques and sources of support for aquaculture nutrition research

The preceding sections have made the case for greater focus on nutrients and the interactions between them in the context of aquafeeds. This also means considering the manufacturing techniques as well, since it is well-established that raw material processing and feed manufacturing can greatly influence the nutrient composition, digestibility and availability, as well as the physical properties and utilization of feeds (Hilton et al, 1981; Gadient and Fenster 1994; Booth et al., 2000; Ljokjel et al., 2002, 2004; Sorensen et al. 2002; Cheng and Hardy 2003; Barrows et al., 2007; Morken et al. 2011; Sorensen 2012). For example, Glencross et al. (2011) observed that digestibility varied substantially when raw materials were processed into aquafeeds using extrusion or pellet-pressing. More specifically, protein digestibility was strongly influenced by manufacturing technique, mostly likely due to the protein-to-protein interactions that occur during extrusion processing. Regrettably, the topic of manufacturing technology is not as frequently addressed as raw material composition, nutrient digestibility, marine ingredient sparing, and so forth. Some of the documented effects of raw materials and diet processing on diet characteristics and fish performance is summarized in Table 4.

Unfortunately, relatively few research labs have access to extrusion equipment comparable to that used in the preparation of industrially compounded aquafeeds. Consequently, most of the research conducted and published in aquaculture nutrition may not be considered directly relevant by feed manufacturers. It is equally important to recognize that not all feed formulations can be effectively manufactured: not all combinations of raw materials can be effectively formed into a pellet with the desired physical characteristics, water stability, durability, or buoyancy profile required for any specific feed type. These factors may not be as evident or problematic in an experimental setting (e.g., defining the requirements for a specific nutrient) or in the manufacturing of steam-pelleted, sinking diets, but they are critical considerations for the commercial-scale manufacturing of extruded feeds. When testing new raw materials or formulations, nutrition researchers are encouraged to ask themselves or—better yet—ask extrusion scientists questions such as "Can this formulation actually be extruded?", "Can it be made to float or sink?", "Will it be durable enough to withstand shipping and on-farm distribution?", or "Will the feed extrusion process change the nutritional value of the raw materials?". Mindful of these needs, modern feed extrusion approaches for aquaculture have been adapted from other manufacturing sectors to accommodate some of these constraints, but they remain pertinent questions to consider (Sorensen 2012).

Regardless of whether research is conducted for the public good or for commercial gains, it requires financial support to be conducted. Extramural funding—provided by industry, government agencies, or other sources—drives innovation in all sectors, including aquaculture. Some nations have recognized aquaculture's potential and have provided research capacity, institutional support, enabling regulations, and various other incentives to encourage its development. In other countries, investment in aquaculture research, including fish nutrition, has been inconsistent and comparatively meager. Though there are a number of public entities that support aquaculture research, aquaculture investments in most countries are minor in comparison with investments in crop and terrestrial animal science or capture fisheries science (Jensen 2008). For example, the U.S. Department of Agriculture invested $294 million in sustainable agriculture research in 2014, but only $10 million of that was dedicated to aquaculture or seafood projects (DeLonge et al., 2016). The funding climate is increasingly competitive and long-term support for foundational science in aquaculture is absent in many contexts. As a result, fish nutritionists must be creative in their approach to identifying sources of funding and blending projects together to advance their research programs and our understanding of feeds and feeding in aquaculture. In aquaculture nutrition, it is quite common to work with commodity groups or specialty ingredient manufacturers to rigorously evaluate the value of their products in aquafeeds. While a welcome and important source of R&D funding for fish nutritionists, the interests of these funding sources can be somewhat narrow: soybean groups want to fund soybean work, animal byproduct groups want to fund byproduct work, etc. This is quite understandable, but drives the unifactorial, single raw material, direct FM or FO replacement approach to fish nutrition. Companies looking to develop markets for their ingredients might do better to entertain research proposals to develop more holistic datasets related to their product—including data on how well their product 'works' with others. For example, in the case of alternative proteins, it's just as important to know how a new raw material measures up against *other raw materials* as it does against fish meal. Further, it is very helpful to know whether a new raw material interacts positively or negatively with others, particularly when subjected to the physical processes of feed manufacturing. Of course, it is primarily the investigators' responsibility to propose and conduct research that is integrative. Researchers may receive funding to test one raw material from company "A", another from company "B", and so forth; their work may prove more fruitful if, when possible, they worked with both companies to evaluate their products in conjunction, in various combinations, and in line with the other recommendations set forth herein.

