ASSESSMENT OF OMEGA-3 LONG CHAIN POLYUNSATURATED FATTY ACID INCORPORATION IN BROILER CHICKEN MEAT FOLLOWING THE CONSUMPTION OF OMEGA-3 RICH VEGETABLE OILS

LILIK RETNA KARTIKASARI

M. Ag.Sc. (Gadjah Mada University, Indonesia)

A thesis submitted in fulfilment of the requirements for the Degree of Master of Agricultural Science (M.Ag.Sc.)

Discipline of Plant and Food Science
School of Agriculture, Food and Wine
Faculty of Sciences
University of Adelaide
Adelaide, South Australia, Australia

February 2009

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ABSTRACT

Dietary omega-3 long chain polyunsaturated fatty acids (n-3 LCPUFAs), eicosapentaenoic acid (EPA, 20:5n-3), docosapentaenoic acid (DPA, 22:5n-3) and docosahexaenoic acid (DHA, 22:6n-3), have beneficial health effects and hence increasing the consumption of these fatty acids is recommended by health authorities. The most common dietary source of EPA, DPA and DHA is seafood, but few Australians habitually consume fish and on average eat less than one meal of fish per week. Thus if Australians are to meet the dietary guidelines for n-3 fatty acid intake, there is a need to develop a source of n-3 rich foods that fit into a typical Australian diet. Feeding fish oils rich in n-3 LCPUFA to chickens has proven problematic due to alteration in organoleptic properties. The incorporation of vegetable oils rich in n-3 PUFA, alpha-linolenic acid (ALA, 18:3n-3) into the diet of chickens is potentially an alternative way to provide meat rich in n-3 LCPUFAs as ALA is the precursor of EPA and DHA. However, most vegetable oils also contain the n-6 (n-6) PUFA, linoleic acid (LA, 18:2n-6) which competes with ALA for the same enzymes in their metabolism to LCPUFA.

This thesis addressed two crucial issues relating to the conversion of ALA into EPA, DPA and DHA of chicken tissues. The objectives of the first experiment were to examine the effects of increasing the ALA content of diets on the conversion of ALA into EPA, DPA and DHA by measuring their accumulation in chicken meat (breast and thigh) and to determine if there was an optimum level of ALA (at a fixed level of LA) in this process. The ratio of LA to ALA of the diets ranged from 10.5:1 to 0.6:1.

The findings in this study demonstrated that there was no optimum level of dietary ALA and as indicated by the observation that EPA, DPA and DHA continued to increase in breast and thigh as the ratio of LA to ALA decreased in the diet. In general, DPA achieved higher levels than DHA. The experimental diets with the lowest LA to ALA ratio elevated the incorporation of EPA and DHA into breast and thigh meat to levels 5 and 4-fold, respectively relative to birds fed the highest LA to ALA ratio. In contrast, arachidonic acid (AA, 20:4n-6) in all groups reduced with decreasing LA to ALA ratio in the diets. The results indicated that the dietary treatments did not significantly change the growth performance of chickens.

The objective of the second experiment was to assess the regulatory effect of dietary LA on the conversion of ALA into EPA, DPA and DHA. While in the first experiment the diets varied in the level of ALA but had a constant LA level, in this experiment the level of ALA in the diets was held constant and the level of LA was varied. The LA to ALA ratio of experimental diets ranged from 1.4:1 to 2.1:1. The results of this study indicated that the highest LA to ALA ratio (2.1:1) resulted in the lowest n-3 LCPUFAs, EPA, DPA and DHA in meat samples. For example, the total n-3 LCPUFA levels in the breast meat of birds fed with the lowest LA to ALA ratio was 16% higher than the n-3 LCPUFA in the breast of birds fed the highest LA to ALA ratio. This study indicated that the strongest influence on EPA, DPA and DHA accumulation in chicken tissues was the level of ALA in the diet. The experimental diets did not appear to affect the growth performance of chickens.

In conclusion, increasing the ALA content of chicken diets may result in a meat source high in n-3 LCPUFAs that may reduce pressure on diminishing marine stocks as well as offering health benefits to Australians.

DECLARATION

I declare that this thesis is a record of original work and contains no material which has been accepted for the award of any other degree or diploma in any university. To the best of my knowledge and belief, this thesis contains no material previously published or written by another person, except where due reference is made in the text.

I give consent to a copy of my thesis being made available in the University Library.

_____ _____

Lilik Retna Kartikasari Date 12 February 2009

POSTER AND ABSTRACT ARISING FROM THIS THESIS

POSTER/ORAL PRESENTATION

Lilik R Kartikasari, Robert J Hughes, Mark S Geier and Robert A Gibson. Effect of Vegetable Oils on Omega-3 Long Chain Polyunsaturated Fatty Acid (LCPUFA) Levels in Broiler Chicken Meat. School of Agricultural, Food and Wine, Research Day. The University of Adelaide, November 2008.

ABSTRACT

Lilik R Kartikasari, Robert J Hughes, Mark S Geier and Robert A Gibson. Effect of Vegetable Oils on Omega-3 Long Chain Polyunsaturated Fatty Acid Levels in Broiler Chicken Meat (Experiment I and II). Postgraduate Symposium. The University of Adelaide, September 2008.

Lilik R Kartikasari, Robert J Hughes, Mark S Geier and Robert A Gibson. Effect of Vegetable Oils on Omega-3 Long Chain Polyunsaturated Fatty Acid Levels in Broiler Chicken Meat (Experiment I). School of Agricultural, Food and Wine, Research Day. The University of Adelaide, November 2008.

ACKNOWLEDGEMENTS

I am very grateful to my supervisors Professor Robert A Gibson and Dr. Robert J Hughes for their support, encouragements, constructive comments and guidance throughout my M.Ag.Sc candidature. In particular, I would like to thank Professor Robert A Gibson for his continuously support, advice and patience.

I would like to thank Professor Maria Makrides, Professor Martine Boulianne and Dr. Mark S Geier for their support, suggestions and comments throughout my study.

I thank David Apps for guiding me in the learning of experimental techniques and Ella Zielinski for technical assistance and advice in the fatty acid analysis. I would also like to thank Varunika Ruwanpura for her assistance in document editing

My gratitude to all the people who in one way or another have helped and supported me during my study, especially Niranjala Seimon, Margaret Cargill, Trevor Hancock, Lisa Smithers, Jo Zhou Derek Schultz, Evelyn Daniels, Kylee Swanson, Natasha Edwards, Sue Walker, Jennifer Washington, and Wei-Chun Tu.

I thank my colleagues in fatty acid group and my fellow postgraduate students and Honours in Functional Food Group for their friendship, comments and help in rearing cultures.

I would like to thank the University of Adelaide and South Australian Research Development Institute (SARDI) for their supports throughout my study. I would also like to thank Sebelas Maret University (UNS), Indonesia for giving me the opportunity to experience studying at Adelaide University.

I gratefully acknowledge the financial support of the Australian Agency for International Development (AusAID/APS) during my studies in Australia, I would also like to thank Directorate General of Higher Education, Jakarta, Indonesia for granting me study leave.

I would also like to express my appreciation to my mother (in memorial, 9 February 2007) and parents in law for their continuous support. Finally my sincere thanks to my dear husband Feri Satria and my lovely daughter Geraldin Noverina for their patience, love and support during my studies.

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
GLA	γ-linolenic acid
GC	Gas chromatograph
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
PUFA	Polyunsaturated fatty acid
LCPUFA	Long chain polyunsaturated fatty acid
RVH	Right ventricle hypertrophy
SARDI	South Australia Research and Development Institute
SFA	Saturated fatty acid
SDA	Stearidonic acid
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

Literature Review

1.1 INTRODUCTION

The beneficial health effects of dietary omega-3 (n-3) polyunsaturated fatty acids (PUFA) have been well documented and emphasise the importance of increasing the consumption of these fatty acids in human diet. The main source of long chain n-3 PUFA (LCPUFA), eicosapentaenoic acid (EPA, 20:5n-3), docosapentaenoic acid (DPA, 22:5n-3) and docosahexaenoic acid (DHA, 22:6n-3) in the human diet is fish and fish products [1], including food enriched with fish oil [2]. However, efforts to encourage people to increase the consumption of marine foods have failed as most Australians do not habitually consume fish [3]. In addition problems have been encountered when foods are fortified with fish oil. These include negative effects on the sensory properties of meat [4,5] and heavy metal contaminants that negatively affect human health [6]. These problems are also encountered in chicken meat when chickens are fed diets supplemented with fish oils [4].

An alternative to the supplementation of chicken diets with fish oil is to enhance the natural production of n-3 LCPUFA in chickens by incorporating vegetable oils rich in the n-3 PUFA alpha-linolenic acid (ALA, 18:3n-3) in their diets. Vegetable oils such as flaxseed and canola oils are rich sources of ALA. Some researchers have conducted supplementation studies using ALA rich vegetable oils at a variety of levels in animals other than chickens including Atlantic salmon [7], lambs [8] and pigs [9]. Animals and humans can convert the ALA in vegetable oils into EPA and DHA

but this conversion rate in certain animals and man is slow. The conversion of ALA into n-3 LCPUFA is influenced by the availability of the desaturation and elongation enzymes. However, these enzymes are also involved in the conversion of the n-6 PUFA, linoleic acid (LA, 18:2n-6), to arachidonic acid (AA, 20:4n-6). So a high consumption of LA can reduce EPA and DHA production, and favour high AA simply by competitive inhibition [10]. An important determinant for the optimal conversion of ALA is the ratio of LA to ALA in the diet. Previous studies have shown an increase in the ALA content of chicken tissues following the consumption of diets rich in ALA [11,12] but failed to demonstrate an increase in EPA and DHA in tissues. The lack of response in such studies may be due to the relatively high ratio of LA to ALA in the experimental diets. Therefore, in order to optimize the conversion of ALA to EPA and DHA in chicken meat, vegetable oils high in n-3 PUFA (ALA) and low in n-6 PUFA (LA) should be tested in chicken diets. This strategy may also result in an improvement in the sensory quality of the meat when compared to chickens raised on diets containing fish products.

The remainder of this chapter will review current knowledge on the effects of ALA rich vegetable oils in chicken diets in order to address the question of whether vegetable oils rich in ALA can increase n-3 LCPUFA (EPA, DPA and DHA) levels chicken meat. The metabolism of fatty acids leading to the synthesis of LCPUFA will be reviewed. In addition this review will also cover the health benefits of n-3 LCPUFA, the recommended intake of n-3 LCPUFA, dietary sources of n-3 fatty acids, the ratio of n-6 to n-3 PUFA in diets, fish oil problems in chicken meat and the conversion of ALA to n-3 LCPUFA.

1.2 FATS

Visible fats and oils are commonly in the form of triglycerides while the so called invisible fats are in the form of phospholipids and are part of every membrane of every cell. The basic structure of all triglycerides consists of glycerol and three fatty acids while all phospholipids have a glycerol backbone, with a head group and only two fatty acids [13]. There are three types of naturally occurring fatty acids associated with all fats: saturated, monounsaturated and polyunsaturated [14]. The structures of these types of fatty acids are shown in Figure 1.1. This classification is based on the number of double bonds present in their fatty acid side chains in the molecule [13,14].

Saturated fatty acids (SFA) have no double bonds in the molecule. A typical SFA is stearic acid, which is labelled as 18:0. This nomenclature indicates that stearic acid contains 18 carbons with no double bonds. The sources of SFA are found mostly in coconut milk and animal fats [13]. In commercial food preparation, saturated fats are used in high proportion because of their stability relative to polyunsaturates. The problem is that saturated fats can have negative health impacts on humans [13] as they raise plasma cholesterol.

Monounsaturated fatty acids (MUFA) have one double bond. Examples of these fatty acids are oleic acid (18:1) and palmitoleic acid (16:1). Monounsaturated fats are good dietary replacements for saturated fats as they can lower plasma cholesterol levels in humans. Moreover, these fats are not antagonistic to n-3 fatty acid metabolism so they are a suitable replacement for dietary n-6 fats [13].

Figure 1.1 Chemical structures of saturated, monounsaturated and polyunsaturated fatty acids (adapted from Baggott, J.[15])

Polyunsaturated fatty (PUFA) acids have two or more double bonds. PUFA are classified into n-6 and n-3 fatty acids [14,15]. This classification is determined by the position of the first double bond in the molecule. A polyunsaturate that has the first double bond after the sixth carbon, counting from the methyl (CH_3) end of the molecule is called n-6 and one that has the first double bond after the third carbon is called n-3. Neither LA nor ALA can be synthesized in the body hence they must be obtained in the diet and are thus known as essential fatty acids [13,14,16].

The most important n-6 fatty acids for the human diet are linoleic acid (LA, 18:2n-6) and arachidonic acid (AA, 20:4n-6). LA can be converted to AA by a desaturase and elongase enzymes [14,17,18]. The main sources of LA are vegetable oils, seeds and nuts [13,14]. 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 [17] which have proinflamatory and prothrombic [16,17] properties and are known to be involved in various pathological processes such as atherosclerosis, bronchial asthma and several inflammatory conditions [17].

Similarly, the important n-3 fatty acids include ALA, EPA, DPA and DHA. ALA can be converted to EPA, DPA and DHA by the same desaturase and elongase enzymes that converts LA to AA [17]. EPA is the precursor of the 3-series prostaglandins and the 5-series leukotrienes [17], a group of eicosanoids that are anti-inflammatory, antithrombotic, antiarrhytmic and vasodilatory [16]. 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 [19]. 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 [20], whereas an elevated intakes of n-3 PUFA could impact positively on human health [20,21].

1.3 THE HEALTH BENEFITS OF N-3 PUFA

Dietary n-3 polyunsaturated fatty acids have health benefits in the prevention and treatment of diseases such as heart disease [22,23], rheumatoid arthritis [20] and positive effects on neural and brain development [24-27]. Several prospective cohort studies have found a negative association between fish consumption and the risk of coronary heart disease [28,29] or sudden cardiac death [30]. Nordoy *et al.* [29] reported that fish consumption of 1-2 meals/wk was associated with reduced coronary heart disease (CHD). Regular fish consumption also gives health benefits related to the reduction of sudden death. Albert *et al.* [30] assessed a relationship between fish consumption and the risk of sudden death. Their studies found that for men who had approximately 1 fish meal per week, the multivariate relative risk of sudden death was 0.48 compared with men who had dietary fish intake less than monthly [30]. Kris-Etherton *et al.* [31] reported that 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 cause mortality.

With regard to coronary heart disease (CHD), treatment with 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 [29]. Studies such as this firmly established that the active ingredient in fish and fish oils is the n-3 LCPUFA.

Dietary n-3 LCPUFA also has beneficial effects on the prevention and therapy of rheumatoid arthritis that is known to have a strong inflammatory component [32,33].

Studies show that the Japanese who consume a high level of n-3 LCPUFA through fish have a lower rate of the disease (0.4%) than Western communities (1%) [13]. Other studies show that daily supplementation with n-3 LCPUFA resulted in significant clinical benefits in rheumatoid arthritis [34]. In addition, there is 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 [33], especially for patients who want a more natural treatment [13]. James and Cleland [33] suggested that fish oil consumption at dosages of at least 3g/day had beneficial effects on rheumatoid arthritis.

DHA is found at a high level in the brain cortex and retina, which indicates that DHA has an important role in the development of neural [26,35] and visual functions [26,36]. 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 [37]. In preterm infants DHA is a clear requirement for full visual and neural development [26]. Makrides and Gibson [27] 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.* [36] 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 three-fold increase in the DHA content of four-month breast milk and that 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 [36]. There is also evidence that increasing n-3 LCPUFA intake during pregnancy

increased maternal n-3 LCPUFA status [38]. Studies show that pregnant women who consume regular amounts of fish oil (0.5g/day DHA and 0.15g/day EPA) increase their maternal EPA and DHA levels [38] and lead to higher DHA supply to the foetus [39]. 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. Infants who were fed with formula milk had lower DHA levels than breast fed infants [13].

1.4 THE RECOMMENDED INTAKE OF N-3 PUFA

Considering the beneficial health effects of n-3 LCPUFA consumption, an increase in these fatty acids, especially EPA and DHA, is important. 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 among studies (Table 1.1 and 1.2).

According to Simopoulos [40] and Kris-Etherton *et al.* [41], the recommended n-3 LCPUFA is 650mg/day, with at least 222mg for both EPA and DHA [40]. Koletzko *et al.* [39] suggests that dietary DHA intake for pregnant and lactating women should be at least 200mg/day. The American Heart Association recommends consuming fish, particularly fatty fish, at least twice a week to provide the necessary n-3 LCPUFA [31]. Wijendran and Hayes [42] suggest EPA and DHA intakes to be 0.25-0.5% of energy. In 2004, the International Society for the Study of Fatty Acids and Lipids recommended intake of EPA and DHA at ≥500mg/day [43].

Table 1.1 Recommended intakes of n-3 LCPUFA

Date	Organization/references	Recommended intakes	Population
2008	Lee *et al.* [44]	EPA + DHA: ≥500mg/day EPA + DHA: 1g/day EPA + DHA: 3 to 4g/day	General population Coronary artery disease Hypertriglyceridemia
2007	Koletzko *et al.* [39]	DHA: ≥200mg/day	General population, pregnant and lactating women
2006	National Health and Medical Research Council [45]	Female: 430mg/day Male: 610mg/day	General population
2006	Gebauer *et al.* [46]	EPA + DHA: ≈500mg/day	Cardiovascular disease risk reduction
2004	International Society for the Study of Fatty Acids and Lipids [43]	EPA + DHA: ≥500mg/day	General population (for cardiovascular health)
2004	UK Scientific Advisory Committee on Nutrition	Minimum2 portions fish/wk	General population
2004	Wijendran and Hayes [42]	EPA + DHA: 0.25 energy %	General population
2002	American Heart Association	Eat fish (fatty fish) at least twice/week	General population
2000	Simopoulos [40]	EPA: ≥220mg/day DHA: ≥220mg/day EPA + DHA: ≥650mg/day	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.* [46]	0.6-1.2 energy %	General population
2004	International Society for the Study of Fatty Acids and Lipids [43]	0.7 energy %	General population
2004	Wijendran and Hayes [42]	0.75 energy %	General population
2002	The Heath Council of Netherlands [47]	1.0 energy %	General population
2000	Simopoulos [40]	2.22g/day	General population

Recommendations are also made for the consumption of n-3 fatty acids to prevent and treat various chronic diseases especially in prevention of heart disease. Lee *et al.* [44] suggest that patients with coronary artery disease can reduce their risk of cardiovascular disease by increasing their EPA and DHA consumption approximately 1g/day. The National Health and Medical Research Council (NHMRC) recommend the consumption of 340mg of n-3 LCPUFA for women and 610mg for men per day in order to prevent chronic disease. Recommended dietary ALA to prevent deficiency symptoms is 0.6-1.2% of energy for ALA [46]. In addition, a diet containing ALA derived from vegetable oils at a level of from 1.5 to 3g/day has substantial benefits to prevent heart disease [31]. This represents an approximately four-fold increase in the consumption of ALA for many Australians. Thus it is apparent that the consumption of n-3 LCPUFA needs to be increased. To achieve the recommended intake of n-3 LCPUFA, it is important to recognize food sources rich in n-3 fatty acids, both n-3 PUFA (ALA) and n-3 LCPUFA (EPA and DHA).

1.5 DIETARY SOURCES OF N-3 PUFA

1.5.1 Sources of 18 Carbon n-3 PUFA: Vegetable oils and nuts

Some vegetable oils are rich sources of ALA, the n-3 PUFA. These oils can be used not only in human diets but also in animal diets. Vegetable oils such as linseed, echium, rapeseed (canola) and soybean oil are the major sources of ALA [13,41,48]. Linseed oil is rich in ALA and low in LA. However, this oil is not commonly used in cooking. The ALA content of linseed, rapeseed and soybean oil is 58.7, 9.2 and 7.8%, respectively [41]. Other sources of ALA include nuts such as walnuts. The content of ALA in walnuts is 6.3% of total fat [13]. 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 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)

Source: James and Cleland [13]

1.5.2 Sources of 20/22 Carbon n-3 LCPUFA: Fish, fish oil, eggs and meat

The major source of n-3 LCPUFA in human diets is fish and fish products [1]. Some fatty fish such as herring, salmon, tuna and whiting, are rich sources of the n-3 LCPUFA, EPA, DPA and DHA (Table 1.4). 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 [41]. Both these oily fish are much higher in n-3 LCPUFA than most white table fish eaten by Australians [49]. N-3 LCPUFA sources 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 [41].

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 [49])

The other sources of n-3 LCPUFA are meat and eggs. The n-3 LCPUFA content of chicken meat and regular chicken eggs is 0.036g/100g and 0.1g/100g, respectively [13]. Howe *et al.* [50] 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.

Although fish and fish products are the major source of n-3 LCPUFA, an increasing variety of alternative food sources enriched with n-3 fatty acids is being developed [2,48,51]. N-3 LCPUFA levels of animal products such as meat and eggs can be improved by changing the fatty acid composition of the animal's diet [2,48]. Several studies have demonstrated the possibility of modifying the n-3 LCPUFA composition of eggs and poultry meat by incorporating sources rich in n-3 fatty acids in diets [2,51,52]. Lopez-Ferrer *et al.* [2] 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 about two-fold in beef, seven-fold in chicken and six fold in eggs [53]. A variety 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.

1.6 THE RATIO OF N-6 TO N-3 FATTY ACIDS IN DIETS

For the purpose of this review I will limit my discussion to 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 [54]. An important determinant for the optimal function of the n-3 PUFA, ALA 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 [55]. Kris-Etherton *et al.* [41] recommends the LA to ALA ratio of 2.3:1. In Western diets, the ratio is in the range of from 10:1 to 20:1 [56]. This indicates that Western diets are deficient in ALA and have excessive amounts of LA. LA consumption has increased from ~3% of energy in the early 1900s, and now contributes to 5-7% of dietary energy in Western nations due to an increase in dietary intake of vegetable oils high in n-6 fatty acids [10]. Increasing the consumption of ALA is important to promote a high level of n-3 fatty acids in tissues.

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 but the conversion rates

vary enormously. ALA can be converted into EPA and DHA [54] and LA can be transformed into AA in the liver of rats to a high degree but conversion rates in humans are less than 6% [57]. ALA and LA have homologous structures, so these fatty acids compete for the same desaturation and elongation enzymes [13]. High consumption of LA can reduce EPA, DPA and DHA production and favour high AA [10]. In other words, a high ratio of LA to ALA leads to decreased metabolism of ALA into EPA, DPA and DHA. Komprda *et al*. [58] showed that increasing dietary LA significantly increased the level of AA. Conversely, diets containing high levels of ALA will promote increasing tissue EPA concentrations. As Garg *et al.* [59] pointed out, diets containing linseed or fish oil, n-3 fatty acid rich sources, inhibit the conversion of LA to gamma-linolenic acid, the precursor of AA. Liou *et al.* [10] examined the effect of replacing for 4 weeks, vegetable oils high in LA, with oils low in LA in foods containing a constant ALA on plasma n-3 LCPUFA in healthy men. Their findings indicated that decreasing LA while keeping ALA constant resulted in an increase in plasma phospholipids EPA. 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 tissues.

1.7 STRATEGY TO INCREASE N-3 LCPUFA IN CHICKEN MEAT

One strategy to enhance n-3 LCPUFA accumulation in chicken meat is supplementation of their diet with fish oil. Many studies have investigated the enrichment of chicken meat through supplementation of fish oil in the diet [4,60-62]. Their findings indicate that there was an increase in the level of n-3 LCPUFA in chicken meat. Lopez-Ferrer *et al.* [2] reported 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 EPA and DHA supplemented with 4% fish oil reached 1.3 and 2.4% of total fatty acids, respectively. Other authors found different results when the feeding period of diet enriched with fish oil did not start at 1 day old [60]. The level of breast EPA and DHA of chickens fed with diets supplemented with 3% fish oil at 21 days old throughout a 21 day growth period was 10.5 and 3.8%, respectively [60]. Another study conducted by Lopez-Ferer *et al.* [61] found 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%). In addition, other investigators [4,63] 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.

