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Fish nutrition research: past, present and future

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Abstract The systematic, scientific investigation of nutrition dates from the eighteenth century, but for many years, there were few studies on fish. As a result, knowledge about fish nutrition still lags behind that of man and his domesticated terrestrial animals. Initially, there were few incentives to collect information about the nutritional requirements of fish, and it is difficult to carry out experiments on aquatic animals. Fish were being farmed, but the extensive rearing methods used meant that there was no pressing need to gather detailed information that could be used for preparing feeds. Research into fish nutrition started in earnest around the middle of the twentieth century. Since then information has accumulated quite rapidly as research efforts have been spurred on by the expansion of aquaculture and developments within intensive fish farming. Nevertheless, the gaining of more knowledge about the nutrition of fish still needs to be given priority to assist in the continued development and improvement of sustainable practices in aquaculture. In this brief overview, fish nutrition research is placed in a historical perspective by considering some of the major challenges faced by fish nutritionists, how these challenges were addressed, the advances made, and knowledge gaps that need to be filled. The spotlight is focused on nutrient requirements, feed ingredients and their evaluation, and the formulation of diets that promote effective production whilst serving to maintain fish health and well-being.

Keywords Nutrient requirements · Feed ingredients · Diet evaluation · Live food organisms · Aquaculture forensics

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Introduction

Nutrition research involves the study of the materials required for the maintenance of life, the formation and repair of body tissues (anabolism) and the production of energy to support this (catabolism). Fish nutrition research currently encompasses studies of feed intake and the physiological mechanisms involved in its regulation, nutrient requirements and interactions, metabolic pathways and nutrient utilization, fish growth, reproduction and early development. The investigation of nutritional influences on the ability of fish to resist environmental stressors and mount an immune response under challenge from pathogens also forms a part of fish nutrition research. As such, modern fish nutrition research covers a broad range of interrelated fields and often requires integration of knowledge gleaned from advances in chemistry, biochemistry, physiology, microbiology, immunology and molecular biology.

Information about nutrition and metabolism has accumulated gradually over a period of many years. Scientific investigation of nutritional principles was underway by the end of the eighteenth century, stimulated by developments in analytical chemistry and physiology. By the middle of the twentieth century, a considerable amount of information had been gathered about energy requirements, the structure and digestion of proteins, lipids and carbohydrates, the nature of vitamins and nutrient metabolism. Much information was also available about the nutrient requirements of humans and a few species of farm animals (Carpenter 2003a, b, c, d; Semba 2012). The available information received practical application in several areas relating to animal production, including the least-cost formulation of feeds, growth prediction and for developing an understanding of nutrient-yield-compositional interactions (Black 2014). More recently, there has also been a focus on the roles that the gut microbiota play in the nutrition, metabolism and health of man and his domestic animals, and how these are influenced by dietary interventions (Nicholson et al. 2012; Tremaroli and Bäckhed 2012; Thomas et al. 2014). In the wake of these studies, this area of research has also attracted the attention of fish nutritionists and immunologists (Merrifield and Ringø 2014).

Studies on the nutrition of fish have lagged some way behind those on humans and terrestrial farm animals, and there are probably several reasons for this. Although fish have been raised in captivity for several centuries, most farming has been carried out extensively in ponds, where the fish generally consume natural prey organisms (Nash 2011). Historically, practitioners needed to have skills in pond management (Huet 1986; McLarney 2013), but there was little need to have detailed knowledge about the nutritional requirements of the fish. There was, therefore, probably little incentive to carry out detailed studies into fish nutrition. In addition, it is much more difficult to carry out controlled experiments and nutritional studies on aquatic animals, such as fish, than it is to conduct similar work on terrestrial animals.

The study of fish nutrition started to gain impetus in the middle of the twentieth century as interest in the intensive farming of fish increased. Intensive farming methods were increasingly used to produce fish for release, re-stocking and for the table. After a few years, a small group of pioneers had collected sufficient research information to justify the publication of compendious works devoted exclusively to fish nutrition (Phillips 1969; Cowey and Sargent 1972; Halver 1972; NRC 1973). Thenceforth, the flow of research publications increased markedly, and the production of overviews and books that aimed to inform about the advances in fish nutrition followed suit (e.g. Halver 1989; Wilson 1991; NRC 1993, 2011; Hertrampf and Piedad-Pascual 2000; Houlihan et al. 2001; Halver and Hardy 2002; Webster and Lim 2002; Holt 2011; Turchini et al. 2011; Merrifield and Ringø

2014). This is an ongoing process, and improved knowledge about fish nutrition is needed to promote sustainable aquaculture practices (Jones et al. 2014).

This brief review is, by necessity, selective and highlights a few key areas within fish nutrition research: determination of nutritional requirements, feed ingredients and their evaluation and the formulation of diets that meet both production criteria and serve to promote the health and well-being of the fish. The presentation largely follows a chronological sequence, making use of some historical references, but relying heavily on books and reviews that give the interested reader a portal to the original literature on specific topics.

Fish nutrition research: the early years to the present day

The earliest studies into fish nutrition often involved finding out whether or not a dietary supply of a particular nutrient, such as an amino acid, was needed for a fish to develop, behave and grow normally. Once a qualitative requirement had been established, this could be followed by experiments designed to determine the amount of the nutrient needed to ameliorate deficiency symptoms and sustain growth; quantitative requirement studies.

The quantitative requirements for essential nutrients are usually assessed by providing the fish with feeds that contain graded levels of the nutrient in question. Responses are then measured in terms of growth, tissue or whole-body accumulation of the nutrient, or by using a secondary indicator, such as enzyme activity. In most nutrient requirement studies, it is growth responses that have been recorded. Dose–response curves are then constructed by equating growth with the amount (concentration) of nutrient supplied in the feed (Fig. 1). The dose–response relationship is then analysed, and the nutrient requirement estimated from the nutrient concentration that gives the desired level of response. Although the basic design of quantitative requirement studies has remained relatively unchanged since the early days, there has been much discussion and debate about the way in which the data should be analysed (Shearer 2000; NRC 2011).

Several methods have been used to analyse the dose–response relationships, including analysis of variance (ANOVA) and a variety of regression techniques. ANOVA, although often used, is not a correct statistical analysis for data of this type, and it also has the

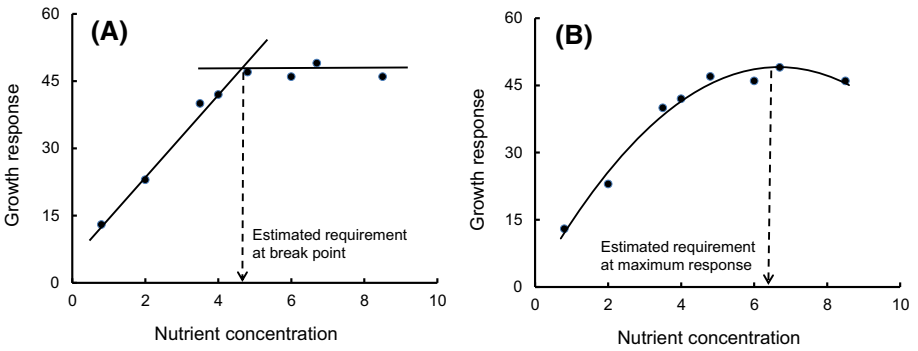

Fig. 1 Dose–response relationships analysed using **A** the broken-line model, and **B** nonlinear polynomial regression. The same data were used to prepare both graphs. The estimated requirement is lower when the broken-line model is used than when using nonlinear polynomial regression to analyse the data

disadvantage that it does not allow accurate estimation of the level of the nutrient required to maximize the growth response. Methods that rely on regression analysis are preferred, and several nonlinear models along with the broken-line model have been used (Fig. 1) (Shearer 2000; NRC 2011). The broken-line model is probably the most widely used method for the estimation of quantitative nutrient requirements of fish. This model is based on the assumption that there is a linear relationship between the growth response and the concentration of a limiting nutrient in the feed. The dietary requirement for the nutrient is determined by finding the break-point in the relationship obtained by plotting the growth of the fish against the concentration of the nutrient in the feed. The break-point is the point at which increased inclusion of the nutrient does not promote any further increase in growth, and a plateau is reached (Fig. 1). The broken-line model, in common with ANOVA, may have a disadvantage that it could provide an underestimation of the requirement for dietary nutrients (Shearer 2000).

Many currently working in aquaculture research would probably find it difficult to compile an accurate list of the fish species used in the earliest nutrition trials. These trials established some nutritional recommendations that are still in use today. The fish used in these early fish nutrition studies were Chinook (*Oncorhynchus tshawytscha*) and coho salmon (*Oncorhynchus kisutch*), rainbow trout (*Oncorhynchus mykiss*), brook trout (*Salvelinus fontinalis*), channel catfish (*Ictalurus punctatus*), Japanese eel (*Anguilla japonica*), carp (*Cyprinus carpio*) and some species of marine flatfish, such as plaice (*Pleuronectes platessa*) and sole (*Solea solea*) (Phillips 1969; Cowey and Sargent 1972; Halver 1972). Intensively farmed species that are familiar to-day, such as Atlantic salmon (*Salmo salar*), European sea bass (*Dicentrarchus labrax*), gilthead seabream (*Sparus aurata*) and Asian sea bass or barramundi (*Lates calcarifer*), along with tilapias (*Oreochromis niloticus* and *O. mossambica*) were comparative latecomers on the scene, and there is still relatively little nutritional information available for some species, such as pangasius (*Pangasianodon hypophthalmus*), that are currently being produced in large quantities (Wilson 1991; Webster and Lim 2002; NRC 2011).

