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

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the requirements of the degree of
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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.

Matthew Robert Miller

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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.

TABLE OF CONTENT

DECLARATION II
AURTHORITY OF ACCESS II
ABSTRACT III
TABLE OF CONTENT VI
LIST OF TABLES X
TABLE OF FIGURES XII
TABLE OF FIGURES XII
ACKNOWLEDGEMENTS XIII
CO-AUTHOURSHIP XV
LIST OF ABBREVIATIONS XVII
CHAPTER 1 1
General Introduction 2
1.1 Introduction 2
1.2 Factors driving replacement oil research 2
1.2.1 Sustainability of wild fish stocks 2
1.2.2 Sustainable Atlantic salmon farming 3
1.2.3 Lipid content and nutrition of aquafeeds 4
1.2.4 Omega 3/omega 6 PUFA 6
1.2.5 Biosynthetic pathway of omega 3 and omega 6 PUFA 8
1.3 Challenges and benefits of replacement oils 10
1.3.1 Advantages/disadvantages with vegetable/plant based oils 10
1.3.2 Phytosterols 12
1.3.3 Regiospecificity of fatty acids 13
1.4 Environmental influences on lipids 14
1.4.1 Temperature 14
1.5 Potential sources of ω3 LC-PUFA for aquaculture 15
1.5.1 Biosynthetic precursors of ω3 LC-PUFA 15
1.5.2 Single cell oils (SCO) 16
1.5.3 Genetic modification (GM) of oils 17
1.6 The aims and hypothesis of thesis 18
1.7 References 20
CHAPTER 2 26
Replacement of dietary fish oil for Atlantic salmon parr (*Salmo salar* L.) with a stearidonic acid containing oil has no effect on omega 3 long chain polyunsaturated fatty acid concentrations. 26
2.1 Abstract 27
2.2 Introduction 28
2.3 Materials and methods 30
2.3.1 Experimental diets 30
2.3.2 Growth experiment 31
2.3.3 Lipid extraction and isolation 34
2.3.4 Chemical analysis 36
2.3.5 Statistical analysis 37
2.4 Results 37
2.4.1 Growth results 37
2.4.2 Lipid class composition 37
2.5.3 Fatty acid (FA) composition 38

2.5 Discussion 43
2.6 Conclusion 53
2.7 Acknowledgements 53
2.8 References 54
CHAPTER 3 58
Effect of stearidonic acid enriched diet on growth, fatty acid composition and elongase and desaturase gene expression in seawater Atlantic salmon (*Salmo salar* L.) 58
3.1 Abstract 59
3.2 Introduction 60
3.3 Materials and methods 63
3.3.1 Experimental diets 63
3.3.2 Growth experiment 65
3.3.3 Digestibility 67
3.3.4 Lipid extraction and isolation 67
3.3.5 Chemical analysis 69
3.3.6 RNA isolation and preparation 69
3.3.7 Reverse transcription 69
3.3.8 Quantitative PCR 70
3.3.9 Relative expression 71
3.3.10 Statistical analysis 71
3.4 Results 72
3.4.1 Growth results 72
3.4.2 Lipid class composition 72
3.4.3 Fatty acid (FA) composition 73
3.4.4 Omega 3 biosynthetic pathway 75
3.4.5 Omega 6 biosynthetic pathway 78
3.4.6 Digestibility 79
3.4.7 Gene expression 79
3.5 Discussion 81
3.5.1 Fatty acid profiles 82
3.5.2 Gene expression 84
3.6 Conclusion 89
3.7 Acknowledgements 89
3.8 References 90
CHAPTER 4 94
Replacement of fish oil with thraustochytrid *Schizochytrium sp. L* oil in Atlantic salmon parr (*Salmo salar* L) diets 94
4.1 Abstract 95
4.2 Introduction 96
4.3 Materials and Methods 99
4.3.1 Experimental diets 99
4.3.2 Growth experiment 101
4.3.3 Lipid extraction and isolation 103
4.3.4 Chemical analysis 105
4.3.5 Statistical analysis 105
4.4 Results 106
4.4.1 Growth results 106
4.4.2 Lipid class composition 107
4.4.3 Fatty acid (FA) composition 107

