OMEGA-3 LONG CHAIN POLYUNSATURATED FATTY ACID (n-3 LCPUFA) LEVELS IN CHICKEN PRODUCTS FOLLOWING CONSUMPTION OF ALPHA-LINOLENIC ACID ENRICHED DIETS

Lilik Retna Kartikasari
M.Ag.Sc. (University of Adelaide)

A thesis submitted in fulfilment of the requirements for the Degree of Doctor of Philosophy

School of Agriculture, Food and Wine
Faculty of Sciences
University of Adelaide
Adelaide, South Australia, Australia
April 2013

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

ABSTRACT

The importance of dietary omega-3 long chain polyunsaturated fatty acids (n-3 LCPUFAs) in human health has promoted interest in developing a range of n-3 rich foods. The inclusion of n-3 FA into chicken products can be achieved by feeding chickens marine n-3 LCPUFA sources such as fish oil. However, this dietary approach has proven problematic due to impaired sensory qualities in the chicken products. The inclusion of the plant n-3 PUFA source alpha-linolenic acid (ALA, 18:3n-3), the n-3 LCPUFA precursor, into the diet of chickens is potentially an alternative way to provide chicken products rich in n-3 LCPUFAs without these detrimental sensory effects.

The objectives of the study were to investigate whether including ALA in the diets of two strains of laying hens and two strains of broilers would increase n-3 LCPUFA accumulation in eggs and meat, without altering production performance or the sensory characteristics of the products. The levels of dietary ALA tested in both laying hen and broiler experiment were 0.3 (low), 3 (moderate) and 6% energy (high; %en), while holding the level of LA constant at around 4%en in the moderate and high ALA diets.

The findings in this study demonstrated that independent of strain, for both eggs and meat, the level of EPA was directly related to the level of ALA in the diet. On the other hand the longer chain fatty acids, DPA and DHA, tended to reach maximal levels when the level of dietary ALA reached 3%en. The level of total n-3 fatty acids

in products from chickens fed ALA enriched diets (3 or 6%en for laying hens and 6%en for broilers) met the requirement needed for labelling as egg and meat n-3 PUFA sources (300mg/egg or 300mg/100g of meat). Fatty acid analysis of lipid fractions in breast meat showed that while ALA was mainly associated with triglyceride (TG) fraction, the n-3 LCPUFA were preferentially deposited in the phospholipids (PL). There was strain dependence in the ability of the chickens to convert ALA into n-3 LCPUFA. Among layers, brown hens were found to be more effective in converting ALA to n-3 LCPUFA than white hens, whereas in broilers, Cobb birds were more effective in the accumulation of n-3 LCPUFA than Ross birds.

Dietary ALA enrichment up to a level of 6%en did not influence the sensory quality of boiled eggs whereas in scrambled eggs, high ALA diets tended to decrease egg aroma. Importantly, a diet enriched with 3%en ALA did not change the consumer acceptance of the eggs compared with eggs purchased from a local supermarket. In broilers, a diet containing ALA 3%en did not affect any of the sensory attributes tested. Importantly, the sensory quality of chicken breast meat from birds fed a dietary ALA of 3%en was comparable to that of commercial breast meat purchased from a local supermarket. There were strain effects on the sensory attributes of the eggs, with boiled brown eggs having a significantly ($P < 0.05$) stronger after-taste than boiled white eggs whereas white eggs had a stronger ($P < 0.05$) sulphur flavour than brown eggs. In scrambled eggs, stronger egg aroma, sulphur flavour, and butter flavour were detected in brown eggs ($P < 0.05$) than white eggs.

In conclusion, a dietary ALA level of approximately 3%en could be recommended as a good ALA level for producing eggs and chicken meat n-3 LCPUFA by industry. The findings of this study demonstrated that incorporating n-3 rich vegetable oils into chicken diets could be an alternative to marine sources to produce eggs and meat higher in n-3 LCPUFA, without influencing either production performance of birds or sensory qualities of the chicken products. This strategy would help to provide consumers with a variety of foods rich in n-3 LCPUFA, and help to achieve recommended intakes for human health.

DECLARATION

This is to certify that the data contained in this thesis is my own work and this work contains no material which has been accepted for the award of any other degree or diploma in any university or other tertiary institution to Lilik Retna Kartikasari and, to the best of my knowledge and belief, contains no material previously published or written by another person, except where due reference has been made in the text.

I give consent to this copy of my thesis when deposited in the University Library, being made available for loan and photocopying, subject to the provisions of the Copyright Act 1968. The author acknowledges that copyright of published work contained within this thesis (as listed below) resides with the copy right holder(s) of those works.

I also give permission for the digital version of my thesis to be made available on the web, via the University's digital research repository, the Library catalogue, the Australasian Digital Theses Program (ADTP) and also through web search engines, unless permission has been granted by the University to restrict access for a period of time.

Publications:

Comparison of omega-3 level in two strains of broilers and layers fed high alpha-linolenic acid diets. Proceedings of 23rd Annual Australian Poultry Science Symposium Sydney, New South Wales, Australia, February 2012.

***Lilik R Kartikasari**, Mark S Geier, Robert J Hughes, Susan EP Bastian, Maria Makrides, Robert A Gibson*

Conference presentations/abstracts:

Omega-3 and sensory properties of chicken meat of two strains of broilers fed high alpha-linolenic acid diets. The Australian Society for Medical Research South Australia Annual Scientific Meeting, Adelaide, 6 June 2012.

***Lilik R Kartikasari**, Robert J Hughes, Mark S Geier, Susan EP Bastian, Maria Makrides, Robert A Gibson*

Omega-3 enrichment and sensory properties of eggs of two strains of laying hens fed high alpha-linolenic acid diets. The 10th Conference of the International Society for the Study of Fatty Acids and Lipids, Vancouver, Canada, May 26-30, 2012.

***Lilik R Kartikasari**, Robert J Hughes, Mark S Geier, Susan EP Bastian, Maria Makrides, Robert A Gibson*

Production performance, omega-3 and sensory properties of eggs of brown and white hens fed diets rich in alpha-linolenic acid. The Australian Society for Medical Research South Australia Annual Scientific Meeting, Adelaide, 8 June 2011.

***Lilik R Kartikasari**, Robert J Hughes, Mark S Geier, Susan EP Bastian, Maria Makrides, Robert A Gibson*

Production performance and sensory properties of eggs of brown and white hens fed diets rich in apha-linolenic acid. The 2010 School of Agriculture, Food and Wine Research Day, University of Adelaide, Australia, 29 October 2010.

***Lilik R Kartikasari**, Robert J Hughes, Mark S Geier, Susan EP Bastian, Maria Makrides, Robert A Gibson*

Optimizing omega-3 long chain polyunsaturated fatty acids in chicken products. The postgraduate student's symposium, School of Agriculture, Food and Wine, University of Adelaide, Australia, 22 September 2010.

***Lilik R Kartikasari**, Robert J Hughes, Mark S Geier, Susan EP Bastian, Maria Makrides, Robert A Gibson*

Omega-3 enrichment of chicken meat following the consumption of omega-3 rich diets. The Australian Society for Medical Research South Australia Annual Scientific Meeting, Adelaide, Australia, 9 June 2010.

***Lilik R Kartikasari**, Robert J Hughes, Mark S Geier, Susan EP Bastian, Maria Makrides, Robert A Gibson*

Lilik Retna Kartikasari

Date : 17 April 2013

ACKNOWLEDGEMENTS

First and foremost, I would like to express my deepest respect and sincere gratitude to my principal supervisor Professor Robert Gibson, for introducing me into the fascinating world of fatty acids, continuous support and guidance throughout my PhD candidature. I am grateful to various opportunities he provided and supported through research topics and scientific international conferences, which greatly expand my knowledge in the field of functional food science. His enthusiasm for my research topic and tremendous expertise is very much appreciated. I am deeply indebted to him for his patience, spending time in discussion and regular meeting despite his busy schedule, and providing me a comfortable space to work conveniently. Without his kind guidance, constant encouragement, valuable suggestions, constructive comments and help in each and every stage of my study, it would have been impossible for me to finish this study. Thank you for all you do Bob.

I would also like to acknowledge my co-supervisor Dr Mark S Geier for his constant support, guidance, and encouragement throughout the program. His contributions, constructive criticism and advice on my thesis and manuscript have been of great value to me. Your commitment, efforts and generosity with your time in helping me during my study have been terrific. I would say that without your sincere assistance this thesis would not have reached its present form. Thank you for all your kind help, Mark.

I would like to thank my co-supervisor, Dr. Susan Bastian, for her encouragement, advice, and sincere efforts to teach me all the techniques of conducting sensory

evaluation in my research project. I greatly appreciate for all your kind guidance and providing me sensory laboratory facilities. Without your support and valuable suggestions in the sensory study, this work is not as detailed and constructive as it looks.

I am grateful to Prof. Maria Makrides for giving me inspiration on how to become a better scholar and sharing valuable knowledge in her field. Many thanks to Ms Anna Seamark for her support during my candidature.

I sincerely thank Dr. Robert Hughes, Pig and Poultry Production Institute (PPPI), SARDI, for his continuous support, providing all facilities for growing chickens, and advices on the laying hen and broiler experiment. I really appreciate him for the knowledge and expertise he shared with me on poultry production. I would also like to extend my gratitude to technical support staff of PPPI, in particular to Mr. Derek Schultz, Ms. Evelyn Daniels, Ms. Kylee Swanson, Ms. Natasha Edwards, and Ms. Sue Walker for their professional and generous assistance in animal experiments.

I would also like to acknowledge many people who have generous supported and helped me dealing with the challenges of a research project and technical aspects. I am sincerely grateful to Dr. Olena Kravchuk for all her valuable suggestions, kind assistance, and teaching me on statistical analysis. I would like to express my gratitude to Dr. Michelle Picard and Dr. Margaret Cargill for their generous support and kind guidance in writing thesis and manuscript, and presentation preparation. I am grateful to Dr. Ronald Smernik for his efforts, valuable comments and expertise that he

contributed to reviewing this thesis. I would also like to thank Ms Ella Zielinski and Mr. David Apps for guiding me in the learning of experimental techniques, technical assistance and advice in the fatty acid analysis. I gratefully acknowledge Ms. Lucy Zuzolo and Ms. Angela Mills for their generous guidance and expertise in endnote and bibliography.

I extend my sincere thanks to all colleagues of the FoodPlus group for their support and assistance in various ways during my study at Waite Campus. In particular, I am much obliged to Dr. John Carragher for his constructive comments in my manuscripts and his caring advice during my study. Many thanks go to Dr. Beverly Muhlhausler for her support in my study and encouragement attending conferences. I would also like to acknowledge Dr. Peter Clements for his valuable comments and reviewing my thesis. I wish to extend my special thanks to Kanch Wikramarachi, Laura Blake, and Liu Ge for their friendship and for keeping me calm and happy during latter-stage of my study. Many thanks to Dr. Jo Zhou, Ms Teresa Fowles, Dr. Weichun Tu, Jing Zhou, Yichau Huang, Pamela Su-Lin Sim, Mini A Vithayathil, and many other staffs and students for their kind support and friendships.

I would also like to thank all the panel members who participated in the sensory evaluation and consumer study and making this research successful. I am grateful to Kapowie Processing and Services, Kapunda, North Adelaide for helping in chicken processing and TAFE SA, Regency Campus, Adelaide, South Australia for their kind helping in providing vacuum packaging facilities. Many thanks to Andrew Dunn for his support and providing facilities for consumer study. I am also thankful to Melrose

Health Supplies for their generous contribution of flaxseed oil and Macadamia for helping in supply macadamia oil.

I gratefully acknowledge Directorate General of Higher Education, Ministry of National Education Indonesia for awarding me a DIKTI scholarship, providing me financial support during my PhD program and granting me a study leave. At this stage, I would like to extend my sincere thank to DEFFST, Australia, which contributed to this research project by partially funding the project.

I would also like to thank Sebelas Maret University (UNS), Indonesia for giving me the opportunity to experience studying at University of Adelaide. In particular, I sincerely thank to Prof. Dr. Ravik Karsidi, M.S. and Prof. Dr. Sutarno for their continuous support and encouragement for successful completion of this study.

Lastly and most importantly with all my heart, my deepest gratitude and appreciation are devoted to my mother and father (in memorial), and my parents in law for their love, unwavering support, and constant encouragement in my study and career. I express my sincere thanks to my dear and loving husband, Feri Satria, and my lovely daughter, Geraldin Noverina, for their boundless love and patience. I would say that without their understanding, caring, and continuous support at each and every moment of this effort, it would have been impossible for me to finish this arduous journey. To them I dedicate this thesis.

ABBREVIATIONS

AA	Arachidonic acid (20:4n-6)
ALA	Alpha (α)-linolenic acid (18:3n-3)
ANOVA	Analysis of variance
BHA	Butylated hydroxyanisol
CHD	Coronary Heart Disease
CVD	Cardiovascular diseases
DHA	Docosahexaenoic acid (22:6n-3)
DPA	Docosapentaenoic acid (22:5n-3)
EFA	Essential fatty acid
EPA	Eicosapentaenoic acid (20:5n-3)
FAME	Fatty acid methyl ester
GC	Gas chromatograph
GLA	γ-linolenic acid
H	Hydrogen
H_2SO_4	Sulphuric acid
ISSFAL	International Society for the Study of Fatty Acids and Lipids
LA	Linoleic acid (18:2n-6)
MUFA	Monounsaturated fatty acid
NS	Not significant
n-3	Omega 3
n-6	Omega 6
n-9	Omega 9
Na_2SO_4	Sodium sulphate
NHMRC	National Health and Medical Research Centre
NNS	National Nutrition Survey
LCPUFA	Long chain polyunsaturated fatty acid
PL	Phospholipids
PUFA	Polyunsaturated fatty acid
SARDI	South Australia Research and Development Institute

SDA	Stearidonic acid
SFA	Saturated fatty acid
TL	Total lipid
TG	Triglycerides
TLC	Thin layer chromatography
UV	Ultraviolet

UNITS

°C	Celcius
cm	Centimetre
d	Day
et al.	and others
g	Gram
h	Hour
kg	Kilogram
L	Litre
mg	Milligram
mL	Millilitre
m^2	Square metre
µ	Micro
v/v	Volume by volume

CHAPTER 1

INTRODUCTION AND LITERATURE REVIEW

1.1 Introduction

Adequate intake of the omega-3 long chain polyunsaturated fatty acids (n-3 LCPUFA), especially eicosapentaenoic acid (EPA, 20:5n-3) and docosahexaenoic acid (DHA, 22:6n-3) have been shown to have a positive impact on human health [1-4] and consequently, increasing dietary EPA and DHA consumption is recommended by health authorities [5-8]. One approach to enhance the dietary n-3 LCPUFA intake of humans is to increase the consumption of fish and fish oil [9, 10]. As many people in Western societies consume very little fish [11], there is a need to provide an alternative source of n-3 enriched foods. Chicken meat and eggs are popular foods worldwide. For example, chicken meat is the main contributor (73%) of meat n-3 LCPUFA intakes in the United Kingdom [12]. The pattern of meat consumption in Australia [13], UK [14], and US [15] shows the increase in chicken meat consumption. Increasing the level of n-3 LCPUFA in chicken products may be a better strategy to increase n-3 LCPUFA intakes of the population without changing existing dietary habits.

One way to increase n-3 LCPUFA accumulation in chicken products (eggs and meat) is to enhance the concentration of dietary n-3 LCPUFA in chicken feeds, through the supplementation of fish meal or fish oil. However, the use of fish oil in the manipulation of meat fatty acid composition may result in negative effects on the sensory properties of chicken products [16-18].

An alternative to fish oil supplementation of chicken diets is to increase n-3 LCPUFA accumulation in chicken products through the dietary inclusion of vegetable oils high in the n-3 PUFA, alpha-linolenic acid (ALA, 18:3n-3), the precursor of all n-3 LCPUFA. This can be achieved by enriching diets with flaxseed or canola oils. While some authors [19-21] have reported an increase in chicken tissue n-3 LCPUFA including EPA, docosapentaenoic acid (DPA, 22:5n-3), and DHA following the consumption of diets rich in ALA, others have failed to demonstrate any effect [22, 23]. The variation of response in such studies may be due in part to a high ratio of dietary linoleic acid (LA, 18:2n-6) to ALA. The age and strain of bird may also contribute to the variation in n-3 LCPUFA levels. Therefore, the focus of this study is to determine the role of dietary ALA on n-3 accumulation in chicken products from two strains of birds.

1.2 Literature review

Both methods of increasing the n-3 LCPUFA content of chicken products, either by direct inclusion of n-3 LCPUFA in the diet or by inclusion of the n-3 LCPUFA precursor, ALA, are covered in this literature review. The remainder of this chapter reviews current knowledge on the effects of diets supplemented with vegetable oils high in ALA in order to address the question of whether high ALA diets can maintain the sensory properties of chicken products. In addition, this review also covers the ratio of n-6 to n-3 PUFA in diets, the health benefits of n-3 LCPUFA, the recommended intake of n-3 LCPUFA, dietary intake of n-3 fatty acids, fish oil problems in chicken products and factors affecting n-3 enriched chicken products.

The last section of the chapter summarises the main gaps in the field and the aims of this research.

1.2.1 Omega-3 polyunsaturated fatty acids

There are three kinds of fatty acids associated with all fats: saturated, monounsaturated and polyunsaturated [24]. This classification is based on the number of double bonds in the fatty acid chains of the molecule [24, 25]. Fatty acids which have no double bonds in the molecule are named saturated fatty acids (SFA); those which include one double bond are termed monounsaturated fatty acids (MUFA), whereas fatty acids with two or more double bonds are called polyunsaturated fatty acids (PUFA). PUFA are grouped into n-3 and n-6 fatty acids [24, 26]. This classification is established by the first site of the double bond in the molecule. A PUFA is defined as an omega-3 (n-3) fatty acid when the first double bond is located after the third carbon, counting from the methyl (CH3) end of the molecule. When the first double bond is placed after the sixth carbon, it is referred to as an n-6 (omega-6) PUFA. The biosynthetic capacity of lipids in the body can provide all of the required fatty acids with the exception of ALA and LA. Neither of the n-3 or n-6 precursors, LA or ALA can be produced by human; therefore they must be attained in the diet and are thus known as essential fatty acids [24, 25, 27].

The biologically important n-3 fatty acids include EPA, DPA and DHA. ALA can be converted to EPA, DPA and DHA by the same desaturase and elongase enzymes that convert LA to arachidonic acid (AA, 20:4n-6)) [28]. EPA is the precursor of the 3-series prostaglandins and the 5-series leukotrienes [28], a group of eicosanoids that

are anti-inflammatory, antithrombotic and vasodilatory [27]. DHA is the precursor of a newly identified series of prostaglandins, known as docosanoids, that includes resolvins that are thought to have potent anti-inflammatory and immunoregulatory actions at concentrations in the nanomolar and picomolar range [29].

Similarly, the most important n-6 fatty acids for the human diet are LA and AA. The main source of LA is vegetable oils, seeds and nuts [24, 25]. LA is considered to have beneficial health effects as it can lower plasma cholesterol; however, AA is the precursor of the 2-series prostaglandins, thromboxanes and the 4-series leukotrienes [28] which have pro-inflamatory and prothrombic [27, 28] properties and are known to be involved in various pathological processes such as atherosclerosis, bronchial asthma and several inflammatory conditions [28]. A high consumption of n-6 PUFA and a very high n-6 to n-3 ratio promote the pathogenesis of many diseases including cardiovascular diseases, cancer, inflammatory and autoimmune diseases [30].

1.2.2 The ratio of n-6 to n-3 PUFA in diets

For the purpose of this review, the discussion of n-6 to n-3 ratio focuses on the 18 carbon PUFA found primarily in fats and oils of vegetable origin (LA and ALA). The positive role of n-3 PUFA in human health has accentuated the need to increase the consumption of these fatty acids [31]. An important determinant for the optimal conversion of ALA to n-3 LCPUFA, is the ratio of LA to ALA in diets. A ratio of LA to ALA of 4:1 has been suggested as optimal for the conversion of ALA to n-3 LCPUFA for humans [32]. Kris-Etherton et al. [7] recommended the LA to ALA ratio of 2.3:1. In Western diets, the ratio is in the range of 10:1 to 20:1 [33], which

indicates that Western diets are deficient in ALA and have excessive amounts of LA. LA consumption has increased from approximately 3% of energy in the early 1900s, and now contributes 5-7% of dietary energy in Western nations due to an increase in dietary intake of vegetable oils that are high in n-6 fatty acids [34]. Increasing the consumption of ALA is important to promote a high level of n-3 fatty acids in tissues [35] and impact positively on human health [30, 36].

1.2.3 The health benefits of n-3 PUFA

Dietary n-3 PUFA have health benefits in the prevention and treatment of heart disease [4, 37] and rheumatoid arthritis [30], while also enhancing neural and brain development [1, 2, 38, 39]. Several prospective cohort studies have found a negative association between fish consumption and the risk of coronary heart disease [40, 41] or sudden cardiac death [42]. Nordoy et al. [41] reported that fish consumption of 1-2 meals/week was associated with a reduced incidence of coronary heart disease (CHD). In addition, the consumption of EPA and DHA in the range of 0.5 to 1.8g/day significantly decreased the number of deaths from heart disease and all causes of mortality [43]. Dietary n-3 LCPUFA (EPA and DHA) has also demonstrated effectiveness for the treatment of CHD. Consumption of 1g/day of ethyl esters of n-3 LCPUFA was found to decrease the risk of cardiovascular and coronary disease and sudden death in patients who had experienced myocardial infarction [41]. Studies such as this firmly established that the active ingredient in fish and fish oils is the n-3 LCPUFA.

Similarly, dietary n-3 LCPUFA also has beneficial effects on the prevention and treatment of rheumatoid arthritis (RA) because n-3 LCPUFA has a strong anti-inflammatory component [44, 45]. A study has shown that the Japanese, who consume a high level of n-3 LCPUFA through fish, have a lower rate of RA (0.4%) than Western communities (1%) [25]. Other studies demonstrated that daily supplementation with n-3 LCPUFA resulted in significant clinical benefits in RA [46]. In addition, there are the potential drug-sparing effects between drug therapy and dietary n-3 PUFA by increasing the consumption of n-3 and decreasing n-6 PUFA [45], especially for patients with a preference for natural treatment [25]. James and Cleland [45] suggested that fish oil consumption at dosages of at least 3g/day had beneficial effects on RA.

Furthermore, there is a positive impact of n-3 LCPUFA on brain development. DHA is found at a high level in the brain cortex and retina suggesting an important role in the development of neural [1, 47] and visual functions [1, 48]. Studies in preterm infants have clearly shown that a dose of approximately 1% of the total dietary fats as DHA is required to prevent neurodevelopmental delays [49]. In preterm infants, DHA is a clear requirement for full visual and neural development [1]. Makrides and Gibson [2] noted that as a result of the positive impacts of DHA on visual and cognitive outcomes, all Australian preterm formulas are now supplemented with DHA. With regard to the effect of DHA during lactation, Lauritzen et al. [48] evaluated an association between fish oil supplements in lactating women and breast milk DHA levels. These studies indicated that the supplementation with fish oil resulted in a 3-fold increase in the DHA content of four-month breast milk and that

full term infants with higher red blood cell levels of n-3 LCPUFA had better visual acuity at four months of age than those with lower levels [48]. Infants who were fed with formula milk had lower DHA levels than breast fed infants [25]. Additionally, Makrides et al. [50] demonstrated that pregnant women who consumed fish oil (0.8g/day DHA and 0.15g/day EPA) increased their maternal EPA and DHA levels which led to higher DHA supply to the foetus. This resulted in fewer children having delayed cognitive development in the DHA group. After birth, the fatty acid status of the mother continues to impact on her newborn via the delivery of breast milk, a naturally rich source of DHA. On the basis of these considerations, an increase in the consumption of n-3 fatty acids is recommended by health authorities in many Western countries, particularly the longer chain fatty acids EPA and DHA.

1.2.4 The recommended intake of n-3 PUFA

Dietary recommendations have been made for n-3 fatty acids (ALA, EPA and DHA) to achieve a nutrient adequacy; however, the recommended n-3 LCPUFA levels vary considerably (Table 1.1 and 1.2). According to Simopoulos [8] and Kris-Etherton et al. [7], the recommended n-3 LCPUFA intake is 650mg/day, with at least 222mg for both EPA and DHA [8]. This recommendation is similar to the International Society for the Study of Fatty Acids and Lipids which recommends an intake of EPA and DHA at ≥500mg/day [5]. In addition, for pregnant and lactating women, dietary DHA intake should be at least 200mg/day [6]. The American Heart Association recommends consuming fish, particularly fatty fish, at least twice a week to provide the necessary n-3 LCPUFA [43].

Recommendations are also made for the consumption of n-3 fatty acids to prevent and treat various chronic diseases, especially for the prevention of heart disease. Lee et al. [51] suggest that patients with coronary artery disease can reduce their risk of cardiovascular disease by increasing their EPA and DHA consumption to approximately 1g/day. The National Health and Medical Research Council (NHMRC) recommends the consumption of 430mg of n-3 LCPUFA for women and 610mg for men per day in order to prevent chronic disease [52]. The recommended dietary intake of ALA to prevent deficiency symptoms is 0.6-1.2% of energy [53]. In addition, a diet containing ALA derived from vegetable oils at a level of 1.5 to 3g/day has substantial benefits to prevent heart disease [43].

Despite these recommendations, the current intake of n-3 LCPUFA in Western countries is still lower than the recommended intake suggested by authorities. The current intake of n-3 fatty acids in the US is 100-200mg/d n-3 LCPUFA (EPA and DHA) and 1.4g ALA [7]. Accordingly, based on a current United States diet, an additional intake of 0.8g of ALA and 0.45g of n-3 LCPUFA is recommended [7]. This intake is similar to the current Australian intake which is at about 200mg/d n-3 LCPUFA and 1.2g/d ALA [5]. Thus, it is apparent that the consumption of n-3 LCPUFA needs to be increased by approximately 3-fold. To achieve the recommended intake of n-3 LCPUFA, it is important to recognise and identify food sources that are rich in n-3 PUFA (ALA) and n-3 LCPUFA (EPA and DHA) that the public would eat in sufficient amounts.

Table 1.1 Recommended intakes of n-3 LCPUFA

Date	Organisation/references	Recommended intakes	Population
2008	Lee et al. [51]	EPA + DHA: ≥500mg/day EPA + DHA: 1g/day EPA + DHA: 3 to 4g/day	General population Coronary artery disease Hypertriglyceridemia
2007	Koletzko et al. [6]	DHA: ≥200mg/day	General population, pregnant and lactating women
2006	National Health and Medical Research Council [52]	Female: 430mg/day Male: 610mg/day	General population
2006	Gebauer et al. [53]	EPA + DHA: ≈500mg/day	Cardiovascular disease risk reduction
2004	International Society for the Study of Fatty Acids and Lipids [5]	EPA + DHA: ≥500mg/day	General population (for cardiovascular health)
2004	UK Scientific Advisory Committee on Nutrition	Minimum 2 portions fish/week	General population
2004	Wijendran and Hayes [54]	EPA + DHA: 0.25 energy %	General population
2002	American Heart Association	Eat fish (fatty fish) at least twice/week	General population
2001	Heath Council of Netherlands [31]	DHA: 150 - 200mg/day	General population
2000	Simopoulos [8]	EPA: ≥220mg/day DHA: ≥220mg/day EPA + DHA: ≥650mg/day	General population
2000	Kris-Etherton et al.[7]	EPA + DHA: 0.5 energy %	General population
2000	Canadian Recommended Nutrient Intake (CRNI)	n-3 PUFA: 0.5 energy %	General population

Table 1.2 Recommended intakes of alpha-linolenic acid (ALA)

Date	Organization	Recommended daily dose of 18:3n-3 (ALA)	Population
2006	Gebauer et al. [53]	0.6-1.2 energy %	General population
2004	International Society for the Study of Fatty Acids and Lipids [5]	0.7 energy %	General population
2004	Wijendran and Hayes [54]	0.75 energy %	General population
2002	Heath Council of Netherlands [31]	1.0 energy %	General population
2000	Kris-Etherton et al. [7]	1.0 energy %	General population
2000	Simopoulos [8]	2.22g/day	General population

1.2.5 Dietary sources of n-3 fatty acids

1.2.5.1 Sources of 18 carbon n-3 PUFA: vegetable oils and nuts

Some plant fats and nuts contain high levels of the n-3 PUFA, ALA. Vegetable oils that are known as the major sources of ALA include perilla, chia, flaxseed, echium, camelina, hempseed, and rapeseed (canola) [7, 25, 55-58]. The ALA content of flaxseed, canola, and soybean oil is 58.7, 9.2 and 7.8%, respectively [7]. Other sources of ALA include nuts such as walnuts, which contain 6.3% of total fat as ALA [25]. The n-3 (ALA) and n-6 PUFA (LA) content of some common oils is represented in Table 1.3. Considering the high level of ALA in some vegetable oils, the incorporation of these oils into chicken diets could be a potential way to increase the concentration of n-3 LCPUFA in chicken tissues.

Table 1.3 The ALA and LA content of some common oils (g/100g)

Food	n-3 PUFA (ALA)	n-6 PUFA (LA)	References
Perilla oil	54-65	14-20	[58, 59]
Chia oil	60.1-64.7	17.6-22.5	[55]
Linseed/flaxseed oil	52-58.7	16	[7, 31, 60]
Camelina oil	27.9-30.7	18.9-20.9	[57]
Hempseed oil	17-19	60	[56]
Canola oil	9.2-11	20	[7, 31, 60]
Soybean oil	6.8-8	54	[7, 31, 60]
Peanut oil	2	32	[60]
Sunflower oil	Trace	60	[60]
Safflower oil	0	75	[60]
Macadamia oil	0.01	2.8	[60]

1.2.5.2 Sources of 20/22 carbon n-3 LCPUFA: fish, meat and eggs

The major sources of n-3 LCPUFA, especially EPA and DHA, are fish and fish products [10], which are naturally found in oily fish [61]. Some fatty fish such as herring, salmon, tuna and whiting, are rich sources of the n-3 LCPUFA, EPA, DPA and DHA (Table 4); however, the n-3 LCPUFA content can vary among fish. For instance, mackerel contains 1.8–5.3g n-3 fatty acids/100g of edible portion, whereas salmon contains 1.0–1.4g n-3 fatty acids [7]. Both of these oily fish are much higher in n-3 fatty acids than most white table fish eaten by Australians [62], for example deep sea bream (Seriolella brama), which only contained 257mg/100g. The sources of n-3 LCPUFA are also available in the form of supplements that are derived from marine oils. A common variety is called "MaxEPA®" and contains 180mg EPA and 120mg DHA per 1g capsule [7].

Although oily fish is rich in n-3 LCPUFA, Western societies consume very little fish so an alternative source of n-3 rich foods is needed. Potential products include meat and hen eggs, which are popular foods and can be alternative sources of n-3 LCPUFA if the birds are fed specialised diets. The typical n-3 LCPUFA contents of chicken meat and eggs are 0.036g/100g and 0.1g/100g, respectively [25]. By modifying the diet, the n-3 LCPUFA levels in these products can be altered to produce higher n-3 fatty acids levels which will have the characteristics that suit the customer's needs.

Table 1.4 Levels of n-3 fats in different types of fish

Fish	Total fat (%)	n-3 (% of total fat)[1]		
		ALA	EPA + DPA + DHA	Total n-3
Barramundi	1.2	1.0	20.8	23.6
Coral Trout	0.7	0.3	40.1	40.6
Deep Sea Bream	0.7	0.1	38.4	38.9
Flathead	0.7	0.1	45.8	46.1
Australian Herring	1.2	0.5	36.4	39.4
Red Snapper	0.7	0.2	48.6	49.3
Atlantic Salmon (farmed)	11.2	1.5	30.9	31.8
Southern Bluefin Tuna	0.7	0.3	34.8	35.4
Whiting	1.0	0.4	28.9	29.1

[1]n-3, omega 3; ALA, alpha-linolenic acid; EPA, eicosapentaenoic acid; DPA, docosapentaenoic acid; DHA, docosahexaenoic acid (Adapted from Soltan and Gibson [62])

1.2.6 Functional food

There has been a sudden increase in consumer interest in the health improving qualities of specific foods that contain physiologically active ingredients [63]. These foods are called functional foods. Foods are categorised as functional foods if they provide beneficial health effects beyond basic nutritional need [64]. Functional foods include enriched or enhanced foods which have potential health benefits when consumed at appropriate levels as part of regular diet [64].

One class of functional food that is of particular interest are those containing n-3 fatty acids, such as n-3 enriched eggs [64-67] and meat [68, 69]. Eggs are rich in nutritional value for humans, and egg composition can be altered to achieve a more functional food through the manipulation of hen diet [70, 71]. Ferrier et al. [72] reported that feeding laying hens a diet containing 10 or 20% flaxseed increased the n-3 PUFA levels in eggs. In the modified eggs, the n-3 PUFA, ALA, increased from

28mg/egg in the control group to 261 and 527mg/egg in the modified groups. The DHA level increased from 51 mg/egg to 81 and 87mg/egg. Similarly, the enrichment of n-3 PUFA into chicken meat can be achieved by modifying the broiler diet. The ingredients which are commonly used to achieve this enrichment include marine sources, marine derived products (fish oil or stabilised fish oil) and vegetables rich in ALA such as canola and flaxseed. As reported by Zelenka et al. [21] the incorporation of flaxseed oil into chicken diets ranging from 0 to 7% elevated the level of ALA, from 28.2 to 215.3mg/100g whereas the DHA content increased from 8.6 to 15.0mg/100g of the meat. Thus it is clear that feed can be modified to significantly alter the fatty acids composition and produce a functional chicken product.

1.2.7 Dietary intake of n-3 fatty acids

1.2.7.1 Intakes of food rich in n-3 LCPUFA

According to the National Diet and Nutrition Survey based on the kinds and amounts of food consumed by individuals in the UK [14], the mean weekly intake of fish, including shellfish is 217g/person, while the weekly intake of poultry and eggs is 374 and 111g/person, respectively. The pattern of meat consumption in Australia over the last 50 years indicates a reduction in the consumption of meat from ruminant animals and a large increase in chicken meat consumption [73]. Based on data from the Australian Chicken Meat Federation (ACMF), chicken meat consumption is forecast to increase to approximately 40kg/person by 2015–16 (Figure 1.1). The increase in poultry meat consumption and the downward trend in ruminant meat consumption

has also been observed in the UK [14] and US [15, 74] (Figure 1.2). In contrast, fish consumption has remained consistently low since the early twentieth century [15].

It appears that even though the level of n-3 LCPUFA in chicken products is lower than in marine foods, chicken meat and eggs contribute significantly to n-3 LCPUFA intake in human diets. Howe et al. [75] assessed the relative contribution of meat and fish to the consumption of n-3 LCPUFA. Their analysis of the 1995 Australian National Nutrition Survey (NNS95) showed that 43% of n-3 LCPUFA intake originated from meat, poultry and game, compared with 48% from fish and seafood. In addition, in Western societies, chicken meat is the main contributor (73%) of meat n-3 LCPUFA intakes in the UK [12]. Givens and Gibbs [14] reported that the estimate of daily intake of n-3 LCPUFA (EPA, DPA and DHA) based on the data from the National Diet and Nutrition Survey (NDNS 2002) together with reported values for the n-3 LCPUFA concentration is 282mg/person/day [14]. Approximately 50% of this amount is derived from oil-rich fish; however, it should be noted that poultry meat contributes the most (61%) compared to other meats. Whilst this indicates that chickens already contribute to dietary n-3 LCPUFA intake, experimental data clearly demonstrate that this contribution could be significantly enhanced by n-3 enrichment of poultry feed.

Figure 1.1 Consumption of various meats per person per year from 1945 in Australia [13].

Figure 1.2 Meat and fish consumption in the USA, 1955-2009 [76].

1.2.7.2 Animal-derived foods as a potential way to increase n-3 fatty acids intakes

As the current intake of n-3 PUFA in most of the Western countries is lower than the recommended intake, this has encouraged interest in enhancing the n-3 LCPUFA concentration of eggs and poultry meat. Hence, an increasing variety of alternative food sources enriched with n-3 fatty acids is being developed [9, 77, 78]. N-3 LCPUFA levels of animal products such as meat and eggs can be improved by changing the dietary fatty acid composition diet [9, 78]. Several studies have demonstrated the capacity to modify the n-3 LCPUFA composition of eggs and poultry meat by incorporating sources rich in n-3 LCPUFA in diets [9, 77, 79].

Fish meal, fish oil and flaxseed are common ingredients used to increase the content of n-3 fatty acids in chicken products. Lopez-Ferrer et al. [9] evaluated the relationship between a diet enriched with fish oil and the fatty acid composition of broiler meat. Their findings indicated that a diet supplemented with fish oil resulted in an increase in n-3 LCPUFA (EPA, DPA and DHA) in the meat. Enrichment of a diet with extracts of fish or algae increased DHA level approximately two-fold in beef, seven-fold in chicken and six-fold in eggs [80]. A source of alternative food rich in n-3 LCPUFA would increase the consumption of these fatty acids for people who do not habitually consume fish and fish products.

1.2.8 Omega-3 LCPUFA enrichment of chicken products using fish oils or sources of n-3 LCPUFA

1.2.8.1 Effect of diets enriched with n-3 LCPUFA on eggs

A number of studies have investigated the influence of dietary n-3 LCPUFA from marine sources, on the fatty acid profiles of eggs [32, 79, 81-83]. n-3 LCPUFA from marine sources offers the benefit of direct inclusion of n-3 LCPUFA into eggs which are metabolically more important than ALA for human health [8]. Hence, one strategy to enhance n-3 LCPUFA accumulation in eggs is supplementation of the layer diet with n-3 LCPUFA sources such as fish meal or fish oil [79, 82-85]. Nash et al. [83] studied the inclusion of menhaden meal into laying hen diets in the range of 0 to 12% and found that the EPA and DHA levels in the egg yolk from hens supplemented with 12% increased 10- and 2.6-fold, respectively. These results were in agreement with a previous study using herring meal, where EPA and DHA levels achieved 7.8 and 100.5mg/yolk, respectively, with the inclusion of herring meal at a level of 12% [82]. In addition, the study conducted by Howe et al. [85] found that incorporation of 5, 10, 15 or 20% stabilised tuna fishmeal to a laying hen diet for 42 days increased the total n-3 LCPUFA content of eggs achieving a maximum level (316mg/egg) at 10% of the weight of the diet. This study demonstrated that the feeding duration from 14 to 42 d at the highest inclusion level (20%) did not affect the n-3 LCPUFA concentration.

Similar results have been reported when fish oils are incorporated into the diet of laying hen. Some authors have reported that increasing the inclusion level of fish oil in the diet results in sequential improvement in n-3 LCPUFA deposition in eggs [79,

86]. Van Elswyk et al. [81] reported that a diet supplemented with 0, 0.5, 1.5 or 3% menhaden oil significantly increased the EPA and DHA content of the eggs. These findings were supported by Garcia-Rebollar [86] who demonstrated that enrichment of layer diets with marine fish oil from 15 to 17g/kg significantly improved egg yolk level of total n-3 fatty acids, EPA and DHA by 3.3, 9.8 and 3.5%, respectively. The incorporation of EPA and DHA into eggs increased further when the inclusion of fish oil was increased up to 30g/kg. As reported by Cachaldora et al. [87], feeding trials using fish oil in laying hen diets, with inclusion from 15 to 30g/kg for 56 days resulted in an increase in n-3 LCPUFA (EPA, DPA and DHA) in the eggs. EPA and DHA levels increased nearly 24-fold and four-fold, respectively. Furthermore, some studies [84, 88] demonstrated that feeding laying hens with fish oil ranging from 0 to 6% caused a linear increase in n-3 LCPUFA in eggs. Lawlor et al. [88] investigated the influence of a microencapsulated fish oil (MFO) product on the fatty acid profiles of eggs. Their results showed that the inclusion of MFO at levels 0, 20, 40 or 60g/kg enhanced EPA, DPA and DHA content of eggs in a linear fashion. DHA concentrations increased from 62mg/total yolk in the 0g MFO/kg group to 96, 129, and 162mg/total yolk in the 20, 40, and 60g MFO/kg group, respectively.

While some studies have successfully demonstrated the incorporation n-3 LCPUFA into eggs using diets supplemented with fish oil, some authors have found that there was an apparent plateau in n-3 LCPUFA yolk accumulation beyond which, further increases in dietary fish oil could not further elevate egg n-3 LCPUFA levels [81, 89]. As pointed out by Van Elswyk et al. [81] and Van Elswyk [89], there was only a small increase in n-3 LCPUFA enrichment in eggs from hens fed diets containing 3%

menhaden oil compared to 1.5%. Moreover, Adams et al. [90] found that hens fed 6% menhaden oil resulted in a numerically lower yolk DHA content compared to those fed 3% menhaden oil. Similarly, Huang et al. [91] reported lower concentrations of yolk DHA in hens fed 3% compared to 2% menhaden oil. This discrepancy is probably due to the intervention of other dietary components on the metabolism and accumulation of n-3 LCPUFA [92].

The incorporation of n-3 LCPUFA into eggs is a gradual process and reaches the maximum n-3 fatty acid incorporation after a number of weeks. Eggs from birds fed dietary menhaden oil ranging from 5 to 30g/kg displayed a gradual increase in n-3 fatty acid deposition over the first three weeks of feeding, with a plateau reached between weeks 3 and 4 [81].

Reduced production performance was observed when laying hens were fed diets containing fish meal or fish oil, in particular, decreasing egg weight [81, 82, 84] and laying rate [79, 83]. Feeding menhaden oil (20, 40 and 60g/kg) to hens reduced egg weight in a dose dependent manner [84]. Although, decreasing egg weight has not been confirmed by others [79, 83], there was an apparent decrease in egg production. As reported by Cachaldora et al. [79], the inclusion of marine fish oil at levels 15, 30, 45 and 60g/kg into diets decreased the laying rate in a dose dependent manner. This was in contrast to other findings [77, 93] in which adding 40g/kg fish oil in the diet did not affect the efficiency of hen production (feed intake, feed conversion, egg production or egg weight).

1.2.8.2 Effect of diets enriched with n-3 LCPUFA on chicken meat

A number of studies have investigated the effects of n-3 LCPUFA supplementation of broiler diets, such as fish meal or fish oil, on bird performance and meat fatty acid profiles [9, 94, 95]. In trying to enhance the incorporation of n-3 LCPUFA into chicken meat, the use of fish meal or fish oils, alone or in combination with ALA sources, is commonly considered [9, 16, 17, 32, 94-96]. Increasing levels of dietary redfish meal increased all n-3 LCPUFA including EPA, DPA and DHA, with the highest level of n-3 LCPUFA observed in birds fed the diet containing the highest level of redfish meal [94, 96]. A study conducted by Ratnayake et al. [96] found that breast EPA and DHA levels of birds fed diet supplemented with 12% redfish meal were 2.3 and 6.0% of total fatty acids, respectively, and had increased 3-fold compared to the levels of birds fed the control diet. A number of studies have also examined the effects of fish oil supplementation of diets on meat fatty acid content [9, 16, 32, 94, 95]. Lopez-Ferrer et al. [9] evaluated the relationship between a diet enriched with fish oil and the fatty acid composition of broiler meat. Their findings indicated that chicken diets with 2 or 4% added fish oil given throughout a five week growth period increased the amount of n-3 LCPUFA, mainly as EPA and DHA. The level of thigh meat EPA and DHA supplemented with 4% fish oil reached 1.3 and 2.4% of total fatty acids, respectively. Another study [95] demonstrated that the enrichment of chicken diets with 8.2% fish oil increased all breast n-3 LCPUFA mainly as EPA (6.4%), DPA (3.1%) and DHA (7.8%). The accumulation of EPA, DPA and DHA was higher in breast meat than in thigh meat, with the total concentration of breast fatty acids achieving 17% of total fatty acids in birds receiving 8.2% dietary fish oil [95]. Chekani-Azar et al. [17] observed that replacing

typical dietary fat sources with fish oil increased all n-3 LCPUFA in breast meat and decreased the n-6:n-3 ratio. Furthermore, other investigators [16, 97] pointed out that a diet containing fish oil produced meat with the highest levels of EPA and DHA compared with diets supplemented by other fat sources.

The literature is inconsistent in this area and it is unclear as to whether diets supplemented with n-3 LCPUFA affect performance production of birds or not. Hulan et al. [94] demonstrated that increasing the dietary levels of red fish meal (7.5, 15, and 30%) or red fish oil (2.1 and 4.2%) resulted in a linear decrease in body weight, lower feed consumption and poorer feed conversion. In a separate study, the feed conversion ratio of birds was not affected by 4% dietary fish oil; however, the resulting final weights were higher than those for birds fed a control diet [9]. Lopez-Ferrer et al. [95] reported that feeding 8.2% fish oil reduced feed intake compared to birds that consumed diets enriched with vegetable oils; however, no significant differences in the other production parameters were observed. In contrast, some studies have reported that production performance is not influenced by the addition of fish oil to the diet [16, 91]. The different results might be due to different diets, feeding periods, different levels of oil used, or differences in the amount and type of antioxidant used to protect the PUFA in the feed. Moreover, studies measuring production performance may need large trials in order to obtain good performance data.

1.2.8.3 Adverse effect of using fish oils or sources of n-3 LCPUFA on chicken products

Several researchers have reported impaired sensory quality, particularly fishy off-flavours, in the meat and eggs from birds fed fish products [16, 17, 83, 88]. This decrease in sensory quality is likely to reduce consumer acceptability. Kjos et al. [98] demonstrated that there was a linear relationship between the inclusion levels of dietary fish oil and the off-taste intensity of egg yolk. In addition, a more intense yolk flavour and a slight fishy off-flavour were detected in eggs when hens were fed a diet containing 8% menhaden oil or higher [83].

Similarly, the use of fish oil in the manipulation of tissue fatty acid composition may result in negative effects on the sensory properties of meat [17, 18] such as off-tastes and off-odours [16]. Marine n-3 LCPUFA sources may also supply the human diet with highly toxic chemicals, including methyl mercury, which is a neurotoxic agent for humans and many species of animals at high doses [99]. Studies conducted by Chekani-Azar et al. [17] indicated that a chicken diet supplemented with 3% of fish oil had the least normal smell and flavour when compared to lower doses and 0%. This finding was in accordance with Lopez-Ferrer et al. [95] who compared the effects of chicken diets containing fish oil, rapeseed (canola) or linseed oils on the sensory quality of breast and thigh meat. Their results showed that the poorest sensory characteristic scores corresponded to the diet which included the greatest proportion of fish oil. In addition, Schreiner et al. [18] noted that the use of fish oil in the form of ethyl esters resulted in a negative effect on the sensory quality of the chicken meat.

In order to achieve an increase in the amount of n-3 LCPUFA in chicken products without negatively affecting sensory quality, several approaches could be taken when adding fish oil or fish meal to the diet. One strategy is the use of the minimum amount possible to achieve the desired increase. Other approaches include the addition of antioxidants in the diet containing a high level of fish oil or the replacement of fish oil with other lipid sources such as vegetable oils rich in ALA. Nash et al. [83] suggested the use of the inclusion of between 4 an 8% menhaden meal to diets of hens. A review paper showed that in terms of organoleptic quality of n-3 enriched eggs, adding fish oil to the diet at, or above, 3% should be avoided due to risk of negative sensory properties [100]. However, problems concerning fishy off-flavour of eggs were still noted even when fish oil levels were lower than 3%. Ceylan et al. [101] investigated the relationship between different dietary oil sources including fish, sunflower, rapeseed and linseed oil with inclusion levels of 1.5 and 3.0%. Their findings showed that a lower score in taste, appearance and odour was observed in eggs from hens fed a diet with 3% added fish oil compared to those fed other diets and sensory panellists scored as unacceptable. These results were in accordance with a study conducted by Gonzales-Esquerra and Leeson [84] which found that diets enriched with either regular marine oil or deodorised marine oil at 2% decreased the sensory properties of eggs including aroma, taste, flavour, acceptability, aftertaste and off-flavours. Moreover, the increase in a fish-like flavour (fishy taste, fishy aroma and fishy aftertaste) in the eggs was observed when laying hens were fed diets containing menhaden oil 10 to 30g/kg [89]. In addition, while EPA and DHA concentrations increased in a linear manner by increasing levels of dietary microencapsulated fish oil [88], the increase in sulphur flavour and off-

flavour were observed for boiled eggs. In relation to the use of antioxidants, while some studies [102, 103] demonstrated that antioxidant supplementation did not increase the acceptability of eggs from birds fed a diet with added flaxseed up to 10%, Huang et al. (90) noted that adding up to 3% fish oil to the diet stabilised with 0.1% ethoxyquin can enhance n-3 LCPUFA in the egg yolk without causing a fishy flavour. With regard to replacing the use of fish oil with vegetable oils in the diet, this seems a good strategy since it resulted in the improved sensory characteristics of chicken meat [95].

1.2.8.4 Summary of marine source or fish oil studies

The fatty acid profiles of eggs and chicken meat are influenced by the fatty acid composition of the diet. As such, eggs and meats that are rich in n-3 LCPUFA can be produced when chickens are fed diets high in n-3 LCPUFA. A large number of studies have demonstrated that dietary addition of fish oil, fish meal or algae can result in an increase in the levels of n-3 LCPUFA, EPA, DPA and DHA in eggs and chicken meat [16, 20, 32, 79, 86, 95, 104, 105]; however, the levels are dependent on the source of n-3 LCPUFA and the amount included [106]. Moreover, n-3 LCPUFA addition is often associated with negative sensory traits [16, 17, 32, 82, 83, 101]. In order to enhance the n-3 fatty acid content in eggs and chicken meat without compromising the optimal sensory characteristics, several strategies have been investigated. These approaches include using the minimum dose of fish oil required to achieve a desired level of n-3, combining antioxidants with fish oils, blending fish oil with vegetable oils and/or seeds, or substituting fish oil with other lipid sources prior to slaughter. Replacing fish oil with linseed oil was considered a good strategy

as it resulted in the lowest decrease in n-3 LCPUFA and an increase in total n-3 PUFA [77, 95]. The substitution of fish oil with vegetable oil noticeably prevented any detrimental effect on sensory properties [95].

1.2.9 ALA enriched diets as an alternative source of n-3 LCPUFA

1.2.9.1 Conversion of ALA to n-3 LCPUFA

The dietary essential fatty acids in human diets are ALA and LA. Humans cannot synthesise these fatty acids *de novo* so they must be provided in the diet. These essential fatty acids need to be metabolised to their respective LCPUFA to derive full benefits. ALA can be converted into the more biologically active n-3 LCPUFA, EPA, DPA, and DHA (Figure 1.3).

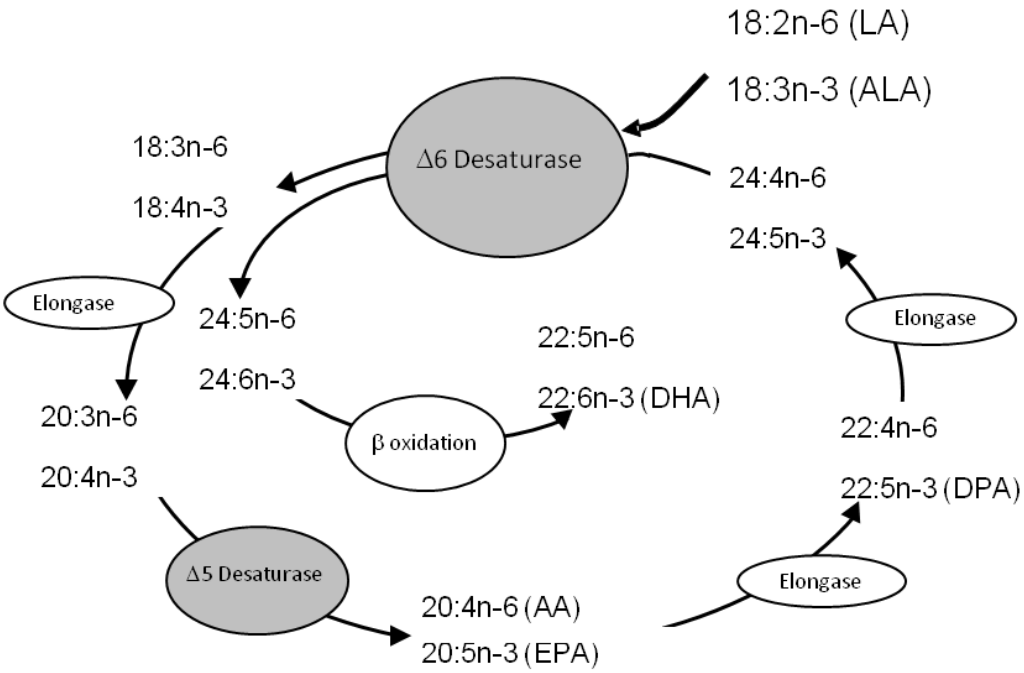


Figure 1.3 Metabolic pathway of n-3 and the n-6 fatty acids representing the existing view of the second use of the delta 6 desaturase for the synthesis of DHA (22:6n- 3) and DPA (22:5n-6) (Adapted from Gibson et al.[107]).

