Replacing fishmeal and fish oil in industrial aquafeeds for carnivorous fish

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8.1 Introduction

World food fish production has increased very significantly, from 32 million tonnes in 2000 to more than 60 million tonnes in 2011 (FAO, 2012). Most of this production (33.7 million tonnes, corresponding to 56% of the total) was based on freshwater fishes, while diadromous (3.6 million tonnes) and marine fish (1.8 million tonnes) production represented only 9.1% of total production. This increasing production of fisheries products follows closely the increasing demand of fisheries products due to the world population growth and the increasing per capita fish consumption. As fisheries captures have stagnated in the past decades, the additional demand of fisheries products will have to be fulfilled by aquaculture production (Klinger and Naylor, 2012).

Feeding habits of fish exploited worldwide in the various environments present substantial differences, with most freshwater fish species being omnivorous or herbivorous and most diadromous and marine species being carnivorous. These differences in feeding habits are also reflected in the nutritional requirements of these fish. For instance, whereas omnivorous and herbivorous fish have relatively low protein requirements, ranging from 25% to 35% of the diet, carnivorous fish have high protein requirements, ranging from 40% to 55% of the diet (Wilson, 2002; NRC, 2011). Also, whereas some species efficiently use diets with carbohydrate levels up to 40−60%, other species do not tolerate more than 10−20% dietary carbohydrates (Wilson, 1994; Enes et al., 2009; Figure 8.1).

Most importantly, there are also qualitative differences in the essential fatty acid (EFA) requirements between freshwater fish and diadromous salmonids and marine fish species (Table 8.1). Whereas EFA requirements of freshwater species and salmonids in general are met with C18-polyunsaturated fatty acids (namely 18:3*n*-3 and/or 18:2*n*-6), EFA requirements of marine fish are typically met only with long-chain polyunsaturated fatty acids (LC-PUFAs; namely 20:5*n*-3 and/or 22:6*n*-3). This is so because marine fish have lost or have low capacity to express the enzymes responsible for elongation and/or desaturation of PUFAs to LC-PUFAs (Sargent et al., 2002; NRC, 2011). This imposes additional limitations on the potential lipid sources to be used in aquafeeds for marine fish species.

Fish oil (FO) is a rich source of LC-PUFAs, being for the moment the only commercially competitive source of these EFAs (Turchini et al., 2009). FO inclusion is

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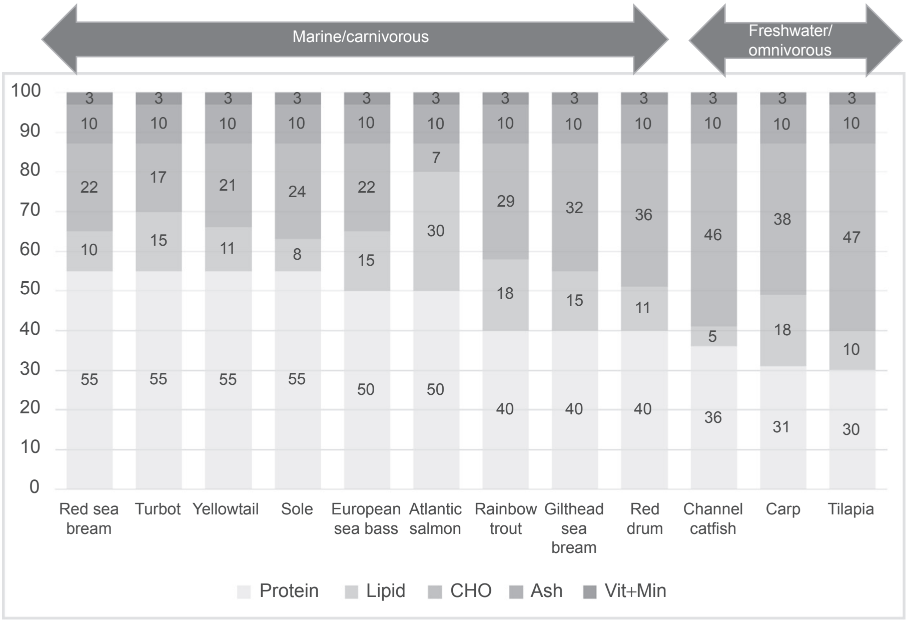

Figure 8.1 Proximate composition of diets for various fish species. CHO, carbohydrates.

therefore mandatory to assure coverage of EFA requirements in diets for marine fish, whereas it is not required in diets for freshwater species (De Silva et al., 2010). Also, fishmeal (FM) is considered the most adequate protein source for fish, as it has a high protein content, with adequate amino acid profile, high protein digestibility and high palatability; it is a rich source of taurine, minerals (including phosphorus) and vitamins (including choline); and it has no anti-nutritional factors (Hardy, 2010). Therefore, it is not surprising that both FM and FO have been used as the main protein and lipid sources in aquafeeds, particularly in diets for carnivorous fish.

However, as world capture fisheries are limited and have even decreased in past years, the world availability of FM and FO is also limited. From an environmental standpoint, overfishing of wild stocks for production of FM and FO is unsustainable (Nordahl, 2011). According to the International Fishmeal and Fish Oil Organisation (Tacon et al., 2011) it is estimated that in 2010 c. 63% of FM production went to aquaculture and that 25% of that went to salmonids and another 25% to marine fish. For the same year, it was estimated that 80% of FO went to aquaculture and that 68% of that went to salmonids and 20% to marine fish.

Thus, owing to the increase in aquaculture production and competition with other industries, enormous pressure is being put on the use of FM and FO for inclusion in aquafeeds, with prices increasing accordingly (Tacon and Metian, 2008). For instance, the FM and soybean meal price ratio increased from 2:1 in the 1990s to 4:1 in 2010 (Shepherd and Jackson, 2013). Further, because of its role in providing EFA for marine fish and also its importance regarding the maintenance of the ‘fishy’ quality of fillets, FO is a key feedstuff for the growth and sustainability of the aquaculture

Table 8.1 **Essential fatty acid requirements of selected fish species (NRC, 2011)**

Species	Environment	18:2*n*-6	18:3*n*-3	*n*-3 LC-PUFA
Common carp	F	1.0	0.5−1.0	
Channel catfish	F		1−2	
Tilapia zilli	F	1.0		
Nile tilapia	F	0.5		
Rainbow trout	F, B, M		0.7−1.0	0.4−0.5
Atlantic salmon	F, B, M		1.0	0.5−1.0
Turbot	M			0.8
European sea bass	M			1.0
Gilthead sea bream	M			0.9 (DHA: EPA = 1) 1.9 (DHA: EPA = 0.5)
Red sea bream	M			0.5−1.0
Red drum	M			0.5−1.0
Grouper	M			1.0

F, freshwater; B, brackish water; M, marine; EPA, eicosapentaenoic acid; DHA, docosahexaenoic acid.

industry (De Silva et al., 2010), risking becoming a bottleneck for the growth of marine fish production.

Reducing aquafeed dependency on FM and FO is of the utmost importance and it is recognized by stakeholders as a priority for the sustainable development of fed aquaculture. Dependency on FM and FO is almost non-existent for most omnivorous fish, and nowadays practical diets for carp, tilapia or catfish are almost devoid of FM and FO. Even during the initial growth phases, which are usually more exigent in terms of nutrient requirements, there is no apparent advantage of including animal protein in the diets of omnivorous species (Sink et al., 2010). Reduction of fisheries products use in aquafeeds is more challenging for carnivorous fish, namely marine fish and salmonids (Tacon, 2004). Even so, it is expected that substantial reductions in FM (40−50%) and FO (30−35%) in aquafeeds will be achieved in the near future (Tacon et al., 2011; Table 8.2).

