

There is mounting evidence that most fish and crustacean species can be considered "obligate carnivores" (of sort) and that some nutrients rich in animal products may often be overlooked in formulation and nutritional requirement studies.

Formulating diets for fish can be complicated as today's aquaculture farms have many new fish species. Of some of them, we still don't know exactly what they require. Investment in knowing more about the different nutritional requirements is therefore pivotal.

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n a context of significant competition and low profit margins, aquaculture feed manufacturers are required to formulate to increasing lower or narrower nutritional specifications ('specs') in order to minimise feed cost. Decreasing specs for certain nutrients (e.g. lysine, methionine, DHA, available phosphorus) can significantly reduce the cost of feeds. The formulation of cost-effective aquaculture feeds adequately meeting the nutrient requirements of animals, while not being too wasteful, can be a very delicate balancing act. Perhaps a relatively unique feature of aquaculture feeds is that they are characterised by a wide range of nutritional specifications. This is expected given the very large number of fish and crustacean species produced around the world using feed-based production systems. However, the protein, lipid, starch and digestible energy contents of feeds can significantly vary not only as a function of species and life stages for which they are formulated (trout vs. tilapia feed, starter vs. grower vs. finisher feed), but also as a function of a myriad of other factors, such as production systems, farmers' or feed manufacturers' preferences, environmental constraints,

and socio-economic conditions (e.g., fish price, access to credit, degree of risk). Most fish feed manufacturers have to serve a large client base cultivating numerous fish and invertebrate species in very different production systems (ponds vs. cages, marine vs. freshwater environment, etc.) and socio-economical contexts (small farmers vs. large vertically integrated corporations). Formulating "on the edge" is therefore very complicated in this complex sector.

More fish species

Significant efforts have been invested over the past six decades on the definition of the nutrient requirements of numerous fish and crustacean species and the body of knowledge is growing significantly every year. Reviews of the literature and nutritional recommendations are provided on a relatively regular basis by different groups of researchers

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or committee of experts. The relatively new NRC (2011) 'Nutrient Requirements of Fish and Shrimp' and other reference documents are providing feed manufacturers with a reasonably good basis for the formulation of feeds meeting the requirements of many of the commercially important aquaculture species. However, the number of fish and crustacean species studied by different investigators is staggering and this leads to dilution of research efforts. Globally, there is need for significant improvements in the focus of nutritional studies, and the scope and quality of the experimental efforts invested in the definition of essential nutrient requirements of commercially important species. It would be recommendable to increasingly focus the research efforts on the 15 or so fish and crustacean species (e.g., Chinese carps, Indian major carps, Nile tilapia, Pangasid catfish, Atlantic salmon, Pacific white leg shrimp, etc.) that represent the bulk of the global farmed fish and crustacean production. Studies have suggested that some of these nutrients, such as phospholipids, cholesterol, nucleotides and arachidonic acid (ARA, 20:4 n-6), abundant in fish meal and other animal feedstuffs, are essential to some species and/or for the larval stage of certain species. Nonetheless, there is mounting evidence that most fish and crustacean species can be considered "obligate carnivores" (of sort) and that some nutrients rich in animal products may often be overlooked in formulation and nutritional requirement studies.

Factors affecting requirements

Estimates of requirements are generally derived from studies with young fish fed diets containing purified and chemically defined ingredients that are highly digestible and, generally, represent minimum nutrient concentrations required for maximising performance of these young animals under laboratory conditions. While this type of approach and definition of 'requirement' may sound relatively simple and straightforward, reality is a lot more complicated. Significant differences may exist in the experimental conditions (diet composition, experimental design, duration of study, fish trains, life stages), measured parameters (live weight gain, protein gain, enzyme activity, body stores, histological changes), performance

