THE EFFECTS OF DIETARY FISH OIL REPLACEMENT ON GROWTH, TISSUE FATTY ACID COMPOSITION AND FATTY ACID METABOLISM OF RED HYBRID TILAPIA AND GENETICALLY IMPROVED FARMED NILE TILAPIA

TEOH CHAIW YEE

UNIVERSITI SAINS MALAYSIA

2013

THE EFFECTS OF DIETARY FISH OIL REPLACEMENT ON GROWTH, TISSUE FATTY ACID COMPOSITION AND FATTY ACID METABOLISM OF RED HYBRID TILAPIA AND GENETICALLY IMPROVED FARMED NILE TILAPIA

by

TEOH CHAIW YEE

Thesis submitted in fulfillment of the requirements for the degree of Doctor of philosophy

UNIVERSITI SAINS MALAYSIA

December 2013

ACKNOWLEDGEMENTS

First and foremost I would like to express my sincere acknowledgement to my supervisor, Professor Dr. Ng Wing Keong for his patience, support and mentorship throughout the course of this research. Without his assistance, completion of this thesis would not have been possible. Special thanks go to Dr. Giovanni M. Turchini for his guidance and advice especially on the wholebody-fatty-acid-balance method.

I am also grateful for the assistance from my labmates, Dr. Kim Young Chul, Mr. Lee Kuan Shern, Ms Lim Chia Ling, Mr Leow Tze Chin, Mr Wang Yan and the wife, Ms Qian Yun Yun, with special heartfelt thanks to Mr. Koh Chik Boon for helping me in pelleting and cutting the diets, and to Dr. Nicholas Romano for his suggestions and corrections of my manuscripts.

Thanks to IPS for the USM fellowship which sustains my living expenses and tuition fees. Without this financial aid, I would not be able to concentrate on my research works entirely.

Cordial thanks are also extended to my friends, particularly Ms. Doreen Yu for moral support and valuable friendship.

I would like to share this moment of happiness with my family. No words can adequately express my profound gratitude for their trust, encouragement and motivation throughout my whole studies. Last, but not least, this work is dedicated to my boyfriend for his support, inspiration and understanding.

TABLE OF CONTENTS

		Page
ACKNOWLEDGEMENTS		ii
TABLE OF CONTENTS		iii
LIST OF TABLES		ix
LIST OF FIGURES		xii
LIST OF PLATES		xiv
LIST OF ABBREVIATIONS		xv
LIST OF SYMBOLS		xvi
ABSTRAK		xvii
ABSTRACT		xx
CHAPTER 1: INTRODUCTION		1
CHAPTER 2: LITERATURE REVIEW		7
2.1	Tilapia aquaculture	7
2.2	Lipid classes	10
	2.2.1 Functions of lipids	10
	2.2.2 Dietary lipid requirement in tilapia	10
2.3	Fish oil resource and production	11
2.4	Fatty acid	13
	2.4.1 Saturated fatty acid	14
	2.4.2 Monounsaturated fatty acid	14
	2.4.3 Polyunsaturated fatty acid	15

2.5 Essential fatty acids 16

2.5.1 Essential fatty acids requirement in tilapia 17

2.6 Fatty acid composition in fish tissue 18

2.7 Dietary alternative lipid sources 20

2.7.1 Effect of fish oil replacement on growth 21

2.7.2 Effect of fish oil replacement on fatty acid composition 24

2.8 Lipid and fatty acid digestibility 28

2.9 Fatty acid synthesis and metabolism 30

2.9.1 *De novo* fatty acid synthesis 31

2.9.2 Δ-9 Desaturation 33

2.9.3 Δ-6 and Δ-5 Desaturation 36

2.9.4 Elongation 38

2.10 Fatty acid β-Oxidation 41

2.11 Whole body fatty acid balance method 45

CHAPTER 3: GENERAL MATERIALS AND METHODS 47

3.1 Experimental diets 47

3.1.1 Composition and formulation of the experimental diets 47

3.1.2 Dietary oil sources 48

3.1.3 Protein source, carbohydrate source and other ingredients 49

3.1.4 Vitamin premix 52

3.1.5 Mineral premix 52

3.1.6 Preparation of the experimental diets 53

3.2 Fish and experimental conditions 53

3.2.1 Fish conditioning 53

3.2.2 Experimental set-up 54

3.3 Sample collection 55

3.3.1 Initial sampling 55

3.3.2 Fecal collection 55

3.3.3 Final sampling 56

3.4 Blood collection and hematological analysis 57

3.5 Chemical analysis 57

3.5.1 Proximate analysis 57

3.5.2 Chromic oxide determination 57

3.5.3 Lipid and fatty acid analysis 57

3.6 Evaluation of growth performance and feed utilization efficiency 59

3.7 Evaluation of fish body indices 60

3.8 Apparent Digestibility coefficient 61

3.9 Whole-body fatty acid balance method 62

3.10 Statistical analysis 66

CHAPTER 4: THE IMPLICATIONS OF SUBSTITUTING DIETARY FISH OIL WITH VEGETABLE OILS ON THE GROWTH PERFORMANCE, FILLET FATTY ACID PROFILE AND MODULATION OF THE FATTY ACID ELONGASE, DESATURASE AND OXIDATION ACTIVITIES OF RED HYBRID TILAPIA, *Oreochromis* sp. 67

4.1 Introduction 67

4.2 Materials and methods 69

4.2.1 Experimental diets 69

4.2.2 Fish and experimental conditions 69

4.2.3 Sample collection 70

4.2.4 Chemical analysis 70

4.2.5 Calculations and statistical analysis 70

4.3 Results 71

4.3.1 Experimental diets 71

4.3.2 Growth performance and biometry 71

4.3.3 Nutrient digestibility and whole-body proximate composition 75

4.3.4 Net intake, fatty acid composition of fillet and fish whole-body 78

4.3.5 Fatty acid appearance and disappearance 86

4.3.6 *In vivo* fatty acid metabolism 89

4.4 Discussion 100

4.4.1 Growth performance and biometry 100

4.4.2 Nutrient digestibility and whole-body proximate composition 101

4.4.3 Fatty acid composition of fillet and fish whole-body 103

4.4.4 *In vivo* fatty acid metabolism 104

CHAPTER 5: COMPARATIVE EFFECTS OF REPLACING DIETARY FISH OIL WITH VEGETABLE OIL BLEND ON GROWTH PERFORMANCE, TISSUE FATTY ACID COMPOSITION AND FATTY ACID METABOLISM OF GENETICALLY IMPROVED FARMED NILE TILAPIA (GIFT) AND RED HYBRID TILAPIA 112

5.1 Introduction 112

5.2 Materials and methods 114

5.2.1 Experimental diets 114

5.2.2 Fish and experimental conditions 115

5.2.3 Sample collection 115

5.2.4 Chemical analysis 116

5.2.5 Calculations and statistical analysis 116

5.3 Results 117

5.3.1 Experimental diets 117

5.3.2 Growth performance, body indices, nutrient digestibility and whole-body composition 119

5.3.3 Fatty acid composition of fillet 120

5.3.4 Fatty acid composition of liver 127

5.3.5 Comparison of fatty acid composition between fillet and liver 128

5.3.6 Fatty acid digestibility 131

5.3.7 Fatty acid net intake, fatty acid and lipid content of fish whole-body 134

5.3.8 *In vivo* fatty acid metabolism 139

5.4 Discussion 151

5.4.1 Growth performance and biometry 151

5.4.2 Nutrient digestibility 152

5.4.3 Whole-body proximate composition 153

5.4.4 Fatty acid composition of fillet and liver 154

5.4.5 Fatty acid composition of whole-body fish 157

5.4.6 *In vivo* fatty acid metabolism 158

CHAPTER 6: EVALUATION ON THE IMPACT OF DIETARY PETROSELINIC ACID ON THE GROWTH PERFORMANCE, FATTY ACID COMPOSITION AND EFFICACY OF LONG CHAIN-POLYUNSATURATED FATTY ACIDS BIOSYNTHESIS OF GENETICALLY IMPROVED FARMED NILE TILAPIA 163

6.1 Introduction 163

6.2 Materials and methods 165

6.2.1 Experimental diets 165

6.2.2 Fish and experimental conditions 166

6.2.3 Sample collection 166

6.2.4 Chemical analysis 167

6.2.5 Calculations and statistical analysis 167

6.3 Results 168

6.3.1 Diet composition 168

6.3.2 Growth performance, nutrient digestibility and whole-body proximate composition 170

6.3.3 Fatty acid composition of fish tissue and whole-body 172

6.3.4 *In vivo* fatty acid metabolism 178

6.4 Discussion 187

6.4.1 Growth performance, nutrient digestibility 187

6.4.2 Fatty acid composition of fish tissues 187

6.4.3 *In vivo* fatty acid metabolism 189

CHAPTER 7: CONCLUSIONS 198

CHAPTER 8: REFERENCES 201

APPENDIXES 225

LIST OF PUBLICATIONS 232

LIST OF TABLES

		Page
2.1	Total world fisheries production and utilization (million tonnes) for the period 1950-2011. Data 2011 is an estimate	12
2.2	Global production (thousand tonnes) of fish oil and vegetable oils from 1980 to 2006	21
2.3	The effect of partial fish oil replacement on growth and/or feed utilization efficiency in various fish species	25
2.4	The effect of full fish oil replacement on growth and/or feed utilization efficiency in various fish species	26
2.5	Dietary C_{18} PUFA and the 18:3n-3/18:2n-6 ratio on the growth performance of various fish species	27
3.1	Fatty acid composition (% of total fatty acids) of various oil source used	50
3.2	Proximate composition (% dry weight basis) of main feed ingredients used in Experiment 1	51
3.3	Proximate composition (% dry weight basis) of main feed ingredients used in Experiment 2	51
3.4	Proximate composition (% dry weight basis) of main feed ingredients used in Experiment 3	51
4.1	Ingredient and proximate composition (g/kg diet) of experimental diets	72
4.2	Fatty acid composition (% of total fatty acids) of experimental diets with different lipid sources	73
4.3	Growth performance, feed utilization efficiency and biological indices of tilapia fed diets with different lipid sources for 75 days	74
4.4	Apparent nutrient digestibility (%) in tilapia fed diets with different lipid sources	76
4.5	Whole-body composition (% wet weight basis) of tilapia fed diets with different lipid sources for 75 days	76
4.6	Fatty acid digestibility of fish fed diets with different lipid sources[1]	77
4.7	Total fatty acid net intake of tilapia (mg of fatty acid per fish)	79

