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Sustainability, Security and Safety in the Feed-to-Fish Chain: Focus on Toxic Contamination

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Abstract: The paper discusses the issue of feed ingredients in aquaculture as a telling example of implementation of a sustainable food safety strategy, aimed at protecting the health of next generation, under the One Health paradigm. Finfish and fishery products are a main nutrition security component as a valuable source of animal protein, particularly in developing countries. In addition, they are a critical source of essential oligo-nutrients, such as polyunsaturated fatty acids (PUFAs) and iodine. Production and consumption of fish has greatly increased in the last decade, mostly due to the growth of aquaculture. While the demand for aquaculture products continues to increase, there is the need to address consumers' concerns related to the nutritional quality and safety. In fact, both wild and farmed finfish can represent a significant source of exposure to contaminants for the consumer: noticeably, caught and farmed fish have a comparable content of nutrients and contaminants. Aquaculture feeds made of fish meal and fish oil are the main vehicle for transfer of environmental pollutants to farmed fish. The main fish contaminants (e.g., methylmercury, PCBs, PBDE) can bioaccumulate and affect development in humans. Feed ingredients as well fish species have a different liability to contamination depending, e.g., on the lipophilicity of the specific chemicals. Up-to-date risk-benefit assessments show that high intake of fish may lead to an undesirable intake of pollutants which is not sufficiently balanced by the concurrent intake of protective nutrients, such as PUFA. The use of vegetable-based feed ingredients in aquaculture has been explored from the standpoints of economic sustainability and fish productivity to a greater extent than from those of food safety and nutritional value. Available data show that vegetable oils can significantly modulate the lipid profile in fish flesh, depending on the oil and fish species. The use of vegetable ingredients can drastically reduce the accumulation of the main contaminants in fish; likewise the presence of other "unconventional" contaminants (e.g. PAHs) and the nutritional value of fish flesh could deserve more attention in the assessment of novel aquaculture feeds.

Keywords: Aquaculture, Risk-to-Benefit Assessment, Environment, Diet, Nutrition, Toxicology

1. Introduction

1.1. The Health Viewpoint: Key Tools for Sustainability, Security and Safety

The concept of sustainability has seen limited applications in the field of food safety still now. The concept of sustainable food safety [1] deals with the components of today's food safety that can impact on the health and well-being of our progeny's adulthood, including its ability to produce a healthy next generation. Indeed, the new concept of food safety features increasingly guarantee and promote health and wellbeing of such vulnerable groups as the unborn and the child. Food safety itself is a framework integrating the assessment and management of many factors, from the welfare of the living organisms used for food production, the quality of their living environment through to the management of production and distribution processes, and food processing and consumption at household level [1]. Any action aimed at improving the intake of nutrients essential to healthy prenatal and neonatal development and/or at reducing the impact of dietary developmental toxicants in foods is relevant to the sustainable food safety. Accordingly, this strategic public health perspective pivots on the dietary exposure of women of childbearing age, as well as pregnant

and breastfeeding women, especially in the case of high consumers of certain foods. Moreover, nowadays it is recognized that any aspects of sustainability must integrate the One Health (and One Prevention) scenario. Therefore, the health of the future generation is strictly linked to today's environmental quality, today's health and welfare of food-producing animals, today's farming practices, as well as today's food security, i.e., the sufficient access to safe and nutritious food [2]. In particular, the One Health perspective points to toxic contaminations of foods of animal origin as a novel aspect of zoonosis [3]. In this frame, and according to the European strategy for food safety "from farm to fork" [4], the quality and safety of animal feed stuffs is a major crossroad, encompassing the environment from which feed ingredients are derived, farming practices, animal health and food safety [5; 6; 7; 8]. For instance, the One Health paradigm is developed in the opinions of the European Food Safety Authority (EFSA) that recommended the reduction of the maximum legal limits of some nutrients in animal feeds; based on an integrated assessment. Such limits were evaluated to be far above the physiological requirements for animal welfare and productivity, and in the meanwhile as cause of excessive deposition in edible tissues or products, as in the case of iodine [9] and vitamin A [10]. In other instances, unnecessary high maximum authorized levels of trace nutrients could pose a risk for the health of farm workers, exposed to dusts, [11] or for ecosystems exposed to large outputs of animal excreta [12]. The scientific implementation of One Health actions may actually allow an integrated protection of animal welfare, humans and the environment, whereas Sustainable Food Safety actions may actually protect the chances of health of the next generation.

In the ensuing sections we will discuss the issue of feed ingredients in aquaculture as a telling example of implementation of a sustainable food safety strategy under the One Health paradigm.

1.2. Food Fish and Aquaculture

The term "food fish" includes finfishes (mostly teleosts, but also selacians), crustaceans, molluscs, amphibians, freshwater turtles and other aquatic animals (such as sea cucumbers, sea urchins, sea squirts and edible jellyfish) intended for use as human food. An impressive number of aquatic species are cultured worldwide in a variety of farming systems: 530 animal species are registered in FAO statistics, including finfishes (354 species, with 5 hybrids), molluscs (102), crustaceans (59), other aquatic invertebrates (9) and amphibians and reptiles (6) [13]. Albeit it might be justified when taking into account a large sector of water-related economic activities, the term "food fish" is a very sweeping one from the scientific standpoint: it includes organisms with completely different biological and ecological characteristics, which are bred or collected in very different ways and, last but not least, provide greatly diversified food commodities.

Global "food fish" production has grown steadily in the last five decades [13]: supply increased at an average annual rate of 3.2 %, thus outpacing world population growth at 1.6%. World per capita apparent fish consumption increased almost two-fold from the 1960s (approximate average 10 kg) to 2012 (approximate average 19 kg) [13]. A combination of factors, such as population growth, rising incomes and urbanization contribute to this substantial development, supported also by the marked expansion of fish production and by an increased efficiency of distribution channels. China, with a "fish food" consumption of about 35.1 kg in 2010, has been responsible for most of the growth in fish availability, owing to the great development in farmed fish production. In 2010, per capita fish consumption was estimated at 23.3 kg in developed countries, where an important and growing share of fish consumed consists of imports; on the other hand, in developing countries fish consumption is mainly based on locally and seasonally available products, with supply driving the fish chain [13].

Fish and fishery products are also main nutrition security components as a valuable source of animal protein: a portion of 150 g of fish provides about 50–60% of the daily protein requirements for an adult. In 2010, fish accounted for 16.7% of the global population's intake of animal protein and 6.5% of all protein consumed; in particular, fish provided more than 2.9 billion people with almost 20 % of their average per capita intake of animal protein [13]. The role of fish proteins in the diet is usually higher in developing countries, especially in some densely populated countries where total protein intake levels may be low. In fact, despite their relatively lower levels of fish consumption, developing countries have a higher share compared with developed countries. In 2010, fish accounted for about 19.6% of animal protein intake in developing countries; conversely, in developed countries the share of fish in animal protein intake has weakened from 13.9 % in 1989 to 11.8% in 2010 face to an increased consumption of other animal proteins [13]. In addition fish, and finfish in particular, is critical for nutrition security as a source of essential oligo-nutrients (Table 1). In Europe it is the critical dietary source of n-3 long chain polyunsaturated fatty acids (n-3 LCPUFAs) [14; 15]. The mean n-3 LCPUFA content can be remarkably different among common edible fish species, as it varies from 200 mg/100 g (cod and whiting) to 2500 mg/100g (herring and tuna). Among farmed fish, Atlantic salmon provides n-3 LCPUFA in high amounts (1800mg/100g), whereas the most consumed freshwater fish, i.e. carp and trout, have acontent of around 300 and600mg/100g, respectively [15] (Table 2). Saltwater fish is an important source of iodine [15; 16], content varying from 30 to 160 µg/100 g, especially cod (160 µg/100 g), hake and mackerel (110 µg/100 g each) are rich sources. Conversely, the commonly consumed freshwater fish species, such as carp and trout, have a markedly lower content (around 2 - 12 µg/100 g) [15]. Other nutrients for which finfish is an important source include selenium [15; 17], with a mean concentration between 21 µg/100 g (trout) and 75 µg/100 g (tuna) [15], vitamin D [18], the content of which varies in different fish species, from around 0.5 - 2 µg/100 g in carp, hake, mackerel and plaice to around 10 - 18

µg/100 g in trout, anchovies and herring [15], and also vitamin B12 [19], with highest average levels (1.1-1,3

µg/100 g) in pike fillet as well as in herring and sardine [20].

