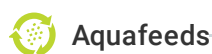




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Aquafeeds

Assessing the risk of alternative aquafeed ingredients, part 2

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By Brett D. Glencross, Ph.D. , Johanna Baily, DVM , Marc H.G. Berntssen, Ph.D. , Ronald Hardy, Ph.D. , Simon MacKenzie, Ph.D. and Douglas R. Tocher, Ph.D.

Genetically modified organisms, changes to product qualities and perspectives



Many plant protein concentrates produced from corn, faba beans, peas, rapeseed and lupins (field of rapeseed in image) have value as potential aquaculture feed ingredients. Photo by Richard Bartz, Munich aka Makro Freak, via Wikimedia Commons.

This second and final part discusses genetically modified organisms, changes to product qualities, and an overall perspective of all the risks discussed and their management.

Genetically modified organisms

A genetically modified organism (GMO) or transgenic organism is one whose genome has been altered using genetic engineering techniques in contrast to other genetic approaches such as more traditional selective breeding programs. The Cartagena Protocol on Biosafety to the Convention on Biological Diversity [<https://bch.dost.gov.ph/transparency/biosafety-protocol>], an international agreement to protect biological diversity from the potential risks posed by organisms resulting from modern biotechnology uses the term “Living Modified Organisms” (LMO), although this is regarded as equivalent to GMO. As well as being a research tool, GMOs have practical and commercial applications in the production of pharmaceutical drugs, experimental medicine (e.g., gene therapy) and agriculture.

Although there have been numerous studies investigating the replacement of marine ingredients with plant products, relatively few have focused on the possible GM origin of the ingredients or on determining the specific effects of GM versus the equivalent non-GM ingredients. In assessing GM products in fish feeds, there are two main areas of possible interest and/or concern including (i) production: Does the GM product alter the growth performance (growth rate and feed efficiency) of the fish; and (ii) safety: Does the GM product affect fish health or welfare and/or the safety of the farmed product. Regarding food safety, one possible issue could be whether transgenic sequences can be transferred to the fish and found in tissues including muscle and thereby possibly further transferred to human consumers.

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So far, the GM materials tested in fish have been developed for agricultural/agronomic purposes, but GM technology can be applied to specifically tailor crops for aquaculture through reduction in anti-nutritionals and/or modification of the levels of nutrients such as essential amino and fatty acids. Improving nutrient levels in crops is currently an area of great interest to aquaculture and fish feeds specifically in relation to the provision of the omega-3 long-chain polyunsaturated fatty acids (LC-PUFA) eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA). The omega-3s are important nutrients with key metabolic and functional roles in fish and humans, but they are only present in fish oil and fishmeal.

Therefore, the major problem in replacing fish oil in feeds is maintaining omega-3s in farmed fish at the high levels required for the farmed products to retain their role as beneficial and healthy components of the human diet. The overall strategy is to genetically modify existing organisms that have oil deposition as a major trait and thus combine this with the omega-3 biosynthesis trait. Potential candidates include other oleaginous microorganisms or conventional oilseed crops to produce entirely novel sources of de novo omega-3s.

Oilseed crops dominate world oil production and there is a highly organized and well-established infrastructure for the cultivation, harvest, processing, distribution, marketing and utilization of vegetable oils. Therefore, oilseed crops are highly practical platforms from which to develop a novel, renewable supply of omega-3s. However, conventional plant breeding strategies cannot be used as the genes required for omega-3 synthesis are simply not present in higher plants, leaving transgenesis as the only option for modification of oilseeds to contain omega-3s. Therefore, arguably the only currently viable approach to developing a novel, renewable supply of EPA and DHA is the metabolic engineering of oilseed crops with the capacity to synthesize omega-3s in seeds. The production of omega-3s in terrestrial plant seeds has been demonstrated in the model plant *Arabidopsis* and in an oilseed crop, camelina (*Camelina sativa*).



The production of omega-3s in terrestrial plant seeds has been demonstrated in the oilseed crop *Camelina sativa*. Photo by Bliesgauoele, via Wikimedia Commons.