At present, information about the nutritional requirements of farmed fish is far from complete, and there are few, if any, species for which the requirements have been defined for all life history stages. Why should this be the case? It is possible that the discovery that feeds based on fishmeal and fish oils promoted good growth of most fish species reduced the incentive to conduct comprehensive requirement studies. The expense of carrying out trials on large fish and the nature of the feeds needed for such studies were also probably important contributory factors. There may also be differences in nutritional requirements for fish being grown directly for consumption and those intended to be used as brood-stock to produce the eggs used to give the next generation of juvenile fish for on-growing. An additional problem relates to the difficulty of carrying out controlled nutritional studies on the earliest life history stages of many fish species.

Brood-stock and larval nutrition are amongst the most poorly understood areas of fish nutrition even though their importance is recognized. Nutrition has an influence on all aspects of reproduction from the determination of the timing of the onset of puberty, through gametogenesis and the determination of fecundity to the production of viable eggs and sperm. Most fish species have yolk-rich (telolecithal) eggs and are lecithotrophic during early development. This means that the developing embryo and newly hatched larva of farmed fish depend upon nutrients deposited in the oocyte by the female during vitellogenesis for their growth and survival. At the time of spawning, the eggs are composed primarily of water, protein and lipids, along with smaller proportions of micronutrients and maternal developmental factors. There are, nevertheless, marked interspecific differences

in the types, form and quantities of nutrients deposited in fish eggs, but a few generalizations can be made. For example, lipid content tends to be highest in eggs with long incubation times, and the proportions of free amino acids are usually much higher in pelagic eggs than in demersal eggs. The consequences of these compositional differences for embryonic and larval metabolism, development and survival have been much discussed, and there is a rich literature covering the question 'what makes a good fish egg?' (Kjørsvik et al. 1990; Wiegand 1996; Brooks et al. 1997; Kamler 2008; Finn and Fyhn 2010).

The realization that nutrients in the egg yolk have profound consequences for embryonic and larval development has stimulated some research into the nutritional requirements of maturing fish. Feeds developed for brood-stock usually contain more protein than grow-out feeds, and a good supply of the amino acid tryptophan seems to be particularly important for successful completion of reproduction. Tryptophan, the precursor of serotonin, may have influences on gonad maturation in both sexes. Dietary supplementation with taurine also seems to be beneficial for improving brood-stock performance of marine fish species. Taurine, a sulphur-containing amino acid-like compound, has several physiological roles, including osmoregulatory and antioxidant functions, and as a neuro-modulator (Salze and Davis 2015). There has also been study of the influences of micronutrients, such as vitamin C, vitamin E, carotenoids and certain trace elements, and other feed additives on brood-stock performance, but the most-studied area seems to be lipid and fatty acid nutrition (Holt 2011; Tocher 2010; NRC 2011; Merrifield and Ringø 2014).

Egg fatty acid composition is influenced by the quantities, types and proportions of fatty acids present in brood-stock feeds. The eggs must contain n-3 highly unsaturated fatty acids (n-3 HUFAs) if the larvae are to develop normally. The two most important n-3 HUFAs required during embryonic and larval development are docosahexaenoic acid (DHA; 22:6 n-3) and eicosapentaenoic acid (EPA; 20:5 n-3) (Watanabe and Kiron 1994; Tocher 2010; Holt 2011). DHA is required for development of the brain and neural tissues, and deficiencies or imbalances in n-3 HUFAs (i.e. DHA/EPA) lead to survival being compromised. There is also a requirement for arachidonic acid (ARA; 20:4 n-6) for the completion of oogenesis and egg maturation, and for embryonic development and survival. Consequently, the balance of DHA/EPA/ARA in the eggs seems to be more important for ensuring normal development than the amounts of n-3 HUFAs *per se* (Bell et al. 1997; Tocher 2010; Holt 2011). Indeed, recent work on the Atlantic cod (*Gadus morhua*) showed that there were beneficial effects on egg production and larval survival when brood-stock feeds were supplemented with ARA provided that this resulted in a low EPA/ARA (Røjbek et al. 2014).

Despite the fact that the start-feeding phase is critical for fish rearing, there is a comparative dearth of quantitative information about the nutritional requirements of fish at this stage in their life cycle. A major stumbling block relates to the difficulty of making nutritionally stable, homogeneous micro-diets that are accepted, ingested and digested by small fish larvae (Watanabe and Kiron 1994; Holt 2011; NRC 2011). At present, the start-feeding phase of many farmed fish species depends on live food organisms, such as brine shrimp (*Artemia salina*), rotifers (*Brachionus* spp.), copepods and other zooplanktonic organisms (Fig. 2). Rotifers and brine shrimp are not natural food for marine fish larvae, but they are relatively easy to produce at high densities, and their nutritional profile can be improved using enrichment procedures (Watanabe and Kiron 1994; Støttrup and McEvoy 2002; Conceicão et al. 2010; Holt 2011; NRC 2011; Mæhre et al. 2013; Dhert et al. 2014;

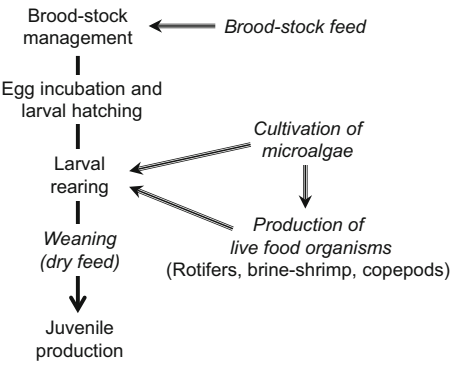

Fig. 2 Feed types (shown in *italics*) required for the production of marine fish larvae and juveniles

Merrifield and Ringø 2014; Rasdi and Qin 2014; Richard et al. 2014; Takeuchi 2014; Yoshimatsu and Hossain 2014).

Rotifers and brine shrimp have concentrations of n-3 HUFAs, taurine and essential elements, such as selenium, that are much lower than those in copepods that are the natural prey of marine fish larvae (Conceicão et al. 2010; Mæhre et al. 2013; Dhert et al. 2014; Rasdi and Qin 2014; Takeuchi 2014; Yoshimatsu and Hossain 2014). The n-3 HUFAs are incorporated into cell membranes, are required for neural and sensory development and are precursors for synthesis of several biologically active molecules. Marine microalgae may be able to supply some of these essential nutrients, and using microalgae to enrich live food organisms often gives increased survival of the fish larvae (Conceicão et al. 2010; Holt 2011; Takeuchi 2014; Yoshimatsu and Hossain 2014). Marine microalgae may be added directly to the water containing the fish larvae (green-water rearing technique), or be used as food for rotifers and other zooplanktonic organisms before they are added to the tanks containing the fish larvae. Reliance on live food organisms for start-feeding requires the establishment of an artificial food chain involving the culture of microalgae and zooplankton that are then fed to the fish larvae; this involves the investment of considerable time and expense (Fig. 2).

There are several commercial products that can be used to enrich and modify the nutritional profile of rotifers and brine shrimp. These closed-formula products are usually emulsions of marine oils that contain n-3 HUFAs and lipid-soluble vitamins or they are dried products or extracts produced from marine algae (NRC 2011; Rasdi and Qin 2014). Enrichment of rotifers and brine shrimp with n-3 HUFAs has beneficial effects on fish larval survival, development and growth (Conceicão et al. 2010; Holt 2011; NRC 2011; Takeuchi 2014), and the form in which the n-3 HUFAs are delivered may be of major importance. Larvae of several species seem to be better at utilizing phospholipid (PL) n-3 HUFAs than those present in triacylglycerols (TAGs), so PLs are usually considered to be mandatory in feeds for marine fish larvae (Holt 2011; NRC 2011; Olsen et al. 2014; Takeuchi 2014).

The enrichment of rotifers and brine shrimp with lipid-soluble vitamins, such as vitamins A, D and K, and taurine, may also be required to promote normal development and growth of marine fish larvae (Holt 2011; Richard et al. 2014; Takeuchi 2014; Yoshimatsu and Hossain 2014; Salze and Davis 2015). Rotifers and brine shrimp contain only trace amounts of taurine, possibly leading to deficiency symptoms that include mal-pigmentation, behavioural abnormalities and reduced growth (Takeuchi 2014; Salze and Davis 2015). Providing the larvae with food organisms that have been enriched with taurine is

often an effective way to alleviate the deficiency symptoms. Similarly, the enrichment of live food organisms with essential elements, such as selenium, copper, manganese and zinc, is often required to ensure adequate survival and growth of marine fish larvae, and there may also be growth and survival benefits to be gained by manipulating the gut microbiota of the developing fish (Merrifield and Ringø 2014; Yoshimatsu and Hossain 2014). The enrichment methods and media that are currently in use give variable results (Mæhre et al. 2013; Rasdi and Qin 2014), so the development of protocols that perform consistently would represent a considerable advance (Dhert et al. 2014).

