

**Week 11. Fish Nutrition Facts**

Aquaculture requires optimisation of nutrition to efficiently raise fish for the purpose of food production. Fish nutrition is the study of nutrients and energy sources essential for fish health, growth and reproduction. Global consumption of seafood is increasing, while the amount of captured fish is declining, therefore it is predicted that aquaculture will provide the most reliable supply of seafood in the coming years. With the world's rapidly expanding population, it is important to provide safe and nutritious fish; however there are many issues related to fish nutrition that need to be considered in order to achieve balance in food production and sustainability. Sustainability of the aquaculture industry is an environmental, economic and social concern; however, this review focused specifically on environmental sustainability in regards to fish nutrition. Certain issues related to fish nutrition have become controversial because they impact the environment and/or affect the final product for consumption. Some of these issues include: feed and nutrient efficiency, overfeeding and waste, unsustainable feed ingredients, fish health issues, biotechnology and human health concerns. Ultimately, each of these issues can affect the final product for human consumption, either nutritionally, environmentally or economically. Achieving a balance between efficient and safe food production with environmental sustainability will be a challenge for the industry. The following is a review of several issues in farmed fish nutrition, relating to providing quality food products while maintaining environmental sustainability, emphasizing limitations of this balance and strategies for improvement. Fish nutrition and nutrient efficiency in aquaculture

The essential nutrients for fish are amino acids, fatty acids, vitamins, minerals and energy-yielding macronutrients (protein, lipid and carbohydrate). Diets for fish must supply all essential nutrients and energy required to meet the physiological needs of growing animals. Guidelines for nutrient adequacy for some farmed fish species suggest the minimum nutrient requirement to promote growth and prevent signs of nutrient deficiency [1]. Protein is required in the diet to obtain amino acids, which are utilized to synthesize new proteins or maintain existing proteins in tissues while excess protein is converted to energy. Lipids supply essential fatty acids and energy in the diet. The requirement of essential fatty acids can only be met by supplying Long Chain (LC) Polyunsaturated Fatty Acids (PUFA) in the diet, specifically  $\alpha$ -Linolenic Acid (LNA, 18:3 $\omega$ 3) and Linoleic Acid (LA, 18:2 $\omega$ 6), with varying requirements for eicosapentaenoic acid (EPA, 20:5 $\omega$ 3) and docosahexaenoic acid (DHA, 22:6 $\omega$ 3) depending on

species. Dietary lipids are also important structural components of membranes, and act as precursors of steroid hormones and prostaglandins in fish. Dietary carbohydrates can be a source of energy for fish; however their ability to utilize dietary carbohydrate for energy varies depending on the species and their natural diet. Therefore, depending on species, protein and lipid are the main source of energy for fish. Feeds in aquaculture are formulated with a balance of nutrients in order to meet specific nutrient requirements for different species, life stages and other purposes. The digestibility of nutrients in the feed can affect aquaculture production efficiency and impact the environment. The bioavailability or digestibility of the diet is the proportion of nutrients in the feed that is digested and absorbed by the fish. Data on the digestibility and available digestible energy of feed ingredients in fish diets are essential for optimization of feed formulations [1]. Feeds that are poorly digested result in limited growth and feces with high nutrient content, which pollutes the environment. Therefore, the digestibility of nutrients and the potential for nutrient retention and waste must be considered for efficient and sustainable animal production when considering feed formulations. Growing fish accrete new tissues and some of the energy supplied in the diet is stored as protein, lipid and some glycogen. Protein deposition depends on the balance of available amino acids in protein and the digestible protein-to-digestible energy ratio. Excess energy intake and low protein levels result in the deposition of lipid as recovered energy, which does not equate to faster growth and is an inefficient use of nutrients. Fish have the ability to utilize lipids for energy, saving protein for deposition and growth [2]; therefore inclusion of lipids in diets for fish is important for both growth and energy purposes. Most commercial feeds today are formulated to increase growth performance by exploiting the protein-sparing effect of high energy lipid, allowing as much of the dietary protein as possible to be converted into muscle protein. As a result, the production efficiency of farmed salmon has significantly improved over time [3]. Today, the use of more highly digestible nutrient-density extruded feeds (46 to 50% protein, 20 to 24% fat) allows commercial farmers to achieve a Feed Conversion Ratio (FCR) of about 0.9-1.2 for rainbow trout (*Oncorhynchus mykiss*) grown to market size [2]. The improved FCR over time has been due to increased digestible nutrient and energy content of the feeds, as well as feed extrusion which has resulted in the production of feeds with higher lipid levels, improved starch gelatinization (increases digestible energy content and utilization), and improved pellet characteristics (durability, buoyancy, etc.) [2,3]. Further research to improve feed ingredients and feed