1.8 PROBLEMS WITH USING FISH OIL IN CHICKEN DIETS

The use of fish oil in the manipulation of meat fatty acid composition may result in negative effects on the sensory properties of meat [5,60] such as off-tastes and off-odours [4]. 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 [6]. Studies conducted by Chekani-Azar *et al.* [60] indicated that chicken diet supplemented with 3% of fish oil had the least normal smell and flavour. This finding agreed with Lopez-Ferrer *et al.* [61] who examined the effects of substituting of chicken diets containing fish oil with rapeseed (canola) and linseed oils on the sensory quality of breast and thigh meat. Their results showed that the poorest sensory quality scores corresponded to the diet which included the fish oil ingredient for a greater time. In addition Schreiner *et al.* [5] 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. One of the goals of food science is to increase the nutritional value of foods without compromising sensory quality [4], as this may reduce consumer acceptability. It is therefore important to introduce alternative n-3 fatty acid sources, such as vegetable oils in order to increase tissue n-3 LCPUFA concentrations without influencing taste and meat quality [1].

1.9 CONVERSION OF ALA TO N-3 LCPUFA

1.9.1 Vegetable Oils High in ALA as an Alternative Source of LCPUFA

The dietary essential fatty acids in human diets are ALA and LA [6]. Humans cannot synthesize these fatty acids *de novo* so they must be provided in the diet. These essential fatty acids need to be metabolized to their respective LCPUFA to derive full benefits [17]. ALA can be converted into the more biologically active n-3 LCPUFA, EPA, DPA and DHA [6] (Figure 1.2). Incorporation of vegetable oils rich in ALA into chicken diets can be an alternative supply of n-3 LCPUFA to fish oil if chickens convert ALA to n-3 LCPUFA in sufficient quantity. Some researchers have conducted studies with regard to the supplementation of ALA rich vegetable oils at a variety of levels in animals such as Atlantic salmon [7], lambs [8] and piglets [9]. Incorporating n-3 rich vegetable oils into chicken diets could help to provide a greater variety of foods rich in n-3 LCPUFA if broilers convert ALA to n-3 LCPUFA in sufficient quantity.

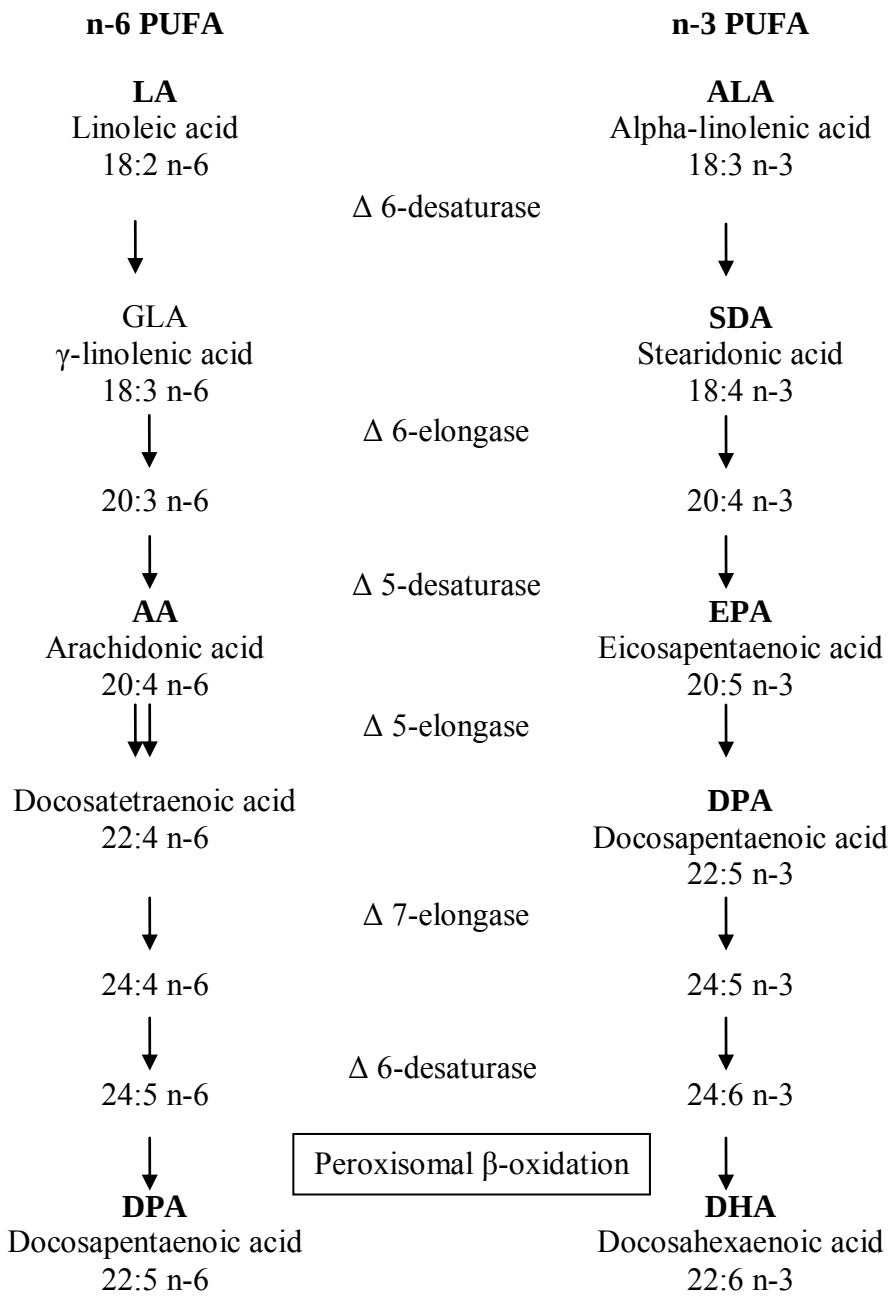


Figure 1.2 The metabolic pathway of the n-3 and the n-6 fatty acids (Adapted from Whelan *et al.* [54] and Mantzioris [64])

1.9.2 Current Evidence

Previous studies showed that the inclusion levels of vegetable oils rich in the n-3 PUFA, ALA, increased production performance of chickens, reduced fat deposition [12] and enhanced the level of ALA and n-3 LCPUFA in tissues [12,52,65]. Studies involving the inclusion of 4% linseed oil plus tallow up to 8% added fat (a higher polyunsaturated and lower saturated diet than control) resulted in the increase in weight in grams per bird per day, feed intake and final body weight [12]. Ferrini *et al.* [66] investigated the effect of different dietary fatty acid profiles on abdominal fat and skin fat deposition of broiler chickens. In this study, diets were supplemented with 10% of tallow, sunflower oil rich in oleic acid, sunflower oil rich in LA, linseed oil rich in ALA, or a blend of fats. Their results showed that diets containing linseed oil reduced abdominal fat and skin fat deposition.

Several studies have shown that feeding diets rich in ALA increased tissue ALA content [4,8]. Studies conducted by Skrivan *et al.* [67] showed that chicken diets supplemented with 50g/kg of rapeseed (canola) oil increased the ALA content of breast muscles and decreased the ratio of LA to ALA. These results were supported by other studies. An *et al.* [68] found that increases in the level of linseed oil gradually elevated ALA, and decreased the levels of LA and AA in tissue lipids. In addition, Lopez-Ferrer *et al.* [61] assessed the effect on the fatty acid composition of tissues when diets containing fish oil were substituted with linseed oil. Their findings indicated that the substitution of fish oil with 8.2% linseed oil increased tissue ALA concentrations.

Similar findings were reported by Gonzales-Esquerra and Leeson [55] 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 (Table 1.5). Their results showed that a diet containing 100g/kg of linseed increased ALA and DPA concentrations and did not affect the sensory quality of breast and thigh tissues. However, these studies have failed to demonstrate an increase in EPA and DHA in tissues. The lack of response in these studies may be due to the high level of LA and the relatively high ratio of LA to ALA in the experimental diets used.

In order to optimize the incorporation of dietary ALA into chicken meat, an effective dietary strategy for altering the ratio of LA to ALA is needed. Studies conducted in humans have shown that ALA rich diets are successful in increasing EPA levels [69,70], and that when the ratio of LA to ALA is lowered either by increasing ALA or decreasing LA, it can result in an increase in DHA [71]. Mantzioris *et al.* [69] investigated the effect on tissue n-3 LCPUFA levels of the consumption of diets containing ALA rich vegetable oils (linseed oil) combined with low levels of LA. Their findings indicated that the inclusion of linseed oil improved EPA concentrations 2.5-fold. In addition, Blank *et al.* [9] examined the effect on the concentration of tissue EPA and DHA of altering the LA to ALA ratio in piglet diets. They found that decreasing the LA to ALA ratio from 10:1 to 0.5:1 increased plasma phospholipid EPA concentrations from 0.4 to 7.1%, as well as DHA levels, which reached a maximum when the ratio of LA to ALA was between 4:1 and 2:1 [9].

Recent studies using chickens indicate that there was an increase in tissue n-3 LCPUFA by decreasing the ratio of LA to ALA in the diet [12,52,65]. Lopez-Ferrer *et al.* [12] assessed the effect on n-3 LCPUFA levels in chicken tissues of diets supplemented with linseed oil. Their findings showed that the supplementation of chicken diets with added animal fat with up to 4% linseed oil (LA to ALA ratio of 0.57:1) increased EPA, DPA and DHA concentrations in thigh tissues approximately 2.5-fold higher than the control diet. In addition, Febel *et al.* [52] found that decreasing LA to ALA ratio of chicken diet by supplementation of vegetable oils (3%) including sunflower oil, soybean oil and linseed oil increased liver and breast n-3 LCPUFA (EPA and DHA). Diets supplemented with linseed oil enhanced the level of EPA and DHA to 1.2 and 1.4% respectively [52] and resulted in the highest level of n-3 LCPUFA. The accumulation of breast n-3 LCPUFA (EPA, DPA and DHA) further increased (Figure 1.3) when the diet was supplemented with 5% of linseed oil [65]. However, the inclusion level of 7% of linseed oil did not change the level of n-3 LCPUFA [65] 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 [52,65]. 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.

Figure 1.3 The effect of decreasing LA to ALA ratio on the accumulation of breast n-3 LCPUFA (adapted from Zelenka *et al.* [65])

Table 1.5 Summary of studies of dietary fats in chickens

Authors and references	Animals	Experimental Diets	Intervention Period	Growth	Results: LCPUFA status	Comment
Febel *et al.* [52] (2008)	1200 1-day-old Male (Ross-308) chicks	Basal diet supplemented with 3% of lard (L), 3% of sunflower oil (SFO), 3% of soybean oil (SBO), and 3% of linseed oil (LSO). Lipid content of diets approximately 6%.	Up to 35 days of age	No effect on growth or feed conversion	LO resulted in higher SFA than SBO LO gave the highest level in n-3 LCPUFA (EPA and DHA)	Without supplementation fish meal/ fish oil
Zelenka *et al.* [65] (2008)	32, 25-day-old Male birds (Ross-308)	Basal grain diet supplemented with 1%, 3%, 5% or 7% linseed oil.	from day 25 to 40 days of age	No effect on basic production parameters. 5 and 7% of oils caused a higher body weight gains and lower feed consumption per unit of body gain	N-3 LCPUFA levels dependent on ALA content of diet There was no differences in n-3 LCPUFA between 5% and 7% LO Highest n-3 LCPUFA = 5.5% in breast and 2.2% in thigh	Diet until day 25 unknown. No fish meal/ fish oil
Haugh *et al.*[72] (2007)	60 1-day-old male Ross 308 chicks	Dietary treatment: 5% rapeseed oil (RO) + 0.50 mg Se/kg (i), 5% RO + 0.84 mg Se/kg (ii), 1% linseed oil (LO) + 4% RO + 0.50 mg Se/kg (iii), and 1% LO + 4% RO + 0.84 mg 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	All diets contain 5% rendered fat Without supplementation fish meal/ fish oil

(Continued on next page)

Authors and references	Animals	Experimental Diets	Intervention Period	Growth	Results: LCPUFA status	Comment
Crespo and Esteve-Garcia [73] (2002)	100 1-day-old female Ross 308 chicks	Control: Basal diet Dietary treatment: Basal diet with added fat including tallow (T), olive (O), sun flower (S) and linseed (L) oil at 10%	28 up to 53 days of age	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	Without supplementation fish meal/ fish oil
Crespo and Esteve-Garcia [74] (2001)	960 21-day-old male and 960 21-day-old female Ross 208 chicks	Control diet: Starter diet Dietary treatment: The inclusion of fat sources including Tallow (TO), olive oil (OO), sunflower oil (SO) 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. SO and LO increased feed efficiency. SO and LO lowered percentages of abdominal fat Diets and level of fat resulted in same fat and protein content of thighs and breasts.	Tallow increased SFA LO produced the highest value of ALA and higher EPA and DHA in thighs and breasts, and the lowest value of LA SO resulted in the lowest value of n-3 fatty acids. LO and SO gave the same value of n-6 fatty acids for abdominal fat.	
Lopez- Ferrer *et al.* [12] (2001)	230 unsexed 1-day-old Cobb chickens	Control diet: 0% linseed oil (T1,1% ALA) Treatment group: LO + tallow up to 8%; 2% LO (T2, 13.5% ALA), 4% LO (T3, 28.1% ALA)	Up to 38 days of age; 24, 28, 54 days for T3 to evaluate the progressive deposition of LCPUFA (liver, thigh)	Little difference, increase in weight in grams/b/d was higher in T3	LO decreased saturated and MUFA, increased PUFA (ALA, LA). EPA, DPA and DHA scored the highest values in T3 0.39, 0.24, and 0.25% of ME of FA, respectively. Longer feeding time of LO did not result in tissue accumulation of n-3 LCPUFA	Without supplementation fish meal/ fish oil

Authors and references	Animals	Experimental Diets	Intervention Period	Growth	Results: LCPUFA status	Comment
Gonzalez-Esquerra and Leeson [55] (2000)	330 1-day-old chickens	Control group: a common diet Treatment group: 0 or 100 g/kg linseed or 7.5 or 15g/kg menhaden oil (MO) and the combination of linseed with either 7.5 or 15g/kg MO	7 or 14 days prior to slaughter	Final body weight (FW) (49) & body weight gain (36-49) were not affected by diets	A diet containing linseed 100g/kg: a major increased in ALA, and a slight increase in DPA No effect on EPA and DHA Not affected sensory quality	
Skrivan *et al.* [67] (2000)	320 1-day-old chickens	Control diet: wheat/maize-soybean meal Treatment group : supplementation 50 g/kg lard (i), 25 g/kg lard + 25 g/kg rapeseed oil (ii), 50 g/kg RO (iii), 50 g/kg RO + 200 mg copper/kg	Up to 39 days of age	The substitution of copper increased final body weight	The substitution of lard by RO increasing breast tissue PUFA, decreased the ratio of n-6:n-3, decreased cholesterol, and no effect on EPA and DHA	Without supplementation fish meal/ fish oil
Lopez-Ferrer *et al.* [61] (1999)	64 unsexed 1-day-old Cobb chickens	Control diet: basal diet Treatment group: basal diet enriched with 8.2% Fish oil (T1), replaced with 8.2% linseed (LO) or canola oil the last week before slaughtering (T2), the last 2 wk (T3), or throughout the trial period (T4)	Up to 35 days of age		Replacement of FO by LO 2 or 3 wk before slaughtering increased ALA, and decreased EPA and DHA	Treatment diets contain fish oil. FO supplementation Increased the deposition of LCPUFA

1.10 CONCLUSION

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. Moreover, there are problems related to negative effects on the sensory properties and on human health of consuming fish and fish products. So efforts to encourage people to increase their n-3 LCPUFA in this way are not always appropriate. In order to increase the consumption of n-3 LCPUFA by humans, vegetable oils can be a source of ALA, the precursor of n-3 LCPUFA but less than 5% of ALA consumed is converted to n-3 LCPUFA in humans. An alternative is to feed vegetable 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 [7], lambs [8] and pigs [9]. These have had positive responses in increasing tissue EPA, DPA and DHA concentrations. Developing studies on chicken diets would help to provide a variety

of food rich in n-3 LCPUFA, and may increase the consumption of n-3 LCPUFA (EPA, DPA and DHA) which would be beneficial to human health.

1.11 AIMS AND HYPOTHESES

1.11.1 Aims

The aims of this research were to investigate the effects of the ALA and LA content of diets on the conversion of ALA into EPA, DPA and DHA by measuring their accumulation in chicken meat and to determine if there was an optimum level of ALA (at a fixed level of LA) in this process.

1.11.2 Hypotheses

The conversion of ALA into EPA, DPA and DHA will be influenced by both dietary ALA and dietary LA.

CHAPTER 2

The Effect of Chicken Diets Varying in ALA on n-3 LCPUFA levels in Chicken Tissues

2.1 INTRODUCTION

One dietary approach to increase the dietary n-3 LCPUFA intake of humans is to increase the intake of n-3 LCPUFA from fish and meat. As most Australians consume very little fish, increasing the level of n-3 LCPUFA in meats including chicken may be a better possibility to increase n-3 LCPUFA consumption without changing dietary habits.

Recent studies conducted in humans [69,70] and piglets [9] have indicated that diets enriched with ALA are successful in increasing EPA levels but they have little effect on longer chain length n-3 fatty acids, including DHA. For example, in piglets, when the ratio of LA to ALA was lowered by increasing ALA it resulted in only a small increase in DHA and the maximum DHA level was achieved at an LA to ALA ratio between 4:1 and 2:1[9]. In chickens, some authors [12,52,65] have reported that there was an increase in tissue n-3 LCPUFA, EPA, DPA and DHA with varying results. Febel *et al.* [52] reported that a diet supplemented with 3% linseed oil enhanced the level of EPA and DHA to 1.2 and 1.4%, respectively and resulted in the highest level of n-3 LCPUFA compared to other vegetable oils. When the inclusion level of linseed oil was increased to 5%, the accumulation of breast n-3 LCPUFA (EPA, DPA and DHA) further increased [65]. Therefore, in order to optimize the conversion of ALA into EPA and DHA, the ratio of LA to ALA should be varied by increasing the level of ALA in the diets and keeping a constant LA

level. Incorporating n-3 rich vegetable oils into chicken diets would help to provide a variety of foods rich in n-3 LCPUFA for human consumption. This strategy may also result in an improvement in the sensory quality of the meat as one of the goals of food research is to increase the nutritional value of foods without compromising sensory quality.

The objective of the first experiment was to examine the effect of dietary ALA content on the conversion of ALA into EPA, DPA and DHA by measuring their accumulation in chicken meat (breast and thigh) and to determine if there was an optimum level of ALA (at a fixed level of LA) in this process.

2.2 MATERIALS AND METHODS

2.2.1 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).

2.2.2 Location

The experiments were conducted at both Roseworthy and Waite Campuses of the University of Adelaide. Chickens were housed 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, Waite Campus the University of Adelaide.

2.2.3 Experimental Design

This experiment was based on a one-way classification design. The variable factor was varying levels of ALA in the diet with a constant LA level. The dietary treatments were based on the ratio of LA to ALA in the diets. There were a total of seven diets comprised of a control diet and six experimental diets.

2.2.4 Birds, Rearing and Management

In this experiment, seventy unsexed one-day-old broiler chickens (Cobb 500) obtained from the Baiada hatchery (Willaston SA, Australia) were randomly allocated to one of the seven diets (n=10 birds/diet). The birds were housed for 28 days and reared at PPPI, SARDI, Roseworthy Campus under controlled environmental conditions (Figure 2.1). Upon arrival from the hatchery, the chickens were immediately weighed in groups of 10 (Libror EB-32KS SHIMADZU) and placed on brown paper in raised rearing pens (1.2 x 0.9m^2 each pen). Feed was provided in a plastic hopper and also scattered over the paper to encourage the chickens to eat immediately after placement.

Figure 2.1 Layout of raised rearing pens

Fresh water was placed in the splash cup under the drinking nipples to encourage the chickens to drink soon after placement. Both feed and water were provided *ad libitum* for the duration of the experiment. Room temperature was maintained at 27°C for 4 days and gradually reduced to 20°C during the experimental period. All birds were subjected to a 24 h light program throughout the growth period. At the same time, the pens were also given heat from infrared lamps (175 watts) for 21 days. 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 comfortable environmental conditions were ascertained by observing the behavior of the chickens. The room temperature was maintained by a logic controller (tempron 606) which managed air flow, cooling and heating. Fresh wood shavings were put on the pen floor at 3 days old. During the experimental period fresh shavings were added three times.

Body weight and feed consumption was recorded on a weekly basis for the 28 day experimental period and body weight gain and feed efficiency (g feed:g weight gain) subsequently calculated. On day 28 all birds were weighed individually.

2.2.5 Diets

The experimental diets were prepared from a basal broiler chicken diet. Fat levels were adjusted by the addition of pure or blended vegetable oils. Blended vegetable oils were obtained by varying the proportion of some vegetable oils including macadamia, linseed, canola and sunflower. The birds were fed a proprietary starter commercial feed (Ridley Agriproducts Pty Ltd, Murray Bridge, Australia) for the first two days after hatch.

The commercial diet was then replaced with experimental diets. Six dietary treatments were prepared by supplementing the basal diet with blended vegetable oils including flaxseed, macadamia, canola, and sunflower oil. The proportion of the basal diet and vegetable oils added to the basal diet is shown in Table 2.1. The fatty acid composition of the oils assigned for experimental diets were then analyzed (Table 2.2).

Table 2.1 Composition of experimental diets from 1-28 days post-hatch

Ingredients (%)	**Experimental diets**							
	Basal diet	**Control**	**Diet 1**	**Diet 2**	**Diet 3**	**Diet 4**	**Diet 5**	**Diet 6**
Starter Basal diet[1]	100.0	97.5	97.5	97.5	97.5	95.0	95.0	92.5
Oil added								
Macadamia oil	0.0	2.5	0.0	0.83	0.0	1.5	0.0	0.0
Flaxseed oil	0.0	0.0	0.25	1.67	2.5	1.6	5.0	7.5
Canola oil	0.0	0.0	2.25	0.0	0.0	1.7	0.0	0.0
Sunflower oil	0.0	0.0	0.0	0.00	0.0	0.2	0.0	0.0
Total	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0

[1]Starter basal diet comprises (g/kg): wheat fine (60.00), oats fine (2.50), triticale fine (6.53), blood meal, 91% CP (2.50), canola meal expeller (1.57), soybean meal, 47% CP (22.80), limestone small (1.30), palphos 88 (rock phos weighed) (0.50), kynofos weighed (1.13), salt (0.19), sodium bicarbonate (0.31), choline chloride 75% (0.05), L-lysine HCL (0.11), L-threonine (0.03), alimet (0.26), feed enzyme premix (avizyme) (0.03), and standard broiler starter premix (0.20)

Table 2.2 Fatty acid composition of oils added in the diets

Fatty acids[2]	Oil and blended vegetable oils[1]						
	Control	**Diet 1**	**Diet 2**	**Diet 3**	**Diet 4**	**Diet 5**	**Diet 6**
Total SFA	16.8	8.8	9.4	11.7	12.2	11.7	11.7
Total MUFA	80.4	55.6	46.4	16.3	51.2	16.3	16.3
Total n-9	59.1	52.2	43.7	15.5	43.9	15.5	15.5
Total n-7	21.3	3.4	2.6	0.8	7.2	0.8	0.8
18:2n-6	2.3	21.2	19.5	16.4	15.7	16.4	16.4
Total n-6	2.3	21.4	19.8	16.6	16.0	16.6	16.6
18:3n-3	0.2	14.0	24.3	55.4	20.5	55.4	55.4
Total n-3	0.5	14.1	24.4	55.4	20.7	55.4	55.4

[1]Composition of oil consisted of: macadamia oil, control diet; 10% flaxseed and 90% canola oil, diet 1; 1:2 blend of flaxseed and canola oil, diet 2; flaxseed oil, diet 3; 32% flaxseed, 34% canola, 30% macadamia and 4% sunflower oil, diet 4; flaxseed oil, diet 5 and diet 6.
[2]SFA: saturated fatty acid; MUFA: monounsaturated fatty acid

The amount of the oils needed for each diet was then weighed and mixed well with the basal diet using a cement mixer. A total of 20kg feed was made for each batch and during the experimental period, a total of 40kg diet was prepared and provided for each dietary treatment throughout the 28-day growth period. This resulted in seven diets with fat content ranging from 5 to 10%. The ingredient composition and nutrient content of the experimental diets are presented in Table 2.3. All diets met or exceeded the requirements recommended by the National Research Council (1994) for broiler chickens.

