Fishmeal is very extensively used in feeds for fish as well as other animals. A recent global survey estimated aquaculture consumption of fishmeal at 3724 thousand tonnes in 2006 (Tacon and Metian 2008). Now it is becoming increasingly evident that such continued exploitation of this natural resource will ultimately become both environmentally and economically unsustainable.

Any satisfactory alternative feed ingredients must be able to supply comparable nutritional value at competitive cost. Conventional land-based crops, especially grains and oilseeds, have been favoured alternatives due to their low costs, and have proved successful for some applications when they were used as substitutes for a portion of the fishmeal. But even when these plant-based substitutes can support good growth they can cause significant changes in the nutritional quality of the fish produced.

Why algae?
The reader may wonder why algae, including both macroalgae (‘seaweeds’) and microalgae (e.g. phytoplankton), and which are popularly thought of as ‘plants’, would be good candidates to serve as alternatives to fishmeal in fish feeds. One fundamental consideration is that algae are the base of the aquatic food chains that produce the food resources that fish are adapted to consume. But often it is not appreciated that the biochemical diversity among different algae can be vastly greater than among land plants, even when ‘Blue-Green Algae’ (e.g. Spirulina), more properly called Cyanobacteria, are excluded from consideration. This reflects the very early evolutionary divergence of different algal groups in the history of life on earth. Only one of the many algal groups, the Green Algae, produced a line of descent that eventually gave rise to all the land plants. Therefore it can be difficult to make meaningful generalisations about the nutritional value of this extremely diverse group of organisms; rather it is necessary to consider the particular qualities of specific algae.

Protein and amino acids
Fishmeal is so widely used in feeds largely thanks to its substantial content of high-quality proteins, containing all the essential amino acids. A critical shortcoming of the crop plant proteins commonly used in fish feeds is that they are deficient in certain amino acids such as lysine, methionine, threonine, and tryptophan (Li et al. 2009), whereas analyses of the amino acid content of numerous algae have found that although there is significant variation, they generally contain all the essential amino acids. For example, surveys of 19 tropical seaweeds (Lourenço et al. 2002) and 34 edible seaweed products (Dawczynski et al. 2007) found that all species analysed contained all the essential amino acids, and these findings are consistent with other seaweed analyses (Rosell and Srivastava 1985, Wong and Peter 2000, Ortiz et al. 2006).

Analyses of microalgae have found similar high contents of essential amino acids, as exemplified by a comprehensive study of 40 species of microalgae from seven algal classes that found that, “All species had similar amino acid composition, and were rich in the essential amino acids” (Brown et al. 1997).

Taurine
One often-overlooked nutrient is the non-protein sulphonic acid taurine, which is sometimes lumped with amino acids in discussions of nutrition. Taurine is usually an essential nutrient for carnivorous animals, including some fish, but it is not found in any land plants. However, although taurine has been much less often investigated than amino acids, it has been reported in significant quantities in macroalgae such as Laminaria, Undaria, and Porphyra (Dawczynski et al. 2007, Murata and Nakazoe 2001) as well as certain microalgae, for example the green flagellate Tetraselmis (Al-Amoudia and Flynn 1989), the red unicellular alga

### Table 1: Nutritional profiles of rotifers enriched using optimized protocols based on culture using Reed Mariculture RotiGrow Plus® and enriched with N-Rich® feeds

<table>
<thead>
<tr>
<th>N-Rich® feed type</th>
<th>Applications</th>
<th>High PRO®</th>
<th>PL Plus®</th>
<th>Ultra PL®</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Moderate PUFA; overnight gut-load maintenance</td>
<td>Overnight or 2-6 hr enrichment</td>
<td>Extreme DHA 2 hr enrichment</td>
<td></td>
</tr>
<tr>
<td>Lipid (Dry wt. % of Biomass)</td>
<td>35%</td>
<td>44%</td>
<td>66%</td>
<td></td>
</tr>
<tr>
<td>DHA (% of lipids)</td>
<td>37%</td>
<td>41%</td>
<td>44%</td>
<td></td>
</tr>
<tr>
<td>EPA</td>
<td>5%</td>
<td>2%</td>
<td>0.5%</td>
<td></td>
</tr>
<tr>
<td>ARA</td>
<td>1.0%</td>
<td>1.0%</td>
<td>1.2%</td>
<td></td>
</tr>
<tr>
<td>Total PUFAs</td>
<td>45%</td>
<td>45%</td>
<td>48%</td>
<td></td>
</tr>
<tr>
<td>Protein</td>
<td>38%</td>
<td>32%</td>
<td>18%</td>
<td></td>
</tr>
<tr>
<td>Carbohydrate</td>
<td>19%</td>
<td>15%</td>
<td>7%</td>
<td></td>
</tr>
<tr>
<td>Ash</td>
<td>8%</td>
<td>9%</td>
<td>10%</td>
<td></td>
</tr>
<tr>
<td>Dry weight Biomass</td>
<td>9%</td>
<td>9%</td>
<td>9%</td>
<td></td>
</tr>
</tbody>
</table>
Porphyridium (Flynn and Flynn 1992), the dinoflagellate Oxyrrhis (Flynn and Fielder 1989), and the diatom Nitzschia (Jackson et al. 1992).

Pigments
A few algae are used as sources of pigments in fish feeds. Haematococcus is used to produce astaxanthin, which is responsible for the pink colour of the flesh of salmon. Spirulina is used as a source of other carotenoids that fishes such as ornamental koi can convert to astaxanthin and other brightly coloured pigments. Dunaliella produces large amounts of beta-carotene.

Lipids
In addition to its high content of high-quality protein, fishmeal provides lipids rich in ‘PUFAs’, or polyunsaturated omega-3 and omega-6 fatty acids. These are the ‘fish oil’ lipids that have become highly prized for their contribution to good cardiovascular health in humans. But it is not always appreciated that algae at the base of the aquatic food chain in fact originate these ‘fish oil’ fatty acids. These desirable algal fatty acids are passed up the food chain to fish, and they are indeed essential nutrients for many fish.

Algae have been recognised as an obvious alternative source of these ‘fish oil’ fatty acids for use in fish feeds (Miller et al. 2008), especially eicosapentaenoic acid (EPA), docosahexaenoic acid (DHA), and arachidonic acid (ARA). There is a substantial literature devoted to analysis of the PUFA content of microalgae, particularly those used in aquaculture, because they have long been recognised as the best source of these essential nutrients for production of zooplankton necessary for the first feeding of larval fish, as well as filter-feeding shellfish.

Many shellfish producers are aware the sterol profile of feed lipids is of critical importance, but much less attention has been paid to the importance of the
Various species of microalgae are used as aquaculture feeds, depending on the cell size and nutritional profile needed for particular applications.

Various species of microalgae are used as aquaculture feeds, depending on the cell size and nutritional profile needed for particular applications. It is not surprising that the biochemical compositions of certain marine microalgae are well-matched to the nutritional requirements some marine fish. Larval feeds are probably deserving of the most attention in efforts to discover how algae can best be used in fish feeds, because microalgae are a natural component of the diet of many larval fish, either consumed directly or acquired from the gut contents of prey species such as rotifers and copepods. Existing protocols that use microalgae to improve the PUFA profile of live prey (Table 1) demonstrate how effectively an algal feed can enhance the nutritional value of these live feeds.

Use of algae in formulated fish feeds

Various species of macroalgae and microalgae have been incorporated into fish feed formulations to assess their nutritional value, and many have been shown to be beneficial: Chlorella or Scenedesmus fed to Tilapia (Tartiel et al. 2008); Chlorella fed to Korean rockfish (Bai et al. 2001); Undaria or Ascomyllum fed to Sea Bream (Yone et al. 1986); Ascomyllum, Porphyra, Spirulina, or Ulva fed to Sea Bream (Mustafa and Nakagawa 1995); Grateluria or Ulva fed to European Sea Bass (Valente et al. 2006); Ulva fed to Striped Mullet (Wassef et al. 2001); Ulva or Porocladia fed to Gilthead Sea Bream (Wassef et al. 2005); Porphyra, or a Nannochloropsis-Ischyrysis combination fed to Atlantic Cod (Walker et al. 2009, 2010). Unfortunately, it has rarely been possible to determine the particular nutritional factors responsible for these beneficial effects, either because no attempt was made to do so, or poor design of the study.

For example, in one of the few studies that has focused on the effects of substituting algal protein for gluten protein, the control and all the test diets contained casein plus added methionine and lysine, no analysis of the algal protein was provided, and the algal protein (a biofuel process by-product) contained very high levels of aluminium and iron (Hussein et al. 2012). More and better-designed studies are necessary before we will have a good understanding of how algae can best be used in fish feeds.

Choosing the right algae

Often the algae chosen for fish feeding studies appear to have been selected largely for convenience, because they are low-cost and commercially available. For example, microalgae such as Spirulina, Chlorella and Dunaliella can be produced by low-cost open-pool technologies and are marketed as dry powders, and their nutritional profiles are well-documented. Macroalgae such as the ‘kelps’ Laminaria, Undaria, and Durvillea, and the brown rockweed Ascomyllum, occur in dense stands that can be harvested economically, and they have a long history of use as sources of iodine, as soil amendments, and animal feed additives to supply trace elements.

In recent years there has been great interest in the potential of algae as a biofuel feedstock, and it has often been proposed that the protein portion remaining after lipid extraction might be a useful input for animal feeds (e.g. Chen et al. 2010). However, the algae chosen for biofuel production may not be optimal for use as a feed input, and the economic pressure for the lowest-cost methods of fuel production is likely to result in protein residues with contamination that makes them unfit for use as feed (e.g. Hussein et al. 2012).

By contrast, the high-value microalgae that are used in shellfish and finfish hatcheries are generally produced in closed culture systems to exclude contaminating organisms, and they cannot be dried before use without adversely affecting their nutritional and physical properties, greatly reducing their value as feeds. Inevitably their production costs are higher, but their exceptional nutritional value justifies the extra expense. Table 2 presents

<table>
<thead>
<tr>
<th>(Dry Weight)</th>
<th>Nannochloropsis oculata</th>
<th>Tetraselmis sp.</th>
<th>Pavlova sp.</th>
<th>Isochrysis (T-Iso)</th>
<th>Thalassiosira weissflogii</th>
</tr>
</thead>
<tbody>
<tr>
<td>Protein</td>
<td>52%</td>
<td>55%</td>
<td>52%</td>
<td>47%</td>
<td>52%</td>
</tr>
<tr>
<td>Carbohydrate</td>
<td>16%</td>
<td>18%</td>
<td>23%</td>
<td>24%</td>
<td>23%</td>
</tr>
<tr>
<td>Lipid</td>
<td>17%</td>
<td>14%</td>
<td>20%</td>
<td>17%</td>
<td>14%</td>
</tr>
</tbody>
</table>

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typical nutritional profiles of algae produced by Reed Mariculture Inc.

