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Vegetable and cereal protein exploitation for fish feed

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Abstract: The current status of cereal and vegetable protein and its potential use in aquaculture feeds to replace fish meal is described in order to provide an insight into the challenges facing its use. The key drivers for exploiting food waste co-products are explored and difficulties arising are identified, e.g. evaluation of the nutritional quality of plant feedstuffs can give different results even when tested for one fish species. Future requirements for standardization of feeding protocols, improvement of the quality of data and the development of useful models that can be used across ingredients and across species are outlined.

Key words: aquaculture feed, fish meal, vegetable protein, cereal protein, fish feed.

17.1 Introduction

Aquaculture has developed over the past 15 to 20 years into a global industry, being one of the fastest growing food production systems in the world. Over 60 countries producing more than 250 species of fish, shellfish, crustaceans and plants are participating in this global development. One of the necessities of a viable aquaculture industry is access to ready-to-use feeds that are affordable, digestible, with a long storage life, and with minimal side-effects such as polluting the water in the aquaculture system. However, as many of these cultivated fish species are carnivorous, it does not improve the environmental pressure if marine protein sources are being used for rearing cultivated fish (Naylor *et al.*, 2000). Therefore, considerable amounts of resources are currently being used worldwide to evaluate the nutritional quality and possible health implications of alternative plant-based feedstuffs with the potential of replacing fishmeal in these feeds and large collaborative international projects such as the Plant Products in Aquafeed Working Group have been initiated (Gatlin III *et al.*, 2007). Various initiatives within the European Union Research Framework programmes also allocated resources to this issue for example the EU FP6 project REPRO (contract no. 006922).

17.2 Key drivers for exploiting food waste co-products

Aquaculture is the fastest growing animal food production sector and today one out of every three fish consumed in the world comes from fish farms (FAO FISHSTAT 2007). The average compounded growth rate for aquaculture is 9.2% per year since 1970 compared with 2.8% for terrestrial farmed meat production and 1.4% for capture fisheries. Of particular concern is the increased need for nutrients in fish feeds, with the need for alternative protein and essential fatty acids being the highest (Gatlin III *et al.*, 2007). Both these nutrient groups were traditionally obtained from marine sources in the form of various types of fish meals and fish oils.

Feed ingredients of plant origin are usually the by-products of processing or milling. The primary driver for the use of a particular feed ingredient is its cost, followed by purity, nutrient availability and digestibility. The cost of the nutrient is not only defined in terms of price per tonne, but also in terms of price per kg of live fish weight generated over the entire growth cycle of the fish (Bum-Kyu and Joong, 2001; Craigh, 2002). Nutrients need to have optimum digestibility and maximum contribution to the feed conversion ratio, in order to have maximum weight gain with the minimum amount of feed and excretory wastes. Water pollution from aquaculture activities is a major concern which necessitates the utilisation of feeds with low residues after digestion (Cho and Bureau, 2001).

Commercial aquaculture feeds require relatively high levels of protein, usually between 25 and 45%, and up to 60% for larvae and small juveniles. Along with that, the protein quality (amino acid profile and availability) must be able to compete with the natural diet of fish, with varying needs between herbivorous, omnivorous and carnivorous fish. As excellent books are available on species-specific information describing the feeding habits and digestion physiology of fish (for example the Kluwer series such as the book on Tilapias by Beveridge and McAndrew, 2000), this chapter will only refer to such publications for further information. In this chapter, the focus will be on the current status of cereal and vegetable protein and its potential use in aquaculture feeds to replace fish meal, and to provide an insight as to the challenges facing its use. Although there is a current drive towards more research on this topic, it is apparent from the literature that evaluation of nutritional quality of plant feedstuffs can give quite different results even when tested for one fish species. A significant emphases will be placed in future on the need for standardisation of feeding protocols, improvement of the quality of data and the development of useful models that can be used across ingredients and across species (Gatlin III *et al.*, 2007).

17.2.1 Environmental pressures on the use of fish meal

The FAO has estimated that the human demand for fish will climb to about 110 million tonnes by the year 2010, with the predicted share of aquaculture to be 38% of total world fish production by 2010. Owing to world-wide overfishing, the supply of fish meal has become a limiting factor for increase in fish production (Aslaksen *et al*., 2007). Generally, capture fisheries as a whole have stabilised at around 85 to 95 million tonnes per year. A disturbing trend is that there is a gradual shift in wild fish capture from large carnivorous fish to smaller less valuable fish that feed in lower trophic levels (Naylor *et al*., 2000). To ensure a continuing supply of fish for human consumption, aquaculture systems rely heavily on fish meal as a protein source, especially for farming with sought after carnivorous species, which tend to have sensory qualities linked to high income niche markets (Naylor *et al*., 2000). However, many intensive and semi-intensive aquaculture systems use two to five times more fish protein in the form of fish meal to feed the farmed species than the amount of protein eventually supplied by the farmed fish itself (Tacon, 1996), in spite of fish having an excellent feed conversion ratio compared with land animals. Fish feed conversion ratios (FCRs) are often less than 1.0 (based on kg live weight gain/kg of dry feed), which is considerably better than for land animals (Naylor *et al*., 2000). There are various arguments against this view, for example the fact that carnivorous fish in the wild rely on protein from small pelagic fish anyway and, thus, naturally caught fish by other fish is an unknown factor in the overall equation which is often overlooked. Regardless of these facts, the true environmental benefit of good FCRs is only applicable to species not reliable on animal or fish protein sources. Overfishing remains a reality and it will only make an already precarious situation worse in the future if feed biomass cannot be generated from other sources too.

Omnivorous fish species such as carp and tilapia can perform very well with well prepared feeds made from plant protein sources. Catfish can utilise feeds made from soybean meal, cottonseed meal and peanut meal (Robinson, 1998) and up to 100% of fish meal can be replaced by soyabean oilcake and maize protein combinations for tilapia (El-Sayed, 1999). Taking into account that carp is the largest group of farmed finsfish and Tilapia the third largest group (with salmonids in second place) (El-Sayed, 1999), it becomes clear that a significant improvement can me made to replace fish meal if the utilisation of vegetable by-products in feed rations can be better understood. Recent developments in the knowledge of such inclusions have resulted into a significant growth in the market for plant protein as a fish feed ingredient, with China already utilising in excess of 5 million tonnes of soya protein per year for farm-raised fish (Gatlin III *et al*., 2007).

Carnivorous fish have an essential requirement for sources of protein and essential fatty acids (eicosapentaenoic acid and docosahexaenoic acid) which are not found in vegetable sources (Naylor *et al*., 2000). Vegetable protein sources can be used to partially replace the protein needs of the fish, but they pose problems such as a lack of essential amino acids in some sources, compromised digestibility owing to the presence of anti-nutrients, and the simple fact that vegetable pellets do not smell like food to the fish, necessitating the need for the development of suitable attractants.