Nutritionists and feed manufacturers are also encouraged to consider other 'down-stream' consequences of their efforts to spare FM and FO. What effect do these formulations have on performance criteria besides growth and survival (Francis et al., 2001; Sitjà-Bobadilla et al., 2005; Desai et al., 2012)? How does the composition of the diet influence the quality and nutritional value of the edible tissues (Fry et al., 2016; Sprague et al., 2016)? How do consumers view the use of traditional vs. alternative ingredients (Mancuso et al.., 2016; Popoff et al., 2017, Shepherd et al., 2017), raw materials derived from GMOs (Lucht 2015), and so on? Nutritionists are understandably preoccupied with the nutritional aspects of feed formulation, but these other questions also merit their attention.

Conclusion

Commercial aquafeed manufacturers formulate aquafeeds based on key limiting nutrients using commercial formulation databases and advanced computer software. More or less, the overall R&D sector is already using a nutrient-based approach. That said, we suggest that greater emphasis on nutrients, including those not considered strictly nutritionally essential, is required to encourage further evolution of the industry and efficiently move aquaculture nutrition beyond the incremental advances achieved in recent years. Of course, nutrients are delivered via raw materials, which cannot be forgotten nor overlooked. Raw materials must be consistent and economical, available in sufficient quantities, possess the needed nutrients, be free of contaminants and other undesirable factors, and be able to withstand a range of processing constraints. While a focus on nutrients should be paramount in the evaluation of new raw materials, we cannot forget these other practicalities. We encourage researchers to investigate the effects of feed manufacturing on raw material suitability and, when possible, to test ingredients in a more integrative, holistic and multifactorial fashion. This will likely require a greater degree of collaboration, between all stakeholders and various specialists. It is our hope that by rethinking or becoming reacquainted with the nutrient-based approach to aquaculture nutrition science, we can spur further innovation within our field and the aquaculture industry and, ultimately, help transform the use of marine-origin resources in aquaculture.

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Table 1. A selection of some of the several available reviews dealing with different aspects of fish meal and/or fish oil replacement in aquafeeds. Within each category, references are sorted chronologically.

Title	Publication type	Reference
General nutrition, feed formulation and manufacturing reviews		
Utilization of conventional and unconventional protein sources in practical fish feeds	Book chapter	Tacon and Jackson 1985
Feed ingredient	Book chapter	Tacon and Akiyama 1997
Raw materials and additives used in fish foods	Book chapter	Métallier and Guillaume 1999
Recent developments in the essential fatty acid nutrition of fish	Journal article	Sargent et al., 1999
Diet formulation and manufacture	Book chapter	Hardy and Barrows 2002
Challenges and opportunities in finfish nutrition	Journal article	Trushenski et al., 2006
A review of processing of feed ingredients to enhance diet digestibility in finfish	Journal article	Drew et al., 2007
A feed is only as good as its ingredients - a review of ingredient evaluation strategies for aquaculture feeds	Journal article	Glencross et al., 2007
Exploring the nutritional demand for essential fatty acids by aquaculture species.	Journal article	Glencross 2009
Protein and amino acid nutrition and metabolism in fish: current knowledge and future needs	Journal article	Kaushik and Seiliez 2010
Fatty acid requirements in ontogeny of marine and freshwater fish	Journal article	Tocher 2010
Nutrient Requirements of Fish and Shrimp	Book	NRC 2011
Omega-3 long-chain polyunsaturated fatty acids and aquaculture in perspective	Journal article	Tocher 2015
Fish meal and oil sparing and alternative ingredient reviews		
Expanding the utilization of sustainable plant products in aquafeeds: a review	Journal article	Gatlin et al., 2007
n-3 oil sources for use in aquaculture - alternatives to the unsustainable harvest of wild fish	Journal article	Miller et al., 2008
Fish oil replacement in finfish nutrition	Journal article	Turchini et al., 2009
Fish Oil Replacement and Alternative Lipid Sources in Aquaculture Feeds	Book	Turchini et al., 2011
A meta-analysis of the effects of dietary marine oil replacement with vegetable oils on growth, feed conversion and muscle fatty acid composition of fish species	Journal article	Sales and Glencross 2011
Benefits of fish oil replacement by plant originated oils in compounded fish feeds, a review	Journal article	Nasopoulou and Zabetakis 2012
Having your omega-3 fatty acids and eating them too: strategies to ensure and improve the long-chain polyunsaturated fatty acid content of farm-raised fish	Book chapter	Trushenski and Bowzer 2013
Feed matters: satisfying the feed demand of aquaculture	Journal article	Tacon and Metian 2015
Fish nutrition research: past, present, and future	Journal article	Jobling 2016