Table 2.3 Ingredient composition and nutrient content of experimental diets

Ingredients (%)	Experimental diets							
	Basal diet[1]	**Control**	**Diet 1**	**Diet 2**	**Diet 3**	**Diet 4**	**Diet 5**	**Diet 6**
Wheat fine	60.00	58.50	58.50	58.50	58.50	57.00	57.00	55.50
Oats fine	2.50	2.44	2.44	2.44	2.44	2.38	2.38	2.31
Triticale fine	6.53	6.37	6.37	6.37	6.37	6.21	6.21	6.04
Blood meal (91% CP)	2.50	2.44	2.44	2.44	2.44	2.38	2.38	2.31
Canola meal	1.57	1.53	1.53	1.53	1.53	1.49	1.49	1.45
Soybean meal	22.80	22.23	22.23	22.23	22.23	21.66	21.66	21.09
Limestone small	1.30	1.26	1.26	1.26	1.26	1.23	1.23	1.20
rock phos weighed	0.50	0.49	0.49	0.49	0.49	0.48	0.48	0.46
Kynofos weighed	1.13	1.10	1.10	1.10	1.10	1.07	1.07	1.04
Salt	0.19	0.19	0.19	0.19	0.19	0.18	0.18	0.18
Sodium bicarbonate	0.31	0.31	0.31	0.31	0.31	0.30	0.30	0.29
Choline chloride 75%	0.05	0.05	0.05	0.05	0.05	0.04	0.04	0.04
L-lysine HCL	0.11	0.10	0.10	0.10	0.10	0.10	0.10	0.10
L-threonine	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03
Alimet	0.26	0.25	0.25	0.25	0.25	0.25	0.25	0.24
Starter premix	0.20	0.20	0.20	0.20	0.20	0.19	0.19	0.19
Feed enzyme premix	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03
Basal diet	100.00	97.50	97.50	97.50	97.50	95.00	95.00	92.50
Added oils	0.00	2.50	2.50	2.50	2.50	5.00	5.00	7.50
Nutrient content (%)								
Crude protein	23.75	23.15	23.15	23.15	23.15	22.56	22.56	21.97
Crude fat	2.52	5.29	5.37	5.13	5.38	6.71	7.26	9.41
Crude fibre	3.00	2.93	2.93	2.93	2.93	2.85	2.85	2.78
Calcium	0.98	0.96	0.96	0.96	0.96	0.93	0.93	0.91
Phosphorus	0.72	0.70	0.70	0.70	0.70	0.68	0.68	0.67
Available phosphorus	0.47	0.46	0.46	0.46	0.46	0.45	0.45	0.43
Na	0.20	0.20	0.20	0.20	0.20	0.19	0.19	0.19
K	0.76	0.74	0.74	0.74	0.74	0.72	0.72	0.70
CL	0.20	0.20	0.20	0.20	0.20	0.19	0.19	0.19
Lysine	1.30	1.27	1.27	1.27	1.27	1.24	1.24	1.20
Methionine	0.56	0.55	0.55	0.55	0.55	0.53	0.53	0.52
Methionine+cystine	0.99	0.97	0.97	0.97	0.97	0.94	0.94	0.92

[1]A standard commercial starter diet (Ridley Agriproducts Pty Ltd, Murray Bridge, Australia).

The fatty acid composition of the final diets is presented in Table 2.4. We attempted to hold the level of fat in the diets constant at 5% (w/w) and increase the level of dietary ALA by altering the balance of vegetable oils added to the basal diet. Due to the level of LA in the basal diet, the maximal level of dietary ALA that could be achieved in this way was 29% of total fatty acids. In order to increase the level of ALA to higher levels we had to increase the total level of oil in the diets (Table 2.4). The ALA levels of the diets varied from 0.26 (control diet) to 7.97% energy (% en; diet 6). LA level of control diet was 2.72% en and that of experimental diets ranged from 3.96 (diet 1) to 4.59% en (diet 6). These resulted in the ratio of LA to ALA varying from 10.46:1 (control diet) to 0.58:1 (diet 6). Each diet was provided *ad libitum* throughout the 28-day growth period.

The control diet was an attempt to mimic the fatty acid composition of commercial diets by adding macadamia oil, which likes tallow, that is often used in commercial diets contained almost no PUFA.

Table 2.4 Fatty acid content of the diets

	Experimental diets							
	Basal diet	**Control**	**Diet 1**	**Diet 2**	**Diet 3**	**Diet 4**	**Diet 5**	**Diet 6**
LA (% en)	2.93	2.72	3.96	3.77	3.67	3.88	4.06	4.59
ALA (% en)	0.30	0.26	1.10	1.61	3.19	2.07	5.82	7.97
LA:ALA ratio	9.76:1	10.46:1	3.60:1	2.34:1	1.15:1	1.87:1	0.70:1	0.58:1
Fat (%)	2.52	5.29	5.37	5.13	5.38	6.71	7.26	9.41
Fatty acids[1]	% of total fatty acids							
SFA	20.7	19.2	14.0	14.7	16.0	15.2	14.4	13.7
MUFA	20.8	53.8	41.1	35.6	20.5	42.1	20.0	20.3
18:2n-6	52.7	24.1	34.6	34.4	33.8	27.6	26.8	24.0
Total n-6	53.0	24.3	34.8	34.6	33.9	27.7	27.0	24.1
18:3n-3	5.4	2.3	9.6	14.7	29.3	14.7	38.4	41.7
Total n-3	5.4	2.6	9.7	14.7	29.4	14.8	38.5	41.7
PUFA	58.4	26.8	44.5	49.3	63.3	42.5	65.4	65.8

[1]SFA: saturated fatty acid; MUFA: monounsaturated fatty acid; PUFA: polyunsaturated fatty acids

2.2.6 Sample Collection

At 28 days of age, six birds from each experimental diet group were weighed individually and prepared for blood collection. A maximum of 2mL of blood was sampled from the brachial vein by venepuncture with a 22 gauge disposable needle fitted to a 5mL disposable syringe and transferred into a lithium heparin tube and transferred into a lithium heparin tube. Blood samples were then kept at 4°C for fatty acid analysis. The birds were then euthanized by intravenous injection of pentobarbitone sodium (325mg/mL) at a dose rate of 0.5mL/kg live weight. Tissue samples (liver, breast and thigh) were then collected and stored at -20°C for fatty acid analysis.

2.2.7 Fatty Acid Methyl Ester (FAME) Extraction Method

Fatty acid profiling was performed on feed, erythrocyte, plasma, liver, breast and thigh. Analyses were also performed on feed ingredients and vegetable oils in order to accurately formulate diets with the desired LA and ALA levels. Total lipids in diet and tissue samples were extracted by the methods of Folch *et al.* [75].The fatty acids were methylated by the procedure described by Blank *et al.* [9].

2.2.7.1 Fatty acid analysis of oils

Oils were shaken vigorously before sampling. Using a short Pasteur pipette, oil (7-10mg) was dispensed into labelled scintillation vials (20mL) to which 5mL 1% H_2SO_4 in methanol was added and the vials 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 gas chromatograph (GC) vial using a short disposable glass Pasteur pipette. A minimal amount of sodium sulphate (approximately 30mg) was included as a dehydrating agent.

2.2.7.2 Fatty acid analysis of feeds

Feed ingredients (0.5g) and experimental diets (0.3g) were weighed (Metler AE 163) and transferred into labelled Kimble glass tubes. Samples were then ground finely using a Polytron homogenizer (a Kinematica PT 2000 fitted with a Kinematica PTA7 Aggregate, Switzerland) in 2mL of cold isotonic saline (0.9% sodium chloride). Chloroform/methanol (2:1, v/v) was used to extract total lipids.

Samples were mixed thoroughly with methanol (3mL) and allowed to stand for 5 minutes. Chloroform (6mL) was added and the glass tubes were shaken vigorously. After standing for 5 minutes at room temperature, the tubes were centrifuged at 1559g for 10 minutes (Heraeus Sepatech, Hanau, Germany) in order to separate the aqueous and organic phases. The organic phase in the bottom chloroform 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 resulting fat obtained was 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 methylated in 5mL of 1% H_2SO_4 in methanol at 70°C for 3 hours. The methyl esters were extracted into 750µL of distilled water and 2mL heptane and stored in GC vials containing approximately 30mg of anhydrous sodium sulphate at -20°C for GC analysis.

2.2.7.3 Fatty acid analysis of blood

Whole blood was spun at 1559g for 10 minutes to separate erythrocytes and plasma. Plasma (top layer) was then removed and placed into labelled tubes using a pipette, and stored at -20°C for later fatty acids analysis. Total lipids of plasma were extracted with methanol/chloroform (1:2, v/v). The remaining erythrocytes were then washed with cold 0.9% isotonic saline three times. Tubes were filled with saline and centrifuged at 1559g for 10 minutes. The top saline layer was discarded.

This process was repeated three times. The resulting washed erythrocytes (1mL) were transferred into labelled Kimble glass tubes and 0.9% of cold isotonic saline (0.5mL) was added to make a total volume of 1.5mL.

Total lipids of erythrocytes were extracted with isopropanol/chloroform (1:2, v/v). Samples were vortexed thoroughly with isopropanol (2mL) followed by chloroform (4mL). The glass tubes were shaken vigorously and allowed to stand for 5 minutes at room temperature. The tubes were then centrifuged at 1559g for 10 minutes (Megfuge 1.0, Heraeus Sepatech, Hanau, Germany) in order 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 to dryness using a vacuum concentrator. 150µL of chloroform/methanol (9:1, v/v) was added to the total lipid extracts which was then spotted onto thin layer chromatography (TLC) plates and developed in petroleum spirit/acetone (3:1, v/v) to separate the lipid components. The plates were analysed under UV light to identify phospholipids. The phospholipids band, including silica, was then scraped into a 5mL glass vial containing 2mL of 1% H_2SO_4 in methanol, sealed and allowed to methylate at 70°C for 3 hours. The resulting methyl esters were extracted into 250µL of distilled water and 0.5mL heptane and stored at -20°C for GC analysis.

2.2.7.4 Fatty acid analysis of tissues

Tissue samples, including liver, breast and thigh, were cleaned from connective tissue and any adipose on a glass plate and heart tissues were washed with saline three times before these samples were used. Liver and heart (0.3g) were broken

prior to homogenization and abdominal fat (10mg), breast and thigh 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 homogenized with a Kinematica PT 2000 fitted with a Kinematica PTA7 Aggregate (Switzerland). Methanol (3mL) was then added and mixed thoroughly followed by chloroform (6mL). 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. The phospholipids fraction of tissue lipids were then separated from other lipids by TLC (see section 2.2.7.3) and then the phospholipids band scraped into 20mL scintillation vials containing 5mL of 1% H_2SO_4 in methanol. Vials were 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 AR heptane. Samples were then stored at 20°C in GC vials with anhydrous sodium sulphate (30mg) for fatty acid analysis (see section 2.2.7.3). All organic solvents contained BHA (0.005%) as an antioxidant.

2.2.8 Gas Chromatograph Analysis of FAME

The fatty acid composition of samples was determined and quantified using a Hewlett-Packard 6890 GC (California, USA) equipped with flame ionization detection and a capillary column (50m x 0.32mm internal diameter) coated with 70% cyanoproply polysilphenylene-siloxane with a film thickness of 0.25μm (BPX-70, SGE, Victoria, Australia). The gas chromatograph was operated with an initial oven temperature at 140°C and programmed to rise by 5°C per minute to 220°C and held for 2 minutes. The temperature was then increased by 20°C per minute to 260°C and held for 8 minutes. Methyl esters were separated using a carrier gas (helium) at a flow rate of 33cm per second and the inlet split ratio was set at 20:1. The injection split and the flame ionization detector temperature was set at 250 and 300°C respectively.

The percentage of fatty acids was integrated and calculated by comparison of retention times to authentic lipid standards (463, Nucheck Prep Inc., Elysian, MN, USA) using the software package Agilent GC Chemstation Rev. B.01.03 (204) 2001-2005 (Agilent Technologies Inc., Palo Alto, CA, USA). However, the program only measured 50 of the 61 FAMEs of the sample analysed leaving 11 FAMEs which were not in the 463 standards (Figure 2.2). Therefore, the other 11 FAMEs needed to identified and labelled manually into the chromatograph by calibrating the peaks of these FAMEs to have the same response as a peak with a similar retention time. At the beginning of every run, an external standard (3mg/mL) from Nucheck Prep was analysed and used as calibration for the samples. Each peak from a trace was expressed as the relative percentage of each fatty acid.

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.

2.2.9 Statistical Analysis

A one-way analysis of variance (ANOVA) using SPSS version 15.0 for Windows was used to examine the effect on final weight and EPA, DPA and DHA concentrations of variables tested of the experimental diets. The analysis was followed by the Tukey-b test if there were significant differences ($P<0.05$) among dietary treatments.

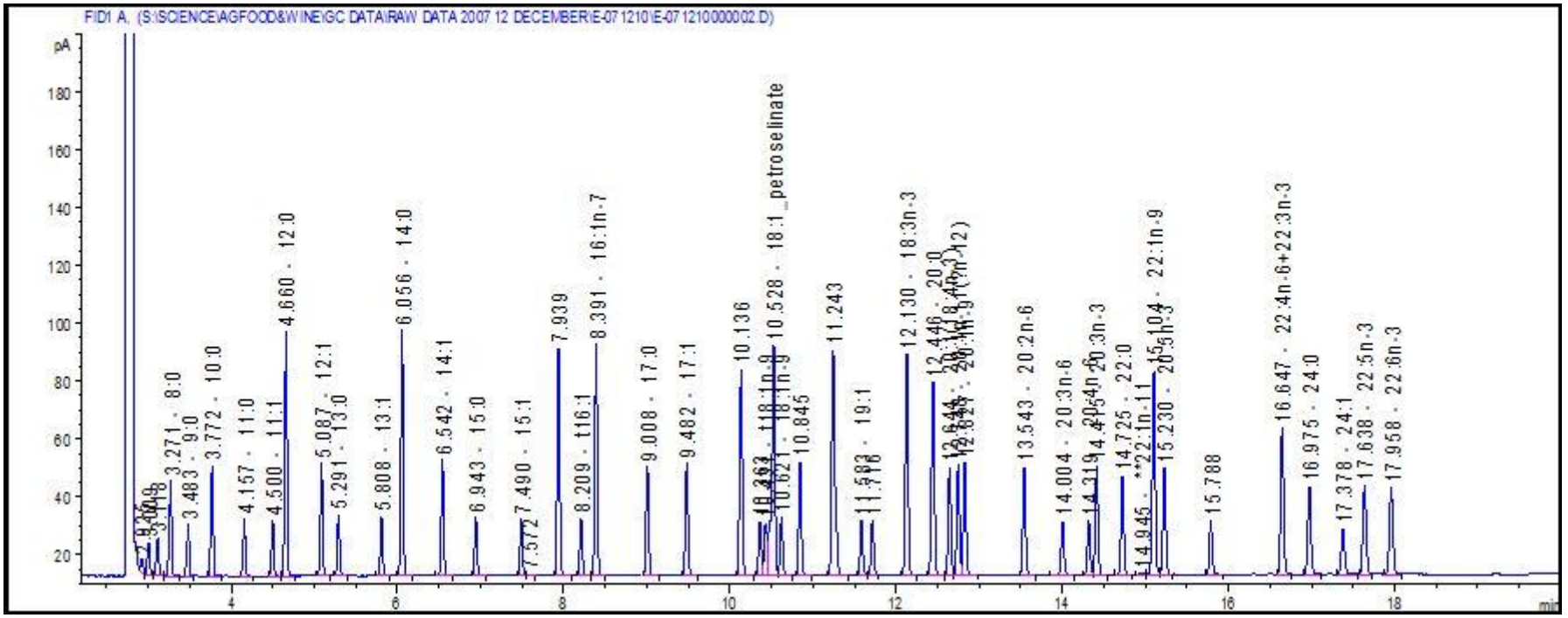


Figure 2.2 Chromatogram of standards obtained from Nucheck Prep Inc. (Elysian, MN)

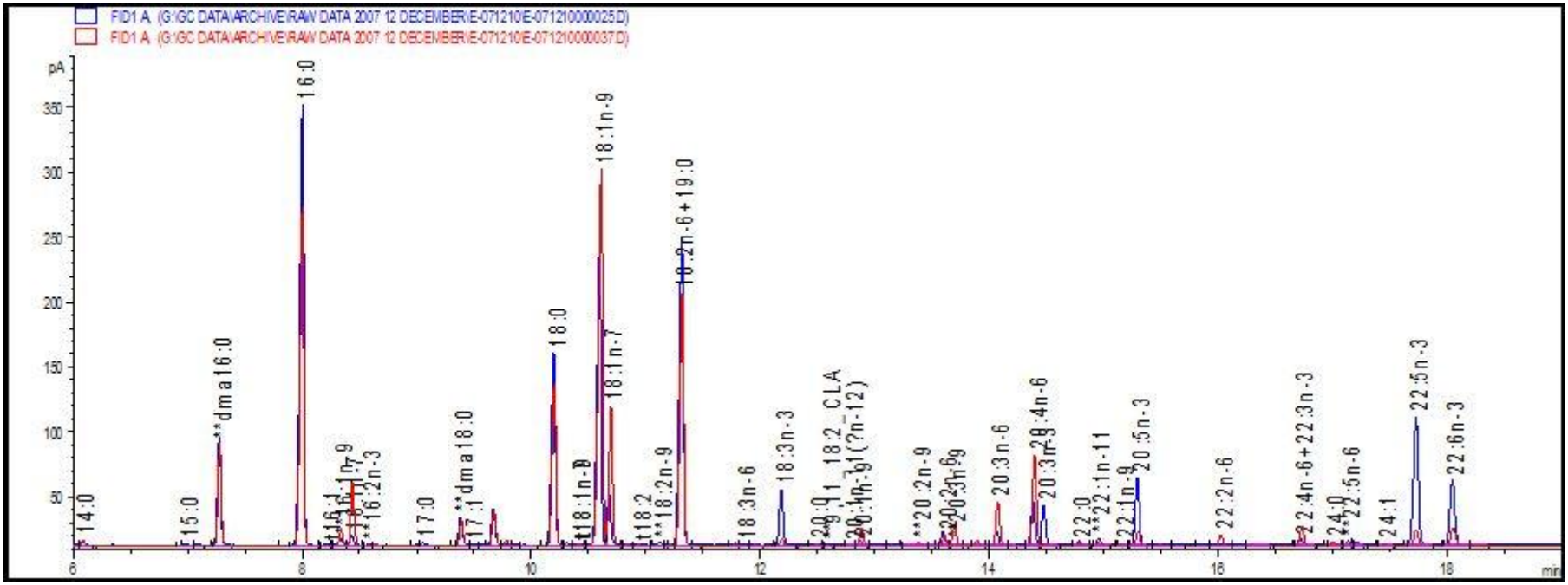


Figure 2.3 A chromatogram of FAMEs derived from a breast sample fed high in ALA content (blue colour) compared to a control sample (red colour)

2.3 RESULTS

2.3.1 Blood Fatty Acids

The fatty acid composition of erythrocytes and plasma are shown in Tables 2.5 and 2.6. The proportion of ALA in both plasma and erythrocytes increased as the dietary LA to ALA ratio was lowered ($P<0.001$); however, the level of ALA in erythrocytes was nearly twice that of in plasma. The metabolites of dietary ALA, EPA, DPA and DHA increased in response to the increased availability of dietary ALA in both blood tissues ($P<0.001$); however, the n-3 LCPUFA levels were generally higher in plasma than erythrocytes (Tables 2.5 and 2.6).

The response to increase in LCPUFA in blood samples was the same either by decreasing LA to ALA ratio (Figure 2.4) or increasing ALA content of diets (Figure 2.5). Dietary treatment with 8% ALA as energy (the lowest LA to ALA ratio) increased the proportion of erythrocyte and plasma DHA which was 2 and 3-fold higher than that of birds fed the control diet (Figure 2.5).

While the level of total n-3 fatty acids increased, the proportion of total n-6 fatty acids of blood samples decreased slightly as a result of lowered ALA level of the diets ($P<0.001$). The lowest ALA dietary content resulted in the lowest level of total erythrocyte and plasma n-6 PUFA (Table 2.5 and 2.6). The LA level of erythrocyte phospholipids decreased slightly ($P<0.001$) by increasing ALA level in the diets while the proportion of LA in the plasma phospholipids was not different among dietary treatments.