Table 1. *Typical composition of fish species most consumed in the EU [15].*

	Anchovy	Carp	Cod and whiting	Hake	Herring	Mackerel	Plaice	Atlantic Salmon	Tuna	Trout
Energy (kJ)	680	498	320	350	778	350	363	787	3682	447
Proteins (g)	21.1	18.1	17.6	17.4	17.3	17.4	17.6	19.7	23.5	18.7
Fat (g)	6.6	5.1	0.6	1.5	13	1.5	1.7	12.1	7.6	3.5
n-3 LCPUFA (mg)	500	296	238	679	2515	679	403	1817	2523	632
EPA (mg)	210	193	66	236	1720	236	224	728	992	139
DHA (mg)	290	103	172	443	495	443	179	1088	1531	493
Vitamn D (µg)	12.5	0.5	3.2	1.4	18.1	1.4	2.1	6.0	7.5	10.1
Calcium (mg)	99	61.4	21.2	24.8	135.1	24.8	47.7	15.0	21.3	47.1
Iodine (µg)	34.3	1.7	158	110	34.4	110	42.2	30.7	33.4	12.4
Selenium (µg)	35.5	27.7	27.1	25.3	27.9	25.3	31.2	26.6	75.2	20.6
Zinc (mg)	2.2	0.7	0.7	0.3	1.1	0.3	0.5	0.4	0.7	0.6

NB: Values are given in units/100 g edible parts (raw) as mean.

Table 2. *Dietary intakes of n-3 LCPUFA from typical servings of different seafood [15].*

Seafood	Serving size (g)	Mean content of n-3 LCPUFA (g/serving)
dark-meat fish such as mackerel, salmon, sardines, bluefish or swordfish	84 - 140	1,5
canned tuna	84 - 112	0,42
other fish	84 - 140	0,48
shrimps, lobster or scallops as the main fish	98	0,37

The increasing fare of fish consumption is driven by the development of aquaculture. Farming of fish is an ancient art, the earliest known examples dating back to China by 2500 BC. Today, thanks to advances in farming and processing technologies, 47% of all fish for human consumption comes from aquaculture [21]. Indeed the growth of productive catch has almost stopped since the mid 1980s, while between 1970 and 2008 the aquaculture sector has maintained worldwide an average annual growth rate of 8.3% [21]; in 2000–2012 production expanded at an average annual rate of 6.2 % from 32.4 million to 66.6 million tonnes. By maintaining such growth rate, aquaculture could bridge the growing gap between fishery supply and global demand for "fish food" [22; 23].

FAO estimates that, overall, fisheries and aquaculture assure the livelihoods of 10–12 % of the world's population. However, aquaculture development is imbalanced and its distribution is uneven, with Asia accounting for about 88 % of world production by volume. Fifteen main producer countries accounted for 92.7 % of all farmed food fish production in 2012; the large majority are Asian countries (China, India, Vietnam, Indonesia, Bangladesh, Thailand, Myanmar, Philippines, Japan, Republic of Korea), two are Latin American countries (Brazil, Chile), the remaining ones are Egypt, Norway and the U.S.A. [13].

World aquaculture production is divided into inland aquaculture and mariculture. Except some operations in saline water, inland aquaculture generally use freshwater, while mariculture includes production operations in the sea and intertidal zones. Out of 66.6 million tonnes of farmed food fish produced in 2012, farmed crustaceans account for 9.7 % (6.4 million tonnes); mollusc production is more than double (15.2 million tonnes) while its value is only half

compared to crustaceans, because a large part are by-products of freshwater pearl culture in Asia; other species provide only 0.9 million tonnes and are farmed mainly for regional markets in a few countries in Eastern Asia. Finfish make up two-thirds (44.2 million tonnes) of 2012 "food fish" production, including species grown from inland aquaculture (38.6 million tonnes, 87.3%) and mariculture (5.6 million tonnes, 12.7%). Finfish grown from mariculture include a large proportion of carnivorous species, such as salmonids and other sea species, which are higher in unit value than most freshwater-farmed finfish [13].

European aquaculture accounts for about 2% of world production [24; 25] and provides 27% of the total production of aquatic organisms in Europe, compared to 47% at the global level [24]. In the European Union aquaculture has grown only to a value close to 0.5% in the period between 2001 and 2008, compared to 7.6% in non-EU countries [24]. The main European producer is Norway (656,000 tons in 2005), followed by France, Spain, Italy and the UK: these countries represent 75% of European production [26]. In France, Spain and Italy the predominant component is represented by shellfish (mussels, clams and oysters); conversely, 90% of the production in Norway, the leading actor in Europe, is represented by salmon.

Italy has a variety of environmental conditions, spanning from the Alps to the Southern Mediterranean; accordingly, the Italian aquaculture is the mirror of almost all species farmed in Europe, distributed in about a thousand production sites. Among finfish, the freshwater species represent about 68% of the production, with rainbow trout (*Oncorhynchus mykiss*) alone accounting for 55.3 % in 2009; the remainder is given by mariculture of sea bass (*Dicentrarchus labrax*, 13.2%) and sea bream (*Sparus aurata*, 12.9%) as well as by a

number of local "niche" productions, e.g., eel [27]. Interestingly, the sector recorded a positive growth trend, parallel to the expansion of national fish consumption, from 15 kg in the 1980s to the current 22 kg per capita per year, which is still below the European average [21]. Nevertheless, the national fishery and aquaculture product is insufficient: Italy imports two-thirds of its fish consumption [27].

While the demand for aquaculture products continues to increase, there is a growing recognition of the need to address consumers' concerns related to the quality and safety of products. Issues such as food safety, traceability, certification and eco-labels are becoming increasingly important and considered as priority policy issues [13].

1.3. Finfish, Aquaculture and Food Safety

As already mentioned, fish is a source of essential nutrients such as iodine, vitamin D and PUFAs, which play a critical role in many biological processes, such as the growth and development of the nervous system, the maintenance of immune response, cardiovascular system and thyroid function. In the meanwhile, both wild and farmed finfish can represent for the consumer a significant source of exposure to contaminants: persistent halogenated compounds, such as polychlorinated biphenyls (PCB), dioxins, brominated flame retardants, perfluorinated chemicals (perfluorooctane sulfonic acid -PFOS- and perfluorooctanoic acid -PFOA), and organic compounds of chemical elements, such as methylmercury (MeHg) and organotins. The type and level of contaminants in finfish may vary depending on the chemicals capacity to persist, bioaccumulate and biomagnificate along the food chain, the fish species, its lipid content and dietary habits, as well as on the place of catch [14]. In most cases, contaminants are slowly and inefficiently metabolized by fish; however in a few cases, fish metabolism does play an important role, as in the telling case of arsenic (As). Whereas inorganic As is an important carcinogenic contaminant of water and cereals, in fish As is metabolized to organic forms, the main ones being of minimal toxicity (arsenobetaine, arsenocholine) while others (arsenosugars, arsenolipids) are less known but plausibly much less toxic than inorganic As. Thus, fishes may accumulate total As, but less than 10% is inorganic As [28]: accordingly, total As in fish may flag environmental pollution but is of low significance for consumer safety.