4.8	Fillet fatty acid composition (% fatty acid) of tilapia fed diets with different lipid sources over 75 days	81
4.9	Fatty acid content (mg/100g fish) of initial and final fish whole-body	84
4.10	Total fatty acid appearance/disappearance during the feeding trial expressed as mg of fatty acid per fish	87
4.11	Total apparent *in vivo* fatty acid neogenesis, β-oxidation, desaturation and elongation (μmol g^{-1} day^{-1}) in tilapia fed diets with different lipid sources for 75 days	90
5.1	Ingredient and proximate composition (g/kg diet) of experimental diets	117
5.2	Fatty acid composition (% of total fatty acids) of the experimental diets	118
5.3	Growth performance, feed utilization efficiency and biological indices of the GIFT and red hybrid tilapia strains	122
5.4	Whole-body composition (% wet weight), apparent dry matter and lipid digestibility (%) of the GIFT and red hybrid tilapia strains after the feeding trial	124
5.5	Fillet fatty acid composition (mg per g of lipid) of initial sample and the GIFT and red hybrid tilapia strains after the feeding trial	125
5.6	Liver fatty acid composition (mg/g of lipid) of initial sample and fish after feeding trial	129
5.7	Apparent fatty acid digestibility (%) of diets with fish oil or blended vegetable oil fed to tilapia	132
5.8	Total fatty acid net intake of fish (mg of fatty acid/fish)	135
5.9	Fatty acid (mg/g of lipid) and lipid (mg/g wet weight) content of initial and final fish whole-body	137
5.10	Total fatty acid appearance/disappearance during the feeding trial expressed as mg of fatty acid per fish	140
5.11	Total apparent *in vivo* fatty acid neogenesis, β-oxidation, desaturation and elongation (μmol g^{-1} day^{-1}) in tilapia of different genotype fed a fish oil (FO) or blended vegetable oil (BVO) diet	142
5.12	Individual apparent *in vivo* enzyme activity of selected fatty acids	149

6.1	Proximate composition (%) of coriander seed oil	166
6.2	Ingredient and proximate composition (g/kg diet) of experimental diets	168
6.3	Fatty acid composition (% of total fatty acids) of experimental diets	169
6.4	Growth performance, feed utilization efficiency and biological indices of fish	171
6.5	Whole-body composition (% wet weight basic) of tilapia fed diets with different lipid sources over 59 days	172
6.6	Fillet fatty acid composition (% fatty acid) and lipid content of tilapia fed increasing dietary petroselinic acid	173
6.7	Liver fatty acid composition (% fatty acid) and lipid content of tilapia fed increasing dietary petroselinic acid	175
6.8	Fatty acid content (mg/ 100g fish) of initial and final fish whole-body	177
6.9	Total apparent *in vivo* fatty acid neogenesis, β-oxidation, desaturation and elongation (μmol g^{-1} day^{-1}) in tilapia fed increasing dietary petroselinic acid	179

LIST OF FIGURES

		Page
2.1	Total global production of tilapia farming (million tonnes) from 1995 to 2010	8
2.2	Total tilapia aquaculture in Malayisa (thousand tonnes) from 1995 to 2010	8
2.3	Fish oil production from 2001 to 2011. Data 2011 is an estimate	13
2.4	Palmitic acid and oleic acid, in the shorthand designation	15
2.5	Linoleic acid and docosahexaenoic acid	16
2.6	The n- (shown in italic) and Δ nomenclature for arachidonic acid	16
2.7	Fatty acid synthesis pathway	33
2.8	Δ-9 desaturation of 18:0 (Stearoyl-CoA) to 18:1n-9 (Oleyl-CoA) by steroyl-CoA desaturase	34
2.9	Desaturation and elongation pathway of n-3 and n-6 PUFA	36
2.10	Enzymatic steps in long-chain fatty acid elongation	39
2.11	Enzymology of mitochondrial and peroxisomal fatty acid β-oxidation systems	42
3.1	Schematic of the whole-body fatty acid balance method applied in the computation for n-3 PUFA balance	65
4.1	The apparent *in vivo* elongase activity on SFA and MUFA in tilapia fed diets with different lipid sources over 75 days	93
4.2	The apparent *in vivo* desaturase activity in tilapia fed diets with different lipid sources over 75 days	94
4.3	The apparent *in vivo* fatty acid β-oxidation in tilapia fed diets with different lipid sources over 75 days	95
4.4	The apparent *in vivo* elongase and desaturase activity of 18:3n-3 pathway in tilapia fed diets with different lipid sources for 75 days	96
4.5	The apparent *in vivo* elongase and desaturase activity of 18:2n-6 pathway in tilapia fed diets with different lipid sources for 75 days	97

4.6	Apparent *in vivo* desaturation, elongation and β-oxidation on 18:2n-6 and 18:3n-3 expressed as % of net intake in tilapia fed diets with different lipid sources for 75 days	99
5.1	The apparent *in vivo* fatty acid β-oxidation in tilapia fed either a fish oil (FO) or a blended vegetable oil (BVO) diet for 14 weeks	145
5.2	The apparent *in vivo* elongase activity in tilapia fed either a fish oil (FO) or a blended vegetable oil (BVO) diet for 14 weeks	146
5.3	The apparent *in vivo* desaturase activity in tilapia fed either a fish oil (FO) or a blended vegetable oil (BVO) diet for 14 weeks	148
5.4	Apparent *in vivo* desaturation, elongation and β-oxidation on 18:2n-6 and 18:3n-3 expressed as % of net intake in tilapia fed either a fish oil (FO) or a blended vegetable oil (BVO) diet for 14 weeks	150
6.1	The apparent *in vivo* elongase activity in tilapia fed increasing dietary petroselinic acid (PSA)	180
6.2	The apparent *in vivo* desaturase activity in tilapia fed increasing dietary petroselinic acid (PSA)	182
6.3	The apparent *in vivo* fatty acid β-oxidation in tilapia fed increasing dietary petroselinic acid (PSA)	183
6.4	The apparent *in vivo* elongase and desaturase activity of 18:3n-3 pathway (A) and 18:2n-6 pathway (B) in tilapia fed increasing dietary petroselinic acid (PSA)	185
6.5	Apparent *in vivo* desaturation, elongation and β-oxidation on 18:2n-6 and 18:3n-3 expressed as % of net intake in tilapia fed increasing dietary petroselinic acid (PSA)	186

LIST OF PLATES

		Page
2.1	GIFT tilapia farmed in Fish Nutrition Laboratory, USM	9
3.1	A: Perilla oil. B: Coriander seed, the powder and the extracted oil	49
3.2	Experimental set-up used in all the three experiments	54
3.3	Fish were individually measured during final sampling	56

LIST OF ABBREVIATIONS

ACP	Acyl carrier protein
ADC	Apparent digestibility coefficient
ANOVA	Analysis of variance
AOAC	Association of Official Analytical Chemists methods
ARA	Arachidonic acid
ATP	Adenosine triphosphate
BVO	Blended vegetable oil
CLO	Cod liver oil
CMC	Carboxymethyl cellulose
CO	Canola oil
CoA	Coenzyme
CPKO	Crude palm kernel oil
CPO	Crude palm oil
CPT	Carnitine palmitoyltransferase
CSO	Coriander seed oil
DHA	Docosahexaenoic acid
DPA	Docosapentaenoic acid
EFA	Essential fatty acids
Elovl	Elongases of very long-chain fatty acids
EPA	Eicosapentaenoic acid
FCR	Feed conversion ratio
FO	Fish oil
GIFT	Genetically improved farmed tilapia
GSI	Gonadosomatic index
HSI	Hepatosomatic index
IPF	Intraperitoneal fat index
LC-PUFA	Long chain polyunsaturated fatty acids
LO	Linseed oil
MUFA	Monounsaturated fatty acids
OO	Olive oil
PeO	Perilla oil
PFAD	Palm fatty acid distillates
PG	Prostaglandins
PPAR	Peroxisome proliferator-activated receptors
PSA	Petroselinic acid
PUFA	Polyunsaturated fatty acids
RBDPO	Refined, bleached and deodorized palm olein
SCD	Steroyl-CoA desaturase
SFA	Saturated fatty acids
SFO	Sunflower oil
SGR	Specific growth rate
SREBP-1C	Sterol regulatory element binding protein-1c
TAG	Triacylglycerols
VLCAS	Very-long chain acyl-CoA sythetase
VLCFA	Very long chain fatty acids
VO	Vegetable oil
VSI	Viscerosomatic index
WBFABM	Whole-body fatty acid balance method