Both ALA and LA, the 18-carbon PUFA in food, can be lengthened in the bodies of human and animals to 20- and 22-carbons, the LCPUFA; however, the conversion rates vary enormously among species. In the liver of rats, the conversion of ALA to EPA and DHA occurs at high rates relative to humans [108]. For example, in the human, it is assumed that the conversion of ALA to EPA is only 0.5 to 10% [109-111]. As ALA and LA have homologous structures, they compete for the same desaturation and elongation enzymes [112]. Dietary LA is identified as suppressing DHA synthesis [107, 113, 114] hence increasing the level of ALA in the diet might enhance DHA accumulation. In contrast, high consumption of LA can reduce EPA, DPA and DHA production and favour high AA [34, 107, 115]. A number of studies

have highlighted that n-6 and n-3 LCPUFA levels in animal tissues can be regulated by modifying the dietary balance of LA and ALA, the precursor of the n-6 and n-3 LCPUFA, in the diet [107, 116-118]. A study conducted by Gibson et al. [107] demonstrated that at corresponding levels of dietary ALA, increasing levels of dietary LA resulted in a reduction in metabolites. As such, a high dietary ratio of LA to ALA, which is typical for broiler diets, leads to decreased metabolism of ALA into EPA, DPA and DHA. Komprda et al. [119] showed that increasing dietary LA significantly increased the level of AA in chicken meat. Moreover, Kartikasari et al. [115] examined the effect of varying dietary LA levels on the conversion of ALA into EPA, DPA and DHA in chicken tissues. In this study, the level of ALA in the diets was held constant at 2.1% energy (%en) while the level of LA varied from 2.9 to 4.4% en. The ratio of LA to ALA of the experimental diets thus ranged from 1.4:1 to 2.1:1. The results indicated that decreasing LA while keeping ALA constant resulted in an increase in the total n-3 LCPUFA levels in the breast meat. The total n-3 LCPUFA content of birds fed with the lowest LA content reached 16% higher than the n-3 LCPUFA in the breast of birds fed with the highest LA content. This highlights the importance of optimising LA: ALA ratios to maximise n-3 LCPUFA conversion.

It is clear that diets containing high levels of ALA will promote elevated tissue EPA concentrations. Garg et al. [120] demonstrated that diets containing linseed or fish oil, both n-3 fatty acid rich sources, inhibit the conversion of LA to gamma-linolenic acid, the precursor of AA. Understanding that the ratio of LA to ALA in the diet determines EPA and DHA production could help design an effective dietary strategy

for altering the dietary ratio of LA to ALA to promote levels of EPA and DHA in chicken products. If chickens could convert ALA to n-3 LCPUFA in sufficient quantity, the dietary incorporation of vegetable oils rich in ALA into chicken diets has the potential to provide an alternative to fish oil for a supply of n-3 LCPUFA.

Chickens confer a relative advantage in converting dietary ALA to n-3 LCPUFA. Unlike mammals (rats, humans), chickens have a rudimentary or lack a defined lymphatic system draining the intestinal tract [121].Therefore, chylomicrons are absorbed directly into the portal blood and are transported to liver for further synthesis and subsequent tissue accumulation [110, 121]. Clearly, this allows direct exposure of the liver to dietary fat, and this unique trait of lipid metabolism in poultry permits the manipulation of fatty acid content of chicken's tissues by dietary means [121]. It appears that first-pass hepatic metabolism plays a greater role in the metabolism of dietary fatty acids, as compared to other species where peripheral metabolism can have a mediating factor.

1.2.9.2 Effect of diets enriched with ALA sources on production performance, fatty acid profiles and sensory quality of eggs

The most recent studies that have examined the effect of dietary ALA on chicken eggs are summarised in Table 1.6.

1.2.9.2.1 Effect of ALA enriched diets on fatty acid profiles of eggs

Studies have shown that the incorporation of n-3 PUFA sources such as vegetable oils rich in ALA enhanced the level of ALA and n-3 LCPUFA in yolk eggs [122-124]. Milinsk et al. [122] assessed the effect of the inclusion of vegetable n-3 PUFA sources including canola, flaxseed, soybean and sunflower oils on the fatty acid composition of eggs. Their findings indicated that the highest level of ALA (3.40%), EPA (0.18%), DPA (0.29%), and DHA (1.55%) was achieved when the diets had added flax meal and flax oil. Bean and Leeson [125] reported that the inclusion of 10% flaxseed resulted in higher n-3 fatty acid levels (ALA and DHA) than the control diet. The levels of ALA, DHA, and total n-3 PUFA in eggs from hens fed 10% flaxseed increased to 8, 1.5, and 4-fold, respectively. These findings were in agreement with a study conducted by Cherian et al. [126] which found that laying hens fed a basal diet supplemented with 17% ground flax (40.7% ALA) increased ALA and other n-3 LCPUFAs such as EPA, DPA, and DHA with a concomitant decrease in AA. Ceylan et al. [101] investigated the effect of different dietary fat sources on fatty acid profiles of egg yolk. In this study, diets were supplemented with two inclusion levels (1.5 and 3.0%) of sunflower, fish, flaxseed, and canola. Their results showed that diets containing canola or flaxseed oil deposited more ALA than those added with sunflower and fish oil. Eggs from hens fed flaxseed oil accumulated the highest level of n-3 PUFA. Moreover, in the study by Grobas et al. [78], in which hens were fed diets supplemented with 5 or 10% tallow, olive, soy or flaxseed oil, they found that diets with added flaxseed oil produced eggs with the highest level of n-3 PUFA and EPA.

It is apparent that when the inclusion levels of n-3 PUFA was increased, the total of n-3 fatty acids of the eggs was enhanced, mainly as ALA. As demonstrated by Souza et al. [124], dietary inclusion of 2% flaxseed oil increased the ALA content of eggs to 3-fold higher than control eggs. Similarly, n-3 fatty acid-enriched diets with 50 and 150g/kg flaxseed, either whole or ground, elevated n-3 fatty acids in yolk as a result of increasing yolk ALA [89].

Some studies found that there was a maximum DHA level achieved by increasing the levels of dietary ALA [78, 123]. Sari et al. [123], evaluating the influence of the addition of flaxseed (5 to 15%) to diets of laying hens, verified that increasing levels of dietary flaxseed increased ALA, EPA, DPA and DHA compared to the control. However, there were no significant differences in the level of DPA and DHA among diets containing flaxseed (5, 10 or 15%). Similarly, Grobas et al. [78] reported that supplementation of flaxseed oil from 5 to 10% did not increase the DHA content of eggs.

It has been well documented that diet composition greatly influences the fatty acid profiles of eggs. The other factors which affect egg composition and fatty acid content include age and strain of the birds [127, 128]. Scheideler et al. [127] reported that when laying hens were fed diets supplemented with 10% flaxseed, the incorporation of ALA and DHA into egg yolk was higher at 58 week than at 36 week of age across all strains (DeKalp Delta, Babcock B300, and Hyline W-36). There was also a significant diet by strain interaction for the ALA level which increased in DeKalp Delta yolk but reduced in Babcock B300 yolk when the hens were fed flax

(10%) plus oats. Moreover, some studies showed the effect of dietary inclusion of ALA on the accumulation of n-3 fatty acids into eggs in different strains of birds [78, 126, 129]. As reported by Bean and Leeson [125], when high ALA was included in laying hen diets, ISA-Brown hens produced higher levels of egg DHA compared to Shaver White hens. This indicates that the extent of n-3 incorporation into eggs is highly strain dependent.

1.2.9.2.2 Sensory characteristics of eggs following consumption of ALA enriched diets

As the use of marine derived n-3 sources caused the reduction in sensory characteristics of the final products [17, 18, 83], the dietary inclusion of n-3 PUFA sources derived from vegetable rich in ALA such as canola and flaxseed might be an alternative. Several studies have assessed the effect of vegetable-derived ALA sources on the sensory characteristics of eggs [130-132]. Feeding flaxseeds to laying hens is one of the alternatives for producing n-3 fatty acids enrichment in eggs. However, it should be taken into account that n-3 fatty acid enriched eggs increase the degree of fatty acid unsaturation, leading to oxidative impairment of yolk lipid and overall decrease in egg quality and preference scores [95]. Jiang et al. [133] reported that a fishy taste was detected in eggs when laying hens were fed diets supplemented with 15% whole flaxseed. The presence of lipid oxidation or the flavour characteristics of flaxseed which was directly transferred into the yolk may have caused this adverse effect [133]. Hayat et al. [130] investigated the effect of flaxseed supplementation on the sensory attributes and consumer acceptance of eggs. In this study, layer diets were supplemented with 10% flaxseed, 10% flaxseed + 100

IU/kg of vitamin E, or 10% flaxseed + 100 mg/butylated hydroxytoluene. Their results showed that trained panellists detected differences in the sensory attributes evaluated including aroma, flavour, off-flavour, and overall difference between control eggs and eggs from flaxseed-fed birds. However, the majority of the untrained panellists could not perceive control eggs from flaxseed eggs for aroma and flavour. In addition, there was no significant difference in consumer acceptance between control eggs and eggs from flaxseed-fed birds.

Some studies have also examined the dietary inclusion of ALA rich vegetable oils on the sensory characteristics of eggs [131, 132]. The dietary incorporation of 3% flaxseed oil did not influence the flavour of hard-boiled eggs suggesting that this level can be added without producing a negative flavour effect [131]. This finding was supported by Parpinello et al. [132] which reported that supplementing the diet with 20g/kg of palm butter, grape seed oil, or flaxseed oil did not change the taste of hard-boiled egg compared to the control eggs.

These findings present evidence that the use of vegetable ALA sources can provide an effective vehicle for improving total n-3 PUFA content of eggs; however, the potential for some adverse sensory characteristics is present at high levels.

1.2.9.2.3 Effect of ALA enriched diets on production performance of laying hens and egg quality

Many studies have reported the effect of ALA supplemented diets on the production performance of laying hens [78, 102, 123]. The inclusion of 10% flaxseed in the diet of brown laying hens did not affect egg production, egg weight, egg mass, or feed conversion ratio compared to the control diet; however, feed consumption was decreased [102]. The reduction in feed intake was in agreement with the results of Sari et al. [123] which included diets with up to 15% flaxseed. A significant reduction in feed consumption was observed when hens were fed with 10 or 15% flaxseed. Increasing levels of dietary flaxseed significantly improved the feed conversion ratio. Similarly, the reduction in feed intake attributable to inclusion of flaxseed also reported by Scheideler and Froning [134] in White Leghorn hens. However, it appears that there are inconsistent results regarding the effect of high flaxseed diet on feed consumption given that Caston et al. [135] reported that feeding flaxseed up to 20% increased feed intake of birds.

However, it seems that the form of flaxseed added in the diet might influence the performance parameters of laying hens. Grobas et al. [78] examined the effects of bird strain and fat source and the level of added fat on performance production. In this study, white and brown hens were fed diets containing tallow, olive, soy, or linseed oil at levels of 5 or 10%. Their findings showed that feed intake, feed efficiency or hen-day production was not affected by fat source. The level of fat supplementation did not change the hen day production or egg weight.

Scheideler et al. [127] investigated the influence of diet, strain, and age on production parameters of hens. In this study, three strains, DeKalp Delta, Babcock B300, and Hyline W-36, were fed a diet supplemented with 10% flaxseed + oats from 30 to 50 week of age. At 50 week of age, the birds were divided into two groups with one group fed flax + oats and the other group fed flax – oats (without added oats). Their results indicated that feed consumption and egg weight was affected by strains where DeKalp Delta had a greater feed intake and egg weight than either Babcock B300 or Hyline W-36. This indicated that some strains perform better on high ALA diets.

1.2.9.3 Effect of diets enriched with high ALA sources on chicken meat

The studies that have examined the effect of ALA on chicken meat are summarised in Table 1.8.

1.2.9.3.1 Effect of ALA enriched diets on fatty acid profiles of chicken meat

As in the case of n-3 enriched eggs, canola, flaxseed or their oils could be a key ingredient available to the poultry diet regime. A number of studies have demonstrated that the inclusion of ALA-rich vegetable oils in the diet enhanced the levels of ALA and n-3 LCPUFA in tissues [19-21]. Studies conducted by Skrivan et al. [136] showed that chicken diets supplemented with 50g/kg of canola oil increased the ALA content of breast muscle and decreased the ratio of LA to ALA. A study by An et al. [137] demonstrated that increases in the level of flaxseed oil gradually elevated ALA, and decreased the levels of LA and AA in tissue lipids. In addition, Lopez-Ferrer et al. [95] assessed the effect of substitution of fish oil with flaxseed oil

on the fatty acid composition of tissues. Their findings indicated that the substitution of fish oil with 8.2% flaxseed oil increased tissue ALA concentrations.

Similar findings were reported by Gonzales-Esquerra and Leeson [32] who evaluated the relationship between the supplementation of dietary sources of ALA in broiler diets and the n-3 fatty acid profiles (ALA and n-3 LCPUFA) of chicken meat. Their results showed that a diet containing 100g/kg of flaxseed (as seed) increased ALA and DPA concentrations; however, these studies have failed to demonstrate an increase in EPA and DHA in tissues. The lack of response in these studies may have been due to the high level of LA and thus the relatively high ratio of LA to ALA in the experimental diets used.

In order to optimise the incorporation of dietary ALA into chicken meat, an effective dietary strategy for altering the ratio of LA to ALA is needed. Recent studies using chickens indicated that by decreasing the ratio of LA to ALA in the diet there was an increase in tissue n-3 LCPUFA [19-21, 138]. Results showed that increasing levels of dietary canola oil up to 4% resulted in an increase in ALA and n-3 LCPUFA content in breast meat compared to the control [139]. Lopez-Ferrer et al. [20] assessed the effect of diets supplemented with flaxseed oil on n-3 LCPUFA levels in chicken meat (Ross 308 strain). Their findings showed that the supplementation of chicken diets with up to 4% linseed oil (LA to ALA ratio of 0.57:1) increased EPA, DPA and DHA concentrations in thigh tissue approximately 2.5-fold higher than the control diet. In addition, Febel et al. [19] found that decreasing the LA to ALA ratio from 20.9:1 to 1.3:1 of chicken (Ross 308 strain) diet by supplementation of

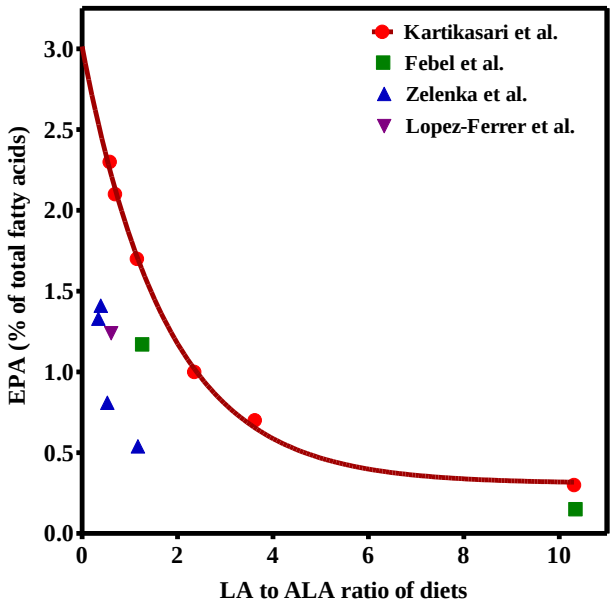
vegetable oils (3%) including sunflower oil, soybean oil and flaxseed oil increased liver and breast n-3 LCPUFA (EPA and DHA). Diets supplemented with flaxseed oil enhanced the level of EPA and DHA to 1.2 and 1.4%, respectively [19] and resulted in the highest level of breast n-3 LCPUFA. The accumulation of breast n-3 LCPUFA (EPA, DPA and DHA) further increased when the diet was supplemented with 5% flaxseed oil [21]. However, the inclusion level of 7% flaxseed oil did not change the level of n-3 LCPUFA [21] indicating that a maximum level of n-3 LCPUFA had been achieved. Conversely, the level of tissue AA was reduced significantly by decreasing LA to ALA ratio in the diet [19, 21].

Several studies also investigated the relationship between diets enriched with ALA and the level of ALA and n-3 LCPUFA in chicken meat phospholipid and triglycerides [92, 138, 140]. A study conducted by Betti et al. [138], in which broiler diets (Ross 308) supplemented with 10 or 17% flaxseed, demonstrated an increase in the n-3 LCPUFA in breast meat mainly deposited in phospholipids. The major n-3 LCPUFA in the phospholipid fraction of breast meat was DPA. However, their findings indicated that there was no change in the concentration of phospholipid ALA and n-3 LCPUFA by increasing levels of dietary flaxseed from 10 to 17%. While n-3 LCPUFA were more readily accumulated in phospholipids, ALA was mainly stored in the breast meat triglycerides [92, 138, 140]. Kartikasari et al. [117] demonstrated that chicken (Cobb 500) diets enriched with ALA ranging from 1 to 8%en, with a constant LA level of 4%en, increased all tissue phospholipid n-3 LCPUFA in a curvilinear manner. The EPA and DHA levels in breast and thigh meat fed the lowest LA to ALA ratio increased to 5 and 4-fold, respectively relative to

birds fed the highest LA to ALA ratio. In contrast, AA (the 20 carbon derivative of LA) reduced in all groups with decreasing LA to ALA ratio in the diets. However, as this study only focussed on Cobb 500 broilers, the possible increasing levels of tissue n-3 LCPUFA of other strains remains unclear.

In general, the increase in the n-3 LCPUFA levels of chicken products by supplementation of n-3 PUFA sources to chicken diets show a similar trend to other studies (Figure 1.4). However, there is a different response in n-3 LCPUFA accumulation in tissues. The variation of response among studies may be due in part to a high ratio of LA to ALA in the experimental diets and may be as a result of the fact that a different strain of birds and age was used. Kartikasari et al. [117] used the Cobb 500 strain while the other studies used Ross 308. Based on this recent study, it is clear that altering the ratio of LA to ALA to keep chicken diets low in LA and high in ALA is important to promote high tissue EPA and DHA concentrations. Furthermore, studies are needed to evaluate two different strains of birds either in broilers or in laying hens in n-3 LCPUFA accumulation and to evaluate sensory quality of the final products.

A



B

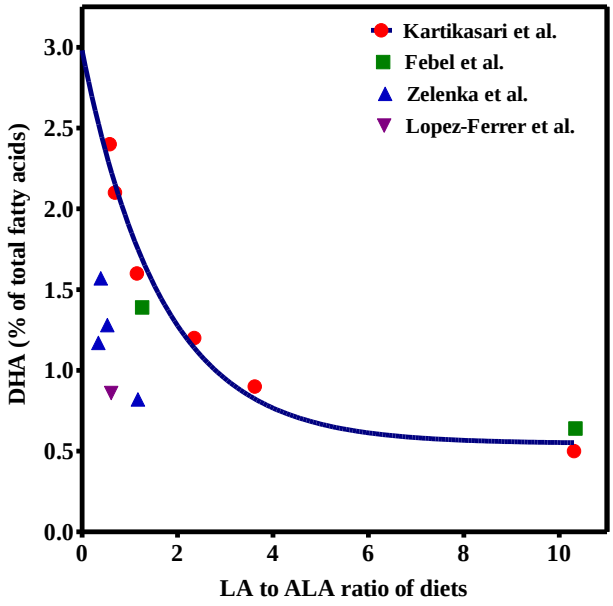


Figure 1.4 Comparison of the effects of varying LA to ALA ratio on fatty acid composition of EPA (A) and DHA (B) in breast tissues in various studies [19, 21, 95, 117].

1.2.9.3.2 Sensory characteristics of chicken meat following consumption of ALA enriched diets

The relationship between diets high in ALA and sensory attributes of chicken meat has also been examined [32, 141]. Zelenka et al. [141] investigated the effect of diets supplemented with 1, 3, 5 or 7% flaxseed oil on sensory characteristics of chicken meat. Their findings showed that odour and taste of breast meat, which contained total n-3 fatty acids 189.4mg/100g of meat, from birds fed a diet containing 5% flaxseed oil (with ALA levels in the diet 31g/kg) did not differ compared to breast meat fed 1 or 3% flaxseed oil. In addition, Gonzales-Esquerra et al. [32] reported that the sensory characteristics of breast meat from chickens fed 100g/kg flaxseed for 14 days were comparable with the control breast meat. These findings were in agreement with Betti et al. [142] which suggested that the incorporation of 10 and 17% ground flaxseed in broiler diets for less than 20 days did not affect the sensory quality of breast meat when presented as ground meat patties.

1.2.9.3.3 Effect of ALA enriched diets on production performance of broilers

Rahimi et al. [143] evaluated the effect of full-fat flaxseed (FS) and canola seed (CS) on broiler performance at 42 days of age. In this study the birds were fed with diets containing 7.5% CS, 15% CS, 10% FS + 10% CS, 7.5% FS or 15% FS. Their results indicated that there was no significant difference in the feed consumption among treatments. However, Najib and Al-Yousef [144] observed a higher feed consumption when flaxseed level in the diet was increased (15 to 20%). Several studies showed that feeding flaxseed above 5% resulted in lower body weight gain and higher feed conversion ratio compared to 5% or 0% [143, 144]. Results indicated

that including vegetable oils rich in ALA may result in better production performance compared to adding flaxseed since the inclusion of vegetable oils in the diet increased production performance of chickens and reduced fat deposition [20]. Moreover, a study conducted by Lopez-Ferrer et al. [20] demonstrated that diets supplemented with 4% flaxseed oil and 4% tallow (a higher polyunsaturated and lower saturated diet than control) resulted in an increase in weight gain and final body weight compared to diets containing 8% tallow. These findings were in agreement with Zelenka et al. [145] which found that the body weight gain of chickens fed a diet supplemented with 5 and 7% flaxseed oil were higher compared to groups receiving 1 and 3% flaxseed oil. In addition, a lower feed intake per unit of body gain was recorded in groups with 5 and 7% of flaxseed oils than in groups fed a diet containing 1 and 3%.

1.2.9.4 Summary of studies including vegetable ALA sources

Some vegetable sources such as canola, flaxseed and their oils are potential sources of ALA for laying hens and broiler chickens. In general, there were no negative effects on production performance of broilers when birds were fed a diet containing flaxseed oil [95, 146] but reduced production performance has been observed in broilers fed diets supplemented with either flaxseed or flaxseed meal [22, 147].

Dietary manipulation using vegetable ALA sources may also be a good strategy to improve n-3 fatty acids into chicken products [19, 21, 122-124, 138, 139] without affecting the sensory quality of the final products. Due to the high content of ALA, flaxseed or flaxseed oil is a good source for incorporating n-3 fatty acids into eggs

and chicken meat. Laying hens and broiler chickens were fed diets enriched with ALA increased the levels of ALA and n-3 LCPUFA into eggs and meat. However, although a significant increase of n-3 LCPUFA, EPA, DPA, and DHA was found in the eggs and chicken meat, the concentration of total lipid n-3 PUFA was mainly as ALA [21, 89, 124, 138]. The small increase in EPA and DHA in the products produced by feeding diets rich in ALA could be due to the fatty acid composition of eggs and chicken meat reflecting the fatty acid profiles of the diets or it might be an inefficient conversion of ALA into n-3 LCPUFA in chickens. N-3 LCPUFA production can be determined by the ratio of LA to ALA in the diet. Diets containing high levels of ALA, while keeping a low LA level, will promote increasing tissue EPA and DHA concentrations. Hence, designing an effective dietary strategy for altering the dietary ratio of LA to ALA to promote levels of EPA and DHA in chicken products is needed.

In relation to sensory quality of the final products, it appears that compared to the inclusion of marine sources, the use of vegetable ALA sources results in superior sensory quality of the final products [95]. This suggests that the incorporation of vegetable ALA sources into the diet of chickens can increase total n-3 PUFA content of chicken products, but the potential for some unpleasant sensory characteristics is considered at the high levels of incorporation.

Table 1.5 Summary of studies involving diets containing n-3 LCPUFA sources on n-3 LCPUFA content of eggs

Authors	Animals	Experimental Diets	Intervention Period	Performance	LCPUFA Status or Sensory Properties
Lawlor et al. (2010) [88]	96 36-week-old Single-Comb White Leghorn	Dietary treatments: Including 0, 20, 40, or 60g/kg diet of a microencapsulated fish oil (MFO).	Up to 21 days (from 36 to 39-week-old)	No effect on feed intake. MFO significantly decreased egg weight and yolk weight.	MFO increased the total n-3 FA yolk, from 141mg/yolk to 299mg/yolk. There were significant differences in sensory quality of hard-boiled eggs among treatments. Off-flavour and sulphur flavour were greater in boiled eggs from birds fed the highest level of MFO. No difference between treatments for scrambled eggs.
Cachaldora et al. (2008) [87]	96 44-week-old ISA Brown laying hens	Four types of fat supplementation of a basal diet (0 or 50g/kg of soybean oil, linseed oil/LO or lard) supplemented with fish oil/FO (0, 15 and 30g/kg). Lipid content of basal diet 23.9g/kg.	From 44 to 55-week-old The experiment started after a pre-experimental period of 21 days and lasted for 56 days.	No effect on production parameters and quality (feed intake, laying rate, egg weight, yolk weight, Haugh units or shell thickness).	LO increased the yolk n-3 FA, predominantly due to an increase in ALA. FO increased n-3 LCPUFA levels (EPA an DHA).

Continued

Authors	Animals	Experimental Diets	Intervention Period	Performance	LCPUFA Status or Sensory Properties
Cachaldora et al. (2006) [79]	96 44-week-old Warren laying hens	Dietary treatments: Incorporation of three types of marine fish oil, with different levels of EPA and DHA (MFO1, MFO2_EPA and MFO3_DHA) with 4 levels of inclusion (15, 30, 45 and 60g/kg). Lipid content of basal diet 21.4g/kg.	from 44 to 55-week-old The experiment started after a pre-experimental period of 21 days and lasted for 56 days.	No effect on feed intake, hen weight gain, egg weight, or Haugh units. Decreased laying rate or shell thickness.	Inclusion of MFO ranging from 15 to 60g/kg enhanced EPA, DPA, DHA and total n-3 fatty acids. EPA and DPA content were highest in eggs from birds fed the diet containing MFO2_EPA (74 and 67%, respectively) whereas DHA and total n-3 fatty acids were highest in the eggs of birds fed the MFO3_DHA diet (27 and 12%, respectively).
Howe et al. (2002) [85]	14 mature laying hens	Dietary treatments: Control diet fed for 42 days or diets supplemented with 5, 10, 15, or 20% PorcOmega (POM), a stabilised tuna fishmeal formulation (42 days). Control diet fed for 14 days followed by POM diet at a level of 20% (28 days); control diet (28 days) continued by 20% POM diet (14 days).	Up to 42 days	Data not shown.	Fatty acid levels: The levels of n-3 LCPUFA increased with increasing levels of POM, achieving a maximum at 10% of the diet (six times higher than control; 316mg/egg). At a level of 20% POM, n-3 LCPUFA content was not influenced by varying the feeding period from 14 to 42 days. Sensory analysis: Compared to commercial and control eggs, the 10% and 20% POM eggs were less desirable in most attributes. 20% POM caused fishy odour.

Continued

Authors	Animals	Experimental Diets	Intervention Period	Performance	LCPUFA Status or Sensory Properties
Gonzalez-Esquerra and Leeson (2000) [84]	224 19-week-old Single Comb White Leghorn Shaver	Control diet: without any fish oil. Dietary treatments: Substitution of fat sources in the basal diet with either regular menhaden oil (RMO) or deodorised MO (DMO) at 2, 4, or 6% of diet. Data were analysed in four experimental periods (2, 4, 6 or 9).	Up to 36 weeks (from 19 to 55-week-old)	Feed intake was not significant in periods 2 or 6; however, a lower feed intake was observed in period 9 for birds fed 4% DMO compared to those fed 2% DMO or the control diet. Increasing levels of MO linearly decreased egg weight in all feeding periods.	Fatty acid levels: EPA, DPA, and DHA levels increased in the eggs from hens fed either RMO or DMO. Feeding diets supplemented with either RMO or DMO at 2% decreased the sensory properties of the eggs (aroma, taste, flavour, acceptability, aftertaste or off-flavour). Sensory analysis: A higher perception of aftertaste and off-flavours was detected in eggs from hens fed the diet containing DMO compared to the control diet.
Baucells et al. (2000) [77]	170 20-week-old LSL-White Leghorn	Control diet: basal diet with 4% added fish oil (FO). Dietary treatments: FO was substituted with linseed oil (LO), rapeseed oil (RO), sunflower oil (SFO), and tallow (T) in proportion of 25, 50, 75, or 100%.	Up to 14 weeks	No effect on feed intake, feed conversion, egg production or egg weight.	When FO was replaced with other fat sources (LO, RO, SFO, or T), EPA and DHA levels of the eggs decreased. Compare to other fat sources used to replace FO, LO achieved the highest levels of EPA, DPA, and DHA in egg yolk.

Continued

Authors	Animals	Experimental Diets	Intervention Period	Performance	LCPUFA Status or Sensory Properties
Nash et al. (1996) [83]	192 140-day-old Two commercial Single Comb White Leghorn (SCWL) genotypes of laying hens	Diets supplemented with either 0. 4, 8 or 12% menhaden meal (MM).	Up to 50 weeks (from 20 to 70-week-old)	No differences on egg production and feed conversion between the genotypes; however, egg production was increased by dietary level of MM in a linear manner and feed conversion was higher in control than in MM diets. No effect on egg quality.	Fatty acid levels: EPA and DHA content increased 10-(9mg/yolk) and 2.6-fold (95mg/yolk), respectively by increasing the level of MM. DHA content was influenced by genotypes, with genotype A having a higher level of DHA compared to B. Sensory analysis: A more intense yolk flavour and fishy off-flavour perception were detected in diets with MM levels of 8% or higher.
Huang et al. (1990) [91]	24 147-day-old White Leghorn laying hens (Shaver, 288)	Control: commercial diet. Three dietary treatments: The commercial diet was added with 1, 2, and 3% menhaden oil, stabilised with 0.1% ethoxyquin to prevent rancidity.	From 180 to 208-day-old (21-week-old birds were kept on a commercial diet for 33 days).	No effect on feed consumption, weight gain, and feed conversion ratio.	Fatty acid levels: EPA and DHA increased with an increase in the level of dietary oil and the weeks of feeding (from week 1 to 4). A greater increase was observed for DHA than EPA. Sensory analysis: Adding up to 3% fish oil did not cause a fishy flavour.

Table 1.6 Summary of studies involving diets containing n-3 PUFA sources (ALA) on n-3 LCPUFA content of eggs

Authors	Animals	Experimental Diets	Intervention Period	Performance	LCPUFA Status or Sensory Properties
Gakhar et al. (2012) [56]	48 19-week-old Bovan White laying hens	Control and dietary treatments. Dietary treatments: Diets containing hemp seed (HS; 12 or 20%) or hemp seed oil (HO; 4, 8, or 12%).	Up to 12 weeks	No effect on average hen-day egg production by feeding HS or HO. Birds fed 4% HO had lower feed intake that that of the controls. Birds fed 20% HS produced higher egg weight than that of the controls.	Increasing levels of dietary alpha-linolenic (ALA) acid provision with HS or HO-based diets increased linearly total n-3 PUFA. Increased levels of ALA resulted in a quadratic response for DHA levels in egg yolk.
Goldberg et al. (2012) [148]	48 19-week-old Bovan White laying hens	Control and dietary treatments. Dietary treatments: Diets containing hemp seed (HS; 12 or 20%) or hemp seed oil (HO; 4, 8, or 12%).	Up to 12 weeks		Total n-3 PUFA content was highest in the 12% HO diet (15.3mg/g of yolk) compared to the control (2.4mg/g of yolk). The n-3 enriched eggs was still not high enough to achieve a nutrient content claim in Canada (300mg/egg) There were no adverse effects (aroma or flavour) on the sensory quality of the cooked eggs.

Continued

Authors	Animals	Experimental Diets	Intervention Period	Performance	LCPUFA Status or Sensory Properties
Ceylan et al. (2011) [101]	640 18-week-old	Dietary treatments: Basal diet with 4 different oil sources: sunflower (SFO), fish oil (FO), linseed oil (LO), and rapeseed oil (RO) at 2 levels of inclusion (1.5 and 3.0%).	Up to 16 weeks (from 18 to 34-week-old)	No effect of FO, SFO, LO, and RO supplementation on production performance (laying rates, egg weight, egg mass output, feed intake, feed conversion).	Fatty acid levels: Diets with RO and LO resulted in more ALA in the egg compared to those supplemented with SFO and FO. Hens fed with FO deposited more DHA compared to other fat sources. Inclusion of LO to diets produced the highest level of egg n-3 PUFA. Sensory analysis: Sensory quality of boiled eggs was affected by fat sources and the levels of oil added, with diets containing 3% FO resulting in the lowest score of the appearance and odour. Inclusion level of 3% FO impaired the egg quality due to oxidation and undesirable changes (egg appearance and odour).

Continued

Authors	Animals	Experimental Diets	Intervention Period	Performance	LCPUFA Status or Sensory Properties
Yalcin et al. (2010) [149]	22-week-old White Bowan laying hens	Control: commercial diet. Dietary treatment: adding 1.5% fish oil (a diet containing fish oil, FOD) or a mixture of 10% flaxseed and 1.5% FO (a diet containing a mixture of flaxseed and fish oil, FFOD) to the commercial diet. Dietary treatments were given to hens for 15 and 30 days.	Up to 30 days (15 or 30 days)	Data not available.	Egg EPA, DPA, and DHA levels increased by feeding FOD or FFO. There was a small increase when the feeding duration was increased from 15 to 30 days. EPA and DHA content of eggs fed FOD were higher than those fed FFOD. EPA content was 0.82% in the FOD group and 0.55% in the FFOD group (30-day feeding time). DHA content of eggs from birds fed FOD and FFOD was 4.92 and 3.91%, respectively (30-day feeding time). Eggs from birds fed with FFOD diet accumulated more ALA compared to those from birds fed FOD.
Rowghani et al. (2007) [150]	120 24-week old Hy-line white layers	Diet 1, basal diet ; diet 2, basal diet with 1% calsium soap of fatty acids (CSFA); diet 3, basal diet with 3% canola oil or diet 4, basal diet with 5% canola oil.	Up to 8 weeks	Adding 5% canola oil produced higher egg-yolk weight than the control diet.	CSFA had no effect on ALA and DHA. Diets supplemented with 3 and 5% canola oil increased ALA by 2.7 to 4.7 fold, respectively. DHA and total n-3 PUFA levels increased by 9.8 and 4.7 fold, respectively, compared to the control diet.

Continued

Authors	Animals	Experimental Diets	Intervention Period	Performance	LCPUFA Status or Sensory Properties
Silversides and Lefrançois (2005) [151]	102 42-week-old DeKalb laying hens	Dietary treatments: Diets containing 50, 100 or 200g/kg hemp seed meal (HSM).	Up to 4 weeks	No effect on hen performance or egg quality by feeding hempseed meal.	Increasing levels of HSM lowered the percentage of palmitic acid and increased the levels of linoleic and alpha-linolenic acid.
Milinsk et al. (2003)[122]	96 Red Lohman (RL) and 96 White Lohman (WL), 20-week-old	Control diet: basal diet. Dietary treatments: Diet 1, 58.2% of corn, 10.1% of canola meal, and 3.2% of canola oil. Diet 2, 59.0% of corn, 2.3% of flax meal, and 3.0% of flax oil. Diet 3, 58.4% of corn, 26.6% of soybean meal and 3.0% of soybean oil. Diet 4, 59.1% of corn, 8.0% of sunflower meal, and 2.9% of sunflower oil.	Up to 16 weeks (from 20 to 36-week-old)		The flaxseed diet resulted in the highest level of ALA (3.40%), EPA (0.18%), and DHA (1.55%).

Continued

Authors	Animals	Experimental Diets	Intervention Period	Performance	LCPUFA Status or Sensory Properties
Ayersa et al. (2002) [152]	450 27-week old (225 white and 225 brown laying hens)	Control diet: a diet without supplementation of chia seed. Dietary treatments: Diets supplemented with 70, 140, 210 and 280g/kg whole chia seed.	Up to 90 days	No significant differences in egg production for brown hens among diets. White hens fed 280g/kg chia diet produced fewer and lighter eggs than the control diet. No significant differences in yolk weight until day 90. White hens fed 70g/kg chia diet produced lighter yolks, whereas brown hens produced heavier yolks compared to the control diet.	Sensory Properties: No significant differences in taste preference or flavour among dietary treatments or between strains.

Continued

Authors	Animals	Experimental Diets	Intervention Period	Performance	LCPUFA Status or Sensory Properties
Ayersa and Coates (2000) [153]	450 27-week old H&N laying hens (225 white and 225 brown laying hens)	Dietary treatments: Diets supplemented with 7, 14, 21 and 28% whole chia seed.	Up to 90 days Cholesterol content, total fat content, and fatty acid composition of the yolks were determined at day 30, 43, 58, 72, and 90.		Total n-3 PUFA content was greater for both strains for all chia enriched diets than the control diet. ALA content increased as the chia content increased, from 0.37 to 15.58% and from 0.29 to 16.60% for both strains at day 90. DHA content was higher in yolks from hens fed the chia diets than in those from the control diet, with exception day 30 with 28% chia diet for the white hens, and days 30 and 72 with 28% chia diet for the brown hens. DHA levels of white and brown egg yolks from hens fed 28% chia at day 90 were 1.27 and 1.58, respectively. The level of AA decreased for both strains as chia levels increased.

Continued

Authors	Animals	Experimental Diets	Intervention Period	Performance	LCPUFA Status or Sensory Properties
Scheideler et al. (1998) [127]	144 30-week-old Three strains: Babcock B300 (B), DeKalb Delta (D), and Hy-Line W-36 (H)	Dietary treatments: 10% flax + 5% oats (diet 1, flax+oats) and 10% flax without oats (diet 2, flax-oats).	Up to 20 weeks. 30-50 weeks (diet 1) 50-60 weeks (diet 1 and diet 2)	B strain had a higher egg production than H and D strain during the first 20 weeks of the feeding duration. From 50 to 60 weeks of age, egg production was lower for hens on diet 1 (flax+oats) than for those on diet 2 (flax-oats). There was a significant diet by strain interaction for feed intake (feeding flax + oats decreased consumption rate for D hens). Egg weight of D strain was higher than B and H.	Age caused significantly increase ALA content. There was interaction between diet and strain on ALA content. Flax + oats increased the deposition of ALA in D, compared to B and no change in H. No significant effects of diet, strain or age on DPA. No significant effects of diet or strain on LA, AA, and DHA. There was effect of age on egg DHA with a higher DHA level at 58 weeks com are to 36 weeks.

Continued

Authors	Animals	Experimental Diets	Intervention Period	Performance	LCPUFA Status or Sensory Properties
Ayersa and Coates (1998) [154]	24 40-week old Isa Brown laying hens	Control diet: commercial diet. Dietary treatments: a diet supplemented with 30% whole chia seed. A commercial diet provided for one week after placement.	Up to 28 days From 41 to 45-week-old	Egg production was greater for the control. No significant differences in egg weight and yolk fat content but yolk weights were greater for the control diet than the chia diet.	Fatty acid levels: Saturated palmitic fatty acid reduced by feeding chia diet. The level of n-3 PUFA, PUFA:SFA and n-3:n-6 ratio increased in eggs from hens fed chia diet. Sensory analysis: Untrained panellists did not detect any differences in taste preferences or off-flavour between eggs from hens fed dietary treatments.
Cherian et al. (1995) [126]	60 hens Six strains: White Leghorn, Brown Leghorn (BL), Light Sussex , New Hampshire , Barred Rock , and Rhode Island Red	Control diet: wheat and soybean meal based diets without supplemented ground flaxseed (11.1% LA and 12.4% ALA) content. Dietary treatments: Basal diet with 17% ground flaxseed (containing 24% LA and 40.7% ALA).	Not reported (Hens were kept as breeding stock).		High ALA diet resulted in an increase in ALA and DHA, and reduced AA. High ALA diets enhance DHA content to two-fold. BL accumulated the lowest level of ALA and DHA.

Table 1.7 Summary of studies involving diets containing n-3 LCPUFA sources on n-3 LCPUFA content of chicken meat

Authors	Animals	Experimental Diets	Intervention Period	Performance	LCPUFA Status or Sensory Properties
Chekani-Azar et al. (2008) [17]	240 21-day-old Ross (male) chickens	Dietary treatments: 3% poultry fat (PF, T1), 2% PF + 1% fish oil (FO, T2), 1% PF + 2% FO (T3), or 3% FO (T4). From 1 to 20 days, birds were fed with a basal starter diet.	Up to 21 days (from 21 to 42-day-old)	Data not shown.	Fatty acid levels: All breast n-3 LCPUFA increased with increasing FO level. Increasing levels of FO increased breast PUFA content and decreased MUFA. Sensory analysis: Breast meat from birds fed the highest content of FO had poor odour characteristics.
Lopez-Ferrer et al. (2001) [9]	250 1-day-old Cobb chickens	Plan 1 Dietary treatments: diets supplemented with 0, 2, or 4% fish oil (FO) plus tallow (T) up to 8% added fat (T 1, 2, and 3). Plan 2 Dietary treatments: T3 was given throughout the experimental period, T4 (3% linseed oil, LO) + 1% FO was given for 1 week or 2 weeks (T5) prior to slaughter.	Up to 5 weeks Or replacing FO with LO for 1 or 2 weeks	Supplementation with FO increased weight gain and final weight. No differences in feed conversion ratio were reported.	The level of n-3 PUFA increased, mainly as n-3 LCPUFA. Replacing FO with LO increased n-3 FA, mainly in the form of ALA.

Continued

Authors	Animals	Experimental Diets	Intervention Period	Performance	LCPUFA Status or Sensory Properties
Gonzalez-Esquerra and Leeson (2000) [32]	330 1-day-old male broiler chickens (a commercial strain)	Control group: commercial diet. Dietary treatments: diets containing 0 or 100g/kg linseed or 7.5 or 15g/kg menhaden oil (MO), and the combination of 100g/kg linseed with either 7.5 or 15g/kg MO. From 1 to 20 days: commercial diet. From 21 to 35 days: grower diet.	From 36 to 49 days: experimental diets. 7 or 14 days prior to slaughter which was 49 days.	Final body weight (FW, 49) & body weight gain (36-49) were not affected by diets.	Fatty acid levels: ALA was preferentially accumulated in dark meat and long chain polyunsaturated fatty acids in white meat. There was a major increased in breast and thigh ALA, and a slight increase in DPA in birds fed 100g/kg flaxseed. No effect on EPA and DHA. Sensory analysis: Sensory quality of breast meat was not affected in birds fed 100g/kg flaxseed for 14 days.
Lopez-Ferrer et al. (1999) [95]	64 unsexed 1-day-old Cobb chickens	Control diet: basal diet. Treatment groups: Basal diet enriched with 8.2% fish oil (FO, T1). FO was replaced with 8.2% linseed (LO) or canola oil at the final week before slaughtering (T2), the final 2 weeks (T3), or throughout the trial period (T4).	Up to 35 days of age		Fatty acid levels: FO supplementation increased the deposition of LCPUFA. Replacement of FO by LO 1 or 2 week before slaughtering increased ALA, and decreased EPA and DHA. Sensory analysis: Replacing 1 or 2 week FO with vegetable oils increased the sensory quality of meat.

Table 1.8 Summary of studies involving diets containing n-3 PUFA sources (ALA) on n-3 LCPUFA content of chicken meat

Authors	Animals	Experimental Diets	Intervention Period	Performance	LCPUFA Status or Sensory Properties
Thacker and Widyaratne (2012) [155]	180 1-day-old Ross-308 male broilers	Contro diet: a diet containing 15% canola meal. Dietary treatments: diets supplemented with 3, 6, 9, 12 or 15% camelina meal at the expense of canola meal.	Up to 21 days (from day 0 to 21)	Weight gain and feed intake were decreased with increasing the levels of camelina meal. Feed conversion ratio was negatively influenced by camelina meal.	Birds fed 15% camelina meal enriched diets had higher levels of PUFA, total n-3 PUFA, total n-6 PUFA and a lower ratio of n-6 to n-3 in the abdominal fat pads.
Rahimi et al. (2011) [143]	324 1-day-old Cobb 500	Control: basal diet. Dietary treatments: Basal diet was supplemented with 7.5% ground canola seed (CS1), 15% ground canola seed (CS2), 10% ground flaxseed + 10% ground canola seed (CS-FS), 7.5% ground flaxseed (FS1), 15% ground flaxseed (FS2).	Up to 42 days	Feed intake was not affected by dietary treatments. Diets with CS and FS had lower body weight gain and higher feed conversion ratio than the control group.	FS and CS diets increased ALA, n-3 PUFA and decreased AA content in breast meat. Breast EPA and DHA results were not reported.

Continued

Authors	Animals	Experimental Diets	Intervention Period	Performance	LCPUFA Status or Sensory Properties
Betti et al. (2009) [138]	128 1-day-old Ross x Ross 308 Mixed-sexed	Control: basal diet. Dietary treatments: Supplemented with low (10%) or high (17%) ground whole flaxseed.	0, 4, 8, 12, 16, 20, 24, and 35 days		Diet supplemented with 10 or 17% flaxseed increased all n-3 LCPUFA in breast phospholipid (PL) and triglyceride (TAG). ALA was mainly deposited in TAG and n-3 LCPUFA in PL fraction, with DPA as the main n-3 LCPUFA. Level of 300mg total n-3 PUFA per 100g of breast meat was achieved at 11.3 and 26.2 d in the high and low flaxseed diets, respectively.
Zuidhof et al. (2009) [156]	800 1-day-old Ross x Ross 308 Mixed-sex broiler	Control: basal diet. Dietary treatments: Supplemented with low (10%) or high (17%) ground whole flaxseed.	0, 4, 8, 12, 16, 20, 24, and 35 days	Added 10 and 17% flaxseed decreased growth rate and body weight (BW), and increased FCR. Feeding 10% flaxseed for 16 days and 17% flaxseed for 8 days decreased BW. Flaxseed level, feeding period, and their interaction influence FCR.	Breast fat content averaged 2.45% and not influenced by diets. In breast meat, the threshold level of 300mg n-3PUFA/100g, for labelling foods as an n-3 fatty acid source, was reached at 24.1and 12.1 days in the low and high flaxseed diets. ALA was the major n-3 PUFA in breast and thigh. In breast and thigh, EPA content increased two-fold after 8 or 12 days, and tripled after 35 days. No change in DHA.

Continued

Authors	Animals	Experimental Diets	Intervention Period	Performance	LCPUFA Status or Sensory Properties
Febel et al. (2008)[19]	1200 1-day-old male (Ross-308) chicks	Basal diet supplemented with 3% of lard (L), 3% sunflower oil (SFO), 3% soybean oil (SO), and 3% linseed oil (LO). Lipid content of diets approximately 6%.	Up to 35 days of age	No effect on growth or FCR.	LO resulted in higher SFA than SBO. LO gave the highest level in n-3 LCPUFA (EPA and DHA).
Zelenka et al. (2008) [21]	32, 25-day-old male birds (Ross-308)	Basal grain diet supplemented with 1%, 3%, 5% or 7% linseed oil. A diet given until day 25 unknown.	From day 25 to 40 days of age	5 and 7% of oils caused a higher body weight gain and lower feed consumption per unit of body weight gain.	The levels of n-3 LCPUFA increased as LO in the diet increased. However, there was no difference in n-3 LCPUFA between 5% and 7% LO. Highest n-3 LCPUFA = 5.5% in breast and 2.2% in thigh.
Haugh et al. (2007) [157]	60 1-day-old male Ross 308 chicks All diets contain 5% rendered fat	Dietary treatment: 5% rapeseed oil (RO) + 0.50mg Se/kg (i), 5% RO + 0.84mg Se/kg (ii), 1% linseed oil (LO) + 4% RO + 0.50mg Se/kg (iii), and 1% LO + 4% RO + 0.84mg Se/kg (iv).	Up to 22 days of age	Final body weight (22) and feed intake (FI) were a little higher in RO, FCR was not different.	LO increased thigh ALA, EPA and DPA and decreased LA and AA. LA to ALA ratio and AA to EPA ratio lowered in LO.

Continued

Authors	Animals	Experimental Diets	Intervention Period	Performance	LCPUFA Status or Sensory Properties
Ryhänen et al. (2007) [158]	196 1-day-old mixed sex Ross chickens	Dietary treatments contained 0 (control diet), 5, and 10% Camelina sativa expeller cake (CSEC).	Up to 37 days	Diets containing CSEC reduced body weight and the growth of birds. The use of CSEC reduced feed intake during the starter phase (1 – 14 days) and feed conversion ratio during the starter period and throughout the study period.	Fatty acid levels: Diets supplemented with 10% CSEC increased n-3 PUFA content of leg samples, from 4.6 and 4.8% to 9.3 and 9.0% in male and female birds, respectively, mainly due to the increase in ALA levels. Diets enriched with CSEC decreased SFA and MUFA levels and increased PUFA content in both female and male. Diets containing 10% CSEC increased the total n-6 PUFA of female broiler leg. The content of n-3 LCPUFA was not reported. Sensory analysis: No any adverse effect on the sensory quality of leg broiler meat.

Continued

Authors	Animals	Experimental Diets	Intervention Period	Performance	LCPUFA Status or Sensory Properties
Crespo and Esteve-Garcia (2002) [159]	100 1-day-old female Ross 308 chicks	Experiment 1 Control: basal diet. Dietary treatments: basal diet with added fat including tallow (T), olive (OO), sunflower (SFO) and linseed (LO) oil at 10%. Experiment 2 Basal diet with fat sources 10% supplemented with full-fat.	Experiment 1 From day 28 up to 48 Experiment 2 From day 28 up to 53	There was no significant in body and carcass fat. LO produced less abdominal fat as a consequence of higher lipid oxidation.	LO had the highest level of all liver n-3 LCPUFA.
Lopez- Ferrer et al. (2001) [20]	230 unsexed 1-day-old Cobb chickens	Plan 1 Control diet: 0% linseed oil (T1, 1% ALA). Treatment groups: LO + tallow up to 8%; 2% LO (T2, 13.5% ALA), 4% LO (T3, 28.1% ALA). Plan 2 Use T3.	Up to 38 days of age (plan 1) 24, 28, 54 days for T3 (plan 2), to evaluate the progressive deposition of LCPUFA (liver, thigh).	The increase in weight in grams/b/d was higher in T3.	LO decreased thigh SFA and MUFA, and increased PUFA (ALA, LA). EPA, DPA and DHA were the highest in T3 0.39, 0.24, and 0.25% of fatty acids, respectively. Longer feeding time of LO did not result in higher tissue accumulation of n-3 LCPUFA.