Complete reliance upon live food organisms for larviculture is undesirable, but over four decades of research into developing micro-diets for start-feeding marine fish larvae have not met with universal success (Watanabe and Kiron 1994; Holt 2011; NRC 2011). Although some success has been achieved, it is clear that the development of micro-diets for a wider range of species would provide tremendous benefits for larviculture: reducing the time, effort and space required for the production of the live food organisms, and ensuring that the fish larvae were being fed a diet of uniform nutritional composition. As such, research directed towards the production of effective micro-diets is one of the key areas within larviculture.

There has been a relative hiatus in nutrient requirement studies in recent years, but there may be a change on the horizon involving a resurgence of interest in such studies. The recent trend of reducing use of fishmeal and fish oils in feeds, with concomitant increases in the amounts plant protein and oil sources (Gatlin et al. 2007; Tacon and Metian 2008; Naylor et al. 2009; NRC 2011; Turchini et al. 2011; Kitessa et al. 2014; Shepherd and Bachis 2014), may provide some of the impetus. This is of particular relevance given that many potential plant-based feed ingredients are likely to have been subjected to some form of genetic manipulation (GM) or metabolic engineering to introduce compositional, or other, changes deemed desirable to improve production and crop yields (Lau et al. 2014; Ronald 2014; Swiatkiewicz et al. 2014; Van Eenennaam and Young 2014; Voytas and Gao 2014). This means that with the continued expansion of intensive fish farming and changes in the composition of feeds, there will be a need to determine the amino acid, fatty acid, vitamin and mineral requirements for a wider range of fish species and life history stages.

In the next generation of requirement studies, fish nutritionists may adopt some ideas from nutritional ecology. Nutritional ecology is the study of how animals relate to their environment through nutritional interactions (Simpson and Raubenheimer 2001; Raubenheimer et al. 2009). It is assumed that the animals use a variety of food-related and metabolic signals to compose their diet from a range of food items that differ in nutrient composition (Simpson and Raubenheimer 2001). Regulation of nutrient intake is postulated to involve integration of assessments of internal nutritional status and the nutrient composition of the food, with the selection of food items being modified by experience and learning (Fig. 3) (Rubio et al. 2003, 2005, 2006; Vivas et al. 2006; Yearsley et al. 2006; Black 2014).

The ability of fish to compose their diet on the basis of nutrient sensing, learning and experience is demonstrated by the results of macronutrient self-selection studies. When given access to feeds with different proportions of macronutrients, fish of various species soon learn to compose a diet that reflects their natural feeding habits. Carnivorous species, such as rainbow trout (*O. mykiss*) (Sánchez-Vázquez et al. 1999), European sea bass (*D. labrax*) (Aranda et al. 2000) and Senegalese sole (*Solea senegalensis*) (Rubio et al. 2009), all compose a protein-rich diet from individual feeds that consist largely of protein, lipid and carbohydrate. In contrast, goldfish (*Carassius auratus*) selects a diet with less protein and more carbohydrate, reflecting its omnivorous nature (Sánchez-Vázquez et al.

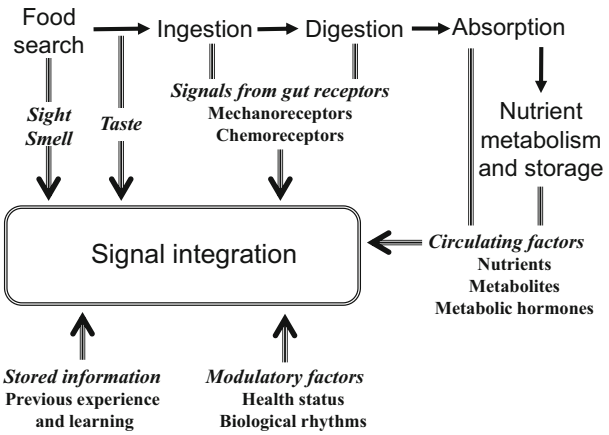

Fig. 3 Signals and factors that influence feeding responses, diet selection and food consumption

1998). Fish may be able to make dietary decisions based upon a range of nutritional criteria (Rubio et al. 2003, 2005, 2009; Almaida-Pagán et al. 2006; Vivas et al. 2006), and dietary adjustments can even be made solely on the basis of the evaluation of post-ingestional signals when macronutrients are encapsulated to mask the textural and chemical properties that stimulate receptors in the mouth (Rubio et al. 2003; Almaida-Pagán et al. 2006).

Selection studies have application in tests of palatability of feeds and feed ingredients. For example, a selection study can be used to examine whether or not a feed ingredient contains anti-nutritional factors (ANFs) that have feeding suppressant or deterrent properties, or that exert negative post-ingestive effects that result in reduced consumption (Kasumyan and Døving 2003). Selection studies can also be used to search for feed ingredients or chemicals, such as amino acids and nitrogenous metabolites, with properties that stimulate the gustatory (taste) receptors in the mouth and invoke ingestion of the food (Hara 1992, 1994; Kasumyan and Døving 2003). In addition, combinations of physiological and behavioural studies can be used to examine which chemicals can be added to feeds as incitants to stimulate the olfactory (smell) and extraoral gustatory (taste) receptors and induce the fish to search for food (Hara 1992, 1994, 2006; Kasumyan and Døving 2003).

Nutritional studies are deemed to be of high priority by both practitioners and researchers (Jones et al. 2014). Although information is available, increased knowledge is needed about the physiological effects of substituting plant protein sources for fishmeals in feeds (Tacchi et al. 2012; Gu et al. 2014a, b; Kortner et al. 2014). Fish nutritionists are accumulating information showing that several amino acids have metabolic and regulatory functions that extend beyond protein synthesis and as components of tissue proteins; these roles require further study (Li et al. 2009). In addition, fatty acids are known to regulate lipid metabolism by modulating gene expression, and the effects of individual fatty acids may differ (Coccia et al. 2014). There are also interactions between fatty acids that have an influence on patterns of biosynthesis and tissue deposition. This is an area for further research as a consequence of changes in the composition of oils in fish feeds resulting from an increased demand for fish oils that has driven the price upwards (Turchini et al. 2011; Kitessa et al. 2014; Shepherd and Bachis 2014).

The use of feed additives and the development of functional feeds for farmed fish are receiving increased attention (Holdt and Kraan 2011; Tacchi et al. 2011; Kortner et al.

2014; Merrifield and Ringø 2014; Newaj-Fyzul and Austin 2014; Salze and Davis 2015). Functional feeds can be defined as feeds that result in physiological benefits beyond fulfilling the basic nutritional requirements of a species. For example, a functional feed could improve health status and reduce disease incidence, and it is known, or suspected, that several feed components have prophylactic properties or act as immune-stimulants (Holdt and Kraan 2011; Merrifield and Ringø 2014; Newaj-Fyzul and Austin 2014; Reverter et al. 2014; Ringø et al. 2014; Song et al. 2014). These observations should stimulate further studies that could lead to the revision of recommendations about dietary inclusion levels of certain amino acids, fatty acids and other feed components.

Feed ingredients: changes in the offing

Many different ingredients are used to manufacture fish feeds, although there are a few staples that are major protein and lipid sources, such as fish products, legumes, oil-seeds and animal by-products (Hertrampf and Piedad-Pascual 2000; Gatlin et al. 2007; NRC 2011; Turchini et al. 2011; Kitessa et al. 2014). Grains, such as maize, usually provide the starch that is needed to bind the feed ingredients together. Fishmeals and marine fish oils have traditionally been used as major ingredients in dry pelleted feeds for intensive farming of carnivorous fish, such as salmonids and high-value marine species, but in recent years, there has been an increasing reliance upon plant-based products as sources of both protein and lipids. Increased information about the potential for, and limitations of, using plant-based, and other novel alternative, ingredients in fish feeds is in demand. Both aquaculture practitioners and researchers consider that studies aimed at improving the knowledge base should be given high priority (Jones et al. 2014).

There are benefits to be gained by including ingredients derived from plants in fish feeds, but there are also problems associated with using them (Hertrampf and Piedad-Pascual 2000; McKevith 2005; Gatlin et al. 2007; NRC 2011; Tacchi et al. 2012; Gu et al. 2014a, b; Kortner et al. 2014). For example, they are deficient in taurine, an amino acid-like compound, and cholesterol, both of which have important physiological functions in fish. Taurine is an essential nutrient for some life history stages of a number of animal species, including several marine fish species (Salze and Davis 2015). In addition, many plants have proteins that are deficient in some of the amino acids, such as lysine and methionine, required by fish. This means that there will often be a need to add amino acid supplements to feeds prepared with protein sources derived from plants. Improvements in micro-binding and micro-encapsulation technologies have made this a viable proposition, and supplementation with amino acids that compensate for deficiencies in plant protein sources is now a cost-effective way to produce feeds for farmed fish (Aragâo et al. 2014; Nunes et al. 2014). An additional disadvantage with plant products is that the oils derived from conventional terrestrial plants do not contain n-3 HUFAs, and many fish species are dependent upon a dietary supply of these fatty acids to develop normally and sustain growth and health (Tocher 2010; NRC 2011; Turchini et al. 2011; Kitessa et al. 2014; Shepherd and Bachis 2014). The increased use of plant oils in feeds for farmed fish also has the effect of reducing the n-3 HUFA concentrations in aquaculture products, with potentially negative marketing implications if the farmed fish are being promoted as health foods (Shepherd and Bachis 2014).

