

REVIEW



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A feed is *still* only as good as its ingredients: An update on the nutritional research strategies for the optimal evaluation of ingredients for aquaculture feeds

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Email: b.d.glencross@stir.ac.uk**Abstract**

The choice of strategies used to assess ingredients can have a strong impact on the interpretation of their quality. In an attempt to standardize the assessment process, a structured approach using five steps for assessing the quality of ingredients was proposed over a decade ago. Since then, there has been considerable progress in the science of ingredient evaluation, and expectations from the users of those ingredients have also evolved. Two additional steps have emerged that formulators require to make appropriate decisions in the use of ingredients. Accordingly, a series of seven steps (and the order in which they should be done) to develop a comprehensive data set on ingredient quality is proposed; Step 1 Characterization, Step 2 Palatability, Step 3 Digestibility, Step 4 Utilization, Step 5 Immunological, Step 6 Processing Effects and Step 7 Product Quality Influences. Once these seven steps had been achieved, a formulator can make the appropriate choice as to whether to use any particular ingredient, and with what constraints to impose on their use. Without any one of these steps, the risk exposure substantially increases as the formulator needs to make assumptions, and this increases the risk of a feed failing in one or more specifications.

KEYWORDS

aquafeed, evaluation, feed ingredient, methodology, raw material, strategy

1 | INTRODUCTION

A variety of strategies can be used to assess the nutritional quality of ingredients; however, the choice of strategies used can have a strong impact on the interpretation of that information. A landmark review paper published over a decade ago has been considered the benchmark approach by which to structure research assessing the quality of ingredients (Glencross, Booth, & Allan, 2007). In that review, a series of five steps (and the order in which they should be done) to develop a comprehensive data set on which to base judgements about ingredient quality were proposed; 1. Characterization, 2. Palatability, 3. Digestibility, 4.

Utilization and 5. Functionality (processability). Once these five steps had been achieved, a formulator could make the judicious choice as to whether to use, and with what constraints to impose, any ingredient that they were presented with. Without any one of these steps, the risk exposure substantially increased as the formulator needed to make assumptions, and this significantly increased the risk of a feed failing in one or more specifications (Glencross et al., 2019). Typically, many studies in this domain have skipped many of these early steps and gone straight to the assessment of utilization (step 4). However, in doing so, many of these studies have ended up with erroneous outcomes and/or misleading assessments of the ingredients that they are testing, not

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due to any limitations of the ingredient per se, but rather failure of the researcher to observe critical formulation constraints that allow the ingredient to be assessed on a basis commensurate with its potential to supply nutrients and energy. The remainder of this review will provide an oversight and some guidance on what steps should be undertaken to assess quality of ingredients and why those steps should be taken in that order and critically, what new steps should be considered for the assessment process in light of recent advances in the science.

1.1 | Step 1—Characterization

The characterization of ingredients is an often overlooked, but critical step in the evaluation process. As such, this initial step in the ingredient evaluation process remains as important as ever. For formulators to make use of technical documentation on ingredients, the users of that information must be able to relate the data to a particular type of ingredient. However, there is substantial variability in both the composition and nutritional values (as defined by the digestibility of nutrients from the ingredient) of most ingredients (Anderson, Lall, Anderson, & McNiven, 1995; Glencross et al., 2008a;b). Details on the species, origins, processing and/or storage history, let alone a chemical characterization, are often absent in a much of the scientific literature. Any chemical characterization needs to include, as a minimum, the basic parameters used to formulate feeds and/or allow clear assessment of the ingredient. An example of what a standard characterization should include, in this case of five different fishmeals, is presented in Table 1. Clearly for other types of ingredients, such as plant-derived materials, there would also be some merit in including data on parameters such as starch, non-starch polysaccharides, acid-detergent and neutral detergent fibre, and lignin (Petterson, Harris, Rayner, Blakeney, & Choct, 1999). The methods for analysis should follow standardized methods such as those recommended by analytical associations such as AOAC, UKAS or similar. Without some form of characterization, the value of the work is somewhat diminished as it becomes difficult for the reader/user of the data to effectively relate the work to their materials. By providing a comprehensive characterization, it becomes much easier to relate the assessment to other materials and/or obtain the same material. Another element to characterization that is gaining importance, and over time may warrant its own step, is an assessment of the sustainability of an ingredient. Various strategies have been examined to define sustainability of ingredients, but life cycle assessment is perhaps the approach gaining most favour (Boissy et al., 2011; Malcorps et al., 2019; Silva, Valente, Matos, Brandão, & Neto, 2018).