Continued

Authors	Animals	Experimental Diets	Intervention Period	Performance	LCPUFA Status or Sensory Properties
Crespo and Esteve-Garcia (2001) [160]	960 21-day-old male and 960 21-day-old female Ross 208 chicks	Control diet: starter diet. Dietary treatments: The inclusion of fat sources including tallow (T), olive oil (OO), sunflower oil (SFO) and Linseed oil (LO), either 6 or 10%.	Male from 21 to 42 days Female from 21 to 49 days of age	Final body weight and weight gain were not affected by dietary treatments. Feed intake reduced as dietary fat increased. SFO and LO increased feed efficiency.	Tallow increased SFA. The LO diets produced the highest level of ALA, EPA and DHA in thighs and breasts, and the lowest level of LA. SFO resulted in the lowest level of n-3 fatty acids. LO and SFO produced similar levels of n-6 fatty acids in the abdominal fat.
Skrivan et al. (2000) [136]	320 1-day-old chickens	Control diet: wheat/maize-soybean meal. Treatment groups: supplementation 50g/kg lard (i), 25g/kg lard + 25g/kg rapeseed oil (ii), 50 g/kg RO (iii), 50g/kg RO + 200mg copper/kg.	Up to 39 days of age		The substitution of lard with RO increased breast tissue PUFA, decreased the ratio of n-6:n-3, decreased cholesterol, and had no effect on EPA and DHA.

1.2 Summary

The health benefits of consuming fish and fish products, the main source of n-3 LCPUFA, have been well documented. However, some people do not habitually consume fish. In order to increase the n-3 LCPUFA status of humans, vegetable oils containing ALA, the precursor of n-3 LCPUFA, can be consumed but the conversion of ALA to n-3 LCPUFA is rather inefficient in humans [109-111]. As reviewed by Burdge and Calder [161], the extent of conversion of ALA to DHA was low, with the highest estimated fractional conversion 4%. An alternative is to feed oils rich in ALA to food animals (such as pigs and chickens) in the hope that this can result in higher levels of tissue EPA DPA and DHA. Many animals can convert ALA to EPA, DPA and DHA far more effectively than humans. This conversion is influenced by the availability of the desaturation and elongation enzymes which are also involved in the conversion of LA, the n-6 PUFA, to AA. The availability of these enzymes and the balance of substrates LA and ALA determine the outcomes of these processes. This is due to the fact that ALA and LA compete for the same enzymes in their metabolism. In order to promote increased EPA, DPA and DHA production, alteration of the ratio of LA to ALA to keep chicken diets low in LA and high in ALA is important.

Studies have been conducted on the supplementation of ALA rich vegetable oils at a variety of levels into diets in other animals, such as Atlantic salmon [162], lambs [163], pigs [116] and chickens [19, 21]. These have had positive responses in increasing tissue EPA, DPA and DHA concentrations. However, some studies in chicken showed that there is variation in the accumulation of n-3 LCPUFA in tissues. The variation of response in such studies may be due in part to a high ratio of LA to ALA in the experimental diets and maybe as a result of the fact that a different strain of birds was

used. Thus, it is important to evaluate the effect of both the LA and ALA content in chicken diets on the conversion of ALA to n-LCPUFA in two different strains of birds both broilers and laying hens.

Since one of the goals of this study is increasing chicken product n-3 fatty acids in order to increase n-3 LCPUFA consumption in human diets, functional properties and sensory quality of the chicken products need to be evaluated to ensure consumer acceptance. Developing studies on chicken diets would help to provide exciting alternative n-3 rich foods without compromising sensory quality and may increase the consumption of n-3 LCPUFA (EPA, DPA and DHA) which would be beneficial to human health.

1.3 Major Research Aims

In order to acquire a better understanding of relationship between ALA content of diets and n-3 LCPUFA accumulation in chicken products, this research has the following aims:

1. To determine whether the levels of dietary ALA, at fixed levels of LA, in chicken diets increases n-3 LCPUFA, EPA, DPA and DHA in chicken products (meat and eggs) without affecting sensory properties.
2. To ascertain if there is a difference in the apparent conversion of ALA to n-3 LCPUFA between Cobb 500 and Ross 308 strains of chickens as measured by the fatty acid composition of the meat.
3. To determine whether there is a difference in the apparent conversion of ALA to n-3 LCPUFA between brown and white laying hens as measured by the fatty acid composition of the eggs.

CHAPTER 2

GENERAL METHODS

2.1 Introduction

The methods used in this thesis are described in detail in this chapter, namely lipid extraction and fatty acid analysis. Modifications to these methods and other methods regarding management of birds and sensory evaluation of the final products are described in each chapter as appropriate.

2.2 Chemicals and reagents

All solvents used for extraction and thin layer chromatography (TLC) were analytical reagent grade. Methanol and diethyl ether were purchased from Chem-Supply (Gillman, SA, Australia). Chloroform and petroleum spirit 40-60°C were obtained from BDH Prolabo, VWR International S.A.S (Fontanay-sous-Bois, France). Butylated hydroxyanisol (BHA) and anhydrous sodium sulphate (Na_2SO_4) were purchased from Sigma-Aldrich (St. Louis, MO, USA). Sulphuric acid (H_2SO_4) was from APS Finechem (Seven Hills, NSW, Australia) and isotonic saline was from Baxter Healthcare (Old Toongabbie, NSW, Australia). Silica gel 60H for thin-layer chromatography was from Merck KGaA (Darmstadt, Germany). Acetic acid glacial was from Ajax Finechem-Unichrom (Auburn, NSW, Australia). Filter papers were obtained from Whatman International Ltd. (Maldstone, England). N-Heptane was from Ajax Finechem (Taren Point, NSW, Australia). Authentic lipid standards and GLC standard were obtained from Nu-Check-Prep Inc (Elysian, MN, USA). The

C17:0 phospholipid (diheptadecanoyl) and C17:0 triglyceride (triheptadecanoin) internal standard was from Sigma (St. Louis, MO, USA). Gases used for gas chromatography (GC) analysis including medical air, high purity nitrogen, high purity hydrogen, and ultra-high purity helium were supplied by Coregas (Thebarton, SA, Australia).

2.3 Methods

2.3.1 Fatty acid methyl ester (FAME) extraction methods

Fatty acid profiling was performed on feed, egg, and tissue samples. Analyses were also performed on vegetable oils in order to accurately formulate diets with the desired LA and ALA levels. Total lipids (TL) in diet, egg, and tissue samples were extracted by the methods of Folch et al. [164] with chloroform/methanol (2:1, v/v). All organic solvents except *n*-heptane contained butylated hydroxyl anisole (0.005%) as an antioxidant. The fatty acids were transmethylated by the procedure described by Tu et al. [118]

2.3.1.1 Fatty acid analysis of oils.

Oils at room temperature were shaken vigorously before sampling. Using a short Pasteur pipette, oil (5-7mg) was dispensed into labelled scintillation vials (20mL) to which 5mL 1% H_2SO_4 in methanol was added. The vials were then heated to 70°C for 2 hours. After cooling, 750µL of distilled water was added, followed by the addition of 2mL *n*-heptane and mixed well by vortex. After standing for a few minutes, the resulting top heptane layer containing fatty acid methyl esters (FAMEs) was transferred into a labelled 2mL GC vial using a short disposable glass Pasteur

pipette. A minimal amount of Na_2SO_4 (approximately 30mg) was included as a dehydrating agent.

2.3.1.2 Fatty acid analysis of feed

Feed samples from each experimental diet was collected after feed mixing and stored at 4°C until analysed. The feed samples were ground using a mortar and pestle and the sample diets (0.3g) were then placed into labelled Kimble glass tubes and the sample weight was recorded (Metler AE 163). Cold isotonic saline (0.9% sodium chloride, 2mL) was added to the tube and then ground finely using a Polytron homogeniser (a Kinematica PT 2000 fitted with a Kinematica PTA7 Aggregate, Switzerland). Total lipids of the diets were extracted with methanol/chloroform (1:2, v/v). Samples were vortexed thoroughly with methanol (3mL) and allowed to stand for 5 minutes at room temperature. Chloroform (6mL) was added and the glass tubes were shaken vigorously. After standing for 15 minutes at room temperature, the tubes were then centrifuged at 3000rpm (1559g) (Heraeus Sepatech, Hanau, Germany) for 10 minutes in order to separate the aqueous and organic phases. The chloroform, organic phase in the bottom layer containing fat, was transferred and placed into a labelled 20mL scintillation vial using a 22.86cm disposable glass Pasteur pipette and then evaporated to dryness under a stream of nitrogen. The lipid extracts were weighed and the percentage of total fat of the samples was calculated as follows:

Total fat (%) = Wt fat (g)/Wt of sample (g) x 100%

Samples were then transmethylated by the addition of 5mL of 1% H_2SO_4 in methanol at 70°C for 3 hours. After cooling, the resulting methyl esters were extracted into 750µL of distilled water and 2mL *n*-heptane. The FAME were then transferred to GC vials containing approximately 30mg of anhydrous Na_2SO_4 as the dehydrating agent and stored at -20°C for GC analysis.

2.3.1.3 Fatty acid analysis of eggs

Egg yolk samples (50mg) were taken and placed into labelled Kimble glass tubes. The tube containing the egg sample was then weighed and the sample weight was recorded (Metler AE 163). Cold isotonic saline (0.9% sodium chloride, 2.25mL) was added to the tube and vortexed vigorously. Chloroform/methanol (2:1, v/v) was added to the homogenate to extract the total lipid. Methanol (3mL) was added to the egg suspension followed by addition of 100µL of triheptadecanoin (C17:0; Avanti Polar Lipids, Inc.; 2.84mg/mL in n-heptane) as an internal standard using an adjustable 40-200µL digital pipette. The glass extraction tubes were then mixed vigorously and allowed to stand for 5 minutes. Chloroform (6mL) was then added to the glass tubes and the tubes were shaken vigorously and then allowed to stand for 15 minutes at room temperature. The tubes were then centrifuged at 1559g for 10 minutes (Megfuge 1.0, Heraeus Sepatech, Hanau, Germany) to separate the aqueous and organic phases. The chloroform layer was removed and placed into labelled 20mL scintillation vials using a disposable glass Pasteur pipette and then evaporated under stream of nitrogen gas to dryness. The lipid extracts were then weighed and total lipid contents were then determined. FAMEs were derivatised from total lipid extract using 1% H_2SO_4 in methanol as the derivatising agent. The dried lipid

extracts were methylated with 5mL of 1% H_2SO_4 in methanol at 70°C for 3 hours and then cooled at room temperature. Distilled water (750µL) was added to the methyl ester and extracted into 2mL of *n*-heptane. The FAME were then transferred to GC vials containing anhydrous Na_2SO_4, sealed and stored at -20°C for GC analysis.

2.3.1.4 Fatty acid analysis of tissues

Tissue samples, including liver and breast, were cleaned from connective tissue and any adipose on a glass plate. Liver (0.3g) was sliced prior to homogenisation and breast tissues (approximately 1g) were scraped to clean away adipose tissues. Samples were then placed into 12mL ground glass tubes and weighed. 0.9% saline (2mL) was added to tissue samples and homogenised with a Kinematica PT 2000 fitted with a Kinematica PTA7 Aggregate (Switzerland). To extract liver and breast total lipid, methanol (3mL) was added and mixed thoroughly, followed by addition of chloroform (6mL) containing triheptadecanoin (C17:0; Avanti Polar Lipids, Inc.) as an internal standard. The concentration of the internal standard for raw and cooked breast meat total lipid was 0.56 and 1.50mg/mL in n-heptane, respectively. The glass tube was shaken vigorously and allowed to stand for 15 minutes at room temperature. Samples were then centrifuged at 1559g for 10 minutes. The chloroform layer was removed and placed into labelled 20mL scintillation vials using a 28.86cm disposable glass Pasteur pipette and then evaporated to dryness using a vacuum concentrator. The total lipid extracts obtained were weighed and recorded to calculate fat content. For fatty acid analysis of liver and breast tissues as total lipid, the lipid extract was then transesterified by methanolysis (1% H_2SO_4 in methanol) at

70^0C for 3 hours and extracted with *n*-heptane (see section 2.3.1.2). For lipid classes, the lipid extract was reconstituted in 150µL chloroform/methanol (9:1, v/v) for further lipid class separation.

2.3.1.5 Lipid classes separation by thin layer chromatography

The phospholipids and triglyceride fractions were separated from other lipids by TLC on 0.3mm silica gel plates (Silica gel 60H, Merck, Darmstadt, Germany) using a developing agent of petroleum spirit/diethyl ether/glacial acetic acid (180:30:2, v/v/v). The TLC were sprayed with fluorescein 5-isothiocyanate in methanol and visualised under ultraviolet light. The phospholipids and triglyceride fractions were identified by comparison to a TLC reference standard 18-5A (Nu-Chek-Prep Inc., Elysian, USA). Cholesterol ester (CE) (fraction 1) migrated to the solvent front, followed by triglyceride (fraction 2), free fatty acid (FFA) (fraction 3), cholesterol (fraction 4) and then phospholipid (fraction 5) (Figure 2.1). The phospholipid and triglyceride bands were individually scraped and transferred into 20mL scintillation vials containing 5mL of 1% H_2SO_4 in methanol. Vials were then sealed and allowed to methylate at 70°C for 3 hours. After cooling, distilled water (750uL) was added to the methyl esters and extracted into 2mL of *n*-heptane. Samples were then stored at 20°C in GC vials with anhydrous Na_2SO_4 for fatty acid analysis. All organic solvents contained BHA at 0.005% (w/v) as an antioxidant.

2.3.2 Gas chromatograph analysis of FAME

The fatty acid composition of samples was determined with a Hewlett-Packard 6890 GC (CA, USA) equipped with a flame ionisation detector (FID), a split injector, and

a BPX-70 capillary column (50m x 0.32mm internal diameter) coated with 70% cyanoproply polysilphenylene-siloxane with a film thickness of 0.25µm (SGE, Victoria, Australia). The operation conditions of the machine were as follows: an initial oven temperature of 140°C was programmed to rise by 3°C per minute to 180°C and maintained for 5 minutes. At a rate of 4°C per minute, the temperature was increased to 220°C and held for 1 minute. The temperature was then increased by 10°C per minute to 240°C and held for 3 minutes. FAMEs were separated using a carrier gas (helium) at a flow rate of 23.3 ml per minute, as the make-up gas, which is to 30ml per minute. The inlet split ratio was set at 20:1. The temperature of injection split and the flame ionization detector was set at 250 and 300°C, respectively.

Each fatty acid was identified by comparing the retention time of each peak against the retention times of a fatty acid standard of known composition (GLC463, 3mg/mL, Nu-Chek Prep Inc., Elysian, MN, USA) using the software package Agilent GC Chemstation (Agilent Technologies Inc., Palo Alto, CA, USA). Each peak from each trace was expressed as the relative percentage of the total FAME in the sample calculated on the basis of the response factors of the external standard. Fatty acid concentrations (mg/100g of breast meat or mg/yolk) were calculated by proportional comparison of gas chromatography peak areas to that of the internal standard (triheptadecanoic acid; 17:0). A typical trace of chromatogram of FAMEs derived from a breast sample is shown in Figure 2.3. The detection limit of each fatty acid was 0.05% of total fatty acids.

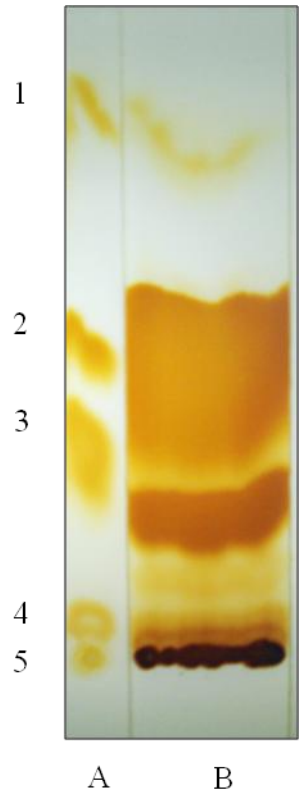


Figure 2.1 Schematic distributions of breast tissue lipids. Total lipids were extracted from breast tissues with chloroform/methanol (2:1, v/v) and separated by thin layer chromatography (TLC) with petroleum spirit/diethyl ether/glacial acetic acid (180:30:2, v/v/v) as a developing agent. The plate was visualised by iodine vapour. TLC reference standard 18-5A (A) were used to identify breast lipids (B) including 1, cholesterol ester; 2, triglyceride; 3, free fatty acid; 4, cholesterol and 5, phospholipids.

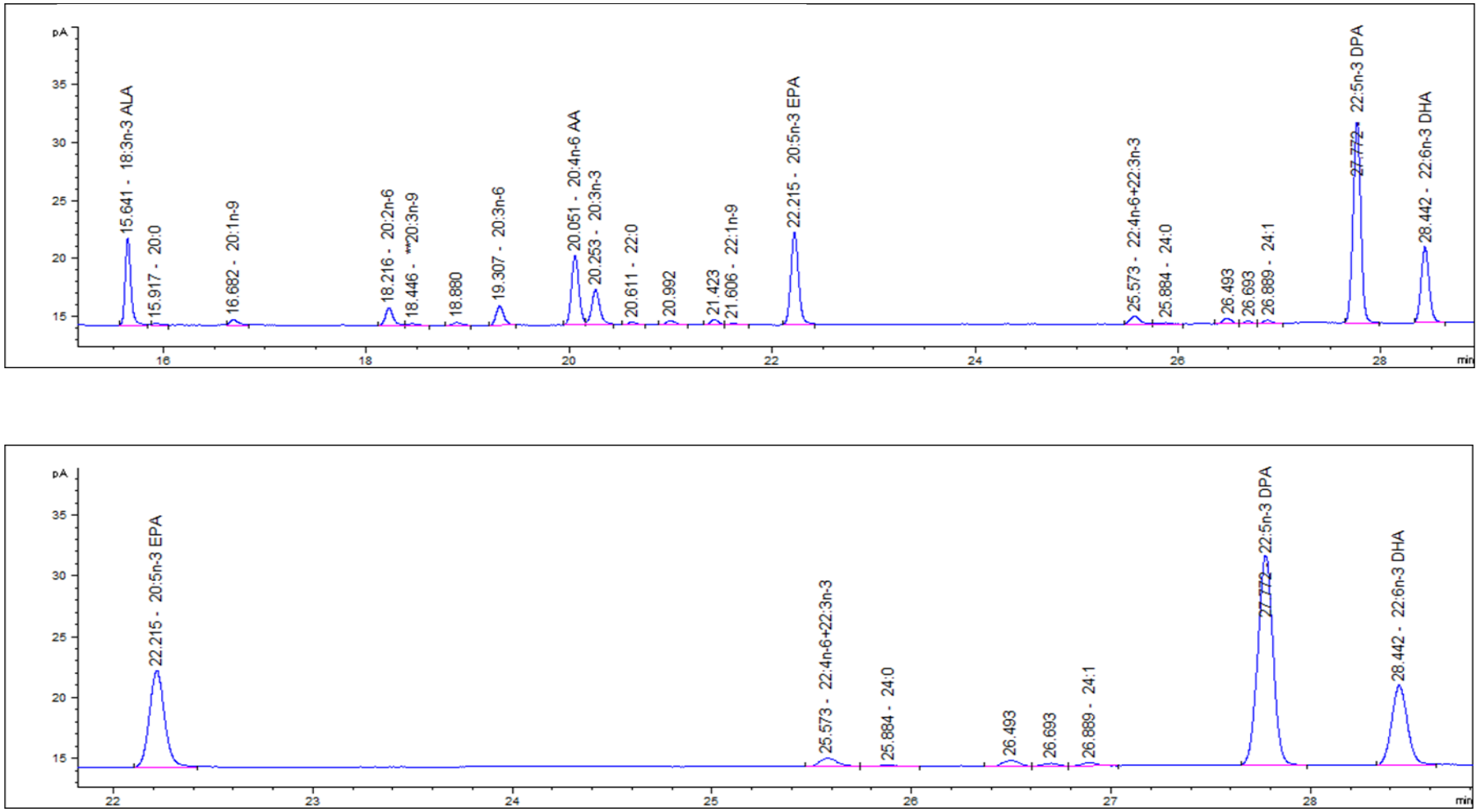


Figure 2.2 A chromatogram of FAMEs derived from a breast phospholipid sample fed diet containing 6%energy as ALA.

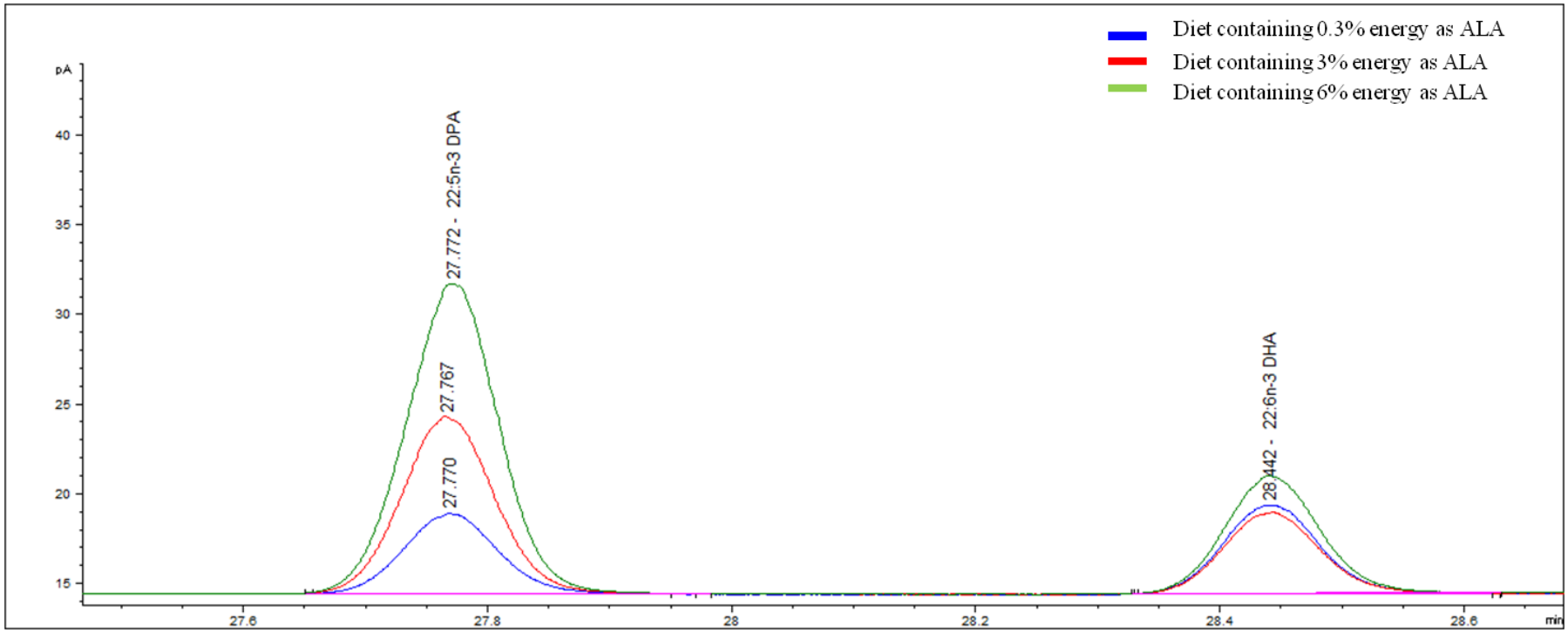


Figure 2.3 A chromatogram of DPA and DHA derived from a breast phospholipid sample of Cobb birds fed diet high in ALA content (6%energy, %en as ALA, green) compared to those of fed low ALA diet (0.3%en, blue).

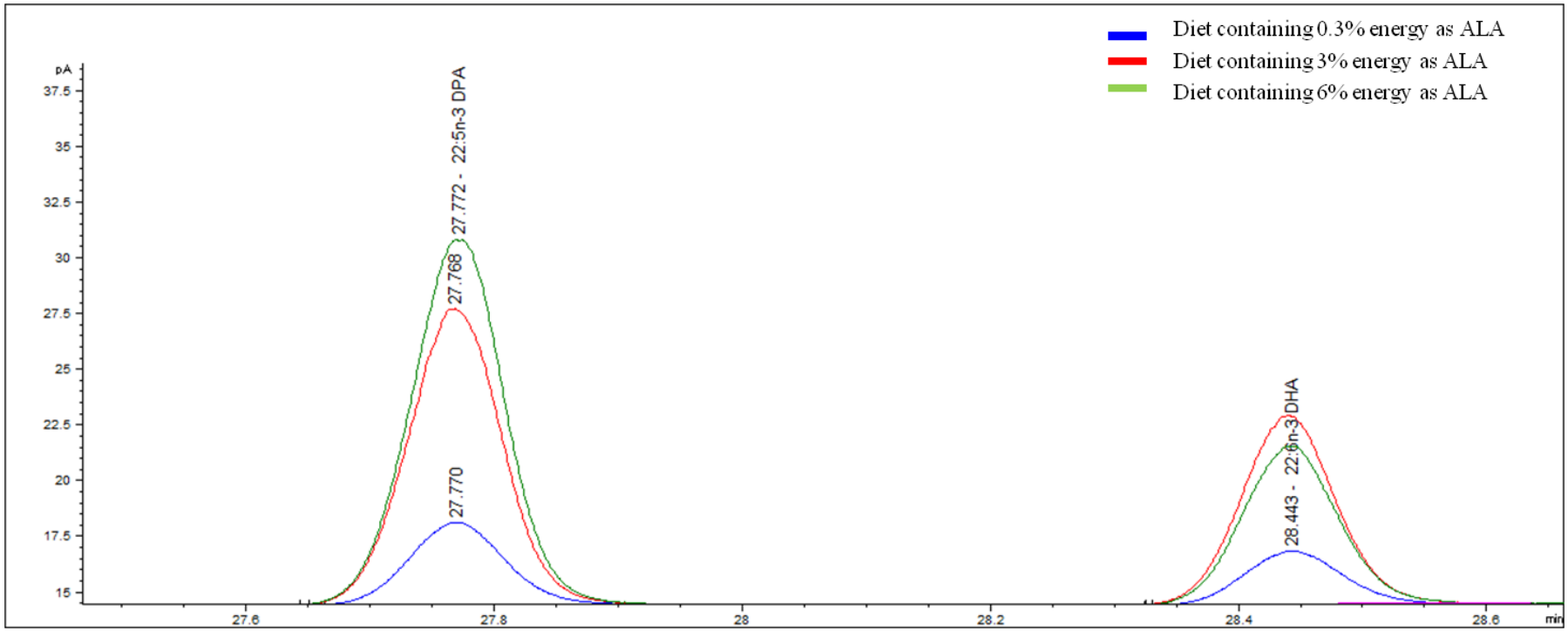


Figure 2.4 A chromatogram of DPA and DHA derived from a breast phospholipid sample of Ross birds fed diet high in ALA content (6%en, green) compared to those of fed low ALA diet (0.3%en, blue).

CHAPTER 3

THE EFFECT OF ALPHA-LINOLENIC ACID ENRICHED DIETS ON PRODUCTION PERFORMANCE, OMEGA-3 FATTY ACIDS AND SENSORY PROPERTIES OF EGGS FROM TWO STRAINS OF LAYING HENS

3.1 Abstract

Including fish meal or fish oil in the diet of chickens results in high levels of n-3 LCPUFA in eggs, mainly in the form of EPA and DHA. However, this dietary approach causes impaired sensory qualities in eggs. The study described in Chapter 3 examined whether including a vegetable source of n-3 fat in the form of ALA in the diets of two strains of laying hens would improve n-3 LCPUFA accumulation without altering egg production performance by hens or the sensory characteristics of the eggs. Diets containing three levels of ALA (0.3, 3 and 6%en) while holding the level of the n-6 fatty acid (LA) constant, were fed to 48 hens (24 Hy-Line white and 24 Hy-Line brown) at point of lay for 13 weeks. Eggs produced prior to the commencement of the trial and at the 4-week, 8-week and 12-week experimental period were collected for fatty acid analysis. Eggs produced during the final week of the trial were collected for the sensory evaluation of the eggs. Results showed that production performance of laying hens was comparable for all experimental diets. Feed intake, feed conversion ratio, egg production and egg weight were not significantly affected by increasing levels of dietary ALA. There was no effect of strain on the production parameters measured. The fatty acid composition of eggs varied in proportion to the levels of dietary ALA and strains of laying hen. Diets high in ALA led to an increase in the levels of EPA (8-fold), DPA (3-fold), DHA (2-fold),

and total n-3 (8-fold) of the eggs. However, most of the increase in the n-3 content was due to enrichment in ALA. For example, while the total n-3 PUFA in yolks of eggs from hens fed the moderate (3%en) and high ALA (6%en) diets was 308 and 533mg/yolk, respectively, the equivalent n-3 LCPUFA levels were 99 and 100mg/yolk. Nevertheless, these eggs met the requirement needed for labelling as n-3 PUFA sources (300mg/egg) in contrast to the control (59mg/egg) or commercial eggs (80mg/egg). Enriching ALA levels in the diets had no effect on aroma, taste, egg flavour or off-flavours of boiled eggs. In scrambled eggs, high ALA diets tended to decrease egg aroma, in particular for white eggs. Increased dietary ALA levels did not change the consumer acceptance of the eggs compared with eggs purchased from a local supermarket. In conclusion, birds fed high ALA diets produced eggs higher in n-3 LCPUFA, which provides an alternative n-3 rich food for consumers without influencing production performance of birds or sensory qualities.

3.2 Introduction

Dietary n-3 PUFA are well known to have beneficial health effects. Therefore, increasing n-3 consumption, in particular EPA and DHA is recommended by health authorities [5-8]. The American Heart Association recommends the consumption of oily fish twice a week and to include oils or n-3 PUFA, ALA enriched foods for patients without documented cardiovascular disease [165]. However, because many people do not eat enough fish, which is the main source of EPA and DHA, it is important to provide alternative foods that are rich in n-3 LCPUFA.

One of the approaches to increase the n-3 LCPUFA content of the diet is the consumption of n-3 enriched eggs. Commercial production of n-3 enriched eggs often utilises diets enriched with n-3 LCPUFA from marine sources. This dietary approach is effective because of the direct incorporation of preformed n-3 LCPUFA into the eggs which are metabolically more important than ALA. The inclusion of fish meal or fish oil into the diets of laying hens to generate n-3 LCPUFA enriched eggs has been investigated widely and results in high levels of n-3 LCPUFA, mainly in the forms of EPA and DHA [32, 79, 82, 83, 88]. The supplementation of layer feed with up to 60g/kg menhaden oil produced eggs containing approximately 45-60mg EPA/egg and 150-200mg DHA/egg [32]. Similar results have been reported by Cachaldora et al. [79]. However, the inclusion of fish products in the diet of laying hens causes impaired sensory quality in the final products, such as fishy odours and fishy off-flavours [16, 17, 88, 132].

Another dietary approach to increase n-3 LCPUFA is feeding laying hens with flaxseed, rich in the n-3 PUFA, ALA. This strategy rests on the ability of laying hens to convert ALA to DHA [92]. Previous studies found that the inclusion of 10% flaxseed (~33% ALA) in the diets of laying hens resulted in a higher accumulation of n-3 fatty acids than a control diet, with levels of ALA, DHA, and total n-3 PUFA in eggs increasing to 8-, 2-, and 4-fold, respectively [102, 123, 125]. However, flaxseed-based diets lead to a preferential retention of ALA and to a lower accumulation of EPA and DHA relative to those fed marine fish oils. Some studies found that the effectiveness of flaxseed in increasing n-3 LCPUFA content was limited after 10% inclusion [123, 135]. Sari et al. [123], who included 5, 10 and 15%

flaxseed in a hen diet, reported no increase in the content n-3 LCPUFA in eggs. In addition, due to competition between ALA and LA for the same enzymes in the metabolic pathways, diets high in LA could inhibit DHA production. This was demonstrated in a previous study conducted by Kartikasari et al. [115] that showed that increasing levels of dietary LA, while maintaining the ALA content of diets reduced EPA and DHA level in broiler tissues. Also, trained panellists detected differences in sensory attributes including aroma, flavour, off-flavour, and overall difference between control eggs and eggs fed flaxseed at the level of 10% [130]. Jiang et al. [133] reported that when laying hens were fed diets supplemented with 15% whole flaxseed, fishy tastes were also detected in the eggs. Finally, the inclusion of high dietary levels of flaxseed (10%) have been associated with a decrease in performance parameters such as reducing feed intake [102, 123, 125], egg production [123], egg weight and yolk weight [135].

The hypothesis for the current study was that ALA rich vegetable oils included against a background of a low LA level in basal diets may lead to the generation of eggs with significant levels of the n-3 LCPUFA and total n-3 fatty acids, without negatively influencing the sensory quality of the egg. There is little information in relation to the use of vegetable oils rich in ALA in different strains of laying hens. Therefore, the objectives of the current study included the evaluation of the effectiveness of altering dietary ALA levels while keeping LA constant for the moderate (3%en) and high (6%en) ALA diets on production performance, incorporation of n-3 fatty acids, and the sensory qualities of the eggs of two strain of laying hens.

3.3 Specific aims of this chapter

The aims of the experiment described in this chapter were:

1. To investigate the effect of varying dietary ALA levels while holding the level of LA constant for moderate and high ALA diets in chicken diets on production performance and n-3 LCPUFA levels in the eggs of white and brown of layers.
2. To determine whether there is a difference in the apparent conversion of ALA to n-3 LCPUFA between brown and white laying hens as measured by the fatty acid composition of the eggs.
3. To identify the level of dietary ALA to achieve enrichment of the egg to a level of 300mg/yolk of fatty acids.
4. To evaluate the sensory properties and consumer acceptance of eggs following consumption of diets enriched with ALA.

3.4 Materials and methods

3.4.1 Ethical considerations

Ethical approval for all experiments was obtained from the Animal Ethics Committees of the Department of Primary Industries South Australia and the University of Adelaide. All procedures complied with the "Australian code of practice for the care and use of animals for scientific purposes" (Australian Agriculture Council, 1997) and the "Australian model code of practice for the welfare of animals Domestic Poultry" (Standing Committee on Agriculture and Resource Management, 1995).

3.4.2 Location

The experiments were conducted at both the Roseworthy and Waite Campuses of the University of Adelaide. Chickens were housed at the Pig and Poultry Production Institute (PPPI, SARDI), Roseworthy Campus. Fatty acid analysis of feed and tissue samples were conducted at the Fatty Acid Laboratory and sensory studies were carried out in the Sensory Evaluation Laboratory, Waite Campus of the University of Adelaide.

3.4.3 Experimental design

The experimental design of this study was a 3 × 2 factorial in a complete randomised block design (8 replications per strain × diet combination). The dietary treatments were based on the level of dietary ALA as %en with a constant LA level.

3.4.4 Birds, rearing and management

A total of 48 laying hens (24 Hy-Line white-36 and 24 Hy-Line brown) were allocated to three dietary treatments. Upon arrival, the hens were immediately weighed and housed one per cage (500mm wide × 550mm deep x 500mm high; Figure 3.1). Each of the six experimental treatments was replicated eight times. The experiment was carried out for 13 weeks. Birds, for both strains, were placed at point of lay and fed *ad libitum* for the duration of the experiment. During the first few days, the chickens were observed at frequent intervals to ensure that they were comfortable with the environmental conditions and that all had access to adequate feed and water. The shed heating, cooling and ventilation systems were designed to provide an ideal environment for the laying hens by a logic controller (Tempron 606,

Agrologic). All birds were subjected to a 16 hour light program throughout the growth period [166, 167]. Feed consumption were measured weekly to determine feed conversion ratio (FCR; kg feed/kg egg). All birds were weighed individually at the beginning and at the end of each 28-day experimental period.

A

B

Figure 3.1 Housing of hens in individual cages. A and B show the layout from different photographic angles.

3.4.5 Diets

Three dietary treatments were assessed in this experiment. These diets were based on a specially formulated layer hen diet (Ridley Agriproducts Pty Ltd, Murray Bridge, SA, Australia), designed to have varying levels of ALA and a low LA level. The dietary treatments were prepared by supplementing a basal diet with pure or blended vegetable oils. The vegetable oil blend was composed of flaxseed (40%) and canola oil (60%) assigned for diet containing 3%en ALA. Pure or blended vegetable oils were added to each diet to create the desired PUFA composition and to balance the fat content to within an accepted level for chickens (Table 3.1). The ALA and LA content of the basal diet were 4.6 and 37.8% of fatty acid, respectively. Fatty acid composition of vegetable oils used in this study is presented in Table 3.2. All experimental diets were identical in nutrient specifications apart from fatty acid compositions. All diets met or exceeded the requirements, the expected feed intake for the strains, recommended by the National Research Council (1994) [168]. The ingredient composition and nutrient content of experimental diets are listed in Table 3.3. The level of fat in the diets was held constant at approximately 8% (w/w), which is within a commercially accepted range, and the ALA levels of the diets were either 0.3% (low ALA), 3% (moderate ALA) or 6%en (high ALA) with the level of LA held constant at approximately 4%en (Table 3.4). These resulted in the ratio of LA to ALA varying from 7.5:1 (low ALA diet) to 0.7:1 (high ALA diet). A low ALA diet was an attempt to mimic the fatty acid composition of commercial diets.

Table 3.1 Composition of experimental diets for the duration of 13 weeks

	Experimental diets (ALA level, %en)		
Ingredients (%)	**0.3 (low ALA)**	**3 (moderate ALA)**	**6 (high ALA)**
Starter basal diet[1]	94	94	94
Oil added			
Macadamia oil	6	0	0
Flaxseed oil	0	2.4	6
Canola oil	0	3.6	0
Total	100	100	100

[1]Starter basal diet comprised (%): wheat fine (19.20), wheat mill vits (0.80), barley (15.00), triticale fine (19.73), peas fine (20.00), meat meal (2.30), canola meal expeller (3.00), soybean meal (5.53), millrun (3.90), limestone large (9.38), monodicalcium phosphate (0.26), salt (0.16), sodium bicarbonate (0.18), choline chloride 75% (0.06), alimet (0.24), layer/pullet premix (0.20), Roxaphyll 112 (0.005), Avizyme 1210 (0.03), Ronozyme P 5000 Layer (0.009). The ALA and LA content of the basal diet were 4.55 and 37.78% of total fatty acids, respectively.

Table 3.2 Fatty acid composition of the vegetable oils included in the diets of laying hens

	Oils and blended oils[1]			
Fatty acids[2]	**Macadamia**	**Canola**	**Flaxseed**	**Blended oils**
	(% of total fatty acids)			
Total SFA[3]	16.7	7.7	12.8	10.3
Total MUFA[3]	80.4	58.5	21.1	47.3
Total n-9	59.7	54.9	20.2	44.7
Total n-7	20.5	3.6	0.9	2.6
18:2n-6 (LA)[3]	2.4	24.1	18.9	18.8
Total n-6	2.4	24.3	19.0	18.9
18:3n-3 (ALA)[3]	0.1	9.4	47.0	23.4
Total n-3	0.5	9.4	47.0	23.5

[1]Composition of oils consisted of: macadamia oil, diet 1; 40% flaxseed and 60% canola oil, diet 2; and flaxseed oil, diet 3.
[2]Fatty acid profiles are presented as % of total fatty acid analysed by GC. Data are means of n = 3.
[3]SFA= saturated fatty acid; MUFA = monounsaturated fatty acid; LA = linoleic acid; ALA = alpha-linolenic acid.

Table 3.3 Ingredient composition and nutrient content of experimental diets

	Experimental diets (ALA level, %en)		
Ingredients (%)	**0.3 (low ALA)**	**3 (moderate ALA)**	**6 (high ALA)**
Wheat fine	18.05	18.05	18.05
Wheat mill vitamins	0.75	0.75	0.75
Barley	14.10	14.10	14.10
Triticale fine	18.55	18.55	18.55
Peas fine	18.80	18.80	18.80
Meat meal	2.16	2.16	2.16
Canola meal expeller	2.82	2.82	2.82
Soybean meal	5.20	5.20	5.20
Millrun[2]	3.67	3.67	3.67
Limestone large	8.82	8.82	8.82
Monodicalcium phosphate	0.25	0.25	0.25
Salt	0.15	0.15	0.15
Sodium bicarbonate	0.17	0.17	0.17
Choline chloride 75%	0.06	0.06	0.06
Alimet	0.23	0.23	0.23
Layer/Pullet premix	0.19	0.19	0.19
Roxaphyll 112	0.00	0.00	0.00
Avizyme 1210	0.03	0.03	0.03
Ronozyme P 5000 Layer	0.01	0.01	0.01
Basal diet[1]	**94**	**94**	**94**
Vegetable oils added	6	6	6
Determined analysis (%)			
Crude fat	8.54	8.48	8.59
Analysis calculated (%)[3]			
Crude protein	16.45	16.45	16.45
Crude fiber	4.12	4.12	4.12
Calcium	3.76	3.76	3.76
Phosphorus	0.51	0.51	0.51
Available phosphorus	0.39	0.39	0.39
Na	0.15	0.15	0.15
K	0.59	0.59	0.59
Cl	0.17	0.17	0.17
Lysine	0.83	0.83	0.83
Methionine	0.41	0.41	0.41
Methionine+cystine	0.72	0.72	0.72

[1]A standard commercial starter diet (Ridley Agriproducts Pty Ltd, Murray Bridge, Australia) with 2555.38 kcal/kg ME. All vitamins/minerals met or exceeded the requirements recommended by the National Research Council (1994) for laying hens [168].
[2]A by-product from the milling of wheat for flour production. It consists of the bran, aleurone, germ and pollard fractions.
[3]Calculated values except for crude fat which was measured in diets.

Table 3.4 Fatty acid composition of the experimental diets

	Experimental diets		
Diets	**0.3 (low ALA)**	**3 (moderate ALA)**	**6 (high ALA)**
Fat content (%)	8.5	8.5	8.6
ALA (%en)	0.3	3.2	6.2
LA (%en)	2.3	4.4	4.4
LA:ALA ratio	7.5	1.4	0.7
Fatty acids (%)[1]			
Total SFA[2]	18.4 ± 0.04	13.7 ± 0.02	15.7 ± 0.02
18:1n-9	48.4 ± 0.13	38.7 ± 0.15	22.3 ± 0.01
18:1n-7	3.1 ± 0.01	2.1 ± 0.01	1.1 ± 0.00
Total MUFA[2]	66.8 ± 0.09	42.3 ± 0.13	24.3 ± 0.02
Total n-9	51.0 ± 0.15	39.8 ± 0.14	22.9 ± 0.02
Total n-7	15.6 ± 0.07	2.4 ± 0.00	1.4 ± 0.01
18:2n-6 (LA)[2]	12.8 ± 0.11	25.1 ± 0.08	24.9 ± 0.03
Total n-6	12.9 ± 0.11	25.2 ± 0.08	24.9 ± 0.03
18:3n-3 (ALA)[2]	1.7 ± 0.04	18.5 ± 0.05	34.8 ± 0.06
20:5n-3 (EPA)[2]	N.D.	N.D.	N.D.
22:5n-3 (DPA)[2]	N.D.	N.D.	N.D.
22:6n-3 (DHA)[2]	N.D.	N.D.	N.D.
Total n-3	1.8 ± 0.04	18.6 ± 0.05	34.9 ± 0.06
Total PUFA[2]	14.6 ± 0.12	43.8 ± 0.12	59.8 ± 0.03

[1]Values are presented as % of total fatty acids. Data are means ± SEM of n = 3.
[2]SFA = saturated fatty acid; MUFA = monounsaturated fatty acid; LA = linoleic acid; ALA = alpha-linolenic acid; EPA = eicosapentaenoic acid; DPA = docosahexaenoic acid; DHA = docosahexaenoic acid; PUFA = polyunsaturated fatty acid.

3.4.6 Sample collection

Egg production was recorded daily throughout the experiment and calculated as hen-day egg production. The collected eggs were classified as perfect, broken, defected or egg with no shell. All of the eggs produced during the last 3 days of every week were saved, and weights per replicate were recorded. All of the eggs produced prior to the commencement of the trial and in the last 3 days by each hen of each 28-day period (week 4, week 8, and week 12) were individually weighed. One egg from each bird was then cracked to separate yolk, and the weight of albumen and yolk was recorded. A total of 48 egg yolk samples of each 28-day period (n = 8 egg yolk for

each treatment) were stored at -20°C to analyse the fatty acid composition of the yolk. Six commercial eggs were also collected and stored for fatty acid analysis. The sensory properties of eggs were determined using the eggs produced at week 12 (boiled egg) and week 13 (scrambled egg).

3.4.7 Fatty acid methyl ester (FAME) extraction

Total lipids (TL) were extracted from feed and egg samples by the methods of Folch et al. [164] with chloroform/methanol (2:1, v/v) solution. The FAME were prepared following the procedures of Tu et al. [118] with 1% H_2SO_4 in methanol at 70°C for 3 hours. The resulting FAME were extracted with *n*-heptane and transferred into gas chromatography (GC) vials containing anhydrous sodium sulphate. Samples were stored at -20°C for GC analysis. Details of procedures for determination of FAME extraction of feed and eggs are given in the Chapter 2 of this dissertation (see section 2.3.1.2 and 2.3.1.3).

3.4.8 Gas chromatograph analysis of FAME

The methyl esters of feed and yolk fatty acids were analysed using a Hewlett-Packard 6890 GC (CA, USA). The procedures are described in detail in Chapter 2 (see section 2.3.2).

3.4.9 Sensory evaluation of eggs

Sensory evaluation of eggs was carried out in two ways, which were (i) descriptive sensory analysis by trained panellists and (ii) consumer study by untrained panellists.

3.4.9.1 Descriptive analysis sensory evaluation of eggs

3.4.9.1.1 Egg collection and storage

Eggs from brown and white laying hens fed diets containing 0.3, 3 and 6%en ALA were collected during the final week of the experimental period for the sensory evaluation. A total of 9 and 21 eggs per each treatment group were used for boiled and scrambled egg evaluation, respectively. For boiled egg evaluation, eggs were refrigerated (4°C) for 15 d to facilitate peeling [132]. Eggs from hens fed macadamia oil, 0.3%en ALA (low ALA), were designated as control eggs whereas eggs from hens fed diet containing ALA at 3% (moderate ALA) and 6%en (high ALA) were n-3 fatty acid enriched eggs.

3.4.9.1.2 Panellist selection

Panellists who had no allergies to eggs and were willing to consume eggs were recruited from students and staff at the School of Agriculture, Food and Wine, University of Adelaide. The number of panellists participating in the evaluation was twelve (n = 12) for hard-boiled eggs and ten (n = 10) for scrambled eggs. Some of these panellists had prior sensory training or descriptive analysis experience. The protocol of the sensory study had been approved by the Human Ethics Committee of the University of Adelaide. A signed written consent form was obtained from each panellist after explaining the details of the experiment. The panellists were blinded to the dietary treatments.

3.4.9.1.3 Panellist training

The selected panellists were trained in four sessions of two-hour duration. The panellists were reimbursed for their travel cost and participation in the egg sensory study. The aims of this training were to develop their ability to distinguish standard aroma, taste and flavour between samples, and to train panellists to have consistency and reproducibility in reporting the intensity of the sensation perceived. Training sessions also defined the final list of sensory attributes to be evaluated by panellists in the formal evaluation sessions. The descriptive vocabulary of the attributes was adapted from Lawlor et al. [88]. The training session reviewed sensory methodology and egg evaluation techniques. During the training sessions, panellists evaluated aroma intensity standards (sulphur, butter, and yogurt) and taste intensity standards (sweet, salt, and sour) at different concentrations to determine the low and high intensity standard levels for each attribute that would be used in the final sensory evaluation. For demonstration and training purposes, egg cooking time (boiled eggs) and the different ratio of yolk and albumen (scrambled eggs) was manipulated to generate sensory discrepancies. Samples presented to panellists were coded with a three-digit random number. Panellists practised evaluating the aroma, taste and flavour of each of the samples using a 15cm-line scale with indented end anchor points of low and high intensity placed at 10% and 90% of the scale, respectively either on paper or on a computer screen in individual booths [169]. Through discussion, a consensus was reached in relation to the low and high levels of the intensity standards and terminology of attributes that would be tested. The final agreed descriptive terminology consisted of four aroma, four taste and four flavour attributes (Table 3.5).

Table 3.5 Descriptive terminology and definitions used by trained panellists to assess boiled and scrambled eggs

Sensory attributes	Definition[1]	Scale anchors[2]	Reference standards
Aroma			
Egg aroma	Aroma typically associated with cooked egg	Low to intense	Low egg aroma (scrambled eggs; yolk:albumen =1:7) to high egg aroma (scrambled eggs; yolk:albumen =7:1)
Sulphur	Aroma associated with products containing sulphur: e.g. eggs	Low to high intensity	Low to high intensity for boiled (2 and 10µg/L) and scrambled eggs (2 and 5µg/L) hydrogen sulphide (H_2S) in distilled water
Butter	Aroma associated with unsalted butter	Low to high intensity	Butter: at room temperature
Off-odour	The presence of something disagreeable differing from the typical egg odour	No off-odour to strong off-odour	
Taste			
Salt	Fundamental taste sensation of which sodium chloride is typical	Low to high intensity	None to medium to high (0, 1.0, and 2.0g/L sodium chloride in distilled water)
Sour	The taste associated with acid (citric acid in solution)	Low to high intensity	None to medium to high (0, 0.1, and 0.15g/L citric acid in distilled water)
Sweet	Basic taste sensation of which sucrose is typical	Low to high intensity	Low to medium to high (2.5, 5, and 7.5g/L sucrose in distilled water)
Aftertaste	The flavour sensation that lingers on the tongue and palate at 5 seconds after swallowing	Low to high intensity	
Flavour			
Egg flavour	Flavour typically associated with cooked egg	Low to intense	
Sulphur flavour	Flavour associated with products containing sulphur: e.g. eggs	Low to high intensity	
Butter flavour	Flavour associated with unsalted butter	Low to high intensity	
Off-flavours	The presence of something disagreeable differing from the typical egg flavour	No off-flavour to strong off-flavour	

[1]The descriptive terminology and definitions used were adapted from Parpinello et al. [132] and Lawlor et al. [88].
[2]A 15cm line-scale with indented anchor points of attribute intensity.

3.4.9.1.4 Sample preparation

Two methods of egg preparation were used: hard-boiled and scrambled eggs. On the day of hard-boiled egg evaluation, three eggs from each treatment (18 eggs in total) were added to a stainless pot containing cold water. The gas range was turned on and the eggs were brought to a boil and simmered for eight minutes [132], then cooled to stop the cooking process under cold running water. The eggs were peeled and then divided into four portions. One quarter-egg from each treatment was placed in a plastic container coded with randomly selected three-digit numbers. The covered containers were kept warm in an oven at about 40°C before the egg samples were presented to panellists.

For scrambled egg evaluation, eggs were collected and refrigerated at 4ºC for one week before use. The egg preparation method was a modification of that described by Lawlor et al. [88]. On the day of evaluation, scrambled eggs were prepared as follows: 21 eggs were cracked and whole eggs from each treatment group were thoroughly mixed with a blender for four seconds till a slight foam formed. A portion (25ml) was poured into eight plastic containers and immediately poured into a pre-heated baking cooker with an anti-adhering pan. Scrambled eggs were cooked for two minutes and then the cooker was turned off and scrambled eggs were allowed to stand on the warm cooker for four minutes. The scrambled eggs were removed from the baking cooker when the egg had reached a minimum temperature of 80ºC. No vegetable oil, cooking spray or salt was added to the scrambled eggs. The samples were placed in a sealed plastic container coded with random three-digit numbers and placed in an oven at 40°C to keep them warm before serving.

3.4.9.1.5 Final descriptive analysis evaluation

On the days of final descriptive analysis sensory evaluation, egg samples were prepared as described above. Each panellist evaluated aroma, taste and flavour of both boiled and scrambled egg samples from six different treatment groups (3 diets × 2 strains combination) with three replications of each of the six treatments. Panellists evaluated 18 boiled egg samples on day 1 and a week later evaluated 18 scrambled egg samples on day 2 (36 samples in total). Panellists were asked to score their perception of each attribute tested on unstructured 15-cm line scales anchored at both ends (at 10 and 90%) with intensity extremes of each attributes [169]. During the evaluation, panellists were encouraged to smell and taste and had access to reference standards of the attributes. In addition, panellists were encouraged to write comments in relation to the major differences in the samples provided.