Owing to the inconsistent data available on the digestibility and utilisation of vegetable protein sources (Jauncey, 2000, Naylor *et al*., 2000, Booth *et al*., 2005, and Gatlin III *et al*., 2007), even compound feeds for herbivorous fish such as tilapia often still contain fish meal, exceeding the required minimum protein levels (Tacon, 1996). Herbivorous, omnivorous and carnivorous finfish all require similar amounts of protein per unit weight. Herbivorous and omnivorous fish can utilise plant-based proteins better than carnivorous fish owing to their longer intestinal tract (Beveridge and Baird, 2000) and minimal supplementation with essential amino acids is necessary (Naylor *et al*., 2000). Yet, manufacturers are compelled to over-formulate, even for a well-known species such as tilapia, owing to inconsistencies in fish nutrition results world-wide and a lack of standardisation (Gatlin III *et al*., 2007).

17.2.2 Value addition to co-products from the food and biofuels industries

Co-products of vegetable origin are abundant from both the food processing and the biofuels industries. In many instances, the economic performance of a company can be enhanced significantly if such co-products can be sold at maximum prices. Generally, these co-products are divided into products from oilseed or cereal origin. Oilseed protein quality tends to be better in terms of sulphur amino acid composition and essential amino acids than cereals. However, oilseeds suffer more severely under a high concentration of anti-nutritional factors than cereals, which often offsets the benefits of the better protein quality (Gatlin III *et al*., 2007). During processing, certain macronutrients are removed from the whole seeds for specific purposes such as oil for biodiesel, or the starch for ethanol production or brewing (Erasmus and Taylor, 2003). In these cases, by-products with a significantly increased protein content are being produced in some cases, while in other cases, especially in high fibre co-products such as brewers spent grain, the residue has a protein content which tend to be on the low side and needs further fractionation in order to increase the protein levels (Musatto *et al*., 2006).

The annual production of barley brewers spent grain in Europe is estimated to be around 3.5 million tonne (dry mass) per year. With a protein content of 23%, this produces around 800 000 tonne of protein. The total need for protein in fish feed for aquaculture in Europe is currently in the order of 600 000 to 700 000 tonne per year (data obtained from EU FP6 project REPRO, contract no. 006922). Production of brewers spent grain is a continuous process meaning that large predictable streams of fresh material are available. Compared with the volumes needed in China, it is already clear that refined protein co-products will have an unsaturated market demand in the fish feed sector in the years to come (Gatlin III *et al*., 2007).

17.3 Technologies for vegetable protein exploitation as fish feed

In order for a specific vegetable protein to be suitable as a replacement for fish feed, it must conform to a certain set of specifications. Apart from the need to have a nutritional profile similar to that of fish meal, it must also be equally digestible, affordable, and most of all, available in sufficient quantities. Technologies for improving the raw material stream focus around improving the nutritional profile (for example breaking down anti-nutrients through fermentation or enzymatic treatments), or improving the nutrient density (for example protein concentration techniques). The protein stream must also be stable in the feed pellet formulation. Fish feed is usually produced by extrusion cooking with companies such as Clextral (Clextral Food Extrusion Machinery n.d.) specialising in custom-built equipment for fish feed. Fish feed need to conform to various needs depending on the management system of the farm. For example, if fish are bottom feeders, the product need to sink and be stable at the bottom of the dams. The extrusion forming process must cater for the required density of the pellets, as well as for the required optimum shape and size, which is, in turn, dictated by the mouth size and morphology of the particular fish species. While conforming to all these physical specifications, the final product must still be nutritious, the nutrients must be available for digestion, and the product must be stable during transport and storage. All of this needs to be implemented without excessive costs, as the feed costs usually make up around 40–75% of the total running cost of rearing fish (Young and Muir, 2000).

As the challenges for utilising legume and cereal protein sources differ, this chapter will focus on soya oilcake as an example of a waste product from legumes, and on barley brewers spent grain (BSG) as an example of a cereal protein waste. Many other plant protein sources also show potential, and will only be referred to in the text.

17.3.1 Fish eating habits and nutrient requirements

The eating habits of fish significantly influences the eventual management of the aquaculture system being implemented. Some fish are very active feeders on the surface of the water (for example tilapia of which the *Oreochromis* spp. are good representatives). As these fish are typically reared in inland freshwater pond systems, where ponds tend to be quite murky as a result of the active encouragement of algal growth as a supplement feed, it becomes vital that the formulated feed pellets are floating on the surface long enough in order for the farmer to assess the 'eating-frenzy' of the fish. Fish will actively swim to the surface to take up as much feed as possible. Therefore, the pellets must also be of optimal size and shape, in order for the fish to take up as many pellets as possible to keep in their mouths. An excellent account of the optimisation of feeding habits in tilapia has been described by various authors in *Tilapias: biology and exploitation* (Beveridge and McAndrew, 2000) where more specific information can be obtained.

Marine finfish tend to have varying feeding habits, depending a lot on the natural water temperature. The various species of mulloway differ greatly in their feeding habits. The tropical species tend to be active feeders while the atlantic species (for example *Argyrosomus inodorus*), are very lazy feeders tending to scavenge feed from the bottom. This poses interesting challenges in cage aquaculture along the coastline because cages typically have perforated bottoms to allow faeces and other contaminants to disperse easily. The fish dwell on the bottom, and while they are attracted to feed pellets during the feeding stage, the fish is often too slow to catch a sinking pellet in time before it falls through the bottom and becomes a loss. Therefore, a very close collaboration between the fish farmer and the feed developer becomes critically important to ensure success, as the management of the feed can ensure success or failure independent of whether the feed actually consists of the best nutrients possible.

Fish feed is usually developed to fit the life cycle as the requirements of the protein contents differ. Larval feeds need the highest amount of protein, usually in excess of 60%, while protein contents for the finishing stages before fish is slaughtered can be as low as 25%. In the later stages, there is a trade-off between protein and energy requirements as the protein should not be utilised as an expensive energy source, yet enough should be available for maintaining lean body mass and health (Jauncey, 2000). Energy is then often added to the feed from cheap sources such as starch.

Many marine finfish, which are carnivorous by nature, utilise carbohydrates very poorly (Koshio, 2002; Krogdahl *et al*., 2004 and Fountoulaki *et al*., 2005). Typical example include the Mediterranean sea bream (*Sparus aurata*), which performed poorly when feeds with high levels of glucose were given (Koshio, 2002), and salmon which developed digestibility problems when precooked maize starch was included in the feeds (Krogdahl *et al*., 2004). The typical symptom often seen is stress on the liver and so-called 'fatty livers' is believed to be linked to an inability to digest carbohydrates properly. It is therefore critically important to understand the digestive system of each fish species before an attempt is being made to develop feeds for that particular fish, in order to prevent disease. The international *Fish Database* describes the feeding habits of most known species including wild species that can become potential candidates for aquaculture. The database can be accessed on www.fishbase.org. Omnivorous and herbivorous species will exhibit a higher tolerance for feeds from plant origin which will make the use of vegetable, cereal and legume wastes more practical for such fish, while feeds will have to be refined for the carnivorous spp. The FAO also provides excellent illustrated study material describing the anatomy and physiology of the various fish gut systems and a series of chapters can be found on www.fao.org/docrep.

