Effects of feeding omega-3 fatty acids and vitamin E on the chemical composition and microbial population of broiler meat

Gerna Coetzee

Thesis presented in partial fulfilment of the requirements for the degree of

Master of Science in Agriculture

at the University of Stellenbosch

Supervisor: Dr L. C. Hoffman

Co-supervisor: Prof J. P. Hayes

March 2000

Stellenbosch

I, the undersigned, hereby declare that the work contained in this thesis is my own original work and that I have not previously in its entirety or in part submitted it at any university for a degree.

Summary

Title: Improving the nutritional value of broiler meat through increased omega-3 fatty acid and vitamin E content.

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Lipids remain one of the most important nutrients required by broilers. The growing awareness that some Western societies have too high a dietary ratio of n-6/n-3 polyunsaturated fatty acids is of direct relevance to broiler nutrition and lipid metabolism. Meaningful quantities of n-3 polyunsaturates have been incorporated into major poultry tissues, so that the production of broiler meat with high n-3 polyunsaturates becomes advantageous for the broiler industry as they are perceived as having a 'healthier' lipid profile. Unfortunately, such broiler meat is rather susceptible to oxidative deterioration, and oxidation often determines shelf life of poultry meat products. The addition of α-tocopherol (vitamin E) to broiler diets is an effective means of improving the oxidative stability of broiler meat. Elevated α-tocopherol levels in broiler feeds increase tissue concentrations thereof resulting in improved stability of membranal structures which may be expected to increase the oxidative stability of broiler meat and meat products.

Three investigations were done at Mariendahl Poultry Research Station in Stellenbosch. The broilers were kept in 1 x 0.4 x 0.5 m cages in a broiler rearing house. All the trials started with day-old chicks, except experiment 1 where 3-week old broilers were used. At the end of trials 2 and 3 the 6-week old broilers were slaughtered and the carcasses prepared for chemical analysis.

Experiment 1: **Metabolisable energy of Canola acid oil and Famarol acid oil for broiler chickens.**

In trials with 21-day-old male broilers the true metabolisable energy value, corrected for nitrogen retention (TMEn) was determined by the balance method for Canola acid oil (CAO) and Famarol acid oil (FAO). The trials were duplicated, each time using different samples of the two oils from the same source (experiment 1 and 2). Each of the two oils were blended in two ratios with a basal diet to form the test diets, viz. 100% Basal; 96% Basal: 4% Oil; 92% Basal: 8% Oil. In experiment 3, 50 % bran was added to the maize to form the basal diet. The balance trials lasted for 3 days after an adaptation period of 4 days. The TMEn values determined by regression for the broilers of CAO did not differ significantly ($P>0.05$) between experiments 1 and 2. However, the value for experiment 3 was significantly ($P<0.05$) higher than those for experiments 1 and 2. The TMEn values of FAO also did not differ significantly ($P>0.05$) between experiments 1 and 2, although the value for experiment 3 was significantly higher than that of experiment 1. The addition of 50 % bran to the basal diet in experiment 3 could have stimulated the digestive breakdown process and hence increase the secretion of digestive enzymes. This could lead to an increase in the utilisation of the test lipid and therefore an increase in the TMEn value. The TMEn values of CAO differed significantly ($P<0.05$) from those of FAO for all three the experiments (exp. 1:30.6 ± 0.399 MJ/kg for CAO vs. 25.9 ± 0.441 MJ/kg for FAO; exp. 2: 31.0 ± 0.633 MJ/kg for CAO vs. 26.1 ± 0.668 MJ/kg for FAO: exp. 3: 32.1 ± 0.867 MJ/kg for CAO vs. 27.1 MJ/kg for FAO).

Experiment 2: **Effects of various dietary n-6/n-3 fatty acid ratios on the performance and body composition of broilers.**