Descriptive analysis was carried out in standard individual booths under yellow sodium lighting to avoid visual differences in yolk colour, at the sensory laboratory at the University of Adelaide. Egg samples were presented to panellists in closed plastic containers which were coded with a randomly generated three-digit number. Randomised tasting order of the samples within days was balanced to account for first order and carry over effects [88]. Samples were presented to each panellist, along with aroma standards and taste references in the booths. Panellists rated the samples on computerised scales and the data was collected by FIZZ software version 2.47b (Biosystèms, Couternon, France). For each testing day, each panellist assessed 18 egg samples in a single session of 2 hours. Filtered water and unsalted crackers were provided as palate cleansers, and panellists had a forced 1 minute break

between samples and were instructed to rinse their palate between every two samples. They were presented samples in groups of six, with 10 minute breaks after every six samples.

3.4.9.2 Consumer acceptance of hard-boiled eggs

3.4.9.2.1 Egg collection and storage

At the time of this study, only brown commercial eggs were available, thus eggs for consumer acceptance testing were obtained from brown egg, laying hens fed diets containing 0.3, 3 and 6%en ALA. Eggs were collected after 12 weeks on the experimental diets and were refrigerated (4°C). A total of 20 brown eggs from each of the 3 dietary treatments and 20 commercial brown eggs were used for boiled egg consumer evaluation.

3.4.9.2.2 Sample preparation

In this study, the hard-boiled egg samples were prepared as described above for descriptive analysis by trained panellists (see section 3.4.9.1.4). One quarter of an egg from each of the dietary treatment and commercial eggs was placed in a plastic container coded with random three-digit numbers. Before the egg samples were evaluated by consumers, the eggs in covered containers were kept warm on a bain-marie at about 40°C.

3.4.9.2.3 Consumer egg acceptance study

Consumer acceptance testing was conducted on brown boiled eggs from hens fed three different dietary treatments (0.3, 3 and 6%en ALA) and commercial brown eggs. The protocol for the consumer study had been approved by the Human Ethics Committee of the University of Adelaide. On the day of consumer testing, egg samples were prepared as described above (see section 3.4.9.1.4). A group of consumers (n = 80) who liked, had no allergies to and were willing to consume eggs, were recruited, from both staff and students at the School of Agriculture, Food and Wine, University of Adelaide. Before the tasting session, the egg consumers were asked to answer a questionnaire concerning their demographic information and egg consumption behaviour. Panellists were then asked to smell and taste four egg samples and rated their liking of the egg samples by considering all characteristics including aroma, taste and flavour using a 9-point hedonic scale ranging from left to right, dislike extremely to like extremely.

Acceptance testing by consumers was conducted at a table in an open plan cafeteria at the School of Agriculture, Food and Wine, the University of Adelaide. The participants completed the paper questionnaire and manually scored their liking of egg samples on a paper ballot provided for this evaluation. Fresh water and unsalted crackers were provided for cleansing their palate between each of the four samples and panellists took approximately five minutes to record their liking of all samples. The evaluation was conducted over two days with 40 participants involved on each day.

3.4.10 Statistical analysis

The data on production performance and fatty acid profiles were analysed by univariate ANOVA with a completely randomised block design of three diets by two strains, with eight replications by using the ANOVA (general analysis of variance) directive in GenStat (Release 14). The experimental unit was a replicate consisting of eight adjacently caged birds fed as one group. There were 16 cages for each diet in the experiment (48 cages in total). The main effects of diet (3 levels), strain (2 levels), and the diet-by-strain interaction were tested for the production parameters of hens and the fatty acid composition of eggs. The analysis was followed by Tukey's multiple comparison test if there were significant differences among treatments. Family-wise significance level was set at $P < 0.05$. The effect of feeding period (the feeding duration of experimental diets) on fatty acid levels was analysed by spit-plot ANOVA with main-plot effects of strain by diet, and sub-plot effect of feeding period. The feeding period-by-strain interaction was also analysed. The main analysis was followed by Tukey's test.

For descriptive sensory analysis, the numerical representation was obtained by measuring the distance between the starting point (zero) of the 15-cm line and the actual score of the panellist intensity rating of each attribute for each egg sample. In the assessment of boiled eggs, the experimental unit was one quarter of an individual egg. The quarters of three eggs were randomised across twelve panellists. An individual egg was not factored into the analysis. The descriptive sensory data for each sensory attribute were analysed as a univariate mixed linear model with panellists as random factors using the ANOVA directive (general analysis of

variance) in GenStat (Release 14). Differences between treatment means were further analysed using Fisher's protected LSD with significance level of $P < 0.05$. In the assessment of scrambled eggs, a portion of cooked scrambled eggs was divided among panellists for each replication of the assessment. The analysis thus additionally included the effect of portions of cooked scrambled eggs. The analysis was conducted with the ANOVA directive in GenStat.

The consumer study was arranged in a randomised complete block design over two days (blocks) with individual assessors reporting on one sample of egg. The data from the consumer study was analysed by one way ANOVA with a completely randomised block design (each assessor is a block) using GenStat (Release 14). Statistically significant attributes were further analysed using Tukey's test at the 95% confidence level ($P < 0.05$).

3.5 Results

3.5.1 Fatty acid composition of n-3 enriched eggs

Fatty acid profiles were determined for eggs produced at day 28 (Table 3.6), 56 (Table 3.7) and 84 (Table 3.8) of the dietary intervention. Inclusion of ALA in the diet enhanced all n-3 LCPUFA (EPA, DPA, and DHA), total n-3 PUFA, and total PUFA content (Table 3.8). In contrast, the level of MUFA and AA were reduced ($P < 0.001$) by increasing levels of dietary ALA. EPA levels of eggs from hens fed diets containing 0.3, 3, and 6%en ALA was 0.0, 0.1, and 0.2% of total fatty acids, respectively (Figure 3.2A). Enrichment of laying hen diets with ALA increased ($P < 0.001$) the DPA content of eggs to about 3-fold higher than those without an ALA

supplemented diet. However, increasing the level of ALA from 3 to 6% did not increase the egg DPA content significantly (Figure 3.2A). Eggs produced by laying hens fed diets enriched with ALA at levels of 3 and 6%en accumulated more DHA ($P < 0.001$) compared to those fed diets containing ALA at 0.3%en (similar to the ALA content of commercial diet). However, we observed that the highest level of egg DHA was achieved when the hens were fed diet at a level of 3%en ALA (Figure 3.2A). The DHA content of the eggs from hens fed diets supplemented with 3 and 6%en ALA increased by 2-fold. The total n-3 content of eggs produced at day 84 from hens fed 3 and 6%en ALA diets was 5- and 9-fold, respectively, higher than those fed a diet low in ALA ($P < 0.001$; Figure 3.2B). Most of the increase in n-3 PUFA was due to enrichment in ALA, which increased by about 33-fold (Table 3.8). It should be noted that DHA and total n-3 content of commercial eggs was similar to those of eggs fed the low ALA diet (0.9 and 1.3% for DHA and total n-3 PUFA, respectively).

Inclusion levels of 3 or 6%en ALA increased the n-6 content of eggs by 2-fold compared to birds fed the diet containing ALA of 0.3%en (Figure 3.2B). The levels of the n-6 PUFA were not different between eggs fed the 3% and the 6% ALA diet. The increase in the n-6 FA was due to an increase in LA. However, while EPA levels increased in a linear manner with increasing levels of dietary ALA, AA content of eggs decreased ($P < 0.001$). Increasing ALA content from 0.3 to 6%en reduced the AA content by more than 2-fold (Table 3.8). Dietary ALA enrichment decreased the n-6 to n-3 ratio in the eggs. The n-6 to n-3 ratio was 6.7, 2.1, and 1.2 for 0.3, 3, and 6%en ALA diets, respectively ($P < 0.001$; Table 3.8). Increasing the ALA content of

diets from 0.3 to 6%en resulted in a 2.5-fold increase in the percentage of PUFA ($P < 0.001$) at the expense, mainly, of MUFA ($P < 0.001$; Figure 3.2B). The percentage of SFA slightly increased with increasing levels of dietary ALA (28.2, 29.2, and 30.3% for 0.3, 3, and 6%en ALA, respectively; $P < 0.001$).

The strain of the laying hens noticeably influenced the fatty acid composition of the yolk but did not affect the percentage of total n-6 or n-3 PUFA (Table 3.8). Significant differences between brown and white laying hens were observed for palmitic acid, stearic acid, oleic acid, MUFA, DPA and DHA. Brown laying hens deposited more ($P < 0.001$) MUFA, DPA and DHA than white hens (51.8 vs. 50.0% for MUFA, 0.3 vs. 0.2% for DPA, 1.5 vs. 1.4% for DHA for brown and white hens, respectively; Figure 3.3). In contrast, it is apparent that white hens deposited more SFA (30.0%; $P < 0.001$), in comparison to brown hens (28.4%; Figure 3.3B). It is interesting to note that a significant interaction was found between ALA levels and strains of hen for deposition of DHA in egg yolk (Figure 3.4A). Brown hens fed a diet containing ALA at a level of 3%en recorded the highest level of DHA in their eggs (2.0%). Accordingly, a higher total n-3 LCPUFA level was observed for brown compared to white laying hens fed diets containing ALA of 3%en (Figure 3.4B). We found that the level of SFA in white eggs increased by elevating the ALA content of the diet (Table 3.8). However, brown laying hens consuming ALA enriched diets (3 and 6%en ALA) deposited comparable SFA level with brown hens fed low ALA diet (0.3%en).

It is interesting to note that the feeding period affected the accumulation of DHA and total n-3 LCPUFA ($P < 0.001$) in eggs. For example, when we plotted the fatty acid content of eggs fed either moderate or high ALA diets, it appears that a feeding period of 28 days resulted in the highest accumulation of DHA ($P < 0.05$; Figure 3.5) compared to other periods (56 and 84 days). Brown hens accumulated more DHA than white hens ($P < 0.01$). A significant interaction was found between feeding period and strains of hen for the deposition of DHA in egg yolk. At day 28, brown laying hens fed diets enriched with 3%en ALA produced the highest level of DHA ($P < 0.001$; Figure 3.5A).

Table 3.6 Fatty acid profiles of eggs produced at day 28 of dietary intervention[1]

ALA level, %en	0.3		3		6			P value[2]		
Strains	Brown	White	Brown	White	Brown	White	SEM	D	S	D x S
Fatty acids (%)[3]										
16:0	20.1	21.7	20.1	21.2	20.2	21.8	0.38	NS	***	NS
18:0	7.2^{b}	6.4^{c}	7.7^{b}	7.8^{b}	8.5^{a}	8.6^{a}	0.16	***	NS	*
Total SFA	27.9	28.7	28.2	29.4	29.1	30.7	0.43	**	**	NS
16:1n-7	4.9	4.9	2.1	1.8	2.2	2.3	0.12	***	NS	NS
18:1n-9	50.2	49.4	43.9	43.8	39.1	37.8	0.51	***	NS	NS
18:1n-7	4.4	4.4	2.2	2.0	1.7	1.7	0.08	***	NS	NS
Total MUFA	61.3	60.2	50.0	49.0	44.2	42.8	0.54	***	*	NS
Total n-9	52.0	51.0	45.5	45.1	40.2	38.7	0.53	***	*	NS
Total n-7	9.3	9.3	4.3	3.8	3.9	4.0	0.18	***	NS	NS
18:2n-6 (LA)	6.8	7.2	12.8	13.2	13.3	13.7	0.32	***	NS	NS
18:3n-6	0.0^{d}	0.0^{d}	0.1^{a}	0.1^{b}	0.1^{c}	0.1^{bc}	0.00	***	NS	*
20:3n-6	0.2	0.2	0.1	0.1	0.1	0.1	0.01	***	NS	NS
20:4n-6 (AA)	1.6	1.6	1.1	1.0	0.8	0.8	0.04	***	NS	NS
Total n-6	9.1	9.5	14.3	14.7	14.3	14.7	0.33	***	NS	NS
18:3n-3 (ALA)	0.3	0.3	4.5	4.5	9.5	9.2	0.32	***	NS	NS
20:3n-3	0.0	0.0	0.1	0.1	0.1	0.1	0.01	***	NS	NS
20:5n-3 (EPA)	0.0	0.0	0.1	0.1	0.2	0.2	0.01	***	NS	NS
22:5n-3 (DPA)	0.1	0.1	0.4	0.3	0.4	0.3	0.03	***	**	NS
22:6n-3 (DHA)	0.9^{c}	0.9^{c}	2.2^{a}	1.8^{b}	1.9^{b}	1.7^{b}	0.06	***	***	**
Total n-3	1.4	1.3	7.3	6.8	12.2	11.6	0.36	***	NS	NS
Total PUFA	10.5	10.8	21.6	21.5	26.6	26.3	0.61	***	NS	NS
LA:ALA ratio	27.8	28.3	2.9	3.0	1.4	1.5	0.56	***	NS	NS

[1]Values are means of eight observations per treatment and their pooled standard error of the mean (SEM). Values in the same row with no common superscript are significantly different ($P < 0.05$).
[2]*$P < 0.05$; **$P < 0.01$; ***$P < 0.001$; NS, not significant; D, diet; S, strain.
[3]SFA, saturated fatty acid; MUFA, monounsaturated fatty acid; LA, linoleic acid; AA, arachidonic acid, ALA, alpha-linolenic acid; EPA, eicosapentaenoic acid; DPA, docosahexaenoic acid; PUFA, polyunsaturated fatty acid.

Table 3.7 Fatty acid profiles of eggs produced at day 56 of dietary intervention[1]

ALA level, %en	0.3		3		6			P value[2]		
Strains	Brown	White	Brown	White	Brown	White	SEM	D	S	D x S
Fatty acids (%)[3]										
16:0	16.2	17.5	16.2	17.8	16.7	18.1	0.31	NS	***	NS
18:0	6.3^{b}	5.2^{c}	6.5^{b}	6.5^{b}	7.3^{a}	7.2^{a}	0.15	***	**	***
Total SFA	22.8^{c}	22.9^{bc}	22.8^{c}	24.5^{ab}	24.2^{abc}	25.5^{a}	0.36	***	**	NS
16:1n-7	3.7	4.0	1.5	1.4	1.7	1.9	0.10	***	NS	NS
18:1n-9	42.6	41.5	36.8	36.8	33.3	31.4	0.35	***	**	NS
18:1n-7	3.6	3.6	1.8	1.7	1.4	1.4	0.05	***	NS	NS
Total MUFA	51.5	50.3	41.4	41.1	37.5	35.5	0.37	***	***	NS
Total n-9	44.2^{a}	42.8^{a}	38.1^{b}	37.8^{b}	34.3^{c}	32.1^{c}	0.37	***	***	*
Total n-7	7.3	7.5	3.3	3.1	3.1	3.3	0.13	***	NS	NS
18:2n-6 (LA)	5.3	6.2	11.0	11.2	11.3	11.3	0.25	***	NS	NS
18:3n-6	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.				
20:3n-6	0.1	0.1	0.1	0.1	0.1	0.1	0.00	***	NS	NS
20:4n-6 (AA)	1.2^{a}	1.4^{a}	0.8^{b}	0.8^{b}	0.6^{c}	0.6^{c}	0.04	***	*	*
Total n-6	7.0	8.1	12.0	12.2	12.0	12.0	0.27	***	*	NS
18:3n-3 (ALA)	0.2	0.2	3.8	3.8	8.1	8.0	0.18	***	NS	NS
20:3n-3	0.0	0.0	0.0	0.0	0.1	0.1	0.01	***	NS	NS
20:5n-3 (EPA)	0.0	0.0	0.1	0.1	0.2	0.2	0.01	***	NS	NS
22:5n-3 (DPA)	0.1	0.1	0.3	0.2	0.3	0.3	0.02	***	*	NS
22:6n-3 (DHA)	0.6^{c}	0.8^{c}	1.7^{a}	1.5^{b}	1.5^{b}	1.4^{b}	0.05	***	*	***
Total n-3	0.9	1.1	6.0	5.6	10.2	10.0	0.20	***	NS	NS
Total PUFA	7.9	8.0	17.9	17.8	22.2	22.0	0.60	***	NS	NS
LA:ALA ratio	31.6	28.5	2.9	2.9	1.4	1.4	0.75	***	NS	NS

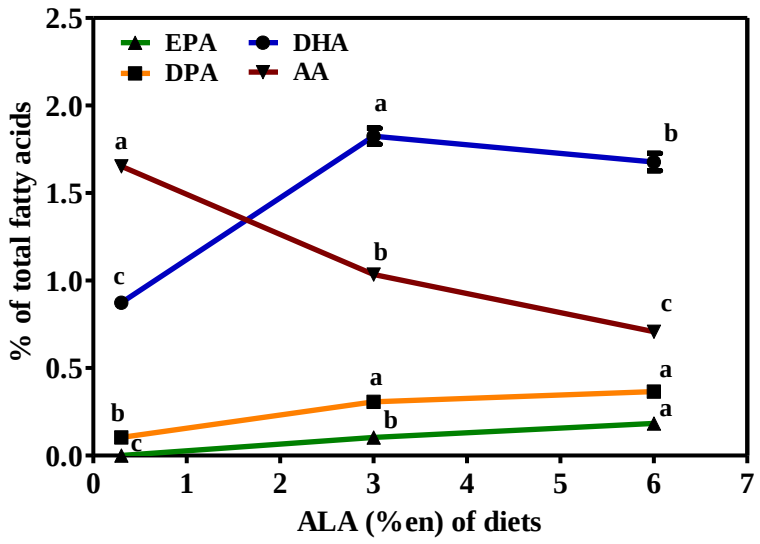
[1]Values are means of eight observations per treatment and their pooled standard error of the mean (SEM). Values in the same row with no common superscript are significantly different ($P < 0.05$).
[2]*$P < 0.05$; **$P < 0.01$; ***$P < 0.001$; NS, not significant; D, diet; S, strain.
[3]SFA, saturated fatty acid; MUFA, monounsaturated fatty acid; LA, linoleic acid; AA, arachidonic acid, ALA, alpha-linolenic acid; EPA, eicosapentaenoic acid; DPA, docosahexaenoic acid; PUFA, polyunsaturated fatty acid.

Table 3.8 Fatty acid profiles of eggs produced at day 84 of dietary intervention[1]

ALA level, %en	0.3		3		6			P value[2]		
Strains	Brown	White	Brown	White	Brown	White	SEM	D	S	D x S
Fatty acids (%)[3]										
16:0	19.9	21.4	20.0	21.7	19.7	22.2	0.37	NS	***	NS
18:0	7.4^{c}	6.5^{c}	8.1^{b}	8.1^{b}	9.1^{a}	9.1^{a}	0.16	***	*	**
Total SFA	27.9^{c}	28.5^{c}	28.3^{c}	30.1^{ab}	29.1^{bc}	31.6^{a}	0.37	***	***	*
16:1n-7	4.7^{a}	4.9^{a}	1.9^{c}	1.7^{c}	2.0^{bc}	2.4^{b}	0.11	***	NS	*
18:1n-9	50.2	49.0	43.7	42.8	39.1	37.0	0.41	***	***	NS
18:1n-7	4.4	4.3	2.1	2.0	1.7	1.7	0.06	***	NS	NS
Total MUFA	61.5	60.0	49.7	48.1	44.2	42.0	0.42	***	***	NS
Total n-9	52.3	50.7	45.5	44.2	40.4	37.8	0.43	***	***	NS
Total n-7	9.1	9.2	4.0	3.8	3.7	4.1	0.13	***	NS	NS
18:2n-6 (LA)	6.8	7.7	13.3	13.6	13.6	13.4	0.34	***	NS	NS
18:3n-6	0.1	0.1	0.1	0.1	0.1	0.1	0.00	***	*	NS
20:3n-6	0.1^{b}	0.1^{b}	0.1^{a}	0.1^{ab}	0.1^{b}	0.1^{ab}	0.00	**	NS	*
20:4n-6 (AA)	1.6	1.7	1.1	1.0	0.7	0.7	0.03	***	NS	NS
Total n-6	8.7	9.7	14.7	14.9	14.6	14.3	0.35	***	NS	NS
18:3n-3 (ALA)	0.3	0.3	4.6	4.6	9.3	9.5	0.24	***	NS	NS
20:3n-3	0.0	0.0	0.1	0.1	0.1	0.1	0.01	***	NS	NS
20:5n-3 (EPA)	0.0	0.0	0.1	0.1	0.2	0.2	0.01	***	NS	NS
22:5n-3 (DPA)	0.1	0.1	0.4	0.3	0.4	0.3	0.02	***	*	NS
22:6n-3 (DHA)	0.9^{c}	0.9^{c}	2.0^{a}	1.7^{b}	1.7^{b}	1.6^{b}	0.05	***	**	**
Total n-3	1.3	1.4	7.1	6.7	11.8	11.8	0.27	***	NS	NS
Total PUFA	10.1	11.0	21.8	21.6	26.4	26.2	0.58	***	NS	NS
LA:ALA ratio	25.9	25.2	2.9	3.0	1.5	1.4	0.37	***	NS	NS

[1]Values are means of eight observations per treatment and their pooled standard error of the mean (SEM). Values in the same row with no common superscript are significantly different ($P < 0.05$).
[2]*$P < 0.05$; **$P < 0.01$; ***$P < 0.001$; NS, not significant; D, diet; S, strain.
[3]SFA, saturated fatty acid; MUFA, monounsaturated fatty acid; LA, linoleic acid; AA, arachidonic acid, ALA, alpha-linolenic acid; EPA, eicosapentaenoic acid; DPA, docosahexaenoic acid; PUFA, polyunsaturated fatty acid.

A



B

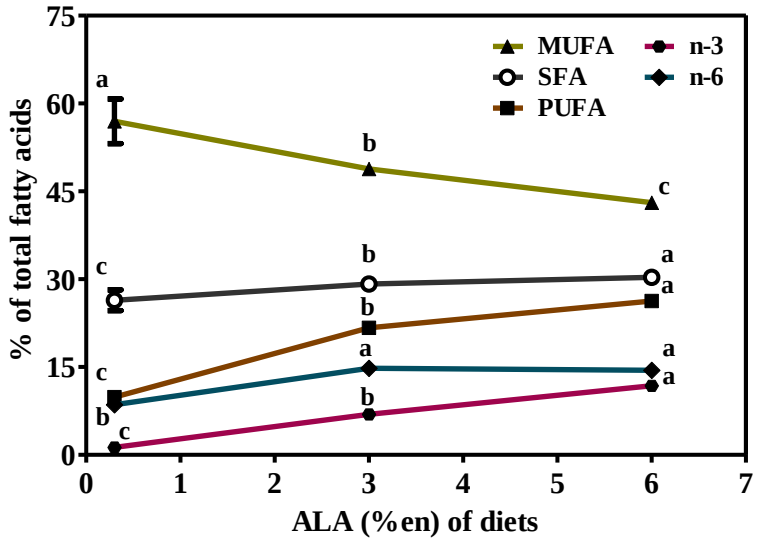


Figure 3.2 Overall effect of increasing dietary alpha-linolenic acid (ALA) on long chain polyunsaturated fatty acid (LCPUFA) content of eggs (A) and fatty acid classes in egg lipids (B) independent of strain (white and brown pooled data) at day 84 of the experimental period. The values presented are means of sixteen replicate analyses ± SEM. There were significant differences in the level of eicosapentaenoic acid (EPA), docosapentaenoic acid (DPA), docosahexaenoic acid (DHA), arachdonic acid (AA; $P < 0.001$), monounsaturated fatty acid (MUFA), saturated fatty acids (SFA), polyunsaturated fatty acids (PUFA), total n-3, and total n-6 in eggs among dietary treatments. Diets enriched with ALA increased all n-3 LCPUFA and decreased AA.

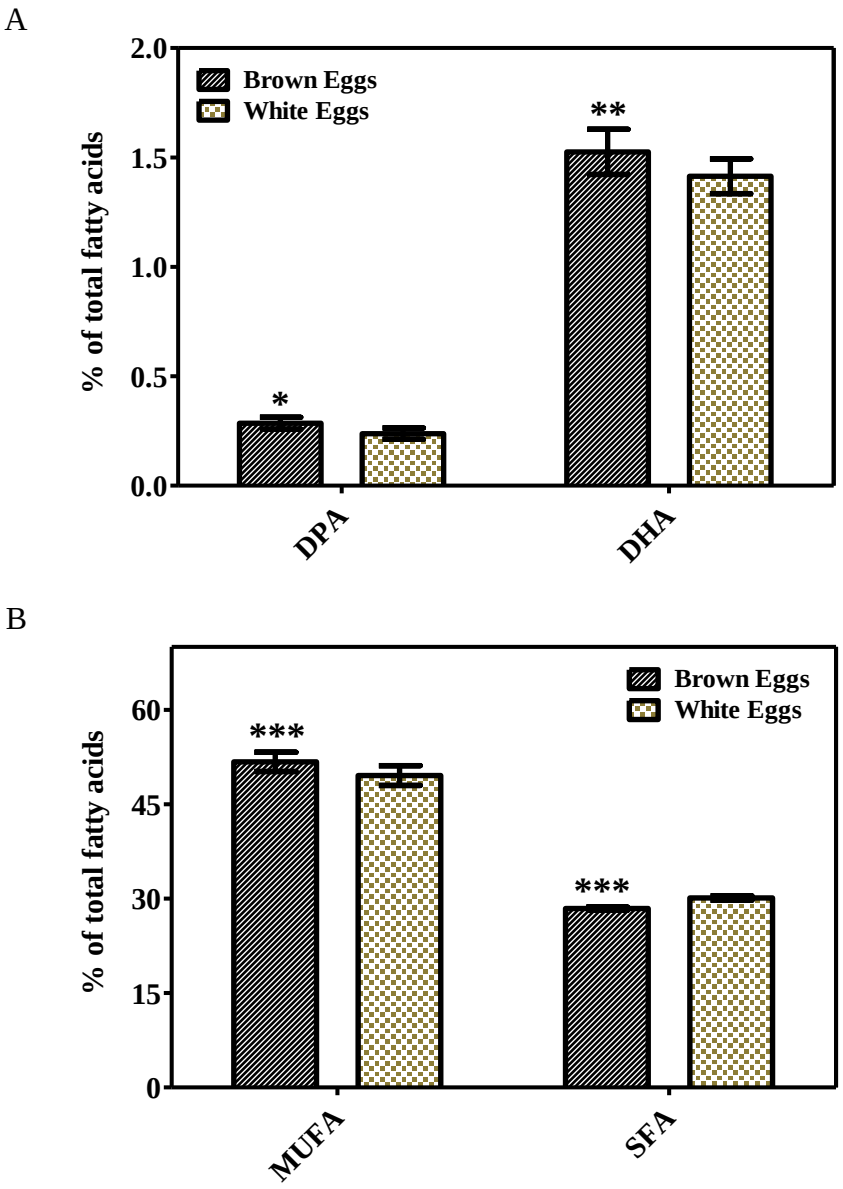


Figure 3.3 Effects of strain on the levels of fatty acids in egg yolks independent of diet. Data from all diets were pooled. Docosapentaenoic acid (DPA) and docosahexaenoic acid (DHA) are shown in (A), and monounsaturated fatty acids (MUFA) and saturated fatty acids (SFA) are shown in (B) for eggs at day 84 of the experimental period. The values presented are means of 24 replicate analyses ± SEM. There were significant differences in the level of DPA ($P < 0.05$), DHA ($P < 0.01$), MUFA and SFA ($P < 0.001$) between brown and white laying hens. Brown hens accumulated a higher level of DPA, DHA and MUFA, and a lower SFA level than white hens.

A

B

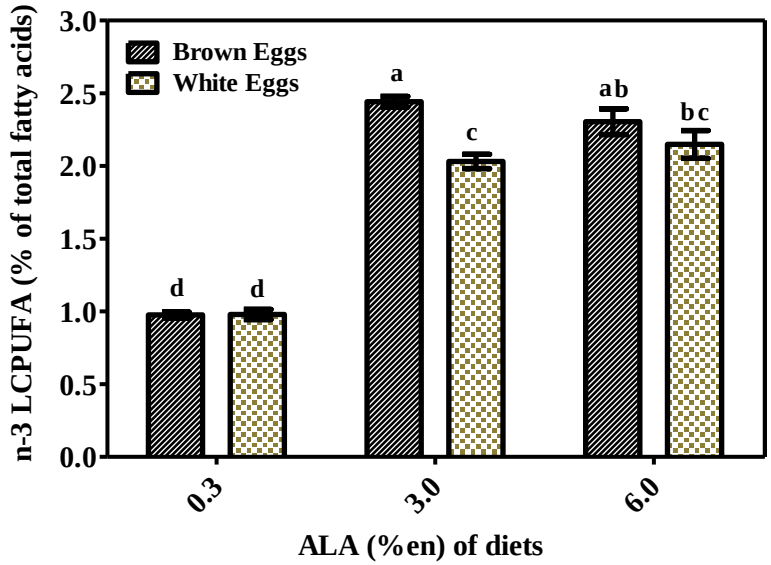
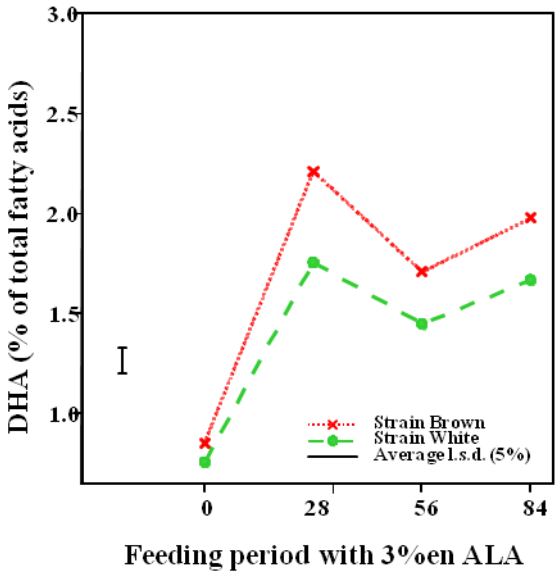


Figure 3.4 Effects of increasing levels of dietary alpha-linolenic acid (ALA) at levels of 0.3, 3, and 6%energy (%en) on docosahexaenoic acid (DHA) (A) and n-3 long chain fatty acid (n-3 LCPUFA) (B) of brown and white eggs at day 84 of the experimental period. The values presented are means of eight replicate analyses ± SEM. There were significant differences in the level of DHA and total n-3 LCPUFA ($P < 0.001$) among dietary treatments. There was a strain effect and interaction between diet and strain of hens for DHA and n-3 LCPUFA. Eggs from brown hens accumulated a higher level of DHA and n-3 LCPUFA than eggs from white hens ($P < 0.001$). Brown hens fed a diet enriched with 3%en ALA deposited the highest egg DHA ($P < 0.05$) content

A



B

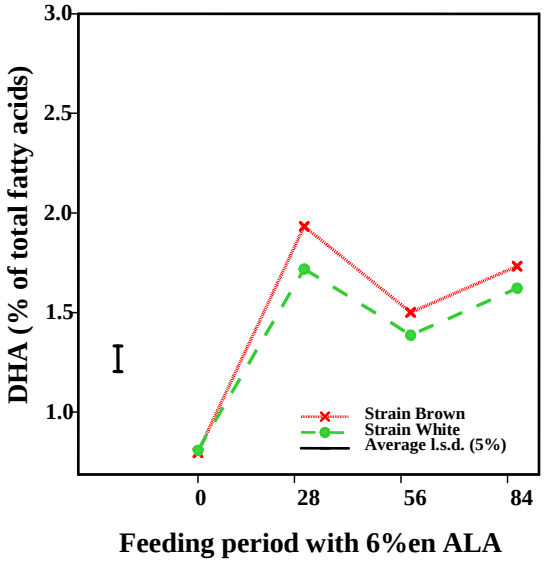


Figure 3.5 Effects of feeding period on DHA levels of brown and white eggs fed moderate (3%energy, %en) (A) and high ALA diet (6%en) (B) measured at day 0, 28, 56, and 84 of dietary intervention. The values presented are means of eight replicate analyses. The 5% LSD are presented separately in each graph. There were significant differences in the levels of DHA ($P < 0.001$) among feeding periods with day 28 producing the highest level of DHA. Eggs from brown hens accumulated more DHA than eggs from white hens ($P < 0.01$). There was an interaction between strain and feeding period on the accumulation of DHA for eggs from hens fed moderate ALA diets. The highest egg DHA content was produced at day 28 by brown laying hens ($P < 0.001$) (A).

3.5.2 Fatty acid content of n-3 enriched eggs (mg/yolk)

We also evaluated the fatty acid profiles of eggs expressed in mg/yolk (Table 3.9). Diets enriched with ALA caused more deposition of ALA, n-3 LCPUFA, total n-3 in egg yolk, while the level of AA reduced. ALA, EPA, DPA and total n-3 increased in a linear manner ($P < 0.01$); however, it appears that the DHA level of eggs reached a maximum level (81 mg/yolk) when laying hens were fed a diet containing ALA of 3%en. The EPA, DPA and DHA content of eggs fed the highest level of dietary ALA increased by about 8-, 3- and 2-fold, respectively. Compared with eggs produced by hens fed 0.3%en ALA, enrichment of diets with 3 and 6%en ALA increased the concentration of total n-3 PUFA by 5- (308mg/yolk) and 10-fold (533mg/yolk), respectively, at the expense of MUFA. As was the case when the data were expressed as a percentage of total fatty acids, a significant interaction was found between the ALA content of diet and strain of hen for DHA concentration in egg yolk. A higher accumulation of DPA and DHA was observed in brown eggs compared to white eggs although no significant difference was observed for DHA. The lack of a significant difference in DHA content when expressed as mg/yolk between brown and white eggs might be because the yolk weight of white eggs was greater ($P < 0.01$) than for brown eggs 15.7 and 16.7g, respectively. The highest level of DHA and total n-3 LCPUFA was found for brown hens fed a diet enriched with 3%en, with levels of 87 and 108mg/yolk, respectively (Table 3.9). Importantly, increasing levels of dietary ALA did not influence the SFA content of eggs while the concentration of n-3 PUFA was increased.

Table 3.9 Fatty acid profiles of eggs produced at day 84 of dietary intervention expressed in mg/yolk or egg[1]

ALA level, %en	0.3		3		6			P value[2]		
Strains	Brown	White	Brown	White	Brown	White	SEM	D	S	D x S
Fatty acids[3]										
Total SFA	1220	1291	1251	1355	1270	1468	59.00	NS	*	NS
Total MUFA	2687	2725	2188	2157	1928	1952	85.70	***	NS	NS
18:2n-6 (LA)	297	341	584	609	592	624	22.34	***	NS	NS
20:4n-6 (AA)	71	77	46	46	31	32	1.84	***	NS	NS
Total n-6	381	432	644	668	633	668	23.81	***	NS	NS
18:3n-3 (ALA)	12	9	200	206	405	444	14.35	***	NS	NS
20:5n-3 (EPA)	0	0	5	5	8	9	0.44	***	NS	NS
22:5n-3 (DPA)	5	4	16	12	17	16	1.15	***	*	NS
22:6n-3 (DHA)	38^{c}	41^{b}	87^{a}	75^{b}	75^{b}	75^{b}	3.13	***	NS	*
EPA+DHA	38	41	92	79	83	84	3.41	***	NS	NS (P<0.1)
n-3 LCPUFA	43	45	108	91	100	100	4.18	***	NS	NS (P<0.1)
Total n-3	59	59	313	303	513	553	17.62	***	NS	NS

[1]Values are means of six observations per treatment and their pooled standard error of the mean (SEM). Values in the same row with no common superscript are significantly different ($P < 0.05$).
[2]*$P < 0.05$; ***$P < 0.001$; NS, not significant; D, diet; S, strain.
[3]SFA, saturated fatty acid; MUFA, monounsaturated fatty acid; LA, linoleic acid; AA, arachidonic acid, ALA, alpha-linolenic acid; EPA, eicosapentaenoic acid; DPA, docosahexaenoic acid; PUFA, polyunsaturated fatty acid.

3.5.3 Sensory properties of n-3 enriched eggs

3.5.3.1 Sensory evaluation of boiled eggs

Panelists did not detect differences in the sensory attributes including aroma, taste, flavour, or off-flavour of the boiled eggs from birds fed dietary treatments (Table 3. 10). This indicates that diets enriched with ALA did not affect the sensory quality of the boiled eggs. There was a difference in the oily odour of boiled eggs but this was only small and there was no consistent effect of increasing levels of dietary ALA. The average scores of the attributes are presented in Figure 3.6.

There was a strain effect on some attributes tested when diets were supplemented with ALA. It appears that brown boiled eggs were generally considered to elicit a higher perception of aftertaste (average of intensity score of 7.58 on a scale from 0 to 15) compared to white boiled eggs (average score of 6.04; $P < 0.05$). In contrast, a higher sulphur flavour was detected in white (average intensity score of 6.66) than in brown boiled eggs (average score of 6.04; $P < 0.05$). A significant interaction was found between levels of ALA in the diet and strains of hen for oily odour. Inclusion of dietary ALA slightly decreased the oily odour of brown eggs ($P < 0.05$) but no differences in sensory panel scores for the oily odour were noted between brown eggs produced by moderate and high ALA diets (Table 3.10). The average score of oily odour of brown eggs from hens fed diets containing 0.3, 3, and 6%en was 1.62, 0.92, and 0.95, respectively. Before the sensory analysis was conducted, panellists were trained intensively to perform a descriptive analysis, and panel performance was evaluated for reproducibility and sensitivity to detect differences due to treatments

Table 3.10 Sensory evaluation by trained panellists of hard-boiled eggs from two strains of laying hens fed high alpha-linolenic acid diet

ALA level, %en	0.3	3	6		0.3 (low)		3 (moderate)		6 (high)			P value[2]				
Strains				SEM	Brown	White	Brown	White	Brown	White	SEM	Diet	Strain	D x S	Assessor	A x T
Sensory attributes[1]																
Aroma																
Egg aroma	8.50	8.13	8.16	0.27	8.06	8.94	8.39	7.88	7.98	8.33	0.38	NS	NS	NS	***	*
Sulphur aroma	7.92	8.03	7.98	0.26	7.45	8.40	7.93	8.13	7.72	8.24	0.37	NS	NS	NS	***	NS
Butter aroma	4.06	4.04	4.26	0.20	3.65	4.47	4.13	3.96	4.53	3.99	0.28	NS	NS	NS	***	***
Oily-odour	1.36	1.21	0.93	0.14	1.62^{a}	1.09^{ab}	0.92b	1.49^{ab}	0.95^{b}	0.92^{b}	0.20	NS	NS	*	***	**
Taste																
Salty taste	7.10	6.48	6.71	0.28	7.13	7.06	6.79	6.17	6.66	6.77	0.39	NS	NS	NS	***	NS
Sour taste	3.96	3.93	4.10	0.15	4.00	3.92	3.81	4.05	4.16	4.03	0.22	NS	NS	NS	***	NS
Sweet taste	3.76	3.77	3.67	0.18	3.78	3.73	3.98	3.55	3.68	3.67	0.25	NS	NS	NS	***	*
Aftertaste	7.13	7.35	7.43	0.21	7.58^{a}	6.69^{b}	7.40^{a}	7.30^{b}	7.78^{a}	7.08^{b}	0.29	NS	*	NS	***	NS
Flavour																
Egg flavour	8.62	8.45	8.80	0.21	8.53	8.71	8.59	8.32	8.72	8.89	0.29	NS	NS	NS	***	NS
Sulphur flavour	6.46	6.30	6.30	0.18	6.40^{b}	6.52^{a}	6.02^{b}	6.58^{a}	5.71^{b}	6.89^{a}	0.25	NS	*	NS	***	NS
Butter flavour	4.67	4.58	4.62	0.15	4.55	4.78	4.76	4.40	4.86	4.38	0.21	NS	NS	NS	***	NS
Off-flavour	1.44	1.69	1.51	0.23	1.47	1.40	1.54	1.85	1.70	1.32	0.32	NS	NS	NS	***	NS

[1]Values are means of three replications per treatment and their pooled standard error of means (SEM) evaluated by 12 panellists. Values in the same row with no common superscript are significantly different ($P < 0.05$).

[2]*$P < 0.05$; **$P < 0.01$; ***$P < 0.001$; NS, not significant; D, diet; S, strain; A, assessor; T, treatment.

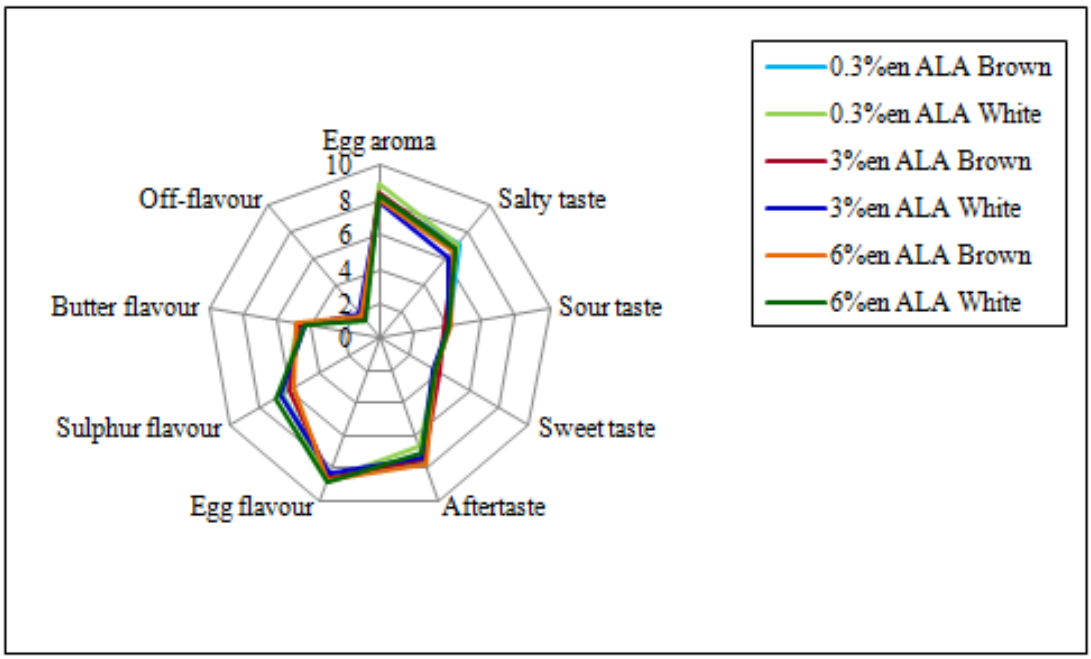


Figure 3.6 Mean levels of egg aroma, taste and flavour in hard-boiled eggs from two strains of laying hens fed diets containing 0.3, 3, and 6%energy alpha-linolenic acid (ALA) as determined by trained panellists (n = 12) using a 15-cm line scale (from low to high intensity); n = 3 replications. Increasing levels of dietary ALA did not affect egg aroma, taste, and flavour of hard-boiled eggs.

3.5.3.2 Sensory evaluation of scrambled eggs

The average scores of the attributes of scrambled eggs are presented in Figure 3.7. Enriching ALA levels in the diet had no effect ($P > 0.05$) on sulphur aroma, butter aroma, oily odour, taste, egg flavour, butter flavour or off-flavour of scrambled eggs (Table 3.11). In relation to egg aroma, scrambled egg samples from hens fed high ALA diets (6%en) gained the lowest score (average intensity score of 8.79). This suggests that eggs from hens fed diets high in ALA have been assessed with less intense egg aroma compared to those fed low (0.3%en) and moderate ALA diet (3%en; average score of 9.55 and 9.45, respectively). Scrambled egg samples from the 0.3%en ALA diet treatment had a lower sulphur flavour (average intensity score of 5.80), compared with diets supplemented with ALA (average scores of 6.80 and 6.96, respectively). However, no differences in sensory panel scores for this attribute were observed between the moderate and high ALA diets.

Strain of birds influenced the sensory attributes of egg aroma, sulphur flavour, and butter flavour. Brown eggs had a significantly ($P < 0.05$) stronger egg aroma, sulphur flavour and butter flavour (average score of 9.60, 6.84, and 4.47, respectively) than white eggs (average score of 8.93, 6.19, and 4.00, respectively). A significant interaction between diet and strain was observed with high ALA diets slightly decreasing egg aroma in white scrambled eggs.

Table 3.11 Sensory evaluation by trained panellists of scrambled eggs from two strains of laying hens fed diets enriched with alpha-linolenic diet

ALA level, %en	0.3	3	6		0.3 (low)		3 (moderate)		6 (high)			P value[2]				
Strains				SEM	Brown	White	Brown	White	Brown	White	SEM	Diet	Strain	D x S	Assessor	A x T
Sensory attributes[1]																
Aroma																
Egg aroma	9.55^{a}	9.45^{a}	8.79^{b}	0.13	9.77^{ab}	9.34^{bc}	10.10^{a}	8.79^{cd}	8.93^{cd}	8.65^{d}	0.19	**	**	*	***	NS
Sulphur aroma	6.33	6.92	7.37	0.37	6.94	5.71	7.08	6.75	7.15	7.59	0.53	NS	NS	NS	***	*
Butter aroma	4.89	5.17	5.07	0.31	5.35	4.44	5.32	5.03	4.97	5.18	0.44	NS	NS	NS	***	NS
Oily-odour	1.20	1.33	1.41	0.12	1.25	1.14	1.42	1.24	1.58	1.24	0.16	NS	NS	NS	***	NS
Taste																
Salty taste	6.98	6.86	7.06	0.23	7.43	6.52	6.79	6.93	7.01	7.10	0.33	NS	NS	NS	***	NS
Sour taste	3.12	3.14	3.30	0.12	3.07	3.18	3.06	3.22	3.17	3.43	0.16	NS	NS	NS	***	NS
Sweet taste	3.59	3.37	3.50	0.19	3.81	3.36	3.32	3.42	3.34	3.66	0.27	NS	NS	NS	***	NS
Aftertaste	6.18	6.48	6.52	0.23	6.04	6.32	6.21	6.76	6.66	6.38	0.32	NS	NS	NS	***	*
Flavour																
Egg flavour	7.93	8.24	7.88	0.23	7.91	7.95	8.64	7.84	8.32	7.44	0.33	NS	NS	NS	***	NS
Sulphur flavour	5.80^{b}	6.80^{a}	6.96^{a}	0.23	6.08^{a}	5.52^{b}	6.98^{a}	6.61^{b}	7.47^{a}	6.45^{b}	0.33	**	*	NS	***	NS
Butter flavour	4.09	4.34	4.29	0.17	4.46^{a}	3.72^{b}	4.61^{a}	4.07^{b}	4.34^{a}	4.24^{b}	0.24	NS	*	NS	***	NS
Off-flavour	1.06	1.24	1.09	0.09	1.05	1.07	1.38	1.11	1.04	1.14	0.13	NS	NS	NS	***	NS

[1]Values are means of three replications per treatment and their pooled standard error of means (SEM) evaluated by 10 panellists. Values in the same row with no common superscript are significantly different ($P < 0.05$).

[2]*$P < 0.05$; **$P < 0.01$; ***$P < 0.001$; NS, not significant; D, diet; S, strain; A, assessor; T, treatment.

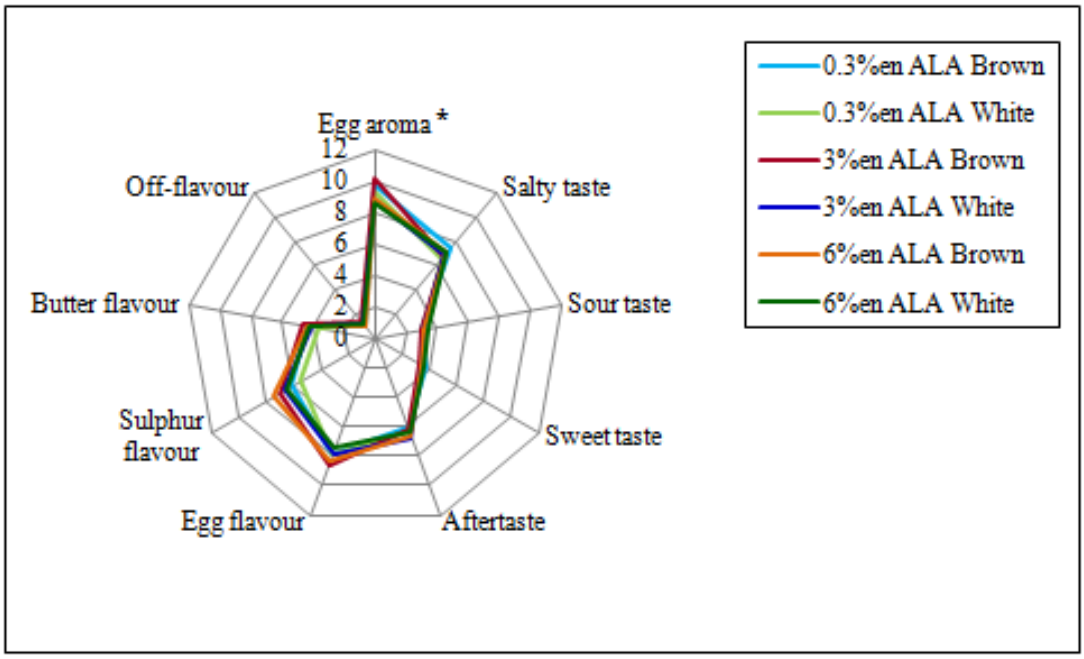


Figure 3.7 Mean levels of egg aroma, taste and flavour in scrambled eggs from two strains of laying hens fed diets containing 0.3, 3, and 6%energy alpha-linolenic acid (ALA) as determined by trained panellists (n = 10) using a 15-cm line scale (from low to high intensity); n = 3 replications. *P < 0.05. There was a significant interaction between diet and strain for egg aroma. Diets enriched with ALA appeared to decrease egg aroma in white scrambled eggs.

3.5.3.3 Consumer acceptance of hard-boiled eggs

The consumers (n = 80) were aged between 19 and 65 and were balanced for gender (Appendix 4). Seventy percent had tertiary qualifications and 74% reported that they did not have any formal food science or consumer education or training. Eighty percent of all consumers had no experience in the food industry and most were students (65%). Almost all consumers (96%) consumed boiled eggs with 70% consuming boiled eggs at least once a month. No consumers reported suffering from an egg allergy. Only two consumers (2.5%) reported they had an anosmia and none recorded any medication affecting their sense or smell.

Importantly, results of consumer testing showed that diets rich in ALA did not change the consumer acceptance of the eggs compared with eggs purchased from a local supermarket (Figure 3.8). It appeared that there was a significant difference in the consumer acceptance between eggs from hens fed diet containing ALA 6%en and commercial eggs; however, all eggs were acceptable.

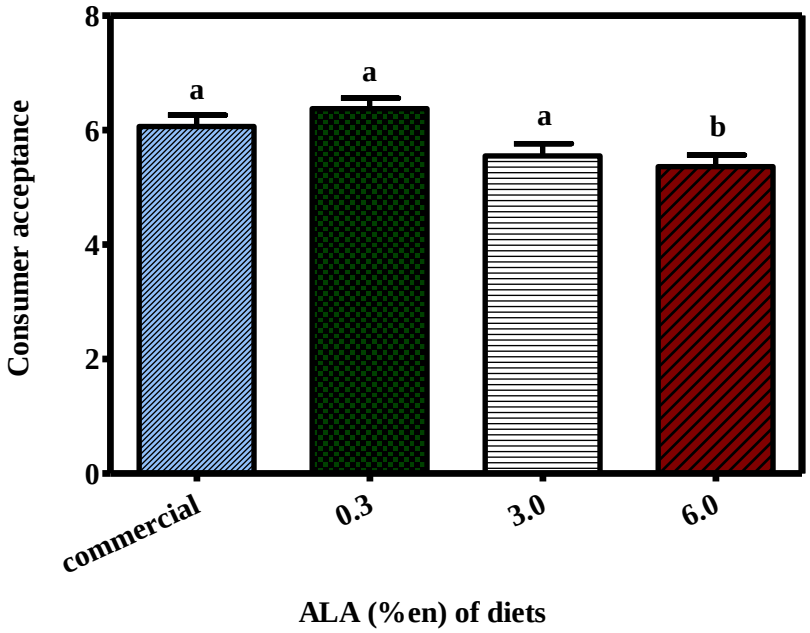


Figure 3.8 Consumer testing of hard-boiled eggs from brown laying hens fed dietary levels of alpha-linolenic acid analysed by 80 egg consumers. There was no difference in the consumer acceptance of the eggs between n-3 enriched eggs from hens fed moderate ALA diets and eggs purchased from a local supermarket (commercial eggs). 1 = dislike extremely; 5 = neither like nor dislike; 9 = like extremely.