Ingredient-oriented reviews		
Handbook on Ingredients for Aquaculture Feeds	Book	Hertrampf and Piedad-Pascual 2000
New development in aquatic feed ingredients, and potential of enzyme supplements	Conference proceedings	Hardy 2000
Antinutritional factors present in plant-derived alternate fish feed ingredients and their effects in fish	Journal article	Francis et al., 2001
Feeding lupins to fish: a review of the nutritional and biological value of lupins in aquaculture feeds	Technical report	Glencross 2001
Use of cottonseed meal in aquatic animal diets: a review	Journal article	Li and Robinson 2006
Alternative Protein Sources in Aquaculture Diets	Book	Lim et al., 2008
Potential, implications, and solutions regarding the use of rendered animal fats in aquafeeds	Journal article	Trushenski and Lochmann 2009
Important antinutrients in plant feedstuffs for aquaculture: an update on recent findings regarding responses in salmonids	Journal article	Krogdahl et al., 2010
A review of using canola/rapeseed meal in aquaculture feeding	Journal article	Enami 2011
Microalgae: a sustainable feed sources for aquaculture	Journal article	Hemaiswarya et al., 2011
Review on the use of insects in the diet of farmed fish: past and present	Journal article	Henry et al., 2015
The supply of fish oil to aquaculture: a role for transgenic oilseed crops?	Journal article	Usher et al., 2015
Species-oriented reviews		
The nutrition of prawns and shrimp in aquaculture - a review of recent research	Book chapter	Fox et al., 1994
Alternative dietary protein sources for farmed tilapia, *Oreochromis* spp.	Journal article	El-Sayed 1999
A review of the development and application of soybean-based diets for Pacific white shrimp *Litopenaeus vannamei*	Journal article	Sookying et al., 2013
Market-, utilization-, and sustainability- oriented reviews		
Feeding tomorrow's fish: keys for sustainability	Journal article	Tacon 1997
Nutrition and feeding for sustainable aquaculture development in the third millennium in: Aquaculture in the Third Millennium	Technical report	Hasan 2001
Fish meal: historical uses, production trends and future outlook for sustainable supplies	Book chapter	Hardy and Tacon 2002
Global overview on the use of fish meal and fish oil in industrially compounded aquafeeds: trends and future prospects	Journal article	Tacon and Metian 2008
Feeding aquaculture in an era of finite resources	Journal article	Naylor et al., 2009
Impact of rising feed ingredient prices on aquafeeds and aquaculture production	Technical report	Rana and Hasan 2009
Utilization of plant proteins in fish diets: effects of global demand and supplies of fish meal	Journal article	Hardy 2010
Demand and supply of feed ingredients for farmed fish and crustaceans	Technical report	Tacon et al., 2011
A limited supply of fish meal: impact on future increases in global aquaculture production	Journal article	Olsen and Hasan 2012
Global fish meal and fish-oil supply: inputs, outputs and markets	Journal article	Shepherd and Jackson 2013

Future availability of raw materials for salmon feeds and supply chain implications: the case of Scottish farmed salmon	Journal article	Shepherd et al., 2017

Table 2. Summary of some selected studies in which the substitution of fish meal with different raw materials, in isolation and/or in combination was tested, in the diet for different commercially important aquaculture species. Entries are sorted alphabetically by common name, finfish first and then crustaceans. Asterisks indicate values are expressed as a percent (%) of the designated reference/control used in each experiment.