4.4.4 Digestibility 110
4.4.5 Saltwater challenge 112
4.5 Discussion 112
4.5.1 Digestion 116
4.5.2 Smoltification 117
4.5.3 ω3/ω6 ratio 119
4.6 Conclusion 122
4.7 Acknowledgements 123
4.8 References 123
CHAPTER 5 127
The digestibility and accumulation of dietary phytosterols in Atlantic salmon (*Salmo salar* L) smolt fed diets with replacement plant oils. 127
5.1 Abstract 128
5.2 Introduction 129
5.3 Materials and methods 131
5.3.1 Experimental diets 131
5.3.2 Growth experiment 131
5.3.3 Sterol extraction and isolation 135
5.3.4 Statistical analysis 136
5.4 Results 137
5.4.1 Growth results 137
5.4.2 Sterol (ST) composition 137
5.4.3 Digestibility of phytosterols 139
5.5 Discussion 140
5.5.1 Digestibility of phytosterols in Atlantic salmon 141
5.5.2 Accumulation of phytosterols in Atlantic salmon 142
5.5.3 Environmental effects of phytosterols 145
5.6 Conclusions 146
5.7 Acknowledgements 146
5.8 References 147
CHAPTER 6 150
Regiospecificity profiles of storage and membrane lipids from the gill and muscle tissue of Atlantic salmon (*Salmo salar L*) grown at elevated temperature 150
6.1 Abstract 151
6.2 Introduction 152
6.3 Material and methods 154
6.3.1 Experimental system 154
6.3.2 Sampling 155
6.3.3 Lipid extraction, fractionation and fatty acid analysis 156
6.3.4 Membrane lipid analysis – ESI-RP-LCMS 158
6.3.5 Storage lipid analysis - ^{13}C NMR spectroscopy 158
6.3.6 Statistical analysis 159
6.4 Results 159
6.4.1 Lipid class 159
6.4.2 Fatty acid profiles of total lipid 160
6.4.3 15^{o}C Fish 162
6.4.4 Lipid class composition of polar lipid 162
6.4.5 Fatty acid profiles of polar lipid 164
6.4.6 Regiospecific analysis of the membrane lipids 166
6.4.7 Regiospecific analysis of storage lipid - ^{13}C NMR 168

6.5 Discussion 170
6.5.1 Membrane Lipids 171
6.5.2 Storage lipids 174
6.5.3 Temperature effects 176
6.6 Conclusion 180
6.7 Acknowledgements 180
6.8 References 181
CHAPTER 7 185
The effect on the cell membrane structure and lipid storage of Atlantic salmon (*Salmo salar* L.) fed high levels of docosahexaenoic acid. 185
7.1 Abstract 186
7.2 Introduction 187
7.3 Materials and methods 190
7.3.1 Experimental diets 190
7.3.2 Growth experiment 192
7.3.3 Lipid extraction and isolation 193
7.3.4 Membrane lipid analysis – ESI-RP-LCMS 193
7.3.5 Storage lipid analysis - ^{13}C NMR spectroscopy 194
7.3.6 Principal components analysis (PCA) 195
7.3.7 Statistical analysis 195
7.4 Results 196
7.4.1 Growth experiment and fatty acid profiles of total lipid extracts 196
7.4.2 Fatty acid profiles of polar lipid extracts 196
7.4.3 Regiospecific analysis of the polar lipids extracts 198
7.4.4 Principal components analysis (PCA) 200
7.5.5 Regiospecificity of storage lipids 205
7.5 Discussion 207
7.5.1 Membrane Lipids 207
7.5.2 PCA 210
7.5.3 Storage lipids 212
7.6 Conclusions 214
7.7 Acknowledgements 214
7.8 References 215
CHAPTER 8 218
GENERAL DISCUSSION 218
8.1 General discussion 219
8.1.1 Capacity for the endogenous production of ω3 LC-PUFA 219
8.1.2 Advanced biochemistry and molecular biology 220
8.1.3 Replacement options for ω3 LC-PUFA oils and their commercial viability 221
8.1.4 Broader issues of sustainable intensive aquaculture 224
8.1.5 Future security of ω3 LC-PUFA oils and sustainable aquaculture 225
8.2 References 226

