aquamax

NEWS

ISSUE N° 06
March 2010

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

NEWSLETTER FOCUS:
Sustainable alternatives to fish meal and fish oil to produce fish feeds

OBJECTIVE: to develop feeds based on sustainable alternatives to fish meal and fish oil and thereby produce healthy and minimally contaminated fish that are highly nutritious and acceptable to consumers

Following survey and analyses of a range of potential feed stuffs, both of the content and availability of nutrients and their levels of contaminants, **new fish feeds have been developed with minimal levels of contaminants and with fish meal and fish oil substantially replaced with sustainable, largely vegetable alternatives.**

The new feeds have been tested with Atlantic salmon, rainbow trout, gilthead sea bream and carps in long term growth trials. A large body of data relating to growth performance and feed conversion efficiencies has been collected. In addition, very large numbers of samples have been collected during the growth trials to assess metabolic responses of fish fed the new diets, and the consequences for their health and well being including the performance of their immune and stress response systems, their ability to process contaminants and veterinary drugs, their nutritional quality, their taste and appearance, and their acceptability to the consumer. Such extensive assessment requires a very wide range of conventional and novel chemical, biochemical, molecular and physiological analyses. These include cDNA microarray assays specifically developed in AquaMax for all four species of fish under study.

Such microarray assays are already yielding new insights into gene regulation in lipid and protein metabolism in fish, e.g. the ready response of key genes in fatty acid and sterol metabolism to substituting fish meal and fish oil with vegetable materials in feeds. Such analyses are also being applied to different strains of particular species of fish to identify and select, with promising initial results, strains of a given species with particular nutritional attributes (e.g. with oil-rich or oil-lean flesh) of importance to both producer and consumer.

Nutrigenomics: is the study of effects of foods and food constituents on expression of a large number of genes. This science aims to develop rational means to optimise animal nutrition with respect to the individual animal's genotype. Nutrigenomics has been defined as the application of high-throughput genomic tools in nutrition research. These tools enable literally millions of genetic screening tests to be conducted at a single time, allowing the examination of how nutrients affect the expression of genes.

Microarray analysis techniques: are used in interpreting the data generated from experiments on DNA, RNA, and protein microarrays, which allow researchers to investigate the expression state of a very large number of genes (in many cases, an organism's entire genome) or proteins in a single experiment.

Family-Specific Response in Atlantic Salmon to Replacing Fish Oil with Vegetable Oil in their Diet: Microarray Analysis of the Liver Transcriptome

S. Morais, J. Pratoomyot, J.G. Bell and D.R. Tocher
Institute of Aquaculture, University of Stirling, Stirling Scotland, UK

Vegetable oils (VO) have the potential to replace, at least partly, fish oil (FO) from wild stocks of fish for the production of more sustainable feeds for aquaculture. However, VO are devoid of the long chain omega-3, or n-3 long-chain polyunsaturated, fatty acids (n-3 LC-PUFA) that are abundant in FO. Thus, growth of fish on VO results in lower levels of n-3 LC-PUFA in their flesh, compromising their nutritional value and health-promoting effects to the human consumer. Given the unavoidable necessity of drastic changes in current commercial feeding practices, stemming from increasingly restricted supplies of wild fish, **one potential strategy is to select and use strains of fish with a higher ability to retain and/or biosynthesise n-3 LC-PUFA (C20 and C22) from shorter chain (C18) n-3 PUFA.**

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.

Project Coordinator:
Professor Oyvind Lie
Project Office:
National Institute of Nutrition and Seafood Research (NIFES)
Postal address:
Post-box 2029 Nordnes
5817 Bergen (Norway)
Tel: +47 55 90 51 00

AQUAMAX is an initiative funded by the European Commission within the Sixth Framework Programme, contract N° 016249-2. The text of the AQUAMAX newsletter represents the authors' views and does not necessarily represent a position of the European Commission who will not be liable for the use made of such information.