The dietary effects of various combinations of Canola acid oil (CAO, a high level of C18:3n-3 and MUFA) and Famarol acid oil (FAO, a high level of 18:2n-6 and SFA) on tissue fatty acid composition were studied in broiler carcasses and abdominal fat pads. From day-old to six weeks, chicks were fed one of six diets containing 100% FAO, 80% FAO-20% CAO, 60% FAO-40% CAO, 40% FAO-60% CAO, 20% FAO-80% CAO, 100% CAO. There were no statistical differences ($P>0.05$) in average daily gain (1.71 ± 0.059 g) or feed conversion ratios (1.97 ± 0.051) among dietary groups. No statistical differences ($P>0.05$) were found in the chemical proximate composition of the carcasses for the moisture (66.20 ± 0.112 %), protein (17.63 ± 0.484 %), lipid (15.92 ± 1.507 %) and ash (0.95 ± 0.115 %) content among dietary groups. No statistical differences ($P>0.05$) were found in the chemical proximate composition of the abdominal fat pads for the moisture (28.77 ± 0.112 %), protein (3.03 ± 0.484 %), lipid (63.32 ± 9.789 %) and ash (0.45 ± 0.135 %) content among dietary groups. With the increase in dietary CAO levels, the percentages of C18:2n-6 and C20:4n-6 in the carcasses decreased respectively with 1.78 % from 20.88 % and 0.35 % from 1.05 %, whilst C18:3n-3 and longer chain n-3 fatty acids such as C20:5n-3 and C22:6n-3 increased respectively with 2.25 % from 1 %, 0.1 % from 0.1 % and 0.67 % from 0.2 %. The same tendency was seen in the abdominal fat pads where C18:2n-6 and C20:4n-6 decreased respectively with 1.55 % from 20.75 % and 0.98 % from 1.2 % with an increase in dietary CAO, whilst C18:3n-3, C20:5n-3 and C22:6n-3 increased respectively with 2.13 % from 1.15 %, 0.45 % from 0.03 % and 0.95 % from 0.05 %. The n-3/n-6 ratio in the carcasses and abdominal fat pads increased respectively with 0.16 % from 0.06 % and 0.19 % from 0.06 % with an increase in dietary CAO. These results clearly indicate that dietary CAO enriched with α-linolenic acid lower saturated fatty acids respectively in broiler carcasses and abdominal fat pads with 4.88 % from 31.6 % and 10.63% from 31.1 %, whilst increasing monounsaturated fatty acids with 3.87 % from 44.95 % and 7.25 % from 46.7 % respectively and polyunsaturated fatty acids with 1.02 % from 23.45 % and 2.38 % from 23.2 % respectively.

Experiment 3: **Effect of dietary vitamin E on the performance of broilers and oxidative stability, colour, microbiological stability, fatty acid composition and pH of broiler meat during refrigerated and frozen storage.**

Experiment 1 was carried out with 220 one-day-old broiler chicks to evaluate the effect of eleven concentrations of vitamin E (0, 20, 40, 60, 80, 100, 120, 140, 160, 180 and 200 mg α-tocopheryl acetate / kg diet) on their production performance and the oxidative stability of their frozen broiler carcasses. The diets with vitamin E levels 0 to 100 mg were fed from day-old to 42 days of age while the diets with vitamin E levels 120 to 200 mg were fed from 21 to 42 days of age. The oxidative stability, evaluated by thiobarbituric acid reactive substances (TBARS) values, was determined after 30, 90, 120 and 150 days of storage at -20°C. There were no statistical differences ($P>0.05$) in average daily gain (1.85 ± 0.111 g) or feed conversion ratios (2.29 ± 0.397) among dietary groups. TBARS values increased significantly ($P<0.05$) with increasing time of storage (basal diet: day 30 = 1.71 ± 0.51; day 150 = 4.89 ± 0.51), but decreased significantly ($P<0.05$) with increasing vitamin E levels (day 150: basal = 4.89 ± 0.51; 100 mg / kg = 1.09 ± 0.27). Experiment 2 was carried out with day-old broiler chicks to evaluate the effect of five concentrations of vitamin E (0, 40, 80, 120 and 160 mg α-tocopheryl acetate / diet) on their performance and the oxidative stability of their refrigerated carcasses. The experimental diets were fed from day-old to 42 days of age. The oxidative stability, evaluated by TBARS values, colour deterioration and microbiological stability were determined after 0, 4, 8, 10 and 12 days of storage at 4°C. Fatty acid analysis was done on the samples of days 0 and 12. There were no statistical differences ($P>0.05$) in average daily gain (1.88 ± 0.117 g) or feed conversion ratios (2.37 ± 0.467) among dietary groups. TBARS values increased significantly ($P<0.05$) with increasing time of storage, but decreased significantly ($P<0.05$) with increasing vitamin E levels. There were no statistical differences ($P>0.05$) in colour measurements for L* (44.97 ± 0.662), a* (5.23 ± 0.315) or b* (12.76 ± 0.321) values between treatments. Microbiological counts increased significantly ($P<0.05$) over time with vitamin E concentration showing no effect. There were no statistical differences ($P>0.05$) for any of the fatty acid groups measured (SFA: Day 0 = 26.1 ± 1.13%, Day 12 = 26.1 ± 1.17%; MUFA: Day 0 = 41.4 ± 1.46%, Day 12 = 40.2 ± 2.28%; PUFA: Day 0 = 32.4 ±