LIST OF SYMBOLS

α	Alpha
β	Beta
Δ	Delta
γ	Gamma

KESAN PENGGANTIAN MINYAK IKAN DALAM DIET KE ATAS PERTUMBUHAN, KOMPOSISI ASID LEMAK TISU DAN METABOLISMA ASID LEMAK DALAM TILAPIA HIBRID MERAH DAN TILAPIA NILE YANG DITAMBAHBAIK BAKA

ABSTRAK

Tiga kajian yang berkaitan telah dijalankan untuk menentukan kesan penggantian minyak ikan (FO) dalam diet ikan tilapia. Eksperimen yang pertama dijalankan untuk menyiasat kesan-kesan empat minyak sayuran (VO) yang berbeza sebagai alternatif kepada minyak ikan (FO) ke atas pertumbuhan, komposisi asid lemak dan perubahan metabolisme asid lemak dalam tilapia hibrid merah (*Oreochromis* sp.). Penggantian sepenuhnya FO dengan VO tersebut tidak menjejaskan ($P>0.05$) prestasi pertumbuhan ikan. Sumber lipid diet mempengaruhi komposisi asid lemak dalam fillet dan seluruh badan tilapia serta metabolisme asid lemak dalam tilapia secara keseluruhan; ikan yang diberi diet VO menunjukkan aktiviti desaturasi Δ-5 dan Δ-6 yang tinggi. Antaranya, ikan yang diberi diet minyak canola merekodkan aktiviti biosintetik n-3 asid lemak politaktepu rantai panjang (LC-PUFA) yang terbanyak dan mempunyai kandungan asid eicosapentaenoik (EPA) + asid docosahexaenoik (DHA) yang lebih banyak jika dibandingkan dengan ikan yang diberi diet VO yang lain. Namun demikian, sintesis LC-PUFA yang dicetuskan oleh diet VO adalah tidak setanding dengan kandungan n-3 LC-PUFA dalam ikan yang diberi makan diet FO.

Dalam eksperimen yang kedua, satu campuran minyak sayur (BVO) telah dirumus supaya mencerminkan kelas asid lemak utama dalam FO dan digunakan sebagai alternatif kepada FO dalam pemakanan tilapia dengan tujuan untuk membandingkan metabolisme asid lemak bagi dua genotip tilapia yang berbeza

[tilapia Nile yang ditambahbaik baka (*Oreochromis niloticus*, GIFT) dan tilapia hybrid merah]. Di bawah keadaan eksperimen, genotip tilapia, sumber lipid pemakanan atau interaksi kedua-duanya tidak menjejaskan kadar penukaran suapan atau indeks badan biometrik secara ketara. Walau bagaimanapun, GIFT tilapia sama ada diberi diet yang mengandungi FO atau BVO menunjukkan nilai peratus penambahan berat yang lebih tinggi berbanding dengan tilapia hibrid merah yang diberi diet FO atau BVO. Tilapia yang diberi diet BVO memaparkan penukaran 18:2n-6 kepada n-6 LC-PUFA yang cekap. Hal ini menunjukkan bahawa metabolisme asid lemak tilapia dapat memampas sepenuhnya kekurangan n-6 LC-PUFA dari diet apabila diberi diet berumusan BVO. Tanpa mengira diet, tilapia GIFT mencatatkan kadar neogenesis asid lemak yang lebih tinggi bersama dengan kadar elongasi, desaturasi Δ-5 dan Δ-6 yang lebih menonjolkan bagi kedua-dua PUFA n-6 dan n-3. Maka, penternakan tilapia GIFT adalah pilihan yang lebih baik kerana ia mengandungi n-3 LC-PUFA yang lebih tinggi berbanding dengan tilapia hibrid merah.

Dalam eksperimen ketiga, minyak biji ketumbar telah digubal dengan VO yang lain pada tahap yang berlainanan dan ditambah dalam diet tilapia untuk menyiasat potensi pemakanan asid petroselinik (PSA) dalam meningkatkan kandungan n-3 LC-PUFA dalam tisu-tisu lemak tilapia GIFT. Pertumbuhan ikan dan penghadaman nutrien tidak dipengaruhi oleh tambahan PSA dalam makanan secara ketara. Secara umum, pemakanan PSA mempengaruhi komposisi asid lemak tisu-tisu dan seluruh badan tilapia, di mana nisbah asid lemak diet dicerminkan serta menpengaruhi metabolisme asid lemak *in vivo*. Diet PSA meningkatkan pengoksidaan-β secara ketara, terutamanya bagi 18:3n-3 dan 18:2n-6, malahan mengurangkan aktiviti desaturasi Δ-6 bagi kedua-dua C_{18} PUFA ini. Di samping itu, pemakanan PSA tidak menyekat biosintesis n-3 dan n-6 LC-PUFA. Jadi, hal ini menunjukkan kecekapan penukaran

dalam tilapia bukan sahaja dimodulasi oleh enzim desaturasi Δ-6 tetapi juga enzim asid lemak yang lain. Akan tetapi, tambahan PSA dalam diet tilapia lebih cenderung meningkatkan kandungan n-3 LC-PUFA dalam fillet ikan, walaupun hanya sedikit. Kesimpulannya, eksperimen-eksperimen ini telah memberi sumbangan ke atas pengetahuan asas tentang metabolism asid lemak dalam tilapia dan membolehkan ahli nutrisi ikan menghasil diet yang mesra alam dan kos efektif sementara memastikan pertumbuhan ikan yang optimum dan kualiti produk ikan yang dapat memanfaatkan kesihatan pengguna manusia.

THE EFFECTS OF DIETARY FISH OIL REPLACEMENT ON GROWTH, TISSUE FATTY ACID COMPOSITION AND FATTY ACID METABOLISM OF RED HYBRID TILAPIA AND GENETICALLY IMPROVED FARMED NILE TILAPIA

ABSTRACT

Three related experiments were carried out to investigate the effects of dietary fish oil replacement in the feeds of farmed tilapia. The first feeding trial was conducted to investigate the effects of four different individual vegetable oils (VOs) with varying fatty acid profile as FO alternatives on the growth performance, fatty acid composition and changes in the fatty acid metabolism of red hybrid tilapia (*Oreochromis* sp.). Full FO replacement by these VOs did not compromise ($P>0.05$) fish growth performance. In general, dietary VO influenced fatty acid composition of tilapia fillet and whole-body as well as regulating the overall fatty acid metabolism in tilapia. Δ-5, Δ-6 desaturase activity were high in fish fed the VO diets. n-3 long chain-polyunsaturated fatty acid (LC-PUFA) biosynthetic activities were highest in fish fed the canola oil diet and resulted in a superior eicosapentanoic aicd (EPA) + docosahexaenoic acid (DHA) content than the other VO diets. However, endogenous LC-PUFA synthesis triggered by the VO diets was insufficient to rival the n-3 LC-PUFA content of fish fed the FO diet.

In the second experiment, a novel blended VO (BVO) was formulated to mimic the major fatty acid classes of dietary FO, and used as alternative to FO in tilapia diets with the aim of comparing the fatty acid metabolism in two different tilapia genotypes [Nile tilapia (*Oreochromis niloticus*, GIFT strain) and red hybrid tilapia]. Under the study conditions, tilapia genotype, dietary lipid source or their interaction did not significantly affect feed conversion rate or body biometric indices. However, GIFT

tilapia fed diets containing FO or BVO showed numerically higher percent weight gain compared to red hybrid tilapia fed FO or BVO. Tilapia fed the BVO diet exhibited efficient bioconversion of 18:2n-6 to n-6 LC-PUFA indicating that the fatty acid metabolism of tilapia is able to fully compensate for the lack of dietary n-6 LC-PUFA when fed a VO-based diet. Irrespective of diet, GIFT tilapia showed higher rates of fatty acid neogenesis along with higher rates of elongation, Δ-5 and Δ-6 desaturation of both the n-6 PUFA and n-3 PUFA. Thus, GIFT tilapia are indeed a better selection for tilapia farming as they contain greater human-beneficial n-3 LC-PUFA as compared to red hybrid tilapia.

In the third experiment, graded inclusions of coriander seed oil in the diets were formulated to investigate the potential role of dietary petroselinic acid (PSA) in enhancing the health beneficial n-3 LC-PUFA content in tissue lipids of GIFT tilapia. Fish growth and nutrient digestibility were not significantly influenced by dietary PSA supplementation. In general, dietary PSA affected the fatty acid composition of tilapia tissues and whole-body, which reflected dietary fatty acid ratios, as well as influenced *in vivo* fatty acid metabolism. Dietary PSA significantly increased β-oxidation, particularly on 18:3n-3 and 18:2n-6, and reduced Δ-6 desaturase activity on both these C_{18} PUFA. The n-3 and n-6 LC-PUFA biosynthesis was not significantly inhibited by dietary PSA, indicating the bioconversion efficiency is not only modulated by Δ-6 desaturase but also other fatty acid enzymes. Nevertheless, the supplementation of PSA in tilapia diets tended to improve the content of health-beneficial n-3 LC-PUFA in their fillet, albeit at only slightly higher levels. In conclusion, these experiments provided fundamental findings of fatty acid metabolism in tilapia and enable fish nutritionists to develop eco-friendly and cost effective diets to ensure optimal fish growth and fish products with beneficial health qualities for human consumers.