Species	**Raw Materials**	**Experiment Constraints and Observations**	**Gain***	**Intake***	**FCR***	**Reference**
Atlantic Salmon *Salmo salar*	Soy concentrate (S), Poultry Meal (P), Corn Concentrate (C)	• Diets formulated to equivalent crude protein and energy and balanced for lysine, methionine and taurine. • Fish meal inclusion in plant protein (PP) diet constrained to 20%. • Diets fed to transgenic/non-transgenic and diploid/triploid fish. • Data presented is only for the non-transgenic diploid fish. • Replacement strategy (PP) fed fish sustained better performance compared to a fish meal reference (FM). • Growth was linked to a better feed intake and improved feed conversion associated with the PP diet.	FM: 100 PP: 113	FM: 100 PP: 107	FM: 100 PP: 95	Ganga et al., 2015
Atlantic Salmon	Wheat gluten, Corn Gluten, Soy Concentrate	• Diets were balanced for both crude protein and energy, as well as being balanced for most amino acids. • No fish meal, only fish solubles (FS and SW) and hydrolysates (SQ) included in any of the test diets. • Reference had 49% fish meal. • All alternative diets had poorer feed intake leading to poorer growth. • Feed conversion was unaffected by replacement.	R: 100 FS: 82 SW: 82 SQ: 87	R: 100 FS: 81 SW: 84 SQ: 86	R: 100 FS: 98 SW: 102 SQ: 99	Espe et al., 2006
Barramundi *Lates calcarifer*	Lupin kernel Meals (L), Wheat gluten (W), Poultry Meal (P), Canola Meal (C), Blend (B) and Fish meal (F) reference	• Diets formulated to same digestible protein (DP) and digestible energy (DE) basis and balanced for amino acids according to ideal protein concept. • Fish meal minimum inclusion constrained to 15%. • Both single replacement and multiple replacement strategies sustained performance equivalent to a fish meal reference and that growth was largely linked to feed intake variability. • In some cases, use of alternative raw materials stimulated enhanced feed intake.	F: 100 L: 151 W: 116 P: 128 C: 158 B: 113	F: 100 L: 125 W: 111 P: 126 C: 127 B: 112	F: 100 L: 83 W: 95 P: 89 C: 80 B: 101	Glencross et al., 2011

Barramundi	Poultry Meal, Soybean Meal	• Diets formulated to same DP and DE basis and balanced for amino acids according to ideal protein concept. • Fish meal inclusion constrained to 30%, 20%, 10% or 0%. • Feed conversion was consistent across the 30% to 0% inclusion of fish meal. • Variation in growth was in response to a decline clearly linked to changes in feed intake.	30%: 100 20%: 93 10%: 94 0%: 84	30%: 100 20%: 89 10%: 92 0%: 87	30%: 100 20%: 97 10%: 98 0%: 104	Glencross et al., 2016
European Seabass *Dicentrarchus labrax*	Blood Meal, Soy Concentrate, Rapeseed Meal, Corn Gluten, Wheat Gluten	• Diets formulated to same crude protein and lipid basis and balanced for amino acids. • Fish meal inclusion constrained to 58%, 20%, 10%, 5% or 0%. • Some treatments were varied with either 6% or 3% fish oil addition. All data presented is with the 6% fish oil. • A deterioration in performance was largely linked to a decline feed intake associated with the replacement strategy diet.	58%: 100 20%: 96 10%: 86 5%: 81 0%: 51	58%: 100 20%: 93 10%: 91 5%: 87 0%: 67	58%: 100 20%: 98 10%: 106 5%: 107 0%: 131	Torrecillas et al., 2017
European Seabass	Blood Meal, Soy Concentrate, Rapeseed Meal, Corn Gluten, Wheat Gluten	• Diets formulated to same crude protein and lipid basis and balanced for amino acids. • Fish meal inclusion constrained to 68% (F), 34% (W19) or 19% (W41, W+P, W+S). • Growth unaffected by treatment, but a deterioration in FCR linked to an increase in feed intake associated with the replacement strategy in some diets.	F: 100 W19: 99 W41: 95 W+P: 99 W+S: 99	F: 100 W19: 97 W41: 95 W+P: 102 W+S: 109	F: 100 W19: 98 W41: 100 W+P: 103 W+S: 110	Messina et al., 2013
Gilthead Seabream *Sparus aurata*	Corn Gluten Meal, Wheat Gluten, Pea Meal, Rapeseed Meal, Lupin Meal	• Diets formulated to same crude protein and lipid basis and balanced for amino acids according to ideal protein concept. • Fish meal inclusion constrained to 70%, 35%, 18% or 0%. • Growth decline with increasing FM replacement linked to a decline in feed intake associated with the replacement strategy diet used. • FCR improved with increasing FM replacement, linked to a decline in feed intake.	F: 100 P50: 93 P75: 87 P100: 73	F: 100 P50: 83 P75: 75 P100: 66	F: 100 P50: 89 P75: 86 P100: 90	Gomez-Requeni et al., 2004
Rainbow Trout *Oncorhynchus mykiss*	Lupin Meal, Faba Bean Meal, Pea Meal, Maize Gluten, Soy Meal, Colzapro, Meat Meal	• Diets formulated to same crude protein and energy basis and balanced for lysine and methionine only. • A blend of plant proteins used in each diet. • Fish meal varied from 54%, 40%, 20% to 0% (C0 to C100 respectively).	C0: 100 C33: 101 C66: 101 C100: 85	C0: 100 C33: 105 C66: 98 C100: 86	C0: 100 C33: 105 C66: 97 C100: 102	Gomes et al., 1995)