efficiency in the future will help improve sustainability in aquaculture. For example, genetically modified (GM) salmon have better FCR than non-GM salmon (see Biotechnology section). Feeding and the environment Feed is the main source of waste and is responsible for most of the environmental impact of aquaculture [4] feed composition and the FCR affect the amount of waste produced, as well as its physical and chemical composition. Excess feed results in immediate eutrophication of the surrounding environment; while consumed feed yields products of metabolic processes, such as ammonia, phosphorus and carbon dioxide [5]. The quantity and quality of the waste excreted by fish depend on intake, digestion and metabolism of dietary compounds [6]. Excess feed waste has a much greater capacity than fecal material to impact the environment, in terms of energy content and degradation rate. The particulate organic matter sinks and disperses, which results in environmental toxicity and anoxia [7]. The degree of impact from effluent wastes depends on feed quality, digestion and metabolism of the diet, species, culture method and the nature of the surrounding environment in terms of physics, chemistry and biology [8]. This has a direct impact on marine benthic habitats, with effects such as reducing sediments, hypoxia in the water overlying the sediment, increased sulphate reduction and changes in benthic fauna assemblages in terms of species number, diversity, abundance and biomass [7]. Feeding methods and technologies have advanced in recent years to minimize and eliminate waste, but the issue remains. Improved digestibility, feed utilization and feeding practices should be implemented by the feed and production industry to reduce losses from waste. One example is the use of devices to monitor feeding activity from below the surface in order to feed to satiation without overfeeding and consequent feed wastage, a technology that has been used in recent years by several commercial farms [2]. Nutritional strategies to reduce waste include improvements in feed formulations without affecting growth and production efficiency, inclusion of feed ingredients with high phosphorus bioavailability, use of feed additives to improve the apparent digestibility of phosphorus, and processing-refining of ingredients [9]. These efforts have resulted in a significant reduction of waste outputs (per unit of fish produced) by fish culture operations in Canada over the past four decades [6]. However, feeds that are fully digested by the fish cannot totally resolve the impact of fecal waste because the scope of digestion in fish is limited and there will always be a fraction of undigested feed [10]. Removing the solid waste before it is discharged can be a solution for reducing the environmental impact of wastewater [9]. Eco-certification may be a tool to set standards with

criteria aimed at reducing eutrophication through the level of inclusion; e.g., specific allowed amounts of nitrogen and phosphorus release from the system and a set limit for solids in the effluent water [11]. Integrated multi-trophic aquaculture (IMTA) of fish in combination with invertebrates and plants can help reduce environmental impacts and maximize the usage of food input. IMTA shows the most promise in terms of new and innovative systems for waste mitigation and production efficiency. The IMTA system reduces organic waste by mimicking trophic relationships found in nature; the waste from one organism is food for another, resulting in decreased organic particle concentrations with increased distance from farm sites as they are consumed by other farmed organisms [12]. The carbon, nitrogen, and phosphorus compositions of feed, fish and faeces were studied at an Atlantic salmon (*Salmo salar*) farm to estimate the release rates of wastes from salmon cages and the qualities of particulate wastes as food resources for integrated multi-trophic aquaculture. The study found that both salmon feed and faeces were adequate food for blue mussels (*Mytilus edulis*) and sea cucumbers (species unknown), and the nutrient content may meet their nutritional requirements, including DHA and EPA contents of feces, which were comparable to those of some phytoplankton species [13]. Other studies have also reported successful incorporation of nutrients from salmon waste feed into the tissue of blue mussels [14,15]. Choice of the extractive species and distance from the feeding location is an important consideration. For example, in a study on a commercial salmon farm in British Columbia, Canada, mussels had significantly higher amounts of DHA compared with other molluscs (chitons, clam, limpets, periwinkles and whelks), which indicates their potential in IMTA. The levels of DHA in mussels showed a significant breakpoint at 339 m from the farm, which suggests that distance should be considered to optimize certain nutrients [16]. However, organic fish waste captured by mussels is limited by the time available to intercept solid wastes contained in the horizontal particle flux, the velocity of the current, available IMTA farm space, and any negative feedback effects on fish culture from flow reduction caused by mussel culture [17]. New research is focused on expanding novel fish and extractive species. A study recently demonstrated that green sea urchins (*Strongylocentrotus droebachiensis*) actively ingest and absorb organic material from the waste produced by sablefish (*Anoplopoma fimbria*) culture. Further research was recommended to determine the effect of the sablefish waste diet on green sea urchin survivorship, growth, and gonad quality for urchin production [18]. Multi-species

production in an IMTA system must be optimized and better utilized in the future to increase productivity and improve sustainability.