3.5.4 Production performance of laying hens

Production performance of brown and white laying hens fed diets supplemented with different levels of ALA for the entire experimental period is shown in Table 3.12. The level of dietary ALA did not affect any of the production parameters measured including feed intake, feed conversion ratio, egg production, egg weight, yolk weight, and weight gain of laying hens. The average feed intake of laying hens fed ALA content 0.3, 3 and 6%en was 120, 120 and 118 g/day, respectively. There was no effect of strain for the production parameters studied throughout the trial. In addition, there was not a significant diet by strain interaction for the performance parameters of the laying hens. Egg production (egg laying rate) was more than 95% for all treatments except the white laying hens fed the diet containing 0.3%en ALA which was slightly lower (87%). The highest average feed intake (123g/day) and egg production (98%) were achieved for brown laying hens fed a diet supplemented with 3%en ALA but the differences were not significant and changes were not consistent with increasing the ALA levels of the diets.

Table 3.12 Effect of diets and strains of laying hens on egg production performance

ALA level (%en)	0.3 (low ALA)		3 (moderate ALA)		6 (high ALA)			F pr.			P value[1]		
Strains	Brown	White	Brown	White	Brown	White	SEM[2]	D	S	D x S	D	S	D x S
Production parameters													
Feed intake (g/day)	119	122	123	117	115	120	3.79	0.79	0.86	0.35	NS	NS	NS
Feed conversion ratio	2.05	2.23	2.03	1.97	1.90	2.10	0.08	0.13	0.10	0.18	NS	NS	NS
Egg production (%)	97	87	98	97	97	98	3.26	0.18	0.25	0.23	NS	NS	NS
Egg weight (g)	59	62	60	61	61	61	1.42	0.83	0.29	0.72	NS	NS	NS
Weight gain of hens (g)	188	138	181	144	188	144	33.40	0.99	0.12	0.98	NS	NS	NS

[1]NS, not significant (P > 0.05).
[2]Standard error of means.

3.6 Discussion

This study was conducted to examine whether the inclusion of different levels of ALA to the diets of two strains of laying hen enhanced the level of n-3 fatty acids of eggs, without adversely affecting production performance or the sensory quality of the eggs. Previous studies have focussed on how diets supplemented with ALA in the form of flaxseed or flaxseed oil affect production parameters and fatty acid profiles in white or brown laying hen strains [101, 102, 123, 170, 171]. In addition, a small number of studies [78, 125, 172] have investigated the fatty acid composition of eggs from different strains of hens; however, there have been no reports regarding the laying hen performance, fatty acid composition, and sensory evaluation of eggs using both brown and white-egg strains fed ALA enriched diets while holding the level of LA constant.

3.6.1 Fatty acid composition of n-3 enriched eggs

The major changes in the fatty acid composition of egg yolk by the dietary ALA can be summarised as an increase in the level of PUFA and a decline in MUFA. The results presented in this chapter show that there was a direct relationship between the ALA levels of the diet and the n-3 content of eggs, mainly as ALA and n-3 LCPUFAs. This finding confirms the established idea that the proportion of n-3 fatty acids in the dietary fat is directly responsible for the type of fat accumulation in the egg yolk, and that the fatty acid profiles of the yolk can be manipulated to varying extents by modifying the fatty acid composition of the diet [77]. The increase in the n-3 LCPUFA content when high ALA diets were included in the diet suggests a limited but effective conversion of ALA to n-3 LCPUFA [77], in particular the

capacity of the laying hens to convert ALA into DHA [78, 102, 123, 172]. In addition, it appears that there is a relative advantage in the mechanisms of the conversion of dietary ALA to n-3 LCPUFA in poultry compared to mammals [111, 173]. The effort required to maintain the metabolic formation of fatty acids in yolk is centred in the organised synthesis and transport system [110]. For example, the fatty acid transport in the birds is different from mammals since chickens have a simple lymphatic system draining the intestinal tract [110, 121]. Hence, chychlomicrons are absorbed directly into the portal blood and transported to the liver for further synthesis [121]. The low incorporation rates of EPA (0.2%) and DPA (0.4%) observed in eggs in the current experiment may be due to a limited ability to accumulate EPA and DPA in egg lipids. Gonzalez-Esquerra and Leeson [84] reported that the EPA, DPA and DHA levels of eggs from hens fed a diet supplemented with 2% menhaden oil containing 3.9% EPA, 1% DPA, and 1.4% DHA were 0.3, 0.2%, and 1.7%, respectively. This corresponded to an amount of DHA in eggs of 87mg/egg which is identical to the level found in eggs from laying hens fed 3%en ALA. This indicates that ALA rich vegetable oils supplemented against a background of a low level of LA in basal diets may lead to the generation of eggs with significant levels of the n-3 LCPUFA. It appears that there was a maximal ALA level for conversion into DHA. The highest amount of DHA (1.82%) was produced by laying hens fed a diet containing 3%en ALA; there was no further increase in the levels of DHA for dietary ALA levels beyond 3%en. These findings are consistent with reports that diets containing high levels of PUFA, including both ALA and LA, inhibit DHA synthesis [107]. Further studies, that include more levels of dietary ALA, should be carried out to more precisely determine the optimal level

of dietary ALA. Moreover, no positive response in the accumulation of DHA by increasing levels of dietary ALA could be explained as a consequence of competition between both 18-carbon PUFA, LA and ALA, for Δ-desaturase in the synthetic pathway [107, 174]. These findings are in agreement with previous studies which found that there was a maximum DHA level achieved by increasing levels of dietary ALA [78, 123]. For example, Sari et al. [123], evaluated the influence of addition of flaxseed (rather than oil) ranging from 5 to 15% to diets of laying hens and found that there was no increase in the level of DPA and DHA above 5% flaxseed. It is difficult to compare these results to the current study since these authors did not report the ALA content of the diets. However, Grobas et al. [78] reported that supplementation of linseed oil from 5 to 10% did not changed DHA content of eggs, which is consistent with my observations.

In this study, the fatty acid profiles of yolk were measured as the total fatty acids. A previous study reported that yolk fatty acids are predominantly in the TG fraction (63 - 65%) while a lesser amount is in phospholipids (27 – 30%) [100, 175]. However, since the DHA tends to be preferentially deposited in the phospholipid fraction of the yolk [111, 173], it seems that the PL pool size could limit the accumulation of DHA. This may explain why the amount of n-3 LCPUFA in the eggs is not changed to any great extend when the ALA levels of diets is above 3%en [111]. Schreiner et al. [111] fed increasing levels of seal blubber oil to laying hens and showed that increasing the levels of blubber oil above 1.25% of the diets, a plateau effect of n-3 LCPUFA content of the eggs was observed. However, the plateau in the Schreiner study was reached when the n-3 LCPUFA were around 7% of the PL fatty acids (4%

of total fatty acids). Because the level of n-3 LCPUFA achieved in eggs in my study did not exceed 2.5% of total fatty acids, it may indicate that the rate of conversion of ALA into n-3 LCPUFA may be the major regulator of n-3 LCPUFA in eggs from hens fed vegetable oils rather than any 'ceiling' for n-3 LCPUFA accumulation in egg PL.

We observed a slightly higher deposition of DHA in eggs than those achieved by Grobas et al. [78], who also used flaxseed oil. They found that the level of DHA in eggs increased to 1.5% of total fatty acids by an inclusion level of either 5 or 10% flaxseed oil in the diet (40 – 48% ALA of total fatty acid in the diet); however, in the current study, the DHA level of eggs from hens fed a diet containing 3%en ALA, for which ALA represents 18% of total fatty acid in the diet, reached 1.82% of total fatty acids. The different results may be due to the low LA level of the basal diet used in the present study and/or the fact that the level of LA was held constant in the moderate and high ALA diets. The competition between LA and ALA to utilise the same desaturase and elongase enzymes for the bioconversion into n-3 LCPUFA is well known [171]. Kartikasari et al. [115] reported that a higher amount of LA in the diets can reduce the deposition of n-3 LCPUFA in chicken tissues.

In the present study, DHA levels of eggs of laying hens fed diets enriched with 3%en ALA reached similar levels to those of eggs from laying hens fed diets supplemented with 2% of either regular or deodorised menhaden oil [32]. However, inclusion levels of 2% menhaden oil resulted in a poorer scores of aroma, taste, flavour, and acceptability of eggs compared to control eggs. Similarly, although it appeared that

diets enriched with a high level of flaxseed (10%) increased DHA content 2-fold [102, 176], which is comparable to the level achieved in this study, the diets containing 10% flaxseed resulted in a negative impact on the sensory properties of the eggs including aroma, flavour and off-flavour [130, 177].

When levels of n-3 LCPUFAs were elevated in the eggs, a significant reduction of AA content was observed. These results are consistent with those of other authors, who showed that an increase in ALA content in the diet decreased the accumulation of AA in eggs [78, 123, 178]. In general there was an inverse relationship between dietary ALA and AA content in egg yolks. The decrease in AA content of eggs might be as a result of the biosynthetic competition between precursors ALA and LA for the Δ-6 desaturase [77, 179] but may also be due to competition between n-3 LCPUFA and AA for incorporation into egg lipids. The LA content of eggs increased in the two test diets, most likely due to the higher LA content of these diets.

There is some controversy in relation to the effect of strain on the fatty acid composition of eggs. On the one hand, Scheideler et al. [127] reported that there was no significant effect of hen strain on the accumulation of ALA or DHA into the egg yolk. On the other hand, several workers have reported greater accumulation of n-3 LCPUFA in eggs from brown hens fed ALA-rich diets [78, 125, 172]. The reasons for this are not understood but it seems likely that we can conclude that brown layers are better converters of ALA to n-3 LCPUFA.

In the present study, the enrichment of laying hen diets with 3 and 6%en ALA resulted in a doubling in the accumulation of DHA. The lack of a significant difference in the DHA levels between brown and white eggs when the data was expressed as mg/yolk could be due to the difference in yolk weight between brown and white eggs. However, it is interesting to note that the highest level of DHA (87mg/yolk) and total n-3 LCPUFA (108mg/yolk) was reached when brown laying hens were fed dietary ALA at a level of 3%en. This indicates that the inclusion of dietary ALA at a level of 3%en can be adopted for brown laying hens for producing eggs higher in n-3 LCPUFA, which is important for human health. This dietary approach can also provide an alternative n-3 rich food for consumers. The DHA and total EPA and DHA content of eggs achieved in this present study are similar to those for eggs of hens fed diets supplemented with 15% flaxseed [170]. Feeding hens with moderate (3%en) and high (6%en) ALA diets led to enrichment of total n-3 up to 308 and 533mg/yolk, respectively. This suggests that eggs produced by hens fed the moderate and high ALA-enriched diet achieved the minimum requirement needed for labelling the eggs as a source of n-3 PUFA (300mg/egg) [171, 180].

Over the past four decades, because of concerns over coronary heart disease associated with high dietary cholesterol, the public has been recommended to avoid the consumption of eggs, [67, 181]. However, the data from free-living populations as reviewed by Kritchevsky [181] show that there is no association between egg consumption and higher serum cholesterol level, and the idea that the consumption of eggs is a risk factor for coronary heart disease is not supported by the epidemiologic literature. Furthermore, Makrides et al. [182] reported that weaning infants were able

to consume up to four n-3 enriched egg yolk/week without influencing their plasma cholesterol levels. Importantly, several studies show that diets supplemented with either flaxseed [102, 176] or flaxseed oil [101] had no effect on the cholesterol level of eggs. The cholesterol level of eggs from hens fed 10% flaxseed was approximately 180mg/egg [102, 176]. In addition, Basmacioglu et al. [183] reported that eggs from hens fed diets enriched with 8.6% flaxseed had a lower cholesterol level compared to the control group. Considering the recommended intake of dietary cholesterol of <300mg/day [184], daily consumption of an n-3 enriched eggs can supplement dietary n-3 LCPUFA intake, especially DHA to get the expected benefits for human health.

3.6.2 Sensory properties of n-3 enriched eggs

Sensory characteristics are among the most crucial quality parameters in determining consumer acceptance of any food item. The addition of marine sources, such as fish meal and fish oil, into the diets of laying hens has been found to produce the important n-3 LCPUFA; however, such diets also impair the sensory quality of eggs [32, 88]. The fishy off-flavour of the eggs could be due to the changes in volatile concentrations in n-3 fatty acid-enriched eggs, the presence of trimethylamine (TMA), lipid oxidation products [92, 133] or the methods of egg preparation [89]. Adverse sensory attributes in eggs were also reported when laying hens were fed diets high in flaxseed (10%) either as whole flaxseed or as ground flaxseed [103, 130, 133, 135], and this caused the lower acceptability of the eggs [103, 177].

However, in this study, diets enriched with ALA sources in the form of vegetable oils doubled the level of DHA and total n-3 LCPUFA without affecting the sensory quality of the boiled eggs. This is consistent with the results of Parpinello et al. [132] who reported no significant difference in terms of odour and taste between boiled eggs from hens fed 2% flaxseed oil and those from a control diet. Reportedly, feeding the flaxseed diet resulted in off-flavour in eggs, in contrast, in this study panellists found no difference in the perception of off-flavour in eggs from hens fed high ALA diets compared to low ALA diet. Since the storage condition of eggs before sensory assessment and the method of egg preparation in this study are similar to those studies which included flaxseed in the diet [103], this discrepancy might be due to the difference of forms of dietary fat sources used, which are whole or ground flaxseeds versus flaxseed oils [133] or the amount of ALA sources included in the diet [103, 130, 132, 133].

Although there is a significant strain-by-diet interaction effect for the oily aroma, the intensity perceived was low, less than 2cm (SE = 0.2cm) on the intensity scale, and the assessors were not in the agreement when rating this aroma (there was a significant assessor-by-treatment interaction). This effect can thus be spurious and is treated with caution. Additionally, only one assessor gave high scores (>5cm) for the oily odour note, but the assessor was not consistent in his judgement between replications.

The sensory assessment of scrambled eggs showed that trained panellists were able to detect some differences in egg aroma and sulphur flavour from scrambled eggs fed

ALA enriched diets compared to low ALA diets. However, the differences were negligible. For example, no significant difference in egg aroma was perceived in scrambled eggs from hens fed a diet supplemented with moderate ALA (3%en) and those from hens fed low ALA diet (0.3%en). The discrepancy in sensory quality between boiled eggs and scrambled eggs might be due to the difference of egg preparation methods [88, 132], which may cause differences in thermal treatment, [132] influencing taste evaluation. There were some differences in the perception of sensory attributes between brown and white eggs; however, there were only small changes, with brown eggs giving a slightly higher perception of aftertaste, egg aroma, and butter flavour compared to white eggs. These differences might be related to a higher n-3 LCPUFA content, in particular DPA and DHA, in brown eggs compared to white eggs. The differences in the sensory characteristics among strains have been reported in previous studies in terms of flavour scores [172].

Similarly, the consumer acceptance test conducted on brown hard-boiled eggs showed that based on taste and flavour, consumers did not have any liking differences between eggs purchased from a local supermarket and eggs from hens fed diets enriched with up to 3%en ALA. However, eggs enriched up to 6%en ALA were significantly less liked than all other eggs. As the descriptive analysis did not significantly discriminate between the egg samples, the reason for the reduction in liking of the 6%en ALA eggs in this study is unclear. It is possible that since the descriptive analysis and consumer tests were conducted in different conditions (sensory laboratory versus open plan central location consumer testing), the sensory properties of egg samples may have been perceived slightly differently. Despite this,

all eggs were accepted by consumers. These findings are in agreement with the results of previous researchers [130, 185].

3.6.3 Production performance of laying hens

Importantly, under our experimental conditions, ALA enrichment did not adversely affect the performance parameters of laying hens over the 12 week experimental period. These findings are similar to those reported by other investigators, who studied the effect of diets containing different fat sources on the performance of laying hens [77, 101, 131, 186]. The hens in all treatment groups consumed a similar amount of feed throughout the trial period, which suggests that there was no palatability problem as reported when the diets were supplemented with high flaxseed [130, 187]. The results of this present study are in line with the findings of Baucells et al. [77] and Lelis et al. [186], who reported no significant effect on feed intake by adding oil sources at a level of 4%. Similarly, increasing ALA levels of diets did not influence feed conversion ratio. No effect on feed conversion ratio was also reported in previous studies when ALA rich sources such as flaxseed [102, 188, 189] or flaxseed oil [131] were included in the diet. In this study, egg production and egg weight were not affected by the inclusion of dietary ALA, suggesting that the use of 6% vegetable oil can be applied without affecting the production performance of hens. The absence of an effect on egg production and egg weight by high ALA diets is in accordance with the previous studies [78, 102, 190]. In this study, no significant strain effect on performance parameters was observed. These results are in agreement with the findings of Grobas et al. [78], who found no difference in feed consumption between brown and white strains, although they observed that brown

laying hens ate slightly more than white laying hens. In the current study, brown laying hens fed diets enriched with 6%en ALA consumed less feed (115g/day) compared to other groups.

3.7 Conclusion

The findings of this study suggest that while ALA derived from vegetable oils can be included in commercial laying hen feed up to 6%en ALA without affecting laying hen performance, sensory characteristics of eggs, and the consumer acceptance of the eggs, 3%en ALA in the diet was found to be optimum with respect to n-3 fatty acid content and the sensory quality of the eggs. Eggs from hens fed the moderate (3%en) and high (6%en) ALA-enriched diet achieved the 300mg/egg minimum content required for labelling the eggs as a source of n-3 PUFA [180]. Brown laying hens were found to be more effective in converting ALA to n-3 LCPUFA, and consequently, accumulated more DPA and DHA than white hens. This suggests that there is a strain dependence in the ability of the hens to convert ALA into n-3 LCPUFA. The amounts of n-3 LCPUFA in eggs were influenced by feeding period of dietary intervention. This work contributes to the understanding of the effect of hen strain and dietary level of ALA on n-3 LCPUFA accumulation. At the highest level of ALA inclusion tested, panellists did not detect differences in the sensory attributes of boiled eggs and only minor impacts on the sensory attributes of scrambled eggs were noted. Diets enriched with ALA did not change the consumer acceptance of the eggs compared to the commercial eggs.

Based on this information, a dietary ALA at a level of 3%en could be recommended as a suitable ALA level for producing n-3 LCPUFA enriched egg by the chicken industry; however, further studies are needed to identify the optimal level. The findings of this study show that vegetable oils, as ALA sources, could be an alternative to marine sources and flaxseed. As laying hens fed moderate and high ALA diets produced eggs higher in n-3 LCPUFA without affecting sensory quality of the eggs, this provides an alternative n-3 rich food for consumers, and may help them approach recommended intake for human health.

CHAPTER 4

PRODUCTION PERFORMANCE, OMEGA-3 ENRICHMENT AND SENSORY PROPERTIES OF CHICKEN MEAT FROM TWO STRAINS OF BROILERS FED ALPHA-LINOLENIC ACID ENRICHED DIETS

4.1 Abstract

The important role of n-3 LCPUFA in human health has promoted interest in expanding the range of foods that are enriched with n-3 LCPUFA. The inclusion of n-3 FA into chicken meat can be achieved by feeding broilers n-3 LCPUFA sources such as fish oil. However, negative aspects in various production parameters and sensory quality of the final product may occur. The supplementation of chicken diets with plant-based n-3 PUFA sources such as ALA may enhance the levels of n-3 LCPUFA in meat, without these detrimental effects. The study described in Chapter 4 investigated the effects of varying dietary ALA levels while attempting to limit the level of LA in the diets of two strains of broilers on production performance, n-3 LCPUFA accumulation, and sensory quality of chicken meat. The experimental design of this study was 3 × 2 factorial in a complete randomised block design (4 pens per strain × diet combination). The levels of dietary ALA tested were 0.3, 3 and 6%en, while holding the level of LA constant at around 4%en. The diets were fed to 233 male broilers (116 Cobb 500 and 117 Ross 308), which were randomly placed in 24 raised rearing pens in groups of 9 or 10 for a 41-day experimental period. At 40 days of age, tissue samples of three birds from each pen (total 72) were collected for fatty acid analysis. At 41 days of age, three birds from each pen (total 72) were processed to carcasses (ready to cook) for sensory analysis. Results showed that the

incorporation of vegetable oils rich in n-3 PUFA (ALA) did not influence production parameters including feed intake, feed conversion ratio and final weight of birds. The body weight gain of Cobb 500 birds was slightly greater than for Ross 308. The fatty acid composition of chicken meat varied with the levels of dietary ALA and strains of broiler chickens. Diets high in ALA led to an increase in the levels of EPA (5-fold), DPA (4-fold), DHA (2-fold), and total n-3 (11-fold) of the chicken meat; however, most of the increase in the n-3 content was due to enrichment in ALA. Fatty acid analysis of lipid fractions showed that the LCPUFA (AA, n-3 LCPUFA) were preferentially accumulated in the PL fraction whereas ALA mainly deposited in the TG fraction. There was a strain effect of birds in the conversion of ALA into n-3 LCPUFA, with Cobb birds being more effective at accumulating n-3 fatty acids than Ross. Total n-3 PUFA of breast meat from Cobb birds fed the high (6%en) ALA-enriched diet achieved the 300mg/100g of meat minimum content required for labelling as a source of n-3 PUFA. Feeding broiler chickens up to 6%en ALA had no real effect on the sensory quality of meat. Moreover, the sensory quality of chicken meat from birds fed 3%en ALA was comparable to those of commercial breast meat purchased from a local supermarket. Based on these results, a dietary ALA level of 3%en appears to be a suitable ALA level for producing meat that is high in n-3 LCPUFA. The findings of this study demonstrated that incorporating n-3 rich vegetable oils into chicken diets could be an alternative to marine sources to produce meat higher in n-3 LCPUFA, without affecting sensory quality of the meat. This strategy would help to provide consumers with a variety of foods rich in n-3 LCPUFA, and help to achieve recommended intakes for human health.

4.2 Introduction

As more consumers become aware of the beneficial effect of n-3 PUFAs, there is an increase in the interest and demand for food products containing these fatty acids. Providing n-3 LCPUFA enriched chicken meat may be a preferable way to achieve the recommended intake of n-3 fatty acids for people who do not habitually consume fish. There have been numerous studies aimed at increasing the n-3 LCPUFA content in chicken products by the n-3 enrichment of chicken feed. One of the dietary approaches to increase n-3 LCPUFA content in chicken meat is by including marine sources such as fish meal or fish oil [9, 17, 94]. This dietary approach has successfully increased the concentration of EPA and DHA in the meat [9, 17, 94]. As reported by Chekani-Azar et al. [17], supplementation of 4% fish oil increased EPA and DHA by 10- and 25-fold, respectively. However, the enrichment of chicken meat with n-3 fatty acids raises concerns of sensory quality changes [17, 95]. Therefore, there is a need to introduce alternative sources of n-3 PUFA to broiler diets.

An alternative to the supplementation of chicken diets with marine sources is to increase the n-3 LCPUFA production through including vegetable oils rich in n-3 PUFA into the chicken feed. Vegetable oils such as flaxseed and canola are rich sources of ALA and dietary manipulation by incorporating these oils may be a better strategy to increase n-3 fatty acids in chicken meat. Several studies have found that it is possible to increase the level of n-3 PUFA in chicken meat through supplementation of ALA rich sources to the feed [19, 20, 22, 32, 141]. However, the meat n-3 PUFA content varied among studies and some studies even failed to demonstrate an increase [22, 23]. The variation of response in such studies may be

due in part to the relatively low dosage of dietary ALA or a high ratio of LA to ALA in the experimental diets. In the metabolic pathway, LA competes for the same desaturation and elongation enzymes so there is potential for competitive inhibition between n-6 and n-3 PUFA in the diet [34, 191]. Kartikasari et al. [115] reported that a high consumption of LA has the potential to reduce chicken meat EPA and DHA production from ALA, and favour high AA. Different strains of birds may also play a role.

Kartikasari et al. [117] has demonstrated significant improvements in n-3 LCPUFA (EPA, DPA and DHA) enrichment of chicken meat by increasing levels of dietary ALA. It is interesting to note that the EPA and DHA level increased sharply when the dietary ALA was increased up to 6%en; however, above this level, the accumulation of these fatty acids did not increase substantially further. Importantly, the levels of n-3 LCPUFA obtained in chicken meat in these studies were higher than those seen in reports that used other poultry strains (mainly Ross strains). However, as this study only focussed on Cobb 500, the possibility of increasing levels of tissue n-3 LCPUFA in other strains remain unclear.

Therefore, in this study, the reasons for the differences in n-3 LCPUFA accumulation were explored by comparing Cobb 500 with Ross 308 strains. In this study, the ALA levels of the diets were varied from 0.3 to 6%en using grain-based diets, in which the level of competing substrate, LA, was kept constant. The diets were given for periods that matched commercial production times.

The objectives of this study were to evaluate the effectiveness of varying dietary ALA levels while holding the LA level constant for the moderate (3%en) and high (6%en) ALA diets on production performance, n-3 LCPUFA accumulation, and the sensory qualities of the meat of two strains of broiler chickens. In this study, the distribution of fatty acids in the total lipid, TG and PL fractions was analysed. The fatty acid composition of cooked breast meat expressed as mg/100g of meat was also analysed because TG in meat can be lost from meat during cooking. As consumers only eat cooked chicken meat, the information regarding the amount of n-3 fatty acids in cooked breast meat is important.

4.3 Specific aims of this chapter

The aims of the experiment in this chapter were:

1. To compare the n-3 LCPUFA concentration in the total lipid, TG and PL fractions of chicken tissues of Cobb 500 and Ross 308 strains following consumption of diets enriched with ALA while holding the level of LA constant for moderate and high ALA diets.
2. To evaluate the effect of cooking on the n-3 PUFA content of chicken breast meat.
3. To identify the dietary ALA content of broiler diets required to achieve an n-3 PUFA level of 300mg/100g of meat.
4. To evaluate the sensory properties of breast meat following consumption of diets enriched with ALA.

4.4 Materials and methods

4.4.1 Ethical considerations

Ethical approval for this study was obtained from the Animal Ethics Committees of the Department of Primary Industries South Australia and the University of Adelaide. The procedures in rearing and slaughtering birds complied with national regulations (see section 3.4.1).

4.4.2 Location

The experiments were carried out at both the Roseworthy and Waite Campuses of the University of Adelaide. Broiler chickens were reared at the Pig and Poultry Production Institute (PPPI), Roseworthy Campus. Lipid extraction and fatty acid analysis of feed and tissue samples were conducted at the Fatty Acid Laboratory, and sensory studies of the breast meat was conducted at the Sensory Evaluation Laboratory, School of Agriculture, Food and Wine, Waite Campus of the University of Adelaide.

4.4.3 Experimental design

In this study, the design was 3 × 2 factorial in a complete randomised block design (4 pens per strain × diet combination). The dietary treatments were similar to those used in the egg study (Chapter 3), which was based on the level of dietary ALA as energy (0.3, 3 and 6%en) while holding the LA level constant.

4.4.4 Birds, rearing and management

A total of 233 male one-day-old broiler chickens (116 Cobb 500 and 117 Ross 308) were obtained from the Baiada hatchery (Willaston SA, Australia). The chickens were immediately weighed in groups of 9 or 10 and randomly allocated to one of 24 raised rearing pens (1.2 × 0.9m^2 each pen). All birds in one pen were of the same strain. The birds were housed for 41 days and reared under controlled environmental conditions (Figure 4.1**)**.

After placement, chickens were fed one of three experimental diets, which were provided in a plastic hopper and also scattered over the paper to encourage the chickens to eat immediately. Clean, fresh water was put in the splash cup under the drinking nipples to encourage the chickens to drink. Both feed and water were provided *ad libitum* for the entire 41-d trial. There were four replicates for each diet × strain combination. During the first few days following allocation to the pens, the chickens were monitored regularly to ensure that they were secure with the environmental conditions and that all had access to sufficient feed and water. The shed heating, cooling and ventilation system were designed to provide an ideal environment for the chickens. Room temperature was kept at 27°C for four days and gradually decreased to 20°C during the experimental period. A lighting program of 24 hour a day was used throughout the entire growing period. The environmental conditions were adjusted at least daily and the chickens were observed twice daily. Group body weight was determined on all pens on day 0, 7, 14 and 21. On day 28, 35 and 40 all birds were weighed individually. Body weight and feed intake were

recorded on a weekly basis for the 41 day experimental period to calculate body weight gain and FCR (kg feed/kg body weight gain).

A

B

Figure 4.1 Layout of raised rearing pens.

4.4.5 Diets

Three dietary treatments were assessed in this experiment. The diets were prepared from a specially formulated basal broiler diet (Ridley Agriproducts Pty Ltd, Murray Bridge, Australia). The basal diet was designed to have a low fat content (2.1%) and low LA levels in order to create the experimental diets with relatively low LA level. To achieve this, grain-based diets containing low LA level were chosen to prepare the basal diet. The ALA and LA levels of the basal diet were 5.02 and 44.16% of total fatty acids, respectively. The dietary treatments were formulated by adding 6% pure or blended vegetable oils to the basal diet to achieve the desired PUFA composition. The composition of the vegetable oil blend for broiler diets was the same as those for laying hen diets (Table 4.1). The fatty acid profiles of the oils used for experimental diets were then analysed (Table 4.2). The amount of the vegetable oils used for each diet was then measured and mixed well with the basal diet using a cement mixer. This resulted in three dietary treatments with a fat content of approximately 8%, which is within a commercially acceptable range. The ingredient composition and nutrient content of the experimental diets are shown in Table 4.3.

The nutrient specifications of all experimental diets were identical apart from fatty acid compositions. All the dietary treatments met or exceeded the requirements recommended by the National Research Council (1994) for broiler chickens [168].

Table 4.1 Composition of experimental diets from 0-41 days post-hatch

	Experimental diets (ALA level, %en)		
Ingredients (%)	**0.3 (low ALA)**	**3 (moderate ALA)**	**6 (high ALA)**
Starter basal diet[1]	94	94	94
Oil added			
Macadamia oil	6	0	0
Flaxseed oil	0	2.4	6
Canola oil	0	3.6	0
Total	100	100	100

[1]Starter basal diet comprised (%): wheat fine (62.85), wheat mill vitamins (0.80), peas fine (10.00), blood meal (1.27), canola meal expeller (5.00), soybean meal (13.53), millrun (2.43), limestone large (1.72), monodicalcium phosphate (0.92), salt (0.18), sodium bicarbonate (0.31), choline chloride 75% (0.05), L-threonine (0.07), alimet (0.29), broiler starter premix (0.20), L-lysine sulphate auto (0.28), avatec (0.05), Avizyme 1210 (0.04), Ronozyme P 5000 broiler (0.02). The ALA and LA content of the basal diet were 5.02 and 44.16% of total fatty acids, respectively.

Table 4.2 Fatty acid composition of vegetable oils included in the diets of broiler chickens

	Oils and blended oils[1]			
Fatty acids[2]	**Macadamia**	**Canola**	**Flaxseed**	**Blended oils**
	(% of total fatty acids)			
Total SFA[3]	16.7	8.8	12.7	10.3
Total MUFA[3]	80.4	64.9	21.1	47.0
Total n-9	59.7	61.3	20.2	44.4
Total n-7	20.5	3.7	0.9	2.5
18:2n-6 (LA)[3]	2.4	18.6	18.9	19.1
Total n-6	2.4	18.8	19.1	19.3
18:3n-3(ALA)[3]	0.1	7.4	47.0	23.4
Total n-3	0.5	7.4	47.1	23.4

[1]Composition of oil consisted of: macadamia oil, diet 0.3%en ALA; 40% flaxseed and 60% canola oil, diet 3%en ALA; and flaxseed oil, diet 6% en ALA.
[2]Fatty acid profiles are presented as % of total fatty acid analysed by GC. Data are means of n = 3.
[3]SFA, saturated fatty acid; MUFA, monounsaturated fatty acid; LA, linoleic acid; ALA, alpha-linolenic acid.

Table 4.3 Ingredient composition and nutrient content of experimental diets

	Experimental diets (ALA level, %en)		
Ingredients (%)	**0.3 (low ALA)**	**3 (moderate ALA)**	**6 (high ALA)**
Wheat fine	59.08	59.08	59.08
Wheat milled	0.75	0.75	0.75
Peas fine	9.40	9.40	9.40
Blood meal	1.19	1.19	1.19
Canola meal	4.70	4.70	4.70
Soybean meal	12.72	12.72	12.72
Millrun[2]	2.29	2.29	2.29
Limestone	1.61	1.61	1.61
Monodicalcium phosphate	0.87	0.87	0.87
Salt	0.17	0.17	0.17
Sodium bicarbonate	0.29	0.29	0.29
Choline chloride 75%	0.05	0.05	0.05
L-threonine	0.07	0.07	0.07
Alimet	0.28	0.28	0.28
Broiler starter premix	0.19	0.19	0.19
L-lysine	0.26	0.26	0.26
Avatec	0.04	0.04	0.04
Avizyme 1210	0.03	0.03	0.03
Ronozyme P 5000	0.01	0.01	0.01
Basal diet[1]	94	94	94
Vegetable oils added	6	6	6
Determined analysis (%)			
Crude fat	8.07	8.13	8.16
Analysed calculated (%)[3]			
Crude protein	19.73	19.73	19.73
Crude fiber	3.43	3.43	3.43
Calcium	0.94	0.94	0.94
Phosphorus	0.56	0.56	0.56
Available phosphorus	0.42	0.42	0.42
Na	0.18	0.18	0.18
K	0.67	0.67	0.67
Cl	0.17	0.17	0.17
Lysine	1.13	1.13	1.13
Methionine	0.52	0.52	0.52
Methionine+cystine	0.88	0.88	0.88

[1]A standard commercial starter diet (Ridley Agriproducts Pty Ltd, Murray Bridge, Australia) with 2800.26 kcal/kg ME. All vitamins/minerals met or exceeded the requirements recommended by the National Research Council (1994) for broilers [168].
[2]A by-product from the milling of wheat for flour production. It consists of the bran, aleurone, germ and pollard fractions.
[3]Calculated values except for crude fat which was measured in diets.

The fatty acid composition of the diets was determined and is presented in Table 4.4. The dietary ALA level was 0.3 (low ALA), 3 (moderate ALA) or 6%en (high ALA) while holding the level of LA in moderate and high ALA diets constant at approximately 4%en. These resulted in the ratio of LA to ALA varying from 9:1 to 0.7:1. The low ALA diet was designed to be similar to the fatty acid composition of commercial diets by including macadamia oil, which contains almost no PUFA. Each diet was fed to broiler chickens from day 0 post-hatch up to 41 days of age. A single diet was used throughout the whole experiment.

Table 4.4 Fatty acid composition of the experimental diets

	Experimental diets		
Diets	**0.3 (low ALA)**	**3 (moderate ALA)**	**6 (high ALA)**
Fat content (%)	8.07	8.13	8.15
ALA (%en)	0.25	3.05	5.89
LA (%en)	2.32	4.38	4.41
LA:ALA ratio	9.34	1.44	0.75
Fatty acids (%)[1]			
Total SFA[2]	17.63	12.97	14.85
18:1n-9	48.46	39.19	22.01
18:1n-7	3.23	2.20	1.16
Total MUFA[2]	66.84	42.65	23.83
Total n-9	50.86	40.09	22.43
Total n-7	15.89	2.48	1.35
18:2n-6 (LA)[2]	13.89	26.03	26.16
Total n-6	13.91	26.08	26.19
18:3n-3 (ALA)[2]	1.49	18.11	34.94
20:5n-3 (EPA)[2]	0.00	0.00	0.00
22:5n-3 (DPA)[2]	0.00	0.00	0.00
22:6n-3 (DHA)[2]	0.03	0.02	0.03
Total n-3	1.52	18.17	35.01

[1]Values are presented as % of total fatty acids. Data are means ± SEM of n = 3.
[2]SFA = saturated fatty acid; MUFA = monounsaturated fatty acid; PUFA = polyunsaturated fatty acid.

4.4.6 Sample collection

At 40 days of age, three birds from each pen (total 72) were weighed individually and euthanised by cervical dislocation. Tissue samples (20g) from liver and breast were then collected and stored at -20°C for fatty acid analysis. At 41 days of age, three birds from each pen (total 72) were processed to carcasses (ready to cook) for sensory analysis (left breasts).

4.4.7 Fatty acid methyl ester (FAME) extraction

In order to evaluate n-3 LCPUFA in chicken tissues, the following methods were used for lipid extraction, fatty acid methylation and gas chromatography analysis. Feed and vegetable oils were also analysed in order to precisely formulate diets with the desired LA and ALA levels. Lipids in oils, diets and tissue samples were extracted using methodology described by Folch et al. [164] with chloroform/methanol (2:1, v/v) solution. The lipids were then methylated with 1% H_2SO_4 in methanol at 70°C for 3 hours following the procedures of Tu et al. [118]. The procedures for determination of FAME extraction of oil, feed, and chicken tissues are detailed in Chapter 2 (see section 2.3.1).

4.4.8 Gas chromatograph analysis of FAME

The methyl esters of oil, feed and chicken tissue fatty acids were measured using a Hewlett-Packard 6890 GC (California, USA). The details of the analysis procedures are described in Chapter 2 (see section 2.3.2).

4.4.9 Descriptive analysis sensory evaluation of meat

4.4.9.1 Chicken meat for sensory evaluation

At 41 days of age, 72 male broilers were processed into ready-to-cook carcasses at a commercial processing facility. The broilers were electrically stunned, manually cut using the killing cone (severed left carotid artery and jugular vein) and then bled for two minutes. After scalding (63°C) for 45 seconds, carcasses were mechanically defeathered and were manually eviscerated. The breast, without skin, was separated from carcasses and deboned after chilling. The breast fillets were then placed into freezer bags and stored on ice until rigor shortening passed (24 hours). After aging, the fillets were vacuum-packed (Webomatic, West Germany), frozen, and stored at -20°C until the sensory analyses were carried out. For sensory evaluation, six chicken breasts (left side) from each of the six treatment groups (3 diets × 2 strains) were used.

4.4.9.2 Panellist selection

Thirteen potential panellists (two males and eleven females) were recruited from students and staff at the School of Agriculture, Food and Wine, University of Adelaide. Panellists were recruited on the basis of the following criteria: not allergic to any foods, interested in and willing to sample meat products, enjoy working in a group and able to participate during training and testing. Some of these panellists had previous sensory training or descriptive analysis experience, and had participated in the sensory assessment of eggs. The protocol of this study was approved by the Human Ethics Committee of the University of Adelaide. Details of the experiment were explained to prospective panellists and panellists were selected after the

completion and signing of the consent form. The panellists were not informed about the dietary treatments and were asked to evaluate the sensory attributes of chicken breast meat.

4.4.9.3 Panellist training

Prior to formal sensory evaluation, the selected panellists were trained in three sessions of 2.5 hours duration in a group setting in an open plan sensory laboratory. The panellists were reimbursed $10/hour for their participation in the study. The aims of the training were detailed in Chapter 3 (see section 3.4.9.1.3). During the training session, panellists were familiarised with the scaling system (15cm-line scale), and decided the sensory methodology and meat evaluation techniques. Training sessions also defined the final list of sensory attributes to be assessed by panellists in the formal evaluation sessions. The sensory characteristics and descriptive vocabulary of the attributes were selected and adapted from those previously reported by other studies in the area [32, 192-194], and confirmed during training sessions. Panellists were presented with reference standards for the selected aroma, taste, and flavour attributes at different concentrations, and instructed to evaluate the low and high intensity of each standard that would be used in the formal sensory evaluation. Reference standards for chicken aroma, texture, taste and flavour in this study were developed using commercial foods. No reference standards were provided for fibrousnesses, aftertaste and off-flavour but panellists had extensive training in these attributes. Commercial chicken meat was also used during the training session to familiarise panellists with the sensory attributes.

To demonstrate and train panellists in possible textural and flavour differences in chicken breast meat, meat cooking time and temperature were manipulated. Panellists were trained to perform a descriptive analysis using a 15cm-line scale, with two anchor points placed at 10 and 90% of the scale [169, 195], either on paper or on a computer screen in individual booths. Samples were coded with a three-digit random number. In the last two training sessions, panel performance was evaluated by assessing breast meat samples from each dietary treatment in triplicate, using a standard set of sensory descriptors. Panellists also assessed breast meat samples as a group to ensure that panellist ratings were in the same range. Through discussion, a consensus was reached in relation to the levels of the intensity standards and the terminology of the sensory attributes (list and terminology). The final agreed descriptive vocabulary consisted of two aroma, four texture, five flavour, and three taste attributes (Table 4.5). A final discussion session also outlined what would arise during formal evaluation including the order in which sensory attributes would be assessed and negotiated agreeable formal assessing schedules.

4.4.9.4 Sample preparation

Six chicken breasts (left side) from each of the six treatment groups were used. Before cooking, the frozen meat was thawed for 24 hours under refrigerated conditions (4°C). The cooking method of breast meat followed the procedures as described by Lopez-Ferrer et al. [20] and Zelenka et al. [141]. On the day of evaluation, cooked breast meat was prepared as follows: the breast meat was individually wrapped in a double layer of aluminium foil, placed on an oven-plate and cooked in a pre-heated convection oven at 200°C until a final core temperature

of 85°C was reached, as determined by inserting a digital thermometer into the meat. After cooking, meat samples were taken out from the oven and cut into 12 pieces in cubes of approximately 1.5cm. One piece of breast meat was placed in a closed plastic container, coded with three-digit random numbers and placed in an oven at about 40°C for 30 – 90 minutes to keep warm until evaluated by panellists. One piece of cooked breast meat from each treatment group used for sensory evaluation was also collected for fatty acid analysis.

4.4.9.5 Final descriptive analysis evaluation

A descriptive analysis was carried out to quantitatively define differences in the sensory characteristics between chicken breast meat samples from birds fed diets enriched with ALA and those fed low ALA diets. On the day of sensory evaluation, breast meat samples were prepared as described above. The sample set presented for descriptive profiling ($3 \times 2 = 6$) was assessed for aroma, texture, taste, and flavour by each panellist, with six replications. Panellists also evaluated sensory profiles of six commercial breast meat samples as comparison to breast meat from birds fed diet enriched with ALA. During the evaluation, panellists had access to reference standards of the attributes. A 15-cm unstructured line scale with two anchor points, as described in the training session, was used. The anchor points were labelled with intensity extremes of each attributes (Table 4.5). Panellists evaluated the breast samples using FIZZ software version 2.47b (Biosystèms, Couternon, France) [196].

Sensory evaluation was performed by a 12-member panel and was carried out in a sensory laboratory [197], with individual booths under fluorescent light at the

University of Adelaide. Sample treatments were randomised across 12 panellists and were presented to the panellists in a sequence ensuring that each assessor received and evaluated the same part of the breast meat every time [141]. Panellists were instructed to rinse their palate with water and unflavoured crackers between two samples to minimise the carry-over effect. In total, each panellist evaluated 42 samples over a two day period. Each panellist tested 21 samples over two hours on one day, and they were presented samples in groups of seven, with 10 minutes break after the first seven samples.

Table 4.5 Aroma, texture, taste and flavour attribute list with agreed definitions and reference standards

Sensory attributes	Definition[1]	Scale anchors[2]	Reference standards
Aroma			
Aroma	Odour of the meat (stimuli strength experienced over the sample when it is near the nose)	Low to high intensity	
Chicken aroma	Steamed chicken / chicken & corn soup /corn	Low to high intensity	Four levels (0.26-2.08g/L) chicken stock in distilled water
Texture			
Tenderness	Force required to bite against the grain (through fibre) in the middle of the meat sample with incisors	Very tough to very tender	Jelly bean and marshmallow
Chewiness	Number of chews required to chew the samples to point of swallow (4–28 chews)	Not chewy to very chewy	Jelly bean and marshmallow
Fibrousnesses	Chew sample up to five times with molars. Determine whether the sample breaks into stringy pieces (extremely fibrous like tough steak muscle fibres) or small powdery particles (finely fibrous)	Finely fibrous to extremely fibrous	
Juiciness	Amount of moisture coming from the sample during the first 5 chews (range from banana = 1, very dry; zucchini = 7, juicy; to snow pea = 14, very juicy)	Very dry to very juicy	Banana, zucchini, and snow pea
Taste			
Savoury taste	The sensation produced on the tongue and palate by the meat taken into the mouth (taste) associated with gustatory perceptions (umami) caused by sample	Low to high intensity	Two levels (0.25 and 0.75g/L) L-glutamic acid in distilled water
Metallic taste	Taste associated with gustatory perceptions (metallic/iron) caused by sample	Low to high intensity	Two levels (0.00125 and 0.0025 g/L) Iron (II) sulfate heptahydrate in distilled water
Aftertaste	The sensation that lingers on the tongue and palate (the flavour remaining in the mouth) for up to five seconds after swallowing (savoury/mushroom/broth)	Low to high intensity	
Flavour			
Chicken flavour	The distinctive aroma and taste of the poultry meat (steamed chicken /boiled chicken)	None to intense	Four levels (0.26-2.08g/L) chicken stock in distilled water
Corn flavour		None to intense	Two levels, low (31.25ml corn solution + 468.75ml distilled water) and high level (150ml corn solution + 450 ml distilled water). Corn solution prepared from 248g sweet corn in can + distilled water and make up to 600ml.
Broth flavour	Chicken stock	None to intense	Four levels (0.26-2.08g/L) chicken stock in distilled water
Mushroom flavour	Shitake mushroom broth /savoury	None to intense	Four levels (2.5-10g/L) slice dried mushroom in distilled water
Off-flavour	Undesirable or unusual smell or taste of the chicken meat	None off-flavour to strong off-flavour	

[1]Definitions of sensory attributes were based on publications in the area [32, 192-194] and confirmed during training sessions.
[2]A 15cm line-scale with indented anchor points of attribute intensity.

4.4.10 Statistical analysis

The data on production parameters and fatty acid profiles of tissues were analysed by univariate ANOVA with a completely randomised block design by using the ANOVA (general analysis of variance) directive in GenStat (Release 14). The experimental unit was a pen of birds with four pens as a replicate fed as one group (24 pens in total). There were four blocks in the experiment. Main effects of diet (3 levels), strain (2 levels), and the diet-by-strain interaction were tested for the production performance of birds and the fatty acid composition of the liver tissue and breast meat. The analysis was followed by Tukey's multiple comparison test if there were significant differences among treatments. Family-wise significance level was set at $P < 0.05$.

For descriptive sensory analysis, the line scale used and numerical representation of each attribute for each breast meat sample were as described in Chapter 3. Panellists assessed six replicate samples of breast meat from six different treatment groups. The descriptive sensory data for each sensory attribute were analysed as a univariate mixed linear model with panellists and chicken breasts as random factors using the ANOVA directive (general analysis of variance) in GenStat (Release 14). For the convenience of discussion, differences between treatment means were further analysed using Fisher's protected LSD with significance level of $P < 0.05$. The differences between the commercial and treatment groups were further analysed using contrast for comparing commercial breast meat and breast meat from birds fed diet containing ALA.

4.5 Results

4.5.1 Fatty acid composition of liver tissue phospholipids (% of total fatty acids)

The fatty acid profiles were measured from chicken liver tissue at day 40 of the dietary intervention (Table 4.6). Increased dietary ALA resulted in increased PL ALA levels in liver tissues ($P < 0.001$). In general, increased dietary ALA increased the level of the major n-3 LCPUFA, EPA, DPA and DHA in liver tissue PL (Figure 4.2A). It appeared that n-3 LCPUFA was accumulated in higher amounts in liver tissue PL than in liver tissue TL (Appendix 1), with EPA and DHA being the greatest responder to dietary ALA (Figure 4.2A). In general, any increase in the n-3 LCPUFA levels in tissues was offset by a decrease in the MUFA content of liver tissues. The changes in MUFA, PUFA and SFA contents as a result of increased dietary ALA content are summarised in Figure 4.2B. A significant strain effect on the fatty acid composition of the liver tissue PL was almost absent. A significant difference between Cobb and Ross birds was only observed for SFA. The liver PL SFA content of Cobb birds was slightly higher than Ross as a result of the higher stearic acid content in Cobb birds compared to Ross ($P < 0.05$).

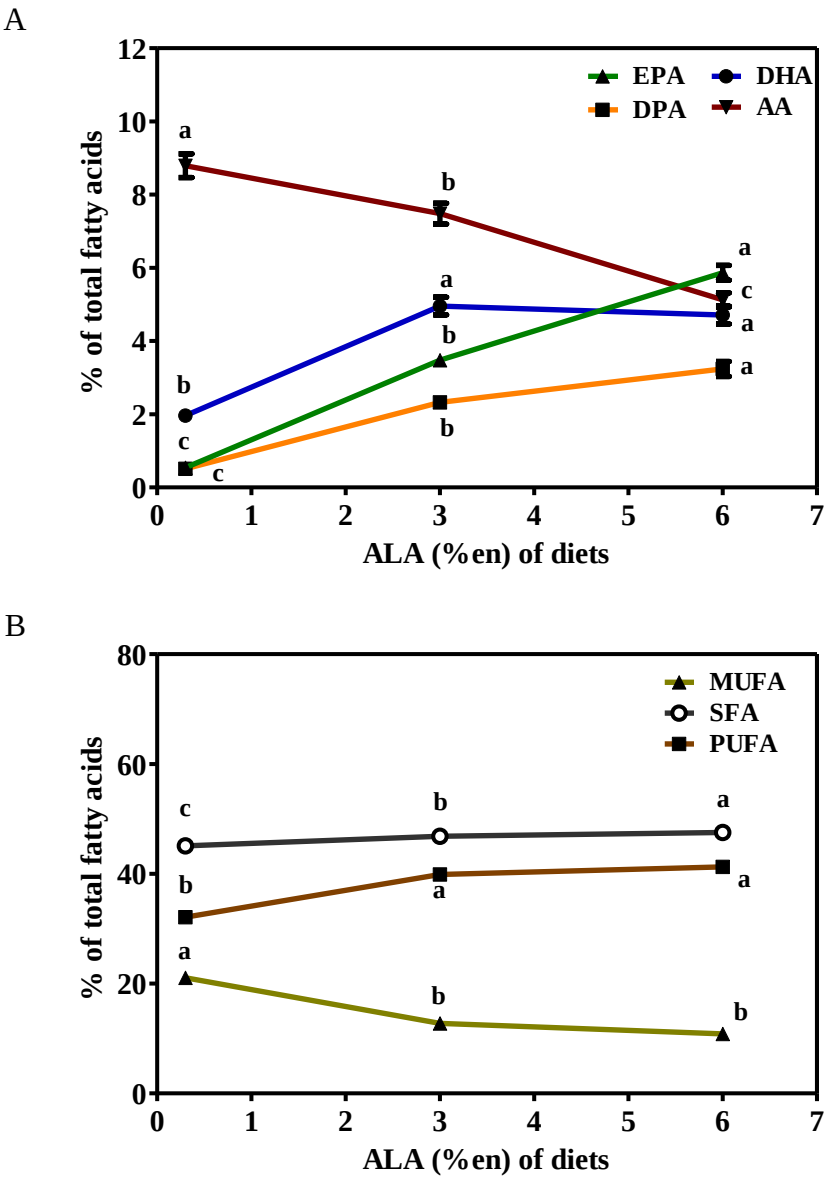


Figure 4.2 Overall effect of increasing dietary alpha-linolenic acid (ALA) on long chain polyunsaturated fatty acid (LCPUFA) content of liver tissue phospholipids (PL) (A) and fatty acid classes in liver PL (B) independent of strain (Cobb and Ross pooled data) at 40 days of age. The values presented are means of 24 replicate analyses ± SEM. There were significant differences in the level of eicosapentaenoic acid (EPA), docosapentaenoic acid (DPA), docosahexaenoic acid (DHA), arachdonic acid (AA), monounsaturated fatty acid (MUFA), saturated fatty acids (SFA), and polyunsaturated fatty acids (PUFA; $P < 0.001$) in liver tissue PL among dietary treatments. ALA enriched diets increased all n-3 LCPUFA but DHA content achieved a maximum level when birds were fed a diet enriched with 3%en ALA.