		• Performance unaffected by alternative diets except at 0% fish meal inclusion, where the poorer feed intake led to a reduced growth. • Feed conversion unaffected by fish meal replacement.				
Rainbow Trout	Peanut Meal (PM), Soybean Meal (SB), Soy Concentrate (SC), Soy Flour (SF) Blood Meal (BM)	• Diets formulated to same crude protein and energy basis and balanced for amino acids. • No fish meal included in any of the test diets, with the treatment protein being the predominant protein in each respective diet. • All alternative diets had poorer performance linked predominantly to lower feed intake leading to poorer feed conversion and growth.	CTL: 100 PM: 57 SB20: 67 SC1: 66 SC2: 57 SF: 86 SB40: 86 BM: 58	CTL: 100 PM: 78 SB20: 85 SC1: 82 SC2: 78 SF: 98 SB40: 100 BM: 77	CTL: 100 PM: 136 SB20: 127 SC1: 125 SC2: 137 SF: 115 SB40: 116 BM: 133	Adelizi et al., 1998
Hybrid Striped Bass *Morone chrysops* x *M. saxatilis*	Grain Distillers Dried Yeast (G), Corn Gluten Meal (C), Distilers Dried Grains with Solubles (D), Poultry By-Product Meal (P), Soybean Meal (S), Soy (SC) Concentrate, Soy Isolate (SI)	• Diets formulated with inclusion of a single "test" ingredient to the same crude protein and energy basis and balanced for methionine. • Fish meal kept constant (~10%) with inclusion of each of the single alternatives and compared to a reference with 30% fish meal. • Some significant effects noted on consumer preference relative to ingredient use.	FM: 100 G: 75 C: 88 D: 85 P: 106 S: 95	FM: 100 G: 86 C: 92 D: 100 P: 94 S: 97	FM: 100 G: 101 C: 99 D: 109 P: 91 S: 99	Trushenski and Gause 2013
Giant Tiger Prawn *Penaeus monodon*	Poultry Meal, Lupin kernel Meal, Microbial Biomass	• Diets formulated with 45% to 0% fish meal, but to same crude protein and energy basis and not balanced for amino acids. • Clear decline in performance associated with decreasing fish meal inclusion linked to poorer conversion with a higher feed intake. • Growth loss could be offset using a microbial biomass supplement. • Effects of different environmental systems also observed.	45%: 100 20%: 95 15%: 91 10%: 79 5%: 84 0%: 82	45%: 100 20%: 156 15%: 137 10%: 133 5%: 127 0%: 118	45%: 100 20%: 159 15%: 146 10%: 139 5%: 134 0%: 114	Glencross et al., 2014
Whiteleg Shrimp *Litopenaeus vannamei*	Poultry Meal, Soybean Meal, Corn Gluten	• Diets formulated to same crude protein and energy basis and not balanced for amino acids. • Trial conducted in outdoor tanks mimicking pond system environment. • Replacement of fish meal (9% to 0%) with combined alternatives had no impact on feed intake, feed conversion or growth.	9%: 100 6%: 101 3%: 102 0%: 94	9%: 100 6%: 100 3%: 100 0%: 100	9%: 100 6%: 99 3%: 98 0%: 106	Amaya et al. 2007)