Terrestrial plant oils in aquaculture feeds Terrestrial plant oils will likely be the main choice when replacing FO in aquaculture diets; however they are fairly limited in their ability to fully replace FO in diets for fish. Most plant oils are relatively poor sources of  $\omega$ 3 PUFA in comparison to marine FO, and completely lack LC  $\omega$ 3 PUFA. Rather, they are rich sources of  $\omega$ 6 and  $\omega$ 9 fatty acids, mainly LA and 18:1 $\omega$ 9, with the exception of some oilseeds. Although considered an excellent energy source, feeding terrestrial plant oils inevitably results in lower levels of DHA and EPA in tissues of fish fed plant oils [21,28-32], which is detrimental to fish health and compromises the health benefits for humans that consume these fish. Extensive replacement of FO with terrestrial plant oils, particularly those high in LA, cause a high incidence of cardiovascular disorders in fish [33] and also has been suggested to be detrimental to human health after consumption of fish fed soybean oil [34], which is discussed in fish nutrition and human health section in this review. As a result, the immediate thought is that plant oils best suited as a substitute for FO should contain high levels of  $\omega$ 3 PUFA (LNA) and lower amounts of LA, in order to increase the  $\omega$ 3/ $\omega$ 6 ratio. However, a study by Francis et al. [35] contradicts this idea because it was found that  $\omega$ 6 PUFA (sunflower oil diet) appeared to 'spare' the catabolism of  $\omega$ 3 LC PUFA and, as such, resulted in the highest retention of these fatty acids by rainbow trout. These results suggest new nutritional approaches to maximise the maintenance of the qualitative benefits of fish oils when they are used in feeds for aquaculture species [35]. Other recent studies have described that diets that contain high levels of LNA are relatively wasteful, because the fish did not extensively utilize the LNA toward  $\omega$ 3 PUFA biosynthesis, but rather catabolized the  $\omega$ 3 PUFA for energy. A similar observation is true for some fatty acid classes, particularly saturated fatty acids and monounsaturated fatty acids, which also appear to enhance the retention of  $\omega$ 3 LC PUFA in the fillets of some fish species. These observations have been found in Atlantic cod (*Gadus morhua*) [31], sunshine bass (*Morone chrysops* x *Morone saxatilis*) [36], Murray cod (*Maccullochella peelii peelii*) [37] and rainbow trout [35]. This approach has been given the more specific definition of the ' $\omega$ 3 LC PUFA sparing effect' as found in Atlantic salmon [36]. Consequently, it could be argued that plant oils high in LNA do not have any nutritional advantage over other commercially available oils with lower levels of  $\omega$ 3 PUFA, provided that DHA and EPA are spared through catabolism of other abundant fatty acids. These results also

indicate that feeding a diet based on FO only is an inefficient practice anyway, because substantial amounts of the nutritionally valuable  $\omega$ 3 LC PUFA are oxidized for energy, particularly EPA [38]. Therefore, the use of plant oils in fish diets conserves LC  $\omega$ 3 PUFA in FO for critical physiological functions only. This has been the focus of studies in this area recently, specifically on the dynamics of dietary DHA/EPA/ARA and its effect on fish performance, tissue concentration and immunity (Table 1). The ability of fish to synthesize LC PUFA may allow for plant oils to fully replace FO in aquaculture feeds without lowering levels of key fatty acids in the flesh such as DHA and EPA that are significant for fish and human health. The saturated fatty acids 16:0 and 18:0 can be biosynthesized by all known organisms, including fish. Desaturases and elongases are the critical enzymes in the pathways for the biosynthesis of the LC PUFA from the shorter-chain fatty acids to longer, more unsaturated chains. Fish can desaturate 16:0 and 18:0 to yield 16:1 $\omega$ 7 and 18:1 $\omega$ 9 by  $\Delta$ 9 desaturase. However, all vertebrates lack  $\Delta$ 12 and  $\Delta$ 15 desaturases, which are necessary to form LNA and LA, so these fatty acids are considered essential. Subsequently, LNA and LA can be desaturated and elongated to form the physiologically essential EPA, DHA and ARA. However, the degree to which an animal can synthesize these fatty acids from LNA and LA depends on the activities of the elongase and desaturase enzymes ( $\Delta$ 6 and  $\Delta$ 5) in their tissues. Fish species differ in the extent to which they can tolerate diets without FO, and this trait appears to be evolutionarily related to the fatty acid profile of the natural diet. Consequently, carnivorous marine fish have lost much of the capacity to synthesize these fatty acids during evolution since they remained in an environment where such a conversion is not necessary. Freshwater fish have a greater ability to biosynthesize EPA and DHA from LNA, since the natural prey of many freshwater fish is not rich in EPA and DHA, but rather LNA and LA. The fatty acid desaturation and elongation pathway has been extensively studied in fish at both the molecular and enzymatic level, with fatty acyl elongase (ELOVL) and fatty acyl desaturase (FAD) identified and functionally characterized in several marine and freshwater species. The dietary fatty acid profile is influential to the expression of ELOVL and FAD genes. The tissue fatty acid profile has been found to be significantly correlated with FAD and ELOVL gene expression in Atlantic cod fed diets without FO; while FAD and ELOVL expression were significantly correlated with each other [38]. This is evidence that the regulation of these genes is signalled by a change in the fatty acid profile of the tissue. However, up-regulation of these genes is not necessarily reflected phenotypically because levels of DHA and EPA in fish fed plant oil diets are