Just as it would be senseless to arbitrarily substitute one conventional crop plant for another (e.g. potatoes for soybeans) when formulating a feed, the particular attributes of each alga must be carefully considered. In addition to the protein/amino acid profile, lipid/PUFA/sterol profile, and pigment content, there are important additional considerations.

The type and quantity of extracellular polysaccharides, which are very abundant in certain algae, can interfere with nutrient absorption, or conversely be useful binding agents in forming feed pellets. The thick cell walls of microalgae such as Chlorella can prevent absorption of the nutritional value of the cell contents. Inhibitory compounds such as the phenolics produced by some kelps, and brominated compounds produced by red algae such as Laurencia, can render an alga with an excellent nutritional analysis unsuitable for use in a feed. Depending on growth and processing conditions, algae can contain high concentrations of trace elements that may be detrimental.

Further careful study of the properties of numerous algae will be necessary in order to optimally exploit the great potential offered by this diverse group of organisms. But it is already apparent that algae will play an important part in the effort to move the formulation of fish feed “down the food chain” to a more sustainable future.

References available on request.

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Fishmeal & fish oil
and its role in sustainable aquaculture

by Dr Andrew Jackson, Technical Director, IFFO, UK

The annual global production of fishmeal and fish oil is currently around five million tonnes of meal and one million tonnes of oil (Figure 1), except in years when the fishing in the South Pacific is disrupted by the warm waters of an El Niño, most recently in 2010. Around 22 million tonnes of raw material is used, of which approximately 75 percent comes from whole fish and 25 percent from by-products of processing fish for human consumption (IFFO estimates).

The majority of the whole fish used are small pelagic fish such as anchovy, menhaden, sardines and sandeels for which there are limited markets for direct human consumption. In addition to the estimated 11.5 million tonnes of small pelagic fish used in fishmeal there is also an estimated five million tonnes of other fish, the majority from mixed tropical trawl fisheries in East Asia.

Going forward

The prospects for increasing the production of fishmeal and fish oil are very limited, since most of the underlying fisheries are now being well managed, using the precautionary principle with tightly set and monitored quotas. Also increasingly, markets are being found for at least a proportion of the catches to go for direct human consumption.

In addition there is concern that some of the mixed tropical trawl fisheries are not being well managed and that catches will therefore decrease in the coming years as these become severely depleted. The prospects for increasing volumes of fisheries by-products do however look better as fishing becomes concentrated at fewer landing sites and aquacultural production also becomes more concentrated. This will be further encouraged by the rising price of fishmeal and stricter laws against the dumping of waste material. So on balance the production of both fishmeal and fish oil over the next few years is likely to remain about where it is or possibly decrease slightly, which will certainly happen in El Niño years.

The lack of growth in the production of marine ingredients has led some to speculate that the growth of aquaculture would in turn be limited by the shortage of such key ingredients – the so-called fishmeal trap. It is certainly true that during the 1990s and early 2000s as aquaculture grew, it used more and more fishmeal, mostly by taking volumes that in the past had gone into pig and poultry feeds.

However, since around 2005 aquaculture requiring feed has continued its strong annual growth of around seven percent but the volumes of fishmeal used in aquaculture have remained steady at around 3.2 million tonnes and those of fish oil have even reduced to around 600,000 tonnes. (Figure 2). This has led the FAO to state in their recently released report on the State of Fisheries and Aquaculture (FAO 2012): “Although the discussion on the availability and use of aquafeed ingredients often focuses on fishmeal and fish-oil resource, considering the past trends and current predictions, the sustainability of the aquaculture sector will probably be closely linked with the sustained supply of terrestrial animal and plant proteins, oils and carbohydrates for aquafeeds.”

Becoming a strategic ingredient

This growth in aquaculture production,
whilst not increasing the total amount of fishmeal used, is coming through the partial replacement of fishmeal in the diets of almost all species (Tacon et al 2011, Figure 3). This drive to replace fishmeal is being driven by the rise in the price of fishmeal and improving nutritional knowledge, but also by concern about the fluctuating supply due to El Niño, etc. Of course the price of all commodities has risen steeply in recent years and it is important to compare the price of fishmeal with the alternatives.

The most commonly used alternative to fishmeal is that of soymeal. Figure 4 shows that over the last twenty years the price ratio of fishmeal to soymeal has increased significantly, which is indicative of the fact that fishmeal is being reduced in less critical areas such as grower feeds, but remains in the more critical and less price-sensitive areas of hatchery and broodstock feeds. Fishmeal is therefore becoming less of a commodity and more of a strategic ingredient used in places where its unique nutritional properties can give the best results and where price is less critical.

Fish oil and its fatty acids

As has been well documented, during the period 1985-2005 fish oil usage moved from being almost exclusively used to produce hydrogenated margarines to being almost exclusively used in aquaculture. Within aquaculture by far the biggest user was in salmon feed, indeed it reached the point, in around 2002, when over 60 percent of the world’s fish oil production was being fed to salmon.

The reason for this very high usage in salmon feeds was that salmon were found to perform best on diets with in excess of 30 percent fat and at the time fish oil was one of the cheapest oils on the market. In addition it also gave the finished salmon fillets a very high level of long chain Omega-3 fatty acids, specifically EPA and DHA.

During the last 10 years increasing evidence has been published on the very important role these two fatty acids play in human health. EPA has been shown to be critical in the health of the cardiovascular system and DHA in the proper functioning of the nervous system, most notably brain function.

This growing awareness within the medical profession and the general public has led to many governments producing recommended daily intakes for these fatty acids and companies launching a large number of health supplements, including pharmaceutical products, with concentrated EPA.

The importance placed on EPA and DHA in the human diet has had a number of profound effects on the fish oil market. Firstly over the last ten years a significant market has
developed for the sale of crude fish oil for its refinement and inclusion into capsules etc.

This has grown from almost nothing, to the point where today around 25 percent of the world’s production of crude fish oil is sold to this market. This has occurred at a time when the demand for salmon feed has gone from 1.8 million tonnes to nearly three million tonnes. The other critical factor is that to obtain fish oil of the right quality (freshness, lack of oxidation products and levels of EPA and DHA) the nutraceutical market pays a premium of 25-30 percent over that for feed oil (current price for feed-grade fish oil is approximately $1,800/tonne).

In order to increase the production of salmon feed in-line with the market (as well as trying to minimise any price effect) feed producers have been increasingly substituting fish oil with vegetable oil. The vegetable oil of choice is rapeseed (or canola) oil, which, while not having any EPA or DHA, does at least have short-chain omega 3 fatty acids and fewer omega-6 fatty acids than most other commonly available vegetable oils such as soya oil. The point has now been reached where over 50 percent of the added oil in salmon diets comes from vegetable sources and this trend seems likely to continue.

As salmon are poor converters of short-chained omega-3 fatty acids to long-chain fatty acids the fatty acid profile of the finished salmon fillet is very much a reflection of the fatty acid profile in the feed. The result is that the EPA and DHA content of farmed salmon is decreasing and the omega-6 content is increasing.

This trend seems set to continue in the years to come. It seems likely that the salmon market will differentiate into ‘high EPA and DHA’ salmon demanding a price premium and regular salmon, which, while still containing some EPA and DHA will have levels well below that found in wild salmon.

Is it sustainable?

One of the most often asked questions about fishmeal and fish oil is whether or not the practice is sustainable. This is a huge topic for discussion and one that is not easily covered in the last section of a short article. To answer the question one has to go back and look at the source of the raw material and look at the matter, fishery by fishery. The most widely accepted measure of sustainability for a fishery is the Marine Stewardship Council’s standard. However, whilst this has been adopted by a growing number of fisheries which can be eco-labelled at the point of sale, there are currently no substantial volumes of whole-fish from MSC certified fisheries being made available to fishmeal plants.

Back in 2008 IFFO became aware that the fishmeal and fish oil industry needed an independently set, third-party audited standard, which could be used by a factory to demonstrate the responsible sourcing of raw material and the responsible manufacture of marine ingredients. IFFO convened a multi-stakeholder task force including feed producers, fish farmers, fish processors, retailers and environmental NGOs who over the next 18 months compiled the standard which was launched late 2009.

The IFFO RS standard has been quickly adopted by the industry and the point has now been reached where over one third of the world production comes from certified factories. The standard requires that any whole fish must come from fisheries that are managed according to the FAO Code of Conduct for Responsible Fisheries. The standard also demands that the factory can demonstrate good manufacturing practice including full traceability from intake to finished product.

There are now around 100 certified factories in nine different countries producing IFFO RS fishmeal and fish oil. Many of the world’s major feed fisheries have been approved for use, although some have yet to produce sufficient evidence to convince the auditors. Full details of certified plants and approved raw materials can be found on the IFFO web site, www.iffo.net.

A continuing area of concern is Asia where, as discussed earlier, there are considerable volumes of fishmeal produced from trawled mixed species. IFFO is working with a number of different organisations including the FAO and the Sustainable Fisheries Partnership to investigate how to bring about fisheries improvement in this critical area. Asia
"Fishmeal and fish oil production is expected to remain around current levels, but this is unlikely to limit the growth of aquaculture which will continue to have reducing inclusion levels of marine ingredients in the diets of most farmed fish."

Conclusions

Fishmeal and fish oil production is expected to remain around current levels, but this is unlikely to limit the growth of aquaculture which will continue to have reducing inclusion levels of marine ingredients in the diets of most farmed fish. Fishmeal will increasingly become a strategic ingredient used at critical stages of the life-cycle when optimum performance is required.

The growing importance of EPA and DHA in human health will ensure that there is a strong demand for fish oil, either for direct human consumption or via farmed fish, such as salmon.

There is a growing need for fish feed producers and farmers to demonstrate that all the raw materials in their feeds are being responsibly sourced. This is best achieved by using an internationally recognised certification standard. Increasing volumes of certified marine ingredients are now coming onto the market which will allow fish farmers to demonstrate their commitment to responsible aquaculture.

References


More Information

Website: www.iffo.net
Feed for fish and shrimp raised in aquaculture needs high levels of protein and energy. Traditionally feed for carnivorous or omnivorous fish and for shrimp provides these mainly as fishmeal and fish oil, which also contributes to the health promoting aspects of fish and shrimp in the human diet.

Aquaculture of fed species today takes 60–80 percent of the fishmeal and 80 percent of the fish oil produced, mainly from the industrial pelagic fisheries or, in a growing trend, from the trimmings produced during processing for human consumption. Trimnings are defined as by-products when fish are processed for human consumption or if whole fish is rejected because the quality at the time of landing does not meet requirements for human consumption. The International Fishmeal and Fish Oil Organisation estimates trimmings are now used for around 25 percent of fishmeal production.

The industry is, therefore, heavily dependent on marine resources but production from these resources cannot be increased sustainably, either for human consumption or the industrial fisheries. At best, sustainably managed fisheries will continue to yield around the current harvest of five million tonnes of fishmeal and one million tonnes of fish oil.

