aquamax

NEWS

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Sustainable Aquafeeds to Maximise the Health Benefits of Farmed Fish for Consumers

In this issue

Colophon

AQUAMAX aims to replace as much as possible of the fish meal and fish oil currently used in fish feeds with sustainable, alternative feed resources. Fourteen countries with together 32 partners are participating in this project.

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SIXTH FRAMEWORK PROGRAMME

NEWSLETTER FOCUS:

Assessment of the safety of fish farmed on the new feeds developed by the project

Controversy remains regarding the safety of consuming certain types of seafood, particularly during pregnancy. While seafood is rich in vital nutrients it may be an important source of environmental contaminants, such as persistent organic pollutants (POPs) and methylmercury (MeHg). Most POPs are man-made, and these are mostly chemicals either used for consumer products and industrial processes or they may be industrial by-products. POPs are typically fat-soluble and are in many cases dispersed globally and readily accumulated in aquatic food chains. Because of these properties, POPs may be found at elevated levels in oily fish. Mercury is naturally present at low concentrations in the environment as a result of geological activity. However, it can also enter the environment from human activities. Mercury is modified by bacteria to a form known as methyl-mercury (MeHg), which is easily accumulated by organisms and concentrated in predatory animals.

Traditional aquaculture feeds are based on protein and oil extracted from marine fish. These protein and oil ingredients are the main sources of MeHg and POPs, respectively, in aquaculture feeds. This is one of the reasons why a principal aim of the AquaMax project has been to reduce the amounts of marine protein and oil used in aquaculture feeds. Thus, aquaculture as a controlled means of farmed food production can assure the safety of seafood through the choice of secure feed and feed-ingredients.

Although POPs and heavy metals may be present in seafood, fish is an important source of nutrients and there is insufficient knowledge regarding the toxicity of POPs and the interactions between contaminants and nutrients in seafood.

AquaMax has achieved major advances in knowledge in how to drastically and sustainably reduce the levels of POPs in fish feeds so as to maximise the safety of farmed fish.

To assess the safety of fish farmed traditionally and on the new feeds, this project has deployed conventional technology with novel molecular genetic technology to mount detailed transcriptomic and proteomic analyses of the toxicological consequences of known and possible future contaminants in farmed fish.

This ambitious project has been conducted in mammalian model systems in cell cultures and by feeding animals fish produced in AquaMax. It has encompassed not only direct toxic effects of known and putative contaminants in farmed fish but also, possible modulating effects of beneficial nutrients in fish on contaminants in consumers.

The developmental effects after exposure to environmentally relevant levels of the brominated flame retardant, BDE47, during the brain growth spurt, were investigated using two nutritionally different maternal diets.

Early neurobehavioural development, spontaneous behaviour and cerebral gene expression were evaluated in a murine model with regards to effects of low and relevant doses of BDE47. Further, the study aimed to examine whether any differences in effects could be ascribed to the differences in nutrient composition between standard rodent diets and fish-based diets.

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"Stomach contents of BDE47 were significantly lower in offspring of the fish-fed dams compared to casein-fed dams"

Effects of contaminants and nutrients in Atlantic salmon on tissue composition, gene expression and development in mice offspring

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The effects of contaminants and nutrients in fish on mice offspring were studied as part of AquaMax. Seafood intake has been a subject of dilemma for consumers, especially pregnant women, owing to the reported risks and benefits associated with its consumption.

***In vivo* studies have shown that nutrients may ameliorate the negative effects of contaminants. Studies were conducted to examine the interactions between nutrients and contaminants in salmon on mice exposed during gestation and lactation. Persistent organic pollutants POPs polychlorinated biphenyl (PCB153) and polybrominated diphenyl ether (PBDE47) and methylmercury (MeHg) were investigated since these compounds are associated with certain types of seafood.** Oily fish are a rich source of n-3 fatty acids such as decosahexaenoic acid (DHA) which has neuroprotective effects, and which plays an important role during the prenatal development of the central nervous system. **Parallel diets were fed to female mice during gestation and lactation. The difference consisted of the sources of protein and fat, which were standard rodent feed or a feed with a high inclusion of salmon fillet.**