17.3.2 Protein and amino acid measurements in fish feeds

Proteins are described as complex organic polymers consisting of many amino acids linked together through peptide bonds and cross-linked between chains by sulfhydryl bonds, hydrogen bonds and van der Waals forces. The protein specificity of living organisms is conferred by the genetic sequences coding for a specific amino acid sequence (Lloyd *et al*., 1978). Various classes and classification systems for proteins exist, and the health, growth and reproduction of a living organism depends on the ability of that organism to break down and utilise proteins ingested through diet and to re-synthesise the obtained amino acid from the digestion process into the required proteins for their livelihood. Typical requirements are for muscle tissue, cell biology (enzymes), structural needs (collagen) and respiration (for example haemoglobin). Monogastric animals such as fish must derive all protein needs from the diet, and the needs differ between omnivorous and carnivorous species relating to the ability to utilise proteins from different sources such as plants. Carnivorous monogastric fish can depend solely on other live fish (for example the *Piscivorous* spp.), and with its short digestive tract, the carnivorous species have lost the ability to utilise large amounts of carbohydrates as an energy source, and may also depend on proteins for that purpose, hence the typical requirements for very high protein contents in fish feeds for piscivorous fish.

Proteins can be classified broadly into four categories namely:

(i) Simple proteins which yield only amino acids after hydrolysis (and sometimes small carbohydrate compounds). Typical examples include albumins, globulins, prolamines and glutelins.
(ii) Conjugated proteins which yield non-protein material as well as amino acids when hydrolysed, and includes nucleoproteins, glycoproteins and haemoglobins.
(iii) Derived proteins are typically obtained from a physical or chemical process, such as denaturation or partial hydrolysis.
(iv) Enzymes, which are catalysts for biological processes in cells and may consist of simple and conjugated proteins.

For the purpose of fish feed ingredients, proteins are usually obtained from the most abundant groups such as storage and structural proteins found in cereals, legumes and animal wastes. Many of these proteins can be derived as a result of pre-treatments of the particular raw material, and waste products are very seldom free of the effects of such pretreatments. For example, proteins undergo characteristic bonding with other proteins in the so-called plastein reaction, and it will combine with free aldehyde and hydroxyl groups of carbohydrates to form Maillard-type compounds, mostly induced by heat. These reactions may render a particular amino acid unavailable for absorption in the body after digestion because it cannot be hydrolysed cleanly by pepsin or trypsin during digestion (Lloyd *et al.*, 1978).

17.3.3 Protein requirements and quality

Data on the requirements of protein for fish is grouped into two broad areas namely the gross protein requirements (total protein content of a feed) and the amino acid profiles (protein quality of a feed). Further important aspects in feed nutrition revolves around digestibility and nutrient availability, as well as the optimal balance between available protein and available energy. The use of protein must be maximised for bodily growth without a loss to energy needs which can be fulfilled by fat or carbohydrate sources (Jauncey, 2000).

In fish, the general gross protein requirement is the highest in initial feeding of the larvae and fry and it decreases as the fish size increases. Maximum growth rate for fry is obtained when the diet contains around 50% of digestible balanced protein in the diet. For salmon, the protein requirement decreases to 40% when the fish is six to eight weeks old and drops to 35% for yearling salmonids (Storebakken, 2002).

Estimated dietary protein requirements for fish has been determined by many authors, however, one of the persistent problems seems to be a lack of research standardisation methodologies, which is being addressed by the Plant Product in Aquafeeds Working Group project (www.aquafeed.com). Until such research is standardised, protein requirements can only be estimated, and true values must be determined for each species and each environment. Environments differ vastly between different countries, and a wide range of fish is being evaluated for aquaculture and fisheries worldwide owing to wild fish shortages. Estimated dietary protein requirements of some fish species are shown in Table 17.1. The objective of this table is to only show summarised values as an overall indicator and for detailed lists the appropriate reference papers need to be consulted. Many lists of data have been developed, and values tend to be influenced by individual differences between experiments and environments, however, in general, fish protein needs tend to vary between 20 and 55% depending on the digestive system (carnivorous *versus* herbivorous *versus* omnivorous).

In general, protein quality is measured by a selection of standard assays listed below (summarised from Webster and Lim, 2002):

Table 17.1 Estimated dietary protein requirements of fish

Species	Crude protein level needed for optimal growth (g kg^{-1}) (Values adapted from Cowey, 1978 and Jauncey, 2000)
Rainbow trout (*Salmo gairdneri*)	400–460
Carp (*Cyprinus carpio*)	380
Chinook salmon (*Oncorhynchus tshawytscha*)	400
Eel (*Anguilla japonica*)	445
Plaice (*Pleuronectes platessa*)	500
Gilthead seabream (*Chrysophrys aurata*)	400
Grass carp (*Ctenopharyngodon idella*)	410–430
Brycon sp.	356
Red sea bream (*Chrysophrys major*)	550
Yellowtail (*Seriola quinqueradiata*)	550
Oreochromis mossambicus	300–500
Oreochromis niloticus	190–400 (depending on age, protein source etc.)
Oreochromis aureus	300–560
Tilapia zilii	350–400

- Apparent protein utilisation (APU), which is the amount of protein gain of fish fed an experimental diet, divided by the amount of protein being fed.
- Protein efficiency ratio (PER), which is defined as the total weight gain of fish divided by the total protein intake over the period of the feeding trial. PER assumes that all protein is used for growth and no allowance for maintenance (turnover) is made.
- Net protein value (NPU), which is the protein gain of a group of fish fed the experimental diet minus the protein loss of a similar group fed a protein free diet, divided by the weight of protein consumed.

An assay that is often done in conjunction with protein measurements is the apparent digestibility coefficient. Digestible protein refers to the proportion (%) of the ingested protein that is hydrolysed in the gastrointestinal tract and subsequently absorbed. It is measured as the difference between protein ingested and protein voided and it is referred to as the ‘apparent digestibility’ because it does not include endogenous losses in the faeces in the calculation. The apparent digestibility needs to be done on a species-specific level. For some species, such as tilapia, some comprehensive results exist for apparent digestibilities of vegetable protein sources. Jauncey (2000) provides a detailed summary of the apparent protein digestibilities of most known cereal and legume wastes streams for tilapia. Values for barley brewers spent grain vary between 62 and 63%, while soybean meal digestibilities vary between 91 and 94%. It must be remembered, however, that the digestibility of plant proteins is strongly linked to the type of processing and it will have a very large effect on the results. Also, any digestibility data for fish feed is at its best only indicative owing to the fundamental problem of quantitatively collecting fish faeces. Faeces start leaching into the water almost immediately after excretion which makes it almost impossible to do quantitative collections, unless implementing very tedious and expensive systems whereby all water is being collected as well. As most research studies cannot afford the systems, the results will remain subject to large experimental errors (Jauncey, 2000).