CHAPTER 1

INTRODUCTION

Fish is a unique source of protein for balanced nutrition and good health. In 2009, fish contributed to 16.6% of the global population's total intake of animal protein and 6.5% of all protein consumed (FAO, 2012). Meanwhile, fish is an important source of essential nutrients including various vitamins, minerals and n-3 (omega-3) long chain polyunsaturated fatty acids (LC-PUFA) which are known to be beneficial for the well-being of humans (FAO, 2012). Increasing awareness of the nutritional value of fish consumption has increased the demand for seafood. Total worldwide seafood supplies have increased with an average annual growth rate of 3.2% in the period 1961-2009 and reached about 148 million tonnes with a value of US$ 217.5 billion in year 2010. World per capita food fish supply is estimated to increase to 18.8 kg in 2011 (FAO, 2012).

Nowadays, worldwide capture fisheries is a "sunset" industry as the total production has remained static at about 90 million tonnes and is not expected to increase. Thus, the aquaculture industry has become more important to meet the increasing demands of worldwide seafood supplies. The global aquaculture industry is rapidly expanding with an average annual growth rate of 8.8% over the last three decades, attaining a total production of 59.9 million tonnes valued at US$ 119.4 billion in 2010, and current estimates suggest that aquaculture production provides about 41% of total world fisheries in 2011 (FAO, 2012). The rapid expansion of aquaculture industry is partly driven by the wide use of aquaculture feeds (aquafeeds).

Total global aquafeed production was at 29.2 million tonnes, accounting for 4.1% of total industrial compound animal feeds in 2008, and the production is

projected to increase to 51.0 million tonnes by 2015 and further to 71.0 million tonnes by 2020 (FAO, 2012). Fish oil (FO) is a major dietary lipid source used in commercial aquafeeds to provide energy and essential fatty acids for growth and biological function. It is estimated that a total of 764 thousand tonnes of FO was utlized by the aquaculture industry for aquafeed production in 2010 which accounted for 86.8 % of global FO production (Tacon et al., 2011; Shepherd and Jackson, 2012). Therefore, increasing demand for its use in aquafeeds coupled with the stagnation in global FO production, which are derived from finite wild resources, have greatly inflated FO prices. Moreover, oceanic phenomena, such as El Niño, exacerbates this situation and increased the FO price to high levels (Turchini et al., 2009).

In 1995, global tilapia aquaculture production was only 703 thousand tonnes. Over the past decade, research on nutrition and culture systems, along with market development and processing advances had led to a rapid expansion of this industry. Global tilapia production is about 3.5 million tonnes valued at US$ 5.7 billion in 2010 (FAO, 2011) and is estimated to reach 8.9 million tonnes by year 2020 (Tacon and Metian, 2008). Currently, tilapia are the second most important farmed fish in the world after carps. The increased global production is fuelled, in part, by the introduction of improved strains of Nile tilapia as well as the use of commercial pelleted feeds in modern farming systems.

In consideration of the global FO shortages impacting the sustainability of aquaculture, it is imperative to find expedient lipid alternatives in aquafeeds. Among all the alternative lipid sources, vegetable oils (VO) appear to be predominant candidates as they are more readily available, cost effective, sustainable and environmentally friendly (Gunstone, 2010). Many studies have shown that substituting dietary FO with VO has no deleterious effect on fish growth performance including

tilapia (Turchini et al., 2009). However, since the use of VO can reduce the content of n-3 polyunsaturated fatty acids (PUFA) in fish fillets, human health issue comes into focus as n-3 LC-PUFA are known be beneficial to human health. VOs have their own specific fatty acid profile, but lack n-3 LC-PUFA which is rich in FO. Given that fish tissue often mirrors the dietary fatty acid content (Bell et al., 2002; Caballero et al., 2002; Bahurmiz and Ng, 2007; Francis et al., 2007c; Stubhaug et al., 2007), the most stringent constraints of dietary FO replacement is a reduced n-3 LC-PUFA content of fish fillets (Turchini et al., 2009). Moreover, it has been well established that fatty acid metabolism in fish is affected by dietary fatty acids (Bell et al., 2001a; Turchini and Francis, 2009; Torstensen and Tocher, 2010).

Tilapia is known to have the ability to convert α-linolenic acid (18:3n-3) to eicosapentaenoic acid (20:5n-3, EPA) and docosahexaenoic acid (22:6n-3, DHA) and linoleic acid (18:2n-6) to arachidonic acid (20:4n-6, ARA) (Olsen et al., 1990). However, this ability was reported to be suppresed by dietary LC-PUFA (Olsen et al., 1990). Therefore, the present project was designed to elucidate the differences and changes in the fatty acid metabolism of tilapia under different dietary conditions. These fundamental findings will serve as the basis for current and future fish nutritionists to develop newer concepts and methods for improving the efficiency of conversion of fish feed to food and the healthiness of fish products for human consumers.

Several methods have been used to answer the fundamental questions as to lipid and fatty acid metabolism. Unlike *ex-vivo* methods which involve expensive and sophisticated approaches, an *in vivo* method that employs a whole-body approach is a simple and effective method for assessing fatty acid metabolism (Turchini et al., 2007a, 2011). Thus, this *in vivo* whole body fatty acid balance method was adapted to examine

the C_{18} fatty acid balance and quantify the changes in fatty acid elongase, desaturase and oxidation activities in tilapia when fed various dietary lipid sources.

Although there were previous studies testing the effect of FO replacement by VO, but there is no information on the fatty acid metabolism of red hybrid tilapia under different VO treatment. Thus, the first phase of the project, Experiment 1 (Chapter 4) was carried out to investigate the effects of different dietary VOs on the growth performance, fatty acid composition and changes in the fatty acid metabolism in red hybrid tilapia (*Oreochromis* sp.), and seek to better understand if the C_{18} polyunsaturated fatty acid (PUFA) elongase and desaturase capabilities of tilapia can compensate for reduced dietary LC-PUFA levels when VOs are used.

The success of tilapia as the second most cultured fish species in the world is largely fuelled by their rapid growth rates, hardiness and high adaptability to various culture systems (FAO, 2012). In Malaysia, red hybrid tilapia is the main cultured species with its outstanding red color and better marketability (Ng and Hanim, 2007). Genetically Improved Farmed Tilapia (GIFT) strain is one of the more popular and successfully introduced Nile tilapia. However, information concerning the nutrient requirement and utilization of high performing tilapia strains compared to non-improved strains are lacking. Also, no information on the fatty acid desaturation and elongation capability of the GIFT tilapia is currently available.

Δ-6 desaturase is known as a rate-limiting enzyme since it is involved in the first step of biosynthesizing LC-PUFA by desaturating the C_{18} PUFA precursor, 18:3n-3 and 18:2n-6 to stearidonic acid (18:4n-3) and γ-linolenic acid (18:3n-6), respectively (Nakamura and Nara, 2004). Thus, dietary ratios of 18:3n-3/18:2n-6 have a crucial impact on modulating Δ-6 desaturase enzymatic activity (Izquierdo et al., 2008) as

excessive dietary 18:2n-6 in VOs will compete with 18:3n-3 for the Δ-6 desaturase enzyme, and therefore inhibit the bioconversion which will subsequently result in minimal activities along the n-3 PUFA biosynthetic pathway (Miller et al., 2007). Furthermore, the opposite is true where excessive dietary 18:3n-3 inhibits the bioconversion of 18:2n-6 to ARA (Miller et al., 2007). In addition, the final fatty acid composition and nutritional quality of fish fillets are greatly affected by dietary 18:3n-3 to 18:2n-6 ratios (Senadheera et al., 2010).

It is believed that with a better understanding of how dietary lipid source modulates *in vivo* fatty acid metabolism, better diets can be formulated for these fast growing tilapia strains while maintaining the healthy benefits for the human consumer in regards to the fillet EPA and DHA content. Therefore, a novel blend of VOs was formulated to match the major fatty acid classes of FO with dietary 18:3n-3/18:2n-6 ratio designed to equal 1, and used as an alternative to FO in tilapia diets in Experiment 2 (Chapter 5) to determine the interaction between tilapia genotype and dietary lipid source on growth performance and proximate composition in the GIFT tilapia strain and red hybrid tilapia. The results was then used to evaluate the differences in overall efficiency of fatty acid metabolism as it relates to genotype and diets. Tilapia strains with higher capability to biosynthesize EPA and DHA from feeds lacking in LC-PUFA should also be selected for aquaculture.

Petroselinic acid (PSA, cis-6-octadecenote, 18:1n-12) is a major monounsaturated fatty acid (MUFA) of coriander seed oil. It has been reported that PSA inhibited the Δ-6 desaturase activity on 18:2n-6 with its Δ6-cis-double bond, a pseudo-product mimicking the structure of 18:3n-6 through feedback inhibition, and which then reduced the ARA content in tissue lipids of rats (Weber et al., 1995, 1997, 1999). It is speculated that reduced Δ-6 desaturation on 18:2n-6 will stimulate more

Δ-6 desaturase activity on 18:3n-3, and ultimately enhance the biosynthesis of health-promoting n-3 LC-PUFA content in fish fillets. Thus, the objective of Experiment 3 (Chapter 6) was to investigate the potential role of dietary PSA in enhancing the health beneficial n-3 LC-PUFA content in fish tissue.

In summary, the overall objectives of this project is to provide fundamental information as to the effects of dietary fish on replacement in the feeds of farmed tilapia.