Overall, it can be assumed that replacing half of the FM in carnivorous fish diets with plant protein feedstuffs is relatively simple (Naylor et al., 2009). However, reaching low levels or complete elimination is more complicated without reducing growth performance and animal health. Thus, continued research efforts to overcome these

Table 8.2 **Estimated fish meal (%) and fish oil (%) incorporation in aquafeeds**

	Fish meal		Fish oil	
	2010	2020	2010	2020
Carp	2	1	0	0
Tilapia	3	1	0	0
Catfish	3	1	0	0
Salmonids	22	12	12	8
Marine fishes	26	12	6	4

After Tacon et al. (2011).

problems and to further reduce the amount of FM used in aquafeeds are required if sustainable growth of global aquaculture is to be ensured. However, with judicious replacement of FM with available alternative feedstuffs it is reasonable to foresee that FM availability will not be a major obstacle to sustained growth of aquaculture production (Olsen and Hasan, 2012). In contrast, world availability of FO may become an important bottleneck for the sustainable growth of marine aquaculture. Thus, it is mandatory to continue research efforts to replace FO in aquafeed as well as developing feeding strategies that minimize the negative impact of dietary FO reduction on the quality of the final product to the consumer. Further, in addition to promoting performance similar to that obtained with FM- and FO-based aquafeeds, the alternative aquafeeds (low in FM and FO) must ensure good fish health and welfare and a final product that is nutritionally adequate, safe to eat and well accepted by the consumer (New and Wijkstrom, 2002).

8.2 Fishmeal sparing in aquafeeds

When considering FM alternatives in aquafeeds aspects such as price, protein content, amino acid profile, digestibility, essential amino acid (EAA) deficiencies, anti-nutritional factors and palatability must be addressed (Gatlin et al., 2007; Hardy, 2010). Caution must be also taken to avoid unintended consequences in fish health, intestine homeostasis, immunological parameters and disease resistance.

Plant feedstuffs are the most abundant alternative protein sources to use in aquafeeds (Tacon et al., 2011). However, plant feedstuffs have a highly variable protein content and present several EAA inadequacies and anti-nutritional factors, and these characteristics impose some limitations to their use in diet formulations.

Other than a few plant protein concentrates, such as soy protein concentrate or potato protein concentrate, most alternative protein sources have an EAA profile that presents imbalances in one or more EAA (Table 8.3). Within the most used

Table 8.3 EAA profiles of fishmeal and selected alternative protein sources with limiting amino acids (Feedpedia: http://www.feedipedia.org/)

	Amino acids (% protein)															Limiting amino acids		
Feedstuffs	**Protein**	**ARG**	**CYS**	**HIS**	**ILE**	**LEU**	**LYS**	**MET**	**PHE**	**THR**	**TRY**	**TYR**	**VAL**	**MET + CYS**	**PHE + TYR**	**1°**	**2°**	**3°**
Fish EAA requirements – Average values[a]		4.5		2.3	3.1	4.7	6.2			3.4	0.9		3.8	3.1	5.4			
Maize distillers wet grains and solubles	44.0	3.4	2.0	2.4	3.5	12.0	2.6	1.9	4.6	3.2	0.5	4.1	4.4	3.9	8.7	Lys	Try	Arg
Maize distillers dried grains and solubles	29.5	4.3	2.0	2.7	3.8	11.6	3.0	2.0	4.8	3.7	0.8	3.9	5.1	4.0	8.7	Lys	Try	
Brewer's yeast, dehydrated	48.6	4.4	0.9	2.0	4.6	6.2	6.3	1.5	3.6	4.4	1.1	2.7	4.9	2.4	6.3	M + C	His	
Earthworm, dehydrated	61.0	4.5	1.0	2.2	3.5	6.3	7.4	4.0	5.1	4.3		4.0	5.2	5.0	9.1	Hys		
Feather meal	85.7	6.7	4.3	0.8	4.9	8.0	2.1	0.7	4.7	4.6	0.6	2.5	7.2	5.0	7.2	Lys	His	Trp
Blood meal	94.1	4.2	1.1	6.2	1.1	12.1	8.7	1.2	6.9	4.7	1.4	3.0	8.5	2.3	9.9	Ile	Trp	His
Poultry offal meal	60.2	6.6	2.5	1.8	3.9	7.0	4.4	1.4	3.9	3.9	0.7	2.6	5.4	3.9	6.5	Lys	Trp	His
Meat and bone meal, low fat	62.0	6.7	1.2	2.7	2.8	6.2	5.0	1.4	3.5	3.4	0.8	2.3	4.5	2.6	5.8	Lys	M + C	Trp
Meat and bone meal, high fat	54.9	6.9	1.1	2.1	2.9	6.0	5.0	1.3	3.4	3.3	0.6	2.2	4.4	2.4	5.6	Trp	M + C	Lys
Fishmeal, 60–68% protein as fed	70.6	6.2	0.8	2.4	4.2	7.2	7.5	2.7	3.9	4.1	1.0	3.1	4.9	3.5	7.0			
Fishmeal, high protein	75.4	5.8	0.8	2.2	4.3	7.0	7.5	2.8	3.8	4.1	1.1	2.9	4.9	3.6	6.7			
Maize gluten meal	67.3	3.1	1.7	2.1	4.1	16.1	1.7	2.4	6.2	3.4	0.5	5.1	4.6	4.1	11.3	Lys	Trp	Arg
Maize grain, Europe	9.4	4.5	2.3	2.8	3.5	12.0	3.1	2.1	4.8	3.6	0.7	3.7	4.8	4.4	8.5	Lys	Tre	
Wheat grain	12.6	4.7	2.2	2.3	3.4	6.5	2.9	1.6	4.5	2.9	1.2	2.7	4.3	3.8	7.2	Lys	Tre	

Continued

Table 8.3 **Continued**

Feedstuffs	Amino acids (% protein)															Limiting amino acids		
	Protein	ARG	CYS	HIS	ILE	LEU	LYS	MET	PHE	THR	TRY	TYR	VAL	MET + CYS	PHE + TYR	1°	2°	3°
Faba bean (*Vicia faba*)	29.0	9.0	1.2	2.6	4.1	7.1	6.3	0.8	4.0	3.5	0.8	2.7	4.6	2.0	6.7	M + C	Trp	
Lupin (*Lupinus angustifolius*), blue, seeds	33.8	11.0	1.5	2.7	4.2	6.9	4.7	0.7	4.0	3.4	0.8	3.6	3.9	2.2	7.6	M + C	Lys	Trp
Pea seeds	23.9	8.4	1.4	2.5	4.2	7.1	7.2	1.0	4.7	3.8	0.9	3.1	4.8	2.4	7.8	M + C		
Linseed meal, expeller-extracted	34.2	9.6	1.8	2.6	4.4	5.9	3.9	1.9	4.8	3.8	1.6	2.4	5.2	3.7	7.2	Lys		
Cottonseed meal, low fibre, low oil	45.0	11.1	1.6	2.9	3.2	5.9	4.2	1.4	5.1	3.3	1.1	2.9	4.2	3.0	8.0	Lys	M + C	Tre
Sunflower meal, solvent-extracted, dehulled and partially dehulled	37.7	8.5	1.7	2.5	4.1	6.2	3.5	2.3	4.4	3.6	1.2	2.4	4.9	4.0	6.8	Lys		
Canola meal, solvent-extracted	39.0	5.9	2.5	2.6	4.0	6.8	5.6	2.0	3.9	4.2	1.2	2.9	4.9	4.5	6.8	Lys		
Rapeseed meal, solvent-extracted, low erucic, low glucosinolates	38.3	6.1	2.3	2.6	4.0	6.7	5.5	2.1	3.9	4.4	1.3	3.1	5.1	4.4	7.0	Lys		
Soybean meal, high oil (expeller)	49.3	7.5	1.6	2.7	4.6	7.7	6.3	1.4	5.1	3.7	1.4	3.5	4.5	3.0	8.6	M + C		
Soybean meal, high protein (dehulled)	53.5	7.3	1.6	2.7	4.6	7.7	6.3	1.4	5.1	3.8	1.4	3.5	4.8	3.0	8.6	M + C		