17.3.4 Amino acid requirements (true *versus* profiles of whole fish)

Amino acid requirements of marine finfish is a complex field and the experimental work for its determination is a costly exercise. In order to save time, many researchers determine the amino acid requirements of feeds based on the amino acid composition of whole minced fish of the target species. However, Halver (1978) conducted an experiment using Chinook salmon where amino acids were tested individually against a reference diet, and the maximum growth rate was determined for each amino acid level. By doing so, they were able to determine the amino acid requirement of Chinook salmon. In Fig. 17.1, a comparative amino acid profile is given of the Chinook salmon amino acid requirements, the amino acid profile of fish meal used as a control in a feed experiment, as well as the amino acid profiles of egg protein which is often used as a protein standard in feeding. The profiles of selected cereals typically used in feed formulations are also shown. Only the profiles of the ten essential amino acids are given here. Of particular importance is the large difference in amino acids such as lysine and arginine between the profile of fish meal and the profiles of the true requirements. Lysine is typically deficient in cereals when compared with that of fish meal and eggs, which is clear in this comparison. The other amino acids that are deficient are methionine and arginine. However, when compared with the fish meal and the egg amino acid profiles, the true requirement seems to be less stringent, and the barley BSG is only slightly deficient in arginine, lysine and methionine for maximum growth, indicating that the cost of supplementing the BSG will therefore be significantly less. There seems to be a significant research gap in the literature for the systematic studies of true amino acid requirements in order to replace a general assumption approach of analysing the amino acid profile of the whole fish. It is also necessary to evaluate whether the amino acids are biologically available, as opposed to the chemical profile only.

17.3.5 New methods

In spite of many years of research on the adverse effects of soybean protein streams on salmon for example, the causing agent has never been identified.

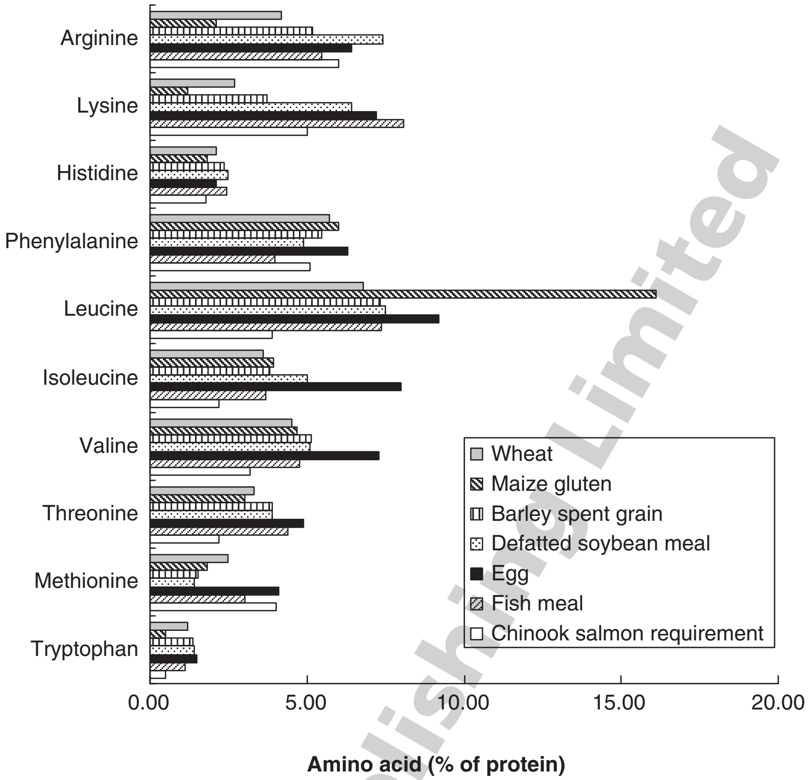

Fig. 17.1 Amino acid profiles of reference protein sources and plant proteins as compared with the true protein requirement of Chinook Salmon (a carnivorous finfish). Barley spent grain analysed by CSIR Biosciences, South Africa (EU FP6 project REPRO contract no. 006922), and other data obtained from Halver, 1978 & Pereira & Oliva-Teles, 2003.

Much less is even understood from the other plant protein sources (for example sunflower protein oilcakes which have a lot of potential, yet expressing an unknown trypsin inhibitory factor which has not yet been identified). Various authors have proposed that new approaches will be needed to solve these problems, especially taking into account the wide selection of protein streams available as well as the wide selection of fish species. Authors such as Francis *et al*. (2001) and Pezzato *et al*. (2004) have stressed the need for understanding of detailed chemical information of secondary metabolites formed during digestion of feed in the fish. A metabolomic approach, where the metabolic profile of cell, tissue, fluid or organs of an organism is studied based on the end products of gene expression may provide a solution. Metabolic profiling and the identification of various volatile, semi-volatile and flavour compounds may provide a clue as to the identification of unknown metabolites that may have a positive or negative impact on digestibility and health of farmed fish (Grigorakis *et al.*, 2001). Such an approach can lead to the discovery of new unknown anti-nutrients, which can help to explain observed effects in fish *in vivo* and on the farm. Comprehensive reviews of proposed new analytical approaches were published by Wilkes *et al.* (2000) and Bino *et al.* (2004) for further information.

17.3.6 Cereal by-product streams

The cereal by-product streams available in the biggest quantities for fish feeds are barley spent grain, maize and wheat. Smaller streams of sorghum by-products are also available, mostly regional and concentrated in certain countries. Brewers spent grain or BSG is a by-product from beer production where, on average, 20 kg of BSG (dry matter) is produced for every 100 L of beer (Mussatto *et al.*, 2006). During the brewing process, the starchy portion of the barley is removed by malting, extraction and fermentation, leaving behind the husk, the aleurone layer and the germ portions. BSG contains significantly more protein than the native barley, with the starch portion removed.

The use of brewers spent grain as a feed ingredient can partly obviate the problem of carbohydrate intolerance in carnivorous finfish. If the malting process is optimised, residual starch levels can be as low as 5% (Musatto *et al.*, 2006) as opposed to native barley which contains 63–65% starch (MacGregor and Fincher, 1993). At the same time, the protein content of the BSG is increased to an average of 25%.

Referring to the protein requirements of fish (Table 17.1), it becomes clear that even after the protein has been concentrated during the malting stage, the protein content in the BSG is still inadequately low for application as a fishmeal replacer. Furthermore, high fibre contents in the BSG will preclude it from being used, as fish cannot digest non-starch polysaccharides (Koshio, 2002; Krogdahl *et al.*, 2004 and Fountoulaki *et al.*, 2005). In Table 17.2 a typical proximate composition of a dried BSG stream from laager beer in South Africa is shown (EU FP6 project REPRO contract no. 006922).