1.95%, Day 12 = 33.8 ± 2.52%) among dietary groups. Similarly, none of the fatty acids showed statistical significant (P>0.05) concentration changes over time. There were no statistical differences (P>0.05) in pH (6.01 ± 0.206) among dietary groups.

Opsomming

Titel: Verbetering van die voedingswaarde van braaikuikenvleis deur die verhoging van die omega-3 vetsuurinhoud en dié van vitamine E.

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Lipiede is steeds een van die mees belangrike voedingstowwe wat deur braakuikens benodig word. Die groeiende bewuswording dat sekere Westerse gemeenskappe 'n te hoë verhouding van n-6/n-3 poli-onversadigde vetsure in hul dieet het, is direk relevant vir braaikuikenvoeding en lipiedmetabolisme. Betekenisvolle hoeveelhede n-3 poli-onversadigde vetsure is geïnkorporeer in die belangrikste hoendersnitte, met die gevolg dat die produksie van braaikuikenvleis met hoë n-3 poli-onversadigde vetsure voordelig is vir die braaikuikenindustrie en geag word 'n meer "gesonde" beeld te hê. Ongelukkig is sodanige braaikuikenvleis redelik vatbaar vir oksidatiewe bederf, en oksidasie bepaal dikwels die rakleeftyd van hoendervleisprodukte. Die byvoeging van α-tokoferol (vitamine E) by braaikuikendiëte is 'n effektiewe manier om die oksidatiewe stabiliteit van braaikuikenvleis te verbeter. Verhoogde α-tokoferol vlakke in braakuikenvoere verhoog die weefselkonsentrasie wat verhoogde stabiliteit van die membraanstrukture en derhalwe moontlike verhoogde oksidatiewe stabiliteit van braakuikenvleis en –produkte tot gevolg het.

Drie ondersoeke is onderneem by Mariendahl Pluimvee Navorsingstasie te Stellenbosch. Die braakuikens is aangehou in 1 x 0.4 x 0.5m hokke in braaikuikenhuise. In al die proewe is dagoud kuikens gebruik, behalwe eksperiment 1 waar drie-week oue kuikens gebruik is. Aan die einde van proewe 2 en 3 is die ses-week oue braaikuikens geslag en die karkasse voorberei vir analise.

Eksperiment 1: **Metaboliseerbare energie van Canola voergraadolie en Famarol voergraadolie vir braaikuikens.**

Die ware metaboliseerbare energie waarde van Canola voergraadolie (CAO) en Famarol voergraadolie (FAO), gekorregeer vir stikstof retensie (WMEn), is by wyse van proewe op 21 dae oue braaikuikenhaantjies bepaal deur van die balansmetode gebruik te maak. Die proewe is tweemaal herhaal vir verhoogde akkuraatheid, met die gebruik van verskillende monsters van die twee olies vanaf dieselfde bron. Die olies is in twee verhoudings met 'n basale diëet gemeng om die proef dieet te vorm, nl. 100% Basaal; 96% Basaal: 4% Olie en 92% Basaal: 8% Olie. Die balans proewe het 3 dae geduur na afloop van 'n aanpassingsperiode van 4 dae. Die WMEn waardes van CAO, bepaal deur middel van regressie analise, het nie betekenisvol verskil ($P>0.05$) tussen eksperimente 1 en 2 nie. Die waarde van eksperiment 3 was betekenisvol hoër ($P<0.05$) as die van die eerste twee eksperimente. Die WMEn waardes van FAO het ook nie betekenisvol verskil ($P>0.05$) tussen eksperimente 1 en 2 nie, maar die waarde vir eksperiment 3 was betekenisvol hoër as dié van eksperiment 1. Die WMEn waardes van COA het betekenisvol verskil ($P< 0.05$) van dié van FAO vir al die eksperimente (exp. 1: 30.6 $\pm$ 0.399 MJ/kg vir CAO vs. 25.9 $\pm$ 0.441 MJ/kg vir FAO; exp. 2: 31.0 $\pm$ 0.633 MJ/kg vir CAO vs. 26.1 $\pm$ 0.668 MJ/kg vir FAO: exp. 3: 32.1 $\pm$ 0.867 MJ/kg vir CAO vs. 27.1 MJ/kg vir FAO).