Feed producers such as Skretting require their marine raw material suppliers to document that the fishmeal and fish oil are derived from responsibly managed and sustainable fisheries and do not include endangered species. Therefore, to meet a growing demand for fish, aquaculture must identify alternatives to these marine ingredients.

**Rising demand**

Analyses of global demographics, widely publicised by the Food and Agriculture Organization of the United Nations (FAO), indicate a continuing expansion of the population passing nine billion by 2050. In parallel, economic development is providing a greater proportion with an income that permits them to be more selective about their diet. The main trend is to switch from vegetable staples to animal and fish protein. A third, but lesser, factor is the growing awareness of the health benefits of fish in the diet, providing long chain omega-3 polyunsaturated fatty acids (LC PUFAs) EPA and DHA, fish proteins and important vitamins and minerals such as iodine and selenium.

At the same time, a growing proportion of the pelagic catch, which includes the industrial fisheries, is going to the more lucrative markets of processing for human consumption, as processing technology improves and as new consumers with different tastes enter the market. Simultaneously, the omega-3 supplements industry is competing for the best quality fish oils and readily outbids the feed producers.

According to the FAO report ‘The State of World Fisheries and Aquaculture 2012’, aquaculture is “set to remain one of the fastest growing feed sectors”. Having doubled in the past decade to almost 60 million tonnes globally, it is expected to grow by up to 50 percent in the next. This makes identifying alternative, sustainable sources of protein and energy a major priority. Researchers are looking for alternatives that will provide low feed conversion ratios, maintain high fish welfare and produce fish that are good to eat, both in terms of eating experience and nutrition. It has been a main focus at Skretting Aquaculture Research Centre for the past decade, for example determining the nutritional value of more than 400 raw materials. These investigations led to AminoBalance™, where balancing of amino acids increases the contribution such proteins make to muscle growth.
Recent advance

Research progress to date means fishmeal levels in feeds for species such as Atlantic salmon have been reduced. Until recently 25 percent appeared to be the limit below which performance suffered, in terms of growth rate and feed conversion ratio.

In 2010 researchers at Skretting ARC finalised a new concept known as MicroBalance™. MicroBalance™ technology is based on the identification of several essential micro-nutrients in fishmeal that were shown to be the limiting factors, not the amount of fishmeal. Supplementing the diet with the right balance of essential micro-nutrients and other functional micro-ingredients helped reduce fishmeal content in fish feed.

Applying the concept enabled Skretting companies to produce commercially successful feeds with as little as 15 percent fishmeal without detracting from feed performance, fish welfare or end product quality. A key advantage of MicroBalance is the flexibility to adapt the raw material combination in response to prices, lessening for farmers the impacts of price volatility.

Today Skretting can formulate fish feed with levels of fishmeal as low as 5–10 percent. Fishmeal can be replaced solely by vegetable raw materials or by a combination of vegetable raw materials and non-ruminant processed animal proteins (PAPs). It should be noted that PAPs are widely used in countries outside the EU and provide extremely good quality, safe nutrition to supplement fishmeal.

Typical examples include blood meal also known as haemoglobin meal, poultry meal, and feather meal. PAPs were banned from animal feed and fish feed in the EU following the BSE crisis in the 1990s. Recently a proposal for the reintroduction of PAPs in fish feed was approved by a qualified majority of EU member states, meaning that non-ruminant PAPs will be authorised for fish feed from June 1, 2013.

Trial results

A 22-month trial with Atlantic salmon in a commercial scale farm in Norway demonstrated the practicality of MicroBalance. It followed a complete generation of salmon from smolt to harvest. The trial was jointly organised by Marine Harvest and Skretting and conducted at the Centre for Aquaculture Competence (CAC) in Norway from May 2009 to February 2011 inclusive. CAC is a commercial-scale R&D farm managed by Marine Harvest and is equipped to measure all operational parameters just as precisely as in a small-scale research station. A total of 780,000 Atlantic salmon provided were divided and fed on one of three feeds:

- Conventional grower feed (pre MicroBalance): 25 percent fishmeal and 13 percent fish oil with EPA + DHA comprising about 10 percent of total fatty acids.
- OptiLine from Skretting Norway (using MicroBalance): 15 percent fishmeal and 13 percent fish oil with EPA + DHA comprising about 10 percent of total fatty acids.
- OptiLine from Skretting Norway (using MicroBalance): 15 percent fishmeal and 13 percent fish oil with EPA + DHA comprising about 10 percent of total fatty acids.
percent fish oil with EPA + DHA comprising about 10 percent of total fatty acids.

Experimental OptiLine (using MicroBalance): 15 percent fishmeal and nine percent fish oil with EPA + DHA comprising about eight percent of total fatty acids.

The parameters monitored were growth, FCR, quality, health, sustainability and food safety. The total harvest weight was 3,517 tonnes. After the harvest the taste, smell and texture of the fillets were tested by a panel of professional tasters. The results showed that both low fishmeal feeds gave the same growth and FCR as the control diet. There were no observed differences in fish health, or in the quality parameters.

The salmon fed with the lowest proportion of marine products (15% fishmeal, 9% fish oil) only needed 1.07 kg of fish in their feed to produce 1 kg at harvest. Calculating protein alone showed a positive ratio, with fish out exceeding fish in.

MicroBalance is now applied in the diets of several other commercial species, including sea bass, sea bream, rainbow trout, turbot and yellowtail.

**Fish oil**

Research to date has enabled producers of fish feed to supplement fish oil with vegetable oils in the diets of carnivorous species by as much as 50 percent. Lower levels have been tested in experimental diets with no negative effects. Much of the progress results from the EU RAFOA project. RAFOA stands for Researching Alternatives to Fish Oil in Aquaculture and the project focused on four species: Atlantic salmon, rainbow trout, sea bass and sea bream. Led by the Institute of Aquaculture at the University of Stirling, partners include NIFES (the National Institute of Nutrition and Seafood Research) and Skretting ARC, in Norway, the INRA (National Institute for Agronomic Research) in France and the University of Las Palmas, in the Canary Islands (Spain). The main challenge is to maintain adequate levels of EPA and DHA, both for the fish and for the health benefits of fish as food.

Secondly the EU AquaMax project, coordinated by NIFES in Norway with 32 international partners around the world including Skretting ARC, addressed this issue directly, developing diets with low levels of both fishmeal and fish oil and thus reducing the fish-in-fish-out ratios. This complements work at Skretting ARC to develop the LipoBalance™ concept, which allows combinations of oils to be prepared that will provide the correct balance of energy and nutrients, including EPA and DHA, at lowest cost.

**Performance ratios**

Feed conversion ratios (FCRs) have advanced significantly over the past three decades. In Atlantic salmon, for example, the FCR has decreased from 1.30 in the 1980s to slightly above 1.00 today, mainly due to the development of high-nutrient-dense diets and to improvements in feed management (reducing feed waste). This represents more efficient use of feed raw materials; especially as fishmeal and fish oil contents were reduced in the same period (Table 1).

Another contributor here is the emergence of functional diets that maintain or even improve performance in adverse conditions such as high or low water temperatures and outbreaks of disease. Better growth, reduced FCR and higher survival will all contribute to improve the utilisation of feed resources.

Feed Fish Dependency Ratio (FFDR) is the quantity of fish meal used per unit of cultured fish produced. This measure can be weighted for fishmeal or fish oil, whichever component creates a larger burden of wild fish in feed. In the case of Atlantic salmon for example, following the introduction of the MicroBalance concept, the fish oil will certainly be the determining factor for the FFDR. The dependency on wild forage fish resources should be calculated for both FM and FO using the following formulae.\[
\text{FFDRm} = \frac{(\% \text{ fishmeal in feed from forage fisheries}) \times (\text{eFCR})}{22.2} \\
\text{FFDRo} = \frac{(\% \text{ fish oil in feed from forage fisheries}) \times (\text{eFCR})}{5.0}
\]

Where:
- eFCR is the Economic Feed Conversion Ratio; the quantity of feed used to produce the quantity of fish harvested.
- Only fishmeal and fish oil that is derived directly from a pelagic fishery (e.g. anchoveta) is to be included in the calculation of FFDR.
- The amount of fishmeal in the diet is calculated back to live fish weight by using a yield of 22.2%. This is an assumed average yield. If the yield is known to be different that figure should be used.
- The amount of fish oil in the diet is calculated back to live fish weight by using a yield of five percent This is an assumed average yield.

**Table 1:** Total production of fed species in 2000, 2005, 2010, with total feed used, total fishmealand total fish oil (x 1,000 tonnes).

<table>
<thead>
<tr>
<th>Year</th>
<th>Total production of fed species</th>
<th>Total of feeds used</th>
<th>Total fishmeal used</th>
<th>Total fish oil used</th>
</tr>
</thead>
<tbody>
<tr>
<td>1995</td>
<td>4,028</td>
<td>7,612</td>
<td>1,870</td>
<td>463</td>
</tr>
<tr>
<td>2000</td>
<td>7,684</td>
<td>14,150</td>
<td>2,823</td>
<td>608</td>
</tr>
<tr>
<td>2010</td>
<td>21,201</td>
<td>35,371</td>
<td>3,670</td>
<td>764</td>
</tr>
</tbody>
</table>

Source: Tacon et al. FAO Fisheries and Aquaculture Paper 564

**Figure 2:** Supply and use of fish oil (Source IFFO and Skretting).
On average 100 g of salmon fillet has around 16 g of fat of which at least four to five percent is omega-3 EPA and DHA (DHA being the main fatty acid in the phospholipid fraction). Thus a 130 g portion would provide around 930 mg of EPA and DHA. That is equivalent to several supplement capsules. Two portions a week adequately provide the recommended dietary levels of LC-PUFAs and important vitamins and minerals in an easily assimilated form.

A second approach is to explore ways of formulating feed so that the LC-PUFAs are retained in the fillet flesh. Further research at Skretting ARC into the functions of micro-ingredients recently led to a new salmon feed that significantly improves the feed conversion ratio and fillet yield. Fillet analysis revealed the micro-nutrients also raised the proportion of EPA and DHA in the muscle.

The third approach is to identify alternative resources. There are two major contenders: genetic modifications to crop plants and micro-algae. Progress is being monitored by feed producers keen to reduce their dependence on marine ingredients. Some plants produce PUFAs, for example rape (canola) or soya, but the carbon chains are too short. The EPA carbon chain has 20 carbon atoms and DHA 22. The ambition is to introduce genes to extend 18-carbon chains already present. Limited progress has been with EPA. DHA is a greater challenge.

Some micro-algae species are natural synthesisers of the longer chain fatty acids. The challenge here is economic; to grow them in bulk, either by sea farming or in vats on land, in sufficient volumes to make them competitive as a feed ingredient. There are also reports of extracting LC-PUFAs from yeast cultures and these would face the same economic challenge.

Conclusion

Aqua feed producers must find alternatives to the marine ingredients fishmeal and fish oil while maintaining fish welfare and aquaculture performance as a highly efficient means of producing nutritious protein. Eating quality and health benefits are equally important.