In Table 17.2, it can be seen that the protein content is just above 20%, and the crude fibre content is high, at 16.8%. Fish meal contains less than 2% fibre (Gatlin III *et al.*, 2007), with crude protein contents of 62–72%, thus clearly showing the shortcomings of the BSG waste stream as a fish feed ingredient.

It is possible to separate BSG into protein-rich and protein-poor fractions by physical treatments, such as sieving. Cheng *et al.* (2004) were able to increase the protein content from 21 to 32% (DM) using this technique. In a feeding trial with rainbow trout, a basal fish feed diet was replaced by a diet containing 30% dry BSG or dry high protein BSG (HP-BSG). The

Table 17.2 Proximate composition of a typical dried barley brewers spent grain from Laager beer

Nutrient	Analytical method	Percentage (dry base)
Protein	Protein (Dumas), AACC 46-30, 1999	21.7
Ash	Ash, In house method	3.3
Fat	Fat (Soxhlett), AACC 30-25, 1999	9.0
Carbohydrates	Carbohydrates – by difference	49.2
Crude fibre	Fibre (crude), AACC 32-10, 1999	16.8

Source: CSIR Biosciences, South Africa.

results from the trial showed that the dry matter digestibility increased from 67–68% in the BSG diets to 75% in the HP-BSG diets. The results showed an overall increase in the amino acid digestibility, and the authors commented on a relatively high lysine availability.

There are some alternatives to sieving. Kanauchi and Agata (1997) managed to increase the protein content of a protein-rich BSG fraction to 48% by using a combination milling and sieving process. The focus of this work was to produce a high-protein foodstuff for treatment of ulcerative colitis. This HP-BSG fraction was shown to improve gut health and resulted in a patent being filed (Bamba *et al*., 2002). A few wet separation methods have also been developed, based on washing and sieving processes. The negative aspect of these processes is the additional cost element added to the final product, because of the complexity of multiple processing stages. It is therefore preferable to have a simple process that separates the fractions in one step immediately after receival from the brewery. A single stage wet separation technique was evaluated in the REPRO project (EU FP6 project REPRO, contract no. 006922) and a provisional patent filed (PCT/IB2007/052769, published in February 2008). Results of the high protein fraction obtained from the process are given in Table 17.3. The process consists of a wet brushing separation step immediately after the BSG is received from the brewery. A small amount of water is added to the BSG, and the protein washed out in a single step and collected as the through fraction from a sieve. The pasty protein concentrate is dried afterwards.

The amino acid profiles of these three BSG products are shown in Fig. 17.2. The profiles are compared with the profiles of a typically formulated fish meal feed (CSIR Biosciences, South Africa) and of unmodified (raw) BSG before extraction of the protein concentrate. The BSG profile is adequate for most essential amino acids except for arginine and lysine which is better than the general profile for cereals. One of the reasons for this is the inclusion of the germ fraction from the barley in the protein concentrate, together with the concentration effects obtained after removal of the starch in the original grain.

Table 17.3 Proximate composition of dried high protein BSG produced from three BSG separation runs

Analysis	Protein stream 1	Protein stream 2	Protein stream 3	Average	Standard Deviation
Moisture[1]	3.5	4.2	4.2	3.97	0.40
Protein[1]	40.26	39.76	39.69	39.90	0.31
Ash[1]	4.11	4.6	4.68	4.46	0.31
Fat[1]	13.8	13.2	13.5	13.50	0.30
Crude fibre[1]	6	5.7	5.9	5.87	0.15
Dietary fibre[1]	32.3	32.5	32	32.27	0.25
Energy (kJ)	1712	1685	1687	1694.67	15.04

[1] g/100 g sample.
Source: Provisional Patent no. PCT/IB2007/052769, CSIR Biosciences, South Africa.

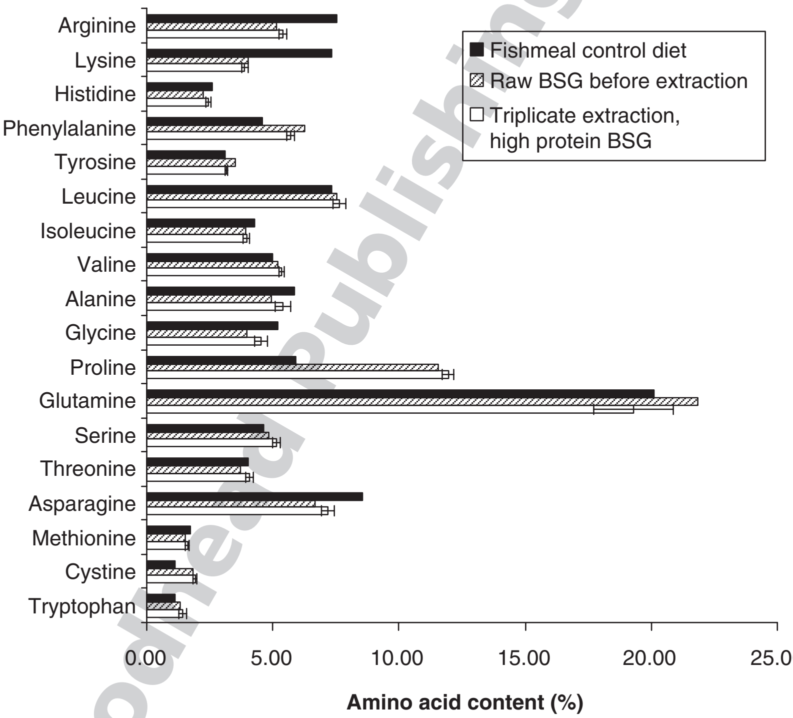

Fig. 17.2 The amino acid profiles of barley spent grain, fish meal-based fish feed and barley spent grain protein concentrate.

Cereal and vegetable protein is commonly deficient in certain essential amino acids such as lysine, tryptophan, methionine, threonine and valine and depending on the source, the ratios between the essential amino acids can vary quite a bit. On the other hand, these plant by-products generally have a high protein digestibility when properly processed.

Fish feeding trials with silver cob (*Argyrosomus inodorus*), which is a mulloway family carnivorous fish species occurring along the South African West coast, yielded feed conversion ratios of 1.3 for a fish meal based diet and 0.9 for a diet where 46% of the fish meal was replaced with the BSG concentrate after four months of feeding. Although this was only a preliminary trial, it showed promising results for barley spent grain protein concentrate as a possible ingredient in marine finfish feeds.