achieved (growth rate, feed efficiency), and methods of analysis of the results for 'similar' studies. Consequently, very different 'estimates' of requirement can be derived from similar studies. Moreover, the same dataset (e.g. data from one single study) can also be interpreted in very different ways through the use of different mathematical models to analyse data or by simply putting more emphasis on different parameters (body stores vs live weigh gain vs enzyme activity). Moreover, how requirements evolve with changes in the genetic, weight, growth rate or feed conversion achieved, or health status of the animal is something that, in my humble opinion, has not been adequately studied for aquaculture species. Defining a nutrient requirement value is clearly not a straightforward thing and yet published estimates of requirements are too often taken at face value and/or misunderstood. It must be recognised that published estimates of nutrient requirement are derived from consensus among 'experts' and are thus very much products of opinion and not some sort of unchallengeable truth. Feed formulators should ideally dig in the primary research literature for the real data and develop their own opinion. Feed manufacturers should also focus a significant part of their R&D efforts toward verifying the adequacy and suitability of their nutritional specifications.

Considering safety margins

The mode of expression of requirement is an issue that has not received sufficient attention. There are numerous diverging opinions with regards to appropriate modes of expression of essential nutrient requirements. It is especially the case for essential amino acid (EAA) for which very different modes of expression of requirement are used, often interchangeably, in the literature. These different modes of expression are based on different, often diametrically opposed, assumptions. In practice, the use of different modes of expression of EAA requirement can often result in dramatically different nutritional recommendations. Individual EAA levels deemed adequate may be very different depending on the mode of expression adopted and the composition of the diet formulated. This is a significant issue since feeds for a given species are formulated to widely different

protein, lipid, starch, and digestible energy levels. The root cause of these conflicting views is our limited understanding of how endogenous and dietary factors affect EAA utilisation and requirements of fish. Finally, requirements are generally amount of nutrient in a biologically available form that needs to be delivered to the animal. It is important in this context to consider a reasonable safety margin to account for potentially lower digestibility or bioavailability of nutrients in practical ingredients, for losses of nutrients during manufacturing and storage of the feed, and for potential 'changes' in nutrient requirements imposed by various environmental or endogenous factors. What represents a reasonable safety margin is again something up for discussion.

The potential value of modelling

Decades of use in different animal production (dairy, beef, swine, poultry) sectors have demonstrated the value of nutritional modeling as an effective way of compiling, integrating, and interpreting available information (research-based or farm-specific information) and enabling the development of practical and reliable tools for feed formulation and/or production, feeding, and waste outputs management. A relatively large number of nutritional models have been developed for fish and crustaceans over the past four decades (Bureau et al., 2002; Dumas et al., 2010). However, the nutritional models developed so far for fish all present important limitation and are not sufficiently flexible and reliable to be applied to a wide range of conditions (Bureau et al., 2002; Dumas et al., 2010). More comprehensive and pragmatic frameworks that incorporate the latest information in terms of nutrient requirements and utilisation by fish and crustaceans need to be developed (Hua et al., 2010; 2012). Future nutritional models need to be robust and increasingly mechanistic and rational.

Alternatives to marine ingredients

Up to about 10 years ago, fish meal and fish oil represented about 70% of the weight of most commercial salmon and marine fish and crustacean species feeds sold worldwide. Its use is declining and fish meal is not a major ingredient anymore. Manufacturers have been relying on the use of an increasingly diverse array of alternative feedstuffs of plant, terrestrial animal or microbial origins, each of which having their own characteristics and limitations. While a number of research trials have shown that feeds can be formulated without fish meal provided the feed is formulated with high quality ingredients (including ingredients of animal origin and various nutritional supplements). However, several studies have shown that formulating fish feeds without fish meal is not always an easy feat. Accurate assessment of nutritive value of feed ingredients is extremely important for the formulation of cost-effective feeds with lower levels of high quality fish meal. Better characterisation of the nutrient composition of feedstuffs is essential to improve their "valorisation" by leastcost feed formulation programmes. More efforts need to be invested in systematically investigating the effects of numerous factors that can affect the nutritive value of feed ingredients. Studies have suggested that some of these nutrients provided by fish meal and other feedstuffs of animal origins (eg. phospholipids, cholesterol, and arachidonic acid) may be essential or conditionally essential for some species or at specific life stages of some species. Recent studies have shown significant benefits from supplementing plantbased "grower" fish feeds with cholesterol, taurine, and hydroxyproline, three nutrients also abundant in fish meal and other animal products. More research needs to be carried out to determine if these nutrients are truly essential or under what circumstances they are required or beneficial. Nonetheless, replacing fish meal may also means paying attention to once overlooked nutrients present in animal products.