Table 2.5 Fatty acid composition of erythrocytes phospholipids from chickens fed experimental diets varying in LA to ALA ratio for 28 days[1]

	Experimental Diets								
	Control	**D1**	**D2**	**D3**	**D4**	**D5**	**D6**		
LA (% en)	2.72	3.96	3.77	3.67	3.88	4.06	4.59		
ALA (% en)	0.26	1.10	1.61	3.19	2.07	5.82	7.97		
LA:ALA ratio	10.46:1	3.60:1	2.34:1	1.15:1	1.87:1	0.70:1	0.58:1		
Fat Content (%)	5.29	5.37	5.13	5.38	6.71	7.26	9.41		
Fatty acid[2]	(% of total fatty acids)[1]							PSEM	P value
16:0	18.63^{a}	20.59^{b}	21.07^{bc}	22.55^{c}	20.81^{b}	21.10^{bc}	19.53^{ab}	0.391	***
18:0	11.11^{a}	10.84^{a}	11.10^{a}	12.07^{ab}	11.87^{ab}	13.24^{bc}	14.26^{c}	0.392	***
SFA	38.24^{bc}	39.06^{c}	34.65^{a}	36.09^{ab}	38.76^{bc}	41.75^{d}	42.12^{d}	0.683	***
18:1n-9	17.73^{c}	12.72^{b}	12.83^{b}	11.26^{b}	11.29^{b}	7.49^{a}	6.82^{a}	0.626	***
18:1n-7	2.11^{e}	1.49^{cd}	1.67^{d}	1.34^{c}	1.40^{c}	0.84^{b}	0.65^{a}	0.022	***
MUFA	23.44^{d}	16.66^{bc}	18.07^{c}	15.10^{b}	15.10^{b}	9.72^{a}	8.64^{a}	0.684	***
20:3n-9	0.36^{d}	0.30^{cd}	0.27^{bc}	0.24^{bc}	0.22^{b}	0.12^{a}	0.10^{a}	0.019	***
18:2n-6	29.18^{a}	33.74^{b}	31.90^{ab}	32.41^{b}	31.24^{ab}	29.61^{a}	29.15^{a}	0.679	***
20:3n-6	0.75^{c}	0.58^{b}	0.59^{b}	0.57^{b}	0.49^{ab}	0.41^{a}	0.38^{a}	0.035	***
20:4n-6	3.79^{c}	3.39^{bc}	3.32^{abc}	3.09^{abc}	2.91^{abc}	2.31^{ab}	2.21^{a}	0.265	**
Total n-6	34.83^{ab}	38.59^{c}	39.42^{c}	36.92^{bc}	35.35^{ab}	32.95^{a}	32.28^{a}	0.812	***
18:3n-3	0.67^{a}	1.69^{b}	2.44^{c}	4.76^{e}	3.96^{d}	7.24^{f}	8.54^{g}	0.126	***
20:3n-3	0.03^{a}	0.05^{ab}	0.08^{b}	0.12^{c}	0.10^{c}	0.17^{d}	0.18^{d}	0.007	***
20:5n-3	0.29^{a}	0.58^{a}	0.97^{b}	1.69^{c}	1.52^{c}	2.49^{d}	2.83^{d}	0.100	***
22:5n-3	0.60^{a}	1.09^{b}	1.42^{c}	1.86^{d}	1.76^{d}	2.39^{e}	2.45^{e}	0.068	***
22:6n-3	1.08^{a}	1.60^{b}	2.11^{c}	2.70^{d}	2.68^{d}	2.80^{d}	2.57^{cd}	0.245	***
Total n-3 FA	2.72^{a}	5.03^{b}	7.07^{c}	11.17^{e}	10.06^{d}	15.13^{f}	16.60^{g}	0.245	***
Total PUFA	37.55^{a}	43.62^{b}	46.49^{bcd}	48.09^{cd}	45.41^{bc}	48.08^{cd}	48.89^{d}	0.798	***

[a,b]Values in the same row with no common superscript are significantly different (P<0.05)
[1]Values are means of six observations per treatment and their pooled standard error of the mean (PSEM)
[2]SFA=saturated fatty acid; MUFA=monounsaturated fatty acid; PUFA= polyunsaturated fatty acid
NS=not significant
**P<0.01
***P<0.001

Table 2.6 Fatty acid composition of plasma phospholipids from chickens fed experimental diets varying in LA to ALA ratio for 28 days[1]

	Experimental Diets								
	Control	**D1**	**D2**	**D3**	**D4**	**D5**	**D6**		
LA (% en)	2.72	3.96	3.77	3.67	3.88	4.06	4.59		
ALA (% en)	0.26	1.10	1.61	3.19	2.07	5.82	7.97		
LA:ALA ratio	10.46:1	3.60:1	2.34:1	1.15:1	1.87:1	0.70:1	0.58:1		
Fat Content (%)	5.29	5.37	5.13	5.38	6.71	7.26	9.41		
Fatty acid[2]	(% of total fatty acids)[1]							PSEM	P value
16:0	20.48^{a}	22.21^{b}	21.91^{b}	23.94^{c}	21.83^{ab}	21.99^{b}	21.06^{ab}	0.345	***
18:0	24.84^{ab}	23.70^{a}	24.62^{ab}	23.32^{a}	24.26^{ab}	25.95^{bc}	27.29^{c}	0.429	***
SFA	46.67^{a}	47.32^{b}	47.82^{b}	48.76^{c}	47.47^{b}	49.49^{d}	49.91^{d}	0.175	***
18:1n-9	16.09^{c}	13.05^{b}	12.54^{b}	11.51^{b}	11.39^{b}	7.70^{a}	6.61^{a}	0.725	***
18:1n-7	2.64^{c}	1.90^{b}	1.95^{b}	1.72^{b}	1.77^{b}	1.11^{a}	0.88^{a}	0.074	***
MUFA	21.39^{c}	16.79^{b}	16.30^{b}	15.01^{b}	14.95^{b}	9.96^{a}	8.48^{a}	0.850	***
20:3n-9	1.07^{d}	0.93^{cd}	0.84^{c}	0.59^{b}	0.61^{b}	0.34^{a}	0.22^{a}	0.057	***
18:2n-6	20.84^{ab}	22.42^{b}	21.27^{ab}	20.12^{a}	21.77^{ab}	21.31^{ab}	21.07^{ab}	0.514	NS
20:3n-6	1.34^{c}	1.12^{bc}	1.18^{bc}	0.97^{ab}	0.84^{a}	0.77^{a}	0.72^{a}	0.068	***
20:4n-6	4.78	5.61	4.88	3.98	4.74	3.53	3.47	0.526	NS
Total n-6	28.12^{ab}	30.38^{b}	28.42^{ab}	25.99^{a}	28.20^{ab}	26.35^{a}	25.87^{a}	0.728	**
18:3n-3	0.30^{a}	0.74^{b}	1.02^{b}	1.84^{c}	1.67^{c}	3.41^{d}	3.89^{e}	0.096	***
20:3n-3	0.05^{a}	0.08^{ab}	0.13^{ab}	0.20^{b}	0.16^{ab}	0.36^{c}	0.43^{c}	0.028	***
20:5n-3	0.35^{a}	0.84^{a}	1.48^{b}	2.60^{c}	2.11^{c}	3.68^{d}	4.67^{e}	0.137	***
22:5n-3	0.35^{a}	0.84^{b}	1.01^{b}	1.76^{c}	1.52^{c}	2.49^{d}	2.96^{e}	0.127	***
22:6n-3	1.00^{a}	1.56^{a}	2.50^{b}	2.83^{bc}	2.95^{bc}	3.68^{bc}	3.37^{c}	0.234	***
Total n-3 FA	2.12^{a}	4.10^{b}	6.16^{c}	9.24^{d}	8.43^{d}	13.63^{e}	15.34^{f}	0.340	***
Total PUFA	30.24^{a}	34.48^{b}	34.59^{b}	35.23^{b}	36.63^{bc}	39.98^{cd}	41.20^{d}	0.936	***

[a,b]Values in the same row with no common superscript are significantly different (P<0.05)
[1]Values are means of six observations per treatment and their pooled standard error of the mean (PSEM)
[2]SFA=saturated fatty acid; MUFA=monounsaturated fatty acid; PUFA= polyunsaturated fatty acid
NS=not significant
**P<0.01
***P<0.001

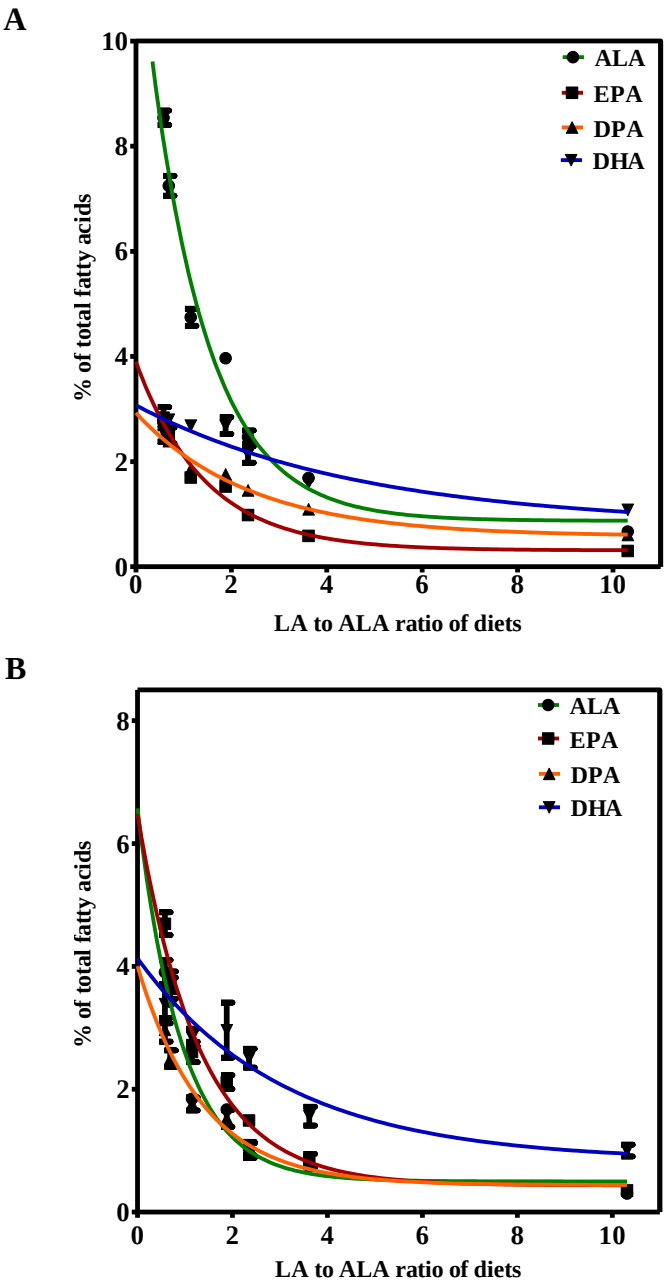


Figure 2.4 Effects of lowered LA to ALA ratio of diets on n-3 LCPUFA of erythrocyte (A) and plasma (B) samples. The values presented are means ± SEM (n=6/diet). There were significant differences on the level of all n-3 LCPUFA ($P<0.001$).

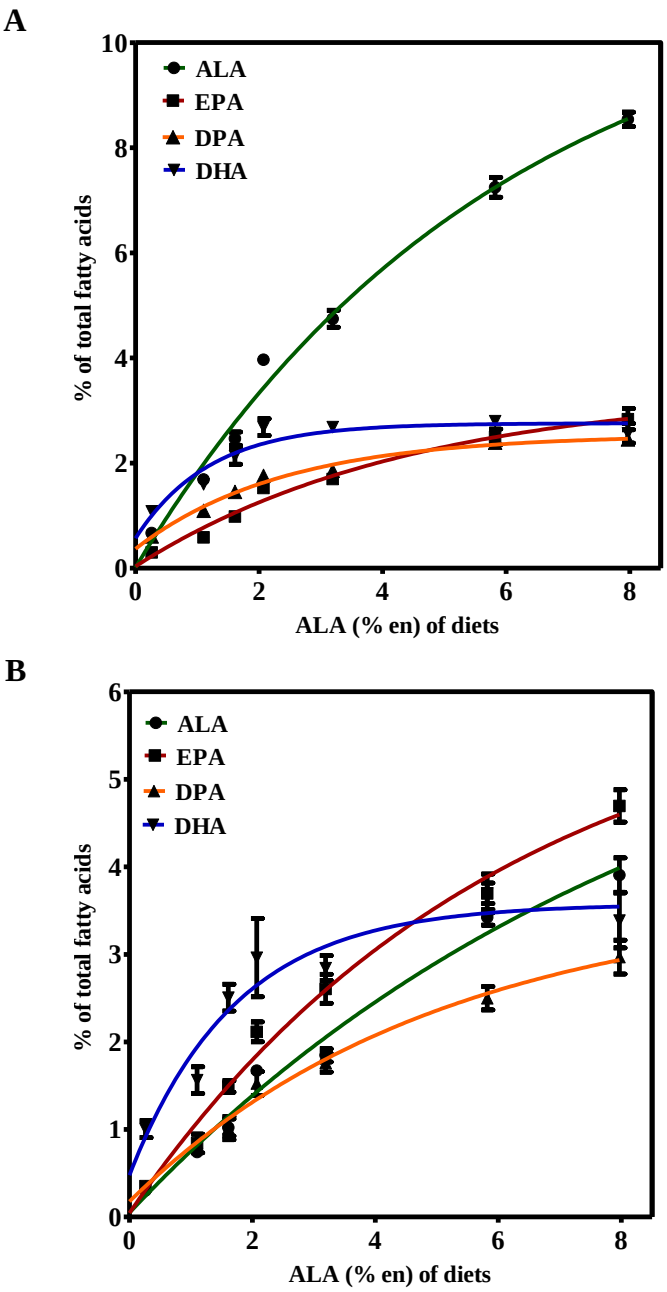


Figure 2.5 Effects of increasing levels of dietary ALA on n-3 LCPUFA of erythrocyte (A) and plasma (B) samples. The values presented are means ± SEM (n=6/diet). There were significant differences on the level of all n-3 LCPUFA ($P<0.001$).

As expected, the AA content of erythrocytes decreased significantly when EPA levels were increased ($P<0.01$). The level of erythrocyte AA in birds fed diet 6 with an LA to ALA ratio of 0.6:1 was almost half of those fed with control diet (LA to ALA ratio of 10.5:1). The level of plasma AA was not different among dietary treatments.

Supplementation of chicken diets with blended vegetable oils containing increased levels of ALA resulted in a modest increase in saturated fatty acid content of erythrocyte and plasma samples ($P<0.001$). The highest saturated fatty acid level was achieved when the ratio of LA to ALA of the diets was the lowest. Stearic acid (C18:0) was the saturated fatty acid which contributed greatest to the observed changes. The MUFA content of erythrocyte and plasma phospholipids decreased in response to decreasing the LA to ALA ratio ($P<0.001$). The lowest LA to ALA ratio (0.6 to 1) resulted in the lowest MUFA content, which was 2.5-fold lower than in birds fed the control diet. Conversely, the total PUFA content of erythrocyte and plasma samples increased ($P<0.001$) as a result of increasing the ALA content of the diets achieving 48.89 and 41.20% of the total fatty acids, respectively when the LA to ALA ratio of diets was at the lowest level. The increase in total PUFA was mainly due to an increase in total n-3 PUFA. In fact, the increased proportion of ALA in the diet caused a decrease in the level of total n-6 PUFA.

The exchange between the various unsaturated fats in the plasma and erythrocyte samples is summarised in Figure 2.6 The increase in total n-3 PUFA, mainly in the

form of ALA in erythrocyte samples and n-3 LCPUFA in plasma samples was offset mainly by a decrease in MUFA content (Figure 2.6).

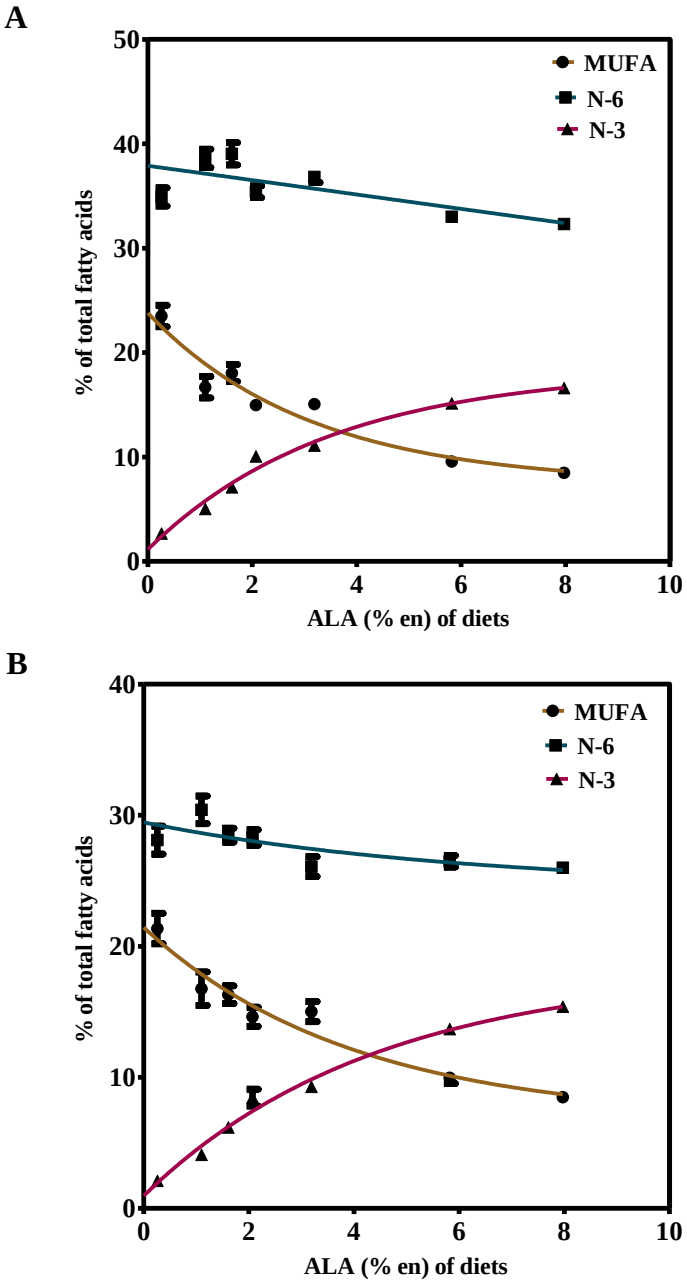


Figure 2.6 The balance of erythrocyte (A) and plasma (B) MUFA, n-6 and n-3 fatty acids of birds fed different dietary levels of ALA. The values presented are means ± SEM (n=6/diet). There were significant differences on the level of MUFA, total n-6 and n-3 PUFA ($P<0.001$).

2.3.2 Tissue Fatty Acids

The fatty acid content of liver, breast and thigh samples are shown in Table 2.7, 2.8 and 2.9, respectively. We observed that there was no effect on fat content of tissue phospholips including liver, breast and thigh with increasing dietary levels of ALA.

Table 2.7 Fatty acid compositions of liver phospholipids from chickens fed experimental diets varying in LA to ALA ratio for 28 days[1]

	Experimental Diets								
	Control	**D1**	**D2**	**D3**	**D4**	**D5**	**D6**		
LA (% en)	2.72	3.96	3.77	3.67	3.88	4.06	4.59		
ALA (% en)	0.26	1.10	1.61	3.19	2.07	5.82	7.97		
LA:ALA ratio	10.46:1	3.60:1	2.34:1	1.15:1	1.87:1	0.70:1	0.58:1		
Fat Content (%)	5.29	5.37	5.13	5.38	6.71	7.26	9.41		
Fatty acid[2]	(% of total fatty acids)[1]							PSEM	P value
16:0	16.36^{ab}	17.45^{bc}	17.22^{b}	18.75^{c}	16.91^{ab}	16.66^{ab}	15.59^{a}	0.356	***
18:0	26.82^{ab}	26.62^{a}	26.82^{ab}	25.64^{a}	27.12^{ab}	28.15^{b}	30.07^{c}	0.359	***
SFA	44.71^{a}	45.50^{ab}	45.38^{ab}	45.78^{bc}	45.30^{ab}	46.36^{c}	47.20^{d}	0.206	***
18:1n-9	14.69^{c}	11.87^{b}	11.58^{b}	10.58^{b}	10.31^{b}	7.17^{a}	5.88^{a}	0.723	***
18:1n-7	2.68^{c}	1.85^{b}	1.93^{b}	1.72^{b}	1.79^{b}	1.12^{a}	0.88^{a}	0.086	***
MUFA	20.23^{c}	15.80^{b}	15.56^{b}	14.21^{b}	13.91^{b}	9.47^{a}	7.70^{a}	0.916	***
20:3n-9	1.77^{e}	0.95^{cd}	1.03^{d}	0.64^{cd}	0.59^{bc}	0.25^{ab}	0.10^{a}	0.099	***
18:2n-6	18.15^{ab}	19.63^{b}	18.69^{ab}	17.99^{ab}	18.86^{ab}	18.10^{ab}	17.78^{a}	0.409	*
20:3n-6	1.67^{c}	1.51^{bc}	1.54^{bc}	1.32^{ab}	1.24^{ab}	1.26^{ab}	1.00^{a}	0.079	***
20:4n-6	8.88^{cd}	9.34^{d}	8.20^{cd}	6.71^{abc}	7.62^{bcd}	5.58^{ab}	4.96^{a}	0.586	***
Total n-6	29.96^{de}	31.95^{e}	29.65^{cde}	27.15^{bc}	28.80^{cd}	25.97^{ab}	24.54^{a}	0.654	***
18:3n-3	0.16^{a}	0.45^{b}	0.63^{b}	1.15^{d}	0.93^{c}	1.85^{e}	2.20^{f}	0.057	***
20:3n-3	0.06^{a}	0.11^{ab}	0.15^{ab}	0.24^{b}	0.21^{b}	0.55^{c}	0.65^{c}	0.031	***
20:5n-3	0.51^{a}	1.15^{b}	1.95^{c}	3.51^{e}	2.83^{d}	5.32^{f}	6.76^{g}	0.164	***
22:5n-3	0.41^{a}	1.09^{b}	1.24^{b}	2.27^{c}	2.07^{c}	3.53^{d}	4.16^{d}	0.184	***
22:6n-3	1.64^{a}	2.57^{ab}	4.03^{bc}	4.73^{c}	5.08^{cd}	6.50^{d}	6.53^{d}	0.412	***
Total n-3 FA	2.82^{a}	5.39^{b}	8.02^{c}	11.93^{d}	11.14^{d}	17.77^{e}	20.31^{f}	0.499	***
Total PUFA	32.78^{a}	37.34^{b}	37.67^{b}	39.08^{b}	39.94^{b}	43.74^{c}	44.86^{c}	0.986	***
	Fat Content (% fresh weight)								
	4.49	4.44	4.69	4.38	4.90	4.40	4.20	0.254	NS

[a,b]Values in the same row with no common superscript are significantly different (P<0.05)
[1]Values are means of six observations per treatment and their pooled standard error of the mean (PSEM)
[2]SFA=saturated fatty acid; MUFA=monounsaturated fatty acid; PUFA= polyunsaturated fatty acid
NS=not significant
*P<0.05
***P<0.001

Table 2.8 Fatty acid composition of breast phospholipids from chickens fed experimental diets varying in LA to ALA ratio for 28 days[1]

	Experimental Diets								
	Control	**D1**	**D2**	**D3**	**D4**	**D5**	**D6**		
LA (% en)	2.72	3.96	3.77	3.67	3.88	4.06	4.59		
ALA (% en)	0.26	1.10	1.61	3.19	2.07	5.82	7.97		
LA:ALA ratio	10.46:1	3.60:1	2.34:1	1.15:1	1.87:1	0.70:1	0.58:1		
Fat Content (%)	5.29	5.37	5.13	5.38	6.71	7.26	9.41		
Fatty acid[2]	(% of total fatty acids)[1]							PSEM	P value
16:0	19.01^{a}	21.39^{b}	20.72^{ab}	21.80^{b}	20.87^{ab}	22.46^{b}	22.07^{b}	0.473	***
18:0	8.78^{a}	8.82^{a}	9.53^{a}	9.53^{a}	9.17^{a}	10.17^{ab}	11.25^{b}	0.324	***
SFA	35.53^{a}	38.08^{b}	38.54^{bc}	39.86^{cd}	38.47^{bc}	40.94^{d}	41.41^{d}	0.418	***
18:1n-9	26.53^{d}	22.01^{c}	22.04^{c}	18.71^{b}	19.66^{bc}	15.17^{a}	15.22^{a}	0.685	***
18:1n-7	6.51^{c}	4.58^{b}	4.62^{b}	4.17^{b}	4.12^{b}	2.59^{a}	2.29^{a}	0.126	***
MUFA	38.86^{d}	30.32^{c}	30.27^{c}	25.82^{b}	26.46^{b}	19.64^{a}	18.77^{a}	0.840	***
20:3n-9	1.01^{c}	0.45^{b}	0.45^{b}	0.25^{ab}	0.20^{a}	0.09^{a}	0.05^{a}	0.055	***
18:2n-6	13.39^{a}	15.85^{ab}	14.33^{ab}	15.08^{ab}	14.98^{ab}	16.01^{b}	15.79^{ab}	0.570	*
20:3n-6	1.57^{c}	1.34^{c}	1.26^{bc}	0.92^{ab}	0.83^{a}	0.79^{a}	0.64^{a}	0.096	***
20:4n-6	3.75^{b}	4.15^{b}	3.80^{b}	3.19^{ab}	3.37^{ab}	2.49^{a}	2.32^{a}	0.291	***
Total n-6	20.81^{a}	23.99^{b}	21.53^{a}	20.84^{a}	20.85^{a}	20.73^{a}	19.89^{a}	0.667	**
18:3n-3	0.45^{a}	0.98^{b}	1.21^{bc}	1.98^{d}	1.69^{cd}	3.16^{f}	2.61^{e}	0.134	***
20:3n-3	0.15^{a}	0.46^{b}	0.57^{bc}	0.88^{d}	0.78^{cd}	1.70^{e}	1.55^{e}	0.080	***
20:5n-3	0.69^{a}	1.21^{a}	1.80^{b}	2.86^{c}	2.81^{c}	3.82^{d}	3.83^{d}	0.145	***
22:5n-3	0.90^{a}	2.30^{b}	2.93^{b}	4.20^{c}	4.72^{c}	6.09^{d}	7.36^{e}	0.247	***
22:6n-3	1.02^{a}	1.73^{ab}	2.28^{abc}	2.98^{bcd}	3.69^{d}	3.52^{cd}	4.22^{d}	0.333	***
Total n-3 FA	3.30^{a}	6.74^{b}	8.84^{c}	12.95^{d}	13.75^{d}	18.32^{e}	19.60^{e}	0.534	***
Total PUFA	24.11^{a}	30.72^{b}	30.37^{b}	33.79^{c}	34.60^{c}	39.05^{d}	39.49^{d}	0.751	***
	Fat Content (% fresh weight)								
	0.84	0.83	0.82	0.81	0.88	0.93	0.95	0.1077	NS