Stomach contents of BDE47 were significantly lower in offspring of the fish-fed dams compared to casein-fed dams. This was also seen in the liver and fat, with the same trend in brain, and indicates that maternal lactational transfer is affected by the dietary composition. However BDE47 had only subtle developmental effects on gene expression in the brain and on behaviour. In the mercury study **supplementation with DHA in the feed reduced MeHg accumulation in brains of mice offspring, and DHA accelerated the development of the grasping reflex in these mice.** Concomitant exposure of MeHg and DHA affected functional groups of genes related to the cytoskeleton suggesting that DHA influences genes involved in maintaining the cellular architecture of the brain, possibly to compensate for the neurotoxic insults of MeHg. **Taken together, the results indicate modulatory effects of dietary composition on accumulation, transcriptomic and neurobehavioural endpoints impacted by environmental contaminants.**

Innovative food safety toxicity tests have been developed by identifying and characterising signature effects of key toxicants in fish

Built on the hypothesis that effects of chemicals on living organism starts with the interaction between a chemical and one or several biomolecules the assumption was made that each such interaction should result in changes in production of proteins and that toxic chemicals with similar mechanism of action would therefore have similar gene expression (mRNA) and protein expression profiles.

In the AquaMax project mice were fed on salmon-based diets spiked with model toxicants. Different toxicants when fed at high doses generated unique mRNA and protein expression profiles.

However, when mice were fed daily salmon, containing environmentally realistic concentrations of single model toxicants, few effects on gene expression remained.

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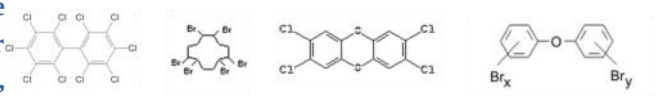
Actions of key toxicants and their amelioration by fish nutrients in developing animals and cultured cells have been elucidated

Technology was used to measure expression of thousands of genes (mRNA) and proteins in brains of mice and cultured mouse brain cells, along with pathological indices on tissues, changes in hormone levels, and neurodevelopment, to elucidate the mode-of-action of toxicants commonly found in fish. These studies focused primarily on MeHg and the emerging group of brominated flame retardants. **It was confirmed that high concentrations of MeHg resulted in oxidative damage in specific brain cells, but other more diverse effects including disturbance of immune protein levels and competition for zinc in proteins appeared to be present. Different brominated flame retardants showed related effects on the brain, with disturbance of calcium and thyroid hormone signaling, unfolding of proteins, and activation of neurodegenerative pathways being prominent.**

At the whole organismal level, even environmentally realistic concentrations of brominated flame retardants resulted in subtle changes in tissues and hormone levels, but this was a worst-case scenario as the mice were eating the contaminated fish every day for four weeks. The brominated flame retardant, BDE47, accumulated in the brains of maternally exposed offspring and accumulation reflected the maternal intake. The accumulation of BDE47 was significantly lower in offspring from dams which had been fed a fish-based feed compared to a casein-based feed. Only a few early reflexes were significantly impaired by exposure to BDE47, and no ameliorating effects of a better nutrition could be seen from behavioural tests. There were also few observed effects on neurodevelopment in mouse pups of dams consuming diets containing MeHg.

Seafood is rich in many nutrients, such as selenium and omega-3 polyunsaturated fatty acids, that may counteract effects of environmental pollutants.

Both selenium and omega-3 fatty acids were shown to protect against MeHg toxicity and that omega-3 also protects against toxic effects in cultured cells from the brominated flame-retardant, HBCD, which is also commonly found in oily fish. Thus, it was concluded that protective effects from nutrients in fish should be taken into account when assessing the risk of consumption of toxicants.



Effects of 4 contaminants (2,3,7,8-TCDD, CB-153, BDE-47 AND HBCD) commonly found in seafood

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Fish is an important food source. However, fish with high fat content tend to accumulate lipophilic contaminants and are therefore a source of human exposure to such compounds. Four contaminants that may be found at elevated concentrations in salmon were studied. These toxicants were a dioxin (2,3,7,8-TCDD), a non-dioxin PCB (CB-153), and two polybrominated flame retardants (BDE-47 and HBCD).