1.2 | Step 2—Palatability

Before the impact of the nutrients within a feed can be measured on animal performance, the animal clearly must ingest that feed. A decision hierarchy framework has been suggested as a means of defining the nature of the responses to feeds to aid defining how the feed is specifically

impacting the response (Figure 1). In this figure, the influence of a feed (and by inference its ingredients) on an animal's response can be assessed to define the specific aspect of palatability that is affected (and note that this response might be positive or negative). It is clear that any factor(s) that negatively impact this hierarchy are going to limit the potential of the ingredient. Therefore, one of the qualities of any ingredient that is critically important to a feed is its effect on palatability of the feed. Clearly, if an ingredient reduces feed intake due to negative effects on palatability, it has some limitations as a potential feed ingredient. Conversely, those ingredients that can stimulate intake, and thereby improve palatability, have added value as ingredients. It can be seen from some studies how this variation in palatability can affect greater than 80% of the variability in growth response to diets trialling alternative ingredients (Figure 2) Kousoulaki et al., 2018). In studies where fish are fed a fixed ration, it becomes impossible to assess the impact of diet (and by extension the test ingredient) on palatability responses. It should also be noted that effects on palatability of diets can often be detected within days of introduction and are usually at their most sensitive point of assessment within the first 10 days (minus days 1 to 3) of an animal being fed that new diet (Figure 3). After this period, the animal may begin to adapt to the diet and the ability to discriminate palatability effects accordingly becomes diminished (Arndt, Hardy, Sugiura, & Dong, 1999; Glencross et al., 2006, 2016; Kousoulaki et al., 2018; Suresh & Nates, 2011, Nunes et al., 2019).

1.3 | Step 3—Digestibility

The assessment of the digestibility of nutrients and energy from diets and ingredients provides one of the clearest ways of unambiguously defining the nutritional value of an ingredient to an animal (Aslaksen et al., 2007; Glencross et al., 2004; 2007; Sugiura, Dong, Rathbone, & Hardy, 1998). By measuring the relative disappearance of nutrients between the feed and faeces, those nutrients available to an animal can be effectively measured. It is these nutrients that are used to drive feed intake (in response to digestible energy demands) and underpin growth. Therefore, variability in this digestibility is one of the critical points of variability in feed quality (Aslaksen et al., 2007; Bureau, Harris, & Cho, 1999; Glencross, Blyth, et al., 2017; Glencross, Blyth, Wade, & Arnold, 2018; Glencross et al., 2008b). There are a range of methods that can be used to assess diet digestibility, and these can all potentially influence the absolute values determined in any given assessment. The important element to the reliable and consistent assessment of digestibility is to minimize non-nutritional effects (i.e. minimize effects of environment or sampling method). For a comprehensive review of the range of strategies and their limitations, see Glencross et al. (2007).

1.4 | Step 4—Utilization

The assessment of nutrient and/or energy utilization is mostly commonly undertaken by a growth/feeding trial approach (Glencross

TABLE 1 An example of the characterization of different types of fishmeal. All values g/kg as received unless otherwise stated

Species	Blue whiting	Atlantic Mackerel	Anchoveta	Capelin	Sandeel
Supplier	Havsbrun	Eskja HF	TASA	SVN	TripleNINE
Material	Whole	Trimblings	Whole	Whole	Whole
Origin	Faroese	Iceland	Peru	Iceland	Denmark
Drying/Processing	steam	steam	steam	steam	Con-Kix™
Dry matter	923	921	918	930	898
Moisture	77	79	82	70	102
Protein	675	672	670	667	695
Lipid	107	115	109	157	84
Ash	159	133	139	116	119
Energy (MJ/kg)	20.1	20.4	20.1	21.9	19.7
<i>Sum Amino Acids</i>	676	626	620	642	693
Alanine	44	42	41	41	43
Arginine	46	40	39	41	43
Aspartic acid	66	61	58	62	71
Cysteine	11	4	9	8	8
Glutamic acid	99	89	83	96	99
Glycine	51	45	43	41	41
Histidine	14	15	25	14	17
Isoleucine	30	27	30	27	31
Leucine	54	51	50	54	57
Lysine	57	52	53	53	61
Methionine	22	21	20	22	25
Phenylalanine	28	27	27	27	30
Proline	30	30	27	29	29
Serine	30	30	22	32	33
Taurine	6	7	10	7	6
Threonine	29	29	25	30	33
Tyrosine	24	24	21	23	28
Valine	36	32	37	36	39
<i>Fatty Acids</i>					
C14:0	2.1	4.0	3.4	5.5	2.4
C15:0	0.3	0.3	0.3	0.4	0.4
C16:0	9.7	10.9	13.1	14.8	9.7
C18:0	1.7	2.2	3.0	1.6	1.9
C20:0	0.1	0.1	0.2	0.1	0.1
C22:0	0.1	0.1	0.1	0.0	0.0
C24:0	0.1	0.0	0.2	0.0	0.1
Total Saturates	14.0	17.6	20.2	22.3	14.5
C16:1n-9	0.2	0.2	0.1	0.3	0.1
C16:1n-7	3.1	3.1	3.8	6.9	5.5
C18:1n-9	9.0	8.7	6.0	9.6	4.0
C18:1n-7	2.2	1.9	1.8	2.8	1.4
C20:1n-11	0.1	0.7	0.1	0.0	0.2
C20:1n-9	5.1	6.6	0.6	11.2	1.7
C20:1n-7	0.2	0.2	0.1	0.4	0.1

(Continues)



TABLE 1 (Continued)