CHAPTER 2

LITERATURE REVIEW

2.1 Tilapia aquaculture

Tilapia are members of the Cichlidae family and native to Africa. Owing to their rapid growth rates, hardiness and adaptability to a variety of culture systems, tilapia is one of the most important farmed freshwater fish in more than 100 countries around the world (FAO, 2012). Several species of tilapia are cultured commercially, including Nile tilapia (*Oreochromis niloticus* L.), Mozambique tilapia (*O. mossambicus*), blue tilapia (*O. aureus*), three spotted tilapia (*O. andersonii*), Sabaki tilapia (*O. spilurus*), and their hybrids (FAO 2012). Tilapia aquaculture is rapidly growing, the global production of 0.7 million tonnes in year 1995 increased to 3.5 million tonnes in 2010 with an average annual growth rate of 11.4% over the last 15 years (Figure 2.1).

Currently, Nile tilapia is the predominant species farmed worldwide, accounting for 80% of the total production. The wide use of commercial pelleted feeds and the introduction of improved Nile tilapia strains contributed to the rapid expansion in tilapia farming (Ng and Hanim, 2007). GIFT strain developed by the WorldFish Center is one of the more popular and successfully introduced Nile tilapia. Research studies conducted in different countries (China, Bangladesh, Philippines, Thailand and Vietnam) reported that the GIFT strain has superior growth performance than the local farmed Nile tilapia. The initial development of the GIFT strain was centered in the Philippines (1988-1997) but with the move of the Worldfish Center to Malaysia, the F_6 generation of GIFT Nile tilapia was transferred from the Philippines to Malaysia for further research and development. The F_7 generation of the GIFT strain was locally

bred at the Jitra Aquaculture Extension Center (Department of Fisheries), Kedah, in 2002 (Ponzoni et al., 2005).

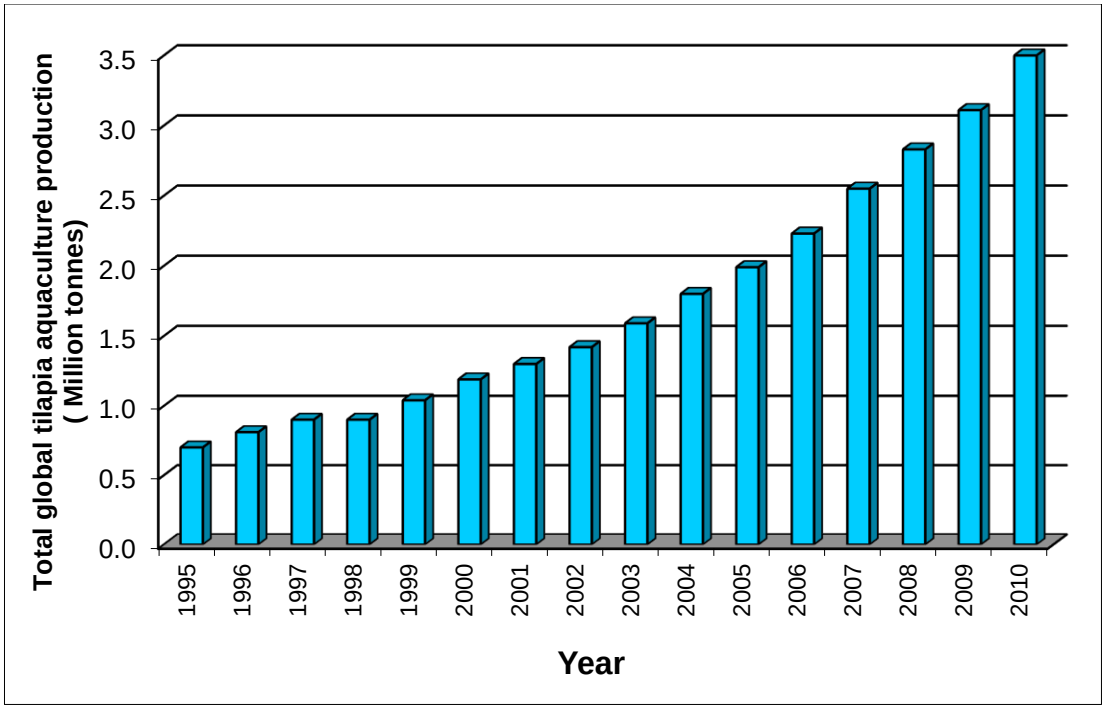


Figure 2.1 Total global production of tilapia farming (million tonnes) from 1995 to 2010 (FAO, 2011).

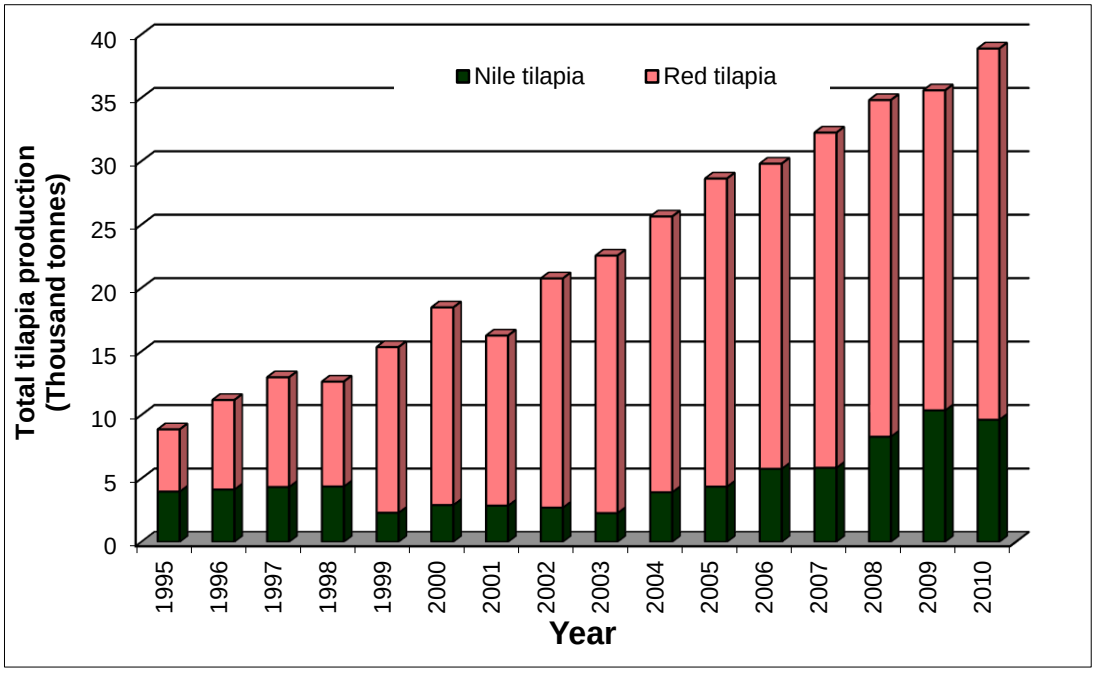


Figure 2.2 Total tilapia aquaculture in Malayisa (thousand tonnes) from 1995 to 2010 (FAO, 2011).

Tilapia farming in Malaysia has risen greatly with the total production of 8.866 tonnes in the year 1995, increased more than 300% and reached 38,886 tonnes in 2010 (Figure 2.2). Unlike other Asian countries, red hybrid tilapia (*O.* sp.) is the major farmed species in Malaysia accounting about 85% of the total tilapia aquaculture production due to its favourable red coloration and high market value. The original red hybrid tilapia introduced into Malaysia was probably a hybrid of *O. niloticus* with *O. mossambicus* (Peters). The enhanced appearance and marketability are attributed by the crossover of different species bred. However, high frequencies of inbreeding led to a gradual genetic deterioration which ultimatetly depressed their growth performance. With the introduction of GIFT tilapia (Plate 2.1) this provides local tilapia farming with an improved strain of tilapia with higher productivity (Ng and Hanim, 2007).

Plate 2.1 GIFT tilapia bred at the Fish Nutrition Laboratory, USM.

2.2 Lipid classes

Animal lipids, can be categorized into two groups based on their polarity and classified as neutral lipids and polar lipids. Neutral lipids such as triacylglycerols (TAG), wax esters, sterols and free fatty acids, which are hydrophobic and totally soluble in organic solvents. Polar lipids, including phospholipids (or phosphoglycerides), sphingolipids, glycolipids and sulpholipids, having a wide range of solvent solubility with their nonlipid head groups (Sargent et al., 2002; Bell and Koppe, 2010).

2.2.1 Functions of lipids

Dietary lipids are important sources of concentrated energy in fish at 39.5 kJ g^{-1}, which is markedly greater than protein (23.6 kJ g^{-1}) or carbohydrates (17.2 kJ g^{-1}) (NRC, 2011). Moreover, lipids are a source of essential fatty acids (EFA) required by fish for regular growth, development, health and physiological functions. Lipids, as main structural components of cellular membranes, are essential for maintenance of homeostasis. In addition, lipids serve as carriers of fat-soluble nutrients including vitamins (A, D, E, K) and carotenoid pigments, and help in their absorption. Lipids are also precursors for a range of metabolic products, such as steroid hormones and vitamin (Sargent et al., 2002; Glencross, 2009; Turchini et al., 2009; Bell and Koppe, 2010; NRC, 2011).