Whereas TCDD has a well-described mechanism of toxicity, acting via the Ah receptor, the toxicities for the other three compounds are less understood. Effects of the four contaminants were investigated in juvenile female balb/c mice fed either of the toxicants for 28 days as a part of a salmon-based diet. Abundance profiles of mRNA and protein were measured in brain along with histological metrics on endocrine glands and reproductive tissues. In addition, transcriptome profiling was carried out on neuroblastoma (N2) cells exposed to BDE-47 or HBCD.

Results showed that all the toxicants induced changes in gene expression and protein abundance in brain. Many of the genes were uniquely expressed in 2,3,7,8-TCDD exposed mice while the non-dioxins showed less distinct profiles from each other. **Histopathological aberrations were observed in thyroid, spleen, thymus and liver, but not in brain, adrenals or uterine tissues. Dietary exposure to BDE-47 and HBCD also showed disturbances in serum thyroid hormone levels. Gene expression in cultured N2A cells exposed to BDE-47 or HBCD revealed bias of regulated genes involved in cell development and differentiation, genes encoding membrane-bound proteins, and genes implicated in cellular metabolic processes.**

"all the toxicants induced changes in gene expression and protein abundance in brain"

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Food safety tests have been conducted in animals fed fish farmed on traditional and new diets

During the course of the project, several genetic, physiological, and morphometric biomarkers have been identified that are symptomatic of exposure to groups of environmental contaminants commonly found in fish. However, in all previous studies exposures have involved addition of single toxicants to a salmon-based diet. In the final study of the AquaMax project feeds have been made from heavily contaminated Atlantic salmon raised on Baltic herring, traditionally farmed salmon, and salmon produced with AquaMax technology and mice have been fed these diets for a period of four weeks. Novel methods of food safety assessment are currently being used to test if consumption of any of these diets on a daily basis for four weeks had detectible effects in mice. **The extensive data on the interaction between nutrients and environmental contaminants in seafood generated by AquaMax will contribute to the European Food Safety Autority's (EFSA) work to formulate scientifically based dietary advice to vulnerable consumer groups, such as pregnant and lactating women and young children, and to develop methodologies for the "risk benefit analysis" of consuming fish, whether wild or farmed.**

The European Food Safety Authority is the keystone of European Union (EU) risk assessment regarding food and feed safety. In close collaboration with national authorities and in open consultation with its stakeholders, EFSA provides independent scientific advice and clear communication on existing and emerging risks.

European Food Safety Authority: Guidance on human health risk benefit assessments of foods

The European Food Safety Authority asked its Scientific Committee to prepare a guidance document for performing risk benefit assessments of food related to human health risks and human health benefits, which was published in February this year for public consultation. As foods provide health benefits but can sometimes also present health risks it is important for decision-makers to be able to take into account the net health impact of different foods.

The guidance document highlights that risk-benefit assessment is a complex process that presents many challenges, such as limited data on benefit assessment.

EFSA's Scientific Committee recommends a three -step approach consisting of: an initial assessment which considers whether a risk-benefit assessment is actually needed or, alternatively, if the health risk clearly outweighs the health benefit (or vice versa); a refined assessment aimed at quantifying estimates of risk and benefit at relevant exposure levels; and finally, a full comparison of the combined risk and benefit to establish a net health impact value.

All stakeholders and interested parties were endorsed to provide their comments by the 15th of April 2010. In line with its policy on openness and transparency, EFSA will publish a summary report of the comments received on its website and will take these comments into consideration before finalising the guidance document.

Moreover, the data generated by AquaMax have been considered in risk-benefit assessments in order to provide guidance to national food safety authorities and the Codex Alimentarius Commission in their work on managing risks taking into account the existing data on the benefits of eating fish.

"The extensive data generated by AquaMax will contribute to the European Food Safety Autority's (EFSA) work to formulate scientifically based dietary advice to vulnerable consumer groups"

Codex Alimentarius

The **Codex Alimentarius** is a collection of internationally recognized standards, codes of practice, guidelines and other recommendations relating to foods, food production and food safety. Its texts are developed and maintained by the Codex Alimentarius Commission, a body that was established in 1963 by the Food and Agriculture Organization of the United Nations (FAO) and the World Health Organization (WHO). The Commission's main aims are stated as being to protect the health of consumers and ensure fair practices in the international food trade. The Codex Alimentarius is recognized by the World Trade Organization as an international reference point for the resolution of disputes concerning food safety and consumer protection.