Several contaminant groups (PCB, dioxins, and brominated flame retardants) specifically accumulate in the fat fraction of the tissues due to their high lipophilicity. Accordingly, populations that consume greater quantities of fish show higher levels of persistent lipophilic contaminants in serum, breast milk and adipose tissue [29]. Biomagnification along the trophic chain implies that species occupying higher positions in the food pyramid (e.g., salmonids and tuna fish among teleosts) are exposed to the contaminant concentration present in the environment as well as in their preys. The risk to the consumer is related to the long-term exposure: these contaminants are mainly developmental toxicants, thus the most vulnerable groups are represented by women of childbearing age, which may build-up a body burden, as well as pregnant and breastfeeding women, and children [30]. Although generally considered less susceptible than the embryo and foetus, children are considered as an especially vulnerable group of direct consumers, due to their not yet fully mature organism as well as higher exposure than adults because of the increased intake of food per kg of body weight: a specific susceptibility to certain toxicants, like carcinogens or endocrine disrupters (ED) is pointed out up to the pre- and peri-pubertal phase [31; 32]. The risk assessment and management of main finfish pollutants should therefore pay special attention to the protection of the developing organism: indeed, fish pollutants are good examples of the sustainable food safety concept [1].

Since wild fish are exposed to bioaccumulating contaminants through the ecosystem, it has been held that farmed fish would show a lower level of contamination. However, analysis of available data made in 2005 by the EFSA showed no significant differences between the concentrations of both nutrients and contaminants in wild and farmed fish. In particular, the degree of contamination of farmed fish is equal and in some cases greater than the wild species due to the use of feed made from animal ingredients (oil and fishmeal) that are highly liable to contamination and may give rise to bioaccumulation [14]. In feed stuffs for farmed fish, PUFAs have to be supplemented, generally through the use of fish oil. Fish meal is another key ingredient, as it is a source of high-quality protein with adequate proportions of essential amino acids, as well as of essential minerals and also PUFAs present in lipid residues [14]. The typical diet of an omnivorous fish (e.g., sea bream, sea bass, etc.) generally contains 10% fish meal and 2% fish oil, while that for carnivorous fish (e.g., salmonids) 50% fish meal and 25% of fish oil [14].

Currently, small pelagic scombroids (anchovies, herring, mackerel, sardines, etc.) are the main species used for the production of fishmeal and fish oil used in aquaculture. Therefore, the usual aquaculture feeds do mimic the biomagnification process occurring in aquatic ecosystems, and the quality and composition of the feed is a pivotal factor for the presence of contaminants in fish as in all foods of animal origin, hence, for the protection of human health [7; 33]. The contamination of wild fish can be controlled with monitoring programs and, in the long term, with global measures to reduce the release of pollutants into the environment. In farmed fish, contamination levels in animal feed may be managed in the course of the production process or, as a more effective option, can be prevented through the widespread use of new feed ingredients [14] with proven lower liability to bioaccumulation of toxic chemicals.

2. Sustainable Food Safety: the Toxicology of Main Chemical Contaminants of Finfish

Methylmercury. Mercury (Hg) is released from both

natural and anthropogenic sources into the environment: in water bodies the inorganic Hg is methylated by sulphate-reducing bacteria, becoming MeHg, the most toxic organic form, which bioaccumulates in marine organisms and biomagnifies through the food chain [34; 35]. Therefore, fish is by far the main dietary vehicle of MeHg [36].

Concerns about MeHg mainly rely on its developmental neurotoxicity [37; 38]. Experimental and epidemiological studies support that exposure levels occurring in high-intake areas (e.g., New Zealand, Far Oer) are related with deficits in language, attention and memory in the offspring [39; 40; 41; 42; 43]; noticeably a study conducted in the Seychelles indicated that when intake of MeHg occurs through fish high in PUFAs, the developmental neutoxicity is significantly mitigated [44]. In 2012, the EFSA has established a tolerable weekly intake (TWI) of 1.3 μg/kg b.w., based on neurodevelopmental effects after prenatal dietary exposure and estimating the maternal intake and body burden through the concentration of Hg in maternal hair [38]. The consumption of fish contaminated with MeHg may enhance the oxidative stress-related vascular damage in adults, thus enhancing the risk of neurological ischemia and cardiovascular disorders [45; 46; 47; 48]; this aspect was considered by EFSA as potentially important, but the evidence was not sufficiently robust for deriving a health-based guidance value.

Bioaccumulation in fish occurs via binding to tissue proteins. Generally, about 80 - 100 % of total Hg in fish muscle is MeHg, albeit with variations related to age and species [49]. The concentration in fish flesh is not changed by cooking; actually, due to moisture loss, Hg concentrations are often slightly higher in cooked fish. The amount of Hg is related to the age of the fish and the position of the fish species within the food chain; predatory fish and older fish have higher concentrations [49]; specific ecosystem characteristics also contribute to the variability in Hg concentration [50].

Mercury concentrations may exceed 1 mg/kg in shark, swordfish, marlin and tuna. In farmed rainbow trout the ratio of Hg concentrations in feed and in fish is about 1:1; in trouts aged 10–14 months, muscle Hg concentrations were not related to fish weight [51].

Methylmercury has a rather unique place among contaminants, because there are no major dietary sources for all age classes other than fish. In particular tuna, swordfish, cod, whiting and pike were major exposure contributors in adults, including women of child-bearing age; for children, hake was an additional major contributor: most of the above species are not major PUFAs sources. Noticeably, the dietary exposure estimations in high and frequent consumers of finfish are about two-fold higher in comparison to the total population [38]. According to the EFSA estimates, the mean dietary exposure in European Union does not exceed the TWI, with the possible exception of toddlers and other children in some surveys. However, the medians of 95th percentile dietary exposures across surveys are close to or above the TWI for all age groups; high and frequent fish consumers,

which might include pregnant women, may exceed the TWI by up to approximately six-fold. Unborn children constitute the most vulnerable group for developmental effects of MeHg exposure. Biomonitoring data from blood and hair indicate that MeHg exposure is generally below the TWI in Europe, but higher levels are also observed [38].

The most important source of Hg in feed is fishmeal, where it is mainly present as MeHg. A European survey showed the average concentrations of total Hg in complete fish feeds is 0.06 mg/kg and approximately 8% exceeded the maximum allowable level of 0.1 mg/kg [52]. Limited data indicate that the proportion of MeHg to total Hg in aquaculture feeds is consistently over 80% [53; 54; 55].

Dioxins. The term "dioxin" refers to a group of 210 polychlorinated aromatic compounds, including dibenzo-p-dioxins (PCDDs or properly "dioxins", 75 congeners) and dibenzo-p-furans (PCDFs or "furans", 135 congeners): both PCDDs and PCDFs are unwanted by-products from combustion of organic material (waste incineration, metallurgy) and other chemical reactions [56; 57]. Whereas all dioxins are stable in the environment, only 17 congeners, (7 PCDDs and 10 PCDFs), are of particular toxicological concern: the most toxic one is 2,3,7,8-tetrachloro-dibenzo-p-dioxin (TCDD) [58; 59].

Dioxins are poorly water-soluble, but they may be absorbed onto mineral and organic particles and undergo air-borne or water-borne transport far away from the emission sources and enter into the food webs [56]. The bioaccumulation in fish species depends on both biomagnification and the fat content of the organism. Except for some highly polluted areas, in the general human population about 95% of the exposure to dioxins occurs through the diet, in particular fatty foods of animal origin [60].