2.2.2 Dietary lipid requirement in tilapia

A study on juvenile hybrid tilapia (*O. niloticus* × *O. aureus*) reported that 5% dietary lipids appeared to meet the minimal requirement of tilapia, and dietary lipid of

12% is optimal for maximum growth (Chou and Shiau, 1996). In blue tilapia, dietary 10% menhaden oil led to superior performance compared to other three lower lipid levels, 2.5%, 5.0% and 7.5% (Stickney and Wurts, 1986). However, higher dietary lipid levels (8%) did not improve growth of Mozambique tilapia × blue tilapia hybrids compared to those fed the 3% dietary lipid, suggesting lower dietary lipid can be used in tilapia diets in an intensive recirculating system if the dietary energy is supplied by appropriate carbohydrates (Fitzsimmons et al., 1997). By increasing dietary lipid and carbohydrates from 5.7% to 9.4% and from 31.9% to 36.9%, respectively, the dietary protein for Nile tilapia can be reduced from 33.2% to 25.7%, which results in a reduced protein-to-energy (P:E) ratio (Li et al., 1991). Given that the optimum dietary lipid level for various tilapia species is affected by the dietary protein and energy content (Chou and Shiau, 1996; Lim et al., 2011), dietary lipid level in a range of 5% to 12% is suggested to be optimal in diets containing 26–40% crude protein and with a P:E ratio of 80–120 mg protein/kcal of digestible energy (Lim et al., 2011)

2.3 Fish oil resource and production

Total world capture fisheries production continues to remain static at about 90 million tonnes, however, human consumption of fish is increasing due to growing knowledge of the nutritional value of n-3 PUFA, accounting for 87% of the total fish seafood supply in year 2010 and is estimated to increase to 131 million tonnes in 2011 (Table 2.1). Thus, the increasing demand of seafood coupled with the stagnation of wild capture fisheries places a dependence on the aquaculture industry. In the last three decades (1980-2010), global aquaculture had grown with an average annual growth rate of 8.8% and attained 12-fold increase in total aquaculture production (FAO, 2012).

Table 2.1 Total world fisheries production and utilization (million tonnes) for the period 1950-2011. Data 2011 is an estimate (Source: FAO, 2012).

	2006	2007	2008	2009	2010	2011
	(Million tonnes)					
PRODUCTION						
Capture						
Inland	9.8	10.0	10.2	10.4	11.2	11.5
Marine	80.2	80.4	79.5	79.2	77.4	78.9
Total capture	90.0	90.3	89.7	89.6	88.6	90.4
Aquaculture						
Inland	31.3	33.4	36.0	38.1	41.7	44.3
Marine	16.0	16.6	16.9	17.6	18.1	19.3
Total aquaculture	47.3	49.9	52.9	55.7	59.9	63.6
TOTAL WORLD FISHERIES	137.3	140.2	142.6	145.3	148.5	154.0
UTILIZATION						
Human consumption	114.3	117.3	119.7	123.6	128.3	130.8
Non-food uses	23.0	23.0	22.9	21.8	20.2	23.2
Population (*billions*)	6.6	6.7	6.7	6.8	6.9	7.0
Per capita food fish supply (*kg*)	17.4	17.6	17.8	18.1	18.6	18.8

FO is a major lipid source in aquafeeds which is deemed a major factor for the rapid expansion of aquaculture. Global fish meal and FO supplies have fluctuated between 4.57 and 7.48 million tonnes over the last 33 years and is now relatively stable, and indeed stagnant, at 5.0-6.0 million tonnes per year. In contrast, the use of FO in aquafeeds has risen from 0.46 million tonnes to 0.78 million tonnes between 1995 and 2008 (FAO, 2012). It is estimated that a total of 0.76 million tonnes of FO were consumed by the aquaculture industry for aquafeed production (Tacon et al., 2011) in 2010 which constituted about 86.8 % of the global FO supplies (Figure 2.3). In consideration of the finite resources of FO, this cannot sustain further aquaculture expansion and therefore alternatives are increasingly being investigated.

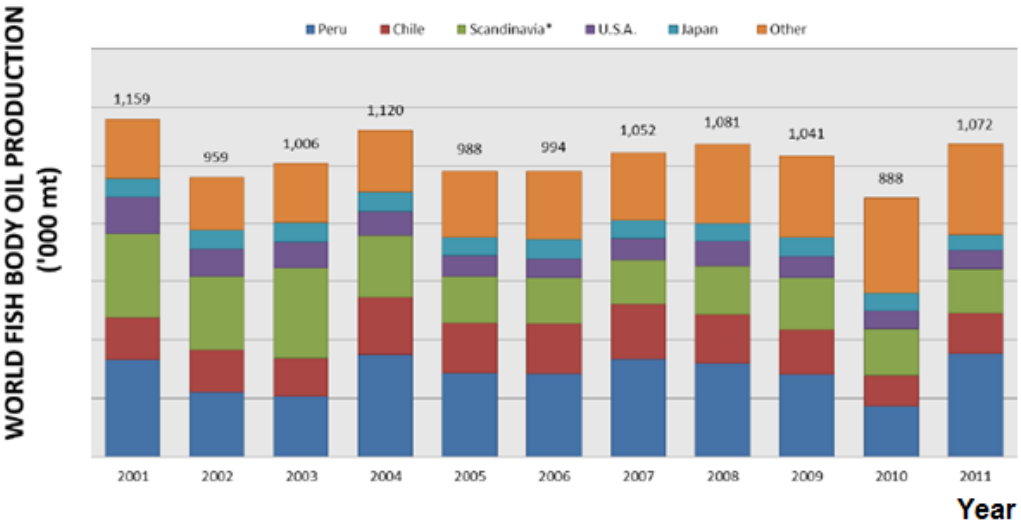


Figure 2.3 Fish oil production from 2001 to 2011. Data 2011 is an estimate (Source: Shepherd and Jackson, 2012).

2.4 Fatty acid

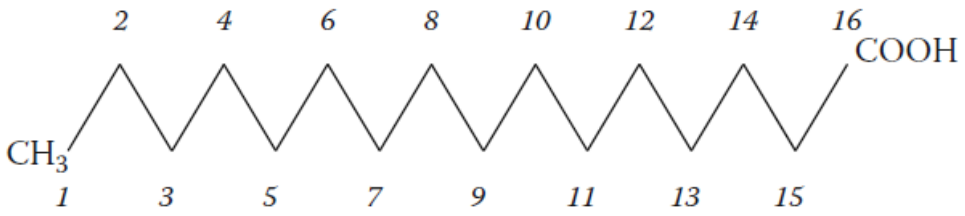
Fatty acids are carboxylic acids with a long aliphatic tail chain. Based on the International Union of Physical and Applied Chemists (IUPAC) standards, fatty acid nomenclatures are designated based on a sequence where the first number denoting the number of carbon chain length; the second (following a colon) is the number of double bonds; and the third is the position of the first double bond relative to the methyl terminal (Glencross, 2009; NRC, 2011). Beside this n- terminology, Δ nomenclature defines fatty acid structure by describing the position of a double bond from the carboxyl end of the chain (Bell and Koppe, 2010). Fatty acids can be divided into saturated fatty acids and unsaturated fatty acids depending on the presence or absence of these double bonds.

2.4.1 Saturated fatty acid

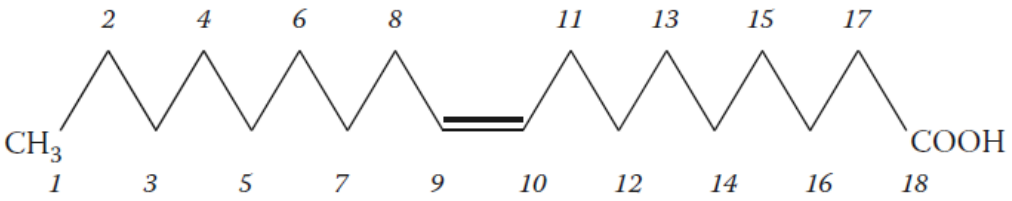
Saturated fatty acids (SFA) are the simplest fatty acids, as the carbon chains have no double bond. For example, palmitic acid, 16:0, contains 16 carbon atoms and no double bond (Figure 2.4). SFA are found naturally in animals with carbon chain length ranging 14 to 24. Due to the unchangeable geometric of the phospholipid bilayers in animal cells, phospholipids only contain 16:0 and stearic acid (18:0) (Sargent et al., 2002; Bell and Koppe, 2010). Plant oils such as palm oil is a rich source of dietary SFA, where palm oil contains high amount of 16:0 and 18:0, while palm kernel oil is abundant in 12:0 and 14:0 (Ng and Gibon, 2010).

2.4.2 Monounsaturated fatty acid

MUFA, is a term to designate fatty acids with a single double bond. One of the major MUFA found in both animals and plants is oleic acid, 18:1n-9, which has 18 carbon atoms and single double bond located at the ninth carbon from the methyl end (Figure 2.4). Using Δ nomenclature system, 18:1n-9 is defined as Δ9-18:1. MUFA with the chain lengths from C_{14} to C_{24} are found in animals but the MUFA in phospholipids are limited to a range of C_{16} to C_{20} ascribed to the geometric restraint of membrane bilayers (Sargent et al., 2002; Bell and Koppe, 2010). Rapeseed oil/canola oil (CO), olive oil (OO) and peanut oil are examples of oils naturally rich in MUFA that are commercially produced worldwide.