Species	Blue whiting	Atlantic Mackerel	Anchoveta	Capelin	Sandeel
C22:1n-11	6.4	10.4	0.4	11.5	2.3
C22:1n-9	0.8	0.7	0.1	1.2	0.2
C24:1n-9	1.1	1.0	0.6	1.1	0.5
Total Monounsaturates	28.0	33.6	13.5	44.8	16.1
C18:2n-6	0.9	1.2	0.6	1.4	1.4
C18:3n-6	0.1	0.1	0.1	0.1	0.1
C20:2n-6	0.2	0.2	0.1	0.2	0.1
C20:3n-6	0.1	0.1	0.1	0.1	0.1
C20:4n-6	0.6	0.5	0.6	0.5	0.3
C22:4n-6	0.0	0.0	0.0	0.0	0.0
C22:5n-6	0.2	0.2	0.3	0.2	0.2
Total n-6 PUFA	1.9	2.3	1.9	2.5	2.2
C18:3n-3	0.5	0.8	0.5	0.8	0.5
C18:4n-3	1.0	2.4	1.4	1.9	1.4
C20:3n-3	0.1	0.2	0.1	0.1	0.1
C20:4n-3	0.3	0.9	0.5	0.5	0.3
C20:5n-3	5.2	5.9	9.5	11.0	6.7
C21:5n-3	0.2	0.3	0.4	0.4	0.2
C22:5n-3	0.5	0.9	1.4	1.2	0.5
C22:6n-3	9.4	10.2	14.6	16.7	9.5
Total n-3 PUFA	17.2	21.5	28.4	32.5	19.1
SFA	14.0	17.6	20.2	22.3	14.5
MUFA	28.0	33.6	13.5	44.8	16.1
PUFA	3.0	4.6	4.8	5.2	3.8
LC-PUFA	16.0	19.3	25.5	29.7	17.5
Total Fatty Acids	61.1	75.7	63.9	102.1	51.9
<i>Minerals (mg/kg)</i>					
Ca	40.952	33.173	35.339	16.731	20.490
Cu	0.003	0.006	0.006	0.003	0.005
Fe	0.027	0.251	0.117	0.045	0.044
K	13.620	10.047	13.999	13.475	10.916
Mg	1.948	2.420	2.340	2.601	2.215
Mn	0.003	0.010	0.011	0.002	0.010
Na	9.044	12.350	7.673	15.423	7.888
P	23.571	22.418	25.317	16.893	17.664
Se	0.002	0.003	0.002	0.001	0.003
Zn	0.047	0.068	0.062	0.062	0.106

et al., 2007). In this strategy, a diet (or series of diets) is fed to the target species and the phenomic responses typically assessed. While primarily responses such as feed intake and weight gain are the main points of assessment, many other nutritional responses can also be measured. For a more comprehensive assessment of the vagaries of different growth trial strategies, read Glencross et al. (2007).

While these trials are the most commonly encountered among the literature, their outcomes are largely predictable subject to diet specification choices, digestibility of ingredients used and the

palatability of the diets produced. In those situations where the diets are formulated to the same specifications and on a digestible nutrient basis, the growth responses that result from the feeding study are usually largely just a reflection of feed intake (palatability) variation. Figure 2 shows a typical such response from the evaluation of a suite of alternative ingredients fed to Asian seabass (*Lates calcarifer*), where the feed intake alone accounts for over 80% of the variation in the growth response. Occasionally, unexpected responses do occur, and this is arguably where the true value of the utilization

FIGURE 1 Decision hierarchy based on the nature of the response of a fish to the presence of a feed

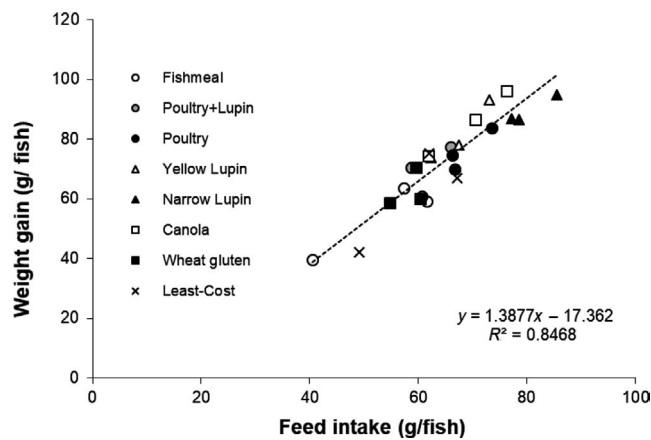
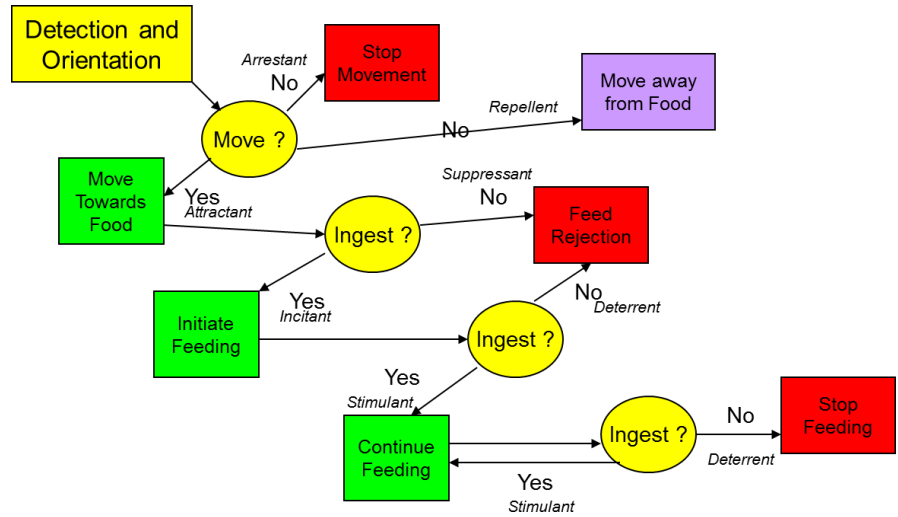