Subscribe for the newsletter:
www.aquamaxip.eu

SIXTH FRAMEWORK PROGRAMME

aqua max
NEWS
ISSUE N° 06
March 2010

NEWS FOCUS

However, this requires that the trait "n-3 LC-PUFA flesh content" is under genetic influence and, in order to investigate whether this is indeed the case, large-scale studies exploring genotype-nutrient interactions are essential. This is one of the objectives of AquaMax and, in this context; we have performed an experiment investigating the effect of genotype on adaptation of Atlantic salmon to sustainable feeds based on plant products. A feeding trial was performed in which smolts drawn from families predicted as either "fat" or "lean", based on muscle adiposity (selected by Landcatch Natural Selection, Scotland), were grown from 90g to 3kg over 55 weeks using feeds containing 44% plant meals and 25% fish meal, with the oil sources being 100% FO or 100% of a VO blend. We then analysed the relationship between the phenotype "fat deposition in muscle" ("fat" vs. "lean") and gene expression in liver, using the TRAITS/SGP salmon 17k cDNA microarray.

This study revealed that liver gene expression was differentially affected in the "lean" and "fat" families.

In general, the dietary change affected mostly metabolism-related genes, particularly those related to lipid metabolism but also some related to glucose and intermediary metabolism. In addition, genes involved in oxidative stress were affected, indicating potentially lower stress in fish fed VO. Finally, genes related to the immune response were altered, possibly related to the recognised anti-inflammatory effect of FO due to the omega-3 fatty acids.

When comparing families independent of diet, i.e. individuals belonging to the same family fed identical diets, fewer differences in expression were observed in genes involved in metabolism with the majority of altered genes being involved in signalling and gene transcription. However, some important interactions were found between family and diet for several inter-related genes that participate in lipid and lipoprotein metabolism, and the biosynthesis and transport of cholesterol. Moreover, the expression of LC-PUFA-biosynthesis genes (i.e. genes converting C18 to C20 and C22 PUFA) was increased in both families when dietary FO was replaced by VO (due to decreased dietary LC-PUFA), with the magnitude of change being considerably higher in the "lean" family than the "fat" family. This indicates that, for some metabolic pathways, the effects on gene expression when dietary FO was replaced by VO depend on the genetic background.

"This opens up the possibility of identifying strains or families of fish that may be better adapted to utilise sustainable feeds"

Conclusions: different families of salmon showed different changes in gene expression when FO in the diet was replaced by VO; thus different families may potentially have different capacities to "adapt" to such dietary changes. **This opens up the possibility, in the future, of identifying strains or families of fish that may be better adapted to utilise sustainable feeds, including higher levels of dietary substitution of FO by VO; in this way genetic selection may be combined with changes in commercial diet formulations as a strategy to meet worldwide growing demands for aquaculture products, without any loss in fish welfare or the nutritional value of farmed fish.**

Changes induced by a plant based diet compared to a marine based diet in two lines of rainbow trout selected for muscle fat content

Stubhaug I[1]., Corraze G[1]., Panserat S[1]., Guernec G.[2], and Médale F.[1]
[1] INRA, Pôle d'hydrobiologie, NUAGE, Saint-Pee-Sur-Nivelle, France
[2] INRA, Scribe, Rennes, France

Two fish lines of rainbow trout (*Oncorhynchus mykiss*), selected for high and low lipid content in their muscle (flesh), were fed either a diet containing a mix of fish oil/plant oil (70/30) and fish meal/plant protein (30/70), called control diet (CD), or a diet containing only plant raw materials, called plant diet (PD). The feeding experiment lasted for 7 months. **Fish fed PD had significantly lower growth performance compared to fish fed CD, irrespective of the line (fat or lean).** In addition, **fish selected for high lipid content (fat line) had significantly lower growth performance than fish selected for low lipid content (lean line). This reduced growth was mainly due to lower feed efficiency which was significantly affected both by line and diet. The muscle lipid content was higher in the fat line compared to the lean line, but there was no difference between the two diets. Further, the visceral adipose index (VSI) was significantly higher in the lean line compared to the fat line, indicating that the lean line specifically stores lipids in the visceral adipose tissue rather than in the muscle. The VSI in the lean line increased when fish were fed PD.**

A generic rainbow trout cDNA nylon array containing 9216 spots was used to measure gene expression levels in the livers and visceral adipose tissue of the fish. **45 genes in liver were different in given lines due to diet, 81 genes were different between the lean and fat lines and 68 genes were different due to interaction of line and diet.** Within the genes differentially expressed due to diet, 38% were involved in metabolism, followed by 11% involved in cellular processes, 10% in transport, 9% in transcription and 32% in other/unknown functions.

aqua max
NEWS
ISSUE N° 06
March 2010

NEWS FOCUS

In general, genes were up-regulated when fish were fed PD. Genes encoding for delta 6 fatty acid desaturase, fatty acid synthase, acyl-CoA binding protein, and FABP3 were confirmed differently expressed in liver, by using quantitative RT-PCR. Differences between the two lines in liver were much more diverse, with metabolism constituting only 16% of the genes that were differentially expressed.

