

Cobia feed diversification

Douglas Tocher and Xiaozhong Zheng



Dr Xiaozhong Zheng, Dr Douglas Tocher, Professor Zhaokun Ding and Professor Youqing Xu enjoying a moment's relaxation during a recent programme in Nanning

The cobia is an exciting new species currently being developed for aquaculture in several parts of the world including China. It is a warm-water marine fish with a very high growth rate, growing from egg to 6kg in one year, a rate around 3-fold higher than in Atlantic salmon. However, this development is taking place against a background of diminishing global marine resources dictating that the traditional fat source in aquaculture diets, fish oil (FO), be replaced with sustainable alternatives, namely vegetable oils (VO). A major problem though, is that VO lack the long-chain omega-3 polyunsaturated fatty acids found in FO that are essential nutrients for marine fish and highly-prized in human nutrition. Thus, feeding VO may impact on growth rates, fish health and the nutritional value of the final product for the human consumer. Consequently, a team of researchers at IOA, led by Dr Douglas Tocher, are investigating fatty acid metabolism in cobia focussing on the pathways of long-

chain omega-3 synthesis. Key to this work is collaboration with the research team of Professor Zhaokun Ding at Guangxi University, Nanning City, PR China, initiated by the cobia project leader in Stirling, Dr Xiaozhong Zheng, who obtained funding from the Royal Society of Edinburgh (RSE) in the form of an RSE-NNSFC (National Natural Science Foundation of China) Joint Project Grant. This scheme aims to develop and promote research links between Scotland and China. Drs Tocher and Zheng travelled to China in 2007 and trained Prof Ding and his team of post-docs and PhD students in the methods of RNA extraction, purification, and PCR gene cloning. Back in Stirling, Dr Zheng was supported by Drs Oscar Monroig and Sofia Morais, and continued to direct the work in China via e-mail and telephone. As a result of these joint efforts, fatty acyl desaturase and elongase gene cDNAs have been successfully cloned and will be further studied to determine their function and tissue expression in cobia during the reciprocal visit of Prof Ding to Stirling later this year.

While in China, Dr Tocher (with Xiao translating) and Dr Zheng both gave presentations on the application of molecular technologies to the study of lipid and fatty acid metabolism in fish at Guangxi University and also at the Chinese Ministry of Agriculture Feed Industry Centre at the China Agricultural University in Beijing on the return journey. The Feed Industry Centre was a well-funded and equipped facility having

a range of departments, and analytical and research portfolios reflecting our own at the Institute of Aquaculture including external facilities such as an experimental farm, albeit their target species was the pig! However, the Centre also has a pilot-scale feed plant and so the possibility of future collaboration in developing sustainable fish diets was discussed. China is, of course, the world's largest producer of aquaculture products albeit largely through extensive culture. However, intensification of fish farming activities in China must be achieved using sustainable feeds, which will necessitate the use of plant meals and VOs. The present work and the existing and, hopefully, future collaborations with research groups in China will contribute to this aim and help to maintain the pre-eminent and global role of the Institute of Aquaculture, University of Stirling as leading providers of research and teaching activities in aquaculture. Dr Zheng's unique knowledge of both UK and, especially, Chinese systems and customs will be an invaluable asset in this goal.

This research is co-funded by the Royal Society of Edinburgh and the National Natural Science Foundation of China to develop and promote research partnerships between Scotland and China. Our Chinese partner is Professor Zhaokun Ding at Guangxi University, Nanning, Guangxi Province, PR China.

AquaMax Update

Following on from the introductory article in the last edition of *Aquaculture News*, Gordon Bell gives us a further insight into the project which is looking at alternatives to fish meal and fish oil in fish diets.

AquaMax starts from the premise that fish play a unique role in human nutrition and well being, not only for the well known beneficial effects of their long chain omega 3

fatty acids, but also because they are a source of high quality readily digested protein, vitamins including especially vitamins A, D and E, and minerals including selenium and iodine. Fish are quite simply a unique and high quality human food. The specific objectives of the project are being pursued in four separate but inter-related Programmes, each comprised of a series of defined Work Packages (WPs), as follows.

Programme 1, the bed rock of AquaMax, is directed by Professor Sachi Kaushik of the National Institute for Food Research (INRA) at

its laboratories in St Pee-sur-Nivelle in France. The Programme, consisting of 13 individual WPs and 23 participants (detailed on www.aquamaxip.eu), seeks to develop feeds based on sustainable alternatives to fish meal and fish oil to produce healthy and minimally contaminated fish that are highly nutritious and acceptable to consumers.

The Nutrition Group at Stirling is involved in 9 of these 13 WPs and includes the following; The first involves a 55 week ongrowing trial, the "Lean/Fat trial", and uses strains of salmon smolts from Landcatch Natural Selection

that are selected as being either lean or fat in terms of flesh adiposity. These are compared to a Marine Harvest strain used in previous studies. The three strains have been fed diets with a 50% replacement of fish meal with plant proteins and the oil component provided either by fish oil (FO) or a blend of vegetable oils (VO) comprising rapeseed, palm and Camelina oil. The final sample was collected in June 2007 when fish had attained a weight of ~3kg. Thereafter, a sub-group of fish from each pen and treatment will be fed a diet containing FO but in this case the FO was "cleaned" to remove almost all organic contaminants. This finishing diet will be used to monitor restoration of n-3 highly unsaturated fatty acid (HUFA), EPA and DHA, in the fish flesh and at the same time produce a fish that is low in organic contaminants.