FIGURE 2 Growth responses of Asian seabass (*Lates calcarifer*) fed one of eight diets based on a range of alternative ingredients. Each of the diets focussed on the assessment of a particular ingredient when included in diets balanced for digestible protein and digestible energy. Clear effects of each diet on palatability were noted. This effect of palatability resulted in greater than 80% of the variation in weight gain response being explained by vagaries in feed intake. Data derived from

study comes into effect. It can also be argued that such studies allow for subtle effects to amortize over time and then these minor variables can be then be observed more clearly. Certainly, such trials are critical for determination of nutrient requirement and metabolic response studies but are arguably less valuable for ingredient evaluation studies.

The formulation strategy used to develop test diets can have a strong bearing on the interpretation of the assessment of ingredients. While most commercial diets are now days formulated on a digestible protein and energy basis, a majority of scientific literature still presents diets formulated on a crude basis (Mock et al., 2019; Turchini, Trushenski, & Glencross, 2019). The reason why this is problematic can be demonstrated via the hypothetical example in Figure 4. In this example, we consider two diet formulation strategies, one formulated solely to crude nutrient specifications and the other to a digestible nutrient specifications. In this

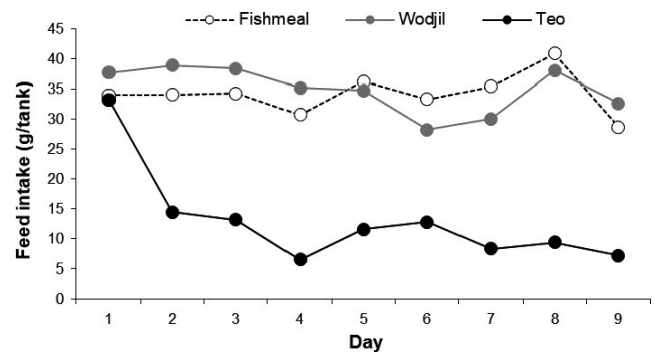


FIGURE 3 Variation in daily feed intake in different treatments of fish (rainbow trout, *Oncorhynchus mykiss*) fed diets with a 30% inclusion of each of two different cultivars of yellow lupin kernel meal compared to a diet using fishmeal as its only protein source. All diets were formulated to the same digestible protein and energy specifications. Data derived from Glencross et al. (2006)

example, we can see the impact of the inclusion of an alternative ingredient containing 650 g/kg protein, with a digestibility of 75%, added to each series of diets at 100 g/kg increments, with the remaining diet protein 85% digestible. From this model, we can see that in the crude specification scenario that the level of digestible protein in the diet declines from 340 g/kg, with no alternative inclusion, to about 310 g/kg when the alternative is included at 400 g/kg. By contrast in the digestible specification scenario, the digestible protein level stays constant at 340 g/kg, while the crude protein level increases from 400 g/kg to about 430 g/kg. These differences in digestible protein levels in each series of diets would have direct impact on the digestible energy density in each scenario as well. In response to these differences, if we were to feed such diets to a species like Atlantic salmon, we would see a clear difference in feed conversion based on the fact that such species are clear responders to dietary energy density, typically responding with a 0.15 decline in FCR for every MJ increase in digestible energy content in a diet (Einen& Roem, 1997; Hillestad & Johnsen, 1994; Mock et al., 2019). Accordingly, we would then see quite different feed conversion ratio (FCR) responses in each