However, although the supply of marine ingredients from the wild catch is limited, with appropriate controls they will continue to be available. A key task for the industry is to ensure they are used in a manner that spreads the benefits through a combination of supplementation, feed formulation and feed management on farm. This way the growing demand for fish can be met and the benefits shared sustainably for generations to come.

About the author

Alex Obach has held the position of Managing Director at Skretting Aquaculture Research Centre since May 1, 2007. Originally from Barcelona, Spain, he is a veterinarian with a Master in Aquaculture from the University of Girona (Spain) and a PhD in fish pathology and immunology from the University of West Brittany (France). He started working at Skretting Aquaculture Research Centre in 1993 as a researcher, initially within fish health then as a nutritionist. He previously was Manager of ARC’s Fish Health department. Between 1993-1995, he was also engaged as lecturer at the University of Barcelona, and worked for two years as Manager of the Marine Harvest Technical Centre.
The world demand for seafood is increasing dramatically year by year, although an annual upper limit of 100 million tons is set so as not to exhaust reserves. It is for this reason that there is a considerable move towards modernising and intensifying fish farming. To be economically viable, fish farming must be competitive, which means that feed costs amongst others must be carefully monitored as the operational cost goes 60 percent for feed alone. Therefore selection of cheaper and quality ingredients is of paramount importance for sustainable and economical aquaculture. Identification of suitable alternate protein sources for inclusion in fish feeds becomes imperative to counter the scarcity of fishmeal.

In addition to its scarcity and high cost, often fishmeal is adulterated with sand, salt and other undesirable materials. All these factors have forced fish feed manufactures all over the world to look for alternate sources. In this context they have been left with no protein but to substitute animal protein with plant protein sources. A variety of plant protein sources including soybean meal, leaf protein concentrate and single cell protein have been tested. The tests have shown that these can be included as alternatives to fishmeal (Ogino et al, 1978, Appler and Jauncy, 1983).

Of various plant protein sources, soybean meal (SBM) is one of the most promising replacements for part or whole of fishmeal. Soybean meal is the by-product after the removal of oil from Soya beans (glycine max). At present soybean meal is the most important protein source as feed for farm animals and as partial or entire replacement of fishmeal?

The products obtained from soybeans and their processing are as follows:-

- Soybean meals, solvent extract
- Soybean meal from dehulled seeds, solvent extracted,
- Soybean expeller
- Soybean expeller from dehulled seeds
- Full fat soybean meal
- Full fat soybean meal from dehulled seeds

The chemical composition of soybean meal is fairly consistent (Figure 1).

The crude protein level depends on the soybean meal quality. Soybean has one of the best amino acid profiles of all vegetable oil meals. The limiting amino acids in soybean meal are methionine and cystine while arginine and phenylalanine are in good supply (New, 1987).

The fat content of the solvent extracted soybean meal is insignificant but soybean expeller has oil content between six and seven percent, while full fat soybean expeller has oil content between 18 to 20 percent. Soybean meal and soybean expeller are lower in macro and trace elements than fishmeal. There is no substantial difference between the individual soybean meal products. The calcium content is low and the phosphorus level is rather higher. However, the phosphorus is bound to phytic acid and its availability for aquatic animals is, therefore, limited.

Soybean meals and expellers are reasonable source of B-vitamins. For most vitamins there are insignificant differences between the different products. However the full fat soybean meal tends to be higher in some vitamins. While the products are mainly higher in choline content, the vitamin B12 content is low and pantothenic acid is mainly damaged by heat treatment.

The digestible energy of soybean meal over all fish species ranges from 2572 to 3340 Kcal/kg (108 to 140 MJ/kg). The metabolisable and digestible energy of full soybean meal increases with the increase heating temperature at a given time due to the inactivation of trypsin inhibitors.

**Deleterious constituents of soybean products**

Trypsin inhibitors - About six percent of the total protein of soybeans reduces activities of trypsin and chymotrypsin, which are pancreatic enzymes and involved in protein digestion (Yen et al, 1977). The activity of trypsin inhibitor is not fully understood, but is responsible for the poor performance of certain fish species (Alexis et al, 1985, Balogum and Ologboho, 1989).

Lectins - This type of toxic protein is chemically hem agglutinin, which causes agglutination of RBC’s (Liener, 1969). There are indications that lectins reduce the nutritive value of soybean meal for Salmonids but are inactivated by treatment of the meals (Ingh et al, 1991).

Other properties - Soybean is unpalatable for some fishes such as Chinook salmon. While as herbivorous and omnivorous species are less choosy. The size or age of the fish may also affect the palatability of soybean meal.

**Utilisation of Soybean Products in Aquaculture**

Comprehensive research work has been done to evaluate soybean meals as a replacement of animal protein sources in diets for fishes but the replacement of all fishmeal by soybean meal has not been very successful perhaps due to the limiting amino acids and insufficient heat treatment of the soybean meals. Smith et al (1980) claimed success in feeding rainbow trout a diet based almost entirely on raw materials of vegetable origin containing 80 percent full fat roasted soybean. In a similar report, Brandt (1979) evaluated a diet based entirely on plant ingredients (containing 50 percent heated full fat soybean + 10 percent maize gluten meal to overcome a possible deficiency of S-amino acids).

Reinitz et al (1978) observed that rainbow trout fry fed a diet containing 72.7 percent full fat soybean had a greater daily increase in length and weight with an improved feed conversion ratio compared with those fed a control diet based on 25 percent herring meal, five percent fish oil 20 percent soybean oil meal. The mortality rate for both groups was similar. Taste panel studies indicated that there was no effect of dietary treatment on firmness and flavour of the fish.

Kaneko (1969) reported that 1/3rd of white fishmeal could be replaced by soybean meal with no negative effects on growth of warm

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**Figure 1: The chemical composition of Soyabean meal**

The chemical composition of soybean meal is shown in the figure. The oil content ranges from 18% to 20% in soybean meal and soybean expeller, with full fat soybean expeller having an oil content of 18% to 20%. The protein content in soybean meal is around 40%, and the amino acid profile is rich, with methionine and cystine being the limiting amino acids. The calcium content is low, and the phosphorus level is higher, but it is bound to phytic acid, reducing its availability for aquatic animals.

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**References:**
water fishes. Viola (1977) iso-nitrogenously reduced the fishmeal content in the diet of carps containing 25 percent protein supplemented by soybean meal with the addition of amino acids, vitamins and minerals and opined that soybean diet did not induce good growth in carp. Similarly Atack et al (1979) reported poor utilisation of soybean protein by carp when it formed the sole protein source. Gracek (1979) used different qualities of soybean meal to supplement ground maize for feeding carp fry and recorded better survival.

No difference in growth was observed when common carp (Cyprinus carpio) were fed either with 45 percent soybean meal (+10 percent fishmeal) or 20 percent soybean meal (+22 percent fishmeal). Other trials however showed that the growth performance and feed efficiency of common carp were reduced when dietary fishmeal was replaced by soybean meals.

There were no differences in performance between extruded full fat soybean meal and oil reconstituted soybean meal (Inghet et al; 1991). A better weight gain was reported when soybean meal was incorporated in the diets of carp fish (Cristoma et al; 1984). Similarly sklyrov et al (1985) successfully used soybean meal in rearing carp fish commercially. It is claimed that soybean meal is deficient in available energy and lysine as well as methionine for carps. Supplementation of soybean meal diets with methionine coated with aldehyde treated caesin significantly improved utilisation of amino acid by common carp (Murai et al; 1982).

Lack of phosphorus rather than the sulphur amino acids may be the cause for poor performance of common carps when 40 percent soybean meal diets were fed to them. Addition of 2.0 percent sodium phosphate did not improve their performances (Viola et al; 1986). Kim and Oh (1985) attributed the poor performance of common carp fed with a diet containing 40 percent soybean to lack of phosphorus rather than sulphur and amino acids, since addition of two percent sodium phosphates to soybean meal diet improved their performance to a level obtained with the best commercial feed.

Nour et al (1989) studied the effect of heat treatment on the nutritive value of soybean meal as complete diet for common carp by autoclaving the soybean seeds for 0, 15, 30, or 90 minutes and recorded maximum average daily weight gain with diets containing soybean seeds autoclaved for 30 minutes. Nandeesh et al (1989) incorporated soybean meal in the diets of Catla and indicated the possibility of utilising soybean meal in carp diets.

Keshavapa et al (1990) used soybean flour in the diet of carp fry and recorded better survival. Senappa (1992) studied protein digestibility from soybean-incorporated diets and recorded better digestibility when fed to fingerlings of Catla. Naik (1998) studied the effect of Soya flour and fishmeal based diets in the diet of Catla catla & Labeo rohita and observed a better growth and survival of carps when reared together and also in combination with fresh water prawn.

Channel cat fish (Ictalurus punctatus) fed on all plant protein diets grew significantly less than fish fed diets containing fishmeal (Lyman et al, 1944). Growth was substantially reduced when menhaden fishmeal was replaced by soybean meal at an isonitrogenous basis (Andrews and Page, 1974). Full fat soybean meal diet treated differently replaced fishmeal at low levels in diets for channel cat fish showed that replacement gave satisfactory results (Saad, 1979).

Growth and feed efficiency of fingerling hybrid tilapia (Oreochromis niloticus) was significantly depressed when soybean meal replaced fishmeal at the optimum level (30 percent) in their diet (Shiau et al, 1988). The growth depression of the hybrid tilapia was reduced when a 30 percent crude protein diet containing soybean meal but by adding two to three percent dicalcium phosphate to the diet. Growth rate of tilapia was comparable to the control (Viola et al, 1986). Soybean meal with supplemental methionine could replace up to 67 percent fishmeal in the diets for milk fish (Chanos chanos) (Shiau et al, 1988).

Growth, feed conversion and survival of tiger prawn (Penaeus monodon) juveniles fed two levels of soybean meal under laboratory conditions were lower with higher levels of soybean meal (Piedad, Pascual and Catacutan, 1990). No significant differences in growth and survival could be established when soybean meal at levels from 15- 55 percent replaced partially or completely fish meal in the diets for tiger prawns stocked in cages in ponds at 10 to 20 shrimps per square meter (Piedad, Pascual et al, 1991). Lim and Dominy(1991) obtained comparable results in feeding Penelus Vannamal with diets containing up to 17 percent of dry extruded full fat soybean meal as a partial replacement for fish protein. Generally the studies outlined above together with several others indicate that there is an advantage to be gained from using properly processed soybean products for formulating diets for fish due to their better quality protein and higher dietary energy value in full fat soybean which is more advantageous with cold water fish species because warm water fish (Carp, Catfish etc) can utilise carbohydrates more efficiently. The only recommendation relating to the limit of inclusion of full fat soybean in fish diets is not to exceed the known practical limits relating to fats in general in order to avoid problems of feed preparation and to reduce the risk of high fat levels in the meal.