[a,b]Values in the same row with no common superscript are significantly different (P<05)
[1]Values are means of six observations per treatment.
[2]SFA=saturated fatty acid; MUFA=monounsaturated fatty acid; PUFA= polyunsaturated fatty acid
PSEM = pooled standard error of the mean
NS=not significant
*P<0.05
**P<0.01
***P<001

Table 2.9 Fatty acid composition of thigh phospholipids from chickens fed experimental diets varying in LA to ALA ratio for 28 days[1]

	Experimental Diets								
	Control	**D1**	**D2**	**D3**	**D4**	**D5**	**D6**		
LA (% en)	2.72	3.96	3.77	3.67	3.88	4.06	4.59		
ALA (% en)	0.26	1.10	1.61	3.19	2.07	5.82	7.97		
LA:ALA ratio	10.46:1	3.60:1	2.34:1	1.15:1	1.87:1	0.70:1	0.58:1		
Fat Content (%)	5.29	5.37	5.13	5.38	6.71	7.26	9.41		
Fatty acid[2]	(% of total fatty acids)[1]							PSEM	P value
16:0	18.32	19.75	18.80	19.79	18.70	20.07	19.33	0.448	NS
18:0	11.20^{a}	11.26^{a}	11.85^{a}	11.42^{a}	11.68^{a}	12.61^{ab}	13.82^{b}	0.484	**
SFA	37.39^{a}	38.83^{abc}	38.53^{ab}	39.10^{abc}	38.57^{ab}	40.26^{bc}	40.50^{c}	0.422	***
18:1n-9	24.84^{d}	20.79^{bc}	21.20^{c}	17.69^{b}	18.14^{bc}	13.69^{a}	12.81^{a}	0.788	***
18:1n-7	5.71^{c}	4.19^{b}	4.05^{b}	3.81^{b}	3.80^{b}	2.38^{a}	2.09^{a}	0.124	***
MUFA	35.39^{d}	28.03^{c}	28.41^{c}	24.13^{b}	24.38^{b}	17.72^{a}	16.17^{a}	0.969	***
20:3n-9	1.09^{e}	0.46^{cd}	0.50^{d}	0.28^{bc}	0.22^{ab}	0.10^{ab}	0.06^{a}	0.053	***
18:2n-6	15.16^{a}	18.55^{bc}	16.98^{ab}	18.04^{bc}	18.60^{bc}	19.51^{c}	19.92^{c}	0.590	***
20:3n-6	1.16^{d}	1.04^{cd}	1.05^{cd}	0.97^{bc}	0.81^{ab}	0.84^{ab}	0.77^{a}	0.043	***
20:4n-6	4.39^{c}	4.53^{c}	4.22^{c}	3.67^{bc}	3.72^{bc}	2.75^{ab}	2.59^{a}	0.252	***
Total n-6	22.55	26.28	24.06	24.21	24.58	24.35	24.35	0.765	NS
18:3n-3	0.38^{a}	0.90^{ab}	1.30^{bc}	2.11^{d}	1.86^{cd}	3.19^{e}	3.08^{e}	0.165	***
20:3n-3	0.13^{a}	0.40^{ab}	0.47^{bc}	0.76^{c}	0.62^{bc}	1.40^{d}	1.42^{d}	0.079	***
20:5n-3	0.57^{a}	0.93^{a}	1.51^{b}	2.24^{c}	2.17^{c}	3.05^{d}	3.14^{d}	0.119	***
22:5n-3	0.99^{a}	2.24^{b}	2.86^{b}	4.23^{c}	4.37^{c}	6.20^{d}	7.20^{e}	0.213	***
22:6n-3	0.93^{a}	1.44^{ab}	1.93^{bc}	2.63^{cd}	2.93^{de}	3.49^{ef}	3.86^{f}	0.204	***
Total n-3 FA	3.08^{a}	6.01^{b}	8.14^{c}	12.01^{d}	12.00^{d}	17.35^{e}	18.74^{e}	0.432	***
Total PUFA	25.63^{a}	32.29^{b}	32.20^{b}	36.21^{c}	36.58^{c}	41.70^{d}	43.09^{d}	0.954	***
	Fat Content (% fresh weight)								
	1.32	1.30	1.29	1.29	1.32	1.41	1.48	0.1607	NS

[a,b]Values in the same row with no common superscript are significantly different (P<0.05)
[1]Values are means of six observations per treatment and their pooled standard error of the mean (PSEM)
[2]SFA=saturated fatty acid; MUFA=monounsaturated fatty acid; PUFA= polyunsaturated fatty acid
NS=not significant
**P<0.01
***P<0.001

Increased dietary ALA resulted in increased membrane phospholipids ALA in liver, breast and thigh tissues ($P<0.001$) but unlike erythrocytes, the levels rarely exceeded 3% of total fatty acids. The changes in n-3 LCPUFA as a result of the dietary treatments were somewhat tissue specific. For example in liver, the level of EPA and DHA responded most to dietary ALA whereas in breast and thigh the greatest responder to dietary ALA was DPA. The reasons for this are not clear. In general there was a direct relationship between dietary ALA expressed either as LA to ALA ratio or ALA content of diets (Figures 2.7 and 2.8) and the level of the major n-3 LCPUFA, EPA, DPA and DHA. The highest level of n-3 LCPUFA was achieved at the highest level of dietary ALA.

N-3 LCPUFA accumulation in breast and thigh tissues is shown in Figures 2.7 and 2.8. The results show that there was no clear maximum of tissue EPA, DPA and DHA levels by the dietary treatments as shown in Figure 2.7 plotted against the LA to ALA ratio, nor in Figure 2.8 when plotted against ALA content expressed as % energy. Dietary treatment with ALA content of 8% en (also the lowest LA to ALA ratio) increased the proportion of tissue EPA, DPA and DHA including breast and thigh tissue to 5 and 4-fold higher than the control. The data also indicates that there was no optimal amount of total dietary fat content since the highest values were scored with diets containing a higher amount of ALA, diet 6 which contained 9% fat (Figure 2.8). In general any increase in n-3 LCPUFA level in tissues was offset by a decrease in the MUFA content of tissues (Figure 2.9).

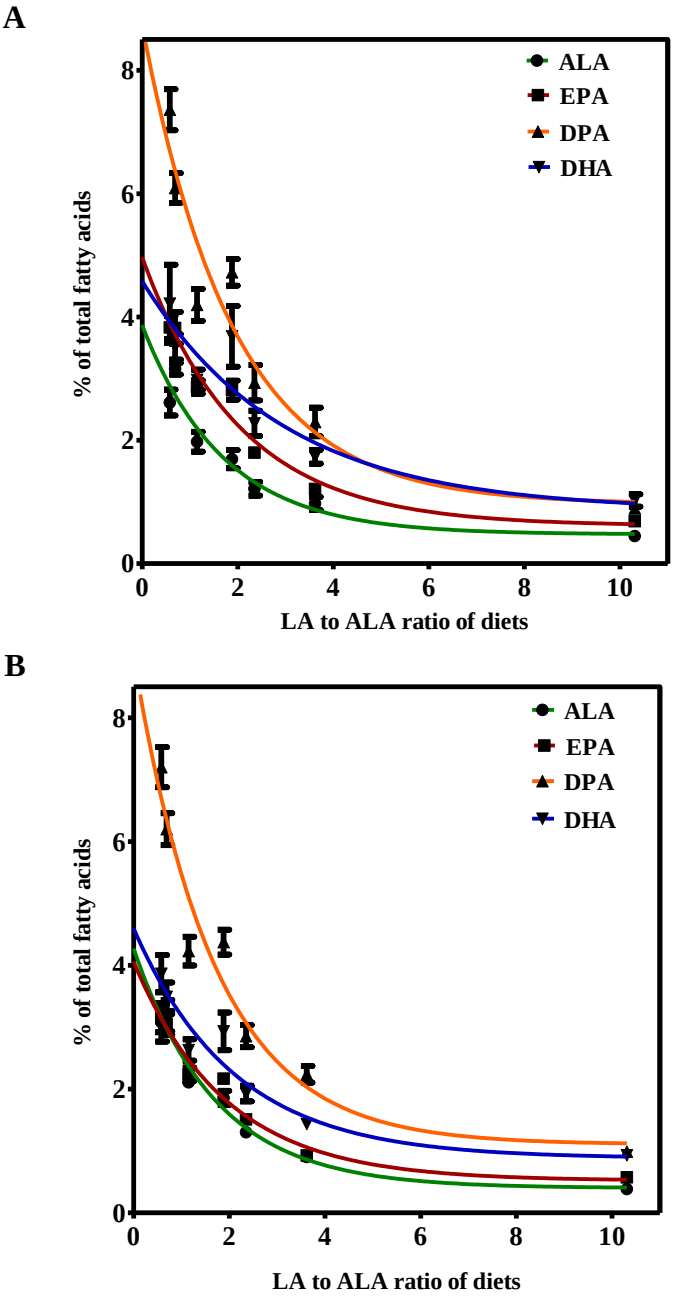


Figure 2.7 Effects of lowered LA to ALA ratio of diets on n-3 LCPUFA of breast (A) and thigh (B) tissue. The values presented are means ± SEM (n=6/diet). There were significant differences on the level of n-3 LCPUFA (P<0.001) among groups.

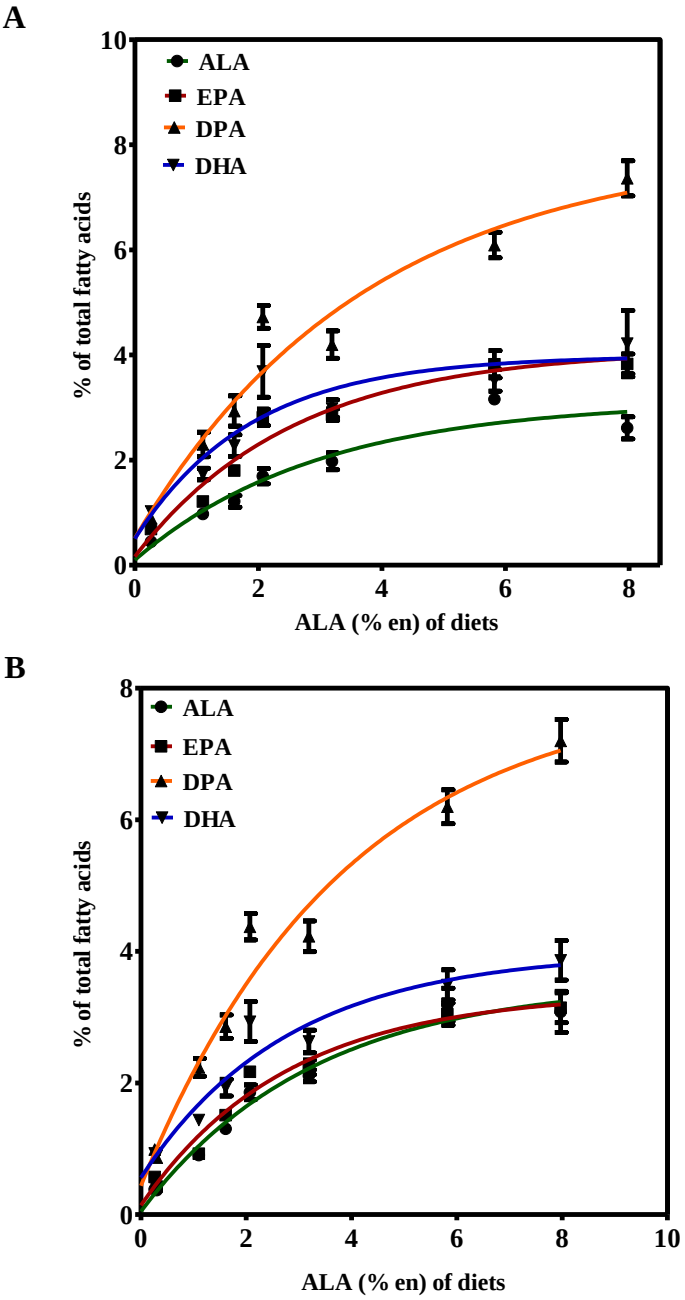


Figure 2.8 Effects of increasing levels of dietary ALA on n-3 LCPUFA of breast (A) and thigh (B) tissue. The values presented are means of six replicate analyses ± SEM. There were significant differences on the level of n-3 LCPUFA ($P<0.001$) among groups.

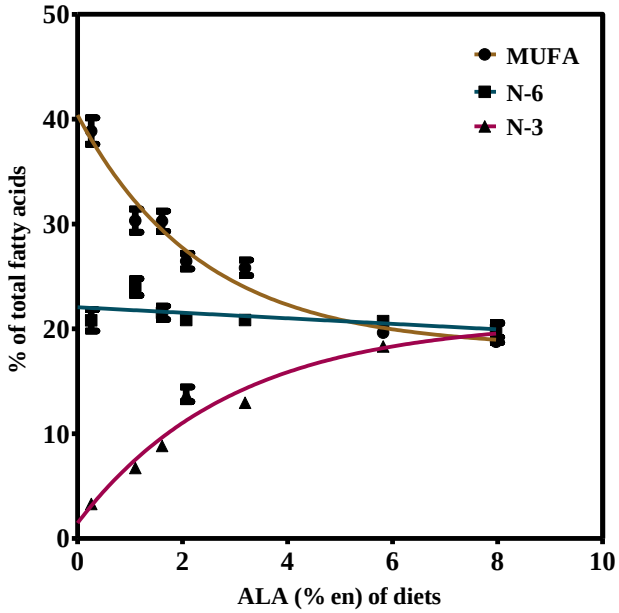


Figure 2.9 The balance of MUFA, n-6 and n-3 fatty acids of breast tissues of birds fed with different dietary levels of ALA. The values presented are means ± SEM (n=6/diet). Dietary treatments increased (P<0.001) total n-3 PUFA and reduced MUFA while total n-6 remained constant.

Our results indicated that there was a strong correlation between breast EPA and erythrocyte EPA (r^2 = 0.84, P<0.001; Figure 2.10, A), as well as breast EPA and plasma EPA (r^2 = 0.81, P<0.001; Figure 2.10, B). We also observed that the proportion of breast phospholipid DHA was significantly and positively correlated with DHA levels both in erythrocyte phospholipids (r^2 = 0.72, P<0.001; Figure 2.11, A) and in plasma phospholipids (r^2 = 0.72, P<0.001; Figure 2.11, B). Similarly, we also observed that there was a significant and positive correlation between plasma (EPA and DHA) and thigh meat (EPA and DHA) (Figure 2.12).

The changes in n-6 fatty acids of liver, breast and thigh tissue to dietary ALA were generally small (Table 2.7, 2.8 and 2.9). Only in liver was there a general towards a decreased (P<0.001) n-6 total PUFA as a result of increased ALA content in the

diets. The n-6 PUFA levels of breast and thigh tissues remained fairly constant. Lowering the LA to ALA ratio slightly increased the LA content of breast and thigh samples.

In addition, AA content in all groups decreased significantly when EPA content increased in the tissue samples ($P<0.001$; Figure 2.13). The decrease in the proportion of AA was almost double in diet 6, which was 2.3 and 2.6% of total fatty acids in breast and thigh samples, respectively.

The saturated fatty acid content of all tissue samples increased modestly ($P<0.001$) but only when the ratio of LA to ALA of the diets was lowest. Stearic acid (C18:0) contributed greatest to the observed changes. The MUFA content of all tissue samples reduced ($P<0.001$) by decreasing the LA to ALA ratio. The lowest LA to ALA ratio (the highest ALA content), which was 0.6 to 1, resulted in the lowest MUFA content. Increasing the ALA content of dietary fats caused an increase in the total PUFA content in tissue samples to 44.9, 39.5 and 43.1% of total fatty acids in liver, breast and thigh samples, respectively ($P<0.001$), and the highest level was achieved when the LA to ALA ratio of diets was lowest (Tables 2.7, 2.8 and 2.9).

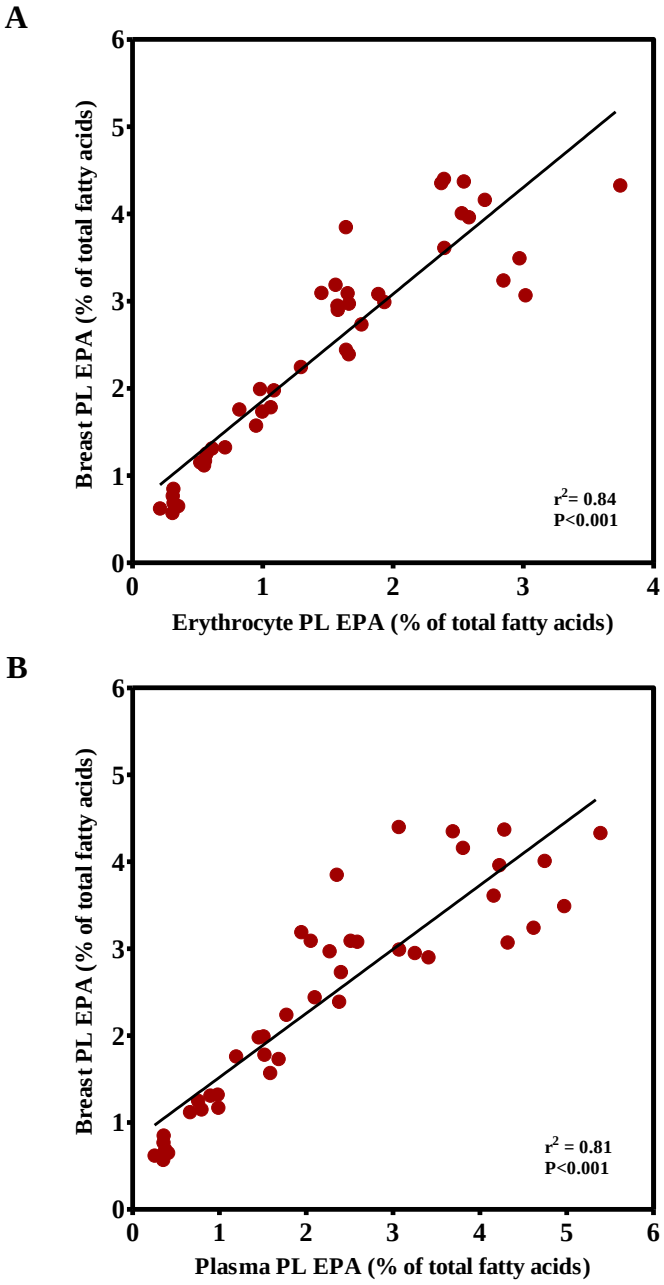


Figure 2.10 A: Relationship between erythrocyte phospholipids (PL) EPA and breast PL EPA. The proportion of breast PL EPA was significantly and positively correlated with the proportion of EPA in erythrocyte PLs. B: Relationship between plasma PL EPA and breast PL EPA. The proportion of breast PL EPA was significantly and positively correlated with the proportion of EPA in plasma PLs. r^2= correlation coefficient.

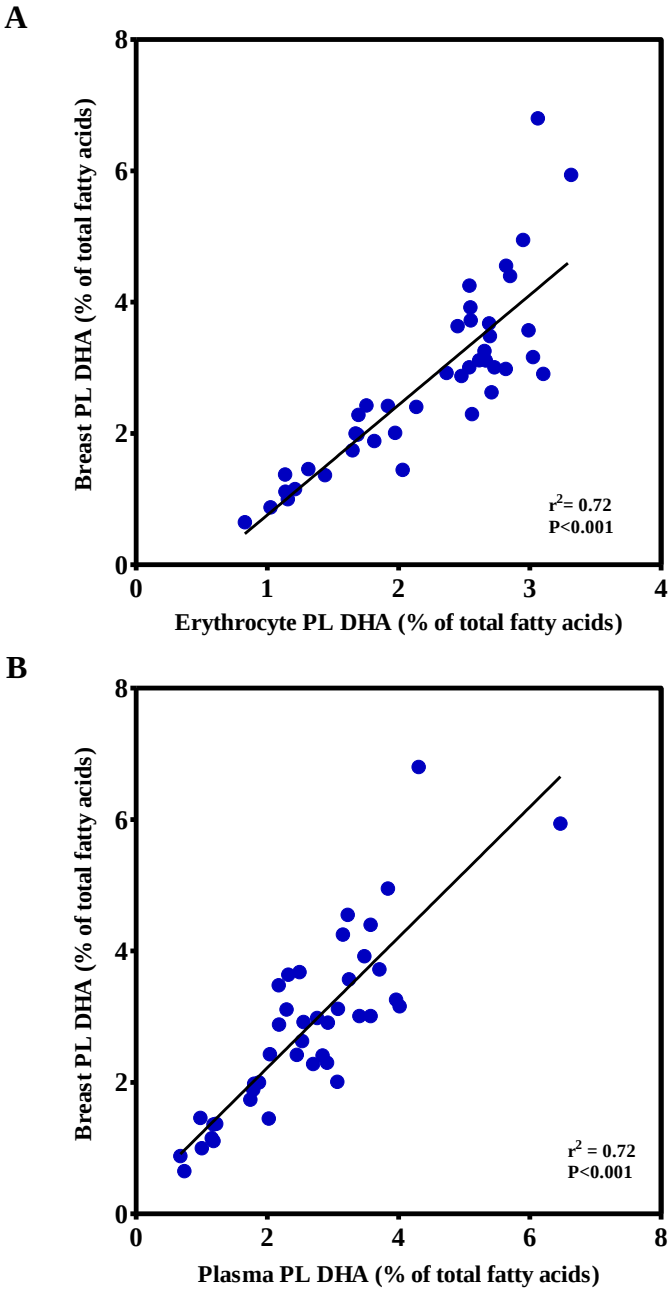


Figure 2.11 A: Relationship between erythrocyte phospholipids (PL) DHA and breast PL DHA. The proportion of breast PL DHA was significantly and positively correlated with the proportion of DHA in erythrocyte PLs. B: Relationship between plasma PL DHA and breast PL DHA. The proportion of breast PL DHA was significantly and positively correlated with the proportion of DHA in plasma PLs. r^2= correlation coefficient.