Table 4.6 Fatty acid profiles of liver tissue PL from broiler chickens fed with ALA enriched diets (% of total fatty acids)[1]

ALA level, %en	0.3		3		6			P value[2]		
Strains	Cobb	Ross	Cobb	Ross	Cobb	Ross	SEM	D	S	D x S
Fatty acids (%)[3]										
16:0	16.3	16.3	17.4	17.8	17.3	17.1	0.48	NS	NS	NS
18:0	27.6	26.5	28.1	27.6	29.0	28.4	0.42	**	*	NS
Total SFA	45.7	44.5	47.0	46.7	47.9	47.2	0.20	***	***	NS
16:1n-7	1.8	1.9	0.5	0.6	0.8	0.4	0.14	***	NS	NS
18:1n-9	14.9	15.3	9.5	10.2	8.7	7.7	0.77	***	NS	NS
18:1n-7	2.6	2.9	1.4	1.4	1.1	1.1	0.11	***	NS	NS
Total MUFA	20.6	21.6	12.5	13.1	11.5	10.2	1.00	***	NS	NS
Total n-9	17.3	18.2	10.7	11.4	9.5	8.6	0.82	***	NS	NS
Total n-7	4.4	4.9	1.9	1.9	1.9	1.5	0.20	***	NS	NS
18:2n-6 (LA)	16.9	17.0	17.5	18.1	17.9	17.4	0.43	NS	NS	NS
18:3n-6	0.1	0.1	0.1	0.1	0.1	0.1	0.01	***	*	NS
20:3n-6	1.3	1.3	1.2	1.3	1.2	1.1	0.06	**	NS	NS
20:4n-6 (AA)	8.8	8.8	7.5	7.5	4.6	5.6	0.44	***	NS	NS
Total n-6	28.8	29.0	27.4	28.0	24.6	25.1	0.77	***	NS	NS
18:3n-3 (ALA)	0.2	0.2	1.2	1.2	2.2	2.1	0.09	***	NS	NS
20:3n-3	0.0	0.0	0.2	0.2	0.4	0.5	0.03	***	NS	NS
20:5n-3 (EPA)	0.5	0.5	3.8	3.1	5.9	5.9	0.30	***	NS	NS
22:5n-3 (DPA)	0.5	0.5	2.3	2.3	2.8	3.7	0.22	***	NS	NS
22:6n-3 (DHA)	2.2	1.8	5.2	4.7	4.3	5.1	0.34	***	NS	NS
EPA + DHA	2.7	2.3	9.0	7.9	10.2	11.0	0.50	***	NS	NS
Total n-3 LCPUFA	3.2	2.8	11.3	10.2	13.0	14.6	0.69	***	NS	NS
Total n-3	3.4	3.1	12.8	11.6	15.6	17.2	0.76	***	NS	NS
Total PUFA	32.2	32.0	40.1	39.6	40.2	42.3	1.12	***	NS	NS
n-6:n-3 ratio	8.5	9.8	2.2	2.5	1.6	1.5	0.33	***	NS	NS
LA:ALA ratio	90.2	97.7	15.3	15.8	8.3	8.6	3.81	***	NS	NS

[1]Values are means of twelve observations per treatment and their pooled standard error of the mean (SEM). Values in the same row with no common superscript are significantly different ($P < 0.05$).

[2]*$P < 0.05$; **$P < 0.01$; ***$P < 0.001$; NS, not significant; D, diet; S, strain.

[3]SFA, saturated fatty acid; MUFA, monounsaturated fatty acid; LA, linoleic acid; AA, arachidonic acid; ALA, alpha-linolenic acid; EPA, eicosapentaenoic acid; DPA, docosahexaenoic acid; PUFA, polyunsaturated fatty acid.

4.5.2 Fatty acid composition of n-3 enriched chicken meat (% of total fatty acids)

4.5.2.1 Breast meat total lipid

ALA enriched diets resulted in higher levels of fat (1.9 and 1.7% for 3 and 6%en ALA, respectively; $P < 0.01$) compared to low ALA diets (1.4%). However, no difference in the fat content was observed between breast meat fed 3 and 6%en ALA diets. The fat content of Cobb birds (1.9%) was significantly higher than Ross birds (1.4%, $P < 0.001$). There was also a significant diet by strain interaction ($P < 0.05$) for the total fat content of the breast tissues. Cobb 500 birds appeared to have a higher fat content (2.1%) than Ross 308 birds (1.3%) when the birds were fed 6%en ALA diets.

The fatty acid composition of breast total lipid expressed as a percentage of total fatty acids is shown in Table 4.7. Increasing levels of dietary ALA enhanced all n-3 LCPUFA (EPA, DPA and DHA), total n-3 PUFA, and paradoxically total n-6 PUFA levels (Figure 4.3). While a direct relationship between ALA enrichment of the diets and the proportion of the EPA, DPA, total n-3 LCPUFA, and total n-3 fatty acids was observed, the maximum level of DHA was achieved in diets containing 3%en ALA, as observed in liver tissues. The high ALA diets increased EPA, DPA, and DHA levels to 5-, 4.5- and 2-fold, respectively, of those without ALA supplemented diets (Figure 4.3A). The most abundant n-3 LCPUFA was DPA, which accounted for up to 2.6% of total fatty acids. The n-3 PUFA content of breast meat from chickens fed diets enriched with 3 and 6%en ALA was 6- and 10-fold, respectively, higher than those fed a diet low in ALA ($P < 0.001$; Table 4.7). Most of the increase in the breast meat n-3 PUFA was due to enrichment in ALA, which increased by about 24-fold.

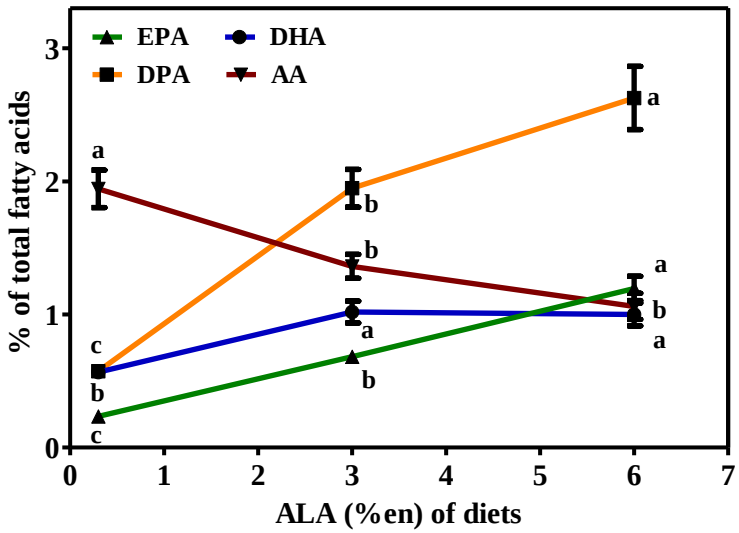
The incorporation of 3 or 6%en ALA into the diets slightly increased total n-6 PUFA content of breast meat compared to those fed diets containing ALA at 0.3%en (Table 4.7); however, no difference in the levels of the n-6 PUFA were observed between breast meat fed the moderate and the high ALA diets. The increase in the n-6 PUFA was due to an increase in LA. In contrast, the proportion of breast meat AA decreased with increasing levels of dietary ALA while the level of EPA increased in a linear manner (Figure 4.3A; $P < 0.001$). Thus, the level of the breast AA in birds fed diets enriched with 6%en ALA was almost half of those fed diets containing low ALA (0.3%en ALA). Dietary ALA enrichment decreased the n-6 to n-3 ratio in the breast meat. The n-6 to n-3 ratio was 6.5, 1.5, and 0.9 for 0.3, 3, and 6%en ALA diets, respectively ($P < 0.001$; Table 4.7).

The changes in SFA, MUFA, and PUFA content are summarised in Figure 4.3B. Oleic, linoleic, and palmitic acid were the most abundant fatty acids in breast TL; they were at levels of 27.5, 17.0, and 16.7% of the total fatty acids, respectively, for birds fed high ALA diets. In general, any increase in the level of n-3 PUFA in breast meat TL was largely offset by a reduction in the proportion of MUFA. High ALA diets decreased the MUFA content in breast meat TL by almost half. ALA enriched diets caused an increase in the total PUFA content in breast meat TL from 15.5 to 40.5% of total fatty acids ($P < 0.001$), with high ALA diets achieving the highest level of the total n-3 PUFA. There was no difference in the SFA content of breast meat TL of birds receiving high ALA and low ALA diets.

The strain of broiler chicken noticeably influenced the fatty acid composition of the breast meat but did not affect the percentage of total n-3 PUFA and total PUFA (Table 4.7). Significant differences between Cobb and Ross birds were observed for SFA, MUFA, EPA, DPA, DHA and total n-6 PUFA. Cobb birds deposited more ($P < 0.001$) ALA and MUFA than Ross birds (9.5 vs. 7.8 for ALA, 46.9 vs. 44.4% for MUFA for Cobb and Ross bird, respectively). In contrast, when the data were expressed as % of total fatty acids, Ross birds deposited slightly more SFA, EPA, DPA, and DHA than Cobb birds (23.7 vs. 26.0% for SFA, 0.6 vs. 0.8% for EPA, 1.4 vs. 2.1% for DPA, 0.7 vs. 1.0% for DHA for Cobb and Ross birds, respectively).

A significant interaction was found between ALA levels and strains of birds for deposition of ALA, DPA, DHA and total n-3 LCPUFA in breast meat TL (Table 4.7; Figure 4.4). Ross birds fed diets containing ALA at a level of 6%en had the highest DPA and DHA accumulation in breast meat TL (3.5 and 1.3%, respectively). Accordingly, a higher total n-3 LCPUFA was observed in Ross birds fed diets enriched with 6%en ALA compared to Cobb birds. Conversely, Cobb birds fed diets supplemented with 6%en ALA accumulated the highest level of ALA (16.8%). The level of SFA in Ross birds was slightly increased by elevating the ALA content of the diet (Table 4.7). However, Cobb birds consuming ALA enriched diets accumulated SFA at levels comparable to Cobb birds fed diets containing ALA at 0.3%en.

A



B

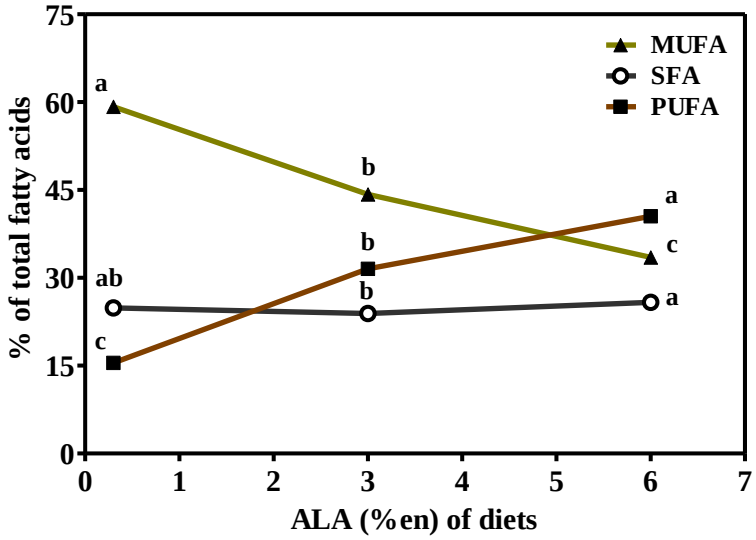
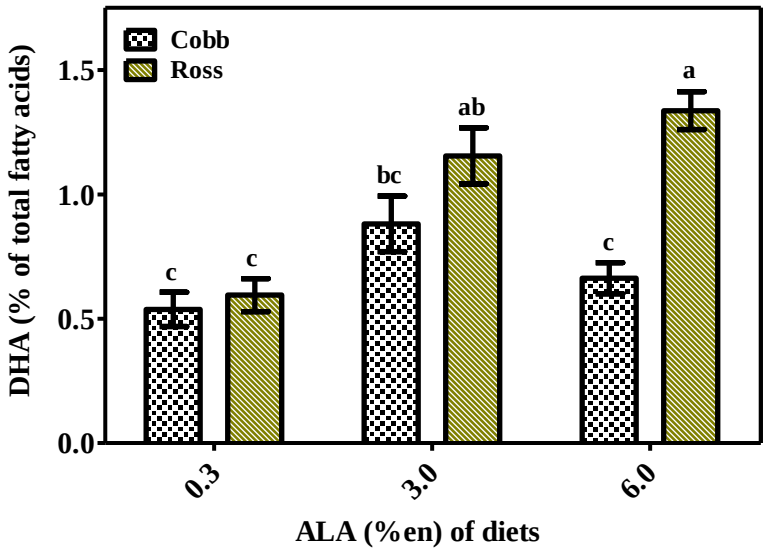


Figure 4.3 Overall effect of increasing dietary alpha-linolenic acid (ALA) on long chain polyunsaturated fatty acid (LCPUFA) content of breast meat total lipid (TL) (A) and fatty acid classes in breast TL (B) independent of strain (Cobb and Ross pooled data) at 40 days of age. The values presented are means of 24 replicate analyses ± SEM. There were significant differences in the levels of eicosapentaenoic acid (EPA), docosapentaenoic acid (DPA), docosahexaenoic acid (DHA), arachdonic acid (AA), monounsaturated fatty acid (MUFA), saturated fatty acids (SFA), and polyunsaturated fatty acids (PUFA; $P < 0.001$) in breast meat TL among dietary treatments. ALA enriched diets increased all n-3 LCPUFA but DHA content achieved a maximum level when birds were fed a diet enriched with 3%en ALA.

A



B

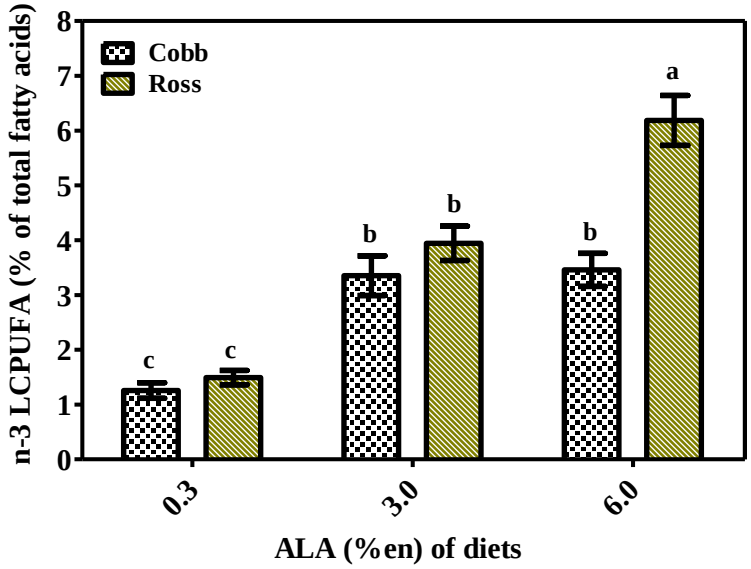


Figure 4.4 Effects of increasing levels of dietary alpha-linolenic acid (ALA) at levels of 0.3, 3, and 6%energy (%en) on docosahexaenoic acid (DHA) (A) and omega-3 long chain fatty acid (n-3 LCPUFA) (B) of breast total lipid (TL) of Cobb and Ross birds at 40 days of age. The values presented are means of 12 replicate analyses ± SEM. There were significant differences in the level of DHA and total n-3 LCPUFA ($P < 0.001$) among dietary treatments. There was a strain effect and interaction between diet and strain of birds for DHA and n-3 LCPUFA. Ross birds accumulated a higher level of DHA and n-3 LCPUFA than Cobb birds ($P < 0.001$). Ross birds fed a diet enriched with 6%en ALA had the highest DHA ($P < 0.05$) and n-3 LCPUFA ($P < 0.001$) content.

Table 4.7 Fatty acid profiles of breast meat TL from broiler chickens fed with ALA enriched diets (% of total fatty acids)

ALA level, %en	0.3		3		6			P value[2]		
Strains	Cobb	Ross	Cobb	Ross	Cobb	Ross	SEM	D	S	D x S
Fatty acids (%)[3]										
Breast fat content (% wet weight)	1.6	1.3	2.0	1.8	2.1	1.3	0.12	**	***	*
16:0	16.5	17.2	16.1	16.6	16.4	16.9	0.29	NS	*	NS
18:0	5.1^{b}	5.4^{b}	5.4^{b}	5.8^{b}	5.5^{b}	7.6^{a}	0.21	***	***	**
Total SFA	24.0^{bc}	25.7^{b}	23.4^{c}	24.5^{bc}	23.8^{bc}	27.9^{a}	0.47	**	***	*
16:1n-7	9.4	8.2	2.7	2.4	3.4	2.0	0.24	***	***	NS
18:1n-9	43.5	42.1	37.8	36.6	29.1	25.9	0.44	***	***	NS
18:1n-7	5.3	5.8	2.9	3.1	1.9	2.2	0.09	***	***	NS
Total MUFA	60.2	58.2	44.9	43.6	35.7	31.3	0.61	***	***	NS
Total n-9	45.7	44.3	39.2	38.0	30.0	26.9	0.44	***	***	NS
Total n-7	14.7	14.0	5.6	5.5	5.4	4.2	0.25	***	**	NS
18:2n-6 (LA)	10.2	9.9	16.7	16.6	16.8	17.2	0.28	***	NS	NS
18:3n-6	0.1	0.1	0.1	0.1	0.0	0.1	0.00	***	**	NS
20:3n-6	0.4	0.5	0.3	0.3	0.3	0.4	0.02	***	***	NS
20:4n-6 (AA)	1.7	2.1	1.2	1.5	0.7	1.4	0.13	***	***	NS
Total n-6	13.2	13.5	18.7	19.0	18.1	19.5	0.37	***	*	NS
18:3n-3 (ALA)	0.7^{d}	0.6^{d}	9.2^{c}	8.5^{c}	18.4^{a}	14.4^{b}	0.41	***	***	***
20:3n-3	0.0^{d}	0.0^{d}	0.2^{c}	0.2^{c}	0.4^{b}	0.6^{a}	0.02	***	**	**
20:5n-3 (EPA)	0.2	0.3	0.7	0.7	1.0	1.4	0.08	***	*	NS
22:5n-3 (DPA)	0.5^{c}	0.6^{c}	1.8^{b}	2.1^{b}	1.8^{b}	3.5^{a}	0.17	***	***	***
22:6n-3 (DHA)	0.5^{c}	0.6^{c}	0.9^{bc}	1.2^{ab}	0.7^{c}	1.3^{a}	0.09	***	***	*
EPA + DHA	0.8^{c}	0.9^{c}	1.6^{b}	1.8^{b}	1.7^{b}	2.7^{a}	0.13	***	***	**
Total n-3 LCPUFA	1.3^{c}	1.5^{c}	3.4^{b}	3.9^{b}	3.5^{b}	6.2^{a}	0.28	***	***	***
Total n-3	2.1	2.2	12.8	12.6	22.3	21.2	0.46	***	NS	NS
Total PUFA	15.3	15.6	31.5	31.6	40.3	40.7	0.70	***	NS	NS
n-6:n-3 ratio	6.6	6.3	1.5	1.5	0.8	0.9	0.11	***	NS	NS
LA:ALA ratio	14.2^{b}	16.5^{a}	1.8^{c}	2.0^{c}	0.9^{c}	1.3^{c}	0.33	***	**	*

[1]Values are means of twelve observations per treatment and their pooled standard error of the mean (SEM). Values in the same row with no common superscript are significantly different ($P < 0.05$).
[2]*$P < 0.05$; **$P < 0.01$; ***$P < 0.001$; NS, not significant; D, diet; S, strain.
[3]SFA, saturated fatty acid; MUFA, monounsaturated fatty acid; LA, linoleic acid; AA, arachidonic acid; ALA, alpha-linolenic acid; EPA, eicosapentaenoic acid; DPA, docosahexaenoic acid; PUFA, polyunsaturated fatty acid.

4.5.2.2 Breast meat phospholipid fraction

In general, the changes in the accumulation of n-3 LCPUFA, total n-3, total PUFA, and MUFA in breast meat PL were similar to those observed in breast meat TL (Table 4.8). While a direct relationship between dietary ALA enrichment and the proportion of the EPA, DPA, total n-3 LCPUFA, and total n-3 PUFA was observed, the DHA content achieved a maximum of 2-fold increase in moderate ALA diets ($P < 0.001$). However, some differences in the levels of ALA and n-3 LCPUFA were found. For example, the increased levels of dietary ALA resulted in an increase in the ALA content of breast meat PL ($P < 0.001$) but unlike in breast meat TL, the ALA levels rarely exceeded 2.5% of total fatty acids. In contrast, breast meat PL contained significant amounts of n-3 LCPUFA, with DPA showing the greatest response to dietary ALA (Table 4.8).

As observed in breast meat TL, when the EPA content was increased in the breast meat PL ($P < 0.001$) as a result of increasing the level of ALA, a significant decrease ($P < 0.001$) in the level of AA was observed although the n-6 PUFA levels remained constant. Increasing the ALA content in the diets slightly increased the LA content of breast meat PL. The increased levels of the dietary ALA changed the composition of SFA, MUFA and PUFA in a similar pattern to those observed in breast meat TL.

Regarding the strain, a significant strain effect was observed in the ALA and MUFA content of breast meat PL ($P < 0.05$). The incorporation of ALA and MUFA was higher for Cobb birds compared to those for Ross birds. In contrast, a higher SFA content was observed in Ross birds than in Cobb birds ($P < 0.01$).

Table 4.8 Fatty acid profiles of breast meat PL from broiler chickens fed with ALA enriched diets (% of total fatty acids)[1]

ALA level, %en	0.3		3		6			P value[2]		
Strains	Cobb	Ross	Cobb	Ross	Cobb	Ross	SEM	D	S	D x S
Fatty acids (%)[3]										
16:0	19.1	19.0	20.6	21.2	21.1	21.2	0.34	***	NS	NS
18:0	9.0	8.7	10.4	10.4	11.2	11.4	0.18	***	NS	NS
Total SFA	35.2	35.5	39.3	39.8	40.5	40.9	0.17	***	**	NS
16:1n-7	3.1	2.8	0.5	0.5	0.7	0.5	0.07	***	**	NS
18:1n-9	26.4	26.3	19.5	18.8	17.4	16.0	0.33	***	*	NS
18:1n-7	7.5	8.0	4.0	4.1	2.8	2.8	0.12	***	*	NS
Total MUFA	38.5	38.5	24.9	24.3	21.5	19.9	0.38	***	*	NS
Total n-9	28.5	28.5	20.5	19.8	18.1	16.8	0.33	***	*	NS
Total n-7	10.5	10.7	4.5	4.6	3.5	3.3	0.17	***	NS	NS
18:2n-6 (LA)	11.8	11.2	14.1	13.9	16.2	15.4	0.50	***	NS	NS
18:3n-6	0.2	0.2	0.1	0.1	0.1	0.1	0.01	***	NS	NS
20:3n-6	1.1	1.1	0.8	0.8	0.8	0.7	0.04	***	NS	NS
20:4n-6 (AA)	5.5	5.6	4.7	4.7	2.9	3.4	0.17	***	NS	NS
Total n-6	21.0	20.5	21.2	21.0	20.9	20.6	0.48	NS	NS	NS
18:3n-3 (ALA)	0.24^{d}	0.22^{d}	1.18^{c}	1.08^{c}	2.36^{a}	1.98^{b}	0.06	***	**	*
20:3n-3	0.2	0.2	0.7	0.7	1.1	1.2	0.03	***	NS	NS
20:5n-3 (EPA)	0.7	0.7	2.4	2.2	3.5	3.2	0.14	***	NS	NS
22:5n-3 (DPA)	1.6	1.7	6.5	6.7	7.0	8.2	0.34	***	NS	NS
22:6n-3 (DHA)	1.7	1.6	3.6	4.0	2.9	3.7	0.23	***	*	NS
EPA + DHA	2.4	2.4	6.0	6.2	6.4	6.9	0.24	***	NS	NS
Total n-3 LCPUFA	4.0	4.1	12.5	12.9	13.4	15.1	0.53	***	NS	NS
Total n-3	4.6	4.6	14.4	14.7	16.9	18.3	0.52	***	NS	NS
Total PUFA	25.5	25.1	35.6	35.6	37.8	38.9	0.39	***	NS	NS
n-6:n-3 ratio	4.7	4.5	1.5	1.4	1.2	1.2	0.10	***	NS	NS
LA:ALA ratio	52.3	53.2	12.3	13.4	7.0	7.9	1.08	***	NS	NS

[1]Values are means of twelve observations per treatment and their pooled standard error of the mean (SEM). Values in the same row with no common superscript are significantly different ($P < 0.05$).

[2]*$P < 0.05$; **$P < 0.01$; ***$P < 0.001$; NS, not significant; D, diet; S, strain.

[3]SFA, saturated fatty acid; MUFA, monounsaturated fatty acid; LA, linoleic acid; AA, arachidonic acid; ALA, alpha-linolenic acid; EPA, eicosapentaenoic acid; DPA, docosahexaenoic acid; PUFA, polyunsaturated fatty acid.

4.5.2.3 Breast meat triglyceride fraction

In general, the fatty acid profiles of SFA, MUFA, total n-6, and total n-3 PUFA in breast meat TG followed a similar pattern to breast meat TL; however, a higher accumulation of ALA and a lower content of n-3 LCPUFA were observed in breast TG compared to breast meat TL (Table 4.9). As expected, when dietary ALA was increased, the level of n-3 PUFA in breast meat TG increased, and was approximately 24 times higher in high ALA diets compared to low ALA diets. The level of ALA represented 94 to 95% of the total n-3 PUFA content in moderate and high ALA diets, respectively. In contrast, the amount of total n-3 LCPUFA in breast meat TG was only 4 and 5% with moderate and high ALA diets, respectively.

The increased dietary ALA at a level of 6%en reduced MUFA content by approximately 2-fold and increased PUFA content by almost 4-fold. No difference between moderate and high ALA diets was observed for SFA. Oleic acid was the main fatty acid of this fraction.

Table 4.9 Fatty acid profiles of breast meat TG from broiler chickens fed with ALA enriched diets (% of total fatty acids)[1]

ALA level, %en	0.3		3		6			P value[2]		
Strains	Cobb	Ross	Cobb	Ross	Cobb	Ross	SEM	D	S	D x S
Fatty acids (%)[3]										
16:0	16.5^{ab}	17.4^{a}	15.7^{b}	15.9^{ab}	16.5^{ab}	15.3^{b}	0.33	**	NS	*
18:0	3.6	3.8	4.4	4.3	4.6	5.1	0.11	***	*	NS
Total SFA	21.0^{ab}	22.2^{a}	20.7^{b}	20.8^{ab}	21.7^{ab}	21.0^{ab}	0.32	*	NS	*
16:1n-7	12.0	11.4	3.4	3.2	4.1	2.9	0.25	***	**	NS
18:1n-9	49.1	49.0	41.5	41.9	31.1	29.8	0.34	***	NS	NS
18:1n-7	4.3	4.4	2.4	2.5	1.7	1.8	0.04	***	*	NS
Total MUFA	67.6^{a}	66.9^{a}	48.9^{b}	49.1^{b}	38.0^{c}	35.6^{d}	0.45	***	*	*
Total n-9	51.0	50.9	42.8	43.2	32.0	30.8	0.34	***	NS	NS
Total n-7	16.3	15.8	5.9	5.7	5.8	4.7	0.28	***	*	NS
18:2n-6 (LA)	9.5^{c}	9.1^{c}	17.6^{ab}	17.7^{ab}	17.0^{b}	18.5^{a}	0.31	***	NS	*
18:3n-6	0.1	0.1	0.1	0.1	0.1	0.1	0.01	***	NS	NS
20:3n-6	0.1	0.1	0.1	0.1	0.1	0.1	0.01	**	NS	NS
20:4n-6 (AA)	0.1^{c}	0.1^{c}	0.2^{a}	0.2^{ab}	0.1^{bc}	0.2^{a}	0.01	***	NS	**
Total n-6	9.9^{c}	9.4^{c}	18.1^{ab}	18.1^{ab}	17.4^{b}	19.0^{a}	0.33	***	NS	*
18:3n-3 (ALA)	1.0	0.9	11.4	11.1	21.8	22.8	0.30	***	NS	NS
20:3n-3	0.0	0.0	0.1	0.1	0.2	0.2	0.01	***	NS	NS
20:5n-3 (EPA)	0.0^{d}	0.0^{d}	0.2^{c}	0.1^{c}	0.3^{b}	0.4^{a}	0.02	***	NS	*
22:5n-3 (DPA)	0.0^{c}	0.0^{c}	0.3^{b}	0.3^{b}	0.4^{b}	0.6^{a}	0.03	***	**	**
22:6n-3 (DHA)	0.0^{c}	0.0^{c}	0.1^{ab}	0.1^{b}	0.1^{b}	0.1^{a}	0.01	***	NS	**
EPA + DHA	0.0^{d}	0.0^{d}	0.3^{bc}	0.2^{c}	0.4^{b}	0.5^{a}	0.02	***	NS	**
Total n-3 LCPUFA	0.0^{c}	0.0^{c}	0.6^{b}	0.5^{b}	0.8^{b}	1.1^{a}	0.05	***	*	**
Total n-3	1.2^{c}	1.1^{c}	12.1^{b}	11.8^{b}	22.8^{a}	24.1^{a}	0.34	***	NS	NS
Total PUFA	11.1^{d}	10.6^{d}	30.2^{c}	29.9^{c}	40.1^{b}	43.1^{a}	0.63	***	NS	*
n-6:n-3 ratio	8.3	8.5	1.5	1.5	0.8	0.8	0.08	***	NS	NS
LA:ALA ratio	9.7	10.0	1.6	1.6	0.8	0.8	0.09	***	NS	NS

[1]Values are means of twelve observations per treatment and their pooled standard error of the mean (SEM). Values in the same row with no common superscript are significantly different ($P < 0.05$).
[2]*$P < 0.05$; **$P < 0.01$; ***$P < 0.001$; NS, not significant; D, diet; S, strain.
[3]SFA, saturated fatty acid; MUFA, monounsaturated fatty acid; LA, linoleic acid; AA, arachidonic acid; ALA, alpha-linolenic acid; EPA, eicosapentaenoic acid; DPA, docosahexaenoic acid; PUFA, polyunsaturated fatty acid.

4.5.3 Fatty acid composition of n-3 enriched cooked chicken meat (% of total fatty acids)

4.5.3.1 Fatty acid composition of cooked breast meat total lipid

The fatty acid profiles of cooked breast meat were measured from Cobb and Ross birds at day 41 of the dietary intervention (Table 4.10). The accumulation of SFA, MUFA, total n-6, and total n-3 PUFA in cooked breast meat TL followed a similar pattern to raw breast meat TL; however, a higher accumulation of n-3 LCPUFA (EPA, DPA, DHA), almost double, was observed in cooked breast meat TL compared to raw breast meat TL. As expected, the EPA, DPA, and DHA content increased by ~7-, 5-, ~3-fold, respectively, in high ALA diets. This resulted in an increase in the level of total n-3 LCPUFA, which was four times higher in high ALA diets compared to low ALA diets. As observed in the raw breast meat TL, an optimum DHA content of cooked breast meat TL was reached in breast meat from chickens fed a moderate (3%en) ALA diet. In contrast, the level of AA was reduced by elevating the ALA content of the diet. There was no strain effect or significant diet by strain interaction in cooked breast meat TL, with the exception of the n-6 to n-3 ratio.

Table 4.10 Fatty acid profiles of cooked breast meat TL from broiler chickens fed with ALA enriched diets (% of total fatty acids)[1]

ALA level, %en	0.3		3		6			P value[2]		
Strains	Cobb	Ross	Cobb	Ross	Cobb	Ross	SEM	D	S	D x S
Fatty acids (%)[3]										
16:0	17.4	17.1	16.4	17.5	18.3	17.0	0.48	NS	NS	NS
18:0	5.7	6.4	7.4	7.1	8.4	8.7	0.31	***	NS	NS
Total SFA	25.9	27.7	26.9	27.5	30.7	29.8	0.97	**	NS	NS
16:1n-7	8.5	7.2	2.0	2.0	2.0	1.6	0.38	***	NS	NS
18:1n-9	40.8	38.4	33.0	33.2	23.7	24.5	1.02	***	NS	NS
18:1n-7	5.2	6.1	3.2	3.2	2.4	2.5	0.23	***	NS	NS
Total MUFA	56.5	53.5	39.5	39.7	29.0	29.5	1.17	***	NS	NS
Total n-9	42.8	40.5	34.2	34.4	24.6	25.4	1.02	***	NS	NS
Total n-7	13.8	13.3	5.2	5.2	4.4	4.0	0.32	***	NS	NS
18:2n-6 (LA)	11.4	10.3	16.3	15.7	16.9	16.0	0.63	***	NS	NS
18:3n-6	0.1	0.1	0.1	0.1	0.1	0.1	0.01	***	NS	NS
20:3n-6	0.4	0.6	0.4	0.4	0.5	0.5	0.05	NS	NS	NS
20:4n-6 (AA)	2.1	3.2	2.3	2.3	1.6	2.1	0.31	NS	NS	NS
Total n-6	14.8	15.4	19.8	19.1	19.6	19.3	0.70	***	NS	NS
18:3n-3 (ALA)	0.7	0.6	7.7	7.5	12.5	12.2	1.05	***	NS	NS
20:3n-3	0.0	0.1	0.3	0.3	0.7	0.7	0.05	***	NS	NS
20:5n-3 (EPA)	0.3	0.3	1.0	1.1	2.1	1.9	0.17	***	NS	NS
22:5n-3 (DPA)	0.7	0.9	2.9	3.0	3.6	4.4	0.38	***	NS	NS
22:6n-3 (DHA)	0.5	0.8	1.6	1.4	1.5	2.0	0.23	***	NS	NS
EPA + DHA	0.8	1.1	2.7	2.4	3.7	3.9	0.37	***	NS	NS
Total n-3 LCPUFA	1.5	2.1	5.5	5.5	7.2	8.3	0.73	***	NS	NS
Total n-3	2.2	2.8	13.6	13.4	20.5	21.2	0.56	***	NS	NS
Total PUFA	17.0	18.2	33.3	32.5	40.1	40.5	1.04	***	NS	NS
n-6:n-3 ratio	6.9[a]	5.7[b]	1.5[c]	1.4[c]	1.0[c]	0.9[c]	0.25	***	*	*
LA:ALA ratio	16.6	18.8	2.2	2.2	1.5	1.4	0.80	***	NS	NS

[1]Values are means of six observations per treatment and their pooled standard error of the mean (SEM). Values in the same row with no common superscript are significantly different ($P < 0.05$).

[2]*$P < 0.05$; **$P < 0.01$; ***$P < 0.001$; NS, not significant; D, diet; S, strain.

[3]SFA, saturated fatty acid; MUFA, monounsaturated fatty acid; LA, linoleic acid; AA, arachidonic acid; ALA, alpha-linolenic acid; EPA, eicosapentaenoic acid; DPA, docosahexaenoic acid; PUFA, polyunsaturated fatty acid.

4.5.3.2 Cooked breast meat phospholipid fraction

Dietary ALA enrichment resulted in increased ALA content in cooked breast meat PL ($P < 0.001$) but unlike cooked breast meat TL, the levels were only small (<2% of total fatty acids). In contrast, cooked breast meat PL contained significant amounts of n-3 LCPUFA reaching ~85% of total n-3 PUFA, with DPA having the greatest response to dietary ALA (Table 4.11). As observed in cooked breast meat TL, when EPA content was increased ($P < 0.001$) as a result of increased levels of dietary ALA, a significant reduction ($P < 0.001$) in the level of AA was observed, although the n-6 PUFA levels remained constant.

Regarding the strain, a significant strain effect was observed in DPA, total n-3 LCPUFA, and total n-3 content of cooked breast meat PL (Table 4.11). The deposition of DPA, total n-3 LCPUFA, and total n-3 was higher for Ross birds (5.8, 10.9, and 12.3% of total fatty acids, respectively) compared to Cobb birds (5.0, 10.0, and 11.5% of total fatty acids, respectively; $P < 0.05$). A significant diet by strain interaction was observed in the proportion of total SFA, with Cobb birds accumulating higher levels of total SFA with increased levels of dietary ALA.

Table 4.11 Fatty acid profiles of cooked breast meat PL from broiler chickens fed with ALA enriched diets (% of total fatty acids)[1]

ALA level, %en	0.3		3		6			P value[2]		
Strains	Cobb	Ross	Cobb	Ross	Cobb	Ross	SEM	D	S	D x S
Fatty acids (%)[3]										
16:0	19.0^{bc}	17.8^{c}	20.1^{ab}	21.3^{a}	21.9^{a}	20.0^{ab}	0.48	***	NS	**
18:0	9.6^{c}	9.1^{c}	11.9^{ab}	11.5^{b}	11.6^{ab}	12.5^{a}	0.22	***	NS	*
Total SFA	37.1^{d}	35.6^{e}	39.6^{c}	41.2^{ab}	42.3^{a}	41.0^{b}	0.25	***	NS	***
16:1n-7	2.4	2.4	0.4	0.5	0.5	0.4	0.05	***	NS	NS
18:1n-9	25.2	25.4	19.9	18.5	17.4	16.9	0.60	***	NS	NS
18:1n-7	7.7	8.2	4.2	4.5	3.1	3.1	0.19	***	NS	NS
Total MUFA	36.8	37.3	25.4	24.3	21.6	21.0	0.63	***	NS	NS
Total n-9	27.1	27.3	20.8	19.5	18.1	17.6	0.61	***	NS	NS
Total n-7	10.2	10.6	4.7	4.9	3.6	3.5	0.20	***	NS	NS
18:2n-6 (LA)	11.4	11.6	13.6	11.7	15.2	14.4	0.69	***	NS	NS
18:3n-6	0.1	0.1	0.0	0.0	0.0	0.0	0.00	***	NS	NS
20:3n-6	1.1	1.1	0.7	0.8	0.8	0.8	0.05	***	NS	NS
20:4n-6 (AA)	5.8	6.3	5.1	5.6	3.0	3.8	0.23	***	**	NS
Total n-6	20.8	21.6	21.0	19.7	20.0	20.1	0.61	NS	NS	NS
18:3n-3 (ALA)	0.2	0.2	0.9	0.7	1.6	1.4	0.06	***	NS	NS
20:3n-3	0.1	0.1	0.6	0.5	1.0	1.0	0.05	***	NS	NS
20:5n-3 (EPA)	0.8	0.8	2.2	2.3	3.4	3.1	0.13	***	NS	NS
22:5n-3 (DPA)	1.9	1.9	6.3	7.4	6.8	8.2	0.29	***	**	NS
22:6n-3 (DHA)	1.6	1.7	3.8	3.5	3.2	3.8	0.28	***	NS	NS
EPA + DHA	2.4	2.4	6.0	5.8	6.6	6.9	0.29	***	NS	NS
Total n-3 LCPUFA	4.2	4.4	12.3	13.2	13.4	15.1	0.45	***	*	NS
Total n-3	4.6	4.8	13.8	14.5	15.9	17.6	0.44	***	*	NS
Total PUFA	25.3	26.3	34.8	34.2	35.9	37.7	0.56	***	NS	NS
n-6:n-3 ratio	4.6	4.6	1.5	1.4	1.3	1.1	0.18	***	NS	NS
LA:ALA ratio	67.6	61.1	15.2	16.4	9.9	10.0	2.43	***	NS	NS

[1]Values are means of six observations per treatment and their pooled standard error of the mean (SEM). Values in the same row with no common superscript are significantly different ($P < 0.05$).

[2]*$P < 0.05$; **$P < 0.01$; ***$P < 0.001$; NS, not significant; D, diet; S, strain.

[3]SFA, saturated fatty acid; MUFA, monounsaturated fatty acid; LA, linoleic acid; AA, arachidonic acid; ALA, alpha-linolenic acid; EPA, eicosapentaenoic acid; DPA, docosahexaenoic acid; PUFA, polyunsaturated fatty acid.

4.5.3.3 Cooked breast meat triglyceride fraction

A higher accumulation of ALA and a lower incorporation of n-3 LCPUFA were observed in cooked breast TG compared to cooked breast meat TL. The ALA levels of cooked breast meat TG represented 92 to 93% of the total n-3 PUFA content in moderate and high ALA diets, respectively (Table 4.12). In contrast, the amount of total n-3 LCPUFA in cooked breast meat TG was only ~7 and 6% with moderate and high ALA diets, respectively. These results were similar to those of raw breast meat TG, suggesting ALA was mainly deposited in TG fraction while n-3 LCPUFA was mostly incorporated into PL.

The increased dietary ALA at a level of 6%en reduced MUFA content by ~2-fold and increased PUFA content by almost 4-fold compared to those without ALA supplementation. No difference between low and high ALA diets was observed for SFA content. Oleic acid was the main fatty acid of this fraction.

A significant strain effect was observed for DPA and total n-3 LCPUFA level of cooked breast meat TG. The deposition of the DPA and total n-3 LCPUFA was higher for Ross birds compared to Cobb birds (expressed as % of total fatty acids). A significant diet by strain interaction was observed for the DPA level, with Ross birds having higher DPA content with increasing levels of dietary ALA.

Table 4.12 Fatty acid profiles of cooked breast meat TG from broiler chickens fed with ALA enriched diets (% of total fatty acids)[1]

ALA level, %en	0.3		3		6			P value[2]		
Strains	Cobb	Ross	Cobb	Ross	Cobb	Ross	SEM	D	S	D x S
Fatty acids (%)[3]										
16:0	17.0	16.9	14.8	15.7	15.9	15.3	0.53	**	NS	NS
18:0	3.9	4.0	4.7	4.7	5.2	5.9	0.20	***	NS	NS
Total SFA	22.2	22.2	20.2	21.2	21.9	21.9	0.60	*	NS	NS
16:1n-7	11.6	11.1	2.7	3.0	3.3	2.3	0.27	***	NS	NS
18:1n-9	48.8	49.4	41.4	41.1	30.0	30.4	0.60	***	NS	NS
18:1n-7	4.2	4.3	2.3	2.5	1.6	1.6	0.09	***	NS	NS
Total MUFA	66.8	67.1	48.0	48.2	36.0	35.7	0.75	***	NS	NS
Total n-9	50.8	51.4	42.8	42.5	30.9	31.5	0.63	***	NS	NS
Total n-7	15.8^{a}	15.4^{a}	5.0^{bc}	5.5^{b}	4.9^{bc}	3.9^{c}	0.27	***	NS	*
18:2n-6 (LA)	9.1	9.1	18.3	17.6	17.6	18.2	0.47	***	NS	NS
18:3n-6	0.1	0.1	0.1	0.1	0.1	0.1	0.01	***	NS	NS
20:3n-6	0.1	0.1	0.1	0.2	0.1	0.2	0.01	**	NS	NS
20:4n-6 (AA)	0.2	0.2	0.2	0.3	0.2	0.3	0.03	*	*	NS
Total n-6	9.7	9.6	18.9	18.3	18.2	18.9	0.50	***	NS	NS
18:3n-3 (ALA)	0.9	0.9	11.8	11.1	22.3	21.5	0.46	***	NS	NS
20:3n-3	0.0	0.0	0.1	0.1	0.2	0.2	0.01	***	NS	NS
20:5n-3 (EPA)	0.0	0.0	0.2	0.3	0.5	0.6	0.04	***	NS	NS
22:5n-3 (DPA)	0.1^{c}	0.0^{c}	0.4^{b}	0.5^{b}	0.6^{b}	0.8^{a}	0.05	***	**	*
22:6n-3 (DHA)	0.0	0.0	0.1	0.1	0.2	0.2	0.02	***	NS	NS
EPA + DHA	0.0	0.0	0.3	0.4	0.7	0.8	0.05	***	NS	NS
Total n-3 LCPUFA	0.1	0.0	0.7	0.9	1.2	1.6	0.10	***	*	NS
Total n-3	1.1	1.0	12.7	12.1	23.8	23.4	0.50	***	NS	NS
Total PUFA	10.8	10.5	31.6	30.5	41.9	42.2	0.96	***	NS	NS
n-6:n-3 ratio	9.2	9.8	1.5	1.5	0.8	0.8	0.20	***	NS	NS
LA:ALA ratio	9.9	10.0	1.6	1.6	0.8	0.8	0.15	***	NS	NS

[1]Values are means of six observations per treatment and their pooled standard error of the mean (SEM). Values in the same row with no common superscript are significantly different ($P < 0.05$).

[2]*$P < 0.05$; **$P < 0.01$; ***$P < 0.001$; NS, not significant; D, diet; S, strain.

[3]SFA, saturated fatty acid; MUFA, monounsaturated fatty acid; LA, linoleic acid; AA, arachidonic acid; ALA, alpha-linolenic acid; EPA, eicosapentaenoic acid; DPA, docosahexaenoic acid; PUFA, polyunsaturated fatty acid.

4.5.4 Fatty acid composition of n-3 enriched chicken meat (mg/100g of meat)

4.5.4.1 Fatty acid composition of raw breast meat total lipid

DPA was the main n-3 LCPUFA in raw breast meat TL (Table 4.13). It is interesting to note that the highest level of DHA (13mg/100g of meat, $P < 0.05$) was achieved from the breast meat of chickens fed a diet containing 3%en ALA. This may be due to breast meat TL from birds fed moderate ALA diet having a higher fat content than those fed low and high ALA diets. In addition, as the concentration of DPA reached plateaus of 24 and 25mg/100g of meat in birds on the moderate and high ALA diets, there was no difference in the total n-3 LCPUFA content between moderate and high ALA diets (~ 46mg/100g of meat; Figure 4.5A). Compared with breast meat TL produced by chickens fed 0.3%en ALA, diets enriched with 3 and 6%en ALA increased total n-3 PUFA levels by 8- (174mg/100g of meat) and 11.5-fold (250mg/100g of meat), respectively, at the expense of MUFA. The level of MUFA was reduced by almost half for a diet enriched with 6%en ALA. While the content of all n-3 fatty acids increased with increasing levels of dietary ALA, AA content was reduced by half in raw breast meat TL in birds fed the high ALA diets (Figure 4.5B).

There were strain effects on the fatty acid composition of raw breast meat TL. For example, Cobb birds contained more MUFA, ALA, EPA, total n-3, total n-6, and total PUFA compared to Ross birds. The concentration of total n-3 in raw breast meat was 184 and 113mg/100g for Cobb and Ross birds, respectively. No differences in DPA, DHA, total n-3 LCPUFA, and AA content between Cobb and Ross birds were observed.

A significant interaction was found between the ALA content of diets and strains of birds for ALA concentration in breast meat TL, with Cobb birds fed a diet enriched with 6%en ALA achieving the highest level of ALA. Consequently, the highest concentration of the total n-3 PUFA in breast meat TL was reached for Cobb birds fed the high ALA diets (338mg/100g of meat). This indicates that the minimum level of 300mg of total n-3 fatty acids/100g of meat required for labelling the meat as a source of n-3 fatty acids was achieved for breast meat from Cobb birds fed diets containing 6%en ALA.

Importantly, while the n-3 PUFA level of chicken breast meat TL was increased by inclusion of ALA in the diets at a level of 6%en, the SFA content was not influenced.

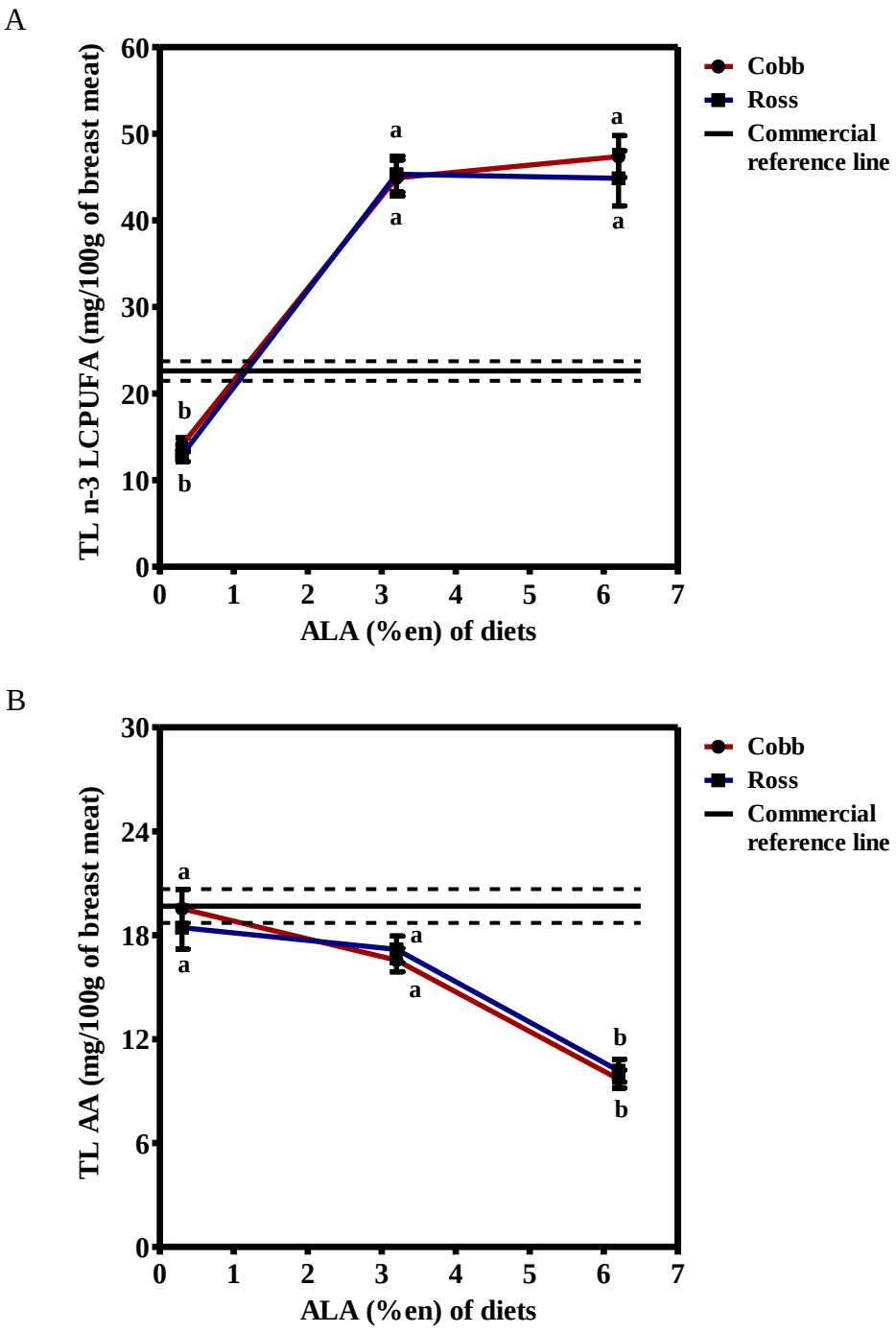


Figure 4.5 Effect of increasing dietary alpha-linolenic acid (ALA) on long chain polyunsaturated fatty acid (LCPUFA) and arachidonic acid (AA) content of raw breast meat total lipid (TL) in Cobb and Ross strains of broilers at 40 days of age. The values presented are means of 12 replicate analyses ± SEM. There were significant differences in the total n-3 LCPUFA and AA levels of cooked breast meat TL among dietary treatments ($P < 0.001$). ALA enriched diets increased total n-3 LCPUFA and decreased AA content in cooked breast meat.