Recommended Inclusion Rates

Soybean may replace animal protein in diets for aquatic animals to a certain extent. However, with increasing substitution of e.g. fish meal by soybean meal the performance of fish decline. Herbivores may tolerate higher levels of soybean meal than carnivores. It appears that full fat soybean meal is more beneficial for cold-water fish than for warm water species due to the better utilization of the energy from the soybean products. Only properly heat-treated soybean products should be used for aquatic feeds. Furthermore, it is advisable to use only soybean meals processed from dehulled seeds in order to reduce the crude fisher content in the diet.
As more of world’s natural fisheries are depleted and demand of fish continues to rise, aquaculture will continue to grow, thus raising demand for healthy, commercially prepared fish.

 Mostly, aquaculture relies upon extrusion cooking to produce feeds that are good mix and nutritionally available, but also in a form that is capable of moving through water column very slowly (floating) to be ingested. Thus, the big dependency in aquaculture is selecting ingredients that when extruded will possess just right buoyancy, not migrate nutrients into water, with high palatability for specific fish species.

 Fishfeed pellets are prepared either by pressed cut sheets or by Extrusion methods. This article will discuss about Ingredients and Extrusion process for producing the pellets.

 Main ingredients include:
1) Fish & Bone Meal
2) Soy protein (though it is not preferred by farmers being not easily digestible by many fish species)
3) Wheat
4) Starch
5) Blood Meal

 Other ingredients like Vitamins, Minerals and Lipids (Fat Oil) are also added in producing pellets.

Fishmeal
Fishmeal is a well-known source of proteins which is strongly demanded by the animal feeds industry. This is due to its balanced amino acid content, which makes it an ideal feed for many domestic animals.

Moreover, its use to adjust (improve) the amino acid content of other dietary protein sources also contributes to increase demand for fishmeal. As its name points out, fishmeal is derived from captured fish, including whole fish, fish scraps from fillets, and preserves of industries.

Most of the main capture fishery producers devote the main part of this activity to fish meal production. The raw materials used in fish meal manufacture come almost entirely from species which are not often used for human consumption (either due their size, or because they are very abundant).

The fishmeal processing system consists of preserving initial fish proteins by means of a controlled dehydration, which extracts around 80 percent of the water and oils contained when fresh from fish.

This leads to the production of a dry product, easy to preserve and easier to transport than the initial product.

Fresh fish entering the manufacturing plant is first ground and then cooked in a continuous heating oven at 90-95 percent, which in-turn coagulates proteins and lose their water-holding capacity. The hot mash is then transported to an...
endless screw or oil expeller that presses it and squeezes out most of the remaining water and oils.

Pressed fish coming out of the press (press cakes) then cut into smaller portions and placed into a dryer on a steam heated surface. During the drying period, the mash is in constant motion and subject to an air jet that removes all the steam emitted. The dried mash obtained is now called ‘fish meal’ and contains from 8 to 10 percent of water.

However, if the moisture level is more than 11 -12 percent, there is a risk of the fish meal developing moulds. Generally, antioxidants are added when fish meal is introduced and taken out of the dryer, and by so doing ensuring the stability of the oils remaining within the fish meal.

**Soy protein**

Not all fish species have easy digestibility of soy protein, primarily due to increased carbohydrate content fraction. It is usually used as supportive additive with other easily digestible protein like fish meal which is rich in fish proteins.

Bean processing consists essentially of extracting the oil so as to concentrate the proteins. This process provides a very important by-product, namely soya oil, which is widely used as a raw material and oil for human consumption. This process also contributes to the elimination of certain anti-nutritional factors present in the raw bean.

The first step in processing involves the removal of the shell (cellulose) from the grain. The ‘bare’ beans are then heated, on the one hand to reduce the activity of certain enzymes, and on the other to break the cellulose strands and facilitate the following steps. The heated beans are then mashed to form thin paste-like slices, which further facilitates the destruction of the cellulose structure and oil extraction.

The product, now termed ‘whole soya cake’, still contains its oil and has around 40 percent protein, and as such is sold directly for animal feeding.

Next, the oil can be extracted from the whole cake by means of a solvent (such as hexane). After total evaporation of the solvent, there remains the solvent extracted soya cake, which in turn is widely used for animal feeding, and contains 45 - 50 percent protein.

**Bloodmeal**

Abattoirs or slaughterhouses produce many important by-products, such as blood and bones, etc which are often difficult to commercialize. Nowadays, however, these by-products constitute the basic raw material of the bone and blood meals widely used in industry for animal feeding.

Considerable amounts of blood are produced by abattoirs, and this product is usually transported to drying ovens and converted into blood meal. Blood from different origins such as, sheep, goat, and poultry are usually stored and processed separately. However, so as to comply with basic sanitary measures, it is generally compulsory to store blood within cooling chambers and to ensure that the level of bacteria is kept within prescribed maximum limits.

**The manufacture of bloodmeal**

Fresh blood is kept cool at the factory, and sizeable particles filtered and the blood mass stirred so as to separate the fibrillar phase from the liquid mass. The fibrin is then heated up to coagulation and the coagulated mass divided and dried through a hot air stream (that is by spray drying). This method is particu-
larly gentle (spraying a product in a hot airstream) and does not denature the proteins because the water evaporation cools down the hot air very quickly, thereby preventing overheating.

**Wheat flour**

Wheat is one of the most important cereals worldwide, and is used for making bread and for many other produces. It is also an essential raw material for livestock feeding, including fish.

**Wheat in fish feeding**

Starch products, especially wheat, are frequently used as binders for the manufacture of pellets; the gelatinizing property of starch when water-heated being useful for this purpose as the starch absorbs water and forms a gel. Moreover, when starch is gelatinized its digestibility improves considerably. Various starch types (wheat, barley, rice, maize or potatoes) can gelatinize but each one will have its own characteristics.

In addition, all three starch types generally have the capacity to form a stable structure when subjected from high to low pressure during the extrusion process.

It is this property that is used for feeds that must have a high lipid content, during the extrusion process the starch forms a cell structure with alveoli that can then be filled with oil instead of air and/or steam.

For carnivorous fish feeding purposes the starch must be considered as a supporting structure that gives the pellets their texture and together with the other dietary ingredients allows the formation of a binded diet.

However, since the natural feeding habits and foods of seabass and/or seabream usually contain very small proportions of carbohydrates (ca. three percent glucose, animal starch - glucose polymer). If excessive quantities of digestible starch are provided in the feed this may result in the accumulation of excess liver glycogen, which in turn may trigger a liver dysfunction.

**Lipids**

Fish oils are co-products of the fishmeal industry. Their nutritional characteristics regarding fatty acids make them indispensable for fish feed manufacture, and in particular their characteristic high content of n-3 unsaturated fatty acids (first double bond linkage in position 3), which are essential for a well balanced food formula for carnivorous fish species.

A large amount of fish oil arising from fish meal manufactures is re-processed in specialized facilities for diverse purposes; part of it being hydrogenated and mixed with other lipids, and transformed into margarine, mayonnaise and bakery compounds, and the other part used directly by the feed industry.

**Minerals**

Minerals are measured as ash in the recipe. Though they serve no functionality in extrusion (on the contrary their abrasive nature will accelerate wear and tear of working parts in extruder), these are usually added in proportions < 5 percent. They include phosphorous, calcium (from calcium carbonate or ground lime stone), sodium chloride (salt), magnesium, potassium, etc.

**Vitamins:**

They can be water soluble or soil soluble. Vitamin B and C are water soluble, A, D, E, and K are fat soluble. They are added in proportions < 0.5-0.6 percent in diet, but due to harsh processing conditions inside the extruder, these get destroyed, hence they are added well in excess of minimum requirements.

Apart from above, the feed may contain, flavors/aromas, antioxidant (preservative) and antimicrobials, dyes & pigments (for human appeal and distinction, rather than for fish itself), etc. It is important to use certified ingredients that does not affect health of fish. Pigments are usually added as a coating step, to minimize losses during harsh extrusion processing conditions.

**Formulation of fishfeed**

As we have seen, feed formulators can resort to a wide assortment of raw materials to make up a food mixture so as to meet the nutritional requirements of the fish for energy, amino acids, fatty acids, carbohydrates, vitamins and minerals.

These raw materials are generally used in flour or liquid form, and will have to undergo binding by means of a technological process to obtain a food mixture in the form of dry pellets, which are easy to use and preserve.

As a guide, salt water marine aquaculture is dependant upon high levels of proteins with high digestibility. The fresh water aquaculture relies upon more carbohydrates, that is high levels of grains coupled with modest to high quality proteins, minerals, vitamins with little or no fiber.

The first factor to be considered for feed formulation is the total energy and protein/energy ratio of the final product. After this, the protein content must be calculated according to the amino acid balance desired, and the lipids included to satisfy the best fatty acid profile for the species concerned and the energy level desired. All this must be considered taking into account the vitamin and mineral requirements of the cultured species.

This formulation is not easily reached and so computerised linear programming techniques must be used. Furthermore, it is also necessary, after covering all the nutritional requirements of the species within the formula to also produce a range of tasty feeds of different pellet sizes for the different age classes.

**Manufacturing stages**

- **Storage**

The raw materials coming into the feed manufacturing plant are generally stored in silos with an ideal height calculated so as to allow the raw material flow to be conveyed downwards, during the manufacturing process, until the final product is produced. This is in order to avoid having to pull the products up by vertical conveyors that usually cause breaks and dust in the final product.

- **Grinding**

Grinding raw materials reduces particle size and increases ingredient surface area, thus facilitating mixing, pelleting and digestibility. The most commonly used grinders are hammer-mills, for fish feed manufacture, as plate-grinders do not generally produce fine enough ground materials.

The Extrusion cooking process utilizes...
wide variety of ingredients that can have varying particle sizes. It is desirable, but not necessary that all ingredients be of uniform particle sizes, to prevent segregation during mixing and transport prior to extrusion.

Uniform particle size of ingredients promotes better mixing and uniform moisture uptake by all particles during the preconditioning step.

If the particle size of raw ingredients is too large, the final product may contain particles which are improperly cooked, which degrade product appearance and palatability.

Also, if particle size is larger than die orifice used at extruder discharge, it may cause plugging of some orifices affecting capacity and appearance. As rule of thumb, it is necessary to maintain size of raw ingredients one third of the die opening die. Hence the need of size reduction equipment and sifting.

In hammer-mills, the grinding chamber consists of a series of mobile hammers on a rotor. The hammers, by centrifugal force, position themselves forming a star on the rotor and split the incoming feedstuff apart, which is then forced by depression through a metal grid composed of appropriately sized meshes.

- Mixing
The ground ingredients must be mixed according to the desired proportions to obtain a homogeneous mixture. If the grinding process is correctly developed, the particles are homogeneous in size and the mixture produces pellets which statistically have the same formulation.

Generally, the dry ingredients (flours) are first mixed, followed by the liquid components. Continuous mixers are designed so that the feedstuff moves along the mixer as it mixes. There are many different types of mixers, including horizontal band-mixers, vertical mixers, conical screw-mixers, and turbine mixers, etc.