Conclusions: Results obtained from microarray analyses of visceral adipose tissue in trout showed that **98 genes were differentially expressed between the two diets, while 58 genes were different between the lines and 110 genes were differentially expressed from the interaction between diet and line. The results showed that liver was more strongly affected by diet than visceral adipose tissue.**

Sea bream performance and health

Food safety - Nutrient metabolism, Nutrigenomics and Flesh quality

L. Benedito-Palos[1], J, Nacher-Mestre[2], A. Saera-Vila[1], A. Bermejo-Nogales[1], I. Estensoro[1], R. Serrano[2], JC. Navarro[1], A. Sitjà-Bobadilla[1], J. Pérez-Sánchez[1]
1Institute of Aquaculture Torre la Sal (IATS), CSIC, Castellón, Spain
2 Research Institute for Pesticides and Water, University Jaume I, Castellón, Spain

Short and long studies have been undertaken with gilthead sea bream at the Institute of Aquaculture Torre la Sal (IATS-CSIC). **Data obtained on growth performance and feed conversion efficiency indicate that both fish meal and fish oil can be substantially replaced by plant ingredients.** This is supported by analyses of the growth endocrine cascade mediated by growth hormone and related growth-stimulatory factors, acting in a systemic and/or local-tissue manner. Feeds with reduced fish meal (80% substitution with plant protein sources) and fish oil (95% substitution with plant oils) were developed and used.

The flesh fatty acid profile markedly reflects the composition of the diet. Thus, when fish are fed a vegetable oil diet and then changed to a fish oil finishing diet, the final levels of long-chain omega-3 fatty acids depend on the total intake of fish oil during the production cycle. **This finding offers the possibility of tailoring the fatty acid composition of fish through the manipulation of feeding regimes. Thus, the reliance on marine wild fishery resources is reduced while still ensuring the final nutritional value of farmed sea bream for human consumption.** Further analyses of the lipid composition of different tissues have allowed the establishment of tissue fatty acid profiles or signatures that can be used as biomarkers to fine tune the aforementioned tailoring process.

"This finding offers the possibility of tailoring the fatty acid composition of fish through the manipulation of feeding regimes"

New molecular technologies have also been developed and used to assess the performance of sea bream fed AquaMax diets. Thus, a sea bream database (www.sigenae.org/iats/) with more than 40,000 sequences has been constructed to assess the expression profile of 7500 unique genes, using a sensitive and reproducible customized oligo-array (Agilent Technologies). This microarray has been validated for randomly selected genes by quantitative real time PCR with excellent correlations, having a slope near to equality. This molecular tool has proved to be very useful for the transcriptomic analysis of the adipose tissue, showing that this tissue is a good marker of nutritional stress. Thus, the progressive replacement of fish oil with vegetable oils provokes strong changes in several pathways and processes (cell differentiation and proliferation, inflammatory response, hypoxia and cold responses), which operate to preserve adipose tissue mass. It has also been established that most estrogenic markers are down-regulated in the livers of fish fed

AquaMax diets, although others are up-regulated indicating the presence of increased levels of phytosterols. At the same time, a large variety of adaptive and counter-regulatory processes, including changes in hepatic lipid uptake and deposition, cholesterol metabolism, phospholipid turnover, biliary excretion and mitochondrial respiration uncoupling, have been considered in an attempt to better understand the ultimate consequences of the combined dietary fish meal and fish oil replacement.

Effects of AquaMax feeds on the oxidative state and innate immunity have also been analyzed Investigations of the mechanisms and processes involved in the different disease outcomes after an intestinal parasite challenge are now underway. Special attention is being focused on multivariate correlation analysis using conventional and customized microarray approaches to determine how the nutritional history can affect the different susceptibility of fish to develop infective diseases. No apparent histopathological damage has been observed in the intestines of fish fed vegetable oil diets; further studies are underway to better characterize other changes in the gut barrier or the leukocyte population, involved in defence against pathogens.