17.3.7 Feed processing

Generally, feeds are processed for two reasons namely increased palatability and improved convenience. The processing method of choice for fish feeds is to produce a ready-to-eat pellet type, with all the nutrients included, and with the correct properties to allow for maximum utilisation by the fish. The typical requirements for a well-processed feed are as follows (Spinelli, 2006):

- composition: must allow for a balanced diet; creating challenges in terms of nutrient density and stability during processing
- physical form: fish perform better if feeds fit the size of their mouths, and approximates their natural feeding habits, for example, bottom feeders perform poorly when feed floats on the surface
- palatability: potential feedstuffs or the effects of processing may render the feed unpalatable to the fish because it may be offensive to their olfactory receptors
- bio-availability of nutrients: processing selection must not cause detrimental effects and rather enhance bio-availability
- stability during storage: critical for the management of farms is that feeds should remain stable during storage in order to ensure continuous supply of quantity and nutrient density
- toxic factors: anti-nutritional factors and toxic substances such as aflatoxins should not be present.

Extrusion cooking is widely used to enhance the palatability and nutritional quality of fishfeeds. The extrusion process can produce pellets with varying densities (Barrows and Hardy, 2001), and increases carbohydrate digestibility (Allan and Booth, 2004). The treatments reduces levels of antinutrients for example trypsin inhibitors in soya feeds.

In Fig. 17.3, a picture is shown of barley spent grain protein concentrate, barley spent grain husks after removal of the protein fraction, and two examples of fish feeds produced by extrusion on a Werner and Pfleiderer

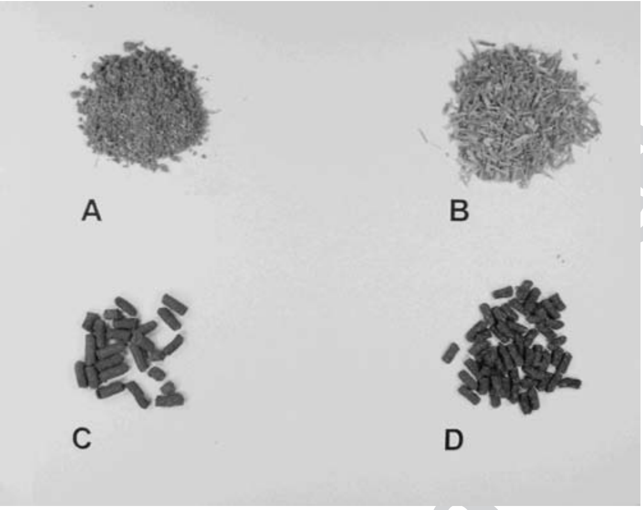

Fig. 17.3 Barley spent grain (BSG) protein concentrate (A), BSG fibre (B), and fish feed pellets produced from BSG protein concentrate and fish meal mixtures for silver cob *Argyrosomus inodorus* (C) and white stumpnose *Rhabdosarghus globiceps* (D), (CSIR Biosciences, South Africa).

co-rotating twin-screw extruder. These feeds were developed as prototypes for feeding cold water marine carnivorous fish in South Africa *Rhabdosarghus globiceps* or white stumpnose, which is a marine sparid, and *Argyrosomus inodorus* or silver cob, which is a species of the mulloway family.

17.3.8 Oilseed co-product streams

Growing amounts of oilcakes are becoming available world-wide owing to an increase in the production of biofuels. Soybeans contain much higher protein contents than cereals and an example of the proximate composition of soybeans is shown in Table 17.4.

From Table 17.4, it is clear that the oil in soya beans is a smaller percentage than, for example, the protein fraction. During oil pressing, the residue is produced as an oil cake which can either be hull-less, or with hulls, which will have an effect on the final fibre concentration and the protein content. Protein contents of oilcake residues can often be as high as 48%. In conjunction with that, the fibre content of dehulled soya oilcakes is lower than for cereal waste streams such as barley spent grain.

Many studies are available on the inclusion of plant-based materials in fish feeds. However, it was generally found that studies tend to be inconclusive, and in many cases, direct comparisons between the results of different authors are suspect because of large differences between feeding

Table 17.4 Typical average composition of soybeans (adapted from Redondo-Cuenca, Villanueva-Suàrez, Rodriguez-Sevilla and Mateos-Aparicio, 2006)

Soybean type	Moisture	Protein	Fat	Dietary fibre	Carbohydrates	Ash
Conventional	10	40	19	17	10	5
Transgenic	8	39	21	14	13	5

Note: Values are rounded off estimates, many exact values in the literature are available and they depend on the cultivar as well as the growing conditions.
Source: adapted from Redondo-Cuenca, Villanueva-Suàrez, Rodriguez-Sevilla and Mateos-Aparicio, 2006.

conditions, feed raw materials and the environment. Studies have been done on a wide range of fish varieties in almost every country in the world. Owing to differences in the design of tanks, ponds and other factors, and differences between the processing conditions employed when preparing feeds, it is difficult in many cases to come to general conclusions about the potential quality of a feed.

Regarding the use of soybean products in fish feeds, again the general impression is that although many studies exists, it does not address specific problems in a systematic way that will be useful for the further development of soybean oilcake as a fish meal replacer. In most studies, the focus was on simply replacing a portion of the fish meal with soya meal (in most cases full fat meal), and recording the results of fish performance against a 100% fish meal control. The exact understanding of the underlying mechanisms responsible for differences between the feeds is not well described, and, according to Francis *et al.*, 2001), this reality contributes significantly to the seemingly conflicting results often seen when comparing fish feed results of different authors. The use of soybean type meals in tilapia feeds has been researched to a certain extent, but very few references were found for carnivorous finfish such as the seabreams. These soybean meal types consist of a variety of products, ranging from full fat untoasted meals to defatted toasted meals. Defatted toasted meals typically originate from the hexane extraction systems for the production of soybean oil.

Another problem arises from the confusion created by the indiscriminate use of the terms ‘oilcake’, ‘soybean meal’ and others. The terms do not necessarily describe the same raw material. Therefore, an article referring to oilcakes, may actually refer to defatted toasted soya flour (most cases are like this), thereby increasing the difficulties associated with the literature studies (Shurtlett and Aoyagi, 2004).

In order to reduce confusion and to define clarity when evaluating the use of soybean products in fish feeds, the following definitions for the different types of soybean products used for animal feeds can be useful:

- Full fat untoasted flour: the flour obtained from milling either the soybeans with hulls, or with the hulls removed without any significant heat treatment applied to the beans/flour either before or after milling.
- Full fat toasted flour: the flour obtained from milling either the soybeans with hulls, or with the hulls removed with significant heat treatment applied to the beans/flour either before or after milling. The heat treatment is of such a nature that all heat-labile anti-nutritional factors are being destroyed completely.
- Defatted untoasted flour: the flour obtained from milling the soybean residue from soybeans with or without hulls, after removal of the oils through solvent extraction, without the application of significant heat treatment before, during or after oil extraction or milling. This excludes mechanical pressing.
- Defatted toasted flour: the flour obtained from milling the soybean residue from soybeans with or without hulls, after removal of the oils through solvent extraction, with the application of significant heat treatment before, during or after oil extraction or milling. This excludes mechanical pressing. The heat treatment is of such a nature that all heat-labile anti-nutritional factors are being destroyed completely.
- Oilcake: the residue obtained after the use of mechanical pressing of soybeans to remove the oil. This includes cold and hot pressing processes. Heat treatments will vary, thereby creating products with varying levels of heat labile anti-nutrients present.
- Oilcake meal: The milled residue obtained after the use of mechanical pressing of soybeans to remove the oil. This includes cold and hot pressing processes. Heat treatments will vary, thereby creating products with varying levels of heat labile anti-nutrients present.
- Press cake: Used sometimes in the South African industry, referring to the by-product of an oilseed after the oil has been expelled or pressed out.