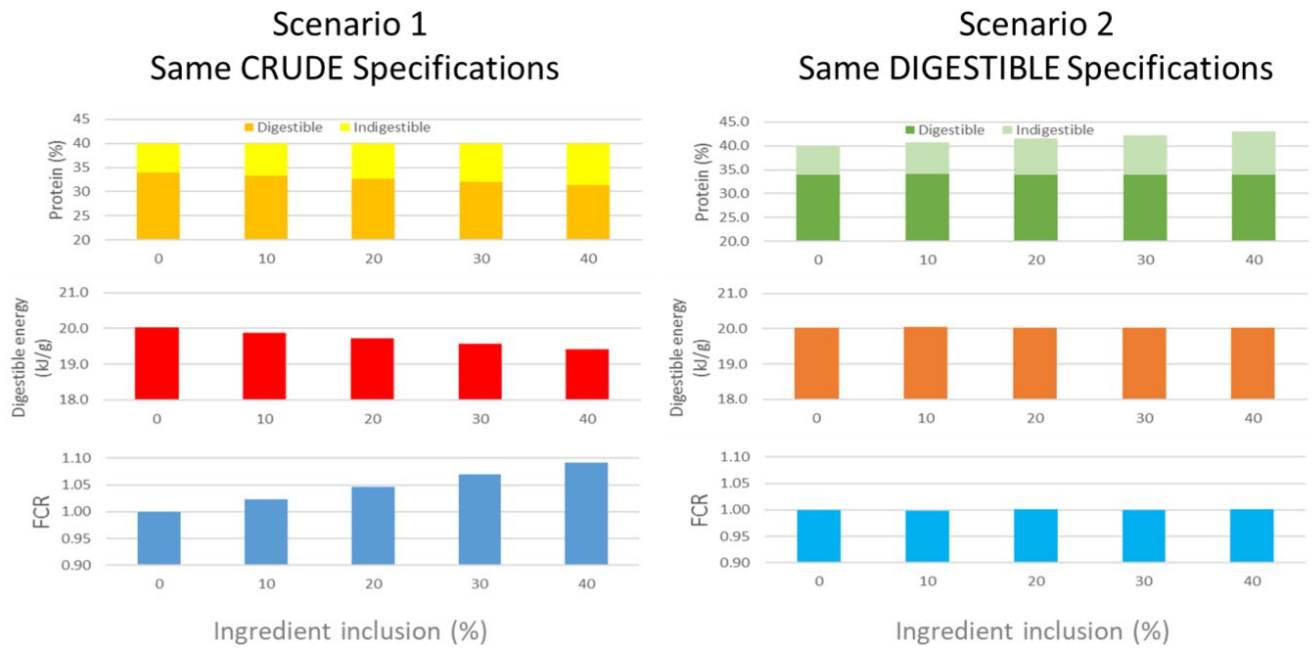


FIGURE 4 Theoretical outcomes of two different series of diets formulated on either a crude specification or digestible specification basis

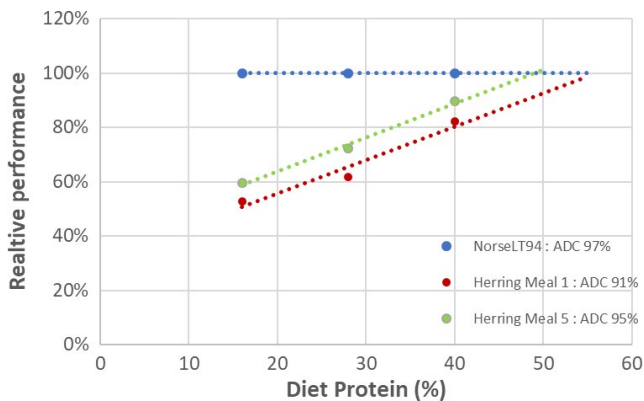


FIGURE 5 Effects of fishmeal quality and diet protein level on relative performance (weight gain) by Atlantic salmon. Data derived from Anderson et al. (1993)

scenario. In the crude specification scenario, it would be reasonable to suggest that the alternative ingredient causes a decline in performance at all inclusion levels and therefore should be considered a low-grade ingredient. By contrast, the digestible specification scenario shows no response in performance to the use of the alternative, and therefore, it would be reasonable to suggest that the alternative ingredient is well utilized and tolerated at all inclusion levels and therefore should be considered a high-grade ingredient. Of course, between each scenario the ingredient is the same, only the formulation strategy has changed.

When diets are formulated to non-constraining specifications (i.e. specifications where no specific nutrients are limiting or close to limitation levels), as like occurs in typical commercial diet specifications, using this approach all too often results in a simple null-hypothesis outcome with no differences observed among the diets. Results

such as this provide little useful information in terms of application of the ingredients being assessed due to the large margins for error built into the diet formulations to specifically limit such responses being observed. An excellent example of how a change in diet specifications changes the interpretation of the assessment of ingredients can be seen from the work of Anderson, Lall, Anderson, and McNiven (1993) as represented in Figure 5. In these figures, the responses of Atlantic salmon to diets made using three different qualities of fishmeal included in diets with one of three different protein levels show clearly the sensitivity of diet specification to interpretation of the impact of ingredient quality. In this work, both the weight gain and feed conversion deteriorated with reducing diet protein levels, as expected. Perhaps the most important feature of this study is seen more clearly when the relative performance (growth) of each of the diets is mapped against each diet protein level (Figure 5). From this, it can be clearly seen that as the diet protein level increases that the differences between the responses to the different fishmeal begin to diminish. This difference between the three fishmeals continues to become negligible as the diet protein level increases up to a point at ~550 g/kg protein content where it would not be possible to discriminate among the three fishmeals in terms using a growth study approach. Important to note is that with the size of fish used in that study (7.5–25 g), the typical commercial diet specification is between 500 g/kg and 550 g/kg protein. As such, the use of a standard commercial diet formulation approach to assess quality in this example would have resulted in a null-hypothesis outcome and no ability to discriminate between the three fishmeals. While the relevance of a commercial style specification might be argued, the lack of sensitivity in using such a specification also needs to be considered. Sometimes a commercial analogy might not be the most appropriate strategy to answer a nutritional question.