Table 4.13 Fatty acid profiles of raw breast meat TL from broiler chickens fed with ALA enriched diets (mg/100g of meat)[1]

ALA level, %en	0.3		3		6			P value[2]		
Strains	Cobb	Ross	Cobb	Ross	Cobb	Ross	SEM	D	S	D x S
Fatty acids (mg/100g)[3]										
Total SFA	293.0	233.6	345.7	304.5	347.2	204.7	21.95	*	***	NS
Total MUFA	761.0	550.0	683.0	566.0	544.0	243.0	56.40	***	***	NS
18:2n-6 (LA)	125.1^{b}	90.4^{b}	251.3^{a}	209.4^{a}	254.6^{a}	129.8^{b}	15.98	***	***	*
20:4n-6 (AA)	19.6	18.4	16.6	17.2	9.7	10.2	1.00	***	NS	NS
Total n-6	159.0^{b}	121.7^{b}	278.3^{a}	237.0^{a}	272.3^{a}	146.5^{b}	16.39	***	***	*
18:3n-3 (ALA)	9.3^{c}	5.8^{c}	141.8^{b}	109.4^{b}	285.2^{a}	113.6^{b}	14.75	***	***	***
20:5n-3 (EPA)	2.4^{c}	2.2^{c}	9.3^{b}	7.9^{b}	13.6^{a}	9.9^{b}	0.72	***	**	NS
22:5n-3 (DPA)	5.7	5.5	23.8	24.2	24.8	25.0	1.45	***	NS	NS
22:6n-3 (DHA)	6.0	5.2	11.8	13.3	9.0	9.9	0.74	***	NS	NS
EPA + DHA	8.4	7.4	21.2	21.2	22.6	19.9	1.09	***	NS	NS
Total n-3 LCPUFA	14.1	12.9	44.9	45.3	47.4	44.9	2.40	***	NS	NS
Total n-3	24.1^{c}	19.3^{c}	189.6^{b}	157.5^{b}	337.9^{a}	162.5^{b}	15.78	***	***	***
Total PUFA	183.0^{de}	141.0^{e}	468.0^{b}	395.0^{bc}	610.0^{a}	309.0^{cd}	30.80	***	***	**
n-6:n-3 ratio	6.6	6.3	1.5	1.5	0.8	0.9	0.11	***	NS	NS

[1]Values are means of twelve observations per treatment and their pooled standard error of the mean (SEM). Values in the same row with no common superscript are significantly different ($P < 0.05$).
[2]*$P < 0.05$; **$P < 0.01$; ***$P < 0.001$; NS, not significant; D, diet; S, strain.
[3]SFA, saturated fatty acid; MUFA, monounsaturated fatty acid; LA, linoleic acid; AA, arachidonic acid; ALA, alpha-linolenic acid; EPA, eicosapentaenoic acid; DPA, docosahexaenoic acid; PUFA, polyunsaturated fatty acid.

4.5.4.2 Fatty acid composition of cooked breast meat total lipid

In this study, the fatty acid composition of cooked breast meat expressed as mg/100g of meat was also analysed because TG in meat can melt and be lost from meat during cooking. Therefore, the information regarding the amount of n-3 fatty acids in cooked breast meat is important. When ALA was included in the diet, a significant incorporation of ALA and all n-3 LCPUFA (EPA, DPA, and DHA) was observed in cooked breast meat (Table 4.14). However, the n-3 LCPUFA levels achieved in cooked breast meat were higher than those observed in raw breast meat. This may be due to removal of TG during cooking. As observed in the raw breast meat TL, there was a linear increase in the concentration of EPA in cooked breast meat, with the greatest EPA content in the cooked breast meat attained in birds receiving a high ALA diet. However, no change was observed in the level of DPA, DHA, n-3 LCPUFA, and total n-3 fatty acids in cooked breast meat TL from birds fed the moderate and high ALA diets (Table 4.14). ALA was responsible for the majority of n-3 PUFA enrichment in cooked breast meat, achieving an 11-fold increase. The maximum concentration of n-3 PUFA was 287mg/100g of meat, which was achieved at a level of 6%en ALA. Interestingly, the minimum level of 300mg/100g of cooked breast meat, required for labelling as a source of n-3 fatty acids, was reached in cooked breast meat from Cobb birds fed a high ALA diet (Table 4.14). This result was consistent with that for raw breast meat. An increase in the n-3 fatty acid content of cooked breast meat resulted in a concomitant reduction in the concentration of cooked breast meat AA, although no change was observed in total n-6 PUFA.

Cooked breast meat PUFA was significantly increased by inclusion of dietary ALA. This increase in cooked breast meat PUFA reflected a significant decrease in MUFA, particularly for oleic acid. Importantly, no change was observed in the SFA content.

Table 4.14 Fatty acid profiles of cooked breast meat TL from broiler chickens fed with ALA enriched diets (mg/100g of meat)[1]

ALA level, %en	0.3		3		6			P value[2]		
Strains	**Cobb**	**Ross**	**Cobb**	**Ross**	**Cobb**	**Ross**	**SEM**	**D**	**S**	**D x S**
Fatty acids (mg/100g)[3]										
Total SFA	555.0	357.0	438.0	482.0	424.0	353.0	60.50	NS	NS	NS
Total MUFA	1197.0	705.0	667.0	726.0	431.0	356.0	127.10	***	NS	NS
18:2n-6 (LA)	236.0	131.0	273.0	282.0	259.0	197.0	44.50	NS	NS	NS
20:4n-6 (AA)	40.5	38.3	35.0	38.1	20.5	24.6	1.86	***	NS	NS
Total n-6	304.0	193.0	325.0	340.0	294.0	235.0	46.10	NS	NS	NS
18:3n-3 (ALA)	26.0	8.0	133.0	140.0	220.0	152.0	44.50	**	NS	NS
20:5n-3 (EPA)	6.5^{d}	4.2^{d}	15.9^{c}	17.7^{bc}	26.4^{a}	21.5^{ab}	1.14	***	NS	*
22:5n-3 (DPA)	15.3	11.2	43.7	50.5	44.9	50.7	2.39	***	NS	NS
22:6n-3 (DHA)	11.5	9.5	24.6	21.8	19.4	22.6	1.90	***	NS	NS
EPA + DHA	18.0	13.7	40.4	39.5	45.8	44.1	2.32	***	NS	NS
Total n-3 LCPUFA	33.4	24.9	84.1	90.0	90.7	94.8	4.13	***	NS	NS
Total n-3	63.0	34.0	223.0	236.0	320.0	254.0	46.70	***	NS	NS
Total PUFA	367.0	227.0	549.0	577.0	614.0	489.0	91.30	*	NS	NS
n-6:n-3 ratio	5.8	5.7	1.5	1.4	1.0	0.9	0.31	***	NS	NS

[1]Values are means of six observations per treatment and their pooled standard error of the mean (SEM). Values in the same row with no common superscript are significantly different ($P < 0.05$).
[2]*$P < 0.05$; **$P < 0.01$; ***$P < 0.001$; NS, not significant; D, diet; S, strain.
[3]SFA, saturated fatty acid; MUFA, monounsaturated fatty acid; LA, linoleic acid; AA, arachidonic acid, ALA, alpha-linolenic acid; EPA, eicosapentaenoic acid; DPA, docosahexaenoic acid; PUFA, polyunsaturated fatty acid.

4.5.5 Sensory properties of n-3 enriched chicken meat

The mean panel intensity scores for each attribute of sensory properties assessed on a 15-cm point scale are presented in Table 4.15. There were some differences in the sensory attributes of chicken breast meat (aroma, aftertaste and off-flavour) but the differences were only detected on breast meat from chickens fed the highest ALA diets. Panelists did not detect differences in sensory attributes including texture, taste, and chicken flavour of the chicken breast from birds fed dietary treatments. The average scores of the attributes of breast meat from birds fed dietary treatments are presented in Figure 4.6 and 4.7. Aftertaste was the only attribute on which the strain of birds had an effect. It appears that breast meat from Ross birds was generally considered to have a higher perception of aftertaste (average of intensity score of 7.9 on a scale from 0 to 15) compared to Cobb breast meat (average score of 7.6; $P < 0.05$). A significant diet by strain interaction ($P < 0.05$) was only observed for metallic taste. Inclusion of dietary ALA slightly reduced the metallic taste of breast meat from Cobb birds; however, no difference in this attribute was noted between breast meat from Cobb birds fed 3 and 6%en ALA diets (Table 4.15). The average score of the metallic taste of breast meat from Cobb birds fed diets containing 0.3, 3, and 6%en was 4.6, 3.5, and 3.7, respectively. Even though there were differences, all scores were quite low on the scale. There was an interaction between assessor and treatment on some attributes tested, which indicates that the panel may have needed more training.

Importantly, panellists did not detect differences in the sensory attributes evaluated between commercial breast meat and the chicken breast from birds fed ALA

enriched diets, with the exception of aroma. However, the discrepancy in aroma perceived was only detected when chickens were fed diets high in ALA, in which high ALA diets appeared to result in chicken meat with a lower perception of aroma compared to commercial breast meat (Table 4.16).

Table 4.15 Sensory evaluation by trained panellists of breast meat of two strains of broilers fed high alpha-linolenic acid diet

ALA level, %en	0.3	3	6		0.3 (low)		3 (moderate)		6 (high)			P value[2]				
Strains				**SEM**	**Cobb**	**Ross**	**Cobb**	**Ross**	**Cobb**	**Ross**	**SEM**	**Diet**	**Strain**	**D x S**	**Assessor**	**A x T**
Sensory attributes[1]																
Aroma																
Aroma	8.01^{a}	7.99^{a}	7.21^{b}	0.194	7.77	8.25	7.58	8.40	7.35	7.07	0.275	**	NS	NS	***	**
Chicken aroma intensity	8.27^{a}	8.64^{a}	7.58^{b}	0.240	8.24	8.30	8.12	9.17	7.66	7.50	0.339	*	NS	NS	***	***
Textures																
Tenderness	9.37	8.87	8.49	0.596	8.85	9.89	9.33	8.42	8.11	8.86	0.843	NS	NS	NS	***	NS
Chewiness	8.66	8.69	8.75	0.462	8.93	8.38	8.55	8.83	8.73	8.77	0.653	NS	NS	NS	***	NS
Fibrousness	5.70	5.88	6.63	0.471	6.21	5.19	5.66	6.09	6.62	6.64	0.666	NS	NS	NS	***	NS
Juiciness	6.00	6.09	6.45	0.532	6.42	5.57	6.40	5.78	6.78	6.12	0.752	NS	NS	NS	***	NS
Taste																
Savoury taste	7.42	7.61	7.08	0.187	7.23	7.62	7.74	7.48	7.03	7.13	0.264	NS	NS	NS	***	NS
Metallic taste	4.22	4.06	3.80	0.202	4.60^{a}	3.84^{ab}	3.54^{b}	4.59^{a}	3.72^{b}	3.88^{ab}	0.286	NS	NS	*	***	*
Aftertaste	7.87^{ab}	8.15^{a}	7.44^{b}	0.184	7.43	8.32	7.92	8.37	7.14	7.74	0.261	*	**	NS	***	*
Flavour																
Chicken flavour	8.42	8.41	8.11	0.182	8.32	8.51	8.37	8.45	8.19	8.03	0.257	NS	NS	NS	***	*
Corn flavour	4.85^{a}	4.64^{a}	4.05^{b}	0.191	4.68	5.02	4.54	4.74	4.12	3.98	0.270	*	NS	NS	***	NS
Broth flavour	5.95	6.35	5.97	0.208	6.20	5.70	6.30	6.40	6.03	5.90	0.294	NS	NS	NS	***	*
Mushroom flavour	4.55	4.62	4.50	0.172	4.45	4.65	4.69	4.54	4.39	4.61	0.244	NS	NS	NS	***	NS
Off-flavour	2.27^{b}	2.51^{b}	3.37^{a}	0.244	2.23	2.30	2.65	2.36	3.77	2.97	0.345	**	NS	NS	***	NS

[1]Values are means of six replications per treatment and their pooled standard error of means (SEM) evaluated by 12 panellists. Values in the same row with no common superscript are significantly different ($P < 0.05$).

[2]*$P < 0.05$; **$P < 0.01$; ***$P < 0.001$; NS, not significant; D, diet; S, strain; A, assessor; and T, treatment.

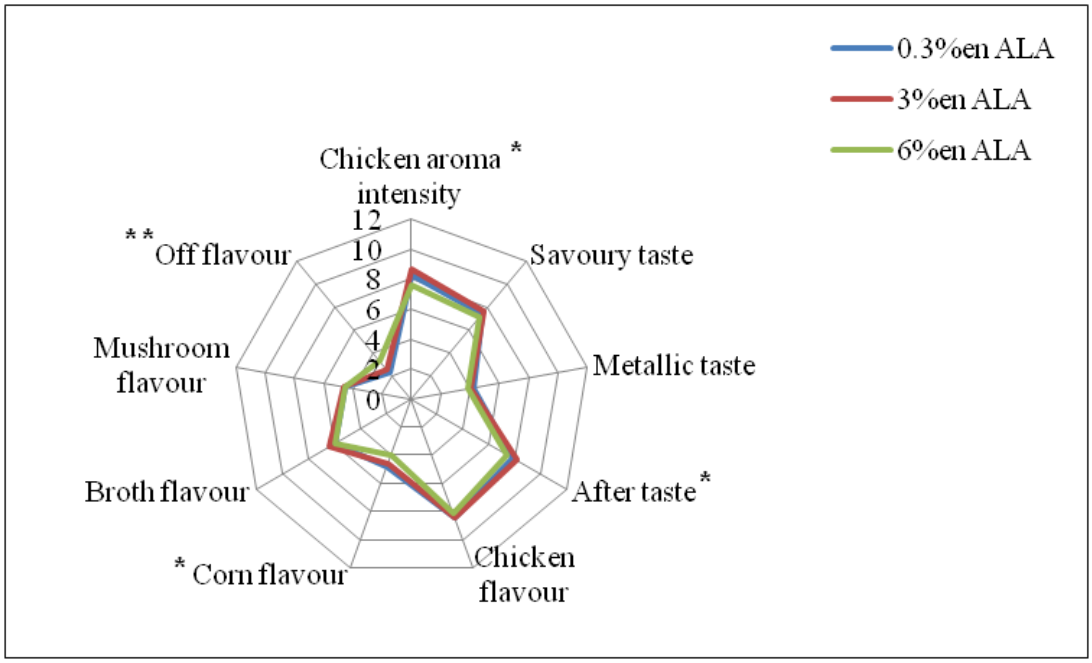


Figure 4.6 Mean levels of aroma, taste and flavour in breast meat from birds fed diets containing 0.3, 3, and 6%energy ALA as determined by trained panellists (n = 12) using a 15-cm line scale (from low to high intensity); n = 6 replications. *P < 0.05; **P < 0.01. There were no differences in the attributes tested between low and moderate ALA diets. Diets high in ALA appeared to result in a lower perception of aroma, aftertaste and corn flavour compared to diets containing low and moderate ALA.

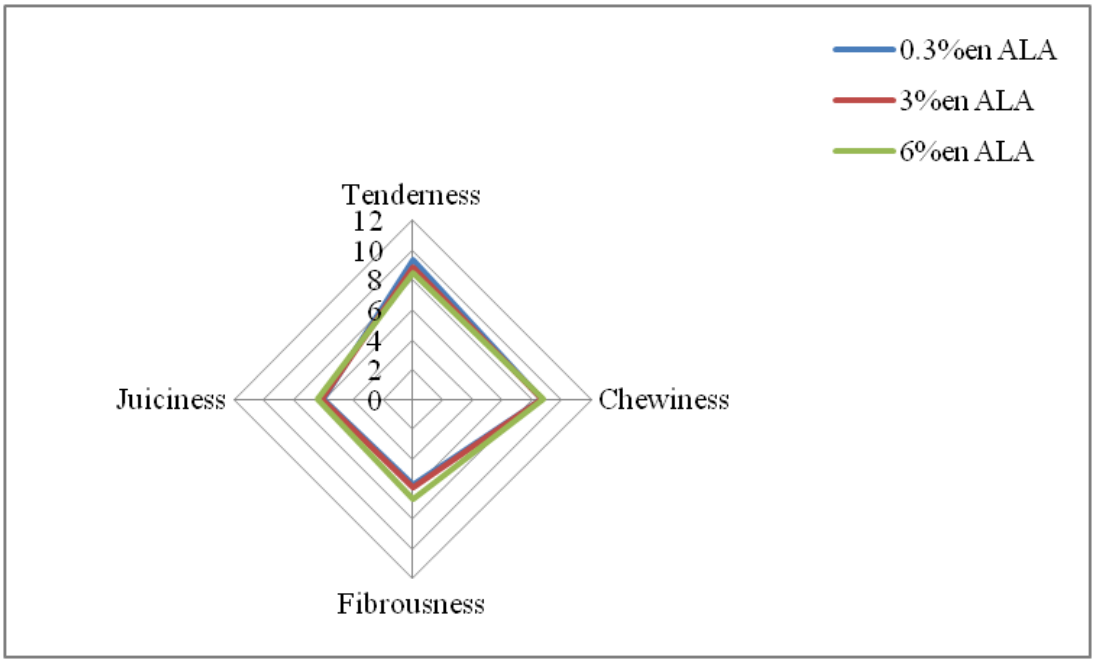


Figure 4.7 Mean levels of textures in breast meat from birds fed diets containing 0.3, 3, and 6%energy ALA as determined by trained panellists (n = 12) using a 15-cm line scale (from low to high intensity); n = 6 replications. Increasing levels of dietary ALA did not affect texture of breast meat.

Table 4.16 Sensory evaluation by trained panellists of commercial breast meat and significance of comparisons to the breast meat of two strains of broilers fed high alpha-linolenic acid diet

		significance of comparisons[2]						
Sensory attributes[1]	**Commercial birds**	**Low-Cb x C**	**Low-Rs x C**	**Moderate-Cb x C**	**Moderate-Rs x C**	**High-Cb x C**	**High-Rs x C**	**SEM**
Aroma								
Aroma[2]	8.49	NS	NS	NS	NS	*	*	0.30
Chicken aroma intensity	8.58	NS	NS	NS	NS	NS	NS	0.35
Textures								
Tenderness	8.44	NS	NS	NS	NS	NS	NS	0.82
Chewiness	9.79	NS	NS	NS	NS	NS	NS	0.63
Fibrousness	6.95	NS	NS	NS	NS	NS	NS	0.68
Juiciness	5.67	NS	NS	NS	NS	NS	NS	0.76
Taste								
Savoury taste	7.50	NS	NS	NS	NS	NS	NS	0.28
Metallic taste	3.98	NS	NS	NS	NS	NS	NS	0.30
Aftertaste	8.04	NS	NS	NS	NS	NS	NS	0.27
Flavour								
Chicken flavour	8.89	NS	NS	NS	NS	NS	NS	0.28
Corn flavour	4.96	NS	NS	NS	NS	NS	NS	0.29
Broth flavour	6.84	NS	NS	NS	NS	NS	NS	0.31
Mushroom flavour	4.65	NS	NS	NS	NS	NS	NS	0.27
Off-flavour	2.73	NS	NS	NS	NS	NS	NS	0.36

[1]Values are means of six replications per treatment and their pooled standard error of means (SEM) evaluated by 12 panellists. Values in the same row with no common superscript are significantly different ($P < 0.05$).

[2]*$P < 0.05$; NS, not significant; C, commercial birds; Low-Cb, Cobb birds fed low ALA diet; Low-Rs, Ross birds fed low ALA diet, Moderate-Cb, Cobb birds fed moderate ALA diet, Moderate-Rs, Ross birds fed moderate ALA diet, High-Cb, Cobb birds fed high ALA diet, High-Rs, Ross birds fed high ALA diet.

4.5.6 Production performance of broiler chickens

Significant differences as a result of ALA content of diet were almost entirely absent from the production parameters throughout the experimental period (Table 4.17). Feed intake and FCR were not significantly influenced by dietary treatment ($P > 0.05$). Although it appeared that Cobb birds consumed more feed than Ross birds, this fact did not result in differences in FCR between strains of birds. There was a slight decrease in body weight gain (BWG) of birds with increased levels of dietary ALA ($P < 0.05$), with high ALA diets resulting in the lowest BWG. However, no differences were recorded in the final weight of the birds. The average BWG of birds fed ALA content 0.3, 3 and 6%en was 2728, 2708, and 2593g, respectively. There was an effect of strain for BWG and final weight, with Cobb 500 birds significantly heavier than Ross 308 birds ($P < 0.05$) at day 40. In addition, a diet by strain interaction was observed that was approaching significance ($P = 0.06$), in which the BWG of Ross 308 birds appeared to reduce as ALA levels in the diet increased.

Table 4.17 Production parameters of broiler chickens fed with ALA enriched diets

ALA level, %en	**0.3**	**3**	**6**		**0.3**		**3**		**6**			**P value[2]**		
Strains				**SEM**	**Cobb**	**Ross**	**Cobb**	**Ross**	**Cobb**	**Ross**	**SEM**	**D**	**S**	**D x S**
Production parameters[1]														
BWG, g/bird	2728[a]	2708[a]	2593[b]	37.00	2782	2674	2890	2526	2750	2436	52.00	*	***	NS
Feed intake, g/bird	4380	4447	4246	67.40	4371	4389	4836	4058	4506	3986	95.00	NS	***	**
FCR, g feed:g gain	1.61	1.64	1.64	0.02	1.59	1.64	1.68	1.61	1.68	1.67	0.03	NS	NS	NS
Final weight, g per bird	2966	2958	2955	45.30	3063	2869	3052	2865	3146	2764	64.00	NS	***	NS

[1]Values are means of four replications per treatment and their pooled standard error of means (SEM).
[2]*P < 0.05; **P < 0.01; *** P <0.001; NS, not significant.

4.6 Discussion

4.6.1 Fatty acid composition of chicken tissues

This study was conducted to examine whether increasing the ALA content in the diet of chickens while holding the LA level constant could result in the accumulation of n-3 LCPUFA in chicken tissues without affecting meat sensory quality. This was tested on two different strains of chickens. The findings of this study clearly demonstrate successful incorporation of n-3 PUFA and n-3 LCPUFA, including EPA, DPA and DHA into the breast meat of broiler chickens as a result of feeding ALA enriched diets. We observed that there was specificity in incorporation ability for each tissue, which resulted in differences in the levels of fatty acids between liver and breast meat. For example, EPA and DHA were found in high amounts (5.9 and 4.7% for EPA and DHA, respectively) in liver phospholipids but appeared in lower amounts in breast phospholipids. In contrast, DPA was present in liver phospholipids in modest amounts but DPA was the main n-3 LCPUFA in breast PL. This may reflect the capacity for DPA to deposit more readily in breast meat, with less being converted to DHA. These findings are consistent with previous studies [20, 21], which reported that DPA was the most abundant n-3 LCPUFA in breast meat. A possible explanation for these findings may be that the conversion of ALA to DPA follows simple zero-order kinetics whereas the synthesis from DPA to DHA is more complex [198], and involves a second use of Δ6-desaturase for the conversion of 24:5n-3 to 24:6n-3 (see Figure 1.3). It is likely that not only LA but also ALA could compete with 24:5n-3 to decrease the production of 24:6n-3, and inhibit DHA production as reported by Gibson et al. [199]. The competition for access to Δ6-desaturase between 24:5n-3 and LA and ALA may explain the complex kinetics between dietary ALA and the level of DHA in tissues [116, 174, 199]. In addition,

the beta-oxidation of 24:6n-3 to DHA in peroxisomes may provide another potential point of regulation.

The findings of this study suggest that DHA content of breast meat TL from birds fed ALA enriched diets doubled, with a maximum level of breast meat TL DHA in both raw and cooked breast meat achieved for diets at a level of 3%en ALA. It is important to note that the fatty acid composition of the low ALA diet was close to that of common commercial feed. Consequently, these chickens produced meat similar to industry standard with regard to n-3 LCPUFA levels. This study further demonstrated that increasing dietary ALA intake beyond 3%en resulted in no further increase in the levels of DHA, suggesting the optimal level of dietary ALA may be in the region of 3%en in this respect. More levels of dietary ALA should be tested in order to determine the optimal level of dietary ALA. The reported inhibition of n-3 LCPUFA synthesis by high levels of both ALA and LA may partly explain the inability of high ALA diets to further increase tissue DHA content [107]. These results are consistent with the concept that chickens convert dietary ALA to n-3 LCPUFA, although the n-3 LCPUFA accumulation in the tissue is relatively low [20, 95, 137, 138]. Similar results have been reported by Gibson et al. [107] who demonstrated that a maximal DHA accumulation was reached in rat plasma phospholipids by feeding diets enriched with ALA. This maximum appears to be animal dependent in terms of dietary ALA level. For example, while in this study, a dietary ALA level of 3%en maximised n-3 fatty acids in chicken meat, in rats, the maximal DHA incorporation was achieved at ~1.0%en ALA [107]. In both studies, the LA to ALA ratio in the diet was low.

The n-3 LCPUFA distribution in breast meat TL followed a similar pattern to previous studies [19, 20, 95, 117, 138]. However, a higher accumulation of n-3 LCPUFA was observed in this study compared to earlier reported levels [21, 138]. For example, while one study observed that when 10% ground whole flaxseed (19mg/g ALA of diet) was added, the total lipid DHA in Ross breast meat was 8mg/100g of meat [138], this study demonstrated an accumulation 13mg/100g of meat in Ross breast meat fed 3%en ALA (18.1% of total fatty acids; 15mg/g ALA of diet). This previous study [138] reported only a small amount of DHA accumulation in breast PL whereas the current study found that DHA content of breast meat PL from chickens fed moderate or high ALA diets more than doubled. This is puzzling because the ALA levels of the experimental diets in this study were similar to those reported by Betti et al. [138]. This discrepancy may be due to different the level of LA in the basal diet, the different forms of dietary flaxseed sources used (ground flaxseed versus flaxseed oils) and/or inhibitors in seeds that are not present in oil. In this study, experimental diets were prepared from a basal diet with a low LA level, while holding the level of LA constant in the ALA enriched diets. In relation to the forms of ALA sources, flaxseed contains substances such as linatine dipeptide, mucilage and phytic acid, which are anti-nutritional factors, and cyanogenic glycosides, which are potentially toxic [92, 200]. Moreover, as reviewed by Gonzalez-Esquerra and Leeson [92], phytic acid is known to decrease the availability of minerals such as calcium, magnesium, zinc and iron, affect proteins, and inhibit proteolitic enzymetic reactions. These substances could impair growth in chickens. In addition, the sex of birds might influence the differences in the PUFA metabolism, although Pourelesmi et al. [201] pointed out that sex had a minor effect on the fatty

acid composition of whole body broiler chickens. In this study, male Ross chickens were used while a previous study conducted by Betti et al. [138] used mixed sex Ross chickens. It seems unlikely that the age of birds could cause the differences in the accumulation of DHA between those achieved in the previous study and this study, since their study used younger birds (35 days of age) and Kartikasari et al. [117] noted that breast meat at 28 days had a higher level of n-3 LCPUFA than at 40 days.

The n-3 LCPUFA levels achieved in the breast meat in this study did not reach levels achieved by Kartikasari et al. [117] or a study conducted by Rymer and Givens [129]. The higher levels achieved by Rymer and Givens [129] may have been due to the higher level of n-3 LCPUFA in the basal diet as a result of fishmeal inclusion; Kartikasari et al. [117] applied lower ratios of LA to ALA and used younger birds. Studies conducted by McMurchie [202] and Kartikasari et al. [115] demonstrated that high LA diets decrease the incorporation of n-3 LCPUFA in tissues.

The levels of TL DHA in breast meat of male Ross chickens fed diets containing 3%en ALA reached levels of DHA ~50% of chickens fed diets supplemented with 2% fish oil over a 21- or 32-d growth period [17, 203]. The TL fraction n-3 LCPUFAs of breast meat from chickens fed moderate ALA diets achieved in this study (45mg/100g of meat) was ~17% of the TL fractions found in two popular Australian white fish, Northen Whiting (Sillago sihama; 287mg/100g) and Barramundi (Lates calcarifer; 271mg/100g) fillet. Enrichment of chicken meat with n-3 LCPUFA using vegetable oils rich in ALA could be a new source for people who

do not habitually consume fish and are already eating chicken. Moreover, the dietary vegetable oils approach applied in this study could partially substitute the use of fish oil in broiler chickens to produce substantial levels of n-3 LCPUFA to consumers.

While ALA mainly deposited in the TG fraction, the LCPUFA (AA, n-3 LCPUFA) were preferentially accumulated in the PL fraction. There was only small deposition of ALA in PL as previously reported by other studies [92, 138]. In breast tissues, TG n-3 LCPUFA represented 4 to 5% of the total TG n-3 PUFA content for both moderate and high ALA diets. This amount was lower than those achieved in the PL fraction of breast meat, in which n-3 LCPUFA accounted for 81 to 87% of total n-3 PUFA content. A higher accumulation of n-3 LCPUFA in the PL fraction is important because PL remains stable during cooking processing while TG tends to be lost from the meat.

There was a consistent result in the AA content of liver and breast meat. While all n-3 LCPUFA were increased by ALA enrichment in the diet, bioconversion of LA to AA decreased. LA and ALA are converted to LCPUFA, mostly in the hepatocytes, and to a smaller amount in intestinal cells [179]. AA was the most important biosynthetic product of LA, while n-3 LCPUFA resulted from ALA bioconversion. Thus, the deposition of AA in PL membranes typically decreases when n-3 LCPUFA increases [117, 119, 138]. The decrease in the AA content of chicken meat was significant when chickens were fed diet containing n-3 fatty acids [204]. The AA reduction in these tissues is consistent with the theory that LA and ALA compete for the desaturase and elongase enzymes in the bioconversion pathway.

Interestingly, the findings showed that there was apparently a genotype effect on n-3 LCPUFA accumulation, with Cobb 500 birds being more efficient in the accumulation of n-3 fatty acids and in the conversion of ALA to n-3 LCPUFA than Ross 308 for EPA. Consequently, the minimum level of 300mg/100g of both raw and cooked breast meat required for labelling as a source of n-3 fatty acid was reached for breast meat only for Cobb birds fed a high ALA diet [138, 180]. However, no differences were observed for the longer chain n-3 fatty acids (DPA and DHA). This finding is partially consistent with Rymer and Givens [129] who reported no significant difference in n-3 LCPUFA accumulation between the two genotypes; although it appeared that Cobb 500 tended to accumulate DHA more efficiently than Ross 308 ($P = 0.053$). Although Ross birds fed high ALA diets resulted in a higher accumulation of n-3 LCPUFA (EPA, DPA, DHA) in breast meat TL when the data was expressed as % of total FA, there was no significant effect on DPA and DHA accumulation when the data was expressed as mg/100g of meat. The higher fat content in Cobb birds compared to Ross may account for this.

Dietary n-3 LCPUFA has been shown to have beneficial health effects such as ameliorating the risks of coronary heart disease [205], improving mental development [49] and potentially having functional benefit for pregnancy and lactating mothers [206]. Therefore, people are encouraged to increase the consumption of n-3 LCPUFA (EPA and DHA). Results from this study clearly show that n-3 PUFA enriched chicken meat produced by including ALA from vegetable oils can help consumers to increase their n-3 PUFA consumption and thus have beneficial health effects. As expected, diets enriched with ALA decreased the n-6 to

n-3 ratio to ~1:1, in particular decreasing the content of breast meat AA, which is reported to have a negative effect on human health. A ratio of n-6 to n-3 of ~1 in a diet is suggested for humans, whereas in Western diets the ratio is ~16:1 [3, 207]. As reported by Simopoulos [3], an n-6 to n-3 ratio 4:1 was associated with a decrease in mortality. In addition, ALA enriched diets did not influence the SFA content of either raw or cooked breast meat TL. This is important due to health concerns of high SFA in chicken products for coronary heart diseases (CHD).

4.6.2 Sensory properties of n-3 enriched chicken meat

As reported in studies in which dietary supplements from marine sources were used to produce n-3 enriched eggs, the use of these sources, such as fish oil, in the manipulation of tissue fatty acid composition increased the n-3 LCPUFA content of chicken meat [9, 17, 18]. However, diets containing fish oil may result in negative effects on the sensory quality of the meat [17, 32, 208].

Alternative sources to fish meal or fish oil are vegetable sources rich in n-3 PUFA such flaxseed or flaxseed oil. For example, Lopez-Ferrer et al. [95] reported that replacing fish oil with a vegetable source rich in ALA resulted in a significant improvement in the sensory quality of chicken meat. In contrast, addition of fish oil to diets enriched with flaxseed impaired the sensory quality of chicken breast meat [32].

Importantly, in this study, diets enriched with ALA sources in the form of vegetable oil did not affect the sensory quality of the breast meat. This is consistent with the

results of Gonzalez-Esquerra and Leeson [32] who reported no significant difference in terms of aroma, taste, and flavour between breast meat from chickens fed 100g/kg flaxseed and those fed a control diet. The only minor consequences on the sensory properties were apparent when chickens were fed high ALA diets (6%en ALA). A possible explanation for these results might be related to the level of ALA achieved in the meat [209, 210], thermal lipid degradation products [57, 211], and concentration of volatile compounds [132, 210]. A study in pigs showed that meat ALA levels of approximately 5% of total fatty acids can produce volatile compounds which seem to adversely impact on meat flavour [212]. In lamb, the ALA levels were positively correlated with odour and flavour intensity scores [210]. In addition, a review paper by Wood et al. [210] reported that beef meat with high ALA levels produced higher concentrations of lipid degradation products (aldehydes, alcohols and ketones). These odour-active volatile compounds, particularly aldehydes such as pentanal and hexanal, may cause relatively undesirable odours and flavours in the meat [57, 210], which are associated with flaxseed or fishy odour [209]. Pentanal and hexanal are the main compounds identified in the head space of flaxseed oil [57]. In contrast, ALA decreased the levels of meaty aroma, such as thiophenes and furans by interacting with the Maillard reaction products [213]. These findings suggest that some compounds in the feed (flaxseed oil) may influence aroma and flavour of the chicken meat, and the oxidation products of meat ALA and its derived volatile compounds are directly responsible for the differences in off-flavour observed from chickens fed high ALA diets.

There was no strain effect on the perception of attributes tested between Cobb and Ross birds, with the exception of aftertaste, in which Ross birds had a slightly higher perception of aftertaste than Cobb birds. This difference in aftertaste is likely to be a reflection of the fatty acid composition of the different strains. As reported by Gonzalez-Esquerra and Leeson [32], differences in n-3 composition and concentration can cause differences in the sensory quality of the edible carcase.

Although there is a significant strain-by-diet interaction effect for metallic taste, the intensity perceived was low, less than 5cm (SE = 0.29cm) on the intensity scale. There was a significant interaction between assessor and treatment indicating that the panellists were not in the agreement when rating the score. Thus this effect may be spurious and should be treated with concern, especially since only one assessor gave high scores (> 7.5 cm) for metallic taste.

In this study, panellists also analysed commercial breast meat in comparison to n-3 enriched breast meat. Importantly, trained panellists did not detect any differences in the sensory quality between breast meat purchased from a local supermarket and those from Cobb and Ross birds fed diets enriched with ALA. Aroma was the only attribute on which the ALA content of the diets had an effect, with high ALA diets causing a decrease in the aroma of breast meat.

4.6.3 Production performance of broiler chickens

Importantly, under our experimental conditions, dietary ALA enrichment did not appear to have major consequences on the performance parameters of chickens.

These findings are in agreement with previous studies [20, 95, 146], which reported that, in general, including ALA sources from vegetable oils did not cause adverse effects on production performance of broilers. For example, in this study there was no significant diet effect on feed intake, FCR, and final weight by feeding ALA enriched diets from vegetable oils, as observed by Lopez-Ferrer et al. [20] including flaxseed oil in their diets. Studies conducted by Lopez-Ferrer et al. [20] and Zelenka et al. [145] actually found that increased levels of ALA sources in the form of vegetable oils up to a level of ~ 5% resulted in an increase in BWG and final weight of birds without effecting FCR.

In contrast, a decrease in performance has been reported in broilers fed diets containing flaxseed as whole or ground flaxseed [22, 156, 214, 215]. For example, Azad et al. [214] reported a 11% reduction in body weight at the end of the experiment when birds were fed diets enriched with 10% ground canola + 10% ground flaxseed, and because feed intake was similar to control birds, a significantly higher FCR was reported. Furthermore, Lee et al. [215] reported that when broilers were fed diets enriched with 10 or 20% flaxseed for six weeks, there was a dose-related reduction in BW and increased FCR. This reduction in performance may be due to the depressed energy utilisation as a result of the lower fat extraction [215], and presence of anti-nutritional factors [200], which could interfere with the use of nutrients. However, future research in this area will include a large-scale commercial validation to evaluate any impact of diet formulation on broiler performance production.

4.7 Conclusion

The research data in this chapter confirm the fact that increased levels of ALA in the diet of chickens against a background of a low LA level in basal diet could be a means to increase the content of meat n-3 LCPUFA (EPA, DPA and DHA). Further, the level of DHA and total n-3 LCPUFA was maximal at a dietary ALA level of approximately 3%en; beyond this level, no further increase in DHA accumulation was achieved. Importantly, diets containing 3%en ALA did not influence production performance. Moreover, the sensory quality of breast meat from broilers fed 3%en ALA diets was comparable to that of commercial breast meat.

There was an effect of strain on the ability of the broiler chickens to convert ALA into n-3 LCPUFA. Cobb birds were found to be more effective in accumulating ALA and in converting ALA to n-3 LCPUFA, and consequently, these birds accumulated more EPA and total n-3 PUFA than Ross birds. In addition, total n-3 PUFA of breast meat from Cobb birds fed the high (6%en) ALA-enriched diet achieved 300mg/100g of meat, the minimum content required for labelling the meat as a source of n-3 PUFA [180]; however, this was due mainly to the accumulation of unmetabolised ALA into the meat.

Based on this information, a dietary ALA level of around 3%en could be recommended as the ALA level for producing meat rich in n-3 LCPUFA by the chicken industry. The findings of this study demonstrate that incorporating n-3 rich vegetable oils into chicken diets could be an alternative to supplements from marine sources. As broiler chickens fed ALA enriched diets produced meat higher in n-3

LCPUFA without affecting sensory quality of the meat, this would help to provide consumers with a variety of foods rich in n-3 LCPUFA and help to achieve recommended intakes for human health.

CHAPTER 5

GENERAL DISCUSSION

The work described in this thesis highlights the efficacy of diets enriched with ALA from vegetable oils as a means of increasing the n-3 LCPUFA levels of eggs and chicken meat. Since the diets did not contain n-3 LCPUFA, it clearly indicates that chickens are capable to converting some dietary ALA to n-3 LCPUFA, producing chicken products that are higher in n-3 LCPUFA. This finding indicates that there is the potential for chicken meat and eggs to provide an alternative source of n-3 LCPUFA, particularly EPA and DHA. The n-3 LCPUFA content of meat and eggs from chickens fed diets enriched with 3%en ALA were close to those seen in chickens fed fish oil. The total EPA and DHA levels of n-3 enriched chicken meat and eggs per serving (mg/100g of meat or mg/yolk) represent approximately 8% and 18% of the recommended daily intake, respectively. Although the n-3 LCPUFA content of chicken products is not as high as the n-3 LCPUFA levels in fish or other marine products, these chicken products can potentially be an alternative n-3 LCPUFA source. Increasing the level of n-3 LCPUFA in chicken products may remain a preferred option to increase n-3 intake without altering existing dietary patterns, in particular for people who consume very little fish. However, this observation is limited to some degree by the strain of bird. For example, brown laying hens appear to be more effective in accumulating n-3 fatty acids compared to white laying hens. Similarly, Cobb birds appear to accumulate higher levels of n-3 fatty acids than Ross birds. These data have relevance to the Australian chicken industry.

It is interesting to note that there may be an optimal level of dietary ALA for the maximum n-3 LCPUFA accumulation into eggs and chicken meat, but it is not yet clear what this optimal level is. Further studies are required to determine whether a level of 3%en ALA gives maximum benefit or if the lower levels of ALA (1 or 2%en) can suffice. If the maximum benefit is achieved by the lower levels of dietary ALA, this would certainly lower the cost for producing n-3 enriched chicken products. A study by Gibson et al. [107] showed that the maximum DHA accumulation in rat plasma phospholipid was observed at approximately 1%en ALA when the ratio of LA to ALA in the diets was low.

Restricting the LA content of the diet was a major focus of the research described in this thesis. An effort to reduce the LA level was made by avoiding the addition of high LA vegetable oils and ingredients high in LA content in the basal diet. Earlier research by Kartikasari et al. [117] demonstrated that dietary treatments with a low LA level promoted higher n-3 LCPUFA accumulation in chicken meat. On the other hand, increasing the levels of dietary LA decreased the accumulation of n-3 fatty acids [115]. Despite efforts to control the LA levels in the diets, it was not possible to reduce the LA content to very low levels as all grains contain LA-rich lipid, and we aimed to use grain based diets that remained as close as possible to industry standards in Australia.

When producing n-3 fatty acid enriched chicken products under commercial conditions, potential increases in formulation cost are also important to consider. The use of vegetable oils as ALA sources will increase feed costs. For example, for diets

supplemented with 6% fat, the cost of producing a diet with 3%en ALA would be approximately AUD$ 50/tonne higher compared to standard chicken feed including tallow as fat sources (Appendix 3). However, considering the benefits of the n-3-enriched chicken products for human health, consumers may be willing to buy these products.

An important aspect of this study has been an evaluation of the sensory characteristics of the final products. Consumers expect chicken meat and eggs to have a familiar taste and not taste of vegetable oils or fish. While chicken diets enriched with as little as 3% fish oil were reported to have negative impacts on the sensory characteristics of the egg or meat, the research described in this thesis has demonstrated that inclusion of vegetable oils in the form of ALA had no major consequences on the sensory properties of the chicken products. However, it is clear that increasing ALA levels to 6%en not only achieves little in terms of increased n-3 LCPUFA content, but also runs the risk on increasing undesirable aroma characteristics.

The observation that ALA enriched diets were capable of increasing n-3 LCPUFA content in chicken products was not novel; however, our work is distinctive in that the levels of n-3 LCPUFA reached were higher than those achieved by other studies using a similar level of ALA. The reasons for the high levels of n-3 LCPUFA achieved are not entirely clear but might be due to the different form of ALA sources used (vegetable oils vs. whole or ground flaxseed) in our study compared to previous reports. Alternatively, it is obvious that LA can interfere with the conversion of ALA

to n-3 LCPUFA, and some of the poor conversions reported in other studies may be due to high levels of LA in the diet. Currently the only oils that are rich in ALA are also rich in LA. A single source vegetable oil that is very low in LA but contains high levels of ALA could be predicted to be very useful for the chicken industry. Furthermore, it is clear that the strain of bird is a major factor, and subsequently, direct comparison of chicken strains on a variety of diets is needed.

Currently the marine stocks that provide the main source of n-3 LCPUFA in the human diet are threatened by overfishing. Thus the use of vegetable oils rich in ALA such as flaxseed and canola oil in the diet of chickens for producing n-3 enriched eggs and meat is important. It is clear that this feeding strategy can be a sustainable alternative source of n-3 fatty acids.

5.1 Study limitations

While the increase in ALA content of diets successfully enhanced n-3 LCPUFA in eggs and chicken meat, there are limitations in this study as follows:

- Broiler chickens used in this experiment were limited to one gender. Thus it was not possible to determine whether responses differed according to gender of the chickens. Previous studies reported that sex had effect on fatty acid profiles in broiler chickens [201, 216].
- The number of chickens was not large enough to comprehensively evaluate the effects of diets on the production or health status of birds.
- Only three levels of dietary ALA were investigated. More levels should be tested in order to determine the optimal level of dietary ALA.

- The panel may have needed more training because there was an interaction between assessor and treatment on some attributes tested.
- A single diet was used throughout the feeding period, which reflects a difference from commercial production where typically a phase-feeding regimen would be used.

5.2 Future directions

These studies have demonstrated that the n-3 LCPUFA content of chicken products can be enhanced by elevating ALA levels in the diet of chickens but many questions remain:

- There is a need to evaluate whether lower levels of dietary ALA ranging from 0.3 to approximately 3%en ALA can also increase the levels of n-3 LCPUFA in chicken products.
- There is a need to determine whether female broiler chickens respond differently to males.
- There is a need to search for strategies regarding the time of feeding ALA-enriched diets to optimise the accumulation of n-3 LCPUFA in chicken products and potentially minimise the period of time where birds need to consume the more expensive ALA rich diets due to cost impact.
- The effectiveness of ALA rich diets on n-3 LCPUFA status needs to be evaluated in other poultry products such as ducks and turkey.
- Chicken diets enriched with ALA may also be potentially valuable to the health of birds so there is a need to examine the health status of birds.

- There is a need to evaluate the production cost of n-3 enriched chicken products from birds fed diets supplemented with vegetable oils.

5.3 Conclusions

This thesis has confirmed that the n-3 LCPUFA content of chicken products can be increased by increasing dietary ALA levels from vegetable oils in chicken diets, although this is influenced to some degree by the strain of bird. As dietary ALA enrichment successfully increased n-3 fatty acids in chicken products without affecting the sensory quality of the final products, this strategy could be an alternative to marine sources, and could help to provide a diversity of n-3 LCPUFA-rich foods that may have beneficial health outcomes for humans. Moreover, chicken meat is the most consumed meat in the world so n-3-enriched chicken products may help consumers to increase the consumption of these health promoting nutrients, and help to achieve the recommended intake of n-3 LCPUFA.

APPENDICES

Appendix 1 Fatty acid profiles of liver tissue TL from broiler chickens fed with ALA enriched diets (% of total fatty acids)[1]

ALA level, %en	0.3		3		6			P value[2]		
Strains	Cobb	Ross	Cobb	Ross	Cobb	Ross	SEM	D	S	D x S
Fatty acids (%)[3]										
Liver fat content (% wet weight)	4.6	7.6	4.8	5.0	7.0	5.8	0.66	*	NS	**
16:0	20.6	21.3	20.6	22.4	23.6	19.8	1.14	NS	NS	NS
18:0	19.4	16.8	19.9	18.4	19.1	20.7	1.21	NS	NS	NS
Total SFA	41.2	39.3	41.7	42.0	43.8	41.7	0.55	**	*	NS
16:1n-7	3.7	4.1	1.4	1.9	2.6	1.4	0.43	***	NS	NS
18:1n-9	27.2	30.6	23.3	25.8	23.3	20.4	2.52	*	NS	NS
18:1n-7	3.3	3.8	1.8	1.8	1.5	1.5	0.12	***	NS	NS
Total MUFA	35.7	40.2	27.6	30.6	28.2	24.2	3.01	**	NS	NS
Total n-9	29.3	32.9	24.5	27.0	24.1	21.4	2.52	*	NS	NS
Total n-7	7.0	7.9	3.3	3.7	4.2	2.9	0.51	***	NS	NS
18:2n-6 (LA)	12.4	10.9	14.3	13.2	12.4	14.3	1.01	NS	NS	NS
18:3n-6	0.1[ab]	0.0[bc]	0.1a	0.1[ab]	0.0[c]	0.1[abc]	0.01	*	NS	*
20:3n-6	0.9	0.8	0.8	0.7	0.6	0.7	0.08	NS	NS	NS
20:4n-6 (AA)	5.5	4.9	4.8	4.2	2.5	3.6	0.60	**	NS	NS
Total n-6	19.9	17.5	20.6	18.7	15.9	19.2	1.71	NS	NS	NS
18:3n-3 (ALA)	0.3	0.2	2.4	2.3	4.2	4.6	0.19	***	NS	NS
20:3n-3	0.0	0.0	0.3	0.2	0.4	0.5	0.05	***	NS	NS
20:5n-3 (EPA)	0.3	0.3	2.4	1.7	3.2	3.8	0.41	***	NS	NS
22:5n-3 (DPA)	0.3	0.3	1.7	1.4	1.8	2.7	0.29	***	NS	NS
22:6n-3 (DHA)	1.3	0.9	3.0	2.5	2.3	3.1	0.39	***	NS	NS
EPA + DHA	1.6	1.2	5.4	4.3	5.5	6.8	0.75	***	NS	NS
Total n-3 LCPUFA	1.9	1.5	7.1	5.7	7.2	9.5	1.04	***	NS	NS
Total n-3	2.2	1.8	9.7	8.2	11.8	14.6	1.21	***	NS	NS
Total PUFA	22.1	19.3	30.3	26.9	27.7	33.8	2.79	***	NS	NS
n-6:n-3 ratio	9.2	10.4	2.2	2.3	1.4	1.3	0.33	***	NS	NS
LA:ALA ratio	49.4	48.8	6.0	5.8	2.9	3.1	1.27	***	NS	NS

[1]Values are means of twelve observations per treatment and their pooled standard error of the mean (SEM). Values in the same row with no common superscript are significantly different ($P < 0.05$).
[2]*$P < 0.05$; **$P < 0.01$; ***$P < 0.001$; NS, not significant; D, diet; S, strain.
[3]SFA, saturated fatty acid; MUFA, monounsaturated fatty acid; LA, linoleic acid; AA, arachidonic acid, ALA, alpha-linolenic acid; EPA, eicosapentaenoic acid; DPA, docosahexaenoic acid; PUFA, polyunsaturated fatty acid.

Appendix 2 Fatty acid profiles of liver tissue TG from broiler chickens fed with ALA enriched diets (% of total fatty acids)[1]

ALA level, %en	0.3		3		6			P value[2]		
Strains	Cobb	Ross	Cobb	Ross	Cobb	Ross	SEM	D	S	D x S
Fatty acids (%)[3]										
16:0	30.2[ab]	30.1[ab]	28.6[b]	31.6[ab]	32.7[a]	28.7b	1.10	NS	NS	*
18:0	11.6	9.4	15.3	13.4	15.2	15.1	1.32	**	NS	NS
Total SFA	42.8	40.5	44.8	45.9	48.8	44.6	1.01	***	*	NS
16:1n-7	4.9	5.2	2.0	2.5	3.3	2.2	0.45	***	NS	NS
18:1n-9	41.0	44.0	37.6	39.4	32.3	35.4	2.06	**	NS	NS
18:1n-7	3.6	4.0	2.0	1.9	1.6	1.6	0.16	***	NS	NS
Total MUFA	51.2	55.0	42.9	44.8	40.2	40.2	2.00	***	NS	NS
Total n-9	42.6	45.5	38.8	40.3	32.9	36.2	2.05	**	NS	NS
Total n-7	8.5	9.2	4.0	4.4	4.9	3.9	0.53	***	NS	NS
18:2n-6 (LA)	4.3	3.3	7.7	5.8	5.4	7.4	0.77	**	NS	NS
18:3n-6	0.0	0.0	0.0	0.0	0.0	0.0	0.00	NS	NS	NS
20:3n-6	0.2	0.1	0.2	0.1	0.1	0.1	0.03	NS	*	NS
20:4n-6 (AA)	0.7	0.4	0.8	0.2	0.1	0.2	0.14	NS	*	NS
Total n-6	5.4	3.9	8.9	6.3	5.8	7.9	0.93	*	NS	NS
18:3n-3 (ALA)	0.2	0.2	2.0	2.1	3.9	5.2	0.45	***	NS	NS
20:3n-3	0.0	0.0	0.1	0.1	0.2	0.3	0.03	***	NS	NS
20:5n-3 (EPA)	0.0	0.0	0.3	0.1	0.2	0.4	0.06	**	NS	NS
22:5n-3 (DPA)	0.0	0.1	0.5	0.4	0.5	1.1	0.18	**	NS	NS
22:6n-3 (DHA)	0.1	0.0	0.2	0.1	0.1	0.2	0.05	NS	NS	NS
EPA + DHA	0.1	0.1	0.5	0.2	0.4	0.6	0.11	**	NS	NS
Total n-3 LCPUFA	0.2	0.1	1.0	0.6	0.9	1.7	0.28	**	NS	NS
Total n-3	0.3	0.3	3.1	2.8	5.0	7.1	0.75	***	NS	NS
Total PUFA	5.7	4.2	12.1	9.1	10.8	15.0	1.60	***	NS	NS
n-6:n-3 ratio	16.1	13.8	3.0	2.3	1.2	1.2	0.73	***	NS	NS
LA:ALA ratio	25.0	20.4	3.9	2.9	1.4	1.5	1.14	***	NS	NS

[1]Values are means of twelve observations per treatment and their pooled standard error of the mean (SEM). Values in the same row with no common superscript are significantly different ($P < 0.05$).
[2]*$P < 0.05$; **$P < 0.01$; ***$P < 0.001$; NS, not significant; D, diet; S, strain.
[3]SFA, saturated fatty acid; MUFA, monounsaturated fatty acid; LA, linoleic acid; AA, arachidonic acid, ALA, alpha-linolenic acid; EPA, eicosapentaenoic acid; DPA, docosahexaenoic acid; PUFA, polyunsaturated fatty acid.