Although a lot of work has been done on the amount and types of antinutrients found in oilseeds, there is still an unknown amount of compounds, as the current knowledge does not explain observations of negative effects to fish growth and fish health, and published literature results are often inconclusive or conflicting. However, the most common classes of anti-nutrients in soyabean oilcake and sunflower oilcake has been summarised by Francis *et al.*, 2001:

- Soybeans: Protease inhibitors, lectins, phytic acid, saponins, phytoestrogens, anti-vitamins, allergens (especially against protein).
- Sunflower: Protease inhibitors, saponins, arginase inhibitors. Owing to colour problems as a result of chlorogenic acid in sunflower, oilcakes from sunflower is less suitable for use in aquaculture feeds than oilcakes from soy. There is also confusion whether chlorogenic

Table 17.5 Classification of anti-nutrients according to factor type

Type	Specific factors
Factors affecting protein utilisation	Protease inhibitors Phenolic compounds including tannin types
Factors affecting mineral utilisation	Phytates Gossypol Oxalates Glucosinolates
Factors affecting vitamin utilisation	Anti-vitamins
Miscellaneous	Mycotoxins Mimosine Cyanogens Nitrates Alkaloids Photosensitising agents Phytoestrogens Saponins

Table 17.6 Oilseed anti-nutrient classification according to heat stability/lability

Classification	Description
Heat labile	Protease inhibitors Phytates Lectins Goitrogens Anti-vitamins
Heat stable	Saponins Non-starch polysaccharides Antigenic proteins Oestrogens Phenolics

exhibits anti-nutritional effects, with some indications that it may have trypsin inhibitor activity, but this has not been proven in the literature.

Francis *et al*. (2001) defined an anti-nutrient as: ‘A substance which by itself, or through its metabolic products arising in living systems, interferes with food utilisation and affect the health and production of animals.’ Classification of anti-nutrients: Two types of classification can be used; as seen in Tables 17.5 and 17.6.

17.3.9 Performance of soya by-products in fish feed

Growth performance of Nile tilapia (*Oreochromis niloticus*) was negatively correlated with an increase in concentration of defatted soybean flour if methionine was not added to the diet; the presence/absence of heat treatment to the soybean flour was not described. An increasing concentration of raw soybean flour (full fat) significantly retarded growth of Nile tilapia (Wee and Shu, 1989); the authors also demonstrated the positive effect of heat treatment by producing a full-fat soybean meal from boiled soybeans. A commercial defatted toasted soybean flour was also tested and the fish showed significantly better growth performance compared with the untreated raw soybean flour control. Mozambique tilapia (*O. mossambicus*) showed poor growth with 50% inclusion levels of raw and heat treated (not clear in article) soybean meal (Jackson *et al*., 1982). Sintayehu *et al*. (1996) demonstrated poor growth in tilapia at a 30% inclusion of a commercial soybean meal (unsure if it was heat treated or not; tilapia species not defined).

Robaina *et al*. (1995) showed a depression of growth performance of gilthead seabream (*Sparus aurata*) at a 30% replacement with raw (untoasted) full fat soya flour, and also showed deposition of lipids in the fish livers.

Most work found was done on trout and salmon, with very limited work done on seabream, while work done on tilapia tend to be inconclusive as poor quality soybean meals were often used (in many cases no heat treatment was given, or the treatment was not described in the paper).

17.4 Identification of research gaps

Many levels of inclusion of soybean meal and flour products exist; with tilapia, it is estimated that 67 to 100% of fish meal can be replaced safely by soybean, providing that it has been thoroughly processed. However, the exact results of many of these trials are confusing because it is not always clear if a particular used soybean meal or cake meal has had sufficient heat treatment or not. Also, most work seems to have focused on larger fish, and it is known that adult fish do tolerate lower protein levels and poorer quality feeds better than the juveniles do. However, problems are usually related rather to the quality of the protein itself than the quantity, as well as the complexity of additional factors having an effect on the bioavailability of the nutrients in the feed (typically the so-called anti-nutritional factors) (Spinelli, 2003). Soybean meal tends to be deficient in sulfur-containing amino acids such as tryptophan and methionine, and usually, these amino acids must be added by fortification of some type either in its pure form or by mixing with other feed ingredients with a high content of the target amino acids (Spinelli, 2003). However, data is inconsistent in many instances for example it is implicated that increased levels of methionine inclusion into the feeds will reduce the negative effects of certain anti-nutritional factors such as trypsin inhibitors, but this has never been proven beyond doubt in finfish types.

Generally, fish feeding trials in the literature are ad hoc, because studies tend to be focusing on localised infrastructure, making general conclusions between studies very difficult. No systematic studies on the effects of soybean inclusion in fish feeds exist for the *Rhabdosarghus* types, and only a few studies exist for tilapia on mainly full fat soya meal, in many cases without heat treatment.

The consensus from authors such as Francis *et al.* (2001) is that levels of anti-nutrients do not necessarily lead to mortality, but it does cloud results and reduces nutritional values significantly.

A systematic approach towards the fundamental understanding of the complex nature of oilcake inclusions in the fish feed rations is needed and, at present, one of the biggest shortcomings is the inconsistency of many results of feeding trials. As oilcakes will become more abundant in the future, it may lead to a market oversupply, and already the price of oilcakes compares extremely favourably to that of fish meal, giving a huge economic benefit providing suitable new technologies for its utilisation can be developed. The prices of fish meal are steadily increasing as it becomes scarcer. It is difficult to predict the long term price movement of oilcakes; in 2006, it was predicted to either stay at current levels or to decrease slightly as a result of increased biodiesel production, but in 2007 and 2008 a world-wide shortage of food caused prices to increase beyond any predicted levels, resulting into a global crisis. The reasons for this increase are not well understood, although links with the international demand for crude oil and biofuels may have stimulated the markets to become unstable.