As well as being easily transformable, camelina has additional desirable traits including modest input requirements (water and pesticides) and ability to thrive in semi-arid conditions. In the United States, several states are actively growing camelina as a biofuels crop, indicating the wide acceptance of this crop platform. Furthermore, wild-type camelina oil has already been shown to be suitable for inclusion in fish feeds and contains no anti-nutritional factors detrimental to fish growth.

Ultimately, all animal production will depend on terrestrial plants/agriculture and this requires land. However, the production of omega-3s in terrestrial oilseed crops should not require additional arable land as the ideal solution would be to switch some vegetable oil production from omega-6 PUFA-rich crops to the new omega-3 crops.

Changes to product qualities

A persistent concern associated with the use of alternative ingredients in feeds for farmed fish is their potential effects on the safety and quality of farmed fish products to the consumer. In terms of the use of alternative ingredients in fish feeds, product safety issues are limited to the potential contamination of ingredients with heavy metals or organic pollutants. To the general public, quality of fish products refers to freshness, but in the context of the topic of this review, quality refers to sensory or organoleptic properties, and nutritional properties.

Sensory characteristics

Sensory attributes of seafood are those detected using the senses, namely sight, taste, smell and touch, and focus on color, taste, odor and texture, each of which can be affected by diet fed to the animal. Sensory characteristics of various foods, including fish products, are typically assessed by taste panels, but instruments can also be used to assess color, odor and texture. Data from Instrumental analysis are

considered quantitative rather than qualitative (or semi-quantitative) as is the case for data obtained by taste panels. Instrumental analyses are also more sensitive than human senses and therefore can distinguish differences between fish samples that are not detected by human senses.

Although researchers have documented definite effects of alternative protein and oil sources on sensory characteristics of farmed fish products, for the most part, the effects are relatively minor and primarily confined to the effects of replacing fish oil with alternative oils. Various studies generally support that replacing part or all fish oil in fish feeds with alternative plant oils (soy, canola, rapeseed, sunflower) or animal fat (poultry, swine) alters fillet odor and/or flavor, and is associated with an increase in consumer/panelist preference in trout, salmon, gilthead red sea bream, sea bass and turbot.

Fillet color is an important attribute for salmon and trout, and the external color is also an important attribute in some markets for fish, such as the red sea bream. Skin, muscle and egg color results from the deposition of carotenoid pigments supplied in the diets of wild or farmed fish. Alternative ingredients containing the carotenoid pigment astaxanthin – such as krill meal, shrimp meals or their oils – enhance the color of fillets and skin. For farmed shrimp, color after cooking is also an important market factor.



For many markets, a darker red color in farmed shrimp is preferred.

Photo by Darryl Jory.

Texture, defined as firmness, is another important sensory characteristic that is evaluated by trained taste panels, with descriptors such as greasy, soft, chewy, grainy and firm being typical descriptors. Although texture is regularly assessed by sensory evaluation of fish products, almost all studies in which fish fed diets with alternative ingredients report no significant differences associated with diet.

Texture is negatively correlated with muscle fiber diameter and by muscle fiber density, meaning the number of muscle fibers in a given cross-sectional area. Muscle fiber numbers in fish can be affected by feeding level at specific life-history stages in fish. However, there are no reports documenting

changes in muscle fiber associated with the use of alternative ingredients. In fact, a number of studies in which fish, mainly rainbow trout, have been fed diets in which fishmeal has been replaced with plant proteins report no differences in a range of sensory attributes, including texture.

Nutritional qualities

The nutritional qualities of fisheries products are associated with their nutritional profiles, namely the contents of protein, fat, vitamins and minerals. Protein and amino acid contents of fish muscle are essentially the same in wild and farmed fish and not affected by feed composition. Muscle amino acid content is associated with the major proteins in muscles and the amino acid contents of these proteins are conserved in vertebrates, including fish species.

Thus, feed ingredient composition has essentially no effect on protein or amino acid profiles of fisheries products even though the amino acid profiles of plant proteins and some animal protein ingredients; for example, blood meal or feather meal differ greatly from that of fishmeal. While the percent protein in fillets changes with the season, fish size and life stage, alternate feed ingredients are not a factor in these changes.