[a] Average values of fish EAA requirements based on EAA recommendations for Atlantic salmon, trout, carp, tilapia and catfish (NRC, 2011).

alternative protein sources in aquafeeds the first limiting EAAs are usually lysine and methionine. Tryptophan, threonine, arginine and histidine may also be limiting in several feedstuffs. Interestingly, within the potential alternative feedstuffs used in aquafeeds, those that have methionine as the first limiting EAA have an excess of lysine, and the opposite is true for feedstuffs deficient in lysine. This increases potential combinations of feedstuffs to include in aquafeeds, as they may complement one another to provide a balanced dietary EAA profile.

The list of limiting EAAs presented in Table 8.3 is based on recommended mean values for EAA inclusion in diets for Atlantic salmon, rainbow trout, common carp, tilapia and catfish, which were compiled by the National Research Council (NRC, 2011). These recommendations seem, however, very conservative, particularly for lysine, for which the dietary allowance and estimated requirement values are, respectively, 6.2% and 5.0% of dietary protein. Thus, there is a safety margin of c. 25% between lysine requirements and dietary lysine allowances, which may have considerable implications for restrictions imposed on diet formulations and for potential combinations of feedstuffs to meet allowances (see Figure 8.2).

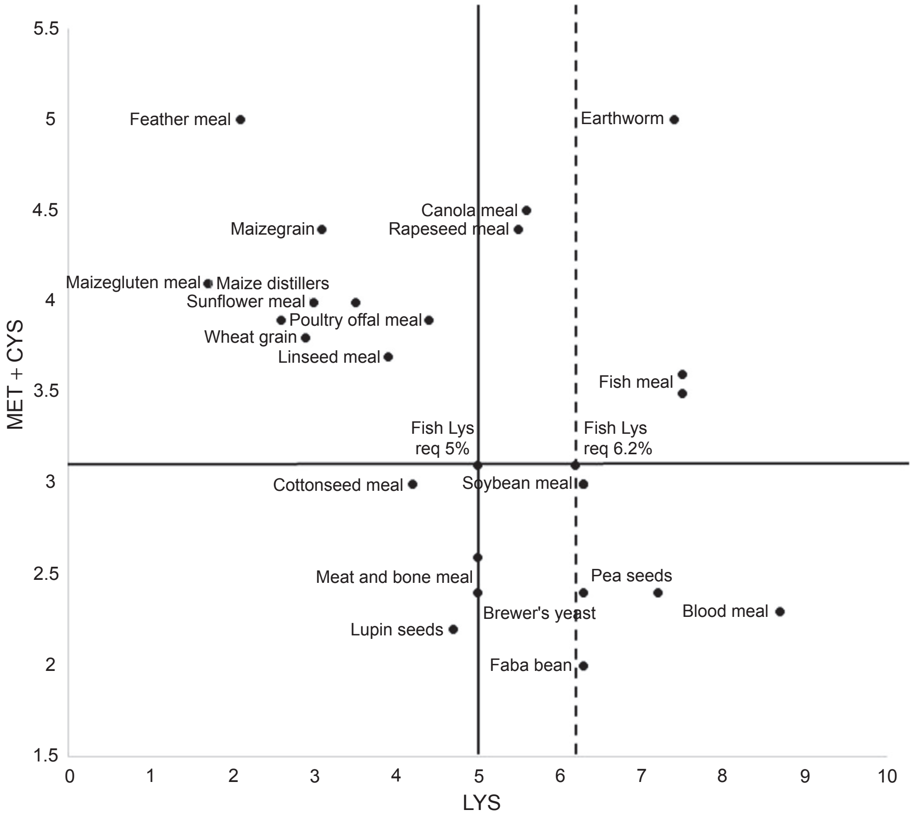

Figure 8.2 Lysine and methionine + cysteine content of selected feedstuffs and mean requirements of fish.

Table 8.4 **Anti-nutritional factors present in plant feedstuffs and means of alleviation (Francis et al., 2001; Krogdahl et al., 2010)**

Anti-nutrients:	Protease inhibitors	Haemagglutinins	Amylase inhibitors	Saponins	Phytate	Glucosinolates	Gossypol	Anti-vitamins	Tannins	Lectins	Alkaloids	Cyanogens	Phytoestrogens
Oilseeds													
Soybean	●	●		●	●	●		●		●			●
Rapeseed	●				●	●			●				●
Cottonseed			●		●		●	●	●				●
Sunflower	●			●				●	●				
Sesame	●				●								
Linseed								●				●	
Legumes													
Lupin	●			●							●		●
Peas	●	●	●	●	●			●				●	
Alfalfa				●				●	●		●		●
Faba bean	●				●					●			
Cereals													
Maize	●				●			●					●
Wheat	●	●	●		●					●			●
Sorghum	●		●		●			●	●	●		●	
Tubers													
Potato	●	●	●							●	●		●
Cassava	●				●			●				●	
Means of alleviation:													
Heat	●	●	●					●		●		●	
Solvent extraction				●					●				●
Enzyme degradation					●								
New varieties						●	●				●		

Anti-nutritional factors in plant feedstuffs are highly abundant and diversified (Francis et al., 2001; Hendricks, 2002; Gatlin et al., 2007; Krogdahl et al., 2010) and various strategies are required to alleviate their negative nutritional impacts (Table 8.4). These include technical treatments such as heat processing, solvent extraction and dehulling or the use of exogenous enzymes such as phytases (Jobling et al., 2001; Glencross et al., 2007; Krogdahl et al., 2010). Breeding new plant varieties with better amino acid (AA) profiles or low phytic acid is also a strategy for improving plant utilization in aquafeeds (Gatlin et al., 2007; Overturf et al., 2003).

Dietary inclusion of exogenous enzymes that improve digestibility of nutrients, particularly of non-starch polysaccharides (NSPs) and phytic phosphorus, is gaining relevance in aquafeeds (Ai et al., 2007; Adeola and Cowieson, 2011; Dalsgaard et al., 2012). Care should be taken, however, regarding the practical efficiency of adding the feed enzymes directly to the diet. Fish, particularly cold water species, are reared at water temperatures that are below the optimal activity of these enzymes, and therefore their efficacy is reduced. Alternatively, pre-treatment of plant feedstuffs at the optimal temperature for enzyme activity may be a better technological strategy and would provide a benefit across animal species.

Comprehensive reviews on the use of alternative protein sources in fish feeding have been published (Gatlin et al., 2007; Barrows et al., 2008; Rana and Hasan, 2009; Kaushik and Hemre, 2010; Tacon et al., 2011). Plant feedstuffs are the major dietary protein sources for omnivorous and herbivorous fish and are second to FM in diets for carnivorous species (Tacon et al., 2009). However, owing to the high dietary protein requirements of carnivorous fish, potential alternative protein sources are limited to a few feedstuffs with high protein content. These comprise mainly plant protein concentrates and oilseeds, animal by-products and unicellular organisms (Figure 8.3).