1.5 | Step 5—Immunological and health allied assessments

A growing priority in the assessment of feeds and ingredients in animal diets is their impact on the immune response and general robustness (health) of the animal. There are a variety of strategies that are being used to examine these parameters, including specific pathogen (disease) challenges, which can be undertaken to assess the impacts of diets on the immunogenic function of fish against a particular pathogen of interest (Martinez-Rubio et al., 2012; Sellars et al., 2015). In such studies, the animals are typically fed the diets and then challenged with a pathogen (usually a virus or bacterium) and the survival and immune responses of the population subsequently assessed. More recently, an alternative use of specific pathogens has been to use a pathogen-associated molecular pattern (PAMP) challenge (Ruyra, Cano-Sarabia, MacKenzie, MasPOCH, & Roher, 2013; Vallejos-Vidal, Reyes-López, Teles, & MacKenzie, 2016). PAMP challenges use a lipopolysaccharide or double-stranded RNA molecule included as part of a vaccine-like adjuvant to simulate the infection of the animal by a pathogen, without having to use live pathogens. Following injection of the PAMP, samples are collected to assess aspects of the immune response. In assessing the responses to such immunogenic challenges, in addition to measuring animal survival, it is also common to measure a suite of cellular, biochemical and molecular parameters. The molecular ones will be considered in a later section. Some common cellular and biochemical assessments include a histological analysis to look at tissue-specific damage arising from the use of any particular ingredient (Caballero et al., 1999; Refstie et al., 2006). Biochemical tests include those such as measuring lysozyme or superoxide dismutase activity (Hartviksen et al., 2014; Metochis et al., 2013, 2017).

Additionally, the recent advent of relatively low cost and reliable DNA sequencing has made it much easier to analyse the microbial diversity of samples. This is now generally referred to as an assessment of the “microbiome” (Llewellyn, Boutin, Hoseinifar, & Derome, 2014; Rimoldi et al., 2018). In this analysis, changes in the microbial diversity and abundance in samples (usually faeces) are assessed by sequencing the 16S rRNA genes using universal primers targeting the V3-V4 variable regions that allows the identity and abundance of the bacteria present any sample to be identified in response to diet/ingredient use (Llewellyn et al., 2014; Lyons, Turnbull, Dawson, & Crumlish, 2017; Mente, Nikouli, Antonopoulou, Martin, & Kormas, 2018; Zarkasi et al., 2017). There has been considerable activity in this space in recent years, with many studies demonstrating responses of the microbiome to changes in the use of ingredients. However, what appears to be lacking is the cause-effect evidence that such changes in the microbiome are relevant to the changes in performance of the diets and/or animals and not just another concomitant change without impact.

1.6 | Step 6—Processing Effects (Functionality)

Physical constraints play an important role in the production of a viable feed with which to feed aquatic species. The fundamental

logistics of handling ingredients play an often-overlooked role in the ingredient assessment process. Most modern aquaculture feeds are manufactured using extrusion processing, where the rheology of a given feed mix is managed to allow the plasticization of the components to produce a well bound and durable product that can be tailored to float or sink and contain a high- or low-oil content (Oterhals & Samuelsen, 2015; Samuelsen & Oterhals, 2016; Sørensen, 2012).

Some of those physical characteristics that are assessed are relatively simplistic but remain fundamental to managing the logistics of using ingredients. Parameters include those such as the flow-figure, which defines the ability of a powder/meal to flow and/or be conveyed. The bulk density, which defines storage demands and hygroscopicity which defines how much moisture the product absorbs from liquid or vapour exposure, is all critical to effective management of ingredients (Samuelsen, Mjøs, & Oterhals, 2013, 2014).

Typically, assessment in this area involves the practical production of feeds using laboratory or pilot-scale extrusion systems (Draganovic, van der Goot, Boom, & Jonkers, 2011; Glencross, Hawkins, Maas, Karopoulos, & Hauler, 2010; Opstvedt et al., 2003; Samuelsen, Mjøs, & Oterhals, 2013, 2014). Variables such as the type of ingredients used and/or their inclusion levels are the main variables being tested in such science, and a range of parameters are subsequently assessed to provide information on the physicochemical properties of the ingredients on the extrusion process (e.g. specific mechanical energy input and expansion among others). The feed pellets produced from such studies usually then go on to other testing (e.g. density and oil absorption capacity) to assess a range of physical parameters important to the feed and feeding management process (Sørensen, 2012). There are other assessments which can now be used to provide alternative assessments of the extrusion process, including phase-transition assessment and rapid-viscosity assessment (Glencross et al., 2010; Oterhals, Ahmad, & Samuelsen, 2019; Samuelsen & Oterhals, 2016). An important feature of these physicochemical properties of ingredients is that they clearly play a role in impacting the physical qualities of feeds. Notably, these physical feed qualities can also directly impact feed intake and nutrient utilization and that this also should be taken into consideration when designing nutritional trials (Sørensen, 2012).

1.7 | Step 7—Product Quality Influences

In most cases, the animal species being fed are intended as a food product. Therefore, the sensory qualities (colour, taste and smell) are important criteria in determining quality of those products. Accordingly, the use of sensory evaluation studies is sometimes included to evaluate the impact of different dietary treatments, including the use of ingredients (Rosenlund, Obach, Sandberg, Standal, & Tveit, 2001; Turchini et al., 2003; Liu et al., 2010). There are various elements to this evaluation, but principal among them is the evaluation of flavour and/or colour (Bjerkeng et al., 1997; Lie, 2001; Wade, Paulo, et al., 2014).