Palmitic acid 16:0

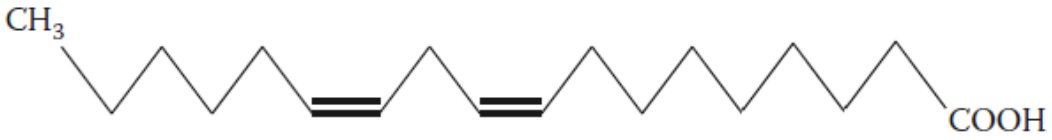


Oleic acid 18:1n-9

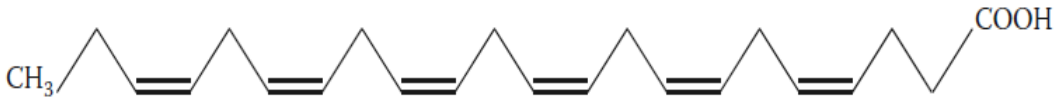
Figure 2.4 Palmitic acid and oleic acid, in the shorthand designation (Source: Bell and Koppe, 2010)

2.4.3 Polyunsaturated fatty acid

PUFA is a term used for fatty acids with two or more saturated double bonds. 18:3n-3 and 18:2n-6 (Figure 2.5) are the representatives of n-3 and n-6 series (omega 6), respectively. PUFA consisting of carbon chain lengths ≥ 20 and three or more double bonds are referred to as LC-PUFA. In freshwater fish, 18:2n-6 and arachidonic ARA, are the main n-6 PUFA and LC-PUFA, respectively. On the other hand, EPA and DHA, (Figure 2.5) are the dominant n-3 LC-PUFA in marine fish (Bell and Koppe, 2010). Using the Δ nomenclature, ARA is defined as Δ-5, 8,11, 14-20:4 as shown in Figure 2.6. VOs like sunflower, soybean, corn, safflower are the oils rich in n-6 PUFA (Brown and Steven, 2010); while linseed, perilla and echium oils are VO sources rich in n-3 PUFA (Tocher et al., 2010).

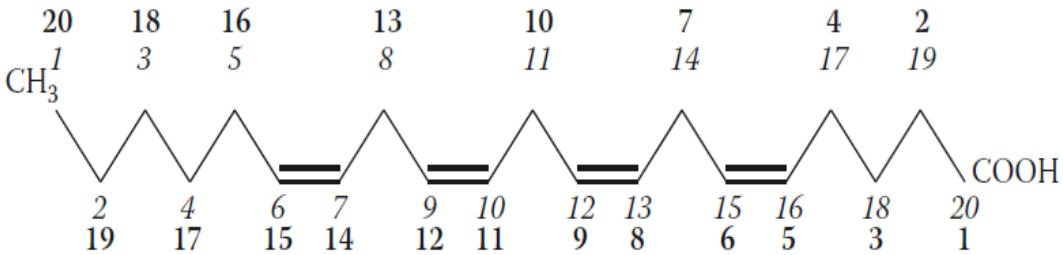


Linoleic acid 18:2n-6



Docosahesaenoic acid 22:6n-3

Figure 2.5 Linoleic acid, is a n-6 PUFA which has two double bonds with the first one located at the sixth carbon from methyl; docosahexaenoic acid, the n-3 LC-PUFA, has three double bonds which starts at the third carbon from the methyl terminal (Source: Bell and Koppe, 2010)



Arachidonic acid 20:4n-6
cis delta-5, 8, 11, 14-eicosatetraenoic acid

Figure 2.6 The n- (shown in italic) and Δ nomenclature for arachidonic acid Source: Bell and Koppe, 2010)

2.5 Essential fatty acids

Fish are unable to synthesize 18:2n-6 and 18:3n-3 *de novo* since they are lack Δ-12 desaturase and Δ-15 desaturase, and thus these fatty acids are regarded as EFA and must be supplied in the diet (Turchini et al., 2009; Bell and Koppe, 2010; NRC,

2011). However, vertebrates are able to produce 18:2n-6 and 18:3n-3 if 14:2n-6 and 14:3n-3 are provided, respectively (Cunnane, 2003). Given that these latter fatty acids rarely occur in nature, 18:2n-6 and 18:3n-3 are deemed the two basic EFA in fish. Like other vertebrates, freshwater fish including tilapia are capable of bio-converting C_{18} PUFA 18:2n-6 and 18:3n-3 to their homologous C_{20} and C_{22} LC-PUFA via a series of desaturation and elongation steps (Nakamura and Nara 2004; NRC, 2011) when fed diets rich in C_{18} PUFA (Kanazawa et al., 1980; Olsen et al., 1990;). To a certain degree, the ability of LC-PUFA biosynthesis is correlated to the EFA in fish (NRC, 2011). Generally, the EFA requirement of fish is influenced by nutritional and environment factors, and can vary within and among fish species based on their physiological stage and trophic level (Turchini et al., 2009; Bell and Koppe, 2010). In various fish species, deficiencies in EFA results in fin rot, shock syndrome, cardiac myopathy, reduced feed efficiency and growth rate and at longer durations, increased mortality (Castell et al., 1972; Takeuchi and Watanabe, 1977; Satoh et al., 1989; NRC, 2011).

2.5.1 Essential fatty acids requirement in tilapia

Like other warm water fish, n-6 PUFA is more preferred than n-3 PUFA for maximal growth of tilapia (NRC, 2011). Previous studies observed that Nile tilapia, *O. niloticus* (Takeuchi et al., 1983b) and red belly tilapia, *Tilapia zillii* (Kanazawa et al., 1980) have a requirement for n-6 PUFA at 0.5 % and 1.0% dietary level, respectively. In addition, a high dietary n-3 PUFA level might depress the growth of red belly tilapia (Kanazawa et al., 1980). Likewise, dietary supplementations of n-3 PUFA or LC-PUFA did not enhance the growth of Nile tilapia (Takeuchi et al., 1983a, b). On the other hand, Santiago and Reyes (1993) demonstrated that FO-based diets, which were

rich n-3 LC-PUFA, resulted in better growth in Nile tilapia, but led to a concomitantly lower (but not significantly) reproductive performance; female tilapia broodfish fed the FO-based diet showed lower reproductive performance than crude palm oil (CPO)-based diets (Ng and Wang, 2011). Nonetheless, Chou and Shiau (1999) reported that both n-3 and n-6 PUFA are essential to maximize growth performance in hybrid tilapia *O. niloticus* × *O. aureu.* Furthermore, Stickney and Hardy (1989) suggested that high dietary requirements of n-6 PUFA in blue tilapia can be reduced when n-3 PUFA are provided.

2.6 Fatty acid composition in fish tissue

Neutral lipids and polar lipid are the two types of lipids that exists in fish muscle. Neutral lipid, mainly TAG, serves as local energy stores and is distributed within the cytoplasm of cells; while phospholipids, which is a polar lipid, is a main component of the cellular membranes (Henderson and Tocher, 1987; Frøyland et al., 1998). In muscle, phospholipids are the major lipid form and consists of high proportions of PUFA while in the liver, TAG (>90%) comprise the major lipid form composition (Dos Santos et al., 1993; Nanton et al., 2001; Tocher et al., 2003a). Thus, white muscle serves as fatty acid storage, and the liver is an energy depot as lipid metabolism and n-3 LC-PUFA biosynthesis occurs in this tissue (Codabaccus et al., 2011a).

The physical composition of TAG and phospholipids are affected by their degree of unsaturated fatty acids (Nakamura and Nara, 2004). LC-PUFA, such as DHA and EPA, are mainly incorporated in muscle phospholipids which is deemed to be the typical fatty acid form when incorporated in fish (Sargent et al., 1989; Nakamura and

Nara, 2004). This is due to the tendency of fatty acyl transferase enzymes towards deposition of DHA into muscle lipid (Codabaccus et al., 2011b). On the other hand, DHA is used for cellular functions and is rapidly incorporated into phospholipis or transferred out of peroxisomes, while the remaining DHA will be degradated in peroxisomes (Nakamura and Nara, 2004). Meanwhile, ARA is more inclined to be transported and deposited in the gonads, followed by the liver and muscle (Norambuena et al., 2013).

In order to function properly at different temperatures, biomembranes must be in a fluid state and the fluidity depends on the balance between SFA and MUFA of membrane phospholipids. During acclimation to cold water, fish change their biomembrane phospholipid fatty acid composition which is known as homeoviscous adaptation (NRC, 2011).

C_{20} PUFA, including ARA, EPA, 20:3n-6 and 20:4n-3 are precursors to eicosanoids, such as prostaglandins, prostacyclins, thromboxanes, and leukotrienes (Leonard et al., 2002; Twibell et al., 2012). Hence, PUFA are important elements that take part in a variety of physiological functions since eicosanoids work as autocrine/paracrine hormones influencing blood coagulation, immune and inflammatory responses, reproduction, fetal growth and brain development, cardiovascular tone, as well as the functions of neural, renal and visual (Leonard et al., 2002; Sargent et al., 2002; Twibell et al., 2012; Nakamura and Nara, 2004). Specifically, EPA and ARA are substrates of prostaglandins (PG) from series III and series II, respectively. PG E II, which is converted from ARA, stimulates testicular testosterone in goldfish and is antagonistic to EPA and DHA which reduces steroidogenesis in the testis (Izqueirdo et al., 2001). Indeed, ARA is a preferred substrate and the ARA-derivate eicosanoid has a higher biological activity than that of

EPA, however the efficiency of ARA is somewhat influenced by the availability of EPA (Bell et al., 1994; Copeman et al., 2002).