Dealing with variability in quality

Sourcing an increasing diverse array of raw materials from different suppliers can also results in greater probability for significant variations in the quality of the raw materials purchased. The high price of certain feedstuffs may also incite unscrupulous suppliers to adopt deceptive practices, such as product adulteration. In this context, the sector needs to have to invest significant resources in the characterisation of the nutritive value of different feedstuffs (and batches thereof) and in quality assurance. Larger manufacturers often need to source the required high volumes of certain raw materials from multiple suppliers. Small manufacturers due to their lesser needs may be able to source from single suppliers but, at the same time, may be even more at the mercy of capriciousness of the markets. The production of highly nutritious and cost effective feeds with an increasingly wide array of feed ingredients obtained from different suppliers is clearly not an easy task. This is certainly keeping some feed formulators awake at night. Sourcing of raw materials from different countries, manufacturers or brokers arguably results in greater probability for significant variations in the quality of the raw materials purchased. The high price of certain feedstuffs (for example fish meal) may also incite (unscrupulous) suppliers to adopt deceptive practices, such as product adulteration (for example blending less expensive raw materials with more expensive raw materials). Feedback from the field indicates that variability in the nutritive quality and adulteration of feedstuffs are not a thing of the past. In this very complex context, quality assurance (QA) plays an extremely important role. QA usually involves the definitions of specifications for the purchasing of the raw materials and for the inspection and analysis of these raw materials as they are received at the feed mill. Most, if not all, aquaculture feed manufacturers have adopted some sort of QA process and invest very significant financial and staff resources in this. The main emphasis of QA systems in place is on chemical composition, mainly on proximate analysis (crude protein, crude lipids, crude fibre, etc.), of the raw materials. Relatively little emphasis is placed on direct measurements of individual nutrient or contaminant levels due to the often prohibitive cost of this type of analysis. Near Infrared Reflectance Spectroscopy (NIRS) is widely used by most aquaculture feed manufacturers around the World to obtain rapid and generally accurate estimation of the proximate and individual nutrient levels of batches of raw materials. However, measurements obtained with these technologies must be calibrated carefully against diet characteristics

that are biologically meaningful, such as content and bioavailability of nutrients, bio-actives and contaminants.

Good diet starts with knowledge

It is not easy to formulate fish diets. Fish meals, feather meals, meat and bone meals and DDGS often come to mind as ingredients that can vary quite significantly in terms of digestibility and nutritional quality. It is therefore important to pay more attention to accurately characterising of the nutritive value of the different types of ingredients (and batches thereof) available on the market, with increasing emphasis on minor nutrients and chemical components. Also more research needs to be carried out to determine if these nutrients are truly essential or under what circumstances they are required or beneficial for different fish species. More data would really help guide QA efforts of aquaculture feed manufacturers. For example, NIRS is highly dependent on the availability of high quality raw data on the composition and nutritive value (for example amino acid digestibility) of different raw materials so that reliable calibration of the instruments can be done. This is one area where academic research groups could play a very important role and yet are virtually absent. Other rapid but more direct ways of assessing the nutritive value of different batches of raw materials are also required. Pepsin digestibility is probably one of the most widely used tests to estimate digestibility of protein. However, there is some controversy as to the proper concentration of pepsin to be used and the applicability of this type of tests to different aquatic animal species and different raw materials. There is very limited published experimental (animal) studies examining the reliability of pepsin digestibility assays and defining their limitations. Other in vitro tests, such as pH-stat protein digestion assays have been developed but they also suffer from a lack of standardisation and lack of validation. Right now, efforts are really disparate and different groups are proposing very different approaches. AAF

This information was presented at Biomin's World Nutrition Forum., held last October in Munich, Germany. References are available on request.