Dioxins are the first group of chemical contaminants that have been assessed and monitored as a mixture, because a) they occur in the same foods, and b) they share the same mechanism of toxicity, the activation of the intracytoplasmic aryl hydrocarbon receptor (AhR) [61]. The toxic equivalency factor (TEF) of each congener is based on the congener AhR-binding affinity compared to that of 2,3,7,8-TCDD, taken as the value reference unit [62]; the total concentration of dioxins in a matrix is measured as toxic equivalent (TEQ), obtained by summing the products between the TEF and the concentrations of each individual congeners. Noticeably, another group of compounds, different from dioxins, is considered to add up to the TEQ and is monitored accordingly: the 12 coplanar, "dioxin-like" (DL) PCBs, which are structurally similar to 2,3,7,8 TCDD and activate AhR [63].

Studies in animals and humans show that critical effects of dioxins include alterations of immune, reproductive and neurobehavioral development as well as porphyrin accumulation in the liver [64; 65; 66; 67] and a potent tumor promoting action [68]. The Scientific Committee on Food of the European Commission set a cumulative dose TWI of 14 pg/kg b.w. of TEQ (SCF Scientific Committee on Food,

2001).

The highest average levels of dioxins in food are found in fish liver oil and its derivatives and in the muscle of eel, one of the edible fish with the highest fat content. Other finfish show levels well below those of the eel, with relatively higher values in salmon and Baltic herring; for a comparative glance, eel flesh had a median of 6.7 ng TEQ/kg total weight whereas the median in salmon was 8.0 ng TEQ/kg on lipid basis, thus resulting in much lower values on total weight. Leaner species showed much lower levels, e.g., 1.20 ng TEQ/kg on lipid basis in farmed trout flesh [56]. Contrary to MeHg, dioxins are present in many other foods, such as liver and milk, that show levels comparable or slightly lower than salmon. The contribution of fish to the average daily exposure is between 11 and 63%, depending on the eating habits of the different countries. The average values of dioxins in the diet in the European Union are between 8.4 and 21 pg TEQ/kg b.w./week; thus, a substantial proportion of the European population would have an intake above the TWI [69].

Fish oil is the most important source of contamination of farmed fish feed with dioxins and dioxin-like PCBs, followed by fish meals [14]. In Europe, overall 8% of fish feed samples exceeded maximum tolerated levels and a further 4% exceeded action levels set as contamination alerts [56]: the complete feed will comply with the regulatory limit (2.25 ng/kg) if the individual components also comply with their respective limits (fish meal, 1.25 ng/kg; fish oil, 6 ng/kg) [14]. Previous data indicated that feed ingredients of fish origin produced in Europe contained higher levels of PCDDs/Fs and DL-PCBs than those of South Pacific origin and that the contribution of such ingredients to the total body burden of farmed fish was markedly higher for carnivorous species (where it could reach 98%) than omnivorous species [62]. The mean transfer rate of PCDDs/Fs from commercial fish feed into the flesh of rainbow trout increased with the duration of exposure and ranged from 11.1 % at 6 months to 30.7 % at 19 months; there was a direct correlation between concentration in the lipid fraction of feed and that in fish flesh [70]. The feed-tissue transfer rate for DL-PCBs was higher than that of PCDDs/Fs in Atlantic salmon [71, 72] and rainbow trout [73]. Interestingly, dioxins might accumulate also in fish eggs [70], a favoured delicacy for several consumer groups.

Non-dioxin-like polychlorinated biphenyls. Polychlorinated biphenyls (PCBs) are a widespread class of persistent and bioaccumulating chemicals that were widely used for many industrial applications; they include 209 congeners defined according to the number of chlorine atoms and their position. The manufacture and use of PCBs has been prohibited in almost all industrial countries since the late 1980s; however, the combination of widespread use and high environmental persistence make them an excellent example of legacy contaminants, which are still an issue decades after the ban [56]. In addition, PCBs still are released into the environment-feed-food chains from ill-managed hazardous waste sites [74].

PCBs do bioaccumulate because of their high lipophilicity. Besides the small group of DL-PCBs, considered in risk assessment together with dioxins, the bulk of PCBs are the non-dioxin like congeners (NDL-PCBs). Even though they might be considered individually less toxic than the DL congeners, NDL-PCBs are much more numerous, more abundant and include the most persistent congeners.

Experimental and epidemiological data suggest that the critical effects of PCBs in adults and children include liver damage, reduced thyroid function, reproductive dysfunctions in both sexes and tumour promotion (especially in liver), even though potency appears much lower than dioxins [14; 75; 76]. The intrauterine development seems particularly vulnerable, mainly concerning thyroid function and neurological development [77; 78; 79]; children born to mothers habitual consumers of PCBs-contaminated fish from Lake Michigan (USA) had a smaller head circumference [80]. Notwithstanding the persisting importance of PCBs in food safety, unfortunately the available data are considered as inadequate to establish a TWI, even a provisional one.

NDL PCBs may have different toxic modes of actions and effects. Several authors proposed a toxicologically-based classification of PCBs in three groups by introducing a distinction within the group of NDL-PCBs on the basis of structure-activity considerations: besides the DL-PCBs (group II), the "estrogenic" congeners (e.g., PCB 52, 101) make up group I, whereas the "highly persistent, cytochrome-P450 inducing" congeners (e.g., PCB 153, 180) make up group III [81; 82]; another approach identifies three clusters characterized by different patterns of mechanisms (androgen receptor antagonism, transthyretin binding and interference with gap junctions) [83]. The further development of NDL PCBs grouping could lead to the definition of TEF-TEQ approaches for clusters of the main congeners present in feeds and foods. In its turn, this could be highly relevant to assess the toxicological significance of a given PCBs mixture as, indeed, PCBs exposure occurs almost exclusively as a mixture of congeners.

Data on occurrence of NDL-PCBs in food and feed are usually reported as sum of three to seven congeners (PCB 138, 153, 180 and others), referred to as "indicator PCBs" and selected both because of their relatively easy analytical quantification and high presence in food matrices. According to EFSA, the sum of six indicator PCBs represents about 50 % of the total NDL- PCBs in food [14].

Infants, toddlers and other children are the population groups with the highest dietary intake of NDL-PCBs: average daily exposures are estimated in the approximate range of 8-25 ng/kg b.w., with 95th percentile ranging approximately 17-60 ng/kg b.w. [14; 84; 85]. Older age groups are less exposed, with daily average exposures between (approximate values) 4 and 17 ng/kg b.w. per day and a 95th percentile between 8 and 45 ng/kg b.w. [14; 84; 85; 86]. Noticeably, more recent data in the French and German adult population suggest some decline of PCBs dietary exposure with daily average or median at 2.7 or 2.8 ng/kg b.w., respectively, and 95th percentile (France) at 7.9 ng/kg b.w. [87; 88].

In the EFSA survey of NDL PCBs in foods and feeds, the highest mean levels of NDL- PCBs in food (whole weight basis) were observed in fish and fish products, with 223 µg/kg in muscle meat of eel, 148 µg/kg in fish liver and 23 µg/kg in muscle meat of fish other than eel. In many studies, fish was the single food commodity providing the highest contribution to exposure (35.9-65.4%) [56].

The pattern of fish contamination parallels that of dioxins, levels being related to lipid content of tissues, biomagnification and area-specific pollution. Some studies have shown that the pitch of the sea is generally less contaminated with PCBs compared to freshwater fish, further pointing out area-specific pollution problems, e.g. from disposal sites [89]. In feed, the highest mean contamination levels were reported in fish oil (59 µg/kg); among complete feeds, fish feed ranked among the most contaminated with a mean of 10 µg/kg [56].