The situation is very different for fillet lipid content and fatty acid composition, especially in fish that store lipid in muscle tissue, such as salmon and trout. Fillet lipid content increases gradually in fish as they grow and can be altered by feed intake, dietary lipid content and protein:lipid or protein:energy ratio. However, there is very little difference in digestibility of various lipid sources to fish and therefore little effect of dietary lipid source on fillet lipid level. As a result, alternative lipid sources have little effect on nutritional quality as far as fillet lipid content is concerned, even though fillet lipid content is an important factor in sensory quality assessment.

Fatty acid content is another matter. For over four decades, it has been well known that the fatty acid profile of fish reflects that of their diet, both in wild and farmed fish. Farmed fish consuming a feed containing fish oil have fatty acid compositions similar to that of fish oil and therefore similar to wild fish. Fish oils used in feeds are produced from several fish species – such as menhaden, anchovy oil, capelin, herring and tuna – and differ somewhat in fatty acid profile.

Of importance to the nutritional quality of fish products is the content of omega-3s, specifically EPA and DHA, as well as the content of omega-6 fatty acids, notably linoleic acid. Differences in EPA and DHA content result from using different fish oils in feeds. However, these differences are minor compared to the effects of replacing a portion or all of the fish oil in fish feeds with plant oils or animal fats. Doing so lowers the content of EPA and DHA in the diet leading to a reduction in levels of these fatty acids and an increase in other fatty acids in fillets.

Although fatty acid profiles of fish fillets reflect that of their diet, the relationship between dietary fatty acids and categories of fatty acids is not exact because fish possess the ability to alter fatty acids to meet their physiological and metabolic needs. However, levels of polyunsaturated fatty acids in fish tissues, especially linoleic acid, are highly responsive to dietary level and can increase greatly when certain alternative lipid sources are present in the diet of fish. Olive, canola and peanut oils are rich sources of oleic acid, whereas corn, cottonseed, safflower, soy and sunflower oils are rich sources of linoleic acid. Substituting fish oil with oils high in linoleic acid significantly increases the level of linoleic acid in fillets and increases the ratio of omega-6 to omega-3 fatty acids, a negative outcome in terms of potential value to nutritional quality from the perspective of human health.

Alternative lipid sources are increasingly required in fish feeds to meet the demands of fish feed production associated with the growth of intensive aquaculture production and given predictions of continued growth of intensive aquaculture, the use of alternative lipids in fish feeds will grow. In recent decades, alternative oils, mainly from oilseeds, have replaced about half of the fish oil in fish feed formulations, leading to a decrease in EPA and DHA levels in fillets, especially salmon fillets. The challenge for the aquaculture industry is to maintain healthful levels of EPA and DHA in farmed fish products as the percentage of alternative oil sources in fish feed formulations increases.

There are several ways this can be accomplished. First, levels of EPA and DHA can be increased by feeding a “finishing diet” during the final stages of grow-out prior to harvest. A second approach is to add high-DHA ingredients, such as products from algae, to the diet. A third approach may be to develop GMO oilseeds that produce EPA and/or DHA. A final approach may be to utilize selective breeding to improve the efficiency with which fish convert linolenic acid to EPA or DHA.

Perspectives on these risks

In terms of a risk assessment, this review mainly identified the key risks in a qualitative sense (i.e., the consequences), but not necessarily in a quantitative manner (i.e., the likelihood). For a more comprehensive risk assessment, both components clearly need to be examined.

Consequences and likelihood

Quantifying the consequences and likelihood of certain risk factors is a challenge fraught with difficulties. Differences in perspective among different use sectors (e.g., ingredient producer cf. ingredient user), countries (e.g., EU cf. USA on GMO crops) and stakeholders (e.g., producers cf. insurers) all complicate the assessment. Because of this variability in perspectives, the authors cover various geographical ranges (EU, USA, Scandinavia and Australia), stakeholders (nutritionists, toxicologists, immunologists and veterinarians), and we attempted to assess the consequences and likelihood of certain risk factors to both the fish and humans fed the fish on a basis independent from the various use sectors. From this approach, we tried to assess the risk relative to an industry standard (fishmeal). Only a generic approach (vegetable vs. animal meals) was considered. A summary of those results is shown in Table 7.