In addition to having proteins that may have an unfavourable balance of amino acids, and oils that lack some nutritionally important fatty acids, most plants contain one or more ANFs. ANFs are compounds that may affect the palatability of the feed, interfere with the

digestion and absorption of nutrients, reduce feed utilization, or have adverse metabolic effects (Francis et al. 2001; Gatlin et al. 2007; NRC 2011). Some of the negative effects of ANFs can be reduced or eliminated by various forms of treatments applied to feed ingredients of plant origin. These include the de-hulling of seeds to remove ANFs present in the seed coat, heat treatment to denature ANFs that are proteins, solvent extraction to remove ANFs that are soluble in water or organic solvents, and the use of bacterial fermentation to destroy the ANFs. In addition, treatment of plant products with exogenous enzymes, such as phytase and carbohydrases, may also have some beneficial effects as a result of enzymatic breakdown of the ANFs (NRC 2011; Castillo and Gatlin 2015).

Some would also consider it a problem that non-transgenic (non-GM) plant products are becoming increasingly scarce in the global market (Flachowsky et al. 2005; Gu et al. 2014a; Swiatkiewicz et al. 2014; Van Eenennaam and Young 2014). This means that it is becoming increasingly difficult to obtain plant-derived feed ingredients, e.g. soya, maize and canola, guaranteed to be free from some form of biotechnological genetic modification. At present, genetically engineered crops are being grown on over 175 million hectares of land in about 30 countries. Even though there is resistance in some quarters to using these crop plants in feeds and food products, various forms of plant genome and metabolic engineering are almost certain to continue, perhaps at an increasing rate (Lau et al. 2014; Ronald 2014; Swiatkiewicz et al. 2014; Van Eenennaam and Young 2014; Voytas and Gao 2014).

Knowledge about the metabolic pathways involved in fatty acid synthesis in marine unicellular organisms has opened a route for the transgenic modification of terrestrial plants to produce oils that resemble those extracted from marine fish species (Guschina and Harwood 2006; Venegas-Caleron et al. 2010; Turchini et al. 2011; Petrie et al. 2012, 2014; Kitessa et al. 2014; Ruiz-Lopez et al. 2014). Alternative strategies to obtain oils rich in n-3 HUFAs would be to utilize microbial fermentation or extract oils directly from microalgae cultured specifically for this purpose under photoautotrophic conditions (Turchini et al. 2011; Kitessa et al. 2014; Ryckebosch et al. 2014), possibly following a genetic enhancement using transgenic technology to modify fatty acid compositions or increase yields of the desired n-3 HUFAs (Gong et al. 2014; Rasala et al. 2014).

A boost on the plus side for the genetic engineering of plants relates to the development of genome editing techniques (Ronald 2014; Voytas and Gao 2014). In the editing techniques, breaks are introduced into the genome at specific sites, and the repair of the break is used to introduce DNA sequence changes or deletions. For example, deletions, or specific gene knockouts, could lead to the generation of plants with reduced concentrations of ANFs, and deletion of certain fatty acid desaturase enzymes allows accumulation of oils that contain high concentrations of monounsaturated fatty acids (MUFAs) rather than n-6 polyunsaturates (n-6 PUFAs) (Voytas and Gao 2014). Genome editing can be used to induce genetic variation without transgenic modification. This may, perhaps, lead to crop plants produced using genome editing techniques being more socially acceptable than those generated by transgenic engineering.

Natural plant products and extracts are being increasingly used as replacers for chemotherapeutics for disease control and management in aquaculture. Some bioactive molecules, such as alkaloids, terpenoids, saponins and flavonoids and various oligosaccharides, act as immune-stimulants, have anti-bacterial or anti-parasitic properties, or lead to changes in the composition of the gut microbiota that promote the health and well-being of the host (Gaggia et al. 2010; Nayak 2010a, b; Ringø et al. 2010, 2014; Lazado and Caipang 2014; Merrifield and Ringø 2014; Newaj-Fyzul and Austin 2014; Reverter et al. 2014; Song et al. 2014). Several plant products influence feeding and digestion, and some

compounds present in plant extracts have growth-promoting properties (Newaj-Fyzul and Austin 2014; Reverter et al. 2014). Work to more fully investigate the potential of these natural plant products as feed additives is likely to be intensified.

Feed ingredient assessment: something old something new

Irrespective of their type and source, ingredients that are thought to have potential for inclusion in fish feeds should be thoroughly assessed before being taken into general use (Houlihan et al. 2001; Glencross et al. 2007; NRC 2011). Assessment involves several stages, the first of which is chemical analysis. The chemical analysis of feed ingredients, feeds and food products has a long history, and the proximate analysis classification was introduced over a century ago. This classification system recognizes six fractions, with each fraction comprising a number of components (Table 1). This system is still used as the standard reference even though there are problems, both with regard to the chemical analyses and the characterization and identification of the different components. For example, the lack of specificity of the Kjeldahl method for analysis of protein, and the potential for fraudsters to exploit this by adulteration of feeds with cheap sources of non-protein nitrogen, has been known for a long time. Addition of nitrogen-rich industrial chemicals, such as urea or melamine, to an animal feed or food product will give inflated values for crude protein (N × 6.25) when samples are analysed using the Kjeldahl method (Moore et al. 2010, 2012; Everstine et al. 2013; Domingo et al. 2014).

Although sporadic cases of adulteration had been reported, and others suspected, this became a significant issue of concern in 2008. It was revealed that melamine (66.7 % N by mass) had been used to adulterate milk produced in China, resulting in renal toxicity and several deaths of young children (Moore et al. 2010, 2012; Everstine et al. 2013; Domingo et al. 2014). Thereafter, there was investigation of a wider range of products, several of which were shown to have been adulterated with melamine; these products included animal feeds, fish and shrimp feeds and pet-foods. This resulted in the initiation of studies

Table 1 Proximate chemical analysis system used for classification of the composition of feed ingredients, feeds and food products

Fraction	Analysis	Main contributory components
Moisture (M)	Oven-dry to constant weight at 100–105 °C	Water (and volatile acids and bases)
Ash	Ignite in muffle oven at 450–550 °C to remove organic compounds	Inorganic salts (minerals) (some sulphur and phosphorus from proteins and other organic compounds)
Crude protein (CP)	Kjeldahl digestion in sulphuric acid followed by distillation and NH_3 determination ($CP = N \times 6.25$)	Proteins, amino acids, amines, nucleic acids, nitrogenous glycosides, glycolipids, B-vitamins
Ether extract (EE)	Reflux extraction with petroleum ether (di-ethyl ether) (alternatively dichloromethane or hexane), sometimes following hydrolysis in strong acid	Fats, oils, waxes, sterols, lipid-soluble vitamins, organic acids, pigments (fatty acids present in polar lipids)
Crude fibre (CF)	Treatment of EE residue with boiling acid and boiling alkali	Cellulose, hemicelluloses, lignin
Nitrogen-free extractives (NFE)	Estimated by difference $NFE = 100 - (M + Ash + CP + EE + CF)$	Starch, sugars, pectins, fructans (some CF, water-soluble vitamins, resins, organic acids)

to examine toxic effects, and pathways of elimination of melamine and related compounds in fish (Liu et al. 2014). The analytical methods used to detect melamine include chromatographic and vibrational spectroscopy techniques, such as near infrared (NIR) and FT-Raman spectroscopy. In addition to NIR techniques, liquid and gas chromatography are commonly used for the investigation of possible adulteration of feeds and food products, and there is also an increasing use of mass spectroscopy in such investigations (Moore et al. 2012; Cubero-Leon et al. 2014).

With the passage of time, the methods used to analyse feed ingredients, animal feeds and food products have been modified, improved and automated; new methods of analysis have been developed and some of these have been adopted for commercial use (Moore et al. 2010; Otter 2012; Cubero-Leon et al. 2014). There may also be a need to analyse feed ingredients, feeds and food products for the presence of rDNA or specific proteins that result from GM events, with polymerase chain reaction (PCR) and enzyme-linked immunosorbent assay (ELISA) being most often used (Van Eenennaam and Young 2014). In addition, some industrial companies and regulatory authorities presently use NIR or FT-Raman spectroscopy for routine analysis of feed ingredients, feeds and food products. Other techniques that are increasingly being used include combinations of mass spectroscopy and gas or liquid chromatography, and nuclear magnetic resonance (Cubero-Leon et al. 2014). These techniques are usually combined with chemometrics to provide an analysis of the data. Chemometrics involves using multivariate data analysis as a reduction tool to group or classify elements within large data-sets (Moore et al. 2012; Cubero-Leon et al. 2014).