Food safety and quality of fish fed new AquaMax diets have also been considered: a) flesh samples have been subjected to electronic nose analyses, b) persistent organic pollutants in feed ingredients and fillets have been analyzed. **DDTs, hexachlorobenzene, polychlorinated biphenyls, polybrominated biphenyls diethyl ethers and polycyclic aromatic hydrocarbons have been detected only at trace levels in the feeds and market fish fed AquaMax diets show only trace or low load-charges** (i.e organochlorine compounds < 0.1 ng/g) of pollutants. **This assures the production of sustainable and safe sea food products in a scenario of global change with increased pressure to assure human health, together with assuring animal health and welfare in production processes.**

aqua max NEWS
ISSUE N° 06
March 2010

NEWS FOCUS

Comparative utilisation of drugs by gilthead sea bream fed fish oil or vegetable oil based diets

G. Rigos, M. Henry, E. Cotou, E. Papoutsi, V. Zonaras, X. Nikoloudaki, D.Nikolopoulou, I. Nengas, M. Alexis

The effects of dietary fat composition (a mixture of fish, rapeseed, linseed and palm oils *vs* fish oil-based diets) on the availability of selected drugs (oxolinic acid, OA; flumequine, FLU, oxytetracycline, OTC) were investigated in gilthead sea bream (170-300g). The dosage schedules were 30 and 75 mg /kg fish of OA or FLU and OTC, respectively (for 5-7 days). Medicated feeds were prepared by mixing and were orally delivered. Experiments were carried out either at 24-26^{0}C (high temperatures for OA and FLU) or 12-14^{0}C (low temperatures for FLU, OTC). A double dosing schedule was also used for OA to determine the effect of dosage on drug uptake and elimination kinetics.

Plasma concentrations of OA were below150 ng/ml even when the fish received high drug doses. The patterns of elimination were similar between the two groups.

With regard to FLU**,** plasma levels were affected by rearing water temperature, being higher at low water temperature than at high water temperature. There was a rapid decline of FLU concentration after treatment completion, which was similar in both groups.

The uptake of OTC was slightly higher in sea bream fed plant oil compared to fish oil-fed group. Plasma OTC levels in both groups remained high even after the completion of the treatment indicating a slow kinetic profile of OTC. Muscle OTC concentrations reflected plasma levels with the plant oil fed group having the highest levels one day post-treatment Removal of OTC from the muscle was slow in both groups, but reached safe levels (below MRL of 0.1 μg/ml) after about 300h.

Concluding remarks

Both groups of fish exhibited relative low levels of both OA and FLU. Dose level had little effect on plasma OA concentrations and OTC had slower absorption and elimination rates compared to the other drugs tested. Differences between plant oil-fed and fish oil-fed gilthead sea bream were observed only with OTC where tissue levels were slightly higher in both plasma and muscle in the former group. There were no distinct differences in patterns of drug clearance between fish fed the different fat sources. Plasma concentrations were higher at low temperatures possibly due to slower metabolic rate/ gastric emptying rate and thus elimination of the drugs.

G. Rigos, H. Morgan, E. Cotou, E. Papoutsi, V. Zonaras, X. Nikoloudaki, D. Nikolopoulou, I. Nengas and M. Alexis, 2009 The effect of diet composition (plant vs fish oil-based diets) on plasma concentration of various antibacterials in gilthead sea bream. 14th International Conference on Diseases of Fish and Shellfish, Prague, September 14-19th.

Consumer acceptance

Sea bream fed the AquaMax diet has been produced under commercial conditions by a private partner SELONDA and distributed through a retailer (Carrefour) outlet in Marousi, Greece. Prior to dispatch, the fish were tagged using barcodes containing traceability data. These tags were used by consumers at the info kiosk in the outlet to retrieve information on the origin of the fish and the conditions under which it was farmed. Consumer feedback after consumption was collected through questionnaires and through telephone interviews.

Some preliminary results of the survey are:

87% of the consumers thought that farmed fish is fresher than wild fish

95% of the consumers thought that AquaMax sea bream tasted better than usual.