LIST OF TABLES

Table 1.1: Fatty acid profiles (g/100g) of possible plant/vegetable replacement oils for fish oil 11
Table 2.1: Ingredient and lipid composition (g/kg dry matter) of experimental diets 32
Table 2.2: Growth and efficiencies of Atlantic salmon fed experimental diets with canola oil (CO), 14% stearidonic acid oil (SO), 1:1 CO:SO (MX) and fish oil (FO) (mean ± SE). 38
Table 2.3: Lipid content and FA and lipid class composition (g $100g^{-1}$) of red muscle samples of Atlantic salmon fed canola Oil (CO), 1:1 mix of canola oil:stearidonic oil (MX), stearidonic oil (SO) and fish oil (FO) diets 39
Table 2.4: Lipid content and FA and lipid class composition (mg g^{-1}) of white muscle samples of Atlantic salmon fed canola Oil (CO), 1:1 mix of canola oil:stearidonic oil (MX), stearidonic oil (SO) and fish oil (FO) diets 41
Table 2.5: Mass balance of absolute amount T_f (g) and total eaten T_e (g) plus total initial T_i (g) of ω3 LC-PUFA in Atlantic salmon fed experimental feeds with canola oil (CO), 14% stearidonic acid oil (SO), 1:1 CO:SO (MX) and fish oil (FO). 42
Table 3.1: Ingredient and lipid composition (g/kg dry matter) of experimental diets 64
Table 3.2: Characteristics of the real-time PCR primers 70
Table 3.3: Growth and efficiencies of Atlantic salmon fed experimental diets with canola oil (CO), 14% stearidonic acid oil (SO) and fish oil (FO) (mean ± SEM). 73
Table 3.4: Fatty acid content and lipid class composition of the whole carcass of Atlantic salmon smolt fed canola oil (CO), stearidonic acid oil (SO) diets and fish oil (FO) (mg g^{-1} total fatty acids) 74
Table 3.5: Fatty acid content and lipid class composition of the white muscle samples of Atlantic salmon smolt fed canola oil (CO), stearidonic acid oil (SO) diets and fish oil (FO) (mg g^{-1} total fatty acids) 76
Table 3.6: Fatty acid content and lipid class composition of the liver of Atlantic salmon smolt fed canola oil (CO), stearidonic acid oil (SO) diets and fish oil (FO) (mg g^{-1} total fatty acids) 77
Table 3.7: Apparent digestibility coefficient (ADC) for the crude protein (N), energy (kJ), and fatty acids for diets containing different oils 78
Table 4.1: Ingredient and lipid composition (g/kg dry matter) of experimental diets 100
Table 4.2: Growth and efficiencies of Atlantic salmon fed experimental diets with palm oil (PO), thraustochytrid oil (TO), 4:1 PO:TO (MX) and fish oil (FO) (mean ± SE). 106
Table 4.3: Lipid content and FA and lipid class composition (as percent of total) of red muscle samples of Atlantic salmon fed palm oil (PO), 4:1 mix of palm oil: thraustochytrid oil (MX), thraustochytrid oil (TO) and fish oil (FO) diets 108
Table 4.4: Lipid content and FA and lipid class composition (as percent of total) of white muscle samples of Atlantic salmon fed palm oil (PO), 4:1 mix of palm oil: thraustochytrid oil (MX), thraustochytrid oil (TO) and fish oil (FO) diets 110
Table 4.5: Apparent digestibility coefficients (ADC) for different fatty acid fractions for diets containing different oil sources 111
Table 5.1: Ingredient, lipid and sterol composition (g/kg dry matter) of experimental diets. 133
Table 5.2: Sterol content of white muscle and livers of Atlantic salmon smolt fed canola oil (CO), Echium oil (SO) and fish oil (FO). 137