Once the chemical composition of a feed ingredient has been analysed, the next stage involves an assessment of how effectively the fish digest and absorb the nutrients it contains. Although several in vitro assays, such as the pH–stat method, have been developed as proxies (Yasumara and Lemos 2014), none of them have been widely adopted for routine use. General acceptance of in vitro assays has been hindered because they have not been adjudged to produce sufficiently consistent and reliable results, and there is also scepticism about how well they mimic the digestive and processes that occur in living fish. Consequently, the assessment is most often done by carrying out a digestibility trial using live fish (Fig. 4).

Usually, an indirect approach is adopted when digestibility trials are conducted (Houlihan et al. 2001; Glencross et al. 2007; NRC 2011). This is because it is extremely difficult to obtain accurate information about the amounts of a feed that are consumed, and it is almost impossible to collect all of the faeces produced. A number of conditions must be satisfied if valid results are to be obtained in a digestibility trial carried out using the indirect method. Firstly, a reference feed containing an indigestible, inert marker that can be used for the calculation of nutrient digestibility is needed. The percentages of digested and absorbed nutrients in the reference feed are then calculated from the ratios of nutrients and marker in the feed and faeces. A test feed is then prepared by adding a given amount of the test ingredient to the reference feed. The test feed is then given to the fish. For assessment of the efficiency with which the fish digests and absorbs the nutrients representative samples of both the test feed and fish faeces are required for analysis (Houlihan et al. 2001; Glencross et al. 2007; NRC 2011). Once the data for the digestibility of nutrients in the test feed have been obtained, these need to be combined with digestibility information for the reference feed for estimation of the digestibility of the nutrients present in the test ingredient (Fig. 4) (Glencross et al. 2007; NRC 2011).

A number of assumptions are made when carrying out digestibility trials, and these may be violated under some test conditions and when certain ingredients are being assessed.

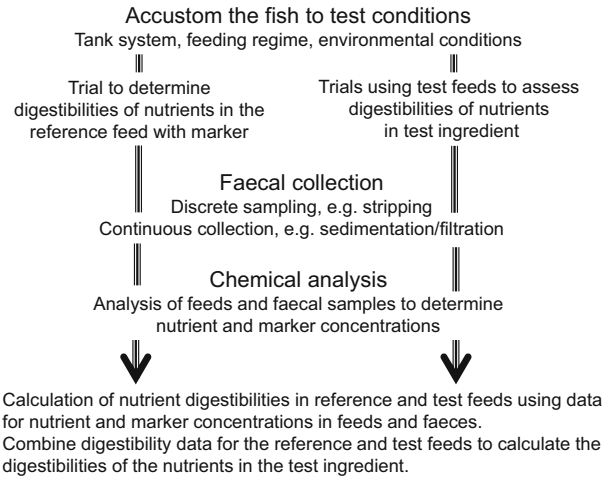

Fig. 4 Procedure for assessment of nutrient digestibilities in feed ingredients using the indirect marker method. Nutrient digestibilities (%) in feeds are calculated as $100 - 100([M_D/M_F] \times [N_F/N_D])$, where M_D and M_F are the concentrations of the marker in the feed (diet) and faeces, and N_F and N_D are nutrient concentrations in the faeces and feed (diet), respectively. The digestibilities of nutrients present in test ingredients can be estimated using the formula $[AD_T \times N_T - (AD_R \times N_R \times P_R)]/(P_I \times N_I)$, where AD_T and AD_R are the digestibilities of the test feed and reference feed, respectively; N_T, N_R and N_I are the nutrient concentrations in the test feed, reference feed and test ingredient, respectively; and P_R and P_I are the proportions of the reference feed and test ingredient used to make the test feed, respectively

One key assumption is that the marker is inert, and a second is that representative samples of faeces can be collected for analysis. An additional assumption is that there is independence in the efficiencies with which the nutrients supplied by different ingredients are digested and absorbed. This means that it is assumed that the feed components do not interact and influence the calculated values of nutrient digestibility when ingredients are combined to form a feed (Houlihan et al. 2001; Glencross et al. 2007; NRC 2011), but this is not always the case (Harter et al. 2014). Interaction effects can be examined by carrying out trials in which several test feeds are prepared using different combinations of the reference feed and test ingredient, e.g. 85:15, 80:20, 75:25, 70:30. Should the calculated values of digestibility of nutrients in the test ingredient differ across feeds, then interaction effects can be suspected. Nutrient digestibility is also influenced by a number of extraneous factors, such as feeding rate, water temperature and feed processing conditions, so the digestibility of a feed ingredient is not a single fixed numerical value (Houlihan et al. 2001; Glencross et al. 2007; NRC 2011). Given the assumptions and prerequisites, it is clear that precautions need to be taken when designing and carrying out digestibility trials, and care must be exercised when interpreting and applying the results obtained.

An assessment of feed palatability should also be made, especially if the test ingredient is plant material suspected to contain ANFs that could interfere with the ingestion of a feed. Palatability can be assessed by measuring ingestion when fish are fed a single type of feed in excess, by carrying out selection studies or by monitoring feeding activity when the fish are using self-feeders (Houlihan et al. 2001). An additional important aspect of ingredient assessment involves the carrying out of growth trials to monitor the utilization of nutrients in the feed ingredient. The carrying out of such studies poses specific practical problems relating to the accurate assessment of feed consumption by the fish, either as

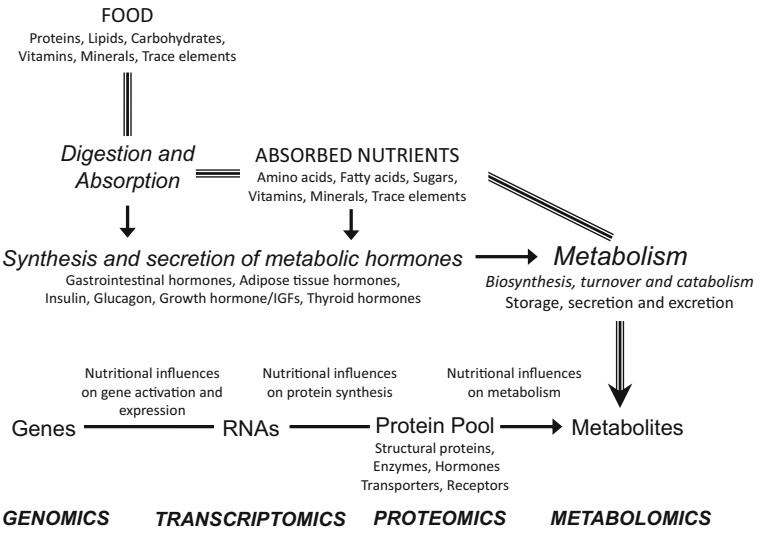

Fig. 5 When food is digested and absorbed, the nutrients influence gene activation and transcription, enzyme activities and metabolism. Gene expression profiling (transcriptomics) along with monitoring of protein expression (proteomics) and metabolites (metabolomics) provide holistic overviews of these nutrient-induced changes

individuals or in groups. Several methods have been developed for monitoring feed intake in fish, including the use of X-radiography, self-feeders and waste-feed collectors of various designs (Jobling et al. 1995; Houlihan et al. 2001; Li et al. 2014).

Growth trials carried out for assessment of feed utilization will often include an evaluation of the physiological consequences that may be associated with the inclusion of an ingredient in a complete feed. These might include reduced digestion and absorption or metabolic disturbances caused by ANFs, or altered hormone secretion that leads to changes in enzyme activity, biosynthesis and tissue growth. Techniques involving genomics, transcriptomics, proteomics, metabolomics and bioinformatics (Fig. 5) are increasingly being used in dietary studies to obtain holistic information relating to the effects of individual nutrients or nutrient groups on gene regulation and the downstream effects that these can exert (Sundell and Power 2008; Zdunczyk and Pareek 2008; Wang et al. 2009; NRC 2011; Tacchi et al. 2011, 2012; Rodrigues et al. 2012; Black 2014). Although a growth bioassay is the preferred method for evaluating nutrient utilization, growth trials are expensive and time-consuming to run. There may, therefore, be good reasons to strive towards developing alternative methods for the assessment of nutrient bioavailability and metabolic utilization (Elango et al. 2012; Rodrigues et al. 2012; Black 2014).

Finally, the investigation of the functional properties of the ingredient may be deemed to be important (Houlihan et al. 2001; Glencross et al. 2007; NRC 2011). Functional properties refer to the effects that the ingredient has on the physical characteristics of a complete feed. The ingredient may, for example, have binding properties that improves the water-stability and durability of the feed and reduces the production of dust and fines, or the ingredient may have characteristics that influence pellet porosity, hardness and sinking rates (Glencross et al. 2007; NRC 2011).

A Parthian shot

We know where we have been in fish nutrition, and we have quite a good understanding of where we are, but what directions can we foresee that fish nutrition research might take in the near future? What are the immediate, pressing challenges and problems that need to be solved?