significantly lower than DHA and EPA levels in fish fed a FO diet. However, these PUFA could be present in even lower amounts if ELOVL and FAD were not facilitating fatty acid biosynthesis. Often this effort cannot fully compensate for low levels of  $\omega$ 3 LC PUFA intake from plant oil diets; although results show that it is this low dietary level that may trigger the up-regulation of genes involved in their synthesis. Using molecular tools to identify ELOVL and FAD gene expression has often been the centre of research studies on FO replacements. However, there are other tools that can be used to quantify LC PUFA biosynthesis and can verify phenotypically the results found at the gene expression level. Mathematically, the level of LC PUFA synthesis can be quantified using the fatty acid mass balance equation. The fatty acid mass balance method was developed for fish by Turchini et al. [40] to quantify  $\omega$ 3 fatty acid synthesis in fish to determine the level of elongation and desaturation that occurred over the course of a feeding experiment. The method involves computation of the fatty acid intake, accumulation, and appearance or disappearance of the selected fatty acids in the  $\omega$ 3 pathway and computes the percentage of synthesized  $\omega$ 3 LC PUFA from dietary intake of LNA. An application of this method revealed that Atlantic cod synthesized 6% of their own LC  $\omega$ 3 PUFA [31], and 12% for rainbow trout [41] after fed a plant oil-based diet. Another interesting method to quantify LC PUFA biosynthesis is the use of compound specific stable isotope analysis. Fatty acid isotopic signatures are frequently used in food web studies to determine the transfer of fatty acids from prey to predator based on their  $^{13}\text{C}/^{12}\text{C}$  ratio [42]. Fatty acids from terrestrial plant oils have distinctly different isotopic signatures than the same fatty acids in marine sources like fish oil due to differences in the source of carbon (terrestrial carbon in the form of  $\text{CO}_2$  gas vs. marine carbon as carbonate) [43]. Using CSIA and a mixing model calculation [42], the proportion of synthesized LC PUFA (i.e., DHA) from LNA can be determined. Using this method it has been found that rainbow trout can synthesize up to 27% of the DHA in the muscle tissue from dietary camelina oil [31]. Quantifying fatty acid biosynthesis in fish using quick and efficient methods will become even more important in subsequent years; particularly if breeding programs are designed to select fish that have superior ELOVL and FAD expression and express these traits phenotypically. Future research in this area will be dedicated to exploring new plant resources that have high levels of  $\omega$ 3 fatty acids, particularly plant sources that contain substantial levels of EPA and DHA. Single-celled microalgae and yeast can produce their own EPA and DHA and are renewable resources [44,45] ; however, high production costs make commercialization very limiting. Research in

this area is needed to optimize time and cost of production. However, the most promising and upcoming FO replacements are genetically modified LC  $\omega$ 3 PUFA enriched crop production [46,47] and is further discussed in the Biotechnology section of this review. The use of plant ingredients in aquaculture inevitable; however it also raises questions regarding sustainability of crop production for aquaculture feeds. Measurements to quantify the amounts of land, water, nutrients and energy required for crops per unit of fish production should be calculated to assess environmental impact and sustainable development, and also could be compared to other types of animal production. Nevertheless, the conservation benefit of substituting plant meal and oil for FM and FO is obvious.