Polybromineted diphenyl ethers. Brominated flame retardants (BFRs) are anthropogenic chemicals added to many consumer products in order to improve their fire resistance; the PBDEs are the group of greater relevance for the contamination of ecosystems and food chains [90]. PBDEs include 209 possible congeners, whose chemical stability is related to the number of bromine substituents, congeners with four to eight bromine substituents showing the highest stability. PBDEs are going to be drastically restricted in the industrialized world but, due to their persistence and lipophilicity, they will remain a legacy issue for food safety, much like PCBs. Based on the presence and persistence in the environment and in food, EFSA has identified eight congeners of primary interest: however, adequate toxicological data for risk assessment exist only for four congeners (BDE-47, -99, -153 and -209) [90].

The available data indicate adverse effects on the liver, the thyroid and the development of the reproductive and nervous systems as well as increased oxidative stress [91; 92; 93; 94; 95; 96]; impaired thyroid regulation and neurobehavioral development are the critical effects used by EFSA for risk assessment [90]. Whereas the available data are not robust enough to define a TWI, the dose-response curves for critical effects were used in order to estimate, for each of the four congener, an exposure causing a minimal increase in response for the critical effects, according to the Benchmark Dose approach (BMD10) [90].

Overall, fish and other seafood are the major commodities with highest PBDEs level, both wild-caught and farmed species. As for PCBs and dioxins, there is a direct relationship between the levels of PBDEs and the fat content of different species of fish [97]. Especially for BDE-47 and -100, the contamination levels in fish with a fat content higher than 8 %, are almost double than in fish with a fat content between 2 and 8 %, and more than 10 times higher than in fish species with a fat content below 2 % [90]. As expected, among main species used for human food herring and eel show the highest levels [98; 99]. The catch area may also play a role, as shown by high levels in brown trout fillets from Lake Mjøsa, a highly contaminated spot in Norway

[100]. In highly contaminated fish species or populations, the levels of the sum of indicator PBDEs may be over 300-400 µg/kg fresh weight [98; 100].

High-level fish consumers are likely to have an elevated dietary intake, as well as consumers of food supplements such as fish oil capsules or fish liver oil. The estimated daily intakes of the different congeners for average European consumers range (approximate values) from 0.1-1 ng/kg b.w. to 0.7-4.6 ng/kg b.w. as minimum lower bound and maximum upper bound values, respectively. However, small children are estimated to have intakes about 3-6 times higher than adults [90]. In the lack of a TWI, EFSA assessed a margin of exposure between the BMD10 and conservative estimates of dietary intakes of the four congeners: a potential health concern was identified only in reference to the current dietary exposure to BDE-99, especially in small children [90]. At the moment, there is no basis for an approach to PBDEs as a whole, comparable to the TEQ adopted for dioxin-like compounds. However, PBDEs occur in the same foods and apparently have similar mechanisms of toxicity, thus a TEQ approach might be envisaged.

Only limited information exist on fish feed contamination with PBDEs [101]. Dietary accumulation of PBDEs has been investigated in feeding trials with different species (Atlantic salmon, trouts, carp, etc.): a wide range of congener-dependent accumulation was reported, ranging from less than 0.02 to 5.2 % for BDE 209 to more than 90 % for BDE 47 [14]. These data, however limited, lend support to the monitoring and reduction of PBDEs in aquaculture feeds and feed materials, also through the definition and enforcement of (yet unavailable) maximum tolerated levels.

There are other BFRs present in fish, for instance the HBCDDs (hexabromocyclododecanes). However, these compounds, albeit affecting similar endpoints as PBDEs, have relatively low toxicological potency [102; 103; 104]. HBCDDs are lipophilic, and fish are the food commodity most affected by contamination; however, levels measured in fish do not indicate that HBCDDs accumulate to a great extent. Therefore, no concerns for consumer safety have been identified [90].

Perfluoroalkylated substances. Perfluoroalkylated substances (PFASs) are fluorinated compounds with high thermal, chemical and biological inertness. Perfluoroalkylated substances are both hydrophobic and lipophilic, and therefore they do not accumulate in fatty tissues as other persistent halogenated compounds [105]. PFASs have been used since decades in a range of industrial and chemical applications [106]. The wide use of certain PFASs led to their global distribution in the environment and biota: PFOS and PFOA are the most known PFASs, as well as those most investigated for their toxicological properties [107].

Both PFOS and PFOA elicit hepatotoxicity, endocrine disruption, and reproductive and developmental toxicity in laboratory animals [108; 109; 110; 111; 112; 113]. Biomonitoring studies on the adult Italian population show that internal exposure to PFOS and PFOA is widespread,

albeit levels were highly variable and partly dependent also on the characteristics of the living environment [114]. In children from Faroe Islands, a community with high fish consumption, exposure to PFASs was associated with reduced humoral response to immunisations [115]. Taking into account effects on liver, prenatal development and the metabolism of thyroid hormones and cholesterol, EFSA has established a TDI) of 150 ng/kg b.w. for PFOS; PFOA has a similar toxicological pattern but is less potent with a TDI of 1500 ng/kg b.w. [105].

Diet is the main exposure route to PFASs in humans: presence of PFASs in the environment is a major factor driving the entry in the feed-food chains [116; 117]. The PFOS concentrations in foods are almost invariably higher than PFOA, which appears to have a lower accumulation potential. While consumption of game and offal can be important for limited "niches" of consumers, fish and fishery products are the major determinants for the PFOS dietary intake of the general population, contributing 50 to 80 % of total intake, while for PFOA the contribution of fish is 7.6 to 27 %, depending on dietary habits [107]. Positive correlations were reported between PFASs body burden and self-reported fish consumption [118]. Concentrations of PFASs were generally higher in fish caught from fresh water compared to marine water [119; 120], pointing out that the fish living environment is an important factor for these pollutants.

Different from previous estimates [105], the most recent European-wide survey showed no concerns for PFOS dietary intake; the most conservative estimates were always below 10% and 20% of the TDI in adults and toddlers, respectively. As for PFOA, the most conservative figure got a bare 2.1 % of the TDI for toddlers [107]. The relatively high levels of PFOS and PFOA found in some biomonitoring studies [114] are in apparent contrast with dietary intake estimates; such high levels might be due to aggregate exposures (diet plus environment) occurring in specific scenarios and/or to the building-up of a PFASs body burden, whose kinetics has yet to be clarified. Noticeably, the highest PFOS concentrations are recorded in fish liver [105; 107], hinting that some attention could be devoted to fish liver oil.

The information on PFASs in aquaculture feeds is very limited: PFOS is by far the PFAS most frequently found [121]. Considering that PFASs are not lipophilic, fish meal may be the main source. No significant PFASs biomagnification occurred upon a 28-day dietary exposure in rainbow trout, even for PFOS; however, it was noteworthy that skin (a potential edible tissue) was among main deposition sites for PFASs [122].

Organotins. Organotin compounds (OTCs) such as Tributyltin (TBT) and Triphenyltin (TPT) have been widely used and are still used to a lower extent as biocides and pesticides [123]. The main factor involved in OTCs contamination of food chains, and especially seafood, is due to their potential for environmental persistence and bioaccumulation.

Experimental studies identified several effects, with an enhanced susceptibility of the developing organism, such as neurotoxicity, endocrine and reproductive toxicity, tumour promotion and especially immunotoxicity, the critical effect in mammals [124]. Limited data showed that OTCs have essentially similar toxicity and toxicokinetics; thus, EFSA established a group TDI of 0.25 μg/kg b.w./day. After the EFSA opinion, experimental *in vitro* and *in vivo* studies on the main OTC, TBT, have indicated an obesogenic action with increased adipocyte proliferation and differentiation [125; 126]. Although the obesogenic effect has still to be defined in the context of OTC risk assessment, these data support the public health relevance of reducing exposure.