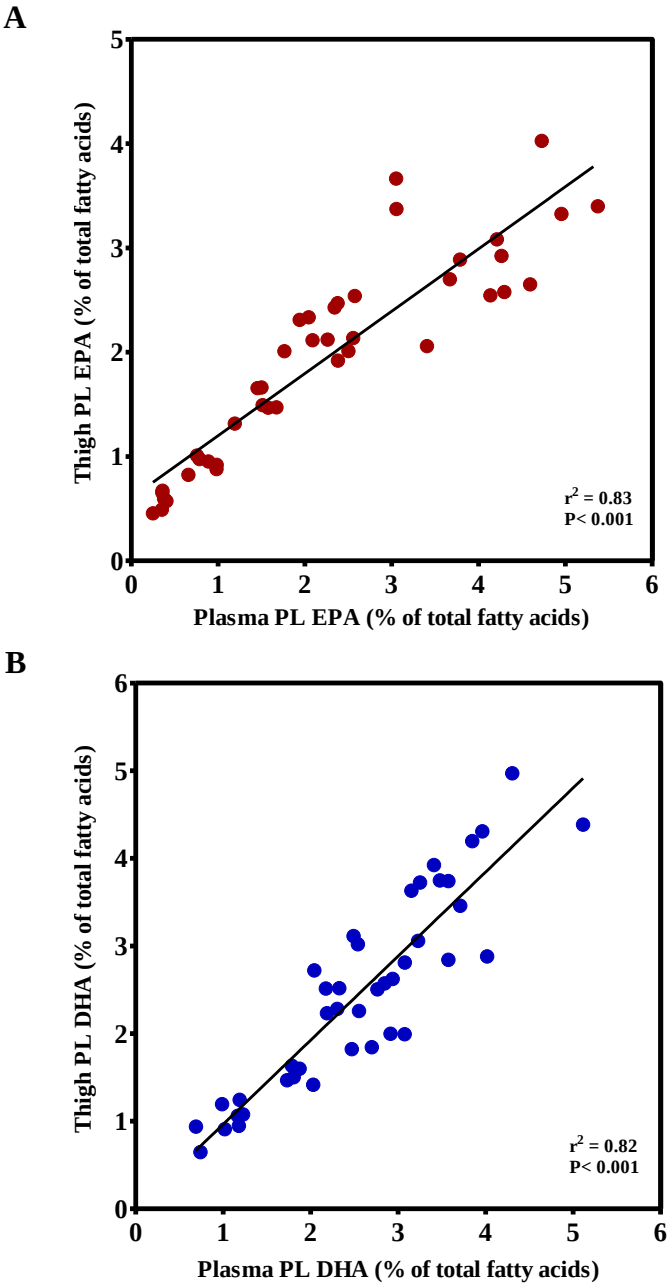


Figure 2.12 A: Relationship between plasma phospholipids (PL) EPA and thigh PL EPA. The proportion of thigh PL EPA was significantly and positively correlated with the proportion of EPA in plasma PLs. B: Relationship between plasma PL DHA and thigh PL DHA. The proportion of thigh PL DHA was significantly and positively correlated with the proportion of DHA in plasma PLs. r^2= correlation coefficient.

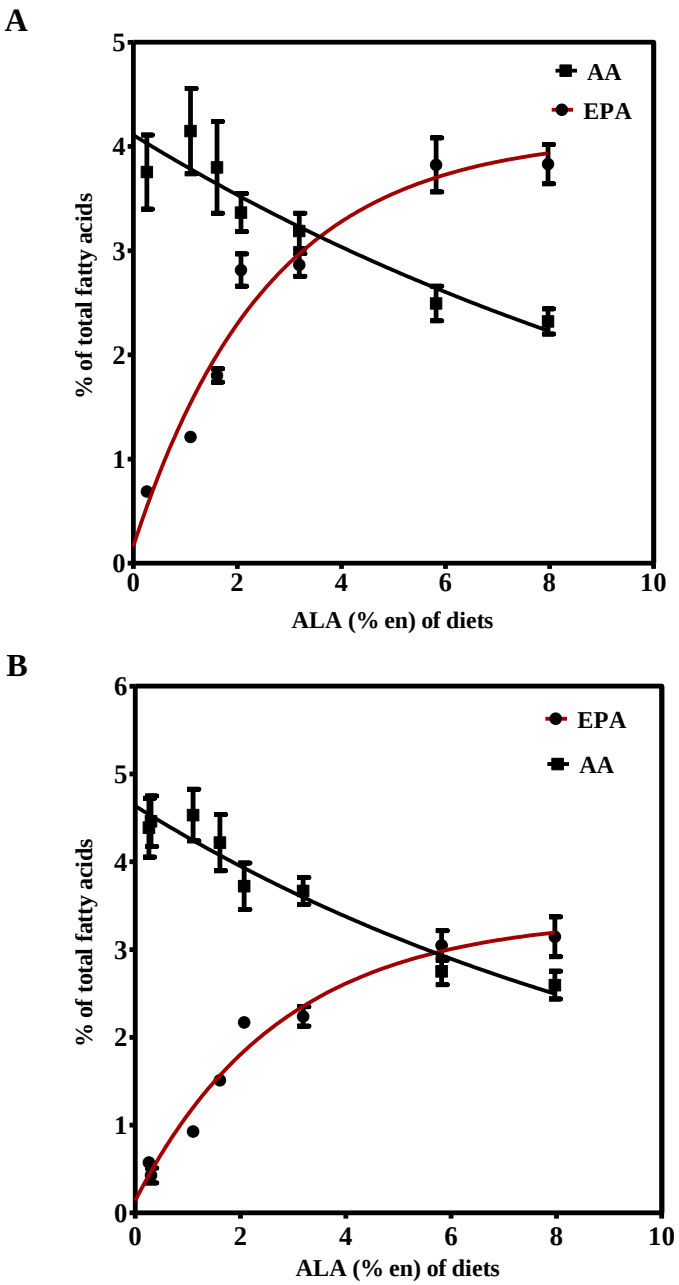


Figure 2.13 Effects of increasing levels of dietary ALA on arachidonic acid (AA) of breast (A) and thigh tissues (B). The values presented are means ± SEM (n=6/diet). There were significant differences on the level of AA (P<0.001) among groups.

2.3.3 Performance Parameters

The values corresponding to production parameters are shown in Table 2.10. The performance parameters including weight gain, feed intake and feed efficiency are shown as the pen average. The increase in levels of dietary ALA as a result of the inclusion level of vegetable oils in the experimental diets did not appear to change the final weight of chickens at 28 days of age.

Table 2.10 Performance parameters from chickens fed experimental diets varying in LA to ALA ratio for 28 days

	Experimental Diets							
	Control	**D1**	**D2**	**D3**	**D4**	**D5**	**D6**	
Fat Content (%)	5.3	5.4	5.1	5.4	6.7	7.3	9.4	P value[2]
LA:ALA	10.3:1	3.6:1	2.4:1	1.2:1	1.9:1	0.7:1	0.6:1	
Variables								
Weight gain, g per bird	1308	1434	1392	1346	1377	1345	1285	
Feed intake, g per bird	2027	2446	2001	2376	2290	2057	2061	
Feed conversion ratio, g:g	1.55	1.71	1.44	1.77	1.66	1.53	1.60	
Final weight[1], g per bird	1393	1504	1482	1434	1454	1471	1361	NS

[1]Values are means of six observations per treatment
[2]NS = not significant

2.4 DISCUSSION

This study was conducted to investigate whether increasing ALA content in the diet influenced the accumulation of n-3 LCPUFA in chicken meat. The results presented in this chapter showed that there was a direct correlation between the ALA content of the diet and the n-3 content of chicken meat, mainly as n-3 LCPUFAs including EPA, DPA and DHA. The observed differences in the levels of the fatty acids in each tissue reflected specificity in incorporation ability for each tissue. EPA was found in high amounts (4.7%) in plasma phospholipids but appeared in lower amounts in erythrocytes, breast and thigh phospholipids. This may be because EPA has a limited ability to accumulate in these tissues. The level of DHA and EPA was approximately the same level in plasma and most tissues. The level of DPA in plasma phospholipids was in modest amounts but DPA was the major n-3 LCPUFA in breast and thigh. This may reflect the capacity for DPA to accumulate more readily in these tissues with less being converted to DHA. Our findings are supported by other studies, which also examined the effect of increasing ALA content with inclusion levels of 1, 3, 5 or 7% [51] or 8.2% of linseed oil in the diet [61]. These studies showed that DPA was the n-3 LCPUFA with the highest levels in breast and thigh samples. A greater deposition of DPA achieved in breast and thigh samples compared to DHA may be because the conversion of ALA to DPA follows simple zero-order kinetics [76], whereas the synthesis from DPA to DHA is more complex [9]. As described by Voss *et al.* [76], the synthesis of DPA from ALA requires only one pass of the Δ6-desaturase, while the synthesis of DHA from DPA needs a second pass after it is elongated to 24:5n-3. Therefore, 24:5n-3 would compete with LA for access to Δ6-desaturase, which may describe the complex kinetics between

dietary ALA and DHA level in tissues [9]. In addition, 24:6n-3 then needs to be beta-oxidised to DHA in peroxisomes, which provides another potential point of regulation.

Increasing levels of dietary ALA from approximately 0.3% energy (the level found in current commercial diets) to 8% energy produced an increase in the accumulation of meat n-3 LCPUFAs, especially EPA, DPA and DHA of between 4 and 8-fold, at the highest level of ALA in the diets (the lowest LA to ALA ratio). These changes were in a similar direction to previous studies [12,52,61,65,77]. However, we observed a higher accumulation of n-3 LCPUFA in breast tissue (Figure 2.14) than those achieved by other workers. Febel *et al.* [52] reported that the level of breast EPA and DHA increased to 1.2 and 1.4% of total fatty acids, respectively by an inclusion level of 3% linseed oil in the diet. However, our results showed that a level of 2.5% linseed oil (diet 3) resulted in a higher breast EPA and DHA (around 3% of each fatty acid) accumulation. The different results may be due to the different strain of birds used in our experiment compared to those used by earlier studies [52,65]. As pointed out by Rymer and Givens [78], White Cobb 500 meat tended to have a higher accumulation of DHA than Ross 308 meat. The effectiveness of our dietary treatments was striking. In our experiment breast EPA and DHA levels of chickens fed diets enriched with 7.5% linseed oil (diet 6) reached 60 and 54%, respectively of the EPA and DHA levels found in breast of chicken fed with 8.2% fish oil [61]. In addition, although it appeared that diet enriched with fish oil resulted in higher levels of breast n-3 LCPUFA in chicken meat compared to our results, the diets containing fish oil caused a negative impact on the sensory properties of the breast

meat [5,61]. When comparing to certain fish as the major source of n-3 LCPUFA, we noted that total n-3 LCPUFA of breast meat achieved 74 and 53% of those of found in Barramundi and Whiting, respectively [49]. Clearly, in the case of Cobb 500 chickens, a diet containing only vegetable oils rich in ALA are capable of attaining levels of n-3 LCPUFA comparable with feeding marine oils.

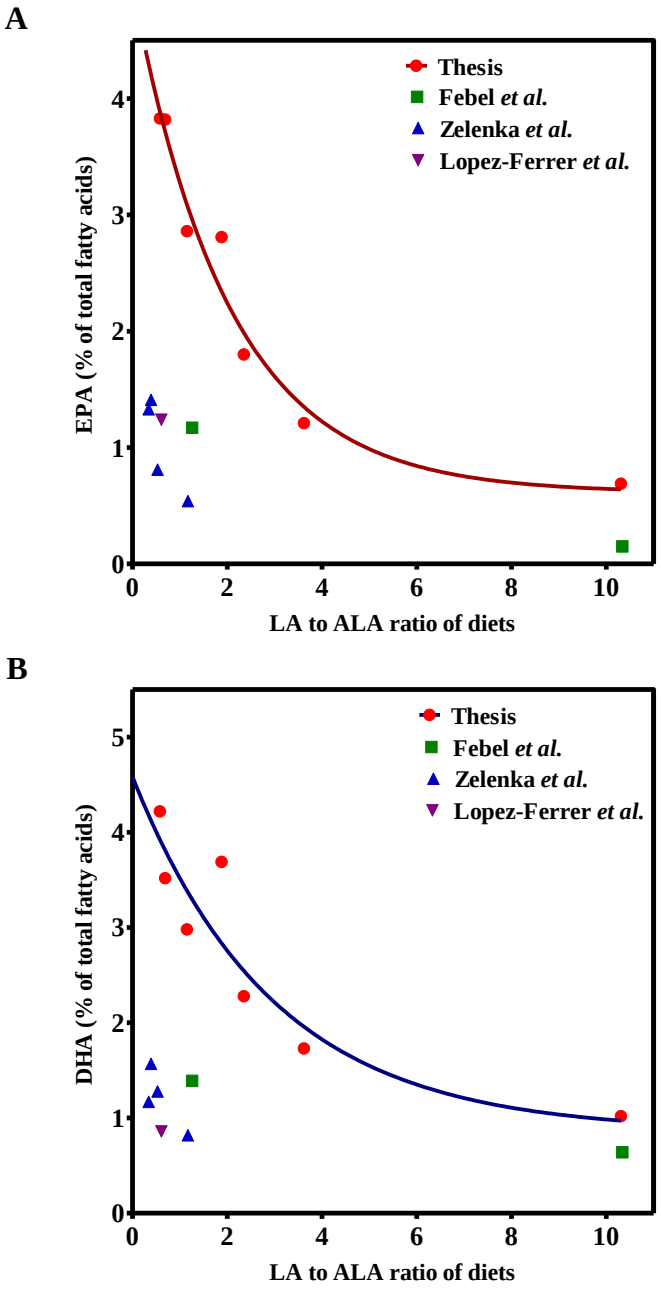


Figure 2.14 Comparison of the effects of varying LA to ALA ratio on fatty acid composition of EPA (A) and DHA (B) in breast tissues in various studies [52,61,65].

We observed a difference in n-3 LCPUFA accumulation between breast and thigh meat. The deposition of these fatty acids in breast tissues was greater than in thigh meat. The increased accumulation of n-3 LCPUFA in breast tissues compared to thigh tissues has previously been observed [61,79]. In addition, Rymer and Givens [78] reported that the response to DHA was much greater in white meat compared to dark meat.

When the n-3 LCPUFAs were elevated in the meat samples, a significant reduction of AA content was observed. These results were consistent with those of other authors, who showed that an increase in linseed oil levels decreased the accumulation of LA and AA in meat samples [68]. The decrease in AA content of tissue samples might be as a result of the biosynthetic competition between precursors ALA and LA but may also be due to competition between n-3 LCPUFA and AA for incorporation into meat membrane phospholipids.

In this study, the strong correlation between n-3 LCPUFA in blood and levels in the meat (breast and thigh) is very interesting. Whilst a study conducted in piglets [9] indicated that the relationship between erythrocyte and tissue (brain) DHA was weak (r^2= 0.18) and suggested the use of erythrocyte DHA as a marker of brain DHA status was not useful, in our poultry study it appeared that both erythrocyte and plasma DHA could provide accurate information regarding tissue fatty acid composition. The increase in EPA and DHA in blood samples (erythrocyte and plasma) was accompanied by increasing EPA and DHA accumulation in breast and thigh meat. It suggests that blood PL EPA and DHA are good markers of EPA and

DHA levels in breast and thigh meat and taking blood samples is an easy and non-lethal way to monitor diet-induced changes in the meat.

The increased levels of dietary ALA did not affect tissue fat content including liver, breast and thigh. This result is in agreement with those of Olomu and Baracos [77] using four levels of dietary linseed oil in combination with animal tallow at 6% added fat and Crespo and Esteve-Garcia [80] who incorporated different fat sources (tallow, sunflower or linseed oils) at the level of 10% into a basal diet.

Importantly, altering the LA to ALA ratio of dietary fats has no negative effect on the growth of birds. Therefore, diets rich in ALA will not reduce production efficiency. This result found is in concordance with those of previous authors [52,77,80], who investigated high inclusion levels of different dietary fats. Specifically, Crespo and Esteve-Garcia [80] using 10% of supplemented tallow, sunflower oil and linseed oil and Kavouridou *et al.* [81] using vegetable oils (coconut, palm, olive soybean and linseed oil) at the same level did not obtain a difference in the final weight of chickens. This finding is similar to studies conducted by Febel *et al.* [52], who used different fat sources with different degrees of ALA. In addition, increased dietary linseed oil in combination with animal tallow to give a total of 6% added fat in the diet [77] did not influence bird performance including weight gain.

The results presented show that the inclusion level of vegetable oils rich in ALA can increase n-3 LCPUFA accumulation in chicken meat and decrease the level of AA whilst only slightly increasing SFA. Overall, our data confirms our hypothesis

that it is possible to significantly increase the n-3 fatty acid content of chicken meat without recourse to feeding marine oils and placing a strain on marine resources.

2.5 CONCLUSION

Increased levels of dietary ALA raised meat n-3 LCPUFA (EPA, DPA and DHA) levels and there was no apparent maximal level of EPA, DPA and DHA achieved in breast and thigh meat. This suggests that in Cobb 500 broilers it is still possible to increase dietary level of ALA further in order to increase n-3 LCPUFA accumulation in chicken meat and there is no inhibitory effect of high levels of dietary ALA on LCPUFA synthesis. The dietary treatments with the lowest LA to ALA ratio elevated the incorporation of EPA and DHA into breast and thigh meat to levels five and four-fold respectively relative to birds fed the highest LA to ALA ratio. In contrast, the n-6 LCPUFA, arachidonic acid, was reduced in all diets as LA to ALA ratio decreased.

Based on our data and the work of others, it appears that increasing ALA content in the diet of chickens could be a means to increase the proportion of meat n-3 LCPUFA. In addition, in order to optimize the conversion of ALA into EPA and DHA, the ratio of LA to ALA should be varied mainly by increasing the level of ALA in the diets but keeping a constant LA level may be of less importance. Incorporating n-3 rich vegetable oils into chicken diets would help to provide humans with a variety of foods rich in n-3 LCPUFA. As chicken is one of the most popular meats consumed in the world, a chicken meat which is rich in n-3 LCPUFA has the capacity to increase the n-3 LCPUFA intake and the health status of most

Australians. This strategy may also result in maintenance in the sensory quality of the meat as one of the goals of food research is to increase the nutritional value of foods without affecting sensory quality.

CHAPTER 3

The Effect of Chicken Diets High in LA Level on n-3 LCPUFA in Chicken Tissues

3.1 INTRODUCTION

There have been a number of reports of n-3 fatty acid enrichment of chicken meat through dietary enrichment with vegetable oils containing the n-3 precursor, ALA [12,52,65]. However, the levels of n-3 LCPUFA found in meat have been low. In Chapter 2, we not only confirmed these findings but extended the observations in that the level of n-3 LCPUFA achieved was much higher than levels reported previously [12,52,65]. The improvement in meat n-3 LCPUFA status was achieved by varying the ratio of LA to ALA by increasing the level of ALA in the diets and keeping a constant LA level in order to optimize the conversion of ALA into EPA and DHA. However, there is competition between LA and ALA for the use of desaturation and elongation enzymes in fatty acid metabolism [13]. The results of the first experiment indicated that the conversion of ALA was mainly driven by the level of ALA in the diet. The lowest LA to ALA ratio of experimental diets resulted in the highest EPA, DPA and DHA in these tissues. These results indicated that increasing the ALA content of the diets was important but it was unclear whether keeping a constant LA level is also important in the regulation of EPA, DPA and DHA accumulation in chicken meat. As reported by Liou *et al.* [10], a high consumption of LA can reduce EPA and DHA production and favour high AA. The objective of the current study was to examine the effect of LA levels in diets on the conversion of ALA into EPA, DPA and DHA into chicken tissues.

3.2 MATERIALS AND METHODS

3.2.1 Experimental Design

The variable factor in the experimental diets was LA while the level of ALA was kept constant. There were a total of four dietary treatments, namely, a reference diet that corresponded to the control diet in Chapter 2 and three experimental diets. These diets were provided to a total of 64 one-day-old mixed chicks housed in eight pens (n=8 birds/pen). Each dietary treatment was assigned to two pens.

3.2.2 Birds, Rearing and Management

One-day-old mixed sex broiler chickens (Cobb 500) obtained from the Baiada hatchery (Willaston, SA, Australia) were randomly placed in eight pens (n=8 birds per pen) and distributed among four dietary treatments (2 pens for each dietary treatment). The location and environmental conditions in rearing the birds were the same as Chapter 2 (section 2.2.4). The procedures in rearing and slaughtering birds complied with national regulations (see section 2.2.1).

3.2.3 Diets

Diets were based on a commercial starter diet, with a low level of fat (Tables 3.1 and 3.2). The experimental diets were formulated by varying the levels of LA, which ranged from 21.6 (reference diet) to 39.8% (diet 3), with ALA levels kept constant at approximately 19% of total fatty acids and the total fat content was kept constant at approximately 5%. Pure or blended vegetable oils were included at a level of 2.8% in order to produce diets with the desired levels of LA and ALA

Table 3.1 Composition of experimental diets from 1-28 days post-hatch

Ingredients (%)	Experimental diets			
	Reference diet	**Diet 1**	**Diet 2**	**Diet 3**
Basal diet[1]	97.2	97.2	97.2	97.2
Oil added				
Macadamia oil	2.80	1.10	0.55	0.0
Flaxseed oil	0.0	1.70	1.70	1.92
Sunflower oil	0.0	0.0	0.55	0.88
Total	100.0	100.0	100.0	100.0

Table 3.2 Ingredient composition and nutrient content of basal diet

Ingredients1	**kg/100 kg**
Wheat fine	43.91
Wheat mil vits	0.80
Barley	10.00
Triticale fine	10.00
Peas fine	10.00
Meat meal	4.60
Blood meal	1.40
Soybean meal	15.00
Millrun	2.00
Limestone small	0.79
Salt	0.18
Sodium bicarbonate	0.27
Choline chloride 75%	0.07
Potassium carbonate	0.01
L-threonine	0.09
Alimet	0.35
Standard broiler starter premix	0.20
Lysine sulphate	0.29
Phyzyme XP5000L broiler	0.01
Feed enzyme premix	0.03
Nutrient content (%)	
ME, Kcal/kg	2787
Protein	22.99
Fat	2.20
Fibre	3.82
Calcium	0.98
Phosphorus	0.74
Available phosphorus	0.5
Na	0.2
K	0.71
Cl	0.2
Lysine	1.3
Methionine	0.59
Methionine + Cystine	0.99

1A standard commercial starter diet (Ridley Agriproducts Pty Ltd, Murray Bridge, Australia).

The different blended vegetable oils were obtained by varying the proportion of some vegetable oils including flaxseed, macadamia and sunflower oil. The oils were sampled for fatty acid analysis and the fatty acid composition of these oils is shown in Table 3.3.

Table 3.3 Fatty acid composition of oils added in the diets

Fatty acid[1]	Oil and blended oils			
	Reference diet	Diet 1	Diet 2	Diet 3
	(% of total fatty acids)			
Total SFA	18.11	12.40	13.10	11.79
Total MUFA	79.28	42.97	31.29	21.60
Total n-9	60.01	34.68	26.95	20.67
Total n-7	19.18	8.27	4.37	0.95
18:2n-6	2.08	12.50	24.40	35.89
Total n-6	2.12	12.61	24.51	35.93
18:3n-3	0.13	31.78	30.88	30.56
Total n-3	0.44	31.93	30.96	30.57

[1]SFA= saturated fatty acid; MUFA= monounsaturated fatty acid

The amount of the oils needed for each diet was then weighed and mixed well with the basal diet into a 20kg feed mixer. A total of 20kg feed was made for each batch. For the duration of the experiment, a total of 60kg diet was prepared for each dietary treatment and the fatty acid composition of the diets produced in each batch was analyzed. The fatty acid content of the experimental diets is presented in Table 3.4.