2.7 Dietary alternative lipid sources

Implementing sustainable alternatives to FO in aquafeeds is inevitable in the aquaculture industry. The use of VO is a viable alternative as VO are more sustainable, available (Table 2.2) and cost-effective than FO. Moreover, the production of major VO continues to increase, particularly palm oil, soybean oil, canola/rapeseed oil and sunflower oil (SFO), attaining more than one million tonnes within 2005-2006 (Turchini et al., 2009). VO can be classified into 4 categories based on their major fatty acid classes: 1) SFA-rich oils, 2) n-6 PUFA-rich oils, 3) n-3 PUFA-rich oils and 4) MUFA-rich oils.

Palm oil is one of the SFA-rich oils, and the use of crude palm kernel oil (CPKO) and various palm oil products in aquafeeds are reported to have various benefits to fish (Ng and Gibon, 2010). With the presence of vitamin E and carotenoids, dietary palm oil can prolong the shelf-life of fish fillet by increasing their oxidative stability. In addition, the expanse of oil palm tillage in tropical countries, especially Malaysia and Indonesia, increases the potentiality of palm oil-based products for aquafeed formulations (Ng and Gibon, 2010). The nutritional value of n-6 PUFA-rich VO such as soybean oil, SFO, cottonseed oil and corn oil as dietary lipid source have been evaluated and results indicate that these have positive impacts on reproduction and larval feeding (Brown and Steven, 2010). Linseed oil (LO), perilla oil (PeO) and echium oil are popular n-3 PUFA-rich VO sources, although these VOs can be more expensive. The inclusion of these n-3 C_{18} PUFA oil are deemed favorable alternative

lipid sources due to their high dietary content of 18:3n-3 and/or 18:4n-3 and the potential for bio-transforming these fatty acids to n-3 LC-PUFA which is beneficial to human health (Tocher et al., 2010). MUFA-rich oils, including canola/rapeseed oil, OO, groundnut oil (also known as peanut oil) are likely to be suitable candidates as MUFA is a good source of available energy, and their deposition into fish flesh is considerably less pernicious than other fatty acid classes for human consumers (Turchini and Mailer, 2010).

Table 2.2 Global production (thousand tonnes) of fish oil and vegetable oils from 1980 to 2006. (Source: Turchini et al., 2009).

Oils	1980	1985	1990	1995	2000	2005	2006
Fish oil	1217	1481	1412	1379	1307	1054	988
Vegetable oils							
Palm oil	4543	6832	11020	20322	25594	33733	36733
Soybean oil	13382	13794	16097	15119	21743	33575	35187
Canola oil/rapeseed oil	3478	6066	8160	10936	14496	16205	18340
Sunflower oil	5024	6564	7869	7003	9808	9661	11094
Cottonseed oil	2992	3942	3782	3312	3815	4989	4917
Groundnut oil	2864	3575	3897	4325	4382	4523	4497
Palm kernel oil	571	868	1450	1877	2620	3975	4308
Coconut oil	2716	2627	3387	3253	3147	3257	3166
Olive oil	1701	1796	1855	1863	2513	2916	2746
Corn oil	-[1]	-	-	1855	1966	2099	2252
Sesame oil	-	-	-	589	705	823	871
Linseed oil	-	-	-	701	705	607	710

[1]Data not available.

2.7.1 Effect of fish oil replacement on growth

Studies on FO replacement had been done on different genotypes of tilapia. In Nile tilapia, the effect of FO replacement was investigated by using five isonitrogenous and isoenergetic diets which were supplemented with 3% of either FO, LO, a mixture of LO with refined palm olein oil (2:1), a mixture of refined palm olein oil with LO (3:2) or CO. The results showed that dietary FO can be totally replaced by LO and its blend with refined palm olein oil without any negative effect on growth and feed

efficiency (Karapanagiotidis et al., 2007). A study on grow-out feeding using either FO, coconut oil, grapeseed oil, LO, or poultry oil as the dietary lipid source, showed that production was unaffected, indicating that these oils can be efficiently utilized by Nile tilapia (Trushenski et al., 2009). Various dietary lipids including SFO, CPO, CPKO, or a mixture of cod liver oil (CLO) with palm fatty acid distillates (PFAD) were used to replace CLO in the diets of red hybrid tilapia, and dietary CLO was found to slightly reduce fish growth and feed efficiency ratio, which is envisaged to the supplementation of 10% CLO exceeding the tilapia biological tolerance limit for n-3 fatty acids (Ng et al., 2001). Furthermore, dietary FO was replaced by different palm oil sources: CPO, PFAD or refined, bleached and deodorized palm olein (RBDPO) in red hybrid tilapia (Bahurmiz and Ng, 2007). The authors demonstrated that fish growth performance, feed conversion ratio, survival, body indices, hematocrit and production yield were not significantly ($P>0.05$) affected by the dietary palm oil source.

Apart from tilapia species, a variety of research on FO substitution had been carried out on other fish species, by either using different VOs or animal fats as the sole lipid source, as well as blending various alternative lipid sources to formulate the desired fatty acid composition (Table 2.3 & 2.4). Many studies on Atlantic salmon (*Salmon salar*) showed that partial or full FO replacement using different dietary VOs did not significantly affect the growth performance (Bell et al., 2001a; Bransden et al., 2003; Menoyo et al., 2005; Miller et al., 2007; Codabaccus et al., 2011b). A study on gilthead seabream (*Sparus aurata*) showed that 80% inclusion of LO did not adversely affect the growth performance, but the same inclusion level of soybean oil significantly reduced the body weight of fish (Menoyo et al., 2004). However, full FO replacement with soybean oil, rapeseed oil or LO in the microdiets for gilthead seabream larvae seems to be possible when the EPA and DHA requirements are met from the dietary

contents of fish or squid meal. In European seabass (*Dicentrarchus labrax*), partial FO replacement with different blends of rapeseed oil, LO and palm oil in the diets showed no significant difference on growth rate or survival (Mourente and Bell, 2006). Different VOs or pork lard was partially included in the diets of rainbow trout (*Oncorhynchus mykiss*) and no differences in growth rate were observed, however, the FCR was negatively affected by the lard oil (Caballero et al., 2002). In African catfish (*Clarias gariepinus*), dietary SFO, RBDPO, CPO or CPKO led to significantly higher growth and feed efficiency ratio than CLO, and blending CLO with PFAD or spent bleaching clay alleviated the growth reduction observed in fish having CLO as the sole lipid source (Ng et al., 2003b). For Murray cod (*Maccullochella peelii peelii*), Senadheera et al. (2010) concluded that full FO replacement significantly depressed growth of this species when the dietary fish meal content was low (<10%). In high energy diets for trout, FO can be replaced by other lipid sources up to 80-90% without any detrimental effects on growth (Caballero et al., 2002). Collectively, dietary FO can be partially or completely replaced by VO without compromising fish growth performance if their essential fatty acid requirements are met.

Moreover, the effect of dietary 18:3n-3/18:2n-6 ratios on fish growth has been evaluated to better understand the nutritional roles and interactions of these EFA in maximizing their performance. The optimal dietary 18:3n-3/18:2n-6 ratio for growth is 1.17 and 2.12 for yellow catfish (*Pelteobragus fulvidraco*), and the relatively low ratio (0.13) led to growth retardation in freshwater fish Tench (*Tinca tinca*) (Turchini et al., 2007b; Tan et al., 2009). However, no significant effect of the 18:3n-3/18:2n-6 ratios on the growth performance of Eurasian perch (*Perca fluviatilis*) or Murray cod were observed (Table 2.5).

2.7.2 Effect of fish oil replacement on fatty acid composition

Generally, the fatty acid composition of the dietary lipid source is mirrored in the fatty acid composition in fish tissue, including gilthead seabream (Menoyo et al., 2004; Izquierdo et al., 2008; Grigorakis et al., 2009), Murray cod (Francis et al., 2006; 2007a; 2007c), rainbow trout (Caballero et al., 2002; Turchini et al., 2009; Thanuthong et al., 2011a), brown trout (*Salmo trutta*) (Turchini et al., 2003), Eurasian perch (Blanchard et al., 2008), European seabass (Mourente and Bell, 2006), tench (Turchini et al., 2007b), Arctic char (*Salvelinus alpinus*) (Tocher et al., 2006), Atlantic halibut (*Hippoglossus hippoglossus*) (Martins et al., 2007), Atlantic salmon (Bell et al., 2001; Bransden et al., 2003; Zheng et al., 2004; Menoyo et al., 2005; Codabaccus et al., 2011b), African catfish (Ng et al., 2003b), yellow catfish (Tan et al., 2009) and red hybrid tilapia (Ng et al., 2001; Bahurmiz and Ng, 2007). As VO does not contain any n-3 LC-PUFA, which are known to be beneficial to human health, this is thus the most stringent drawback of FO replacement with VO due to the profound reduction of n-3 LC-PUFA in the fish fillet (Turchini et al., 2009).

However, dietary lipids high in SFA, such as CPO and CPKO did not significantly increase the SFA content in tilapia tissue (Ng et al., 2001). Other studies also observed that dietary SFA tend to be incorporated into fish fillet within a particular physiological level (Bell et al., 2002; Bahurmiz and Ng, 2007). Besides, a modification of fatty acid composition of fish tissue is not affected to the same magnitude but varied depending on the tissue type. (Bell et al., 2001a; Turchini et al., 2009). The proportion of polar lipids relative to neutral lipids in tissue determines the similarity of the fatty acid profile between diets and fish tissue (Olsen and Henderson, 1997; Turchini et al., 2009). Moreover, the incorporation fatty acids into fish tissue is also influenced by