Plant protein concentrates include corn gluten, wheat gluten and less abundant protein concentrates such as soy protein concentrate, pea protein concentrate, potato protein concentrate and rapeseed protein concentrate. Some of these protein concentrates are still expensive compared to FM, because of processing costs, and, with the exception of corn gluten, their use in aquafeeds is still limited. However, with the increasing price of FM the use of protein concentrates in diets for carnivorous fish species is expected to increase (Naylor et al., 2009; Tacon et al., 2011).

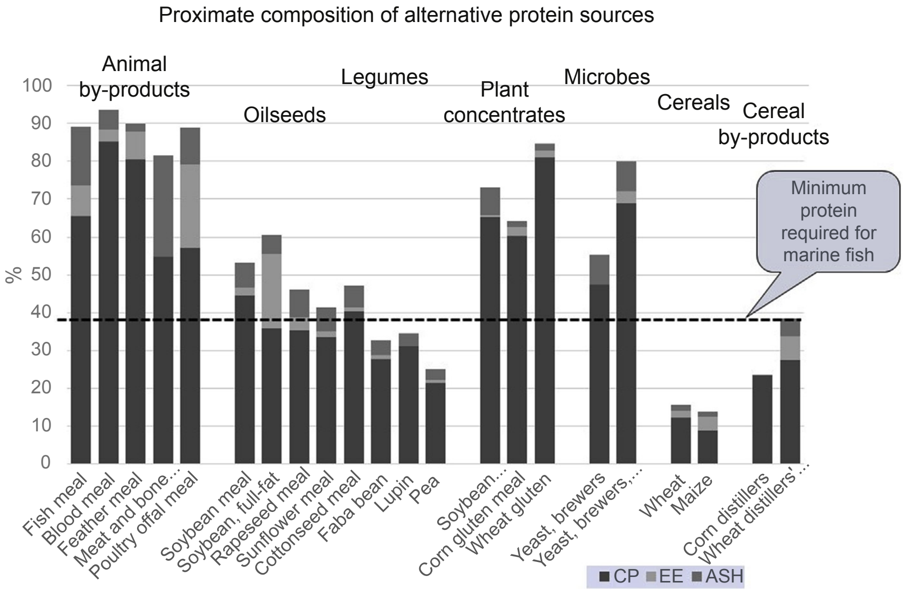

Figure 8.3 Proximate compositions of selected feedstuffs used in aquafeeds. CP, crude protein; EE, ether extract.

Protein concentrates have great potential for use in aquafeeds because of their high protein content (between 60% and 80%) and because they are almost devoid of anti-nutritional factors. Lysine, threonine and methionine are the first limiting AAs in these feedstuffs. Plant protein concentrates may replace FM protein from almost 30% to 100% in experimental diets, corresponding to dietary incorporations of 10–60%. Corn gluten meal is currently used in feeds for carnivorous fish, with upper inclusion limits of 20–25% (Gatlin et al., 2007). Incorporation of other plant protein concentrates in carnivorous fish diets is usually lower than 15% (Tacon et al., 2011). It is worth noting that, when included in the diet at high concentrations, carotenoids present in corn gluten may confer undesirable colour to the flesh. For instance, in rainbow trout dietary inclusion of corn gluten above 10% will impart an undesirable yellow colour to the fillets (Gaylord et al., 2010). Wheat gluten is incorporated in diets at lower levels than corn gluten, partially because of its higher price, but also because of the binding proprieties of its protein, which have undesirable effects on pellet quality (Gatlin et al., 2007; Gaylord et al., 2010). Further, wheat gluten has very high levels of glutamic acid, which represents c. 30% of the protein, and although it may be of value as a nutraceutical if included at moderate levels in the diet, at high levels it may have undesirable effects.

Oilseeds, such as soybean meal, cottonseed meal, rapeseed meal or sunflower meal, have competitive prices and a protein content ranging from 38% to 52%. Soybean meal is the most available oilseed worldwide and it is also the most common plant feedstuff used in aquafeeds. Owing to the relatively low protein content, FM protein replacement by oilseeds is usually limited to 20–40%, corresponding to dietary incorporation of 15–30%. According to Tacon et al. (2009) the mean incorporation of oilseeds in practical diets for carnivorous fish is about 10–20%, except for sunflower meal, the inclusion level of which is usually lower (up to 10%) mainly owing to its high fibre content (Tacon et al., 2011).

Oilseeds have some EAA deficiencies, the first limiting EAA being methionine in soybean meal and lysine in rapeseed, sunflower and cottonseed meals. Oilseeds also have several anti-nutritional factors, some of which are inactivated by heat processing or solvent extraction but others of which cannot be inactivated. Dehulling is routinely used in some oilseeds as an efficient technological treatment to reduce fibre and tannins and to increase protein content. An alternative to eliminating some resistant antinutrients is the selection of new cultivars. This has been successfully achieved for rapeseed/canola, in which levels of glucosinolates and erucic acid have been extremely reduced, and for cottonseed, with a variety almost free of gossypol. Oilseeds are not very palatable for fish and this may affect feed intake. In such cases, addition of feed stimulants or mixture with more palatable feedstuffs may reduce or overcome this inconvenience (Dias et al., 1997).

Animal by-products comprise meat meal, meat and bone meal, poultry by-product meal, feather meal and blood meal, among others, and have high potential as alternatives to FM in aquafeeds as they have acceptable protein content and competitive prices. However, in contrast to plant feedstuffs, which have relatively constant nutritional composition, animal feedstuff composition is highly variable, particularly that of poultry by-products and meat and bone meals, and therefore proximate composition must be closely checked. The use of animal by-products in aquaculture is highly variable depending on the region. For instance, in Australia the use of rendered animal products in aquafeeds is high, whereas in the European Union very strict regulations for their use, and also consumer concerns over the potential risk of disease transmission (due to bovine spongiform encephalopathy), virtually prevent their use in animal feeds (Klinger and Naylor, 2012).

Protein content of animal by-products is usually high, ranging from 50% to 80% or even more, as in blood meal or plasma hydrolysate, but AA deficiencies may occur, particularly for lysine, methionine and tryptophan. Depending on the source and nutritional quality, animal by-products may replace up to 20—40% of FM protein in experimental diets, corresponding to dietary incorporations of 15—60%. According to Tacon et al. (2009) the range of meat meal and poultry by-product incorporation in practical diets for carnivorous fish is 10—30%, whereas that of hydrolysed feather meal is limited to 5—20%, owing to its high methionine deficiency and very high cysteine—methionine imbalance (Tacon et al., 2009). Though having a very high protein content, blood meal is characterized by a severe leucine—isoleucine imbalance that limits its inclusion in aquafeeds to 1—8%, with a mean inclusion level of 2—4% (Tacon et al., 2009).

Animal by-products have good palatability and present no anti-nutritional factors. However, poultry by-products, meat meal and meat and bone meal have high ash contents and high saturated fat levels and this limits their use in aquafeeds. Ash is rich in phosphorus, and high incorporation of animal by-products in aquafeeds may result in excess dietary phosphorus, with the concomitant environmental problems associated with phosphorus loss to the water bodies. Also, saturated fats tend to deposit in carcasses, thus affecting the nutritional and organoleptic quality of fish fillets.