17.5 Future trends

Long term pressures on animal and fish-based feed protein ingredients will ensure a continued focus on the utilisation of alternative proteins. Apart from soybean and barley protein, other plant-based proteins are being evaluated in fish feeds and used in many places. Such examples include canola meal and wheat products (Cheng and Hardy, 2002), lupins (Pereira and Oliva-Teles, 2004), palm kernel meal (Ng *et al*., 2002), cottonseed and sunflower meal (Sintayehu *et al*., 1996) and maize gluten (Pereira and Oliva-Teles, 2003). All these plant ingredients have challenges related to protein digestibility, protein quality and the presence of anti-nutrients. Additionally, plant-based oils commonly associated with these ingredients are also deficient in essential fatty acids, especially for marine finfish species. Gatlin III *et al*. (2007) summarised new trends in processing and addressing these challenges. For example, biological processing of ingredients by fermentation (Ng *et al*., 2002), and the use of feed enzymes to reduce phytate levels and release phosphorus (Cheng and Hardy, 2002) has become a significant trend. However, high costs often prohibit the use of such fermentations, yet the benefit may outweigh the costs and will pose interesting

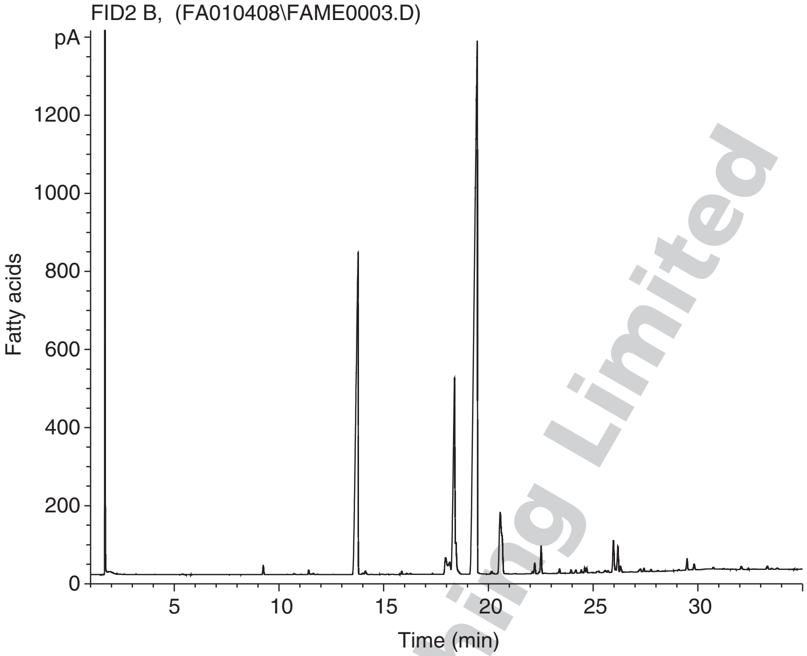

Fig. 17.4 A typical fatty acid profile for brewers spent grain used as a protein source in fish feed (CSIR Biosciences South Africa).

challenges for future feed manufacturers. Other new trends are also emerging. Genetic manipulation of plants has become common in animal feed ingredients and low phytate mutations, high lysine mutations and other specialised mutations such as crops with high oils or modified starch structures are possible (Gatlin III *et al.*, 2007). Apart from that, the development of adapted fish by genetic selection for individuals with improved ability to digest plant materials will further enhance development. The use of pre- and pro-biotic compounds to enhance nutrition is common in farming practices for poultry, yet still a new area for aquaculture. Finally, metabolomics where the end products of gene expression is represented by the metabolome, will become one of the pillars of modern feed ingredient studies. It is becoming possible to evaluate secondary metabolites produced from a specific feed in a specific species of fish as evaluated in a single cell, giving rise to a better understanding of possible harmful volatile, semi-volatile and flavour compounds which may cause changes in the physiology, growth and health of farmed fish (Gatlin III *et al.*, 2007).

An example of the utilisation of fermentation to enhance the nutritional profile of barley brewers spent grain (BSG) is given in Fig. 17.4 and Table 17.7. In this case, *Mortierella* fungi was grown directly on wet BSG. The

Table 17.7 The effect of fermentation of barley spent grain (BSG) using *Mortierella* spp. on the essential fatty acid profile. Values show % fatty acid of total fat. Native BSG refers to unmodified brewers spent grain and Mo. 1, 2 and 3 refers to fermented brewers spent grain with different *Mortierella* strains

Fatty acid	Fatty acid generic name	Native BSG[1]	Mo 1	Mo 2	Mo 3
C14:0	Myristic	0.2			
C16:0	Palmitic	22.6	19.1	21.7	20.0
C16:1	Palmitoleic	0.1			
C18:0	Stearic	2.0			
C18:1n9	Oleic	12.3	12.8	13.3	13.1
C18:2n6	Linoleic	51.4	41.4	48.0	43.2
C18:3n3	Linolenic	5.1	6.5	6.5	7.1
C18:3n-6γ	γ-linolenic	0.0	1.8	1.1	1.2
C20:0	Arachidic	0.4			
C20:1n9	Gadoleic	1.0			
C20:2	Eicosadienoic	0.2			
C20:3n6	*cis*-8,11,14-Eicosatrienoic	0.1			
C20:3n3	*cis*-11,14,17-Eicosatrienoic	0.2			
C20:4	Arachidonic	0.0	8.5	0.0	7.2
C20:5n	Eicosapentaenoic	0.0	2.0	0.5	1.2
C22:0	Behenic	1.4			
C22:1n9	Erucic	1.0			
C24:0	Lignoceric	0.4			
C24:1n9	Nervonic	0.2			

[1] Average of three chromatograms.

Source: CSIR Biosciences South Africa.

fungus is non-toxic and can be used directly in the fish feed. A production of essential fatty acids (eicosapentaenoic acid, γ-linolenic acid and arachidonic acid) was achieved by the fungus. Such fatty acids occur in fish oils, but not necessarily in plant oils (as can be seen by the untreated BSG profiles). Apart from improving the fatty acid fingerprint, which will reduce the need for the addition of fish oil, the fungus also increases protein mass.

The samples (Fig. 17.4 and Table 17.7) were first dried in an oven at 65 °C, then ground in a coffee grinder. Fat extraction was carried out by the Soxhlet method using petroleum ether. Thereafter, the fatty acids in the fat sample were derivatised to the fatty acid methyl esters (FAMEs) using a boron trifluoride–methanol mixture. Extracts were analysed for FAMEs by capillary gas chromatography using a polyethylene glycol-based capillary column with split injection and flame ionisation detection. A reference 37-component FAME mixture was used to identify the FAMEs in the BSG samples. Work was done at CSIR Biosciences, South Africa, as part of the EU FP6 project REPRO contract no. 006922.

17.6 Sources of further information and advice

An excellent general source of information on alternative plant ingredients in fish feeds can be obtained from the aquafeed working group which is an informal group of international experts working collaboratively on research to optimise the use of plant foods in aquaculture diets. The group also develops support for research. The web page is www.aquafeed.com. Other important sources of information can be obtained from the FAO Fisheries Department and FAO website (www.fao.org).

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