Whiteleg Shrimp	Soybean Isolate, Corn Gluten	• Diets formulated to same crude protein and energy basis. • Trial conducted in a recirculating aquaculture system environment. • Replacement of fish meal (56% to 0%) with combined alternatives had no impact on feed intake, feed conversion, survival or growth. • Use of stable isotopes demonstrated differential contributions of the various raw materials	56%: 100 28%: 101 18%: 104 14%: 82 0%: 35	N/A	N/A	Gamboa-Delgado et al., 2013

Table 3. Summary of some selected studies in which the advantages of using blends of oils, evidences of omega-3 sparing effect of different dietary fatty acid classes, and the importance of individual lipid nutrients (essential and non-essential) have been reported. (Within each category, entries are sorted per species, alphabetically; finfish first, and then crustaceans).

Species	**Raw Materials / Individual nutrient**	**Experiment Constraints and Observations**	**Outcomes**	**Reference**
Lipid blends				
Atlantic Salmon *Salmo salar*	Fish oil (FO) Blend of vegetable oils (VO) (rapeseed 55%, palm 30% and linseed 15%)	• FO replaced at two levels (75 and 100%), extruded diets. • Over entire production cycle. • Output measured: fish performances, tissues' fatty acid composition, astaxanthin content, and final product sensorial qualities.	No statistically significant difference in performance, except for 100%VO outperforming control (FO) during seawater, winter period. Fatty acid composition of fish tissues modified and reflective of that of the diet. No effects on pigmentation. 100% VO had less rancid and marine characteristics and was preferred over flesh from the other dietary groups	Torstensen et al., 2005
Atlantic Salmon	Fish oil (FO) Rapeseed oil (RO) Linseed oil (LO)	• Isoenergetic and isoproteic extruded diets, fed over 50 weeks. • 9 experimental diets containing single oils or various blends of two vegetable oils at different inclusion, plus control (FO). • Output measured: fish performances, tissues' chemical and fatty acid composition.	Some differences in performance at 50 week being recorded, but likely due to constrains in feeding methodology. Fatty acid composition of fish tissues modified and reflective of that of the diet. Atlantic salmon can be raised on diets in which FO is replaced with different blends of vegetable oils for the entire seawater culture phase	Bell et al., 2003
European Seabass *Dicentrarchus labrax*	Fish oil (FO) Rapeseed oil (RO) Linseed oil (LO) Palm oil (PO)	• Isoenergetic and isoproteic extruded diets, fed to satiety. • Control (FO) and two experimental diet containing 60% of different blends of the three vegetable oils. • Output measured: fish performances, tissues' fatty acid composition, plasma prostaglandin, blood parameters (haematocrit, leucocytes erythrocytes), kidney macrophage activity, serum lysozyme activity, and tissue histology	Normal immune function can be more successfully achieved when dietary FO is replaced by a blend of VO (with physiologically balanced fatty acid composition), compared to using a single oil.	Mourente et al., 2007)