Aquamax is also investigating whether flesh n-3HUFA content is a heritable trait in salmon, as selection strategies would require the trait to have a phenotypic and quantitative genetic basis. Initially, the ability of different salmon families to retain high levels of n-3HUFA in flesh when fed diets with low n-3HUFA has been studied. Analysis of 50 families showed flesh lipid contents ranged from 3.5 to 6.5% of wet weight, with n-3HUFA contents ranging from 9.5 to 15.6 % of total fatty acids or 428 to 663 mg/100g flesh. There were clear correlations between lipid content and n-3HUFA content, which showed that only families with similar lipid contents can be compared. Four candidate family pairs with identical flesh lipid contents but significantly different relative n-3HUFA contents were identified. This study provides the first evidence that it may be possible to identify salmon families with differences in the ability to retain n-3HUFA in the flesh when fed diets with low n-3HUFA.

Programme 2 is directed by Professor Philip Calder of the University of Southampton in the UK and consists of 2 separate WPs and 8 participants (see www.aquamaxip.eu). The Programme aims to assess health benefits of fish produced on new feeds, focussing on incidences of atopic disease in new born infants.

Atopic diseases, e.g. asthma, hay fever, dermatitis and allergies, have increased dramatically in the last few decades. Such a rapid increase cannot be due to genetic causes alone but must involve changing environmental factors amongst which diet has been strongly implicated. There is a substantial body of evidence pointing to a dietary deficiency of long chain omega 3 fatty acids during pregnancy being a significant factor in infants developing atopic disorders. Thus far it has not been possible to test this experimentally because of ethical constraints in feeding fish contaminated with even low levels of pollutants to pregnant women. This constraint has now been removed by AquaMax research generating farmed salmon rich in long chain omega 3 fatty acids

but with minimal if not negligible levels of pollutants. A major dietary intervention study has been set up at the University of Southampton Medical School in the UK in which salmon farmed on diets developed in AquaMax are being fed to pregnant women. These analyses are also being conducted in specialist laboratories in Norway, France, Sweden and Spain.

It is notable that this study has been subjected to rigorous ethical reviews by independent authorities in the UK, by EU – appointed authorities during the review of AquaMax by the Commission, and by a committee of independent ethical authorities which advises the AquaMax Management Board. Standard operating procedures for this major dietary intervention study and its ethical evaluation comprise a report to the Commission of some 450 pages.

The second WP in Programme 2 is a study by the Chinese participant in AquaMax, the National Institute of Nutrition and Food Safety in Beijing. This study has been developed from the recently completed (though not yet published) first national dietary survey of China. Data from the survey identified three areas of China: first, an area where consumption of marine fish was common; second, an area where consumption of freshwater fish was common; third, an area where fish consumption was negligible. The AquaMax study plans to investigate the food intake and dietary status of pregnant women in the three areas, the dietary status of their new born infants and the incidences of atopic diseases in the infants. Essentially the same analytical procedures and standard operating procedures as developed for the intervention study in Southampton will be applied to the study in China.

Programme 3 is directed by Professor Anne-Katrine Lundbye-Haldorsen of the National Institute for Seafood Research and Nutrition, Bergen, Norway and consists of 3 WPs and 3 participants. The Programme is concerned with assessing the safety of fish farmed on the new feeds.

Thus far, diets based on salmon farmed on the new diets developed in AquaMax have been produced and confirmed as being suitable for feeding to mice in laboratory experiments.

The diets will be spiked with graded amounts of various known and potential marine pollutants prior

to being fed to the mice. A key objective of the Programme is to develop a modern toxicogenomic approach to assessing potential deleterious effects of contaminants in mice. This has necessitated the development of DNA microchips capable of detecting a very wide range of effects elicited by known and potential pollutants and toxicants, and whether such effects are expressed in alterations in the production of messenger RNAs or proteins. Alterations in protein production elicited by pollutants and toxicants are being determined by proteomics techniques, including 2D gel electrophoresis and mass spectrometry of proteins on MALDI- and SELDI-TOF platforms. The feeding studies in mice are being supported by studies in cultured mouse cell models, focussing on neural cells, to enable an innovative feature of toxicogenomics to be investigated, namely the extent to which potentially beneficial nutrients in fish, including long chain omega 3 fatty acids and antioxidant vitamins, can counterbalance the potentially deleterious effects of pollutants and toxicants. This Programme aims to provide objective scientific data to enable the development of an objective risk – benefit analysis of consuming fish with varying levels of pollutants.

Programme 4 of AquaMax is directed by Professor Ragnar Lofstedt of King's College in the University of London, UK. It consists of 3 WPs and 3 participants, and is concerned with assessing public perceptions regarding farmed fish and devising a framework to communicate the risks and benefits of consuming farmed fish to the public and other stakeholders.

Finally, fish farmed on the new diets developed in AquaMax will be marketed through conventional retail outlets and consumer response evaluated by a series of questionnaires and IT based procedures. To this end a call will shortly be released by the Commission to invite a major supermarket concern to participate in AquaMax and to test its farmed fish products in various European countries.



The author with two prize salmon