The dietary treatments had the same nutritional values as the basal diet except for the fatty acid composition of the fats. This resulted in the diets varying in the ratio of LA to ALA which varied ranging from 9.75:1 (reference diet) to 2.05:1 (diet 3).

All diets met or exceeded National Research Council guidelines for broiler chickens.

Each diet was provided *ad libitum* for the duration of the 28-d growth period. Feed consumption and body weight were recorded to calculate performance parameters on days 7, 14, 21 and 28 (see section 2.2.4).

Table 3.4 Fatty acid contents of the diets

	Experimental diets			
	Reference diet	**Diet 1**	**Diet 2**	**Diet 3**
LA (% en)	2.34	2.90	3.78	4.38
ALA (% en)	0.24	2.12	2.06	2.13
LA:ALA ratio	9.75:1	1.37:1	1.83:1	2.06:1
Fat content (%)	5.05	5.11	5.18	5.15
Fatty acid	(% of total fatty acids)			
Total SFA[1]	21.37	19.41	18.53	17.97
Totals Trans	0.39	0.42	0.41	0.43
18:1n-9	40.25	27.11	23.64	19.94
18:1n-7	2.53	1.58	1.26	0.96
Total MUFA[1]	53.85	33.80	27.86	21.93
Total n-9	42.40	28.34	24.59	20.63
Total n-7	11.31	5.36	3.20	1.25
18:2n-6	21.63	26.52	34.09	39.78
Total n-6	21.85	26.76	34.33	40.04
18:3n-3	2.23	19.37	18.63	19.38
Total n-3	2.47	19.52	18.75	19.48
Total PUFA[1]	24.32	46.28	53.08	59.52

[1]SFA= saturated fatty acid; MUFA= monounsaturated fatty acid; PUFA:=polyunsaturated fatty acid

3.2.4 Sample Collection and Fatty Acid Analysis

At 28 days post-hatch, six selected birds from each pen (12 birds per group) were weighed individually and blood and tissue samples, including liver, breast and thigh, were collected for fatty acid analysis (see section 2.2.7).

3.2.5 Statistical Analysis

All data obtained were analysed by one-way analysis of variance (ANOVA) using GenStat-tenth edition version 10.1.0.72. The analysis was followed by the Tukey test if there were significant differences ($P<0.05$) among dietary treatments

3.3 RESULTS

3.3.1 Blood Fatty Acids

Erythrocyte and plasma fatty acid composition are shown in Tables 3.5 and 3.6. There was an effect of increasing the LA to ALA ratio of experimental diets from 1.4:1 to 2.1:1 on the n-3 LCPUFA in both erythrocyte and plasma samples (Tables 3.5 and 3.6). The accumulation of EPA in blood samples was reduced in proportion to the LA content of the diet ($P<0.01$). There was no statistically significant effect of dietary LA on DPA levels and a significant reduction in DHA was only observed in erythrocytes. There were no differences in the results observed between diet 2 and diet 3 indicating a saturation effect of dietary LA.

The level of total n-3 fatty acids tended to decrease and the highest LA to ALA ratio (the highest LA content) resulted in the lowest total n-3 content. The proportion of total n-6 fatty acids increased slightly as a result of increased LA levels of the diets

($P<0.05$). The highest dietary LA content resulted in the highest level of total erythrocyte and plasma phospholipids n-6 fatty acids, which was 39.3 and 32.1% of total fatty acids, respectively. This was mainly due to increasing LA levels.

Increased dietary LA resulted in an increase in LA levels in plasma and erythrocytes but there was no effect on AA levels in either blood tissue.

Supplementation of chicken feeds with blended vegetable oils containing different levels of LA had no effect on the saturated fatty acid content of erythrocyte and plasma samples (Tables 3.5 and 3.6). The dietary treatments significantly decreased MUFA content in erythrocyte phospholipids ($P<0.05$) with the highest LA to ALA ratio of experimental diets (LA to ALA ratio of 2.1 to 1), resulting in the lowest MUFA content, which was 12.7% of total fatty acids.

Table 3.5 Fatty acid composition of erythrocytes phospholipids from chickens fed experimental diets varying in LA to ALA ratio for 28 days[1]

	Experimental Diets					
	Reference	**D1**	**D2**	**D3**		
LA (% en)	2.34	2.90	3.78	4.38		
ALA (% en)	0.24	2.12	2.06	2.13		
LA:ALA ratio	9.75:1	1.37:1	1.83:1	2.06:1		
Fat Content (%)	5.05	5.11	5.18	5.15		
Fatty acid[2]	(% of total fatty acids)[1]				PSEM	P value
16:0	18.17	19.24	19.60	19.57	0.141	NS
18:0	10.42	11.67	11.63	11.66	0.268	NS
SFA	36.61	38.81^{a}	39.22^{ab}	39.47^{b}	0.084	*
18:1n-9	19.09	12.47	11.73	9.75	0.494	NS
18:1n-7	2.27	1.51	1.39	1.15	0.070	NS
MUFA	24.96	16.24^{b}	15.23^{b}	12.73^{a}	0.489	*
20:3n-9	0.76	0.35	0.33	0.21	0.029	NS
18:2n-6	28.23	28.52^{a}	31.08^{b}	32.98^{c}	0.479	*
20:3n-6	0.79	0.72	0.74	0.69	0.035	NS
20:4n-6	4.01	3.75	3.87	4.41	0.144	NS
Total n-6	34.35	33.90^{a}	36.76^{b}	39.26^{c}	0.423	**
18:3n-3	0.67	3.73^{b}	2.73^{a}	2.82^{a}	0.073	**
20:3n-3	0.04	0.10	0.08	0.10	0.006	NS
20:5n-3	0.33	1.60^{b}	1.15^{a}	1.10^{a}	0.022	**
22:5n-3	0.67	1.90	1.64	1.64	0.054	NS
22:6n-3	1.06	2.99^{b}	2.44^{a}	2.27^{a}	0.116	*
Total n-3	2.83	10.34^{b}	8.08^{a}	7.95^{a}	0.054	**
Total PUFA	37.18	44.24^{a}	44.83^{a}	47.21^{b}	0.471	*

[1]Values are means of twelve observations (n = 12) per treatment and their pooled standard error of the mean (PSEM)
[a,b]Values in the same row with no common superscript are significantly different (P< 0.05)
[2]SFA= saturated fatty acid; MUFA= monounsaturated fatty acid; PUFA= polyunsaturated fatty acid
NS= not significant
*P<0.05
**P<0.01

Table 3.6 Fatty acid composition of plasma phospholipids from chickens fed experimental diets varying in LA to ALA ratio for 28 days[1]

	Experimental Diets					
	Reference	**D1**	**D2**	**D3**		
LA (% en)	2.34	2.90	3.78	4.38		
ALA (% en)	0.24	2.12	2.06	2.13		
LA:ALA ratio	9.75:1	1.37:1	1.83:1	2.06:1		
Fat Content (%)	5.05	5.11	5.18	5.15		
Fatty acid[2]	(% of total fatty acids)[1]				PSEM	P value
16:0	19.66	20.94	20.43	21.50	0.562	NS
18:0	21.59	22.40	23.22	22.44	0.727	NS
SFA	42.97	45.22	45.22	45.69	0.251	NS
18:1n-9	19.06	13.26	13.43	11.33	0.632	NS
18:1n-7	2.81	1.84	1.73	1.43	0.082	NS
MUFA	25.25	17.15	17.13	14.37	0.637	NS
20:3n-9	2.06	1.02	1.13	0.61	0.107	NS
18:2n-6	20.84	20.60^{a}	23.11^{b}	24.63^{c}	0.410	*
20:3n-6	1.24	1.11	1.27	1.10	0.064	NS
20:4n-6	3.65	4.63	4.24	5.23	0.457	NS
Total n-6	26.79	27.24^{a}	29.64^{b}	32.06^{c}	0.572	*
18:3n-3	0.40	2.00^{b}	1.41^{a}	1.58^{a}	0.072	*
20:3n-3	0.04	0.14	0.11	0.13	0.010	NS
20:5n-3	0.40	2.14^{b}	1.60^{a}	1.48^{a}	0.041	**
22:5n-3	0.32	1.47	1.02	1.29	0.133	NS
22:6n-3	0.80	3.08	2.13	2.27	0.197	NS
Total n-3	2.02	8.86^{b}	6.30^{a}	6.77^{a}	0.359	*
Total PUFA	28.82	36.10	35.94	38.83	0.879	NS

[1]Values are means of twelve observations (n = 12) per treatment and their pooled standard error of the mean (PSEM)
[a,b]Values in the same row with no common superscript are significantly different (P< 0.05)
[2]SFA= saturated fatty acid; MUFA= monounsaturated fatty acid; PUFA= polyunsaturated fatty acid
NS= not significant
*P<0.05
**P<0.01

3.3.2 Tissue Fatty Acids

Fatty acid composition of liver, breast and thigh are shown in Tables 3.7, 3.8 and 3.9. The results show that the increased levels of dietary LA from 2.9 to 4.4% en did not affect on tissue fat content including liver, breast and thigh.

Elevating the level of dietary LA had an effect that was not quite significant on ALA levels in breast tissues and only reached significance in thigh tissue. The diets caused a consistent reduction in EPA levels in all tissues ($P<0.01$) but DPA levels did not respond ($P>0.05$; Figure 3.1). The levels of DHA were unaffected by dietary LA in liver and breast tissues but were significantly decreased ($P<0.05$) in thigh.

While increased levels of LA resulted in a decrease in the level of total n-3 PUFA, the level of total n-6 fatty acids increased ($P<0.01$). The highest dietary LA content resulted in the highest level of total liver, breast and thigh phospholipids n-6 fatty acids, which was 32.47, 27.28 and 29.15.1% of total fatty acids, respectively. Elevating dietary LA increased the level of LA in all tissues but this was not translated to changes in AA levels (Figure 3.2).

The dietary treatment generally resulted in small increases in total saturated fatty acids and small reductions in total MUFA in breast and thigh tissues but the values were not significantly different in liver.

Table 3.7 Fatty acid composition of liver phospholipids from chickens fed experimental diets varying in LA to ALA ratio for 28 days[1]

	Experimental Diets					
	Reference	**D1**	**D2**	**D3**		
LA (% en)	2.34	2.90	3.78	4.38		
ALA (% en)	0.24	2.12	2.06	2.13		
LA:ALA ratio	9.75:1	1.37:1	1.83:1	2.06:1		
Fat Content (%)	5.05	5.11	5.18	5.15		
Fatty acid[2]	(% of total fatty acids)[1]				PSEM	P value
16:0	16.74	17.38	16.78	17.43	0.462	NS
18:0	24.21	24.79	25.23	24.75	0.483	NS
SFA	42.80	43.87	43.59	43.99	0.191	NS
18:1n-9	17.19	12.05	11.93	10.58	0.703	NS
18:1n-7	2.77	1.75	1.68	1.46	0.102	NS
MUFA	23.43	15.81	15.63	13.74	0.809	NS
20:3n-9	1.82	0.91	1.00	0.61	0.069	NS
18:2n-6	18.55	17.62^{a}	20.35^{b}	20.80^{b}	0.436	*
20:3n-6	1.46	1.36	1.60	1.47	0.071	NS
20:4n-6	7.61	7.55	7.80	8.81	0.620	NS
Total n-6	28.68	27.54^{a}	30.93^{b}	32.47^{c}	0.316	**
18:3n-3	0.21	0.98^{b}	0.76^{a}	0.89^{ab}	0.013	**
20:3n-3	0.05	0.17	0.14	0.18	0.015	NS
20:5n-3	0.60	2.89^{b}	1.97^{a}	1.95^{a}	0.090	**
22:5n-3	0.37	1.92	1.34	1.75	0.249	NS
22:6n-3	1.30	5.51	4.19	4.03	0.342	NS
Total n-3	2.57	11.50^{b}	8.44^{a}	8.82^{a}	0.537	*
Total PUFA	31.25	39.04	39.37	41.29	0.761	NS
	Fat Content (% fresh weight)					
	4.56	5.06	5.43	4.81	0.593	NS

[1]Values are means of twelve observations (n = 12) per treatment and their pooled standard error of the mean (PSEM)
[a,b]Values in the same row with no common superscript are significantly different (P< 0.05)
[2]SFA= saturated fatty acid; MUFA= monounsaturated fatty acid; PUFA= polyunsaturated fatty acid
NS=not significant
*P<0.05
**P<0.01

Table 3.8 Fatty acid composition of breast phospholipids from chickens fed experimental diets varying in LA to ALA ratio for 28 days[1]

	Experimental Diets					
	Reference	**D1**	**D2**	**D3**		
LA (% en)	2.34	2.90	3.78	4.38		
ALA (% en)	0.24	2.12	2.06	2.13		
LA:ALA ratio	9.75:1	1.37:1	1.83:1	2.06:1		
Fat Content (%)	5.05	5.11	5.18	5.15		
Fatty acid[2]	(% of total fatty acids)[1]				PSEM	P value
16:0	19.42	20.86	21.18	21.48	0.274	NS
18:0	8.88	9.78	9.49	9.93	0.246	NS
SFA	35.63	38.36[a]	38.03[a]	39.40[b]	0.089	**
18:1n-9	23.92	17.75	16.94	15.07	0.856	NS
18:1n-7	7.61	5.05	4.59	3.84	0.210	NS
MUFA	38.08	26.51[b]	25.24[b]	21.56[a]	0.750	*
20:3n-9	1.08	0.43[b]	0.41[b]	0.26[a]	0.025	*
18:2n-6	14.33	14.80[a]	16.97[b]	18.31[c]	0.222	**
20:3n-6	1.68	1.27	1.52	1.45	0.083	NS
20:4n-6	3.48	3.62	4.12	4.78	0.235	NS
Total n-6	21.39	21.63[a]	24.98[b]	27.28[c]	0.438	**
18:3n-3	0.40	1.52	1.30	1.34	0.063	NS
20:3n-3	0.15	0.97	0.89	0.96	0.067	NS
20:5n-3	0.62	2.63[b]	2.17[a]	2.00[a]	0.051	**
22:5n-3	0.85	4.24	3.76	4.11	0.244	NS
22:6n-3	0.94	3.23	2.73	2.63	0.117	NS
Total n-3	3.09	12.66[b]	10.90[a]	11.09[a]	0.300	*
Total PUFA	24.48	34.29	35.89	38.37	0.691	NS
	Fat Content (% fresh weight)					
	0.93	0.84	0.85	0.87	0.939	NS

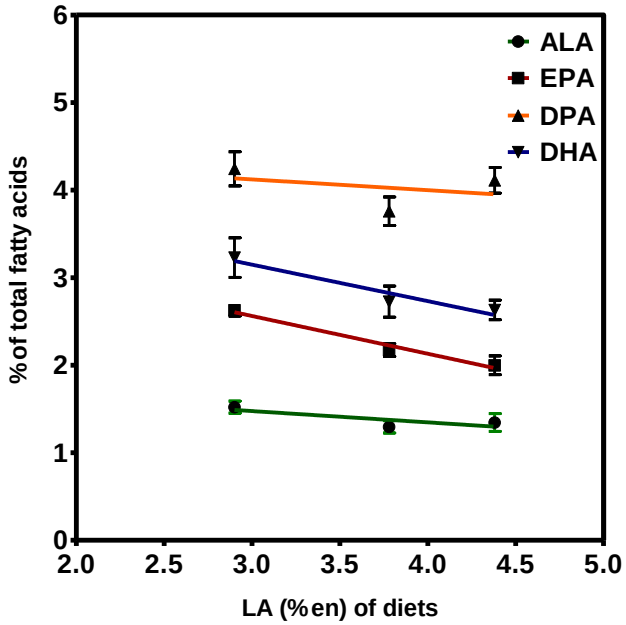
[1]Values are means of twelve observations (n = 12) per treatment and their pooled standard error of the mean (PSEM)
[a,b]Values in the same row with no common superscript are significantly different (P< 0.05)
[2]SFA= saturated fatty acid; MUFA= monounsaturated fatty acid; PUFA= polyunsaturated fatty acid
NS= not significant
*P<0.05
**P<0.01

Table 3.9 Fatty acid composition of thigh phospholipids from chickens fed experimental diets varying in LA to ALA ratio for 28 days[1]

	Experimental Diets					
	Reference	**D1**	**D2**	**D3**		
LA (% en)	2.34	2.90	3.78	4.38		
ALA (% en)	0.24	2.12	2.06	2.13		
LA:ALA ratio	9.75:1	1.37:1	1.83:1	2.06:1		
Fat Content (%)	5.05	5.11	5.18	5.15		
Fatty acid[2]	(% of total fatty acids)[1]				PSEM	P value
16:0	19.16	20.16	21.11	21.17	0.337	NS
18:0	12.01	13.04	12.63	12.91	0.323	NS
SFA	34.81	37.40^{a}	37.94^{ab}	38.52^{b}	0.081	**
18:1n-9	26.70	19.54	18.71	16.42	0.747	NS
18:1n-7	6.71	4.61^{b}	4.26^{b}	3.66^{a}	0.147	*
MUFA	39.10	27.32^{b}	25.95^{b}	22.27^{a}	0.660	*
20:3n-9	1.05	0.37	0.33	0.22	0.028	NS
18:2n-6	14.22	16.67^{a}	18.47^{b}	20.42^{c}	0.324	**
20:3n-6	1.13	0.96	1.04	1.02	0.052	NS
20:4n-6	4.49	4.27	4.66	5.37	0.193	NS
Total n-6	21.67	23.49^{a}	26.13^{b}	29.15^{c}	0.388	**
18:3n-3	0.32	1.48^{b}	1.17^{a}	1.25^{a}	0.028	*
20:3n-3	0.11	0.69	0.66	0.75	0.067	NS
20:5n-3	0.52	2.15^{b}	1.59^{a}	1.46^{a}	0.062	**
22:5n-3	0.98	4.12	3.71	3.93	0.160	NS
22:6n-3	0.83	2.63^{b}	2.18^{a}	2.11^{a}	0.092	*
Total n-3	2.85	11.10b	9.35a	9.53a	0.259	*
Total PUFA	24.53	34.60a	35.48^{a}	38.69b	0.627	*
	Fat Content (% fresh weight)					
	1.24	1.07	0.91	1.14	0.368	NS

[1]Values are means of twelve observations (n = 12) per treatment and their pooled standard error of the mean (PSEM)
[a,b]Values in the same row with no common superscript are significantly different (P< 0.05)
[2]SFA= saturated fatty acid; MUFA= monounsaturated fatty acid; PUFA= polyunsaturated fatty acid
NS= not significant
*P<0.05
**P<0.01

A



B

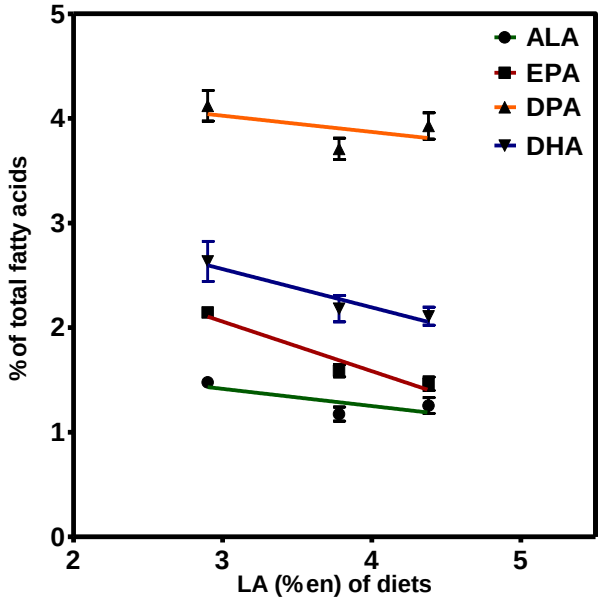
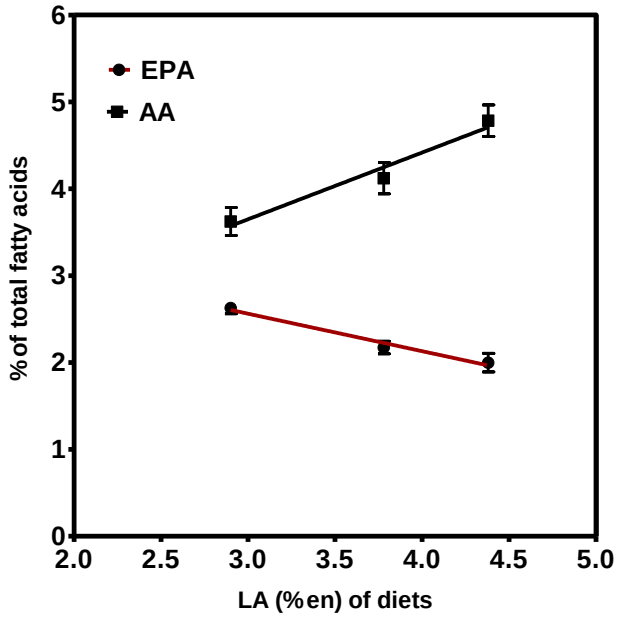


Figure 3.1 Effects of increasing levels of dietary LA on the n-3 LCPUFAs in breast (A) and thigh (B) tissues. Increasing LA content of experimental diets slightly decreased ($P<0.01$) EPA levels both in breast and thigh. The level of DPA was not affected whereas the concentration of DHA tended to decrease and reduced significantly ($P<0.05$) in thigh. The values presented are means ± SEM (n=12/diet).

A



B

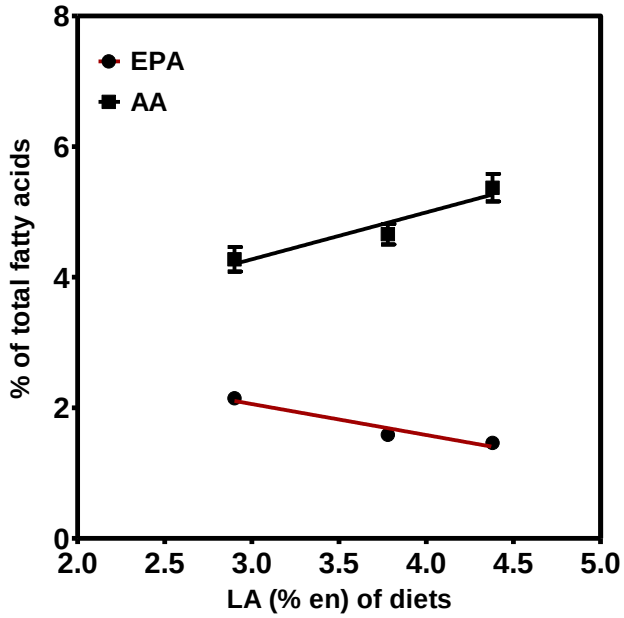


Figure 3.2 Effects of increasing levels of dietary LA on eicosapentaenoic acid (EPA) and arachidonic acid (AA) of breast (A) and thigh tissues (B). Dietary treatments increased ($P<0.01$) EPA level while AA did not change. The values presented are means ± SEM (n=12/diet).

3.3.3 Performance Parameters

The values corresponding to production parameters are shown in Table 3.10. The final weight data of birds at 28 days of age showed that there was no significant difference among dietary treatments. The mean values of the final weight for the reference diet, diet 1, diet 2 and diet 3 was 1610, 1503, 1472 and 1556g respectively.