Gilthead Seabream *Sparus aurata* European Seabass	Fish oil (FO) Soybean oil (SO) Rapeseed oil (RO) Linseed oil (LO) Mixture (Mix) of SO, RO and LO	• Isoenergetic and isoproteic extruded diets, fed to satiety. • Control 100% FO. • Experimental diets 60% of FO replaced by one of the tested oil. • Output measured: fish performances, fatty acid composition and final product sensorial qualities.	No statistically significant difference in performance, but Mix resulted in numerical better values, even compared to FO. Fatty acid composition of fish tissues modified and reflective of that of the diet. No effects on smell, taste and texture of fish fillet, apart from stronger smell and taste recorded for fish fed SO.	Izquierdo et al., 2003
Giant Tiger Prawn *Penaeus monodon*	Fish oil (FO) Several different marine oils, vegetable oils and purified fatty acids.	• Several dietary treatments to assess various dietary fatty acid combinations • Output measured: prawn performances, tissues' fatty acid composition,	The correct balance of dietary fatty acids, particularly C18 PUFA of the n-3 and n-6 series, coupled with the optimal ratio between EPA and DHA, resulted in lower requirement, and more efficient utilisation, of n-3 LC-PUFA; Proper oil blend results also in improved growth performances compared to prawn fed with FO as the main dietary lipid source.	Glencross et al., 2002a, 2002b
Omega-3 sparing				
Atlantic Salmon	Fish oil (FO) Tuna oil (TO) Poultry oil (PoL) Rapeseed oil (RO)	• Isoenergetic and isoproteic diets, fed to satiety. • Control 100% FO, compared to different blends of the other oils • Output measured: fish performances and fatty acid composition.	A DHA:EPA ratio higher than that commonly occurring in FO, resulted in more efficient deposition of n−3 LC-PUFA. Blending FO with PoL increased the efficiency of n-3LC-PUFA retention/deposition compared to a diet based on FO only	Codabaccus et al., 2012
Barramundi *Lates calcarifer*	Fish oil (FO) Olive oil (OO) Palm oil (PO) Palm flake (PF)	• Isoenergetic and isoproteic diets, fed to satiety. • Control 100% FO, and two experimental diets, SFA rich and one MUFA rich blending the different oils. • Output measured: fish performances, fatty acid composition and apparent *in vivo* fatty acid metabolism.	Either dietary SFA or MUFA can influence the in vivo metabolism of fatty acids and the final fatty acid composition of the whole fish Dietary MUFA and SFA are both equally efficient at sparing n-3 LC-PUFA from an oxidative fate.	Salini et al., 2017
European Seabass	Fish oil (FO) Cottonseed oil (CSO) Canola oil (CO)	• Isoenergetic and isoproteic diets, fed to satiety. • Control 100% FO. Each oil tested in isolation at a 50/50 mix at 100% substitution. • Output measured: fatty acid composition and apparent *in vivo* fatty acid metabolism.	European sea bass was able to efficiently use n-6 PUFA for energy substrate, and this minimized the β-oxidation of n-3 LC-PUFA, and increased their deposition into body compartments.	Eroldogan et al., 2013

Murray Cod *Maccullochella peelii peelii*	Fish oil (FO) Linseed oil (LO) Olive oil (OO) Palm oil (PO) Sunflower oil (SFO)	• Isoenergetic and isoproteic diets, fed to satiety. • Control 100% FO. Each oil tested at 100% substitution. • Grow-out plus finishing on FO. • Output measured: fish performances, fatty acid composition and apparent *in vivo* fatty acid metabolism.	Not all alternative oils performed the same, and the actual overall fatty acid composition of the alternative oil used (i.e. SFA, MUFA, PUFA) had a remarkable effect on the final n-3 LC-PUFA content of the fish MUFA, and to a lesser extent SFA, showed an "omega-3 sparing effect", where their abundant availability in the diet decreased the catabolism of n-3 LC-PUFA and resulting in a greater flesh deposition rate.	Turchini et al., 2011
Hybrid Striped Bass *Morone chrysops* x *M. saxatilis*	Fish oil (FO) Coconut oil (CCO) Palm oil (PO)	• Isoenergetic and isoproteic diets, fed to satiety. • Control 100% FO, and CCO and PO either tested at 50% or 100% substitution of FO. • Output measured: fish performances and fatty acid composition.	Dietary inclusion of abundant levels of SFA appeared to improve the retention of n-3 LC-PUFA in the tissues of the fish.	Trushenski 2009
Individual (essential and non-essential) lipids				
Atlantic Salmon Rainbow Trout *Oncorhynchus mykiss*	Individual fatty acid	• Different studies, see references for details.	Individual dietary fatty acids trigger differential responses in regulation of gene transcription	Coccia et al., 2014; Kjaer et al., 2016
Atlantic salmon California Halibut *Paralichthys californicus* Cobia *Rachycentron canadum* Rainbow Trout	EPA (20:5n-3), DHA (22:6n-3) and EPA/DHA ratio	• Different studies, see references for details.	EPA and DHA have different nutritional roles and metabolic fates. DHA appears to be nutritionally more important and preferentially retained into fish tissues, whereas EPA seems to be more metabolically expendable.	Betiku et al., 2016; Codabaccus et al., 2012; Emery et al., 2016; Trushenski et al., 2012; Vizcaino-Ochoa et al., 2010
Atlantic salmon	ARA (20:4n-6)	• Different studies, see references for details.	Dietary ARA plays a series of important roles affecting fish performance, health and	Ding et al., 2018; Glencross and Smith