During this mixing process, the vitamin ‘premix’, the binding agents and other additives are added; they must in turn contribute to one or other particular desired quality of the pellets during the pelleting process.

- Pelleting
Two different types of pellets are generally prepared for aquafeeds, namely pressed and extruded pellets. A third type, designed as ‘expanded feed’, is also marketed by some manufacturers.

The main difference between a pressed and an extruded feed is the cooking of the feedstuff in the case of extrusion, with the added mechanical and biological advantages previously described, especially with regard to starch gelatinization.

Extruded Feeds:
The Extruder can be described as a Bio-Reactor with (mostly) a single, multiple-flighted screw (rotor) rotating at high speed inside a stationary hollow tube (stator).

The raw materials fall from top at one end on the rotating screw which has multiple flights and varying pitches along its length. The barrel (tube) is externally heated/cooled by steam and cold water externally around. Due to this arrangement, a high pressure of around 40-70 bars (Kg/cm2) is developed on the ingredients, temperature of ingredients varies from 110 C to 160 C, which ensures cooking of ingredients into plastic mass which is extruded out of multiple die openings/orifices and cut to produce porous pellets for fish feed.

Pre-Extrusion: Dry ingredients after having been mixed & ground thoroughly in desired proportions, are usually transported to the Single screw Extruder (Cooker) provided with a Pre-conditioner at top.

The Feed Delivery System: It consists of a “Live Hopper” or Bin with a horizontal conveying screw to convey dry ingredients to the Preconditioner from above. The Bin is provided with device which avoids bridging of material (since raw ingredients have low bulk density and poor flow through a normal Hopper) and ensures continuous flow of materials to the Preconditioner below, hence the name “live” bottom bin. It should hold adequate volume to support the extruder operation for minimum 5-8 minutes, as a buffer time for the operator and auto control network to respond.
"As more of world’s natural fisheries are depleted and demand of fish continues to rise, aquaculture will continue to grow, thus raising demand for healthy, commercially prepared fish"

and allow recharging the bin from top. The screw is provided with variable speed motor to properly adjust the flow as per production capacity of the Extruder.

Preconditioning: This step ensures the dry ingredients are constantly added with moisture (water) in desired proportions (25-30%) and steam is also added, at 5-6 bar, for pre-cooking the wet ingredients. As the ingredients move forward towards the Extruder feed opening, they are held at temperature of approximately 100 C and atmospheric pressure. Preconditioning makes the ingredients soft by precooking and it reduces energy requirement in the Extruder. If lipids are to be added, their proportion is limited from 5-7 percent in this stage.

Conventional Preconditioners had only one tank and single agitator, but modern preconditioners have special oval tanks with two agitating shafts with adjustable beaters to control residence time inside the tank. Two agitators result in the better mixing of dry and liquid ingredients. Longer retention time approximately 2-2 ½ minutes are desirable before feeding into the Extruder. Usually lipids are added not more than 5-7 percent by weight here, since it leads to excessive slippages inside the Extruder and poor mixing & expansion/texture of final pellets.

- Extrusion

Usually single screw Extruders with single barrel and screw is used for cooking the preconditioned ingredients, but twin screw extruders are also used. The latter have limited use because of high initial capital costs compared to single screw extruder.

The action of the Extruder allows the free flowing ingredients to bond to each other and remain in pellet form after exiting from shaping (pelletting) die. It does this by the action of rotating screw or spiral inside a stationery barrel by generating high mechanical shear and raised temperature on feed materials.

Extruders for Fish Feed production have Mechanical Energy Input levels between 20-40 Kw-hr/ton of produce. Their screws run between 400-1000 RPM depending on sizes. Output capacities range between 1 t to 20 t per hour.

The Extruder usually employed is "Wet Extruder" since feed materials contain around 25 to 30 percent moisture (water). Both screw and the barrel are made up as separate segments so that individual components could be replaced when worn. Multiple flighted, varying pitch screw elements are usually employed to provide cooking and forward conveying of feed materials. The Volumetric capacity of screw is highest at Feed zone to account of low bulk density of ingredients. However, it reduces (lower pitch) towards the die, which causes compression and cooking of feed material. The final discharge end of screw is usually Conical to generate high pressure and attain maximum expansion of pellet when emerging from die opening. The barrel heads are provided with Steam Heating and water cooling Jackets around, for heating or cooling, as per process demand. The process temperature is held from 110 C to 160 C gradient from Feeding Zone to Final Cooking Zone. Maximum conveyance & mechanical shear of material is ensured by action of multiple flighted screw elements and spirally grooved barrel segments. Water present inside the mixture is held as steam at high temperature and pressure. However, as soon as the cooked mass emerges out of die openings pressure drops to atmospheric

<table>
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<th>Table 1:</th>
<th>FEED A</th>
<th>FEED B</th>
<th>Difference</th>
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<tbody>
<tr>
<td>(1) Growth</td>
<td>1</td>
<td>1.1</td>
<td>10%</td>
</tr>
<tr>
<td>(2) Conversion rate</td>
<td>2</td>
<td>2</td>
<td>0%</td>
</tr>
<tr>
<td>(3) Feed price / kg</td>
<td>5</td>
<td>6</td>
<td>+20%</td>
</tr>
<tr>
<td>(4) Selling price of fish</td>
<td>50</td>
<td>50</td>
<td>0%</td>
</tr>
<tr>
<td>(5) Feed expenditure for 1 kg of fish produced</td>
<td>10</td>
<td>12</td>
<td>+20%</td>
</tr>
<tr>
<td>(6) Profit from fish sales (1) X (4)</td>
<td>50</td>
<td>55</td>
<td>+1%</td>
</tr>
<tr>
<td>(7) Gross margin for feed item (6) - (5)</td>
<td>40</td>
<td>43</td>
<td>+7.5%</td>
</tr>
</tbody>
</table>

"As more of world’s natural fisheries are depleted and demand of fish continues to rise, aquaculture will continue to grow, thus raising demand for healthy, commercially prepared fish"
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and the product expands or “puffs”, being cut continuously by Rotating Die Knives working against the die Face, giving the pellet the specific rounded shape for extruded pellets.

Retention time inside Extruder is from 100-180 seconds, which ensures 70-85 percent starch gelatinization and production of good shape and density.

The above Extruder produces Floating Pellets with low bulk density, e.g. 350-450 g/l that are classified as “Floating” and sink very slowly into water column. Most Extruders have an arrangement, whereby the water vapour present in the mix is released by a vent opening on the barrel so that high density pellets or sinking pellets are produced for certain species of fish.

The following parameters will control the final pellet density: 1. Initial moisture content (usually 25-30 percent on wet basis). 2. Process temperature. 3. Extruder back pressure. 4. Extruder RPM (residence time). 5. Quantity of Fats, vitamins & minerals applied post extrusion.

- Drying

When added to Extruder, the ingredients contain around 25-30 percent moisture (wet basis). Extrusion process evaporates approx. 4-7 percent moisture thus still retaining considerable moisture inside the pellets. After the pelleting process, the pellets usually have a high moisture content (17 to 22%) that must be quickly reduced to avoid spoilage. This is usually achieved by using a hot-air drier, which lowers the moisture level to between 8 and 10 percent depending upon the manufacturing process.

Continuous Belt Dryers are commonly employed that provide heated air to remove excess moisture from wet product, as it travels on multiple decks of perforated steel belting. Since the Drying process is critical and determines the quality of pellets, it needs to be carefully monitored and controlled. The Air Temperature, Humidity and Residence time of products should be carefully adjusted to attain properly dried product that can absorb maximum fats and coatings in the Coating step.

- Sifting

The mechanical manufacturing processes inevitably results in shocks and scorching that partially crumble the pellets at their surface and cause various breaks and dust that must be eliminated. This is achieved by sifting, a process that is generally applied at least twice before the final conditioning (sifting after drying and after coating/cooling).

- Coating

The pellets emerging from the pelleting presses or extruders do not generally contain more than 7 to 10 percent lipid. To achieve higher dietary lipid levels (20-27%), coating is necessary with the appropriate oils, generally using heat. In the same manner, certain heat sensitive vitamins and/or drugs that would not normally withstand the harsh extrusion processes (thermo labile products) can also be added later during the coating process. These ingredients are usually added through spray nozzles fed through dosing pumps which accurately control the weights deposited. They can be vacuum assisted for still more good results.

The Expansion that occurs as a result of extrusion processing makes the product porous with low bulk density and air pockets, so that more oil is absorbed during the spray coating process. Fats could be added in the form of Animal fats, Fish Oil or Vegetable Oil.

- Cooling

On completion of the coating process (generally undertaken with heated material) the pellets are then cooled and sieved before the final conditioning; cooling occurring in a cool-air flow generated by a cooling-machine. Again, this machine usually provides continuous flow of product on perforated steel belts, while cooling air is applied through bed of pellets to lower the temperature.

Cooling is important, since if packed in hot state, moisture will condense in the packing, wetting the outer surface of the pellets, allowing mold growth. It is desirable to cool down within 10 °C of ambient air temp. So that problem of condensation in packing doesn’t occur.

- Bagging

Bagging usually produces different types of feed presentations within the same factory, namely either small bags (20 or 25 kg) on pallets covered with a plastic film, or big-bags (500 or 1000 kg) in bulk.

Viability of Extrusion process

It follows from the higher temperatures and pressures used during extrusion processing that investment and energy costs will be higher than those of conventional pressed feeds. Despite this however, the use of extruded feeds may be more profitable.

Following Table (illustration) summarizes the theoretical results obtained with fish fed a pressed (A) or extruded (B) feed. Table showing Justification for Extrusion Over Press Feed production method for Fish Feed.

It is clear from the example given that despite the fact that the price of the extruded feed is 20 percent higher, the feed which provides 10 percent additional growth provides a 7.3 percent additional gross margin.

Note:

The author is CEO Malik Engineers, Mumbai, which manufactures a wide range of extruders for food processing and aqua/animal feed. He can be reached on info@malikengg.com Tel: +91 22 28830751, +91 250 2390839
gilthead sea bream production in Mediterranean countries increased from 30,000 tons in 1996 to 90,000 tons in 2005, which means that sale prices dropped considerably, from 6.6 €/kg in 1996 to 5 €/kg in 2005, with an historical minimum of 4 €/kg in 2002 (APROMAR, 2006). To maintain the profitability of gilthead sea bream farms, cutting production costs is necessary, mainly through feeding, which represents between 38 and 45% of operational costs (Lisac & Muir, 2000 and Merinero et al., 2005).

Reductions in feeding costs can be obtained by optimizing feeding strategies, nutrient levels in diets, and by using vegetable sources as substitutes for fish oil and fishmeal. This aspect is also very important to improve the sustainability of aquaculture, as it would reduce dependence on fish sources (Martinez-Llorens et al., 2009).