Abstract from the executive summary of the joint FAO/WHO Expert Consultation on the Risks and Benefits of Fish consumption
ftp://ftp.fao.org/FI/DOCUMENT/risk_consumption/executive_summary.pdf

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Established and emerging pollutants in farmed fish: characterisation of critical effects and their possible modulation by diet in juvenile female mice

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In recent years intake of protein from fish has become increasingly important in several countries. Consequently, there is a need to produce more and more fish protein and since wild fisheries are now strictly limited, this can only be done by aquaculture and in a way that is sustainable. **Fish is recommended as an important component of the diet of all the population groups, including children and elderly people, because it contains a range of beneficial nutrients as well as readily digestible, high quality protein.** However, fish, whether wild or farmed, can be potentially contaminated with persistent chemicals (pollutants) that could pose problems for human health, in particular the health of vulnerable subgroups of consumers such as children and pregnant women.

In the present study new fish feeds developed within AQUAMAX, in order to reduce the presence of chemicals in farmed fish and to improve its quality, were tested in juvenile female rodents. These rodents are a model for the most vulnerable group of consumers (in the peri-pubertal phase of life) to test the impact of different food matrices on the emerging and traditional persistent organic pollutants (dioxin, PCB153, the brominated flame retardants BDE47 and HBCD) that are known to interfere with endocrine homeostasis.

Dose levels, two for each compound, were selected as being compatible with the expected human exposure and also as the lowest dose levels able to induce reproducible effects in rodents. Selected organs were examined to elucidate and assess toxicological effects on neuroendocrine and immune systems in juvenile (prepubertal) animals. Such effects were recorded on thyroid homeostasis (with altered tissue structure and function for all the test compounds), on the reproductive system and in particular for sex steroid (estradiol and testosterone) levels in serum and uterus morphology (which was more affected by flame retardants), on the immune system (where the thymus and spleen were affected by all substances), and the liver (where increased vacuolization occurred, possibly implicating alteration of lipid metabolism, for all substances with different potency).

The presence of alterations at the lowest dose levels tested did not allow definition of a NOAEL (no observable adverse effect level) for most of the substances tested.

A further study was conducted with the brominated flame retardants HBCD and BDE47 at two dose levels under the same experimental conditions as the previous study, using two different diets based on either casein or the fish produced in AQUAMAX as the main protein sources.

This study confirmed the spleen, thyroid and liver as the main target organs for both brominated flame retardants with different patterns of effects according to the diet composition.

It is well known that the content of iodine and selenium (important micronutrients in fish) in the diet can modulate thyroid functions: different concentrations of these micronutrients as well as different protein sources in the two diets can influence both their bioavailability and, therefore, thyroid homeostasis. The casein-based diet appeared to exert immunomodulatory actions so that the effects of brominated flame retardants may be masked. Indeed, the immune tissues of control animals on casein diets lacking the retardants showed changes comparable to those on fish diets .

Data from the present study provide evidence that subtle but biologically meaningful effects were still present at dose levels compatible with high range human exposure. Moreover, the immature female mouse has been established as a useful model in toxicological research on the most vulnerable (worst case) group of consumers.

Toxicological research using an animal modell can contribute to improving the risk assessment process for food safety. Effects recorded in such studies can be used to set tolerable doses, at least for vulnerable sub-group of the population, as well as assessing and evaluating emerging contaminants, such as the brominated flame retardants, in monitoring programmes of feed and food of animal origin.

"Toxicological research using an animal modell can contribute to improving the risk assessment process for food safety. Effects recorded in such studies can be used to set tolerable doses for vulnerable sub-group of the population"

From the second trial reported here it can be deduced that a fish-based diet appeared to be partly protective against the effects of brominated flame retardants mainly on immune system and thyroid. However, the active i.e. potentially protective substances present in fish do not fully prevent the effects of persistent contaminants, at least in vulnerable consumers. Thus, the safe and healthy novel feeds developed in AQUAMAX are a positive and substantial advance for aquaculture.