Seafood is by far the main source of OTCs as contaminants of food chains. The OTCs levels in seafood other than finfish (crustaceans and molluscs) are in general higher than those in finfish, possibly because of the greater contact with sediments, that are a critical environmental compart for OTCs pollution. For instance, calculated mean concentration values for TBT in seafood other than fish is 60 μg/kg fresh weight, whereas in finfish the corresponding estimate is 17 μg/kg fresh weight. However, since finfish consumption is on average much higher than other seafood, finfish is the major contributor to OTCs dietary intake, representing 80%-85% and 66-73% when occurrence medians and means are utilized respectively. A conservative estimate calculated that the intakes for high consumers were up to 0.17 microgram/kg b.w./day, i.e. up to approximately 70% of the group TDI. The TDI may be exceeded by the frequent consumption of seafood caught or farmed from highly contaminated area, such as the vicinity of harbors and heavily used shipping routes [124].

Organotin compounds are not usually monitored in feeds; however, the detection of these compounds, and mainly of TBT, in fish feeds suggests that the carry-over of OTC to farmed fish might be an overlooked issue [121].

This review indicates that some contaminants of fish feeds are a recognized issues and regularly monitored (MeHg, dioxins, PCBs); PBDEs are an emerging problem, especially for fish oils; more data on PFASs and OTCs in aquaculture feeds are needed to assess the exposure of farmed fish to these environmental pollutants. Feed ingredients have a different liability to contamination depending, e.g., on the lipophilicity of the specific pollutants. From the standpoint of human risk assessment, the main fish pollutants share remarkable features of concern, such as the ability to make up a body burden that can be transferred to the next generation and the enhanced susceptibility of the developing organism [1].

3. One Health: Issues for Risk-to-Benefit Analysis

In 2010, FAO and WHO convened a Joint Expert Consultation on the Risks and Benefits of Fish Consumption, which considered a restricted panel of major nutrients (PUFAs) and chemical contaminants (MeHg and dioxins) in

a range of fish species. Considering the benefits of docosahexaenoic acid (DHA) versus the risks of MeHg, the consultation concluded that, among women of childbearing age, pregnant women and nursing mothers, fish consumption lowers the risk of suboptimal neurodevelopment in their offspring compared with not eating fish, in most circumstances. Among infants, young children and adolescents, the evidence was insufficient to derive a quantitative framework of health risks and benefits [127]. In 2014, EFSA has dealt with the actual fish intake amount that can be recommended for nutritional purposes, without unduly exposing the consumers to contaminants. EFSA focused on PUFAs and MeHg, since for them both fish is the only significant dietary source, and devoted a specific attention to pregnancy [15].

According to EFSA, the weekly consumption of 3-4 portions of fish in pregnancy may have beneficial effects on the development of nervous system and is definitely recommended compared to the avoidance of fish consumption for fear of MeHg or other contaminants. Considering the mean MeHg levels detected in fish in Europe, the intake associated with up to 4 portions/week would not elicit any significant risk. However, there is no evidence that an intake higher than 4 portions/week would bring any additional benefits. A successive EFSA statement on the benefits of fish consumption compared to the risks of MeHg pointed out that the risk-benefit balance strongly depends on scenarios of seafood consumption, which are highly variable among European countries, in terms of both the total amount and the main species of fish consumed. When the main species consumed have a high MeHg content, only a few numbers of servings (<1–2) can be eaten before reaching the TWI, which may be attained before the desired intake value for PUFAs especially for vulnerable population groups (toddlers, children and women of childbearing age). Better controls on aquaculture feeds may achieve a substantial reduction of MeHg in farmed fish; however, the fish species that contain higher levels of Hg (tuna, swordfish, cod, etc.) are not farmed. Therefore, besides reducing Hg emissions, EFSA recommends issuing recommendations at national/regional levels to increase the intake of fish species with lower MeHg content [128].

It is now recognized that the balance of risks and benefits is not essentially different between wild and farmed fish. Noticeably, farmed fish has a lower mean content of PUFAs per g of weight, as compared to caught fish of the same species. On the other hand, farmed fish is overall more fat, also because it moves less: therefore, it has a higher percentage of the tissue more liable to fat-soluble pollutants as well as containing PUFAs. Consequently, the eaten portions of wild and farmed fish have overall comparable levels of nutrients and contaminants [14].

Producers, food safety operators, and consumers should be aware that finfish are quite diverse and that the physiology and ecology of edible fish species predict the liability to bioaccumulate certain contaminants. As discussed above, predatory fishes (e.g., tuna, sharks, swordfish) are more liable to persistent contaminants such as MeHg, because the biomagnification is related to the place in the food web, whereas fatty fishes (e.g., herring, mackerel, eel) are more liable to fat-soluble halogenated pollutants (PCBs, dioxins, BFRs). Salmon, as a large and fatty fish, indeed is liable to bioaccumulate both kinds of pollutants.

Since not all fish species are exposed in the same way and to the same extent, dietary habits of people do influence exposure. For instance, in New Zealand the exposure to MeHg is higher in the Maoris, traditionally high fish consumers, and in people with lower socio-economic status, who have a frequent consumption of fish'n'chips, made with large cheap fishes like sharks [129]. These simple information may enforce feasible actions for the prevention and control of contaminants, and/or risk communication.

Nutrients and contaminants are concurrently present in fish tissues. Scientific evidence shows that in many cases they can interact, rather than exert independently their beneficial or adverse actions; for instance, several ED interfere with iodine uptake and utilization by the thyroid, thus possibly increasing the physiological requirements for iodine [130]. This may have some relevance for risk-benefit analysis: experimental and epidemiological studies suggest that the concurrent exposure to PUFAs may mitigate the developmental neurotoxicity of MeHg; MeHg intake through PUFAs-rich fish might be of somewhat lower concern [44; 131]. However, when juvenile mice are exposed to levels of persistent lipophyllic pollutants devoid of apparent toxicity by fish-based diets, subtle but clearly adverse effects are observed in the brain, liver, thymus and thyroid [132; 133]. These studies suggest that the intake via fish food matrix could not afford a detectable protection towards main pollutants in the vulnerable direct consumer, the child. In addition, different lipophilic pollutants do have the same toxicological targets in juvenile rodents, albeit chemical structures are diverse and the potency is much different (from very high -as for TCDD- to very low -as for HBCD): therefore, an additive effect should not be ruled out. The scientific evidence consistently indicates that the overall pollution of fish should be further reduced to improve the balance between health benefits and health risks. Under this respect, caught fish could be better controlled, but farmed fish should be produced minimizing the chance of being polluted. The issue of feeds becomes prominent.

4. The Perspective of Novel Vegetable Derived Aquaculture Feeds

In general, fish can uptake and bioaccumulate contaminants from feeds without overt toxicity, unless in the case of very high exposures which are unlikely to occur in the farm; therefore, monitoring of zootechnical parameters, such as growth or reproduction, would not provide meaningful alerts of ongoing contamination in most cases. As there are no established biomarkers of effective dose in farmed fish, the analytical monitoring of contaminants in

feed and fish samples remains the only current control tool in the routine of aquaculture production. Whereas controls are obviously necessary to check the enforcement of good practices and prevention, the sole reliance on controls is not cost-effective; even high-quality analytical monitoring programmes are just a "defensive weapon" and do not indicate any way forward.