Flavour as a parameter has a large degree of complexity to it given that there are components defined by the tongue senses



(taste: salt, sweet, acid, bitter and umami), and there are those components defined by the olfactory senses (smell). The evaluation of these sensory characteristics is typically undertaken using a range of strategies including the use of tasting panels, but increasingly the use of instrumentation is being used to assess colour, odour and texture (Chytiri, Chouliara, Savvaidis, & Kontominas, 2004; Liu, Zeng, & Sun, 2013; Olafsdottir, Högnadóttir, Martinsdottir, & Jonsdottir, 2000; Olafsdottir, Martinsdottir, & Jonsson, 1997; Olafsdottir et al., 2004). Sensory data are commonly considered qualitative in nature rather than quantitative. The advantage of using an instrumentation approach is that the data can be quantitative rather than qualitative. For some aspects of sensory evaluation, modern instrumentation analyses are even considered to be more sensitive than human senses and therefore provide a greater level of assay fidelity. Although in some instances, like with the detection of the muddy taint of geosmin, the advantages of instrumentation over human sensory perception have been questioned (Howgate, 2004).

There have been several studies devoted to examining the influence of different ingredients on the flavour profile of a range of different aquaculture species (Al-Marzooqi, Al-Farsi, Kadim, Mahgoub, & Goddard, 2010; Jónsdóttir, Valdimarsdottir, Baldursdóttir, & Thorkelsson, 2003; Kousoulaki et al., 2020; Mock et al., 2019; Thomassen & Røsjø, 1989; Williams, Paterson, Barlow, Ford, & Roberts, 2003). Although a variety of effects of alternative ingredients on sensory characteristics of animal products have been reported, including changes to meat texture, fatty acid profile, prevalence of melanin spots and pigmentation, the effects are usually quite limited and mostly confined to changes linked to use of different lipid sources (Glencross, Hawkins, & Curnow, 2003; Hardy, Scott, & Harrell, 1987; Kousoulaki et al., 2020; Mock et al., 2019; Thomassen & Røsjø, 1989).

Colour as a quality parameter, like flavour, also has many complexities to it subject to the objectives being sought, which are largely defined by the species being farmed. There are various methods that have been developed to assess colour including the use of colour-fans, colour-meters ($L^*a^*b^*$ meters) and computer-assisted imaging assessment techniques (Liu et al., 2013; Wade, Paulo, et al., 2014; Wathne, Bjerkeng, Storebakken, Vassvik, & Odland, 1998). Most studies examining elements of colour end to focus on the application of carotenoids or carotenoid containing meals (Wade, Budd, Irvin, & Glencross, 2015; Wathne et al., 1998).

2 | OTHER NUTRITIONAL ASSESSMENTS

A range of other nutritional assessment methodologies and strategies exist that either value-add the primary *in vivo* work previously detailed and/or add new dimensions to that work by providing a greater mechanistic assessment of the functionality of the various nutritional responses seen. While many of these appear relatively academic in their intent, some have clear practical application in the ingredient assessment process.

2.1 | Rapid Analysis Technologies

The processes of *in vivo* assessment of diet and ingredient quality assessment are costly, laborious and time consuming. The development of technologies for the rapid analysis of nutritional value of raw materials, such as the use of *in vitro* assays and scanning technologies, like NIR, has been the subject of research since the 1980s (Bassompierre et al., 1997; Carter, Bransden, Van Barneveld, & Clarke, 1999; Dimes & Haard, 1994; Eid & Matty, 1989). This next section will examine some of the various assessment methods that have been attempted.

2.2 | In vitro Assessments

There are several options for the use of *in vitro* assessments of ingredient quality that can be applied. These extend from simple chemical analysis of specific parameters of the ingredient to provide a guide on various quality indices, to more complex *in vitro* assessments that aim to mimic the digestion process and provide an assessment of digestibility.

Perhaps the most commonly used *in vitro* assessment used as a quality criterion is those based on the level of certain indicators of quality/freshness in some ingredients, such as the TVN assay for fishmeals. Such indices have been used for other ingredients, like poultry and blood meals, though are less common, and new more rapid methods are being introduced (Johnson, Atkin, Lee, Sell, & Chandra, 2019; Lewis et al., 2019; Sheng et al., 2016). For fishmeals though, such assays have become so routine these days that they are used to provide demarcations between premium, high and average quality fishmeals (Jensen, Fiskeindustri, & Denmark, 1990). There are various methods that can be used to assess these parameters these days. In particular, the use of capillary electrophoresis (CE) provides a fast and sensitive procedure to simultaneously quantify volatile amines (TVN) and trimethylamine-oxide (TMAO) in samples (Bassompierre et al., 1998; Wu & Bechtel, 2008).

Various *in vitro* digestibility methods have been used to attempt to provide estimates of the nutritional (digestible) value of different raw materials for some time (Bassompierre et al., 1997; Eid & Matty, 1989; Lewis et al., 2019). Most methods use an enzyme-mediated process, but the key variable is often what enzymes are used (purified preparations or crude homogenates) and how the various products of the enzymatic process are interpreted that vary across the different methods. However, critical to the viable use of any rapid assessment method must be their validation against *in vivo* methods of assessment. Without these validations, the *in vitro* responses are merely academic. Although there has been much work done on developing and testing a range of *in vitro* assays, a range of problems surrounding their use persist. While they are clearly quicker than using *in vivo* testing, they are still time-consuming and have continued to have problems surrounding their reliability and inconsistencies in their predictive ability, though recent studies

claim to have made significant gains in this area (Lewis et al., 2019; Yasumaru & Lemos, 2014).