Legumes such as peas, lupins and faba beans have competitive prices and may contribute with some dietary protein to carnivorous fish diets. However, because of their relatively low protein content they cannot be considered a main protein source, being mainly used as complementary protein. Legumes have protein contents ranging from 22% to 30% and may replace 10—30% of FM protein in experimental diets. This implies a dietary incorporation of up to 40%, which is far above the normal range of incorporation in practical diets for carnivorous fish. According to Tacon et al. (2009) legumes are incorporated in diets for carnivorous fish up to 15—25%, with mean values of 10—15%.

Legumes are low in methionine and lysine and present some anti-nutritional factors, including NSPs, which are high in lupin. Lupin is also rich in alkaloids, and new varieties of lupin with reduced alkaloids are being developed. Peas are rich in starch (>40%) and when considering their incorporation into diets this high carbohydrate level must be taken into consideration, as carnivorous fish have limited ability to use dietary carbohydrates.

Cereals have low protein content (8—12%) and are rich sources of starch (~60%). Cereals are the most economical feedstuff and are incorporated in diets mainly as an energy source. Cereal inclusion in carnivorous fish diets is usually limited to 10—20%, thus providing just up to 5% of dietary protein, which is deficient in lysine.

Distilled dried grains with solubles (DDGS) are cereal by-products obtained from the biofuel industry and are becoming increasingly available as feedstuffs at competitive prices. DDGS have a protein content similar to that of legumes (25–30%) and an amino acid profile similar to that of whole grains. DDGS have no starch, but are rich in fibre, and that may limit their incorporation into diets. The range of incorporation of DDGS in carnivorous fish diets is 3–20%, with mean values of 5–10% (Tacon et al., 2009). High-protein-content (more than 40%) DDGS products are being developed, and this may increase their potential use in aquafeeds for carnivorous fish species (Overland et al., 2013; Prachom et al., 2013).

Single-cell proteins (SCPs), such as bacteria or brewer's yeast, are rich protein sources (>50%), though they also contain high levels of nucleotides (12–20% of total N), are very palatable and are devoid of anti-nutritional factors. SCPs may have nutraceutical proprieties and are rich sources of B vitamins (Rana and Hasan, 2009; Oliva-Teles, 2012). Methionine is a potential limiting AA in these feedstuffs, which may replace up to c. 50% of FM protein in experimental diets, corresponding to a dietary incorporation of 30–55%. In practical diets, mean incorporation values are, however, limited to 2–4% (Tacon et al., 2009). Microalgae are a novel source of SCPs, with potential for incorporation in fish diets. However, they are still scarce and their price is very high. Moreover, their protein content is highly variable according to source and processing.

SCPs are mostly included in diets as potential probiotics (Irianto and Austin, 2002; Balcazar et al., 2006; Nakano, 2007; Nayak, 2010). Probiotics are live organisms that may colonize the intestinal tract and contribute to improving health condition, disease resistance, microbiota balance and gut physiology (Irianto and Austin, 2002; Merrifield et al., 2010).

As stated above, FM has an EAA profile that is adequate for most fish species, whereas the EAA profile of alternative ingredients is usually unbalanced. Thus, replacement of FM protein by individual alternative protein sources requires a careful adjustment of the dietary EAA profile to that of animal's requirements. Therefore, supplementation of diets with limiting EAAs is generally required (Watanabe et al., 2001; Fournier et al., 2004; Deng et al., 2006; Alam et al., 2011). This can be done at competitive prices with commercially available feed-grade AAs, which for the moment are limited to a few EAAs. Care must also be taken to guarantee that absorption of free AAs, and therefore their availability for metabolism, occurs simultaneous with that of protein-bound AAs. This may require using technological strategies for delaying intestinal absorption of free AAs, such as coating with agar (Li et al., 2009). In addition, free AAs are prone to leaching into the water and may also be used by intestinal microbiota (Li et al., 2009). This may decrease free AA availability to the animals, and surplus inclusion in aquafeeds may be required to counterbalance this reduced availability.

As alternative feedstuffs are also usually less palatable to fish than fishery products (Glencross et al., 2007), decreased performance observed in fish fed diets including alternative feedstuffs may be related to decreased intake of such less palatable feeds, and not to nutrient imbalances or deficiencies. Thus, inclusion of feed attractants to low-FM-content aquafeeds may be required to improve their utilization by fish (Dias et al., 1997; Kader et al., 2010, 2012; Trushenski et al., 2011).

It is worth noting that when evaluating the potential of alternative feedstuffs for inclusion in aquafeeds, there are a number of strategies. In general, the control diet should have a protein content that meets but does not exceeds the animal's protein requirement, a well-balanced EAA profile and high digestibility. It is also advisable that the control diet is formulated with an FM level that maintains maximum performance. Otherwise, conclusions of dietary FM-replacement studies may be highly biased. For instance, if the control diet has a protein content clearly exceeding the animal's protein requirement, alternative protein sources may be incorporated in the diets at higher levels than what would be possible in a diet with a more suitable protein level. In such cases, the portion of dietary protein that exceeds requirements for growth purposes will be used as an energy source, and protein biological value is not important for energetic purposes. Also, in diets with protein levels above requirements EAA deficiencies are expected to occur only at higher levels of FM replacement with alternative feedstuffs, thus further confusing results. Also, if the control diet does not ensure adequate fish performance, comparison of results obtained with the alternative diets is of no practical value.

Using mixtures of alternative protein sources that may complement one another in terms of AA composition is usually a more adequate strategy for replacing FM protein than using individual alternative protein sources. Several studies have been dedicated to the evaluation of such more practical diets in several carnivorous species (Gomez-Requini et al., 2004; Espe et al., 2006; Hansen et al., 2007; Altan et al., 2010; Cabral et al., 2011, 2013; Booth et al., 2012). For instance, in European sea bass almost total replacement of FM protein (95%) by plant protein was first achieved by Kaushik et al. (2004) with a mix of corn gluten, wheat gluten, soybean meal and rapeseed meal. Even so, it was necessary to supplement the diet with 1% lysine to meet an adequate EAA profile. In the same year, Kissil and Lupatsch (2004) also reported successful replacement of FM protein (100%) by a mixture of plant protein concentrates (corn gluten, wheat gluten, soy protein concentrate) in the diet for gilthead sea bream. In this study it was necessary to supplement the diet with lysine, methionine, threonine and arginine. Owing to the cost of AA supplementation, particularly that of arginine, cost-effective replacement levels of FM by plant feedstuffs were limited to 25%. Since then, successful complete replacements of FM with alternative ingredients in diets for marine fish have been accumulating for other species (Hansen et al., 2007; Silva et al., 2009; Salze et al., 2010; Kader et al., 2012).

In salmonids, individual or combinations of plant protein concentrates (pea, canola, potato and soy) were successfully used to completely replace FM in diets for rainbow trout, provided that the diets were supplemented with the limiting EAAs (Zhang et al., 2012). Further, it was also demonstrated that complete FM replacement by a mixture of alternative protein sources (rapeseed protein concentrate, canola protein isolate, soy protein concentrate, blood meal and crustacean meal) without crystalline AA supplementation promoted performance similar to that of control diets containing 30% FM (Slawski et al., 2012, 2013). In Atlantic salmon, blends of plant protein sources alone or with poultry by-product meal successfully replaced FM in diets for juveniles (over 30 g) without compromising growth performance (Burr et al., 2012). However, for early stage salmonids such alternative diets are not recommended as growth depression was severe.