In the last decade, the increasing demand, price and world supply fluctuations of fishmeal (FM) has emphasized the need to look for alternative protein sources in aquafeeds. Some plant ingredients have been studied in gilthead sea bream (lupin seed meal, extruded peas and rapeseed meal) but Poaceae and Fabaceae seeds and their by-products, among which corn gluten and soybean meal, in particular, are widely used in fish nutrition because of their high protein content (40-60%), low cost and relative widespread availability. Therefore, soybean meal being the most nutritive and it is used as the major protein source in many fish diets. Partial or even total replacement of dietary fishmeal by soybean meal protein sources had successfully accomplished with tilapia diets (Ngahele and Davies, 2002). Some studies with gilthead sea bream have shown that partial replacement of FM by PPs is possible (Robaina, et al., 1995; Hassanen, 1997a, b; 1998; Kissil, et al., 2000; Sitja-Bobadilla et al., 2005 and Martinez-Llorens et al., 2009). Studies with sea bass have also reported some success to partial replacing of FM by PPs (Lanari, 2005 and Tibaldi, et al., 2006). Studies of using corn gluten to feed carnivorous fish (sea bream) are very limited; therefore, the scope of

---

**Table 1:** Composition of the experimental diets

<table>
<thead>
<tr>
<th>Ingredients (g/100g)*</th>
<th>Diet</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>FM</td>
</tr>
<tr>
<td>Fish meal (CP 68%)</td>
<td>63</td>
</tr>
<tr>
<td>Corn gluten meal (62%)</td>
<td>-</td>
</tr>
<tr>
<td>Soybean meal (44%)</td>
<td>-</td>
</tr>
<tr>
<td>Yellow corn</td>
<td>21.5</td>
</tr>
<tr>
<td>Fish oil+ Soya oil (1:1) 1**</td>
<td>12</td>
</tr>
<tr>
<td>Lysine</td>
<td>-</td>
</tr>
<tr>
<td>DL-Methionine</td>
<td>-</td>
</tr>
<tr>
<td>Vit &amp; Min mix2</td>
<td>3</td>
</tr>
<tr>
<td>Cr2O3</td>
<td>0.5</td>
</tr>
<tr>
<td>Total</td>
<td>100</td>
</tr>
</tbody>
</table>

1- Mixture of fish oil and soybean oil (1:1 w/w).
2- Each Kg vitamin & mineral mixture premix contained Vitamin A, 4.8 million IU, D3, 0.8 million IU; E, 4 g; K, 0.8 g; B1, 0.4 g; Riboflavin, 1.6 g; B6, 0.6 g; B12, 4 mg; Pantothenic acid, 4 g; Nicotinic acid, 8 g; Folic acid, 0.4 g Biotin, 20 mg, Mn, 22 g; Zn, 22 g; Fe, 12 g; Cu, 4 g; I, 0.4 g; Selenium, 0.4 g and Co, 4.8 mg.
3- Cr2O3: Chromic Oxide
* obtained from the local market.

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*Sparus aurata*
the present study was to evaluate the effect of partial or complete replacement of fishmeal with increasing levels of plant protein origin like corn gluten and soybean meal on growth performance, feed utilization, body composition and cost production of sea bream fingerlings’ diets.

**Experimental protocol**

**Diet preparation** - Five isocaloric and isonitrogenous diets were formulated based on fishmeal as the only animal protein source or a mixture of PPs (Corn gluten and Soybean meal) as plant protein sources (Table 1). The diets formulated to be almost containing 45% crude protein by replacing 25, 50, 75 and 100% of the FM (fishmeal protein) in control diet. Crystalline amino acids (L-lysine and DL-methionine) were added to diets PPs 25, 50, 75 and PPs100% to become similar to control diets. Fish oil and soybean oil were added as dietary lipid sources (Table 1). The diets were pelleted using a small catering grinder with a 1.5 mm diameter and kept frozen until the experiment was started. During the growth period (120 days), each diet was randomly allocated to triplicate tanks of fish. Feed was offered by hand at two meals/day (8:00h and 15:00) at 3% of body weight daily and the amount of diets were readjusted after each weighing.

**Experimental design** - Sea bream fingerlings were obtained from a private fish farm in Damietta governorate. Fish were acclimated to laboratory conditions for 2 weeks before being randomly distributed into fiberglass tank of 300-L water capacity each, in Ashtom Elgamel, Port-Said governorate. The water was obtained from channel comes from Mediterranean sea. Fish of 10±0.2 g initial body weight were distributed into 15 experimental tanks in triplicate groups of 50 fish each. The photoperiod was regulated to be 12h light:12h dark. Water temperature was maintained at 25°C by a 250-watt immersion heater with thermostat. Water temperature and dissolved oxygen were recorded daily (by Metteler Toledo, model 128. s/No1242), other water quality parameters including pH and ammonia were measured every two days by pH meter (Orion model 720A, s/No 13062) and ammonia meter (Hanna ammonia meter). Water salinity was 34ppt. The average water quality criteria of all tanks are presented in Table 3. All fish in each tank were weighed every 10 days.

**Experimental methodology** - The tested diets and faeces were analyzed for crude protein (CP %), ether extract (EE %), crude fiber (CF %), ash (%) and moisture while whole body composition of sea bream fish samples was also analyzed except for crude fiber (CF %) according to the procedures described by A.O.A.C. (1995) as shown in Table 2 and Table 5. The nitrogen free-extract (NFE %) was calculated by difference. Blood samples were collected using heparinized syringes from caudal vein of the experimental fish at the termination of the experiment. Blood was centrifuged at 3000rpm for 5 minutes to allow separation of plasma which was subjected to determination of plasma total protein (Arms特朗 and Carr, 1964) and plasma albumin (Doumas, et al., 1977). Apparent protein digestibility was determined using the method of Furukawa and Tasukahara (1966). For determination of protein digestibility the diets and faeces were collected during the last 15 days of the experimental period. Any uneaten feed

<table>
<thead>
<tr>
<th>Table 2: Proximate analysis of the experimental diets (% as fed)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Chemical analysis</strong></td>
</tr>
<tr>
<td>Moisture</td>
</tr>
<tr>
<td>Crude protein*</td>
</tr>
<tr>
<td>Crude fat</td>
</tr>
<tr>
<td>Crude fiber</td>
</tr>
<tr>
<td>Crude ash</td>
</tr>
<tr>
<td>Nitrogen free extract</td>
</tr>
<tr>
<td>Gross Energy (kcal/100gm)</td>
</tr>
<tr>
<td>P/E Ratio (mg protein/Kcal)</td>
</tr>
</tbody>
</table>

1. Based on 5.64 Kcal/g protein, 9.44 Kcal/g fat and 4.11 Kcal/g carbohydrate (NRC, 1993).
2. Protein/Energy Ratio (mg Protein/Kcal).

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bream diets with no significant differences \((P \geq 0.05)\) in growth performance compared to the control (Table 4). This conclusion is in agreement with Gomes et al (1995a & b) for rainbow trout. These workers reported that replacement of fishmeal by plant protein sources had no adverse effects on growth. The optimal rate of substitution found in the present research was closed with Lanari (2005), he reported that soybean meal can substitute up to 25% of total protein of the sea bass diets without any negative effect on growth performance. Higher value than reported in the present study was reported by Gallagher (1994) in diets for hybrid striped bass, where soybean meal substituted 44% of fishmeal without evidencing a negative effect on the feed intake and he also reported that up to 75% of fishmeal protein can be replaced with soybean meal. Moreover, Sitja-Bobadilla et al., (2005) reported that up to 75% of fishmeal protein can be replaced by plant protein sources for juvenile sea bream, which also is in agreement with the present study for sea bream fingerlings. In the recent years, significant amount of research has been conducted on the replacement of FM by different PP sources.

More over, statistical analysis - All data of growth performance, body composition and blood parameters were analyzed by one-way analysis of variance (ANOVA) using the general linear models procedure of statistical analysis system (SAS) version 8.02, (1998). Duncan's multiple range test (Duncan, 1955) was used to resolve differences among treatment means at 5% significant level using the following model.

### Results & discussion

The present study indicates that PPs (corn gluten and soybean meal) can replace 25% to 50% of fishmeal protein in sea bream diets without any negative effect on growth performance compared to the control (Table 4). This conclusion is in agreement with Gomes et al (1995a & b) for rainbow trout. These workers reported that replacement of fishmeal by plant protein sources had no adverse effects on growth. The optimal rate of substitution found in the present research was closed with Lanari (2005), he reported that soybean meal can substitute up to 25% of total protein of the sea bass diets without any negative effect on growth performance. Higher value than reported in the present study was reported by Gallagher (1994) in diets for hybrid striped bass, where soybean meal substituted 44% of fishmeal without evidencing a negative effect on the feed intake and he also reported that up to 75% of fishmeal protein can be replaced with soybean meal. Moreover, Sitja-Bobadilla et al., (2005) reported that up to 75% of fishmeal protein can be replaced by plant protein sources for juvenile sea bream, which also is in agreement with the present study for sea bream fingerlings. In the recent years, significant amount of research has been conducted on the replacement of FM by different PP sources.

### Table 3: Average water quality parameters in the experimental tanks used in the study.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Means ± SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Temperature (ºC)</td>
<td>25 ±1</td>
</tr>
<tr>
<td>Oxygen (mg/l)</td>
<td>5.4 ±1</td>
</tr>
<tr>
<td>Ammonia (NH3, mg/l)</td>
<td>0.011 ± 0.0001</td>
</tr>
<tr>
<td>pH</td>
<td>7.1 ± 0.10</td>
</tr>
<tr>
<td>Salinity (ppt)</td>
<td>34.0 ± 0.4</td>
</tr>
</tbody>
</table>

or faeces from each tank was carefully removed by siphoning about 30 min after the last feeding. Faeces were collected by siphoning separately from each replicate tank before feeding in the morning. Collected faeces were then filtered, dried in an oven at 60ºC and kept in airtight containers for subsequent chemical analysis.