If the predicted increase in production of farmed fish is to become a reality, ways will need to be found to fulfil the projected increases in feed demands. It is clear that global supplies of fishmeal and fish oil will be insufficient to meet these demands. There are also ethical reasons for reducing the tonnages of marine fish that are rendered to produce fishmeals and oils for inclusion in animal feeds. Reducing the use of fishmeals and fish oils for feed production means that the use of alternative feed ingredients must be increased. This potentially generates a number of nutritional and marketing problems, several of which have been discussed in previous sections of this review. It is also likely that there will be an increase in the diversity of species that are farmed, and for marine species, this brings urgency to the finding of solutions to the problem of larval nutrition. We are aware that these problems exist and some of them are being addressed.

Irrespective of the challenge and type of nutritional problem under investigation:

- larval feeding and development (Richard et al. 2014).
- alternative protein sources (Tacchi et al. 2012; Gu et al. 2014a, b; Kortner et al. 2014).
- essential fatty acid supplies and metabolism (Betancor et al. 2014; Xue et al. 2014).
- ANFs and nutrient interactions (Gu et al. 2014a, b).
- the effects of dietary components on the composition of the gut microbiota (Merrifield and Ringø 2014; Newaj-Fyzul and Austin 2014; Ringø et al. 2014).
- the influence of diet on the immune system, health and well-being (Lazado and Caipang 2014; Merrifield and Ringø 2014; Newaj-Fyzul and Austin 2014).

new methods and techniques will be used to an increasing extent, and the application of genomics, transcriptomics, proteomics, metabolomics and bioinformatics (Fig. 5) is likely to become routine in fish nutrition research.

There is international legislation aimed at ensuring that animal feeds and food products do not pose health risks for consumers, but there have been many cases of food-related problems that have been traced to the composition of animal feeds. Routine monitoring programmes have been established in a number of countries in an attempt to reduce the risk of contaminated food products reaching the consumer (Sissener et al. 2013; Van Eenennaam and Young 2014). For example, forensic methods have been used to identify, and trace the source of, contaminants, such as dioxins and PCBs, that have been detected in a range of animal food products, including milk, eggs and farmed fish (Hites et al. 2004; Carlson and Hites 2005; Montory and Barra 2006; Malisch and Kotz 2014). The majority of the body burden of dioxins and PCBs in animals is derived from the diet, and these chemicals tend to accumulate in the fatty tissues. Consequently, when dioxins and PCBs are detected in animal food products, the most likely source is the feed. Animal feed components that have been implicated in contamination incidents include kaolinitic clay and ball clay (hydrated aluminium silicate) that are added to animal feeds as anti-caking and flowing agents, recycled cooking oils and fats, and marine fish oils (Perugini et al. 2013; Sissener et al. 2013; Malisch and Kotz 2014).

DNA-based methods are being increasingly used to identify, and trace the source of, biological materials, including fish species (Rasmussen and Morrissey 2008, 2009; Teletchea 2009). The DNA-based identification techniques rely upon there being polymorphisms in the

genetic codes of different species. It is usually mitochondrial DNA (mtDNA) that is used for species identification, with three gene regions serving as the markers of choice; cytochrome *b*, cytochrome oxidase and 16s RNA (Rasmussen and Morrissey 2008, 2009; Teletchea 2009). DNA-based identification techniques have been mostly used to investigate species substitution and mislabelling (Everstine et al. 2013), but these techniques may also have application in the testing of feed ingredients and animal feeds. For example, DNA-based methods, along with NIR techniques, can be used in feed analysis, to check the accuracy of the product declaration and to examine for the presence of prohibited ingredients, such as some rendered terrestrial animal by-products in fish feeds (Tena et al. 2014).

The potential for changing the nutritional requirements and the chemical compositions of fish using transgenic technologies has not been covered in this review, even though proof-of-concept has been demonstrated (Cheng et al. 2014). For example, it is possible to increase tissue concentrations of n-3 HUFAs by manipulating fish to overexpress the elongase and desaturase enzymes required for their synthesis from linolenic acid (18:3 n-3) as the precursor. In addition, double transgenesis, involving the introduction of genes coding for the appropriate desaturases, has the potential to generate fish that are able to synthesize n-3 HUFAs from either linoleic (18:2 n-6) or oleic acid (18:1 n-9) as precursors. This would potentially reduce the dietary need for 18:3 n-3 and preformed n-3 HUFAs (Pang et al. 2014). This is a facet of fish nutrition research that will probably be increasingly explored in the years to come, even though there will be a continuing need to monitor the attitudes of the consuming public to the use of genetically modified (GM) animals in food production. At present, there appears to be low acceptance for the introduction of GM animals into the human food chain, and the use of GM animals in food products is generally viewed as imposing a higher risk than the use of GM plants (Frewer et al. 2014).

References

Almaida-Pagán PF, Rubio VC, Mendiola P et al (2006) Macronutrient selection through post-ingestive signals in sharpnose seabream fed gelatin capsules and challenged with protein dilution. Physiol Behav 88:550–558

Aragão C, Colen R, Ferreira S et al (2014) Microencapsulation of taurine in Senegalese sole diets improves its metabolic availability. Aquaculture 431:53–58

Aranda A, Sánchez-Vázquez FJ, Zamora S, Madrid JA (2000) Self-design of fish diets by means of self-feeders: validation of procedures. J Physiol Biochem 56:155–166

Bell JG, Farndale BM, Bruce MP et al (1997) Effects of broodstock dietary lipid on fatty acid compositions of eggs from sea bass (*Dicentrarchus labrax*). Aquaculture 149:107–119

Betancor MB, Howarth FJE, Glencross BD, Tocher DR (2014) Influence of dietary docosahexaenoic acid in combination with other long-chain polyunsaturated fatty acids on expression of biosynthesis genes and phospholipid fatty acid compositions in tissues of post-smolt Atlantic salmon (*Salmo salar*). Comp Biochem Physiol B 172–173:74–89

Black JL (2014) Brief history and future of animal simulation models for science and application. Anim Prod Sci 54:1883–1895

Brooks S, Tyler CR, Sumpter JR (1997) Quality in fish: what makes a good egg? Rev Fish Biol Fish 7:387–416

Carlson DL, Hites RA (2005) Polychlorinated biphenyls in salmon and salmon feed: global differences and bioaccumulation. Environ Sci Technol 39:7389–7395

Carpenter KJ (2003a) A short history of nutritional science: part 1 (1785–1885). J Nutr 133:638–645

Carpenter KJ (2003b) A short history of nutritional science: part 2 (1885–1912). J Nutr 133:975–984

Carpenter KJ (2003c) A short history of nutritional science: part 3 (1912–1944). J Nutr 133:3023–3032

Carpenter KJ (2003d) A short history of nutritional science: part 4 (1945–1985). J Nutr 133:3331–3342

Castillo S, Gatlin DM III (2015) Dietary supplementation of exogenous carbohydrase enzymes in fish nutrition: a review. Aquaculture 435:286–292