Table 5.3: Sterol composition (mg/100g) of white muscle of Atlantic salmon smolt fed canola oil (CO), Echium oil (SO) and fish oil (FO) diets 138
Table 5.4: Sterol composition (mg/100g) of liver of Atlantic salmon smolt fed canola oil (CO), Echium oil (SO) and fish oil (FO) diets 139
Table 5.5: Apparent digestibility coefficients (ADC) (%) for the different sterols in canola oil (CO), Echium oil (SO) and fish oil (FO) diets fed to Atlantic salmon .. 140
Table 6.1: FA and lipid class composition and content of the total lipid fraction of a commercial formulated diet and white and red muscle and gill tissue of Atlantic salmon *Salmo salar* fed that diet when held at 19oC 161
Table 6.2: FA and lipid class composition and content of the total lipid fraction of white and red muscle and gill tissue of Atlantic salmon *Salmo salar* fed commercial formulated diet when held at 15^{o}C 163
Table 6.3: FA and lipid class percentage composition comparison and content of the polar lipid fraction of white and red muscle and gill tissue of Atlantic salmon *Salmo salar* fed commercial formulated diet when held at 19^{o}C 165
Table 6.4a: Composition of the polar lipid fraction of white and red muscle and gill tissue of Atlantic salmon *Salmo salar* fed commercial formulated diet when held at 19^{o}C 167
Table 6.4b: Composition of the polar lipid fraction of white and red muscle and gill tissue of Atlantic salmon *Salmo salar* fed commercial formulated diet when held at 19^{o}C 168
Table 6.5: Percentage of fatty acid in the *sn-2* position in the triacylglycerol fraction from Atlantic salmon *Salmo salar* tissues when held at 19^{o}C and fed a commercial formulated diet determined by ^{13}C NMR spectroscopy..... 169
Table 7.1: Ingredient and lipid composition (g/kg dry matter) of experimental diets.... 191
Table 7.2: FA content and lipid class composition of the polar lipid extract of white muscle samples of Atlantic salmon fed: palm oil (PO), 1:4 mix of thraustochytrid oil: palm oil (MX), thraustochytrid oil (TO) and fish oil (FO) diets. Initial fish (Int) were fed a FO diet. 197
Table 7.3: Percentage composition of molecular species of phospholipids as determined by ESI RP LC-MS of the white muscle of Atlantic salmon 199
Table 7.4: Frequencies and statistical significance for one way ANOVA for principal component scores..... 205
Table 7.5: FA percentage composition and phosphatidylinositol molecular species of the polar lipid extract of gill samples of Atlantic salmon fed: palm oil (PO) 1:4 mix of thraustochytrid oil: palm oil (MX), thraustochytrid oil (TO) and fish oil (FO) diets. Initial fish (Int) were fed a FO diet. 208

TABLE OF FIGURES

Figure 1.1: Representation of the ω3 and ω6 LC-PUFA biosynthetic pathway from their C_{18} FA precursors in Atlantic salmon. 9
Figure 3.1: Differential gene expression in livers of Atlantic salmon fed diets rich in stearidonic rich oil (SO); canola oil (CO) and fish oil (FO). 80
Figure 3.2: Differential gene expression in white muscle of Atlantic salmon fed diets rich in stearidonic rich oil (SO); canola oil (CO) and fish oil (FO). 81
Figure 4.1: Blood osmolarity (mOsm) of Atlantic salmon on last day of trial (day 63) and 14 days later (day 77) after the salinity was raised from 0 to 32 ppm over four diets. 118
Figure 6.1: Abundances of SFA, MUFA, total ω3 PUFA and total PUFA from the total lipid extract (TLE) for the diet, red muscle, white muscle and gills from Atlantic salmon *Salmo* salar grown at 15 and 19^{o}C. 171
Figure 7.1: Principal component analysis of the fatty acid composition of the total solvent extract of Atlantic salmon: a) red muscle b) white muscle c) liver and d) gill - fed 4 experimental diets; palm oil (PO), 4:1 mix of palm oil: thraustochytrid oil (MX), thraustochytrid oil (TO) and fish oil (FO). 201
Figure 7.2: Principal component analysis of the fatty acid composition of the polar lipid extract of Atlantic salmon: a) red muscle b) white muscle c) liver and d) gill - fed 4 experimental diets; palm oil (PO), 4:1 mix of palm oil: thraustochytrid oil (MX), thraustochytrid oil (TO) and fish oil (FO). 202
Figure 7.3: Principal component analysis of the molecular species from liquid chromatography-mass spectrometry of the polar lipid extract of Atlantic salmon: a) red muscle b) white muscle c) liver and d) gill - fed 4 experimental diets; palm oil (PO), 4:1 mix of palm oil: thraustochytrid oil (MX), thraustochytrid oil (TO) and fish oil (FO). 204
Figure 7.4: Percentage of fatty acids in the *sn*-2 position in the triacylglycerol fraction from Atlantic salmon *Salmo salar* red muscle fed: palm oil (PO) 1:4 mix of thraustochytrid oil: palm oil (MX), thraustochytrid oil (TO) diets and fish oil (FO). 206

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- 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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Barnes, J. (PhD candidate) with Carter, C.G. designed and performed the elevated temperature Atlantic salmon trial in chapter 6. Other than fish weight data no other data from this trial is used in this thesis.

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