These results reflect what has already been found for AquaMax salmon and trout, that is relatively high indices of satisfaction and consumer acceptance.

"95% of the consumers thought that AquaMax bream tasted better than usual"

aqua max NEWS
ISSUE N° 06
March 2010

NEWS FOCUS

Fish In : Fish Out Ratio
An assessment of environmental burdens

Sachi Kaushik
UMR 1067, Nutrition, Aquaculture and Genomics Unit, INRA, 64310 St-Pee sur Nivelle, France

Consequent to world fisheries stagnation and decline, there has been growing concern over the sustainability of wild fish to produce farmed **piscivorous** finfish species and shrimp. One of the most debated issues has been the use of FM and FO in the production of farmed salmon. Fish-In : Fish-Out ratios (FIFO) have been developed to calculate how much wild fish is needed to produce 1 kg of farmed fish.

Based on yields and the proportions of FM and FO in the feeds for farming finfish and shrimp, a "Fish-in : Fish-out (FIFO") ratio can be calculated as follows: (*equation1*)

$$FIFO = \left[\frac{Level\ of\ FM\ in\ the\ feed}{Yield\ of\ FM\ from\ wild\ fish} + \frac{Level\ of\ FO\ in\ the\ feed}{Yield\ of\ FO\ from\ wild\ fish} \right] x\ FGR$$

"FIFO: how much wild fish is needed to produce 1 kg of farmed fish"

Table. Results from the "AquaMax" project showing a significant reduction in FM and FO usage, in "FIFO" ratio calculated applying *equation1*:

FISH-IN FISH-OUT "FIFO" RATIO

Pre-Aquamax

	Feed composition, g/ kg			Wild Fish required, kg			
	FM	FO	FGR	FM	FO	FIFO	FM left, kg
Salmon	350	250	1,2	1750	7500	7,5	1450
Trout	300	200	1,2	1500	6000	6,0	1140
Seabream	400	150	1,2	2000	4500	4,5	680
Carp	200	50	1,2	1000	1500	1,5	160

Aquamax Targets

	Feed composition, g/ kg			Wild Fish required, kg			
	FM	FO	FGR	FM	FO	FIFO	FM left, kg
Salmon	160	120	1,2	800	3600	3,6	704
Trout	50	50	1,2	250	1500	1,5	310
Seabream	150	100	1,2	750	3000	3,0	570
Carp	0	0	1,2	0	0	0,0	0

These data show that we can now include even more plant by products than marine fishery by products in the feeds of such species. From a sustainability perspective, this is intuitively positive, since it decreases the aquaculture sector's demand for wild fish resources.

So rather than relying on ratios such as FIFO, a more comprehensive approach is to use indicators revealing input:output ratios of nutrients /energy /proteins etc. This will guide the aquaculture industry towards a more efficient and ethical production.

References:
Kaushik, S.J., Troell, M., 2010. Taking the Fish-In Fish-out ratio a step further. Aquaculture Europe, 35 (1), 15-17.

aqua max NEWS
ISSUE N° 06
March 2010

NEWS FOCUS

Life Cycle Analysis
Environmental impact assessment: ingredients, feeds and farm

Joël AUBIN, Joachim BOISSY, Abdeljalil DRISSI
INRA - UMR SAS, 65 rue de St Brieuc, CS 84215, F-35 042 Rennes cedex, France
aubin@rennes.inra.fr

One of the objectives of AquaMax is to propose sustainable fish feeds by replacing fish oil and meal by vegetable sources of lipids and proteins. To assess the fulfilment of this objective, an environmental assessment using Life Cycle Assessment (LCA) methodology is being conducted on the ingredients used in fish feeds, on the feeds themselves, and on the fish production at the fish farm.

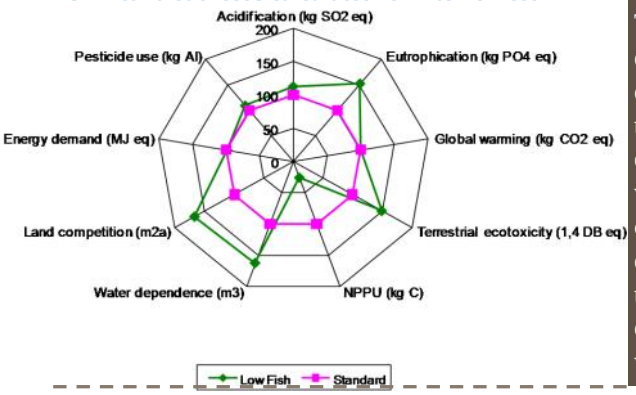
LCA is a technique for assessing the environmental aspects and potential impacts throughout the life of a product or service, from raw material extraction through to production, use and disposal.