Cheng Q, Su B, Qin Z et al (2014) Interaction of diet and masou salmon Δ5-desaturase transgene on Δ6-desaturase and stearoyl-CoA desaturase gene expression and n-3 fatty acid level in common carp (*Cyprinus carpio*). Transgenic Res 23:729–742
Coccia E, Varricchio E, Vito P et al (2014) Fatty acid-specific alterations in leptin, PPARα, and CPT-1 gene expression in the rainbow trout. Lipids 49:1033–1046
Conceicão LEC, Yúfera M, Makridis P et al (2010) Live feeds for early stages of fish rearing. Aquac Res 41:613–640
Cowey CB, Sargent JR (1972) Fish nutrition. Adv Mar Biol 10:383–492
Cubero-Leon E, Peñalver R, Maquet A (2014) Review on metabolomics for food authentication. Food Res Int 60:95–107
Dhert P, King N, O'Brien E (2014) Stand-alone live food diets, an alternative to culture and enrichment diets for rotifers. Aquaculture 431:59–64
Domingo E, Tirelli AA, Nunes CA et al (2014) Melamine detection in milk using vibrational spectroscopy and chemometrics analysis: a review. Food Res Int 60:131–139
Elango R, Levesque C, Ball RO, Pencharz PB (2012) Available versus digestible amino acids—new stable isotope methods. Br J Nutr 108:S306–S314
Everstine K, Spink J, Kennedy S (2013) Economically motivated adulteration (EMA) of food: common characteristics of EMA incidents. J Food Prot 76:723–735
Finn RN, Fyhn HJ (2010) Requirement for amino acids in ontogeny of fish. Aquac Res 41:684–716
Flachowsky G, Chesson A, Aulrich K (2005) Animal nutrition with feeds from genetically modified plants. Arch Anim Nutr 59:1–40
Francis G, Makkar HPS, Becker K (2001) Antinutritional factors present in plant-derived alternate fish feed ingredients and their effects in fish. Aquaculture 199:197–227
Frewer LJ, Coles D, Houdebine L-M, Kleter GA (2014) Attitudes towards genetically modified animals in food production. Br Food J 116:1291–1313
Gaggia F, Mattarelli P, Biavati B (2010) Probiotics and prebiotics in animal feeding for safe food production. Int J Food Microbiol 141:S15–S28
Gatlin DM III, Barrows FT, Brown P et al (2007) Expanding the utilization of sustainable plant products in aquafeeds: a review. Aquac Res 38:551–579
Glencross BD, Booth M, Allan GL (2007) A feed is only as good as its ingredients—a review of ingredient evaluation strategies for aquaculture feeds. Aquac Nutr 13:17–34
Gong Y, Wan X, Jiang M et al (2014) Metabolic engineering of microorganisms to produce omega-3 very long-chain polyunsaturated acids. Prog Lipid Res 56:19–35
Gu J, Bakke AM, Valen EC et al (2014a) *Bt*-maize (MON810) and non-GM soybean meal in diets for Atlantic salmon (*Salmo salar* L.) juveniles—impact on survival, growth performance, development, digestive function, and transcriptional expression of intestinal immune and stress responses. PLoS ONE 9:399932
Gu M, Kortner TM, Penn M et al (2014b) Effects of dietary plant meal and soya-saponin supplementation on intestinal and hepatic lipid droplet accumulation and lipoprotein and sterol metabolism in Atlantic salmon (*Salmo salar* L.). Br J Nutr 111:432–444
Guschina IA, Harwood JL (2006) Lipids and lipid metabolism in eukaryotic algae. Prog Lipid Res 45:160–186
Halver JE (ed) (1972) Fish nutrition. Academic Press, New York
Halver JE (ed) (1989) Fish nutrition, 2nd edn. Academic Press, San Diego
Halver JE, Hardy RW (eds) (2002) Fish nutrition, 3rd edn. Academic Press, San Diego
Hara TJ (ed) (1992) Fish chemoreception. Chapman and Hall, London
Hara TJ (1994) The diversity of chemical stimulation of fish olfaction and gustation. Rev Fish Biol Fish 4:1–35
Hara TJ (2006) Feeding behaviour in some teleosts is triggered by single amino acids primarily through olfaction. J Fish Biol 68:810–825
Harter TS, Heinsbroek LTN, Schrama JW (2014) The source of dietary non-protein energy affects in vivo protein digestion in African catfish (*Clarias gariepinus*). Aquac Nutr. doi:10.1111/anu.12185
Hertrampf JW, Piedad-Pascual F (2000) Handbook on Ingredients for aquaculture feeds. Kluwer Academic, Dordrecht
Hites RA, Foran JA, Carpenter DO et al (2004) Global assessment of organic contaminants in farmed salmon. Science 303:226–229
Holdt SL, Kraan S (2011) Bioactive compounds in seaweed: functional food applications and legislation. J Appl Phycol 23:543–597
Holt GJ (ed) (2011) Larval fish nutrition. Wiley, Oxford
Houlihan D, Boujard T, Jobling M (eds) (2001) Food intake in fish. Blackwell Science, Oxford

Huet M (1986) Textbook of fish culture: breeding and cultivation of fish, 2nd edn. Fishing News Books, Farnham
Jobling M, Arnesen AM, Baardvik BM et al (1995) Monitoring feeding behaviour and food intake: methods and applications. Aquac Nutr 1:131–143
Jones AC, Mead A, Kaiser MJ et al (2014) Prioritization of knowledge needs for sustainable aquaculture: a national and global perspective. Fish Fish. doi:10.1111/faf.12086
Kamler E (2008) Resource allocation in yolk-feeding fish. Rev Fish Biol Fish 18:143–200
Kasumyan AO, Døving KB (2003) Taste preferences in fishes. Fish Fish 4:289–347
Kitessa SM, Abeywardena M, Wijesundera C, Nichols PD (2014) DHA-containing oilseed: a timely solution for the sustainability issues surrounding fish oil sources of the health-benefitting long-chain omega-3 oils. Nutrients 6:2035–2058
Kjørsvik E, Magnor-Jensen A, Holmefjord I (1990) Egg quality in fishes. Adv Mar Biol 26:71–113
Kortner TM, Björkheim I, Krasnov A et al (2014) Dietary cholesterol supplementation to a plant-based diet suppresses the complete pathway of cholesterol synthesis and induces bile acid production in Atlantic salmon (*Salmo salar* L.). Br J Nutr 111:2089–2103
Lau W, Fischbach MA, Osbourn A, Sattely ES (2014) Key applications of plant metabolic engineering. PLoS ONE 12:e1001879
Lazado CC, Caipang CMA (2014) Mucosal immunity and probiotics in fish. Fish Shellfish Immunol 39:78–89
Li P, Mai K, Trushenski J, Wu G (2009) New developments in fish amino acid nutrition: towards functional and environmentally orientated aquafeeds. Amino Acids 37:43–53
Li W, Wei QW, Luo H (2014) Special collector and count method in a recirculating aquaculture system for calculation of feed conversion ratio in fish. Aquacult Eng 60:63–67
Liu H, Xue M, Wang J et al (2014) Tissue deposition and residue depletion in rainbow trout following continuous voluntary feeding with various levels of melamine or a blend of melamine and cyanuric acid. Comp Biochem Physiol C 166:51–58
Mæhre HK, Hamre K, Elvevoll EO (2013) Nutrient evaluation of rotifers and zooplankton: feed for marine fish larvae. Aquac Nutr 19:301–311
Malisch R, Kotz A (2014) Dioxins and PCBs in feed and food—review from European perspective. Sci Total Environ 491–492:2–10
McKevith B (2005) Nutritional aspects of oilseeds. Nutr Bull 30:13–26
McLarney W (2013) Freshwater aquaculture: a handbook for small scale fish culture in North America. Echo Point Books and Media, Brattleboro
Merrifield D, Ringø E (eds) (2014) Aquaculture nutrition: gut health, probiotics and prebiotics. Wiley, Chichester
Montory M, Barra R (2006) Preliminary data on polybrominated diphenyl ethers (PBDEs) in farmed fish tissues (*Salmo salar*) and fish feed in Southern Chile. Chemosphere 63:1252–1260
Moore JC, DeVries JW, Lipp M et al (2010) Total protein methods and their potential utility to reduce the risk of food protein adulteration. Compr Rev Food Sci Food Saf 9:330–357
Moore JC, Spink J, Lipp M (2012) Development and application of a database of food ingredient fraud and economically motivated adulteration from 1980 to 2010. J Food Sci 77:R118–R126
Nash CE (2011) The history of aquaculture. Wiley, Ames
Nayak SK (2010a) Role of gastrointestinal microbiota in fish. Aquac Res 41:1553–1573
Nayak SK (2010b) Probiotics and immunity: a fish perspective. Fish Shellfish Immunol 29:2–14
Naylor RL, Hardy RW, Bureau DP et al (2009) Feeding aquaculture in an era of finite resources. Proc Natl Acad Sci USA 106:15103–15110
Newaj-Fyzul A, Austin B (2014) Probiotics, immunostimulants, plant products and oral vaccines, and their role as feed supplements in the control of bacterial fish diseases. J Fish Dis. doi:10.1111/jfd.12313
Nicholson JK, Holmes E, Kinross J et al (2012) Host-gut microbiota metabolic interactions. Science 336:1262–1267
NRC (National Research Council) (1973) Nutrient requirements of domestic animals; 11 Nutrient requirements of trout, salmon and catfish. National Research Council, Washington
NRC (National Research Council) (1993) Nutrient requirements of fish. National Academy Press, Washington
NRC (National Research Council) (2011) Nutrient requirements of fish and shrimp. The National Academies Press, Washington
Nunes AJP, Sá MVC, Browdy CL, Vazquez-Anon M (2014) Practical supplementation of shrimp and fish feeds with crystalline amino acids. Aquaculture 431:20–27
Olsen Y, Evjemo JO, Kjørsvik E et al (2014) DHA content in dietary phospholipids affects DHA content in phospholipids of cod larvae and larval performance. Aquaculture 428–429:203–214