The cost effectiveness of FM-free or almost FM-free diets is seldom considered in scientific literature, though it is of utmost importance at a commercial level. Lack of economic evaluation in most studies is due to the volatile prices of feedstuffs in contrast to the nutritional data results, which are independent of feedstuff price. Decision-makers, however, have to base their least-cost formulas taking into consideration both nutritional requirements and feedstuff prices, and high replacement levels of FM by plant feedstuffs may not always be the most effective economical choice (Kissil and Lupatsch, 2004; Martinez-Llorens et al., 2012). For instance, in gilthead sea bream, Martinez-Lorenz et al. (2007) concluded that from a nutritional perspective dietary soybean meal might be included in the diet up to 30% for juveniles and up to 50% for grow-out fish without affecting the animals' performance. However, from an economic perspective, considering soybean prices at the date of the study, the optimum dietary soybean inclusion level was only 22%. There are, however, huge differences worldwide in feedstuff market prices and an acceptable FM replacement level for a given country or region may not be economically reproducible in another country or region.

Taurine is not a constituent of proteins but it is abundant in many animal tissues, being involved in, among other functions, the synthesis of bile pigments, cell membrane stabilization and osmoregulation, and it also has antioxidant properties (NRC, 2011). FM is a very rich source of taurine (Divakeran, 2006), whereas plant feedstuffs are devoid of it. In some carnivorous fish it has been shown that performance is hampered with low-taurine diets and that a pathological condition, named green liver syndrome, may develop in certain species under these circumstances (Takagi et al., 2005, 2006; Goto et al., 2001). Though fish are known to be capable of synthesizing taurine, differences in biosynthesis rate, physiological requirement level, life stage or species may explain the essentiality of taurine under certain conditions (Kim et al., 2008; Wang et al., 2014). Taurine inclusion in plant-based diets has been shown to improve the performance of several marine fish species (Lunger et al., 2007; Chatzifotis et al., 2008; Takagi et al., 2008, 2010; Enteria et al., 2011). In contrast, in freshwater species a taurine requirement has been observed only in rainbow trout fed FM-free diets (Gaylord et al., 2006, 2007), though such taurine requirement is still controversial (Boonyoung et al., 2013). In some marine species the estimated taurine requirement is considerably high (Goto et al., 2001; Kim et al., 2005; Qi et al., 2012; Lim et al., 2013) and this may help explain why a taurine requirement has been established essentially in marine fish, even when fed FM-containing diets.

Carnivorous fish are not naturally prepared to deal with plant feedstuffs and therefore, when replacing FM with alternative feedstuffs, aspects such as gut homeostasis, gut integrity, immunological status, health and welfare and carcass composition should also be considered. However, the effect of FM replacement on non-specific defence mechanisms has seldom been assessed in fish (Oliva-Teles, 2012). For instance, Atlantic salmon (and to a lesser extent other salmonids) is particularly sensitive to dietary soybean, and pathological effects occur at the distal intestine even at low dietary soybean inclusion levels (Krogdahl et al., 2010). This so-called soybean-induced enteritis seems, however, to be less important in marine fish such as European sea bass (Couto et al., 2014). It was also shown that in rainbow trout sensitivity to dietary plant feedstuffs varies between strains (Venold et al., 2012) and therefore selective breeding can be a useful strategy to improve tolerance to plant feedstuffs in aquafeeds.

In gilthead sea bream, replacement of FM protein by plant feedstuffs above 75% seems to decrease immune defence mechanisms and also to affect gut integrity (Sitja-Bobadilla et al., 2005; Santigosa et al., 2008; Koukou et al., 2012). In contrast, gut integrity does not seem to be affected in European sea bass (Couto et al., 2014), cobia (Romarheim et al., 2008) or Atlantic cod (Olsen et al., 2007; Colburn et al., 2012). In this last species, enteritis did not develop even with 100% replacement of FM by soybean meal or soy protein concentrate. Thus, sensitivity to plant feedstuffs is species specific and extensive studies are still required to have a clearer picture of the potential negative effects of alternative feedstuffs in gut integrity, immune parameters and overall fish health.

FM replacement by alternative feedstuffs may also modify the AA balance, and this can also affect the immune response, as AAs have a central role in fish defence mechanisms (Li et al., 2009; Kiron, 2012). However, the role of AAs in the fish immune response is still poorly studied (Li et al., 2009).

High dietary levels of FM replacement by plant feedstuffs may also affect fillet composition. For instance, in gilthead sea bream, fish fed an FM-based diet had higher moisture, lower lipid and higher (*n*-3) LC-PUFA levels than fish fed a plant-based diet (De Francesco et al., 2007). Nevertheless, only minor differences in muscle free-AA levels were detected and sensory evaluation of cooked fillets by a panel of judges was unable to discriminate between diets. Also in gilthead sea bream, a lack of differences in sensory analysis tests of fish fed diets with high levels of plant protein concentrates (pea and rice protein concentrates) was observed by Sanchez-Lozano et al. (2009). Similarly, in Senegalese sole 75% FM replacement by alternative plant protein blends did not influence most of the sensory evaluation descriptors of cooked slices (Cabral et al., 2013). Thus, it seems that even at high dietary levels of FM replacement by plant feedstuffs only minor effects on quality traits of commercial size animals are to be expected. Nonetheless, some effects on flesh quality are not to be disregarded, as carotenoids present in plant feedstuffs may affect fillet colour, and lipids in plant feedstuffs may also modify fillet lipid profiles, thus affecting the organoleptic characteristics of fillets.

Replacement of FM with plant feedstuffs may also affect gut microbiota and this may affect the fish health status, as the intestine is an important route for pathogenic bacteria attack (Gatesoupe, 2009; Merrifield et al., 2009). For instance, in Atlantic cod, replacement of FM with soybean meal was shown to affect the autochthonous microbiota community and this might affect the protective potential of indigenous bacteria against pathogenic colonization (Ringo et al., 2006).

8.3 Fish oil sparing in aquafeeds

Vegetable oils (VOs) are cheaper than FO and may be used as the only lipid source for freshwater fish, as they may provide all the EFAs required by these fish (Table 8.1). In salmonids, EFA requirements are met with (*n*-3) PUFAs and therefore VOs may be used as major sources of replacement for FO in diets without negative effects on performance (Naylor et al., 2009; Turchini et al., 2009; Glencross and Turchini, 2010; Tocher et al., 2010). Animal fats are also cheaper than FO and are mainly used as energy source, though poultry and swine fats are a good source of 18:2*n*-6 and therefore may provide the EFA requirements of freshwater species that have only (*n*-6) PUFA requirements (Table 8.5).

Marine fish, however, have absolute requirements for LC-PUFAs and to date the only competitive commercial source of these FAs is FO (Turchini et al., 2009). Unconventional sources of EFAs for marine fish, such as krill, still have prohibitive costs and therefore are not yet real alternatives to FO. Genetic modification of plant feedstuffs to produce LC-PUFAs is being researched, but to date with modest results (Miller et al., 2008; Naylor et al., 2009), although progress has been fast through biological engineering (Nichols et al., 2010; Olsen, 2011; Petrie and Singh, 2011). Industrially produced single-cell biomass, particularly biomass from microalgae, may become an alternative in the future, as some species of microalgae have high LC-PUFA content. Production costs are, however, still relatively high (Olsen, 2011). Therefore, as of this writing, marine aquafeeds still need to incorporate FO as a source of EFAs.