Around 50 different feed ingredients have been described using LCA, based on a precise inventory of physical inputs and pollutants associated with their production and transformation. These inventories concern all ingredients used, both of terrestrial and of marine origin coming from different parts of the world.

Using these data and the description of the feed production process, we have been able to conduct an environmental assessment of the different diets used. **The LCA method has been applied to compare two trout diets, containing the same quantity of protein and energy, but differing in their fish meal content: 24% in the standard diet, 5% in the AquaMax diet.**

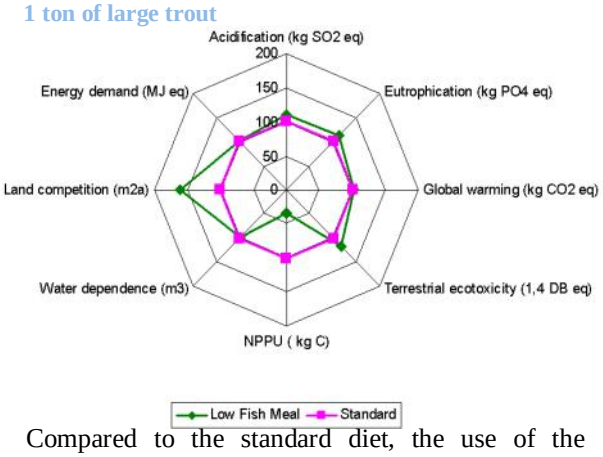
As expected, the Net Primary Production Use (kg C) reflecting the pressure on biotic resources decreases dramatically in the AquaMax diet (-73.5%). The energy demand (MJ) and the global warming potential (kg CO2-eq) remain equal between the diets, despite their different ingredient profiles. An increased level of inclusion of plant by-products leads to increases in: land competition (m2), by 67%; water requirements, by 64% (due to the processing of high protein extracts-wheat and corn glutens); eutrophication potential (kg PO4-eq), by 53%; and terrestrial ecotoxicity (kg 1.4 DB-eq), by 50% (due to intensive agriculture products such as rapeseed oil).

Relative environmental impacts of standard and low fish meal trout feeds calculated for 1 ton of feed



As a next step, animal production data (growth, survival rate, feed conversion ratio) were gathered from a commercial farm of one of the SME partner (Viviers de France) during a production of large trout (3kg) receiving these two feeds. These data were used to calculate the farm-level environmental impact of the production of one ton of fish, taking into account the feeds and the other production factors (farm energy use, liquid oxygen, fingerling production, equipment and infrastructure).

Relative environmental impacts of fish farms using standard and low fish meal trout feeds calculated for 1 ton of large trout



Compared to the standard diet, the use of the AquaMax diet for the production of one ton of trout induces a reduction of the Net Primary Production Use by 64.5%. The energy demand and the global warming potential remain equal. The difference in land competition is still high (60%) as feed production is the major contributor to this impact category. For eutrophication potential and terrestrial ecotoxicity, the difference between the two diets is a lot less (12% and 18% respectively). This can be explained by the influence of other production factors, such as nitrogen and phosphorus emission at the farm outlet, which are more important than the feed effects. Since the river water entering the farm has been taken into account in the calculation, the water demand is no longer influenced by the feed. These two water uses need to be separated in further studies.

Conclusion:
Trout feed environmental assessment shows that, even if we can expect a good saving effect in terms of Net Primary Production Use (and no effect in terms of energy requirement and greenhouse gas emissions), the replacement of fish meal by vegetable sources can induce an increase in land competition as well as impacts such as eutrophication and terrestrial ecotoxicity. This has to be taken into account in the feed formulation in order to minimise these environmental effects, as well as water consumption.

"LCA is a technique for assessing the environmental aspects and potential impacts throughout the life of a product or service "