Otter DE (2012) Standardised methods for amino acid analysis of food. Br J Nutr 108:S230–S237
Pang S-C, Wang H-P, Li K-Y et al (2014) Double transgenesis of humanized *fat1* and *fat2* genes promotes omega-3 polyunsaturated fatty acids synthesis in zebrafish model. Mar Biotechnol 16:580–593
Perugini M, Manera M, Tavoloni T et al (2013) Temporal trends of PCBs in feed and dietary influence in farmed rainbow trout (*Oncorhynchus mykiss*). Food Chem 141:2321–2327
Petrie JR, Shrestha P, Zhou X-R et al (2012) Metabolic engineering plant seeds with fish oil-like levels of DHA. PLoS ONE 7:e49165
Petrie JR, Shrestha P, Belide S et al (2014) Metabolic engineering *Camelina sativa* with fish oil-like levels of DHA. PLoS ONE 9:e85061
Phillips AM Jr (1969) Nutrition, digestion and energy utilization. In: Hoar WS, Randall DJ (eds) Fish physiology, vol I. Academic Press, Orlando, pp 391–432
Rasala BA, Chao S-S, Pier M et al (2014) Enhanced genetic tools for engineering multigene traits into green algae. PLoS ONE 9:e94028
Rasdi NW, Qin JG (2014) Improvement of copepod nutritional quality as live food for aquaculture: a review. Aquac Res. doi:10.1111/are.12471
Rasmussen RS, Morrissey MT (2008) DNA-based methods for the identification of commercial fish and seafood species. Compr Rev Food Sci Food Saf 7:280–294
Rasmussen RS, Morrissey MT (2009) Application of DNA-based methods to identify fish and seafood substitution on the commercial market. Compr Rev Food Sci Food Saf 8:118–154
Raubenheimer D, Simpson SJ, Mayntz D (2009) Nutrition, ecology and nutritional ecology: toward an integrated framework. Funct Ecol 23:4–16
Reverter M, Bontemps N, Lecchini D et al (2014) Use of plant extracts in fish aquaculture as an alternative to chemotherapy: current status and future perspectives. Aquaculture 433:50–61
Richard N, Fernández I, Wulff T et al (2014) Dietary supplementation with vitamin K affects transcriptome and proteome of Senegalese sole, improving larval performance and quality. Mar Biotechnol 16:522–537
Ringø E, Olsen RE, Gifstad TØ et al (2010) Prebiotics in aquaculture: a review. Aquac Nutr 16:117–136
Ringø E, Olsen RE, Jensen I et al (2014) Application of vaccines and dietary supplements in aquaculture: possibilities and challenges. Rev Fish Biol Fish 24:1005–1032
Rodrigues PM, Silva TS, Dias J, Jessen F (2012) Proteomics in aquaculture: applications and trends. J Proteomics 75:4325–4345
Røjbek MC, Støttrup JG, Jacobsen C et al (2014) Effects of dietary fatty acids on the production and quality of eggs and larvae of Atlantic cod (*Gadus morhua* L.). Aquac Nutr 20:654–666
Ronald PC (2014) Lab to farm: applying research on plant genetics and genomics to crop improvement. PLoS ONE 12:e1001878
Rubio VC, Sánchez-Vázquez FJ, Madrid JA (2003) Macronutrient selection through postingestive signals in sea bass fed on gelatine capsules. Physiol Behav 78:795–803
Rubio VC, Sánchez-Vázquez FJ, Madrid JA (2005) Fish macronutrient selection through post-ingestive signals: effect of selective macronutrient deprivation. Physiol Behav 84:651–657
Rubio VC, Sánchez-Vázquez FJ, Madrid JA (2006) Oral serotonin administration affects the quantity and the quality of macronutrients selection in European sea bass *Dicentrarchus labrax* L. Physiol Behav 87:7–15
Rubio VC, Boluda Navarro D, Madrid JA, Sánchez-Vázquez FJ (2009) Macronutrient self-selection in *Solea senegalenis* fed macronutrient diets and challenged with dietary protein dilutions. Aquaculture 291:95–100
Ruiz-Lopez N, Haslam RP, Napier JA, Sayanova O (2014) Successful high-level accumulation of fish oil omega-3 long-chain polyunsaturated fatty acids in a transgenic oilseed crop. Plant J 77:198–208
Ryckebosch E, Bruneel C, Termote-Verhalle R et al (2014) Nutritional evaluation of microalgae oils rich in omega-3 long chain fatty acids as an alternative for fish oil. Food Chem 160:393–400
Salze GP, Davis DA (2015) Taurine: a critical nutrient for future fish feeds. Aquaculture 437:215–229
Sánchez-Vázquez FJ, Yamamoto T, Akiyama T et al (1998) Selection of macronutrients by goldfish operating self-feeders. Physiol Behav 65:211–218
Sánchez-Vázquez FJ, Yamamoto T, Akiyama T et al (1999) Macronutrient self-selection through demand-feeders in rainbow trout. Physiol Behav 66:45–51
Semba RD (2012) The discovery of the vitamins. Int J Vitam Nutr Res 82:310–315
Shearer KD (2000) Experimental design, statistical analysis and modelling of dietary nutrient requirement studies for fish: a critical review. Aquac Nutr 6:91–102
Shepherd J, Bachis E (2014) Changing supply and demand for fish oil. Aquac Econ Manag 18:395–416
Simpson SJ, Raubenheimer D (2001) A framework for the study of macronutrient intake in fish. Aquac Res 32:421–432

Sissener NH, Julshamn K, Espe M et al (2013) Surveillance of selected nutrients, additives and undesirables in commercial Norwegian fish feeds in the years 2000–2010. Aquac Nutr 19:555–572
Song SK, Beck BR, Kim D et al (2014) Prebiotics as immunostimulants in aquaculture: a review. Fish Shellfish Immunol 40:40–48
Støttrup J, McEvoy L (eds) (2002) Live feeds in marine aquaculture. Blackwell Science, Oxford
Sundell K, Power D (eds) (2008) Special issue: functional genomics in sustainable aquaculture. Rev Fish Sci 16(Supplement 1):1–166
Swiatkiewicz S, Swiatkiewicz M, Arczewska-Wlosek A, Jozefiak D (2014) Genetically modified feeds and their effect on the metabolic parameters of food-producing animals: a review of recent studies. Anim Feed Sci Technol 198:1–19
Tacchi L, Bickerdike R, Douglas A et al (2011) Transcriptomic responses to functional feeds in Atlantic salmon (*Salmo salar*). Fish Shellfish Immunol 31:704–715
Tacchi L, Secombes CJ, Bickerdike R et al (2012) Transcriptomic and physiological responses to fishmeal substitution with plant proteins in formulated feed in farmed Atlantic salmon (*Salmo salar*). BMC Genom 13:363
Tacon AGJ, Metian M (2008) Global overview on the use of fish meal and fish oil in industrially compounded aquafeeds: trends and future prospects. Aquaculture 285:146–158
Takeuchi T (2014) Progress on larval and juvenile nutrition to improve the quality and health of seawater fish: a review. Fish Sci 80:389–403
Teletchea F (2009) Molecular identification methods of fish species: reassessment and possible applications. Rev Fish Biol Fish 19:265–293
Tena N, Pierna JAF, Boix A et al (2014) Differentiation of meat and bone meal from fishmeal by near-infrared spectroscopy: extension of scope to defatted samples. Food Control 43:155–162
Thomas LV, Ockhuizen T, Suzuki K (2014) Exploring the influence of the gut microbiota and probiotics on health: a symposium report. Br J Nutr 112(S1):S1–S18
Tocher DR (2010) Fatty acid requirements in ontogeny of marine and freshwater fish. Aquac Res 41:717–732
Tremaroli V, Bäckhed F (2012) Functional interactions between the gut microbiota and host metabolism. Nature 489:242–249
Turchini GM, Ng W-K, Tocher DR (eds) (2011) Fish oil replacement and alternative lipid sources in aquaculture feeds. CRC Press, Boca Raton
Van Eenennaam AL, Young AE (2014) Prevalence and impacts of genetically engineered feedstuffs on livestock populations. J Anim Sci 92:4255–4278
Venegas-Caleron M, Sayanova O, Napier JA (2010) An alternative to fish oils: metabolic engineering of oil-seed crops to produce omega-3 long chain polyunsaturated fatty acids. Prog Lipid Res 49:108–119
Vivas M, Rubio VC, Sánchez-Vázquez FJ et al (2006) Dietary self-selection in sharpsnout seabream (*Diplodus puntazzo*) fed paired macronutrient feeds and challenged with protein dilution. Aquaculture 251:430–437
Voytas DF, Gao C (2014) Precision genome engineering and agriculture: opportunities and regulatory challenges. PLoS ONE 12:e1001877
Wang J, Wu G, Zhou H, Wang F (2009) Emerging technologies for amino acid nutrition research in the post-genome era. Amino Acids 37:177–186
Watanabe T, Kiron V (1994) Prospects in larval fish dietetics. Aquaculture 124:223–251
Webster CD, Lim CE (eds) (2002) Nutrient requirements and feeding of finfish for aquaculture. CABI Publishing, Wallingford
Wiegand MD (1996) Composition, accumulation and utilization of yolk lipids in teleost fish. Rev Fish Biol Fish 6:259–286
Wilson RP (ed) (1991) Handbook of nutrient requirements of finfish. CRC Press, Boca Raton
Xue X, Feng CY, Hixson SM et al (2014) Characterization of the fatty acyl elongase (elovl) gene family, and hepatic elovl and delta-6 fatty acyl desaturase transcript expression and fatty acid responses to diets containing camelina oil in Atlantic cod (*Gadus morhua*). Comp Biochem Physiol B 175:9–22
Yasumara F, Lemos D (2014) Species specific in vitro protein digestion (pH-stat) for fish: method development and application for juvenile rainbow trout (*Oncorhynchus mykiss*), cobia (*Rachycentron canadum*), and Nile tilapia (*Oreochromis niloticus*). Aquaculture 426–427:74–84
Yearsley JM, Villalba JJ, Gordon IJ et al (2006) A theory associating food types with their postingestive consequences. Am Nat 167:705–716
Yoshimatsu T, Hossain MA (2014) Recent advances in the high-density rotifer culture in Japan. Aquacult Int 22:1587–1603
Zdunczyk Z, Pareek CS (2008) Application of nutrigenomics tools in animal feeding and nutritional research. J Anim Feed Sci 17:3–16