Appendix 3 An example of calculation of feed cost comparison between a commercial diet and diet enriched with 3%en ALA based on lipid sources used at a level of 6%[1]

Diets	**6% Tallow**	**6% oils (diet 3%en ALA)**	
		Flaxseed oil (40%)	**Canola oil (60%)**
Oil (kg)	**60**	**24**	**36**
Oil price per tonne (AUD $)[2]	800	2400	1200
Oil price per kg (AUD $)	0.8	2.4	1.2
Oil Price (AUD $)	48	57.6	43.2
Total oil price (AUD $)	48	100.8	
Difference of feed cost between a diet containing tallow and 3%en ALA (AUD $)	52.8		

[1]The calculation of feed cost was based on current prices. Much less added fat may be used in broiler feeds.
[2]Source: Four Leaf Oils Pty Ltd, Tarlee, SA (personal communication in November 2012) and Index Mundi [217].

Appendix 4 Demographic characteristics of consumers recruited to egg consumer study

Consumer demographic item	Number of consumers (n = 80)	Proportion (%)
Gender		
Male	38	47.50
Female	42	52.50
Age		
18 - 25	38	47.50
26 - 35	25	31.25
36 - 45	9	11.25
46 - 55	7	8.75
56 - 65	1	1.25
Highest level of education achieved		
High school certificate	17	21.25
TAFE certificate/diploma,/trade	7	8.75
Bachelor's degree	25	31.25
Graduate/post graduate diploma	7	8.75
Master/doctorate degree	24	30.00
Any formal food science/technology/consumer training		
Yes	21	26.25
No	59	73.75
Occupation		
Student	52	65.00
Lecturer	1	1.25
Research/technical officer, administrator	22	27.50
Others	5	6.25
Experience in food industry		
No	64	80.00
0 - 3 years	9	11.25
4 - 6 year	3	3.75
> 6 year	4	5.00
Consumption of boiled chicken eggs		
Yes	77	96.25
No	3	3.75

Continued

Consumer demographic item	Number of consumers (n = 80)	Proportion (%)
How long eating boiled chicken eggs (n = 77)		
0-10	8	10.39
10-20th	24	31.17
20-30th	25	32.47
> 30th	20	25.97
Kind of boiled eggs consumed (n = 77)		
Soft boiled eggs	35	45.45
Hard-boiled eggs	42	54.55
Frequently consuming boiled eggs (last six month)		
Once or a few times per day	3	3.75
A few times per week	13	16.25
Once a week	12	15.00
Once a fortnight	6	7.50
Once every three to four weeks	12	15.00
Once a month	10	12.50
Once every two months	10	12.50
Less often than once every two months	14	17.50
Suffer from egg allergy		
Yes	0	0.00
No	80	100.00
Suffer from any allergy[1]		
Yes	11	13.75
No	69	86.25
Any anosmias (very limited sensitivity to any odour)		
Yes	2	2.50
No	78	97.50
Any medication affecting sense of smell		
Yes	0	0.00
No	80	100.00

Notes:

1. 96.25% of consumers ate boiled eggs (only 3.75% consumed not boiled eggs).
2. 89.61% (~ 90%) of consumers more than 10 years consumed boiled eggs.
3. 70% consumers at least consumed boiled egg once a month.
4. 91.25 smoking, 7.50 no smoking and 1.25 not reported.
5. [1]Any allergy including pollen, bees, cats, strawberry, grasses, peanuts, dust, hay fever.

REFERENCES

1. Gibson RA, Makrides M: **Long-chain polyunsaturated fatty acids in breast milk: are they essential?** *Adv Exp Med Biol* 2001, **501**:375-383.

2. Makrides M, Gibson RA: **The role of fats in the lifecycle stages: pregnancy and the first year of life**. *Med J Aust* 2002, **176 Suppl**:S111-112.

3. Simopoulos AP: **The importance of the ratio of omega-6/omega-3 essential fatty acids**. *Biomed Pharmacother* 2002, **56**(8):365-379.

4. von Schacky C, Harris WS: **Cardiovascular benefits of omega-3 fatty acids**. *Cardiovasc Res* 2007, **73**(2):310-315.

5. ISSFAL: **Recommendations for intake of polyunsaturated fatty acids in healthy adults**. 2004:1-9.

6. Koletzko B, Cetin I, Brenna JT: **Dietary fat intakes for pregnant and lactating women**. *Br J Nutr* 2007, **98**(5):873-877.

7. Kris-Etherton PM, Taylor DS, Yu-Poth S, Huth P, Moriarty K, Fishell V, Hargrove RL, Zhao G, Etherton TD: **Polyunsaturated fatty acids in the food chain in the United States**. *Am J Clin Nutr* 2000, **71**(1 Suppl):179S-188S.

8. Simopoulos AP: **Human requirement for n-3 polyunsaturated fatty acids**. *Poult Sci* 2000, **79**(7):961-970.

9. Lopez-Ferrer S, Baucells MD, Barroeta AC, Grashorn MA: **n-3 enrichment of chicken meat. 1. Use of very long-chain fatty acids in chicken diets and their influence on meat quality: fish oil**. *Poult Sci* 2001, **80**(6):741-752.

10. Mourente G, Bell JG: **Partial replacement of dietary fish oil with blends of vegetable oils (rapeseed, linseed and palm oils) in diets for European sea bass (Dicentrarchus labrax L.) over a long term growth study: effects on muscle and liver fatty acid composition and effectiveness of a fish oil finishing diet**. *Comp Biochem Physiol B Biochem Mol Biol* 2006, **145**(3-4):389-399.

11. James MJ, Ursin VM, Cleland LG: **Metabolism of stearidonic acid in human subjects: comparison with the metabolism of other n-3 fatty acids**. *Am J Clin Nutr* 2003, **77**(5):1140-1145.

12. Givens DI, Gibbs RA: **Current intakes of EPA and DHA in European populations and the potential of animal-derived foods to increase them**. *Proc Nutr Soc* 2008, **67**(3):273-280.

13. **Industry Facts and Figures** [www.chicken.org.au/page.php?id=4]

14. Givens DI, Gibbs RA: **Very long chain n-3 polyunsaturated fatty acids in the food chain in the UK and the potential of animal derived foods to increase intake**. *Nutr Bull* 2006, **31**(2):104-110.

15. Daniel CR, Cross AJ, Koebnick C, Sinha R: **Trends in meat consumption in the USA**. *Public Health Nutr* 2011, **14**(4):575-583.

16. Bou R, Guardiola F, Barroeta AC, Codony R: **Effect of dietary fat sources and zinc and selenium supplements on the composition and consumer acceptability of chicken meat**. *Poult Sci* 2005, **84**(7):1129-1140.

17. Chekani-Azar A, Shahriar HA, Maheri-Sis N: **Omega-3 fatty acids enrichment and organoleptic charateristic of broiler meat**. *Asian J Anim Vet Adv* 2008, **3**(2):62-69.

18. Schreiner M, Hulan HW, Razzazi-Fazelli E, Bohm J, Moreira RG: **Effect of different sources of dietary omega-3 fatty acids on general performance and fatty acid profiles of thigh, breast, liver and portal blood of broilers**. *J Sci Food Agric* 2005, **85**:219-226.

19. Febel H, Mezes M, Palfy T, Herman A, Gundel J, Lugasi A, Balogh K, Kocsis I, Blazovics A: **Effect of dietary fatty acid pattern on growth, body fat composition and antioxidant parameters in broilers**. *J Anim Physiol Anim Nutr (Berl)* 2008, **92**(3):369-376.

20. Lopez-Ferrer S, Baucells MD, Barroeta AC, Galobart J, Grashorn MA: **n-3 enrichment of chicken meat. 2. Use of precursors of long-chain polyunsaturated fatty acids: linseed oil**. *Poult Sci* 2001, **80**(6):753-761.

21. Zelenka J, Schneiderova D, Mrkvicova E, Dolezal P: **The effect of dietary linseed oils with different fatty acid pattern on the content of fatty acids in chicken meat**. *Vet Med* 2008, **53**(2):77-85.

22. Ajuyah AO, Hardin RT, Sim JS: **Effect of dietary full-fat flax seed with and without antioxidant on the fatty acid composition of major lipid classes of chicken meats**. *Poult Sci* 1993, **72**(1):125-136.

23. Scaife JR, Moyo J, Galbraith H, Michie W, Campbell V: **Effect of different dietary supplemental fats and oils on the tissue fatty acid composition and growth of female broilers**. *Br Poult Sci* 1994, **35**(1):107-118.

24. Defilippis A, Sperling L: **Understanding omega-3's**. *Am Heart J* 2006, **151**:564-570.

25. James MJ, Cleland LG: **Fats and Oils: The Facts**: Meadow Lea foods Ltd.; 2000.

26. Baggott J: **Classification of fatty acids**. 1998.

27. Covington MB: **Omega-3 fatty acids**. *Am Fam Physician* 2004, **70**(1):133-140.

28. Das UN: **Essential fatty acids: biochemistry, physiology and pathology**. *Biotechnol J* 2006, **1**(4):420-439.

29. Serhan CN, Arita M, Hong S, Gotlinger K: **Resolvins, docosatrienes, and neuroprotectins, novel omega-3-derived mediators, and their endogenous aspirin-triggered epimers**. *Lipids* 2004, **39**(11):1125-1132.

30. Simopoulos AP: **The importance of the ratio of omega-6/omega-3 essential fatty acids**. *Biomed Pharmacother* 2002, **56**(8):365-379.

31. Whelan J, Rust C: **Innovative dietary sources of n-3 fatty acids**. *Annu Rev Nutr* 2006, **26**:75-103.

32. Gonzalez-Esquerra R, Leeson S: **Effects of menhaden oil and flaxseed in broiler diets on sensory quality and lipid composition of poultry meat**. *Br Poult Sci* 2000, **41**(4):481-488.

33. Barlow SM, Young FV, Duthie IF: **Nutritional recommendations for n-3 polyunsaturated fatty acids and the challenge to the food industry**. *Proc Nutr Soc* 1990, **49**(1):13-21.

34. Liou YA, King DJ, Zibrik D, Innis SM: **Decreasing linoleic acid with constant alpha-linolenic acid in dietary fats increases (n-3) eicosapentaenoic acid in plasma phospholipids in healthy men**. *J Nutr* 2007, **137**(4):945-952.

35. Mantzioris E, James MJ, Gibson RA, Cleland LG: **Dietary substitution with an alpha-linolenic acid-rich vegetable oil increases eicosapentaenoic acid concentrations in tissues**. *Am J Clin Nutr* 1994, **59**(6):1304-1309.

36. Ruxton CH, Reed SC, Simpson MJ, Millington KJ: **The health benefits of omega-3 polyunsaturated fatty acids: a review of the evidence**. *J Hum Nutr Diet* 2007, **20**(3):275-285.

37. Moyad MA: **An introduction to dietary/supplemental omega-3 fatty acids for general health and prevention: Part I**. *Urol Oncol - Semin Ori* 2005, **23**(1):28-35.

38. Arterburn LM, Hall EB, Oken H: **Distribution, interconversion, and dose response of n-3 fatty acids in humans**. *Am J Clin Nutr* 2006, **83**(6 Suppl):1467S-1476S.

39. Bourre JM: **Where to find omega-3 fatty acids and how feeding animals with diet enriched in omega-3 fatty acids to increase nutritional value of derived products for human: what is actually useful?** *J Nutr Health Aging* 2005, **9**(4):232-242.

40. Hu FB, Bronner L, Willett WC, Stampfer MJ, Rexrode KM, Albert CM, Hunter D, Manson JE: **Fish and omega-3 fatty acid intake and risk of coronary heart disease in women**. *JAMA* 2002, **287**(14):1815-1821.

41. Nordoy A, Marchioli R, Arnesen H, Videbaek J: **n-3 polyunsaturated fatty acids and cardiovascular diseases**. *Lipids* 2001, **36 Suppl**:S127-129.

42. Albert CM, Hennekens CH, O'Donnell CJ, Ajani UA, Carey VJ, Willett WC, Ruskin JN, Manson JE: **Fish consumption and risk of sudden cardiac death**. *JAMA* 1998, **279**(1):23-28.

43. Kris-Etherton PM, Harris WS, Appel LJ: **Omega-3 fatty acids and cardiovascular disease: new recommendations from the American Heart Association**. *Arterioscler Thromb Vasc Biol* 2003, **23**(2):151-152.

44. Gibson RA, Makrides M, Colyer CG: **Essential role of fats throughout the lifecycle: summary and recommendations**. *Med J Aust* 2002, **176 Suppl**:S107-109.

45. James MJ, Cleland LG: **Dietary n-3 fatty acids and therapy for rheumatoid arthritis**. *Semin Arthritis Rheum* 1997, **27**(2):85-97.

46. Sidhu KS: **Health benefits and potential risks related to consumption of fish or fish oil**. *Regul Toxicol Pharmacol* 2003, **38**(3):336-344.

47. Assisi A, Banzi R, Buonocore C, Capasso F, Muzio VD, Michelacci F, Renzo D, Tafuri G, Trotta F, Vitocolonna M *et al*: **Fish oil and mental health: the role of n-3 long-chain polyunsaturated fatty acids in cognitive development and neurological disorders**. *Int Clin Psychopharmacol* 2006, **21**(6):319-336.

48. Lauritzen L, Jorgensen MH, Mikkelsen TB, Skovgaard M, Straarup EM, Olsen SF, Hoy CE, Michaelsen KF: **Maternal fish oil supplementation in lactation: effect on visual acuity and n-3 fatty acid content of infant erythrocytes**. *Lipids* 2004, **39**(3):195-206.

49. Makrides M, Gibson RA, McPhee AJ, Collins CT, Davis PG, Doyle LW, Simmer K, Colditz PB, Morris S, Smithers LG *et al*: **Neurodevelopmental outcomes of preterm infants fed high-dose docosahexaenoic acid: a randomized controlled trial**. *JAMA* 2009, **301**(2):175-182.

50. Makrides M, Gibson RA, McPhee AJ, Yelland L, Quinlivan J, Ryan P: **Effect of DHA supplementation during pregnancy on maternal depression and neurodevelopment of young children: a randomized controlled trial**. *JAMA* 2010, **304**(15):1675-1683.

51. Lee JH, O'Keefe JH, Lavie CJ, Marchioli R, Harris WS: **Omega-3 fatty acids for cardioprotection**. *Mayo Clin Proc* 2008, **83**(3):324-332.

52. NHMRC: **Nutrient reference values for Australia and New Zealand including recommended dietary intake**. *NHMRC* 2006:263-273.

53. Gebauer SK, Psota TL, Harris WS, Kris-Etherton PM: **n-3 fatty acid dietary recommendations and food sources to achieve essentiality and cardiovascular benefits**. *Am J Clin Nutr* 2006, **83**(6 Suppl):1526S-1535S.

54. Wijendran V, Hayes KC: **Dietary n-6 and n-3 fatty acid balance and cardiovascular health**. *Annu Rev Nutr* 2004, **24**:597-615.

55. Ayerza R: **The seed's protein and oil content, fatty acid composition, and growing cycle length of a single genotype of chia (Salvia hispanica L.) as affected by environmental factors**. *J Oleo Sci* 2009, **58**(7):347-354.

56. Gakhar N, Goldberg E, Jing M, Gibson R, House JD: **Effect of feeding hemp seed and hemp seed oil on laying hen performance and egg yolk fatty acid content: Evidence of their safety and efficacy for laying hen diets**. *Poult Sci* 2012, **91**(3):701-711.

57. Krist S, Stuebiger G, Bail S, Unterweger H: **Analysis of volatile compounds and triacylglycerol composition of fatty seed oil gained from flax and false flax**. *Eur J Lipid Sci Tech* 2006, **108**(1):48-60.

58. Kurowska E, Dresser G, Deutsch L, Vachon D, Khalil W: **Bioavailability of omega-3 essential fatty acids from perilla seed oil**. *Prostaglandins Leukot Essent Fatty Acids* 2003, **68**(3):207.

59. Asif M: **Health effects of omega-3, 6, 9 fatty acids: Perilla frutescens is a good example of plant oils**. *Orient Pharm Exp Med* 2011, **11**(1):51-59.

60. James MJ, Gibson RA, Cleland LG: **Dietary polyunsaturated fatty acids and inflammatory mediator production**. *Am J Clin Nutr* 2000, **71**(1 Suppl):343S-348S.

61. Riley P, Enser M, Nute G, Wood J: **Effects of dietary linseed on nutritional value and other quality aspects of pig muscle and adipose tissue**. *Anim Sci* 2000, **71**:483-500.

62. Soltan SSAM, Gibson RA: **Levels of omega-3 fatty acids in Australian seafood**. *Asia Pac J Clin Nutr* 2008, **17**(3):385-390.

63. Hasler CM: **Functional foods: their role in disease prevention and health promotion**. *Food Technol* 1998, **52**:63-147.

64. Hasler CM: **The changing face of functional foods**. *J Am Coll Nutr* 2000, **19**(suppl 5):499S-506S.

65. Hasler CM: **A new look at an ancient concept**. *Chem Ind* 1998(3):84-89.

66. Prates JAM, Mateus C: **Functional foods from animal sources and their physiologically active components**. *Rev Med Vet* 2002, **153**(3):155-160.

67. Stadelman WJ: **The incredibly functional egg**. *Poult Sci* 1999, **78**(6):807-811.

68. Jiménez-Colmenero F, Carballo J, Cofrades S: **Healthier meat and meat products: their role as functional foods**. *Meat Sci* 2001, **59**(1):5-13.

69. Khan MI, Anjum FM, Sameen A, Arshad MS, Gill WT: **Meat as a functional food with special reference to Probiotic sausages**. *Food Res Int* 2011.

70. Hargis PS, Van Elswyk ME: **Manipulating the fatty acid composition of poultry meat and eggs for the health conscious consumer**. *World's Poult Sci J* 1993, **49**(03):251-264.

71. Lewis NM, Seburg S, Flanagan NL: **Enriched eggs as a source of n-3 polyunsaturated fatty acids for humans**. *Poult Sci* 2000, **79**(7):971-974.

72. Ferrier LK, Caston LJ, Leeson S, Squires J, Weaver BJ, Holub BJ: **alpha-Linolenic acid-and docosahexaenoic acid-enriched eggs from hens fed flaxseed: influence on blood lipids and platelet phospholipid fatty acids in humans**. *Am J Clin Nutr* 1995, **62**(1):81.

73. ABARE: **Australian Commodities**. In. Edited by ABARE, vol. 18. Canberra; 2011.

74. Haley MM: **Changing consumer demand for meat: the US example, 1970-2000**. *Changing Structure of Global Food Consumption and Trade Anita Regmi* 2001:41.

75. Howe PR, Meyer BJ, Record S, Baghurst K: **Dietary intake of long-chain [omega]-3 polyunsaturated fatty acids: contribution of meat sources**. *Nutrition* 2006, **22**(1):47-53.

76. Meyer S, Steiner L: **CME: Consumption of Meat, Poultry and Fish Down**. *The Daily Livestock Report* 2011, **9**(22).

77. Baucells MD, Crespo N, Barroeta AC, Lopez-Ferrer S, Grashorn MA: **Incorporation of different polyunsaturated fatty acids into eggs**. *Poult Sci* 2000, **79**(1):51-59.

78. Grobas S, Mendez J, Lazaro R, de Blas C, Mateo GG: **Influence of source and percentage of fat added to diet on performance and fatty acid composition of egg yolks of two strains of laying hens**. *Poult Sci* 2001, **80**(8):1171-1179.

79. Cachaldora P, Garcia-Rebollar P, Alvarez C, De Blas JC, Mendez J: **Effect of type and level of fish oil supplementation on yolk fat composition and n-3 fatty acids retention efficiency in laying hens**. *Br Poult Sci* 2006, **47**(1):43-49.

80. Bourre JM: **Dietary omega-3 Fatty acids and psychiatry: mood, behaviour, stress, depression, dementia and aging**. *J Nutr Health Aging* 2005, **9**(1):31-38.

81. Van Elswyk ME, Dawson PL, Sams AR: **Dietary menhaden oil influences sensory characteristics and headspace volatiles of shell eggs**. *J Food Sci* 1995, **60**(1):85-89.

82. Nash DM, Hamilton RMG, Hulan HW: **The effect of dietary herring meal on the omega-3 fatty acid content of plasma and egg yolk lipids of laying hens**. *Can J Anim Sci* 1995, **75**(2):247-253.

83. Nash DM, Hamilton RMG, Sanford KA, Hulan HW: **The effect of dietary menhaden meal and storage on the omega-3 fatty acids and sensory attributes of egg yolk in laying hens**. *Can J Anim Sci* 1996, **76**(3):377-383.

84. Gonzalez-Esquerra R, Leeson S: **Effect of feeding hens regular or deodorized menhaden oil on production parameters, yolk fatty acid profile, and sensory quality of eggs**. *Poult Sci* 2000, **79**(11):1597-1602.

85. Howe PR, Downing JA, Grenyer BF, Grigonis-Deane EM, Bryden WL: **Tuna fishmeal as a source of DHA for n-3 PUFA enrichment of pork, chicken, and eggs**. *Lipids* 2002, **37**(11):1067-1076.

86. García-Rebollar P, Cachaldora P, Alvarez C, De Blas C, Méndez J: **Effect of the combined supplementation of diets with increasing levels of fish and linseed oils on yolk fat composition and sensorial quality of eggs in laying hens**. *Anim Feed Sci Tech* 2008, **140**(3-4):337-348.

87. Cachaldora P, García-Rebollar P, Alvarez C, De Blas JC, Méndez J: **Effect of type and level of basal fat and level of fish oil supplementation on yolk fat composition and n-3 fatty acids deposition efficiency in laying hens**. *Anim Feed Sci Tech* 2008, **141**(1-2):104-114.

88. Lawlor JB, Gaudette N, Dickson T, House JD: **Fatty acid profile and sensory characteristics of table eggs from laying hens fed diets containing microencapsulated fish oil**. *Anim Feed Sci Tech* 2010, **156**(3-4):97-103.

89. Van Elswyk ME: **Comparison of n-3 fatty acid sources in laying hen rations for improvement of whole egg nutritional quality: a review**. *Br J Nutr* 1997, **78 Suppl 1**:S61-69.

90. Adams R, Pratt D, Lin J, Stadelman W: **Introduction of omega-3 polyunsaturated fatty acids into eggs**. *Poult Sci* 1989, **68**(1):166.

91. Huang Z, Leibovitz H, Lee CM, Millar R: **Effect of dietary fish oil on. omega.-3 fatty acid levels in chicken eggs and thigh flesh**. *J Agr Food Chem* 1990, **38**(3):743-747.

92. Gonzalez-Esquerra R, Leeson S: **Alternatives for enrichment of eggs and chicken meat with omega-3 fatty acids**. *Can J Anim Sci* 2001, **81**(3):295-305.

93. Hargis PS, Van Elswyk ME, Hargis BM: **Dietary modification of yolk lipid with menhaden oil**. *Poult Sci* 1991, **70**(4):874-883.

94. Hulan HW, Proudfoot FG, Ackman RG, Ratnayake WM: **Omega-3 fatty acid levels and performance of broiler chickens fed redfish meal or redfish oil**. *Can J Anim Sci* 1988, **68**(2):533-547.

95. Lopez-Ferrer S, Baucells MD, Barroeta AC, Grashorn MA: **n-3 enrichment of chicken meat using fish oil: alternative substitution with rapeseed and linseed oils**. *Poult Sci* 1999, **78**(3):356-365.

96. Ratnayake WM, Ackman RG, Hulan HW: **Effect of redfish meal enriched diets on the taste and n-3 pufa of 42-day-old broiler chickens**. *J Sci Food Agr* 1989, **49**(1):59-74.

97. Schiavone A, Romboli I, Chiarini R, Marzoni M: **Influence of dietary lipid source and strain on fatty acid composition of Muscovy duck meat**. *J Anim Physiol Anim Nutr (Berl)* 2004, **88**(3-4):88-93.

98. Kjos NP, Herstad O, Skrede A, Øverland M: **Effects of dietary fish silage and fish fat on performance and egg quality of laying hens**. *Can J Anim Sci* 2001, **81**(2):245-251.

99. Mahaffey KR: **Fish and shellfish as dietary sources of methylmercury and the [omega]-3 fatty acids, eicosahexaenoic acid and docosahexaenoic acid: risks and benefits**. *Environ Res* 2004, **95**(3):414-428.

100. Leskanich CO, Noble RC: **Manipulation of the 12-3 polyunsaturated fatty acid composition of avian eggs and meat**. *World's Poult Sci J* 1997, **53**.

101. Ceylan N, Ciftçi I, Mızrak C, Kahraman Z, Efil H: **Influence of different dietary oil sources on performance and fatty acid profile of egg yolk in laying hens**. *J Anim Feed Sci* 2011, **20**:71-83.

102. Hayat Z, Cherian G, Pasha TN, Khattak FM, Jabbar MA: **Effect of feeding flax and two types of antioxidants on egg production, egg quality, and lipid composition of eggs**. *J Appl Poult Res* 2009, **18**(3):541-551.

103. Leeson S, Caston L, MacLaurin T: **Organoleptic evaluation of eggs produced by laying hens fed diets containing graded levels of flaxseed and vitamin E**. *Poult Sci* 1998, **77**(9):1436-1440.

104. Carvalho PR, Pita MCG, Piber Neto E, Mendonça Jr CX: **Efficiency of PUFAs Incorporation from Marine Sources in Yolk Egg's Laying Hens**. *Int J Poult Sci* 2009.

105. Hulan HW, Ackman RG, Ratnayake WM, Proudfoot FG: **Omega-3 fatty acid levels and general performance of commercial broilers fed practical levels of redfish meal**. *Poult Sci* 1989, **68**(1):153-162.

106. Bou R, Codony R, Tres A, Decker EA, Guardiola F: **Dietary strategies to improve nutritional value, oxidative stability, and sensory properties of poultry products**. *Crit Rev Food Sci Nutr* 2009, **49**(9):800-822.

107. Gibson RA, Neumann MA, Lien EL, Boyd KA, Tu WC: **Docosahexaenoic acid synthesis from alpha-linolenic acid is inhibited by diets high in polyunsaturated fatty acids**. *Prostaglandins Leukot Essent Fatty Acids* 2013, **88**(1):139-146.

108. Igarashi M, Ma K, Chang L, Bell JM, Rapoport SI, DeMar JC, Jr.: **Low liver conversion rate of alpha-linolenic to docosahexaenoic acid in awake rats on a high-docosahexaenoate-containing diet**. *J Lipid Res* 2006, **47**(8):1812-1822.

109. Indu M: **n-3 fatty acids in Indian diets—Comparison of the effects of precursor (alpha-linolenic acid) Vs product (long chain n-3 poly unsaturated fatty acids)**. *Nutr Res* 1992, **12**(4):569-582.

110. Pita MCG, de Carvalho PR, Neto EP, de Mendonça Júnior CX: **Modulation of Plasma Levels and Percentages of Incorporation of ω-3 PUFAs in Egg Yolk under the Influence of Supplementation Sources Rich in Omega-3 to Diet of Laying Hens**. *Pak J Nutr* 2011, **10**(8):735-754.

111. Schreiner M, Hulan HW, Razzazi-Fazeli E, Bohm J, Iben C: **Feeding laying hens seal blubber oil: effects on egg yolk incorporation, stereospecific distribution of omega-3 fatty acids, and sensory aspects**. *Poult Sci* 2004, **83**(3):462-473.

112. Barceló-Coblijn G, Murphy EJ: **Alpha-linolenic acid and its conversion to longer chain n-3 fatty acids: Benefits for human health and a role in maintaining tissue n-3 fatty acid levels**. *Prog Lipid Res* 2009, **48**(6):355-374.

113. Sprecher H, Chen Q, Yin FQ: **Regulation of the biosynthesis of 22:5n-6 and 22:6n-3: a complex intracellular process**. *Lipids* 1999, **34 Suppl**:S153-156.

114. Sprecher H, Luthria DL, Mohammed BS, Baykousheva SP: **Reevaluation of the pathways for the biosynthesis of polyunsaturated fatty acids**. *J Lipid Res* 1995, **36**(12):2471-2477.

115. Kartikasari LR, Hughes RJ, Geier MS, Makrides M, Gibson RA: **Diets high in linoleic acid reduce omega-3 long chain polyunsaturated fatty acids in chicken tissues**. In: *the Proceedings of 21st Annual Australian Poultry Science Symposium: 1-3rd February 2010; Sydney*; 2010: 64-67.

116. Blank C, Neumann MA, Makrides M, Gibson RA: **Optimizing DHA levels in piglets by lowering the linoleic acid to alpha-linolenic acid ratio**. *J Lipid Res* 2002, **43**(9):1537-1543.

117. Kartikasari LR, Hughes RJ, Geier MS, Makrides M, Gibson RA: **Dietary alpha-linolenic acid enhances omega-3 long chain polyunsaturated fatty acid levels in chicken tissues**. *Prostaglandins Leukot Essent Fatty Acids* 2012, **87**(4–5):103-109.

118. Tu WC, Cook-Johnson RJ, James MJ, Muhlhausler BS, Gibson RA: **Omega-3 long chain fatty acid synthesis is regulated more by substrate levels than gene expression**. *Prostaglandins Leukot Essent Fatty Acids* 2010, **83**(2):61-68.

119. Komprda T, Zelenka J, Fajmonova E, Fialova M, Kladroba D: **Arachidonic acid and long-chain n-3 polyunsaturated fatty acid contents in meat of selected poultry and fish species in relation to dietary fat sources**. *J Agric Food Chem* 2005, **53**(17):6804-6812.

120. Garg ML, Thomson AB, Clandinin MT: **Interactions of saturated, n-6 and n-3 polyunsaturated fatty acids to modulate arachidonic acid metabolism**. *J Lipid Res* 1990, **31**(2):271-277.

121. Cherian G, Wolfe FW, Sim JS: **Dietary oils with added tocopherols: effects on egg or tissue tocopherols, fatty acids, and oxidative stability**. *Poult Sci* 1996, **75**(3):423-431.

122. Milinsk MC, Murakami AE, Gomes STM, Matsushita M, de Souza NE: **Fatty acid profile of egg yolk lipids from hens fed diets rich in n-3 fatty acids**. *Food Chem* 2003, **83**(2):287-292.

123. Sari M, Aksit M, Ozdogan M, Basmacioglu H: **Effects of addition of flaxseed to diets of laying hens on some production characteristics, levels of yolk and serum cholesterol, and fatty acid compositions of yolk**. *Archiv fur Geflugelkunde* 2002, **66**(2):75-79.

124. Souza JG, Costa FGP, Queiroga R, Silva JHV, Schuler ARP, Goulart CC: **Fatty acid profile of eggs of semi-heavy layers fed feeds containing linseed oil**. *Rev Bras Cienc Avic* 2008, **10**(1):37-44.

125. Bean LD, Leeson S: **Long-term effects of feeding flaxseed on performance and egg fatty acid composition of brown and white hens**. *Poult Sci* 2003, **82**(3):388-394.

126. Cherian G, Li SX, Sim JS: **Dietary alpha-Linolenic Acid and Laying Hen Strain: Fatty Acids of Liver, Adipose Tissue, White Meat, Dark Meat, and Egg Yolk**. *J Agr Food Chem* 1995, **43**(10):2553-2559.

127. Scheideler SE, Jaroni D, Froning G: **Strain and age effects on egg composition from hens fed diets rich in n-3 fatty acids**. *Poult Sci* 1998, **77**(2):192-196.

128. Stadelman WJ, Pratt DE: **Factors influencing composition of the hen's egg**. *World's Poult Sci J* 1989, **45**.

129. Rymer C, Givens DI: **Effect of species and genotype on the efficiency of enrichment of poultry meat with n-3 polyunsaturated fatty acids**. *Lipids* 2006, **41**(5):445-451.

130. Hayat Z, Cherian G, Pasha TN, Khattak FM, Jabbar MA: **Sensory evaluation and consumer acceptance of eggs from hens fed flax seed and 2 different antioxidants**. *Poult Sci* 2010, **89**(10):2293-2298.

131. Mazalli MR, Faria DE, Salvador D, Ito DT: **A comparison of the feeding value of different sources of fat for laying hens: 2. Lipid, cholesterol, and vitamin E profiles of egg yolk**. *J Appl Poult Res* 2004, **13**(2):280-290.

132. Parpinello GP, Meluzzi A, Sirri F, Tallarico N, Versari A: **Sensory evaluation of egg products and eggs laid from hens fed diets with different fatty acid composition and supplemented with antioxidants**. *Food Res Int* 2006, **39**(1):47-52.

133. Jiang Z, Ahn DU, Ladner L, Sim JS: **Influence of feeding full-fat flax and sunflower seeds on internal and sensory qualities of eggs**. *Poult Sci* 1992, **71**(2):378-382.

134. Scheideler SE, Froning GW: **The combined influence of dietary flaxseed variety, level, form, and storage conditions on egg production and composition among vitamin E-supplemented hens**. *Poult Sci* 1996, **75**(10):1221-1226.

135. Caston LJ, Squires EJ, Leeson S: **Hen performance, egg quality, and the sensory evaluation of eggs from SCWL hens fed dietary flax**. *Can J Anim Sci* 1994, **74**(2):347-353.

136. Skrivan M, Skrivanova V, Marounek M, Tumova E, Wolf J: **Influence of dietary fat source and copper supplementation on broiler performance, fatty acid profile of meat and depot fat, and on cholesterol content in meat**. *Br Poult Sci* 2000, **41**(5):608-614.

137. An BK, Banno C, Xia ZS, Tanaka K, Ohtani S: **Effects of dietary fat sources on lipid metabolism in growing chicks (Gallus domesticus)**. *Comp Biochem Physiol B Biochem Mol Biol* 1997, **116**(1):119-125.

138. Betti M, Perez TI, Zuidhof MJ, Renema RA: **Omega-3-enriched broiler meat: 3. Fatty acid distribution between triacylglycerol and phospholipid classes**. *Poult Sci* 2009, **88**:1740-1754.

139. Salamatdoustnobar R, Aghdamshahriar H, Gorbani A, Branch S: **Enrichment of broiler meat with n-3 polyunsaturated fatty acids**. *Asian J Anim Vet Adv* 2008, **3**(2):70-77.

140. Ahn DU, Wolfe FH, Sim JS: **Dietary α-Linolenic Acid and Mixed Tocopherols, and Packaging Influences on Lipid Stability in Broiler Chicken Breast and Leg Muscle**. *J Food Sci* 1995, **60**(5):1013-1018.

141. Zelenka J, Jarosova A, Schneiderova D: **Influence of n-3 and n-6 polyunsaturated fatty acids on sensory characteristics of chicken meat**. *Czech J Anim Sci* 2008, **53**(7):299-305.

142. Betti M, Schneider BL, Wismer WV, Carney VL, Zuidhof MJ, Renema RA: **Omega-3-enriched broiler meat: 2. Functional properties, oxidative stability, and consumer acceptance**. *Poult Sci* 2009, **88**(5):1085-1095.

143. Rahimi S, Azad SK, Torshizi MAK: **Omega-3 enrichment of broiler meat by using two oil seeds**. *J Agr Sci Tech* 2011, **13**:353-365.

144. Najib H, Al-Yousef YM: **Performance and Essential Fatty Acids Content of Dark Meat as Affected by Supplementing the Broiler Diet with Different Levels of Flaxseeds**. *Annu Rev Res Biol* 2011, **1**(2):22-32.

145. Zelenka J, Schneiderová D, Mrkvicová E: **Linseed oils with different fatty acid patterns in the diet of broiler chickens**. *Czech J Anim Sci* 2006, **51**.

146. Olomu JM, Baracos VE: **Influence of dietary flaxseed oil on the performance, muscle protein deposition, and fatty acid composition of broiler chicks**. *Poult Sci* 1991, **70**(6):1403-1411.

147. Ajuyah A, Lee K, Hardin R, Sim J: **Influence of dietary full-fat seeds and oils on total lipid, cholesterol and fatty acid composition of broiler meats**. *Can J Anim Sci* 1991, **71**(4):1011-1019.

148. Goldberg EM, Gakhar N, Ryland D, Aliani M, Gibson RA, House JD: **Fatty Acid Profile and Sensory Characteristics of Table Eggs from Laying Hens Fed Hempseed and Hempseed Oil**. *J Food Sci* 2012, **77**(4):S153-S160.

149. Yalçın H, Ünal MK: **The Enrichment of Hen Eggs with ω-3 Fatty Acids**. *J Med Food* 2010, **13**(3):610-614.

150. Rowghani E, Arab M, Nazifi S, Bakhtiari Z: **Effect of canola oil on cholesterol and fatty acid composition of egg-yolk of laying hens**. *Int J Poult Sci* 2007, **6**(2):111-114.

151. Silversides FG, Lefrancois MR: **The effect of feeding hemp seed meal to laying hens**. *Br Poult Sci* 2005, **46**(2):231-235.

152. Ayerza R, Coates W: **Dietary levels of chia: influence on hen weight, egg production and sensory quality, for two strains of hens**. *Br Poult Sci* 2002, **43**(2):283-290.

153. Ayerza R, Coates W: **Dietary levels of chia: Influence on yolk cholesterol, lipid content and fatty acid composition for two strains of hens**. *Poultry Sci* 2000, **79**(5):724-739.

154. Ayerza R, Coates W: **An ω-3 fatty acid enriched chia diet: influence on egg fatty acid composition, cholesterol and oil content**. *Can J Anim Sci* 1999, **79**(1):53-58.

155. Thacker P, Widyaratne G: **Effects of expeller pressed camelina meal and/or canola meal on digestibility, performance and fatty acid composition of broiler chickens fed wheat–soybean meal-based diets**. *Archives of Animal Nutrition* 2012, **66**(5):402-415.

156. Zuidhof MJ, Betti M, Korver DR, Hernandez FI, Schneider BL, Carney VL, Renema RA: **Omega-3-enriched broiler meat: 1. Optimization of a production system**. *Poult Sci* 2009, **88**(5):1108-1120.

157. Haug A, Eich-Greatorex S, Bernhoft A, Wold JP, Hetland H, Christophersen OA, Sogn T: **Effect of dietary selenium and omega-3 fatty acids on muscle composition and quality in broilers**. *Lipids Health Dis* 2007, **6**:29.

158. Ryhänen EL, Perttilä S, Tupasela T, Valaja J, Eriksson C, Larkka K: **Effect of Camelina sativa expeller cake on performance and meat quality of broilers**. *J Sci Food Agric* 2007, **87**(8):1489-1494.

159. Crespo N, Esteve-Garcia E: **Dietary polyunsaturated fatty acids decrease fat deposition in separable fat depots but not in the remainder carcass**. *Poult Sci* 2002, **81**(4):512-518.

160. Crespo N, Esteve-Garcia E: **Dietary fatty acid profile modifies abdominal fat deposition in broiler chickens**. *Poult Sci* 2001, **80**(1):71-78.

161. Burdge GC, Calder PC: **Conversion of alpha-linolenic acid to longer-chain polyunsaturated fatty acids in human adults**. *Reprod Nutr Dev* 2005, **45**(5):581-597.

162. Bell JG, Tocher DR, Henderson RJ, Dick JR, Crampton VO: **Altered fatty acid compositions in atlantic salmon (Salmo salar) fed diets containing linseed and rapeseed oils can be partially restored by a subsequent fish oil finishing diet**. *J Nutr* 2003, **133**(9):2793-2801.

163. Cooper SL, Sinclair LA, Wilkinson RG, Hallett KG, Enser M, Wood JD: **Manipulation of the n-3 polyunsaturated fatty acid content of muscle and adipose tissue in lambs**. *J Anim Sci* 2004, **82**(5):1461-1470.

164. Folch J, Lees M, Sloane Stanley GH: **A simple method for the isolation and purification of total lipides from animal tissues**. *J Biol Chem* 1957, **226**(1):497-509.

165. Kris-Etherton PM, Harris WS, Appel LJ: **Fish consumption, fish oil, omega-3 fatty acids, and cardiovascular disease**. *Circulation* 2002, **106**(21):2747-2757.

166. Cetingul IS, Inal F: **The effects of utilization of hazelnut oil, sunflower oil and their products on performance and fatty acid composition of yolk in layer hens**. *Vet Res Commun* 2009.

167. Novak C, Scheideler SE: **Long-term effects of feeding flaxseed-based diets. 1. Egg production parameters, components, and eggshell quality in two strains of laying hens**. *Poult Sci* 2001, **80**(10):1480-1489.

168. National Research Council: **Nutrient Requirements of Poultry**, 9th rev. ed. edn. Washington, DC: National Academy Press; 1994.

169. Bastian SEP, Collins C, Johnson TE: **Understanding consumer preferences for Shiraz wine and Cheddar cheese pairings**. *Food Qual Prefer* 2010, **21**(7):668-678.

170. Jia W, Slominski BA, Guenter W, Humphreys A, Jones O: **The effect of enzyme supplementation on egg production parameters and omega-3 fatty acid deposition in laying hens fed flaxseed and canola seed**. *Poult Sci* 2008, **87**(10):2005-2014.

171. Nain S, Renema RA, Korver DR, Zuidhof MJ: **Characterization of the n-3 polyunsaturated fatty acid enrichment in laying hens fed an extruded flax enrichment source**. *Poult Sci* 2012, **91**(7):1720-1732.

172. Ahn DU, Sunwoo HH, Wolfe FH, Sim JS: **Effects of dietary α-linolenic acid and strain of hen on the fatty acid composition, storage stability, and flavor characteristics of chicken eggs**. *Poult Sci* 1995, **74**(9):1540-1547.

173. Shimizu Y, Arai K, ISE S, Shimasaki H: **Dietary fish oil for hens affects the fatty acid composition of egg yolk phospholipids and gives a valuable food with an ideal balance of n-6 and n-3 essential fatty acids for human nutrition**. *J Oleo Sci* 2001, **50**(10):797-803.

174. Gibson RA, Muhlhausler B, Makrides M: **Conversion of linoleic acid and alpha-linolenic acid to long-chain polyunsaturated fatty acids (LCPUFAs), with a focus on pregnancy, lactation and the first 2 years of life**. *Matern Child Nutr* 2011, **7 Suppl 2**:17-26.

175. Walzem RL, Hansen RJ, Williams DL, Hamilton RL: **Estrogen induction of VLDLy assembly in egg-laying hens**. *J Nutr* 1999, **129**(2):467S-472S.

176. Botsoglou NA, Yannakopoulos AL, Fletouris DJ, Tserveni-Goussi AS, Psomas IE: **Yolk fatty acid composition and cholesterol content in response to level and form of dietary flaxseed**. *J Agric Food Chem* 1998, **46**(11):4652-4656.

177. Scheideler SE, Froning G, Cuppett S: **Studies of consumer acceptance of high omega-3 fatty acid-enriched eggs**. *J Appl Poult Res* 1997, **6**(2):137-146.

178. Ayerza R, Coates W: **Omega-3 enriched eggs: The influence of dietary α-linolenic fatty acid source on egg production and composition**. *Can J Anim Sci* 2001, **81**(3):355-362.

179. Bezard J, Blond JP, Bernard A, Clouet P: **The metabolism and availability of essential fatty acids in animal and human tissues**. *Reprod Nutr Dev* 1994, **34**(6):539-568.

180. **Guide to Food Labelling and Advertising**
[http://www.inspection.gc.ca/english/fssa/labeti/guide/ch7be.shtml#7.19]

181. Kritchevsky SB: **A review of scientific research and recommendations regarding eggs**. *J Am Coll Nutr* 2004, **23**(suppl 6):596S-600S.

182. Makrides M, Hawkes JS, Neumann MA, Gibson RA: **Nutritional effect of including egg yolk in the weaning diet of breast-fed and formula-fed infants: a randomized controlled trial**. *Am J Clin Nutr* 2002, **75**(6):1084-1092.

183. Basmacioglu H, Cabuk M, Unal K, Ozkan K, Akkan S, Yalcin H: **Effects of dietary fish oil and flax seed on cholesterol and fatty acid composition of egg yolk and blood parameters of laying hens**. *S Afr J Anim Sci* 2004, **33**(4):266-273.

184. Lichtenstein AH, Appel LJ, Brands M, Carnethon M, Daniels S, Franch HA, Franklin B, Kris-Etherton P, Harris WS, Howard B: **Diet and lifestyle recommendations revision 2006**. *Circulation* 2006, **114**(1):82-96.

185. Ayerza R, Coates W, Lauria M: **Chia seed (Salvia hispanica L.) as an omega-3 fatty acid source for broilers: influence on fatty acid composition, cholesterol and fat content of white and dark meats, growth performance, and sensory characteristics**. *Poult Sci* 2002, **81**(6):826-837.

186. Lelis G, Silva MD, Tavernari FC, Albino L, Rostagno H: **Performance of layers fed diets containing different oils**. *Rev Bras Cienc Avic* 2009, **11**(4):235-240.

187. Bhatty RS: **Nutrient composition of whole flaxseed and flaxseed meal**. *Flaxseed in human nutrition* 1995:22-42.

188. Al-Nasser AY, Al-Saffar AE, Abdullah FK, Al-Bahouh ME, Ragheb G, Mashaly MM: **Effect of Adding Flaxseed in the Diet of Laying Hens on Both Production of Omega-3 Enriched Eggs and on Production Performance**. *Int J Poult Sci* 2011, **10**(10):825-831.

189. Basmacioglu H, Çabuk M, Ünal K, Özkan K, Akkan S, Yalçin H: **The Effects of Using Fish Oil and Flaxseed in Laying Hens Diets on Egg Yolk Cholesterol, Fatty Acid Composition of Egg Yolk and Some Blood Parameters**. *Pak J Nutr* 2003, **2**(2):89-95.

190. Caston L, Leeson S: **Research note: dietary flax and egg composition**. *Poult Sci* 1990, **69**(9):1617-1620.

191. Portolesi R, Powell BC, Gibson RA: **Competition between 24:5n-3 and ALA for u6 desaturase may limit the accumulation of DHA in HepG2 cell membranes**. *J Lipid Res* 2007.

192. Broadway PR, Behrends JM, Schilling MW: **Effect of alternative salt use on broiler breast meat yields, tenderness, flavor, and sodium concentration**. *Poult Sci* 2011, **90**(12):2869-2873.

193. Lawlor J, Sheehan E, Delahunty C, Kerry J, Morrissey P: **Sensory characteristics and consumer preference for cooked chicken breasts from organic, corn-fed, free-range and conventionally reared animals**. *Int J Poult Sci* 2003, **2**(6):409-416.

194. Lee YS, Owens CM, Meullenet JF: **On the quality of commercial boneless skinless broiler breast meat**. *J Food Sci* 2008, **73**(6):S253-S261.

195. Poste LM, Butler G, Cave NA, Burrows VD: **Sensory analysis of meat from broiler chickens fed diets containing hulless oats (Avena nuda)**. *Can J Anim Sci* 1996, **76**(3):313-319.

196. **Cumulative index volume I parts A–G**. In: *Rodd's Chemistry of Carbon Compounds (Second Edition).* Edited by Coffey S, M.Sc, D.Sc, F.R.I.Ca2 - S. Coffey MSDSFRIC. Amsterdam: Elsevier; 1964: 53-344.

197. ISO: **International Standard - ISO 8589: Sensory analysis - General guidance for the design of test rooms**: Dansk Standard; 1988.

198. Voss A, Reinhart M, Sankarappa S, Sprecher H: **The metabolism of 7,10,13,16,19-docosapentaenoic acid to 4,7,10,13,16,19-docosahexaenoic acid in rat liver is independent of a 4-desaturase**. *J Biol Chem* 1991, **266**(30):19995-20000.

199. Gibson RA, Neumann MA, Lien EL, Boyd KA, Tu WC: **Docosahexaenoic acid synthesis from alpha-linolenic acid is inhibited by diets high in polyunsaturated fatty acids**. *Prostaglandins Leukot Essent Fatty Acids* 2012.

200. Madhusudhan KT, Ramesh HP, Ogawa T, Sasaoka K, Singh N: **Detoxification of commercial linseed meal for use in broiler rations**. *Poult Sci* 1986, **65**(1):164-171.

201. Poureslami R, Raes K, Turchini GM, Huyghebaert G, De Smet S: **Effect of diet, sex and age on fatty acid metabolism in broiler chickens: n-3 and n-6 PUFA**. *Br J Nutr* 2010, **104**(02):189-197.

202. McMurchie EJ, Rinaldi JA, Burnard SL, Patten GS, Neumann M, McIntosh GH, Abbey M, Gibson RA: **Incorporation and effects of dietary eicosapentaenoate (20:5(n-3)) on plasma and erythrocyte lipids of the marmoset following dietary supplementation with differing levels of linoleic acid**. *Biochim Biophys Acta* 1990, **1045**(2):164-173.

203. Navidshad B, Boo LJ, Akhlaghi A: **Effect of dietary fish oil on n-3 fatty acid content of meat from broiler chickens fed low protein diets**. *Anim Prod Sci* 2012, **52**(9):799-805.

204. Shin D, Choi SH, Go G, Park JH, Narciso-Gaytan C, Morgan CA, Smith SB, Sanchez-Plata MX, Ruiz-Feria CA: **Effects of dietary combination of n-3 and n-9 fatty acids on the deposition of linoleic and arachidonic acid in broiler chicken meats**. *Poult Sci* 2012, **91**(4):1009-1017.

205. Gibson RA: **The effect of diets containing fish and fish oils on disease risk factors in humans**. *Aust N Z J Med* 1988, **18**(5):713-722.

206. Makrides M, Gibson RA: **Long-chain polyunsaturated fatty acid requirements during pregnancy and lactation**. *Am J Clin Nutr* 2000, **71**(1 Suppl):307S-311S.

207. Simopoulos AP: **Genetic variants in the metabolism of omega-6 and omega-3 fatty acids: their role in the determination of nutritional requirements and chronic disease risk**. *Exp Biol Med* 2010, **235**(7):785-795.

208. Kolanowski W, Laufenberg G: **Enrichment of food products with polyunsaturated fatty acids by fish oil addition**. *Eur Food Res Technol* 2006, **222**(3):472-477.

209. Campo MM, Nute GR, Wood JD, Elmore SJ, Mottram DS, Enser M: **Modelling the effect of fatty acids in odour development of cooked meat in vitro: Part I—sensory perception**. *Meat Sci* 2003, **63**(3):367-375.

210. Wood J, Richardson R, Nute G, Fisher A, Campo M, Kasapidou E, Sheard P, Enser M: **Effects of fatty acids on meat quality: a review**. *Meat Sci* 2003, **66**(1):21-32.

211. Mottram DS: **Flavour formation in meat and meat products: a review**. *Food Chem* 1998, **62**(4):415-424.

212. Myer RO, Johnson DD, Knauft DA, Gorbet DW, Brendemuhl JH, Walker WR: **Effect of feeding high-oleic-acid peanuts to growing-finishing swine on resulting carcass fatty acid profile and on carcass and meat quality characteristics**. *J Anim Sci* 1992, **70**(12):3734-3741.

213. Elmore JS, Campo MM, Enser M, Mottram DS: **Effect of lipid composition on meat-like model systems containing cysteine, ribose, and polyunsaturated fatty acids**. *J Agiric Food Chem* 2002, **50**(5):1126-1132.

214. Azad SK, Rahimi S, Torshizi MAK: **Effect of dietary oil seeds on n-3 fatty acid enrichment, performance parameters and humoral immune response of broiler chickens**. *Iran J Vet Res* 2009, **10**:158-165.

215. Lee KH, Olomu JM, Sim JS: **Live performance, carcass yield, protein and energy retention of broiler chickens fed canola and flax full-fat seeds and the restored mixtures of meal and oil**. *Can J Anim Sci* 1991, **71**(3):897-903.

216. Rondelli SG, Martinez O, Garcia PT: **Effects of different dietary lipids on the fatty acid composition of broiler abdominal fat**. *Rev Bras Ciênc Avíc* 2004, **6**(3):171-175.

217. **Rapeseed Oil Monthly Price**
[http://www.indexmundi.com/commodities/?commodity=rapeseed-oil&currency=aud