Gilthead Seabream Giant Tiger Prawn *Penaeus monodon* Oriental River Shrimp *Macrobrachium nipponense*			reproduction and its dietary availability should be considered in feed formulation.	2001; Koven et al., 2001; Lund et al., 2007; Norambuena et al., 2015; Van Anholt et al., 2004
Atlantic Salmon Rainbow Trout Turbot *Scophthalmus maximus* Yellowtail Kingfish *Seriola lalandi*	Cholesterol	• Different studies, see references for details.	Though not essential, the availability of dietary cholesterol appears to have several physiological important effects, which ultimately may affect fish performance. Diets where FM and FO are abundantly substituted with vegetable alternatives may be limited in their cholesterol availability.	Guerra-Olvera and Viana 2015; Leaver et al., 2008; Norambuena et al., 2013; Yun et al., 2012; Zhu et al., 2014

Table 4. Influence of raw materials and diet processing on diet characteristics and fish performance.

Parameter	Finding	References
Raw materials processing		
- Particle size	- Reducing particle size had no effect on digestibility, but improved FCR	Zhu et al., 2001; Booth et al., 2001; Glencross et al., 2004, 2007, 2008; Ngo et al. 2015; Refstie et al. 1998, Barrows et al., 2007; Opstvedt et al. 2003
- Dehulling grain	- Dehulling (removal) of grain seed coats increases their protein content AND also increases the digestibility of that protein → some non-starch polysaccharides have a clear influence on nutrient absorption from vegetable proteins	
- Solvent extraction	- Solvent-extraction reduces the energy digestibility of canola meals - Solvent-extraction reduces the energy digestibility of soybean meals	
- Extrusion cooking	- Pre-extrusion of soybean meal improved its digestibility	
- Thermal cooking	- Increased thermal cooking reduced digestibility of fish meals - Increased thermal cooking reduced digestibility of canola meals	
Diet processing type		
- Pelleting cf. Extrusion	- Extrusion improved the durability of pellets and digestibility of starch - Extrusion improved the digestibility of energy - Extrusion improved the digestibility of most nutrients in most ingredients - That dry matter and energy digestibilities correlate between pelleting and extrusion, but not nitrogen or sum amino acid digestibilities	Hilton et al., 1981; Vens-Capell 1984; Cheng and Hardy 2003; Glencross et al. 2011
Extrusion constraints		
- Internal lipid levels	- That lipid levels with the extrudate mash cannot exceed a certain level without interfering with gelatinisation/melt → poor pellet binding and low expansion.	Lin et al., 1997; Sørensen, 2012; Oterhals and Samuelsen, 2015; Samuelsen and Oterhals 2016; Draganovic et al. 2013; Glencross et al., 2010, 2012; Samuelsen et al. 2013, 2014; Sorensen et al., 2002; Morken et al., 2011; Oehme et al., 2014; Storebakken et al., 2015
- Soluble protein levels	- Soluble protein content of the extrudate mash cause extrudate plasticisation - Soluble protein content of the extrudate mash improves pellet durability	
- Certain ingredient levels	- Certain ingredients cause acute densification (e.g. wheat gluten) - Certain ingredients cause acute expansion (e.g. tapioca)	
- Temperature	- Certain fish meals improve pellet durability more than others - Increasing temperatures (100°C, 125°C or 150°C) had no effect on nutrient digestibility	
- Inclusion of NaDiFormate	- The use of high extrusion temperature (141 °C) improved nutrient digestibility - Addition of NaDF increased the digestibility of most nutrients	
- Inclusion of water	- There are critical thresholds for water retention in the extrudate → changes in pellet rheology and extrusion operating parameters	
- Screw configuration	- Constrained water addition reduces starch gelatinization - Screw configuration affects pellet durability	