2.3 | Near-infrared and Nuclear Magnetic Resonance Spectroscopy

In contrast to *in vitro* assays, technologies like near-infrared (NIR) spectroscopy and nuclear magnetic resonance (NMR) spectroscopy have allowed the assessment of the nutritional value of raw materials, on a near-real-time basis, and provide significant advancements in the responsiveness and cost savings in diet formulation by the feed industry (Conceição, Grasdalen, & Dinis, 2003; Cozzolino, Murray, & Scaife, 2002; Fontaine, Hörr, & Schirmer, 2001; Glencross et al., 2015). The use of NIR for determining the composition of raw materials is now relatively common in most modern feed production plants throughout the world. However, the use of NIR to assess the digestible value of protein and energy from raw materials is not as well established and reports on its successful application are scarce (Glencross, Bourne, Irvin, & Blyth, 2017; Glencross et al., 2015). To achieve a viable NIR calibration, it is critical that a wide range of samples is obtained from which to determine the nutritional (digestible protein and energy) values of the raw materials and to then correlate this with the NIR spectra of those same samples (Glencross, Bourne, et al., 2017; Glencross et al., 2015). The process of calibration development can be laborious and costly, though the potential gains in functionality through this method are enormous. Not only can NIR be used to determine compositional and nutritional (digestible) parameters of feeds and ingredients, it has also shown some prospect in being used as a discriminatory tool to determine the origins of raw materials to make certain ingredients (e.g. the type of fish used to make fishmeal) (Cozzolino et al., 2002).

2.4 | Nutrigenomic and allied assessments

A variety of “omics” (e.g. transcriptomics, proteomics, metabolomics) applications have emerged in aquaculture research with varying degrees of success in their application and capacity to deliver meaningful outcomes to nutritional science (Panserat & Kaushik, 2010).

2.5 | Transcriptomic analysis

Transcriptomic analysis is an assessment of the gene expression changes that occur in response to some stimulus (Panserat & Kaushik, 2010). In nutrition, this is usually with the use of a nutritional regime involving diets with different nutrients and/or ingredients and the assessment of the expression of specific genes relevant to various metabolic pathways (Panserat et al., 2009; Qian, Ba, Zhuang, & Zhong, 2014). It has become quite routine practice to include some level of transcriptomic analysis in modern nutritional research. However, it can be argued that in many cases, the

practical value of using transcriptomics is questionable at best (Tacchi, Bickerdike, Douglas, Secombes, & Martin, 2011). Responses of the transcriptome (all those genes being expressed at any given time-point) to diet change dramatically postprandially, with tissue samples collected 24h after the animal has been fed arguably having little relevance to the total level of transcriptomic activity for any particular gene (Wade, Skiba-Cassy, Dias, & Glencross, 2014). Perhaps a more pertinent approach would be to identify the postprandial period more responsive to diet and compare treatments from samples collected at that point. However, this requires an assessment of the variability postprandially and then often substantial changes to sampling regimes to allow the comparative sampling of experiments in time frames such as 2h after feeding (Poppi, Moore, Wade, & Glencross, 2019; Wade, Skiba-Cassy, et al., 2014).

2.6 | Proteomic analysis

Proteomic analysis is an assessment of the change protein expression that occurs with the use of a particular nutritional regime (Rodrigues, Silva, Dias, & Jessen, 2012). This “omics” analysis is arguably the next progression from transcriptomics in that it assesses the translation of the RNA produced from the differential gene expression in transcriptomics and allows variables of translational and post-translational effects to be examined and therefore allows a closer look at the functional impact of a gene expression cascade (Seilliez et al., 2008; Wade, Skiba-Cassy, et al., 2014). Additionally, a range of tools can be applied at this level to examine post-translational modifications, like phosphorylation or methylation of proteins to identify whether their functional activity is also being affected (Zhou, Ding, & Wang, 2012).

2.7 | Metabolomic analysis

Metabolomic analysis is an assessment of the changes that occur in the abundance of metabolites within either a whole animal, tissues or cells. In this regard, metabolomics aims to use metabolite profiles to identify biomarkers indicative of physiological responses of organisms to nutritional or other conditions (Bankefors et al., 2011). One of the benefits of the metabolomics approach is that it uses a broad scan of biological conditions to identify often unexpected problem or risk areas upon which to focus attention (Alfaro & Young, 2018). However, to date there has been little application of metabolomics in aquaculture nutrition and even less in studies devoted to ingredient assessment (Viegas et al., 2019).

2.8 | Endnote

Since the review by Glencross et al., (2007), not only has there been considerable progress in the science of ingredient evaluation for aquaculture feeds, but expectations from the users of those



ingredients have also evolved. The original five steps proposed by the original authors still remain highly relevant, though emphasis on other parameters has now come to the fore as we increasingly adopt new ingredients into feeds for aquaculture species. Despite this, it still remains critically important that scientists evaluating ingredients do not skip the foundation steps in the rush to give quick answers.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available in peer-reviewed literature as detailed in the following references section. Where a specific data set was used, the reference for that source is linked to the analysis. All data were derived from resources available in the public domain. The data for Table 1 of this study are available from the corresponding author upon reasonable request.

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