The assessment of omega 3 oil sources for use in aquaculture – alternatives to the unsustainable harvest of wild fish stocks

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DECLARATION

This thesis contains no material which has been accepted for a degree or diploma by any tertiary institution. To the best of my knowledge the thesis does not contain any material written or published by another person, expect where due reference is made.

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ABSTRACT

Worldwide harvest of wild marine fisheries for fish oil cannot increase. However, the demand for fish oil is increasing due to a rapidly expanding aquaculture industry and is further increased by nutraceutical/biomedical and agricultural companies. Aquaculture uses fish oil as a source for essential fatty acids in particular omega-3 long chain-polyunsaturated fatty acids (ω3 LC-PUFA) and for energy. Other novel sources of renewable, environmentally sustainable oil that provide these nutritional requirements for Atlantic salmon (*Salmo salar* L.) are needed. This research looked at alternate sources of oil containing the ω3 LC-PUFA that are associated with the many health benefits of eating Atlantic salmon. This thesis also contributed to the development of three techniques for use in aquaculture lipid nutrition research: 1) advanced chromatography and mass spectroscopy to examine intact molecular membrane lipids; 2) nuclear magnetic resonance (^{13}C NMR) to assess the regiospecific distribution of ω3 LC-PUFA in oil, and 3) molecular RT-PCR to investigate endogenous ω3 LC-PUFA production.

Two ways of supplying the nutritional requirement for ω3 LC-PUFA in aquafeeds for Atlantic salmon were studied in a series of feeding trials. A biosynthetic precursor of ω3 LC-PUFA from Patterson's curse (*Echium plantagineum* L.) was fed in two trials to Atlantic salmon parr and to smolt. It was shown that feeding oil rich in the biosynthetic precursor, stearidonic acid (18:4ω3 SDA), maintained concentrations of ω3 LC-PUFA in the flesh of salmon parr comparable to fish fed a traditional fish oil diet. In smolt, it was demonstrated that dietary SDA elevated the expression of the genes encoding the enzymes responsible for the desaturation and elongation steps involved in the ω3 LC-PUFA biosynthetic pathway. However, with increased expression and bypassing the Δ^6 desaturation step through the provision of SDA, the smolt stage, unlike parr, did not

maintain concentrations of ω3 LC-PUFA. The high concentrations of ω3 LC-PUFA found in traditional fish oil fed adult salmon will likely not be provided by diets rich in SDA.

Single cell organisms such as microalgae, including thraustochytrids, diatoms and other micro-organisms de novo synthesis ω3 LC-PUFA and are the original sources in the marine food web. Thraustochytrids are heterotrophic protists, commonly found in the marine environment and produce high levels of ω3 LC-PUFA rich oils. Thraustochytrid oil was fed to Atlantic salmon parr to investigate the effect of feed containing high concentrations of ω3 LC-PUFA, in particular docosahexaenoic acid (22:6ω3, DHA), on performance and how this important fatty acid is incorporated into cell membranes and stored in the fish. The thraustochytrid oil in the diet significantly increased the amount of DHA in Atlantic salmon muscle and therefore is a candidate for use in oil blends for salmon diets. Thraustochytrid oil also significantly increased the ability of salmon parr to undergo smoltification. Regiospecificity analyses of intact lipids can indicate how diet, in particular high dietary DHA, can affect the membrane structure of muscle tissues. However, in the gill and liver, adaptive changes due to smoltification were the major factors that contributed to differences in membrane structure. The incorporation of high concentrations of dietary DHA into the membrane structure and storage molecules is achieved by adaptation of molecular species. Regiospecific analysis of the storage lipid demonstrated that increased dietary DHA increased its bioavailability to the consumer.