Table 3.10 Performance parameters from chickens fed experimental diets varying in LA to ALA ratio for 28 days

	Experimental diets				
	Reference	**D 1**	**D 2**	**D 3**	
LA:ALA ratio	9.7:1	1.4:1	1.8:1	2.1:1	
Variables					P value[2]
Weight gain, g per bird	1551	1370	1452	1531	
Feed intake, g per bird	2220	2422	2317	2230	
Feed conversion ratio, g:g	1.43	1.77	1.59	1.46	
Final weight[1], g per bird	1610	1503	1472	1556	NS

[1]Values are means of twelve observations per treatment
[2] NS = not significant

3.4 DISCUSSION

The objective of this study was to examine the effect of LA content of diets on the conversion of ALA into EPA, DPA and DHA into chicken tissue. The results observed in this study indicated that increasing the dietary concentration of LA as energy from 2.90 to 4.38% (LA to ALA ratio from 1.37:1 to 2.06:1) whilst keeping a constant ALA level reduced both blood and tissue (liver, breast and thigh) n-3 LCPUFA (EPA and to a lesser extent, DHA) accumulation; however DPA tended to not be affected. The decrease in n-3 LCPUFA levels might be due to competition between ALA, the precursor to n-3 LCPUFA, and LA for Δ6-desaturase. Thus, a high dietary level of LA might depress the conversion of ALA to n-3 LCPUFA [10,82].

Although increasing the proportion of LA in the diet appeared to decrease n-3 LCPUFA accumulation in blood samples (erythrocyte and plasma) and tissue samples, the effect was similar in size to the increases in n-3 LCPUFA caused by increasing dietary ALA by a similar amount. Our findings indicated that the dietary treatments did not influence DPA and DHA level in breast meat. The level of thigh EPA and DHA of chickens fed dietary treatments with the lowest LA to ALA ratio (Diet 1) decreased to 32.1 and 19.8% respectively compared to chickens fed the highest LA to ALA ratio (Diet 3). In addition, the total n-3 LCPUFA levels in breast and thigh of birds fed with the lowest LA to ALA ratio was 15.6 and 18.7%, respectively higher than the n-3 LCPUFA in the breast and thigh of birds fed the highest LA to ALA ratio. These findings indicate that the effect of LA content in the diets is important in regulating the n-3 LCPUFA accumulation in tissues. The changes found were in concordance with other authors [10,65,83]. Zelenka *et al.* [65]

reported that there was no effect on the level of DPA and DHA both in breast and thigh meat by increasing dietary levels of LA in the diet of chickens from 14.2 to 58.4% of total fatty acids . A study in rats conducted by Marangoni *et al.* [83] found that increased levels of dietary LA of 1.9, 3.1, 7.7 and 10.1 en%, respectively, progressively reduced heart DHA. However, the experimental formulas did not significantly affect on n-3 LCPUFA accumulation in the liver tissues. Another study on the application of dietary LA levels was reported by Liou *et al.* [10], who examined the effect of replacing vegetable oils high in LA (LA to ALA ratio 10:1) with oils low in LA (LA to ALA ratio 4:1) in human (male) diet. Their results showed that LA intake did not influence ALA, DPA and DHA.

Conversely, we found that with increasing LA content in the diet, the level of LA rose in blood and tissue samples, but n-6 LCPUFA, AA, did not respond. These findings also agreed with studies reported previously [83,84]. Marangoni *et al*. [83] reported that diets enriched with LA increased LA levels in plasma, liver, heart and kidney but did not change long chain n-6 fatty acids. In addition, a study in humans show that diets enriched with sunflower oil (17g/day LA) induced an increase in LA but no change in AA [84].

The increased levels of dietary LA did not affect tissue fat content. This result is in concordance with those of Zelenka *et al*. using increased levels of dietary LA. Their results show that there was no difference on fat content of breast by increased levels of dietary LA from 14.22 to 58.44g/kg.

Similar to Chapter 2, the final weight of birds was not significantly different among dietary treatments. A number of investigators [52,77,81] have made similar regarding the final weight among broilers fed different types of fat sources involving vegetable oils.

3.5 CONCLUSION

Increasing the levels of dietary LA reduced both blood and tissue n-3 LCPUFA (EPA and to a lesser extent DHA) concentration. In addition, the concentration of LA and total n-6 fatty acids appeared to be strongly influenced by the level of dietary LA while the accumulation of AA blood and tissue samples did not change. We conclude that diets that are lower in LA will allow greater conversion of ALA into n-3 LCPUFA.

CHAPTER 4

The Effect of a Diet High in ALA on Heart n-3 LCPUFA

4.1 INTRODUCTION

Sudden death syndrome is an acute heart failure of birds that affects mainly male fast growing chickens that appear to be in good condition [85]. Julian [86] noted that right ventricular failure following right ventricular hypertrophy (RVH) from pulmonary hypertension causes ascites, which has become a prominent cause of illness and death in broiler chickens. Birds which die of sudden death syndrome showed that all birds were well-fleshed and had general pulmonary congestion [85]. The rate of sudden death ranges from 1.3 to 9.6% depending on the conditions and age of the birds [87]. The sudden death in chickens could have a similarity to sudden cardiac death in humans which has been related to low levels of n-3 fats in heart membranes [30].

Therefore, the accumulation of heart n-3 LCPUFA in chickens was measured in order to examine whether diets high in ALA were potentially beneficial for the health of birds by increasing the level of n-3 LCPUFA in cardiac membranes. Support for this comes from previous reports that feeding chickens diets containing 5% linseed oil reduced right ventricle hypotrophy [88] and pulmonary hypertension was reduced in birds fed a diet containing 10% linseed oil [89].

The primary objective of the research was to examine the effect of diet high in ALA on heart n-3 LCPUFA levels.

4.2 METHODS

4.2.1 Experimental Design

The design of this study was a one-way classification. The variable factor was the ALA level in the diet. The dietary treatments were based on the ratio of LA to ALA of the diets. There were a total of two diets comprised of a control diet and diet high in ALA. These diets were provided to a total of 32 one-day-old chicks housed in four pens (n=8 birds/pen). Each dietary treatment was assigned to two pens.

4.2.2 Birds and Diets

One-day-old mixed sex broiler chickens (Cobb 500) obtained from the Baiada hatchery (Willaston, SA, Australia) were randomly placed in four pens (n=8 birds per pen) and distributed between two dietary treatments (2 pens for each dietary treatment). The location, environmental conditions and the procedures in rearing and slaughtering birds were the same as for both Chapter 2 and 3 (see section 2.2.4).

The diets were prepared as described in Chapter 3 and the composition of experimental diets aligned with the reference diet and diet 1 in Table 3.1 (section 3.2.3). The fatty acid composition of the experimental diets is presented in Table 4.1.

Table 4.1 Fatty acid contents of the diets

	Experimental diets	
	Control diet	**High ALA Diet**
LA (% en)	2.34	2.90
ALA (% en)	0.24	2.12
LA:ALA ratio	9.75:1	1.37:1
Fat (%)	5.05	5.11
Fatty acid[1]	(% of total fatty acids)	
Total SFA[1]	21.37	19.41
Totals Trans	0.39	0.42
18:1n-9	40.25	27.11
18:1n-7	2.53	1.58
Total MUFA[1]	53.85	33.80
Total n-9	42.40	28.34
Total n-7	11.31	5.36
18:2n-6	21.63	26.52
Total n-6	21.85	26.76
18:3n-3	2.23	19.37
Total n-3	2.47	19.52
Total PUFA[1]	24.32	46.28

[1]SFA= saturated fatty acid; MUFA= monounsaturated fatty acid; PUFA= polyunsaturated fatty acid
Each diet was provided *ad libitum* for the duration of the 28-d growth period.

4.2.3 Sample Collection and Fatty Acid Analysis

At 28 days of age, six selected birds from each pen (12 birds per group) were weighed individually and hearts were collected for analysis. A ratio of right ventricle mass (RV) to total ventricle mass (TV) was used to indicate the health status of the heart. The heart analysis of birds followed the procedure described by Julian [90]. After dissection, the heart was removed and placed on a paper plate. Auricles, major vessels and fat were stripped from the heart. The right ventricle (RV) including the valve was carefully removed from the left ventricle (LV) and septum. The right ventricle was weighed and recorded. The left ventricle and

septum were also weighed. These data were used to calculate the percentage values of RV/TV, where TV is total ventricle and septum weight. A ratio of RV/TV of <0.25 (25%) was classified as a normal broiler [90]. Hearts were stored for fatty acid analysis (see section 2.2.7).

4.3 RESULTS

4.3.1 Heart Tissue Fatty Acids

The fatty acid composition of heart tissues is presented in Table 4.2. The two diets contained similar levels of dietary LA (around 2% en) but high ALA group contained 10 times the control level of ALA. The results show that the response to SFA content of heart samples was not different between control and the diet high in ALA whereas the concentration of MUFA was reduced ($P<0.05$). Dietary treatment increased the level of ALA from 0.1% (control diet) to 0.5% ($P<0.01$). The level of heart n-3 LCPUFA (EPA, DPA and DHA) and total n-3 increased 4-5-fold higher than in those chickens fed with the control diet. There was no significant difference observed in the level of heart LA, AA and total n-6.

4.3.2 Heart analysis

The increased levels of dietary ALA did not cause changes in the ratio of right ventricle (RV) to total ventricle (TV). The RV to TV ratio of birds fed the control diet and diet high in ALA was 19.12 and 21.39% respectively (Table 4.2).

Table 4.2 Ventricular characteristics and fatty acid composition of heart phospholipids from chickens fed experimental diets varying in LA to ALA ratio for 28 days[1]

	Experimental diets			
	Control	**High ALA**		
LA (% en)	2.34	2.90		
ALA (% en)	0.24	2.12		
LA:ALA ratio	9.75:1	1.37:1		
Fat Content (%)	5.05	5.11		
Fatty acids[2]	(% of total fatty acids)[1]		PSEM	P value
16:0	16.83	16.97	0.601	NS
18:0	20.68	21.44	0.481	NS
SFA	41.59	42.97	0.323	NS
18:1n-9	13.74[b]	10.31[a]	0.260	*
18:1n-7	3.98	3.02	0.196	NS
MUFA	19.88[b]	14.59[a]	0.446	*
20:3n-9	1.55[b]	0.78[a]	0.063	*
18:2n-6	25.43	25.93	0.729	NS
20:3n-6	1.50	1.33	0.109	NS
20:4n-6	7.46	8.22	0.684	NS
Total n-6	35.57	36.63	0.408	NS
18:3n-3	0.08[a]	0.48[b]	0.017	**
20:3n-3	0.06[a]	0.21[b]	0.010	**
20:5n-3	0.44[a]	2.21[b]	0.090	**
22:5n-3	0.25[a]	1.15[b]	0.053	**
22:6n-3	0.14[a]	0.64[b]	0.012	**
Total n-3	0.99[a]	4.71[b]	0.018	**
Total PUFA	36.56[a]	41.34[b]	0.410	*
RV/TV ratio (%)				
RV/TV	19.12	21.39	1.225	NS

[a,b] Values in the same row with no common superscript are significantly different (P< 0.05)
[1]Values are means of 12 observations per treatment
[2]SFA= saturated fatty acid; MUFA= monounsaturated fatty acid; PUFA= polyunsaturated fatty acid; RV= right ventricle; TV= Total ventricle
PSEM = pooled standard error of the mean
NS=not significant
*P<0.05
**P<0.01

4.4 DISCUSSION

This aspect of the current project was conducted to examine whether increasing the level of dietary ALA was potentially beneficial for the health of fast growing broilers. Our study found that with increasing dietary level of ALA, heart membrane n-3 LCPUFA increased 4-5-fold. The level of EPA was found in the highest amount (2.2%) among n-3 LCPUFA and appears as the major n-3 LCPUFA in heart tissues while DHA was less than 1% of the total fatty acids. This might be because DHA has a limited ability to accumulate in cardiac tissue in chickens. This contrasts with the fatty acid patterns in human hearts where the level of EPA is normally around 0.5% and DHA is nearly 5% of the total fats [91]. Feeding fish oil to human subjects has been shown to increase EPA and DHA levels markedly but feeding ALA rich flax oil had no effect [91].

We observed that there was no difference in the percentage of right ventricle (RV) to total ventricle (TV) of birds with increasing dietary ALA level in the diet using 1.7% flaxseed oil. These results are supported by other investigators. Walton *et al*. [88] conducted a study in broiler chickens fed diets containing 2.5 or 5% flaxseed oil or control diets with equivalent amounts of animal/vegetable blend oil for 4 weeks using hypobaric chambers and control pens. They found that the inclusion level of 5% flaxseed oil in the diet reduced right ventricle hypertrophy in birds exposed to hypobaric conditions compared to the birds fed with control diets. However, there was no change observed in the birds fed with 2.5% flaxseed oil. Thus our inability to demonstrate a change in ventricular size may have been due to the lower level of flaxseed oil tested (1.7%).

The ratio of RV to TV both in the control diet (19.12%) and diet high in ALA (21.39%) was <0.25 (25%). It indicates that the birds were in a normal range as noted by Julian [90]. Studies conducted by other investigators show that increasing dietary ALA seems to be a potential way to reduce RVH leading to ascites [88], mortality and the incidence of ascites at a high altitude [89] and the incidence of pulmonary hypertension syndrome [92]. Potential of diets rich in ALA to prevent sudden death needs to be fully evaluated in a large-scale commercial setting and in an environment more conducive to causing sudden death syndrome.

4.5 CONCLUSION

Increasing levels of dietary ALA raised the level of n-3 LCPUFA in heart tissues by 4-5-fold but did not cause change in the percentage of RV to TV of birds. It appears clear that based on our data and the work of others, increasing ALA content in the diet of chickens could potentially be beneficial for the health of the birds; however, further work is necessary.

CHAPTER 5

General Discussion and Conclusions

The work described in this thesis highlights the efficacy of diets rich in vegetable oils containing ALA as a means of increasing the n-3 LCPUFA levels of chicken meat, although this observation may be variety specific since we only used Cobb 500 birds.

The observation that diets rich in ALA were capable of increasing n-3 LCPUFA in chicken tissues was not novel. However, our work is unique in that the levels of n-3 LCPUFA achieved were extremely high and there was no indication of a maximal effect of dietary ALA on n-3 LCPUFA levels. In fact, the levels of n-3 LCPUFA that we saw in the meat of chickens fed diets with the highest level of ALA were close to those seen in chickens fed fish oil and were also close to the levels seen in some white table fish. The reasons for the high levels of n-3 LCPUFA achieved are not entirely clear but might be due to the different strains of chickens used in our study compared to previous reports. Alternatively, it is clear that LA can interfere with n-3 LCPUFA accumulation and some of the poor conversions reported by other workers may be due to high LA levels in the diet. It is obvious that direct comparison of chicken varieties on a range of diets is important to clarify this issue.

Currently the marine stocks that serve as the main source of n-3 LCPUFA are threatened by being continually depleted. Thus, vegetable oils that can be a sustainable alternative source of these fatty acids when fed to meat-producing

animals are important. The use of vegetable oils rich in ALA may reduce harvesting pressure on the marine environment by providing meat rich in n-3 LCPUFA for the consumers.

The use of vegetable oils rich in ALA in the diet of chickens also has the potential to increase n-3 LCPUFA without reducing sensory quality of meat although this was not tested in the current experiments. The fact that chicken diets enriched with as little as 3% fish oil had negative impacts on the sensory properties of the meat underlines the importance of evaluating whether enrichment of n-3 LCPUFA in chicken meat with ALA could be an alternative way without sensory quality losses.

The levels of n-3 LCPUFA found in blood (erythrocyte and plasma) were highly correlated with the levels seen in liver, breast and thigh tissues. This suggests that fatty acid compositions of blood samples can be used to evaluate the fatty acid composition of chicken meat. Thus blood sampling may be a useful tool for evaluating diets commercially without sacrificing birds.

5.1 STUDY LIMITATIONS

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

- Chickens used in this experiment were limited to one strain of chicken, thus we cannot evaluate whether our observation is universal or not.
- The number of chickens was not large enough to evaluate the effects of diets on the production or health status of birds.

- It was not possible to determine whether responses differed according to gender of the chickens.
- The duration of the studies should be extended to approximately 42 days in order to reflect the current length of broiler chicken production.

5.2 FUTURE DIRECTIONS

These studies have demonstrated that meat n-3 LCPUFA can be increased by increasing ALA levels in the diet of chickens but many questions remain:

- There is a need to determine whether even higher levels of dietary ALA can increase the levels of n-3 LCPUFA in chicken meat even further.
- While increased n-3 LCPUFA of broiler Cobb 500 was successfully achieved, there is a need to explain if the poorer results reported by others are due to the use of other strains of birds.
- There is a need to determine whether males respond differently to females, particularly in respect to cardiac health as male broiler chickens grow faster than females and are more at risk from heart failure.
- The effectiveness of ALA rich diets on n-3 LCPUFA status needs to be evaluated in other poultry products such as eggs.
- As one of the goals of food research is to increase the nutritional value of foods without affecting sensory quality, the sensory properties of chicken meat enriched with high levels of ALA needs to be evaluated.

5.3 CONCLUSIONS

This thesis has highlighted that meat n-3 LCPUFA can be increased by increasing the levels of ALA-rich vegetable oils in chicken diets. This strategy could help to provide a variety of foods rich in n-3 LCPUFA that may have beneficial health effects for humans. Chicken diets enriched with ALA may also be potentially beneficial to the health of birds and may reduce production costs.

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APPENDIX 1

Validation studies

To determine the consistency of the analysis of fatty acid methyl ester samples, one vial of each sample was run on the GC in succession six times. Each chromatogram was analysed and the mean and standard deviation calculated to determine any variation due to the hardware or the analysis of samples. The percentage variation for individual fatty acids was low and rarely exceeded 1%.

Table 1 Intraday variation of erythrocyte fatty acids

Fatty acids (%)	Mean	SD	% Variation[1]
16:0	19.13	0.03	0.17
18:0	11.81	0.07	0.57
Total SFA	36.10	0.12	0.33
18:1n-9	11.89	0.14	1.15
Total MUFA	16.25	0.19	1.14
18:2n-6	25.91	0.14	0.54
20:4n-6	3.51	0.03	0.86
Total n-6	30.93	0.18	0.59
18:3n-3	3.24	0.02	0.64
20:5n-3	1.55	0.01	0.81
22:5n-3	1.63	0.01	0.77
22:6n-3	3.05	0.03	0.84
Total n-3	9.53	0.08	0.79

[1]Variation (%) = SD/Mean x 100

Table 2 Intraday variation of plasma fatty acids

Fatty acids (%)	Mean	SD	% Variation[1]
16:0	19.31	0.01	0.08
18:0	23.26	0.02	0.07
Total SFA	44.06	0.04	0.08
18:1n-9	12.41	0.01	0.05
Total MUFA	16.74	0.07	0.40
18:2n-6	17.88	0.01	0.07
20:4n-6	3.78	0.01	0.20
Total n-6	23.50	0.02	0.06
18:3n-3	1.68	0.01	0.39
20:5n-3	2.36	0.01	0.54
22:5n-3	1.17	0.00	0.10
22:6n-3	2.69	0.00	0.13
Total n-3	8.02	0.01	0.15

[1]Variation (%) = SD/Mean x 100

Table 3 Intraday variation of liver fatty

Fatty acids (%)	Mean	SD	% Variation[1]
16:0	17.48	0.04	0.25
18:0	26.45	0.06	0.22
Total SFA	45.05	0.13	0.29
18:1n-9	10.69	0.03	0.25
18:1n-7	1.63	0.02	1.03
Total MUFA	14.03	0.06	0.42
18:2n-6	16.60	0.04	0.21
20:4n-6	7.54	0.02	0.21
Total n-6	26.22	0.06	0.22
18:3n-3	1.08	0.00	0.29
20:5n-3	3.47	0.00	0.10
22:5n-3	1.71	0.00	0.25
22:6n-3	6.38	0.01	0.20
Total n-3	12.79	0.02	0.17

[1]Variation (%) = SD/Mean x 100

Table 4 Intraday variation of breast fatty acids

Fatty acids (%)	Mean	SD	% Variation[1]
16:0	20.42	0.02	0.11
18:0	10.08	0.01	0.05
Total SFA	37.05	0.06	0.17
18:1n-9	19.69	0.03	0.16
18:1n-7	4.88	0.01	0.19
Total MUFA	28.19	0.04	0.12
18:2n-6	14.22	0.02	0.12
20:4n-6	3.90	0.00	0.09
Total n-6	21.05	0.03	0.12
18:3n-3	1.55	0.00	0.21
20:5n-3	2.84	0.01	0.24
22:5n-3	3.92	0.01	0.23
22:6n-3	3.49	0.00	0.12
Total n-3	12.71	0.02	0.15

[1]Variation (%) = SD/Mean x 100

Table 5 Intraday variation of thigh fatty acids

Fatty acids (%)	Mean	SD	% Variation[1]
16:0	18.09	0.04	0.21
18:0	13.02	0.03	0.26
Total SFA	37.96	0.03	0.07
18:1n-9	18.00	0.04	0.23
18:1n-7	4.20	0.01	0.21
Total MUFA	25.07	0.03	0.10
18:2n-6	17.30	0.04	0.25
20:4n-6	4.97	0.01	0.28
Total n-6	24.49	0.07	0.27
18:3n-3	1.45	0.00	0.22
20:5n-3	2.33	0.01	0.30
22:5n-3	3.71	0.01	0.29
22:6n-3	2.81	0.01	0.32
Total n-3	10.89	0.03	0.24

[1]Variation (%) = SD/Mean x 100

Table 6 Intraday variation of heart fatty acids

Fatty acids (%)	Mean	SD	% Variation[1]
16:0	14.86	0.02	0.11
18:0	23.52	0.04	0.15
Total SFA	42.55	0.04	0.09
18:1n-9	8.63	0.01	0.09
18:1n-7	2.61	0.02	0.75
Total MUFA	12.86	0.03	0.21
18:2n-6	28.65	0.04	0.14
20:4n-6	8.03	0.01	0.15
Total n-6	39.16	0.05	0.14
18:3n-3	0.60	0.00	0.22
20:5n-3	1.59	0.01	0.33
22:5n-3	1.20	0.00	0.09
22:6n-3	0.52	0.00	0.79
Total n-3	4.15	0.01	0.18

[1]Variation (%) = SD/Mean x 100

APPENDIX 2

Composition and amount of vegetable oils

Table 1 The amount of oil needed for 100kg feed for experiment I

Diet details (fat content of basal diet 2.5%)	Oil (kg)				
	Vol.	Macadamia	Flaxseed	canola	Sunflower
Control diet Fat content 5% 2.5% macadamia	2.50	2.50	-	-	-
Diet 1 Fat content 5% 2.5% of 10% Flaxseed and 90% canola oil	2.50	-	0.25	2.25	-
Diet 2 Fat content 5% 2.5% of 1:2 blend of Flaxseed and canola	2.50	-	0.83	1.67	-
Diet 3 Fat content 5% 2.5% flaxseed oil	2.50	-	2.50	-	-
Diet 4 Fat content 7.5% 5% of 32% flaxseed, 34% canola, 30% macadamia and 4% sunflower	5.00	1.50	1.60	1.70	0.2
Diet 5 Fat content 7.5% 5% flaxseed oil	5.00	-	5.0	-	-
Diet 6 Fat content 10% 7.5% flaxseed oil	7.50	-	7.50	-	-

Table 2 The amount of oil needed for 100 kg feed for experiment II

Diet details (fat content of basal diet 2.2%)	Oil (kg)			
	Volume	Macadamia	Flaxseed	Sunflower
Reference diet Fat content 5% 2.8% macadamia	2.8	2.8	-	-
Diet 1 Fat content 5% 1.7% Flaxseed and 1.1% macadamia oil	2.8	1.1	1.7	-
Diet 2 Fat content 5% 1.7% Flaxseed and 1.1% of 50% macadamia and 50% sunflower oil	2.8	0.55	1.7	0.55
Diet 3 Fat content 5% 1.92% flaxseed and 0.88% sunflower oil	2.8	-	1.92	0.88