Aquaculture feeds are traditionally based on fish meal and fish oils; however, the feed grade fisheries that supply fish meal and oil have reached their sustainability limits. Therefore, if aquaculture production must expand in order to meet global demand for fish, alternative materials must be investigated and introduced. Indeed, aquaculture development is becoming increasingly constrained by increasingly limited supplies of the industrial fish that provide the fish meal and fish oil on which aquaculture feeds are so heavily dependent. However, replacement of significant amounts of the conventional feed ingredients by feed ingredients of vegetable origin could be achieved without loss of growth performance or effects on fish health [134; 135; 136; 137]. Growth, health and reproduction of fish are primarily dependent upon an adequate supply of nutrients both in terms of quantity and quality, irrespective of the culture system in which they are grown. Dietary protein and lipid requirements, and carbohydrate utilization have been relatively well investigated for several fish species, while data on the requirements of micronutrients such as amino acids, fatty acids and minerals are only available for few most commonly farmed carnivorous and omnivorous species. Lipids are primarily included in formulated diet to maximize their protein sparing. The degree of unsaturation does not appreciably affect digestibility or utilization of fats and oils as energy sources for coldwater or warmwater fish [138]. Carnivores like trout have natural diets rich in triglycerides and can easily adapt to high fat feeds; lipid levels as high as 35% have been reported in some salmonid feeds [139]. The maximum lipid levels for other freshwater fish appear to be lower: in general, 10-20% of lipids provide optimal growth rates, without producing an excessively fatty carcass [140]. Carbohydrates are the least expensive form of dietary energy and are frequently used for protein sparing in formulated diets; the ability to utilize carbohydrates varies in different fish species as well as with the complexity or chemical structure of the carbohydrate source [141; 142]. The ability of carnivorous species to hydrolyse or digest complex carbohydrates is limited due to the weak amylolytic activity in their digestive tract; thus, for species such as the trout, starch digestion decreases as far as the proportion of dietary starch increases. For salmonids, carbohydrate digestibility also diminishes with increasing molecular weight [138]. Therefore, any alternative feed ingredient of vegetable origin for salmonids, or other carnivorous farmed species, should preferably have a low content of complex carbohydrates. Conversely, farmed warmwater omnivorous or herbivorous fish species (e.g., common carp, channel catfish, eel) are more tolerant of high dietary carbohydrate levels. In common carp, carbohydrate levels up to about 25% of the diet are an energy source as effective as lipids [143; 144]. Finfish do need the same essential amino acids as most other vertebrates. The requirements for individual amino acids were found to be consistent between coldwater fish (rainbow trout) and warmwater fish (channel catfish) when expressed in absolute terms and not as percentage of the protein content [145]. Fish, like all animals, require essential fatty acids (the PUFAs) for basic cellular functions (e.g., maintenance of cell membranes), but cannot synthesize them. Vegetable oils are rich in linoleic series fatty acids (n-6) but contain little or no linolenic series fatty acids (n-3), which, however, are present in marine oils; highly unsaturated fatty acids, or HUFAs (20: 5n-3, 22: 5n-3, 22: 6n-3), are limited to seafood fish [138; 146]. Indeed, marine fish species (e.g., bream, sea bass, yellowtail, turbot, flounder) require also HUFAs, while freshwater or anadromous species require a greater amount of n-3 and n-6 fatty acids in the form of α-linolenic acid and linoleic acid; in general, the requirements for n-3 or n-6 PUFA correspond to about 1-2% of the diet by dry weight. Differently from marine fish species, freshwater fish are provided with enzymes to desaturate and elongate C18 PUFAs to the longer chain C20 and C22 PUFAs, which are the functionally essential fatty acids in vertebrates. Therefore, the specific PUFAs requirements must be considered when including novel feed ingredients of vegetable origin in a complete feed tailored for a given species. Determination of dietary mineral requirements is made complex by the fish ability to absorb essential elements (e.g., iodine) from the surrounding water in addition to the diet. Therefore, the dietary requirement of a fish species for a particular element depends to a large extent upon the concentration of that element in the water medium [138; 147]. Since even subclinical deficiencies of trace elements (e.g., copper, selenium, zinc) may impair the fish ability to cope with stress or diseases, the development of feed ingredients of vegetable origin should consider other factors beyond requirements for individual elements, such as those reducing the bioavailability by binding elements within the feed matrix (e.g., phytochelatins) [148; 149], by impairing absorption (e.g., phytates) [150; 151], or through unbalanced intake of elements (e.g., excess zinc impairing copper uptake and utilization) [152]. Although the disorders related to vitamin deficiency in fish are well investigated, quantitative dietary vitamin requirements are probably the least studied area in fish nutrition. While natural food is usually rich in vitamins, this may not be the case with formulated, energy-intensive feed. Vitamin deficiency may appear, therefore, mainly in intensive culture systems [147]. For instance, it might be hypothesized that formulated complete feed using only or mainly ingredients of vegetable origin would be low in vitamin D, thus prompting for an increased need of vitamin D supplementation; however, much more robust scientific evidence is needed to assess whether and how the use of feed ingredients of vegetable origin might increase the risk of nutritional problems in intensively farmed fish.

Beyond supporting the zootechnical performance, fish nutrition strategies play critical roles in fish health, especially

concerning immunocompetence and disease resistance within intensive and, to lesser extent, in semi-intensive farming systems. The role of nutrition is further emphasized by the fact that fish depend more heavily on nonspecific defence mechanisms than mammals [153; 154].

Plant oils stand out as the most likely candidates to partly substitute fish oils in fish feeds. Their total global production is around 100 times higher than that of fish oils and a number of studies have shown that they can replace significant parts of the fish oil in diets for salmonids without compromising growth, feed efficiency or reproduction [155]. However, the replacement of fish oil may not be so straightforward, due to its unique content of long chain PUFAs, especially EPA and DHA. Mixtures of vegetable oils have been prepared to simulate the total levels of saturated, monounsaturated and polyunsaturated fatty acids, especially omega-3 found in fish oil; such mixtures are able to replace the fish oil for most of the growth period of several farmed fish [156]. Salmonids such as Atlantic salmon and rainbow trout currently account for over 66% of the total fish oil used in aquaculture. However, salmonids have a lipid metabolism characterized by a 'freshwater' fish pattern in their metabolism of ALA to EPA and DHA. In addition, they are able to store fat at high concentrations in their fillets and have an extremely efficient protein-sparing capability, i.e. a highly efficient lipid utilization capability. These characteristics are peculiar to salmonids and, consequently, from a growth and performance viewpoint, fish oil replacement in these species could be easily and effectively implemented [157].

Fish meal is more difficult to replace, because it has the correct balance of all amino acids required by fish and other interesting features, including the excellent palatability. In general, good quality fishmeals used in aquaculture have protein levels higher than 66% of dry matter. The few vegetable ingredients comparable to fish meal are corn or wheat gluten and concentrated soy protein products: these have a similar amount of protein but with different amino acid profiles from fishmeal. Therefore, none of these ingredients, individually, is able to replace it completely and it is necessary to use a mixture of ingredients to get the optimum amino acid profile [158]. In seabram and sea bass, mixtures of plant proteins can successfully replace a large part (up to above 90%) of fish meal [137; 159; 160; 161].

Serious concerns over pollutants in fish meal and fish oil make aquaculture feeds a food safety issue that requires considerable resources for monitoring and control. The growing attention towards the long-term risks associated with human exposure to contaminants, especially during development [1], has prompted attention towards the use of aquaculture feed ingredients of vegetable origin that could be less liable to bioaccumulation of pollutants [7; 162]. Novel aquaculture feeds were considered mostly from the standpoint of animal nutrition, zootechnical performance and economic advantages; however, a One Health standpoint requires to assess their impact on the safety and nutritional quality of fish food.

The European project AquaMax [163] has represented the most comprehensive research effort toward a strategy to replace fish oil and fish meal in feeds for aquaculture with vegetable ingredients, also considering nutritional and safety issues.