Comprehensive reviews on EFA requirements and the use of alternative VO in aquafeeds have been published (Sargent et al., 2002; Turchini et al., 2009; Bell and Koppe, 2010; Tocher et al., 2010; NRC, 2011). As a rule of thumb, it can be considered that EFA requirements of carnivorous fish juveniles are met with 0.5−1% (*n*-3) PUFA for salmonids and 0.5−1% (*n*-3) LC-PUFA for marine fish (Turchini et al., 2009; NRC, 2011). Thus, VO rich in (*n*-3) PUFAs, such as linseed, rapeseed/canola, soybean, sunflower, olive and palm oils, will meet the EFA requirements of salmonids. However, an inclusion of 5−10% FO should be considered in marine fish diets to meet EFA requirements. Above that level of dietary FO inclusion, lipids are essentially used as the energy source and therefore this dietary space may be fulfilled with alternative lipid sources.

Nowadays the trend in carnivorous fish production is to use energy-dense diets that are formulated with high lipid levels. However, in contrast to diets for Atlantic salmon, which include 20−30% or higher lipid levels, marine fish do not seem to tolerate well such high dietary lipid levels. For instance, in European sea bass, though performance was not affected with diets including from 12% to 30% lipids, feed utilization decreased with the 30% lipid diet (Peres and Oliva-Teles, 1999). Significantly, increasing dietary lipid from 12% to 30% had no protein sparing effect. Lack of protein sparing and/or improved performance with increasing dietary lipid levels was also observed in studies with other marine fish (Tibaldi et al., 1996; Jover et al., 1999; Espinós et al., 2003; Sa et al., 2006, 2008). This is different from Atlantic salmon, in which both growth and feed efficiency improved with an increase in dietary lipids from 30% to 47% (Hemre and Sandness, 1999).

From the above, it stands that there is a large margin for decreasing FO in aquafeeds for grow-out carnivorous fish, still meeting EFA requirements and without affecting fish performance (Turchini et al., 2009). In starter feeds it is still advisable to use mainly FO as the lipid source, as during this phase the fish have critical requirements for LC-PUFAs, and FO is the only cost-effective source of

Table 8.5 Fatty acid composition of selected oil sources

	Saturated	**Mono-unsaturated**	**HUFA**		**LC-HUFA**			***n*-6 PUFA**	***n*-3 PUFA**	***n*-3 HUFA**
Oil/fat		18:2*n*-6	18:3*n*-3	20:4*n*-6	20:5*n*-3	22:6*n*-3				
Fish oils										
Anchovy	28.8	24.9	1.2	0.8	0.1	17	8.8	1.3	26.6	25.8
Capelin	20	61.7	1.7	0.4	0.1	4.6	3	1.8	8	7.6
Menhaden	30.5	24.8	1.3	0.3	0.2	11	9.1	1.5	20.4	20.1
Herring	20	56.4	1.1	0.6	0.3	8.4	4.9	1.4	13.9	13.3
Cod liver	19.4	46	1.4	0.6	1.6	11.2	12.6	3	24.4	23.8
Vegetable oils										
Palm	48.8	37	9.1	0.2				9.1	0.2	
Soybean	14.2	23.2	51	6.8				51	6.8	
Rapeseed	4.6	62.3	20.2	12				20.2	12	
Sunflower	10.4	19.5	65.7					65.7		
Cottonseed	45.3	17.8	51.5	0.2				51.5	0.2	
Groundnut	11.8	46.2	32					32		
Corn	12.7	24.2	58	0.7				58	0.7	
Linseed	9.4	20.2	12.7	53.3				12.7	53.3	
Animal fats										
Beef tallow	47.5	40.5	3.1	0.6	0.4			3.5	0.6	
Pork lard	38.6	44	10.2	1				10.2	1	
Poultry fat	28.5	43.1	19.5	1				19.5	1	

Adapted from Turchini et al. (2009). HUFA, highly unsaturated fatty acid; PUFA, polyunsaturated fatty acid; LC, long-chain.

these FAs (Glencross and Turchini, 2010). Moreover, absolute FO needs during this phase of the rearing cycle are relatively small and therefore globally irrelevant. The greatest demand for lipids is during the grow-out phase and it is during this phase of the rearing cycle that most savings can be achieved.

During grow-out, replacement of 60% of the FO with VOs (blends of soybean oil, rapeseed oil, linseed oil) in 20–25% lipid diets for gilthead sea bream (Caballero et al., 2004; Izquierdo et al., 2005; Benedito-Palos et al., 2008) or European sea bass (Montero et al., 2005a,b; Mourente and Bell, 2006; Richard et al., 2006) can be done without affecting growth performance. In these diets 8–10% of lipids were still provided by FO and that ensured that EFA requirements were met. Even though it is not mandatory to include FO in salmonid diets, as they do not require LC-PUFAs as EFAs, it is important to note that in most of the partial FO replacement studies performed with salmonids, the diets still included a minimum of 1.6% LC-PUFAs (Turchini et al., 2009).

In gilthead sea bream long-term feeding with VOs did not affect the gross morphology of the intestinal epithelium (Caballero et al., 2003), though hepatic modifications such as steatosis, liver vacuoles and swollen hepatocytes (Caballero et al., 2004) or modifications in plasma lipoprotein (Caballero et al., 2006) were observed. The histological modifications were, however, non-pathological and were all reversible after a finishing period with FO.

Studies in which both FM and FO were replaced by plant feedstuffs in diets for marine fish are scarcer, and more attention should be given to these most extreme alternative aquafeeds. For instance, in Atlantic salmon, diets with only 10% FM and 50% FO replacement by rapeseed oil promoted similar performance compared to the control FM and FO-based diet, and the fillet quality was considered adequate by consumers (Bendiksen et al., 2011). The authors further concluded that FO supplies impose greater limitations on the formulation of salmon feeds than FM supplies. In gilthead sea bream, diets including only 20% FM and up to 66% VOs (rapeseed, linseed and palm oils) replacing FO (22% in the control diet) were shown not to impair fish performance (Benedito-Palos et al., 2007). In the same study, 100% FO replacement by VO affected performance, and this was most certainly due to EFA deficiencies. Complete dietary replacement of FO by VO also resulted in decreased total protease activity and increased lipid droplet accumulation in the posterior intestine enterocytes of gilthead sea bream, and that may further contribute to explaining the lower fish performance (Santigosa et al., 2011). Also in gilthead sea bream, FO replacement by a VO blend did not provoke damage in the intestine epithelium or massive accumulation of lipid droplets in the enterocytes, though signs of lipoid liver disease were found in fish fed a 100% VO diet (Benedito-Palos et al., 2008).

Although FO sparing up to a certain level does not influence marine fish performance, it may affect carcass composition and fillet nutritional value for human consumption (Rosenlund et al., 2010). Fish have great plasticity regarding carcass fatty acid composition, which tends to reflect that of the diet. As VOs have an FA composition different from that of FO, particularly regarding the PUFA profile, and as deposited lipids tend to match dietary lipid sources, fillet composition reflects these differences in FA profiles. For instance, in gilthead sea bream a significant linear correlation between dietary FA level and FA in muscle was established (Benedito-Palos et al., 2011). Also in gilthead sea bream, 72% replacement of dietary FO by soybean oil affected muscle FA composition, and sensory differences between fillets of those fish and those obtained from fish fed the FO-based control diet were detected by panellists (Martinez-Lorenz et al., 2007). In contrast, 60% replacement of FO by linseed or soybean oil also affected fillet FA composition but did not affect instrumental texture analysis (Menoyo et al., 2004). Also, in Atlantic halibut partial replacement of FO by flaxseed oil did not affect colour, texture, odour or flavour of cooked fillets (Alves-Martins et al., 2011). According to the authors, Atlantic halibut selectively retained LC-PUFAs and therefore could adapt to a lower FO supply without adverse effects on performance or fillet nutritional and sensory quality.