Other factors involved in oil replacement were examined. These included the effect and accumulation of minor components, such as phytosterols in vegetable oil and the effect rising ocean temperature has on the membrane structure and lipid storage in salmon. Phytosterols have a beneficial effect in humans by reducing low density lipoprotein (LDL) cholesterol. The digestibility of natural abundances of phytosterols by

Atlantic salmon was poor compared to cholesterol. However, significantly increased concentrations of the phytosterols were observed in both the liver and white muscle of Atlantic salmon fed vegetable oils which ultimately may provide health benefits to the consumer. Salmon adapt their membrane structures due to an elevated water temperature of 19^{o}C. This temperature now often occurs in Tasmanian waters in summer and autumn and is approaching the upper limit for Atlantic salmon to maintain health and performance. Adaptation of structural and storage lipids at elevated temperatures was shown by a reduction in PUFA, especially eicosapentaenoic acid (EPA 20:5ω3), and an increase of saturated fatty acids in the gill and white muscle. Salmon altered their membrane structure to compensate for elevated water temperature, which could affect dietary FA requirements.

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The following people and institutions contributed to the publication of the work undertaken as part of this thesis

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Miller, M. R., Nichols, P. D., Davies, N. W., Peacock, E. J. & Carter, C. G. (2007) The effect on the cell membrane structure and lipid storage of Atlantic salmon (Salmo salar L.) fed high levels of docosahexaenoic acid. Internal review.

The following people have contributed to the following chapters

- Nichols, P.D. and Carter, C.G assisted with the general supervision of all aspects of this thesis. These included experimental design, interpretation of data and proof reading manuscripts (10% of chapters)
- Bridle, A. R. assisted in the gene expression sample preparation and data collection and contributed to the proofing of chapter 3. (10% of chapter)
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We the undersigned agree with the above stated "proportion of work undertaken" for each of the above published (or submitted) peer-reviewed manuscripts contributing to this thesis.

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LIST OF ABBREVIATIONS

The following abbreviations are used in this thesis:

^{13}C NMR, ^{13}C nuclear magnetic resonance
ARA, arachidonic acid
ADC, apparent digestibility coefficients
ALA, α-linolenic acid
ANOVA, 1-way analysis of variance
BSFTA, N,O-bis(trimethylsilyl)-trifloroacetamide
$CDCl_3$, deuterated chloroform
CHD, coronary heart disease
CMC, carboxymethyl cellulose
DHA, docosahexaenoic acid
DM, dry matter
DPA, docosapentaenoic acid
EPA, eicosapentaenoic acid
ESI-RP-LCMS, electrospray ionization reversed-phase liquid chromatography-mass spectrometry
ETA, eicosatetraenoic acid
FA, fatty acid(s)
FAD5, fatty acid Δ^5 desaturase
FAD6, fatty acid Δ^6 desaturase
FAE, fatty acid elongase
FAME, fatty acid(s) methyl ester
FC, total feed consumption
FER, feed efficiency ratio
FFA, free fatty acids
GLA, γ-linolenic acid
GC, gas chromatography
GC-MS, gas chromatography mass spectroscopy
HPLC, high pressure liquid chromatography
HIS, hepatosomatic index
HNF, hepatic nuclear factors
LA, linolenic acid
LC, long chain ($\geq C_{20}$)
LDL, low density lipoprotein
LXR, liver X receptor
mRNA, messenger ribonucleic acid
MUFA, monounsaturated fatty acid(s)
NOE, nuclear overhauser effect
NRQ, normalised relative quantities
OA, oleic acid
PCB, polychlorinated biphenyls
PC, phosphatidylcholine
PCA, principal components analysis
PE, phosphatidylethanolamine
PG, phosphatidylglycerol
PI, phophatidylinositol

PKS, polyketide synthases
PL, polar lipid
PLFA, polar lipid fatty acid
PPAR, peroxisome proliferators-activated receptors
PS, phosphatidylserine
PUFA, polyunsaturated fatty acid(s)
RT-PCR, real-time quantitative polymerase chain reaction
RXR, retinoid X receptor
SCO, single cell oils
SDA, stearidonic acid
S.E. standard error
SFA, saturated fatty acid(s)
SGP, salmon genome project
SGR, specific growth rate
SREP-1c, sterol regulatory element protein-1c
ST, sterol(s)
TAG, triacylglycerol
TLC-FID, thin layer chromatography-flame ionisation detection
TLE, total lipid extract
tr, trace amounts
UPL undetermined polar lipid
UFA, unsaturated fatty acid(s)
ω3, omega 3
ω3 LC-PUFA, omega 3 long chain ($\geq C_{20}$)-polyunsaturated fatty acid(s)
ω6, omega 6
WW, wet weight