Aqua Max examined a wide range of vegetable ingredients for Atlantic salmon (*Salmo salar*), rainbow trout (*Oncorhynchus mykiss*), sea bream (*Sparus aurata*), common carp (*Cyprinus carpio*) and Indian major carps. In the diets investigated by the project, fish meal and fish oil were still present, but represented a limited portion of complete feeds, in the range of 5-23% and 5-8.4%, respectively: noticeably, the feed for common carp had no fish oil, while the feed for Indian carps was composed of vegetable ingredients only. Main vegetable ingredients in the different formulations included soy, wheat gluten and corn gluten as protein components, and rapeseed oil and linseed oil as lipid components. Rapeseed oil is a potential candidate for fish oil substitution because it has moderate levels of 18:2 (n-6) and 18:3 (n-3), and richness in 18:1 (n-9). In addition, the ratio of 18:3 (n-3)/18:2 (n-6) in rapeseed oil of 1:2 is regarded as beneficial to human health and not detrimental for fish health, provided EPA and DHA are also present from dietary fishmeal [164]. Linseed oil is also a potential candidate for fish oil replacement because it is rich in α-linolenic acid [18:3 (n-3)], it is the substrate for synthesis of (n-3) HUFA, and also contains significant levels of 18:2 (n-6), thus having an 18:3 (n-3)/18:2 (n-6) ratio of 3–4:1. The development of any replacement should also take into account the maintenance of the PUFAs content of fish, as one main nutritional benefit. In fact, the complete substitution of fish oil with either rapeseed oil or palm oil in feeds for Atlantic salmon affected muscle fatty acid composition; the concentrations of 16:0, 18:1 (n-9), 18:2(n-6), total saturated fatty acids and total monoenoic fatty acids increased linearly with increasing dietary palm oil. The concentration of eicosapentaenoic acid (EPA) [20:5 (n-3)] was reduced significantly with increasing levels of dietary palm oil but the concentration of DHA [22:6 (n-3)] was significantly reduced only in fish fed 100% palm oil [165; 166]. When Atlantic salmon was raised on diets with blends of linseed, rapeseed and fish oils, where vegetable oil represented >66% of the added dietary oil, considerable reductions of flesh concentrations of both 20:5 (n-3) and 22:6 (n-3) occurred; however, returning fish previously fed 100% rapeseed or linseed oil to a marine fish oil diet for a finishing period before harvest allowed flesh (n-3) HUFA concentrations to be restored to 80% of salmons fed fish oil throughout the seawater phase, although 18:2 (n-6) remained significantly higher [167]. When soybean, rapeseed or linseed oil, or a mixture of them replaced up to 60% of fish oil in diets for seabream and seabass, the levels of dietary saturated fatty acids in liver were comparable to those in fish fed the fish oil diet; however, in muscle levels were reduced according to that in the diet. Linoleic and linolenic acids accumulated in the liver proportionally to their levels in the diet, suggesting a lower oxidation of these fatty acids in comparison to other 18C fatty acids. The essential fatty acids EPA (20 : 5n − 3), DHA

$(22 : 6n − 3)$ and arachidonic acid $(20 : 4n − 6)$ were reduced in the liver at a similar rate, whereas DHA was preferentially retained in the muscle in comparison with the other fatty acids, denoting a higher oxidation particularly of EPA in the muscle [168]. No detrimental effect on the growth and feed conversion ratio was observed in the rainbow trout, as a result of fish oil substitution with canola and flaxseed oil. However, from the point of view of human nutrition, the reduction of EPA and DHA levels in fish fed the vegetable oil diets could constitute a drawback for plant oils replacement. The content of α-linolenic acid concentrations in the muscle of fingerlings of rainbow trout was lower than that in the vegetable oil diets; probably, a high degree of metabolism of the fatty acid contributed to this effect in fingerlings, through β-oxidation and/or desaturation and elongation [169]. Nevertheless, the available data indicate that the magnitude of PUFA reduction in fish flesh is small, albeit variable, and that the impact on fish nutritional value would be limited. The available studies show that a significant portion (60–75%) of dietary fish oil can be replaced by vegetable oils, preferably by a mixture of them, with just a limited impact on PUFAs content in fish muscle. An improper lipid profile in diet may affect metabolism and endocrine regulation in fish, which in their turn can affect the amount of fatty acids in tissues and their oxidation. Several studies indicate that soybean-based ingredients may promote a healthy lipid metabolism [170]. Notwithstanding some changes in lipid profiles, the novel feeds preserve a large portion of the nutritional value of fish, as far as PUFAs are concerned. A 150 g fillet from salmon fed 80% plant protein and 70% plant oil for 12 months contains 1.4 g of EPA + DHA ; by comparison, the same fillet from a farmed salmon fed with conventional feed provides 1.9 g of DHA plus EPA [14]. Thus, the salmon fed the novel feeds contained 74% DHA+EPA of that fed the conventional, fish-based diet. PUFA have a significant role in protecting health: an average intake of 250 mg/day of EPA plus DHA for healthy adults (as provided by 1–2 servings of oily fish per week) has a protective effect against cardiovascular risk while an additional mean intake of 100-200 mg/day (overall 3-4 servings of oily fish per week) supports the formation of the placenta and the development of the brain and retina in the fetus [14; 21; 171]. The intake during pregnancy, breastfeeding and early infancy positively influences also the growth and cognitive function in childhood [172; 173]. However, further research is needed on the possible modulation, if any, of the content of other relevant nutrients, e.g., iodine.

A most interesting aspect is the impact of novel feeds on the bioaccumulation of contaminants. The replacement of the combined oil and fish meal with vegetable ingredients has led to significant reductions in the fillets of farmed Atlantic salmon of the concentrations of lipophilic pollutants by 51-82%, (dioxins, PBDEs, HBCDs, PCBs and the "legacy" organochlorine pesticides such as DDT metabolites) and of the toxic elements Hg and As by 80-96 %. As already noted, As in fish is usually mainly present as organic compounds of minimal or low toxicity [51]. No speciation was performed to assess whether this pattern of As deposition was maintained by using the novel feeds; nevertheless, the data clearly showed that the feed ingredients of vegetable origin were associated with a much lower overall deposition rate of the most relevant contaminants. For instance, compared to fish fed the conventional fish oil diet, feeding salmon flesh the novel vegetable oil diet for 55 weeks achieved a 4-fold reduction in dioxin-like compounds (PCDDs + PCDFs+ DL-PCBs levels), namely 0.5 ng vs. to 2.0 ng TEQ/kg, and a 6-fold reduction in PBDEs, namely 0.5 ng/kg vs. 3 ng/kg; accordingly, the margin to the current maximum EU level for dioxin-like compounds (8 ng TEQ/kg) increased from 4-fold to 16-fold [174; 175]. On the other hand some shortcomings of vegetable fees deserve attention. The levels of polycyclic aromatic hydrocarbons (PAHs) significant increased compared to conventional feed [176]. Polycyclic aromatic hydrocarbons are moderately persistent and their relevance in foods is mostly due to their role as cooking by-products [177]; PAHs are human carcinogens, and therefore this finding cannot be underestimated. Polycyclic aromatic hydrocarbons are normally not found in Atlantic salmon, but vegetable derivatives can accumulate PAHs from different sources such as atmospheric deposition of contaminated dust and particulate matter in the plants and the processing for oil production [178]. Also, the use of soybean oil may modify the nutritional value of fish by modifying the lipid profile: the long-term intake of farmed Atlantic salmon fed soybean oil in mice increased the levels of linoleic acid in fat, insulin resistance and accumulation of fat in the liver [179].

As a conclusion, with only a modest reduction of PUFA the vegetable feed ingredients may substantially improve the risk-to-benefit balance. However, the different vegetable ingredients should be further investigated for their liability to PAHs contamination and for the potential impact of fatty acid balance on the nutritional value. Preventing PAHs contamination and controlling the lipid profile might be considered among quality criteria of novel aquaculture feeds.

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