Overall, VO blends that better match the FA profile of fish fillets tend to induce fewer modifications in fillet FA composition than single VO sources (Rosenlund et al., 2010). Particular attention should be given to the linoleic acid content of the VO, as it is suggested that the most important factor for using VO blends is to minimize the dietary level of this FA (Tocher et al., 2010). Indeed, a high dietary concentration of linoleic acid may markedly modify fillet composition owing to its incorporation into phospholipids, in addition to storage fats (Tocher et al., 2010). Even a long finishing period with an FO diet may not be enough for flushing deposited linoleic acid and returning its levels in the carcass to basal values.

A finishing period with an FO-finishing diet has been tested as a strategy for ensuring that at the end of the grow-out period fish fed with a VO diet have a nutritional value similar to that of fish fed the whole production cycle with an FO diet (Jobling, 2004a; Roselund et al., 2010). This concept of ‘finishing’ feed for carnivorous fish has been successfully applied to salmonids and several marine fish (Robin et al., 2003; Benedito-Palos et al., 2009; Ballester-Lozano et al., 2011). This allows a predictable management of stocks and feeding practices and guarantees that the nutritional value of the final product meets that required by the market. Nonetheless, differences exist regarding dilution time of specific FAs and this may affect the finishing period strategy. For instance, it was shown in Atlantic salmon, brown trout, and turbot that the neutral lipid fraction is more responsive to the dietary FA than the polar lipid fraction (Robin et al., 2003; Jobling, 2004b). This can be explained by the different functions of these lipid fractions. Whereas neutral lipids have mainly a storage function, polar lipids are main components of cell membranes and are selectively incorporated into phospholipids.

In Atlantic salmon, feeding an FO diet for 20 weeks after feeding a VO-based diet for 50 weeks restored c. 80% of LC-PUFA levels, but (n-6) PUFA levels still remained 50% higher than in fish fed an FO-based diet during the whole period (Bell et al., 2003). In European sea bass and in gilthead sea bream (n-3) PUFAs and docosahexaenoic acid (DHA) recovered to FO values, whereas eicosapentaenoic acid (EPA) was still lower than desired values after 90—98 days of re-feeding an FO-based diet (Izquierdo et al., 2005; Mourente et al., 2005; Mourente and Bell, 2006). Even so, after this flushing period the fish fillets were very well accepted by the panel of judges (Izquierdo et al., 2005). Also in Atlantic cod, after a flushing period with an FO-based diet of fish previously fed soybean oil-rich diets, the fish still showed an altered FA profile (Morkore et al., 2007). Interestingly, although consumers could distinguish the taste of the two fish groups, no particular preference for one group or another was evidenced. In gilthead sea bream, long-term feeding (6 months) of commercial diets low in FM and high in VO (69% FO substitution) showed that soybean oil or rapeseed oil, but not palm oil, did not affect fish performance (Fountoulaki et al., 2009). However, even 4 months of re-feeding with an FO-based finishing diet was not adequate to restore the LC-PUFA profile. Also, although sensory analysis revealed no differences in organoleptic characteristics of the fillets low acceptance scores were obtained for the experimental groups.

Although the dilution model seems to generally apply to changes in FA composition of medium and fat fish (Jobling, 2003), it may not apply to lean fish, as they have a low fillet FA level and most of it comprises polar lipids (Jobling, 2004b). It must also be emphasized that the importance of the flushing/finishing period is particularly relevant in species that contain considerable amounts of fat (fat fish) in their edible portion; otherwise it may be irrelevant regarding human consumption advantages (Rosenlund et al., 2010). For instance, whereas the muscle lipid content of Atlantic salmon is c. 13%, that of cod is only 0.1% and that of pangasius 1.3% (Usydus et al., 2011). This implies that although Atlantic salmon fillets are a good source of lipids, and thus of (n-3) PUFAs, both cod and pangasius are negligible sources of lipids or (n-3) PUFAs. In the mentioned examples, it would be necessary to eat just 26 g of salmon to achieve the daily dose of 1 g EPA + DHA recommended by the American Heart Association, but it would be necessary to eat 2 kg of cod or 4 kg of pangasius to achieve the same values (Usydus et al., 2011).

In addition to aspects related to growth performance and carcass FA profiles, replacement of FO by VO will modify diet FA balance and this may also affect fish health and welfare, immune status, intestinal mucosa morphology and microbiota (Montero and Izquierdo, 2010; Kiron, 2012; Oliva-Teles, 2012). Altogether, it may affect intestine physiology, nutrient utilization and gut-associated immune defence. Indeed, reduction of some non-specific response defences was observed in species such as European sea bass and gilthead sea bream (Montero et al., 2003, 2008; 2010; Mourente et al., 2005, 2007). Nonetheless, in grouper a blend of FO with corn oil (3:1 or 2:1) was shown to enhance non-specific immune responses compared to fish fed an FO diet (Lin and Shiau, 2007). In general, it can also be assumed that high levels of replacement of FO by a single VO will affect immunity parameters more intensely than the replacement of FO by a VO blend (Montero and Izquierdo, 2010).

VOs may also contain phytosterols (Francis et al., 2001), which have cholesterol-lowering properties and thus may also affect an animal's health (Oliva-Teles, 2012). On the other hand, FO may be contaminated with dioxins and it is also a source of persistent organic pollutants in aquaculture fish (Turchini et al., 2009).

Of particular interest, but still poorly studied, is the effect of (n-3:n-6) LC-PUFA ratios in eicosanoid production, inflammatory response and immune function of fish (Montero and Izquierdo, 2010; Torstensen and Tocher, 2010; Kiron, 2012; Oliva-Teles, 2012; Furne et al., 2013). For instance, partial replacement of FO by VO in European sea bass affected non-specific immune response but not prostaglandin production (Mourente et al., 2005). Overall, an adequate balance of n-3:n-6 ratio seems to be the most adequate strategy to ensure fish health, stress resistance and immunological status (Oliva-Teles, 2012). Thus, for instance, feeding a sunflower oil-rich diet to Atlantic salmon may induce cardiovascular disorders, which are attributed to an (n-3:n-6) PUFA imbalance, due to the high levels of (n-6) PUFAs present in sunflower oil (Bell et al., 1991, 1993). Also, in gilthead sea bream and European sea bass, partial replacement of FO by soybean, rapeseed or linseed oil reduced immunocompetence and stress resistance, whereas the use of VO blends did not affect health conditions (Montero et al., 2003; Mourente et al., 2000, 2005). Such effects on immune function are related more to a correct n-3:n-6 balance in fish fed VO blends rather than individual VO sources.

8.4 Conclusions

There is potential for significant sparing of FM and FO in carnivorous fish diets during the grow-out phases without affecting overall fish performance. However, more studies are required evaluating the effects of simultaneous replacement of FM and FO in the diet.

Adequate finishing with 'fishy' diets is the best strategy to guarantee the nutritional and sensory value of fish for consumers, particularly regarding the 'ω-3' recognized value of fish fillets. Studies are still needed to better characterize the best feeding strategies for this finishing period and on the sensorial quality of the final product.

In addition to effects on fish performance and quality traits, aspects related to immunological status, oxidative status, health and disease resistance due to the use of alternative diets are still very scarcely known and should be further considered.

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