### Table 4: Growth performance and feed utilization of sea bream (S. aurata) fed the experimental diets

<table>
<thead>
<tr>
<th>Parameters</th>
<th>FM</th>
<th>PPs 25</th>
<th>PPs50</th>
<th>PPs75</th>
<th>PPs100</th>
</tr>
</thead>
<tbody>
<tr>
<td>Average Initial body weight (g)</td>
<td>10.1±0.05</td>
<td>10.2±0.25</td>
<td>10.3±0.10</td>
<td>10.1±0.10</td>
<td>10.0±0.23</td>
</tr>
<tr>
<td>Average Final body weight (g)</td>
<td>102.6 ±2.2</td>
<td>101.3 ±0.3</td>
<td>97.7±0.20</td>
<td>85.2±0.2</td>
<td>78.9±0.2</td>
</tr>
<tr>
<td>Average Weight gain (g)</td>
<td>92.5 ±1.1</td>
<td>91.1 ±1.2</td>
<td>87.4 ±0.9</td>
<td>75.1±0.10</td>
<td>68.9±1.10</td>
</tr>
<tr>
<td>SGR (% / d) 1</td>
<td>1.93 ±0.02</td>
<td>1.91 ±0.01</td>
<td>1.87 ±0.02</td>
<td>1.78 ±0.09</td>
<td>1.72 ±0.01</td>
</tr>
<tr>
<td>Feed intake (g)</td>
<td>151.93 ±0.20</td>
<td>152.51 ±0.20</td>
<td>153.15 ±0.10</td>
<td>159.62 ±0.2</td>
<td>159.30 ±0.10</td>
</tr>
<tr>
<td>Feed conversion ratio (FCR2)</td>
<td>1.64 ±0.10</td>
<td>1.67 ±0.10</td>
<td>1.75 ±0.10</td>
<td>2.13 ±0.10</td>
<td>2.31 ±0.20</td>
</tr>
<tr>
<td>Protein efficiency ratio</td>
<td>1.35 ±0.01</td>
<td>1.32 ±0.02</td>
<td>1.27 ±0.01</td>
<td>1.04 ±0.10</td>
<td>0.95 ±0.20</td>
</tr>
<tr>
<td>Feed efficiency</td>
<td>0.61 ±0.10</td>
<td>0.60 ±0.10</td>
<td>0.57 ±0.10</td>
<td>0.47 ±0.10</td>
<td>0.43 ±0.12</td>
</tr>
<tr>
<td>HSI (%)</td>
<td>3.2 ±0.10</td>
<td>2.97 ±0.10</td>
<td>2.93 ±0.12</td>
<td>2.71 ±0.01</td>
<td>2.56 ±0.12</td>
</tr>
<tr>
<td>Apparent Protein Digestibility (APD)</td>
<td>88.25 ±0.3</td>
<td>87.39 ±0.2</td>
<td>86.09 ±0.1</td>
<td>73.16 ±0.2</td>
<td>65.32 ±0.1</td>
</tr>
<tr>
<td>PTP (g/dl)</td>
<td>5.21±0.10</td>
<td>5.20±0.12</td>
<td>5.15±0.10</td>
<td>5.03±0.12</td>
<td>5.01±0.10</td>
</tr>
<tr>
<td>PA (g/dl)</td>
<td>2.15±0.11</td>
<td>2.17±0.11</td>
<td>2.17±0.12</td>
<td>2.07±0.02</td>
<td>2.08±0.08</td>
</tr>
<tr>
<td>PTG (g/dl)</td>
<td>3.06±0.12</td>
<td>3.03±0.10</td>
<td>2.98±0.11</td>
<td>2.96±0.09</td>
<td>2.93±0.01</td>
</tr>
<tr>
<td>Survival rate (%)</td>
<td>100</td>
<td>100</td>
<td>98</td>
<td>96</td>
<td>94</td>
</tr>
</tbody>
</table>

Values in the same row with a common superscript letter are not significantly different \((P \geq 0.05)\).

Specific growth rate = \((100 \times [\ln \text{final wt (g)} - \ln \text{initial wt (g)} / \text{days}])\)

Feed conversion ratio (FCR) = feed intake (g) / body weight gain (g).

Protein efficiency ratio (PER) = gain in weight (g) / protein intake (g).

Feed efficiency = body weight gain (g) / feed intake (g).

Hepato-somatic index = 100 x liver wt / fish wt.

6 - Apparent protein digestibility, APD (%)

7 - Plasma Total Protein, PTP (g/dl)

8 - Plasma albumin, PA (g/dl)

9 - Plasma total globulins= plasma total protein - plasma albumin, PTG (g/dl)

10 - Survival rate =No of survive fish/total No. of fish at the beginning X100
Fish. In fish, protein digestibility is generally high ranging from 75% to 95% and the apparent digestible coefficient of proteins from fishmeal is often higher than 90% in salmonids (NRC, 1993). Soybean meal contains various anti-nutritional factors such as the anti-trypsin and an anti-chymotrypsin factors, lectins, oligosaccharides and a low level of methionine. Corn gluten has also a low level of amino acid lysine reduces the protein digestibility and amino acid availability of these plant protein ingredients.

Corn gluten meal (CGM) is considered to have a good digestibility (NRC, 1993). Diets containing 20% of CGM meal had a very good digestibility, in accordance with the results of Morales et al. (1994) and Gomes et al. (1995 a) in rainbow trout fed diets containing about 20% corn gluten meal. In contrast, apparent digestibility of diets with high levels of plant proteins was very low. In common carp, Pongmaneerat et al. (1993) observed that the apparent protein digestibility of diets used in the experiment which showed worst feed utilization related to apparent protein digestibility of diets used in the experiment which showed worst feed utilization of sea bream fed on diets containing high mixture of PPs (corn gluten meal and soybean meal) was possibly due to the low biological value of such based diets, which are in agreement with Robaina, et al. (1995), Boonyaratpalin et al. (1998), Regost et al. (1999), Lanari (2005), Sitja-Bobadilla et al. (2005), and Tibaldi, et al. (2006).

Regarding to feed digestibility (Table 4), several investigations were conducted to evaluate PPs and their digestibility by these results of feed utilization related to apparent protein digestibility of diets used in the experiment which showed worst feed utilization of sea bream fed on diets containing high mixture of PPs (corn gluten meal and soybean meal) was possibly due to the low biological value of such based diets, which are in agreement with Robaina, et al. (1995), Boonyaratpalin et al. (1998), Regost et al. (1999), Lanari (2005), Sitja-Bobadilla et al. (2005), and Tibaldi, et al. (2006).

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Concerning the results of feed utilization in terms of FCR, PER and FE in the present study, the same trend was showed with growth performance. These results of feed utilization related to apparent protein digestibility of diets used in the experiment which showed worst feed utilization of sea bream fed on diets containing high mixture of PPs (corn gluten meal and soybean meal) was possibly due to the low biological value of such based diets, which are in agreement with Robaina, et al. (1995), Boonyaratpalin et al. (1998), Regost et al. (1999), Lanari (2005), Sitja-Bobadilla et al. (2005), and Tibaldi, et al. (2006).

Table 5: Whole body composition (% fresh weight) of sea bream (S. aurata) fingerlings fed the experimental diets

<table>
<thead>
<tr>
<th>Chemical analysis</th>
<th>Initial</th>
<th>Final</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>FM</td>
<td>PPs/25</td>
</tr>
<tr>
<td>Moisture</td>
<td>70.50</td>
<td>59.91 a</td>
</tr>
<tr>
<td>Crude protein</td>
<td>14.25</td>
<td>17.56 a</td>
</tr>
<tr>
<td>Crude fat</td>
<td>10.5</td>
<td>15.62 a</td>
</tr>
<tr>
<td>Crude ash</td>
<td>4.75</td>
<td>6.91 a</td>
</tr>
</tbody>
</table>

Values in the same row with a common superscript letter are not significantly different (P<0.05)

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had to be near 94% in a diet without fishmeal (corn gluten meal, soybean and meat meal).

Results of apparent protein digestibility in the present study recorded that the dietary inclusion of high levels of corn gluten and soybean meal in replacement of fishmeal led to a significant decrease in protein digestibility which are in agreement with Lanari (2005), Tibaldi et al. (2006) and Sampaio-Oliveira and Cyrino (2008). The value of hepato-somatic index was found to be similar to that reported for sea bass by Ballestrazi et al., (1994) and Dias et al., (1998), et al. (2006) and Sampaio-Oliveira and Cyrino (2008) for sea bass D. labrax and Peres and Oliva-Teles (2009) for sea bream S. aurata.

Calculation of the economical efficiency of the tested diets was based on the costs of feed because the other costs were equal for all studied treatments.

As described in Table 6 feed costs (LE) were the highest for the fishmeal diet and gradually decreased with increasing the replacing levels of plant protein sources. These results indicate that incorporation of PPs in sea bream diets reduced the total feed costs.

Table 6: Feed cost (LE) for producing one Kg weight gain by sea bream (S. aurata) fingerlings fed on the experimental diets

<table>
<thead>
<tr>
<th>Experimental diets</th>
<th>Cost (LE/kg)</th>
<th>Relative fishmeal diets</th>
<th>Decrease in feed cost (%)</th>
<th>FCR</th>
<th>Feed cost (LE/Kg) weight gain</th>
<th>Relative to fish meal diet</th>
</tr>
</thead>
<tbody>
<tr>
<td>FM</td>
<td>6.56</td>
<td>100</td>
<td>0.00</td>
<td>1.64</td>
<td>10.76</td>
<td>100</td>
</tr>
<tr>
<td>PPs25</td>
<td>5.66</td>
<td>86.29</td>
<td>13.71</td>
<td>1.68</td>
<td>9.51</td>
<td>88.38</td>
</tr>
<tr>
<td>PPs50</td>
<td>4.77</td>
<td>72.71</td>
<td>27.29</td>
<td>1.89</td>
<td>9.02</td>
<td>83.83</td>
</tr>
<tr>
<td>PPs75</td>
<td>3.96</td>
<td>60.36</td>
<td>39.64</td>
<td>2.13</td>
<td>8.43</td>
<td>78.35</td>
</tr>
<tr>
<td>PPs100</td>
<td>3.12</td>
<td>47.56</td>
<td>52.44</td>
<td>2.31</td>
<td>7.21</td>
<td>67.01</td>
</tr>
</tbody>
</table>

The local market price were 8LE for fish meal, 2.50LE for gluten, 1.70LE for soybean meal, 1.00 LE for yellow corn, 9 LE for oil, 5 LE for Vit. & Min.

they reported that the values of HSI were 2–3% or above. Effect of the experimental diets on hepato-somatic index confirmed that the fish fed on diets containing high levels of corn gluten meal and soybean meal evidenced a significant (Ps0.05) decrement of the HSI in relation to the utilization of glycogen, stored as an energy source. The results are in agreement with Lanari (2005) and Sampaio-Oliveira and Cyrino (2008).

Effects of the experimental diets on whole body protein concentration (Table 5) were very small with exception of fish diet containing FM, 25 and 50%PP which showed a significant difference (Ps0.05) compared to the other experimental diets (75 and 100%PP). Fish body fat content decreased with increasing level of PPs substitution. The low percentage of fat stored with diets containing high level of PPs is due to the limited ingestion of the feed or to probable use of the body fat as energy source and may be also related to the carbohydrate levels and type of the diets. These results are in agreement with Lanari (2005), Tibaldi et al. (2006) and Sampaio-Oliveira and Cyrino (2008) for sea bass D. labrax and Peres and Oliva-Teles (2009) for sea bream S. aurata.

However, high replacing levels of fishmeal by PP (75% and 100%PP) adversely affected all the growth and feed utilization parameters (Table 4), but the incorporation of PPs in sea bream diets seemed to be economic as incorporation of PPs in the diets sharply reduced feed costs by 13.71, 27.29, 39.64 and 52.44% for 25PPs, 50PPs, 75PPs and 100%, respectively. The reduction of feed costs was easily observed for the feed costs per Kg weight gain which decreased with increasing incorporation levels of PPs in agreement with Soltan (2005) for Nile tilapia and Eid and Mohamed (2007) for sea bass fingerlings.

References