Table 7. Perceived risk (less = 1; same = 0, more = 1) to fish and human consumer health when consuming fish fed diets based on the use of either vegetable or terrestrial animal-derived feed raw materials relative to that from marine derived resources (fishmeals and oils).

We noted that compared to fishmeal, both vegetable and terrestrial animal-derived feed raw materials offered a range of risk-reduction opportunities and a small number of increased risk threats. While it was perceived that there were a greater number of potential risks with vegetable materials relative to the terrestrial animal-derived feed raw materials, what such an assessment does not do is give any weighting to one risk over another. In this situation, some risks might be considered of low consequence, but greater likelihood but of lower perceived overall risk than something of low likelihood but catastrophic consequences.

Prevention or cure?

In addition to the consideration of the consequences and likelihood of certain risks, the management of such risks also needs to consider the various options to their control, such as the prevention or remediation (cure).

Prevention is arguably always better than remediation. In the present case, we refer to the prevention of risk entering the feed chain. A range of such strategies exist and are widespread across the sector but vary in their extent and detail. The most common strategy is the simple analysis of ingredients to assess both the type and extent of potential risk. For such analysis, there are certain standards that need to be considered to ensure reliability in the results and these standards and how they are defined vary from country to country. However, exhaustive analytical testing is both cost and time-prohibitive in most cases, so a degree of rationalization is applied subject to the type of ingredients being assessed and potential risk factors of concern. One such point of value of the present review is to highlight those risks across the various ingredients in the aquaculture feed chain. Once data is obtained from such testing it is then used to inform about potential thresholds/exposure and the associated risk.

Both ingredient and feed processing can also be used as a means of prevention of some risks. Ingredient processing is typically used to mitigate some anti-nutritional factors, while the conditions used in modern feed processing (e.g., extrusion) provide a degree of sterilization from microbes. There are also potential remedial actions to address some of the potential risks. A common one presently in use for many contaminants is the use of a withdrawal period before fish enter the human food chain.

Another potential remedial action is the use of binders and adsorbents to bind toxic substances in either the raw materials or feed. Several commercial products are available to mitigate the impacts of some risk factors like mycotoxins. And a third proposed remediation strategy has been to consider the manipulation of the physiology of the animal to enhance the metabolic turnover and excretion of contaminants.

There are also risks about the movement of different raw materials produced in one country which may introduce residues from drugs/chemicals otherwise banned or restricted in another country. With an increasing degree of globalization in the international feed sector, a need to harmonize many of the feed-associated regulations is emerging. Many of the companies now supplying the global aquaculture sector are multinationals and are trading across the world in both developed and developing regions. Accordingly, the trade in aquaculture feed ingredients is also a global activity, with most major companies sourcing from across the globe.

This globalization of the feed sector, like many others seen in the past decades, is also likely to raise a suite of issues. There will be an increasing need for consistency in regulations and standards across the sector, irrespective of international boundaries. For there to be such standards to exist, there will clearly then need to be a degree of objectivity in those standards. However, there will also need to be some consideration of the rate of change in those standards as the international community seeks to obtain this consistency.

Authors



BRETT D. GLENCROSS, PH.D.

Corresponding author
Institute of Aquaculture, University of Stirling
Stirling, UK

b.d.glencross@stir.ac.uk (<mailto:b.d.glencross@stir.ac.uk>).



JOHANNA BAILY, DVM

Institute of Aquaculture, University of Stirling
Stirling, UK



MARC H.G. BERNTSSEN, PH.D.

Department of Feed Safety, Institute for Marine Research
Bergen, Norway



RONALD HARDY, PH.D.

Aquaculture Research Institute, University of Idaho
Hagerman, Idaho, USA



SIMON MACKENZIE, PH.D.

Institute of Aquaculture, University of Stirling
Stirling, UK



DOUGLAS R. TOCHER, PH.D.

Institute of Aquaculture, University of Stirling
Stirling, UK

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