Eksperiment 2: **Die invloed van verskeie rantsoen n-6/n3 vetsuurverhoudings op die produksie en liggaamsamestelling van braaikuikens.**

Die rantsoeneffek van verskeie kombinasies Canola voergraadolie (CAO, 'n hoë vlak van C18:3n-3 en mono-onversadigde vetsure) en Famarol voergraadolie (FAO, 'n hoë vlak van 18:2n-6 en versadigde vetsure) op die weefselvetsuursamestelling is bestudeer in braaikuikenkarkasse en abdominale vetneerlegging. Die kuikens is van dag-oud to op ses-weke ouderdom een van ses diëte gevoer met die volgende samestellings: 100% FAO, 80% FAO-20% CAO, 60% FAO – 40%CAO, 40% FAO – 60% CAO, 20% FAO – 80% CAO, 100% CAO. Daar was geen statistiese verskil tussen die rantsoengroepe ($P>0.05$) in die gemiddelde daaglikse toename (1.71 ± 0.059 g) of die voeromsetverhoudings (1.97 ± 0.051) nie. Geen statistiese verskil ($P>0.05$) is gevind in die chemiese samestelling van die karkasse vir vog (66.20 ± 0.112 %), proteïn (17.63 ± 0.484 %), lipied (15.92 ± 1.507 %) en as (0.95 ± 0.115 %) inhoud tussen die rantsoen groepe nie. Geen statistiese verskille ($P>0.05$) is gevind in die chemiese samestelling van die abdominale vetneerlegging vir vog (28.77 ± 0.112 %), proteïen (3.03 ± 0.484 %), lipied (63.32 ± 9.789 %) en as (0.45 ± 0.135 %) inhoud onder die rantsoengroepe nie. Met die verhoging in die rantsoen CAO vlakke het die persentasie van C18:2n-6 en C20:4n-6 in die karkasse verminder met 1.78 % en 0.35 % respektiewelik, terwyl C18:3n-3 en langer ketting n-3 vetsure soos C20:5n-3 en C22:6n-3 respektiewelik met 2.25 %, 0.1 % en 0.67 % verhoog het. Dieselfde tendens is opgemerk in die abdominale vetneerlegging waar C18:2n-6 en C20:4n-6 afgeneem het met 1.55 % en 0.98 % respektiewelik met die verhoging van rantsoen CAO, terwyl C18:3n-3, C20:5n-3 en C22:6n-3 verhoog het met 2.13 %, 0.45 % en 0.95 % respektiewelik. Die n-3/n-6 verhouding in die karkasse en abdominale vetneerlegging het verhoog met 0.16 % en 0.19 % respektiewelik met die verhoging van die rantsoen CAO. Die resultate toon onomwonde aan dat rantsoen CAO verryk met α-linoleïensuur, verlaag versadigde vetsure in braaikuikenkarkasse en –adbdominale vetneerleggings met 4.88 % en 10.63% respektiewelik, terwyl die mono-onversadigde vetsure met 3.87 % en 7.25 % respektiewelik verhoog word en poli-onversadigde vetsure met 1.02 % en 2.38 % respektiewelik verhoog word.

Eksperiment 3: **Die invloed van vitamine E op die produksie van braaikuikens en die oksidatiewe stabiliteit, kleur, mikrobiologiese stabilitiet, vetsuursamestelling en pH van braaikuikenvleis gedurende verkoelde en bevrore berging.**

Eksperiment 1 is uitgevoer met 220 dagoud braaikuikens ten einde die effek van elf konsentrasies van vitamine E (0, 20, 40, 60, 80, 100, 120, 140, 160, 180 en 200 mg α-tokoferyl acetaat / kg voer) op hul produksieprestasie en die oksidatiewe stabiliteit van hul gevriesde braakuikenkarkasse te evalueer. Die diëte met vitamine E vlakke 0 tot 100 mg is vanaf dagoud tot 42-dae-ouderdom gevoer, terwyl die diëte met vitamine E vlakke van 120 tot 200mg gevoer is vanaf 21 tot 42-dae-ouderdom. Die oksidatiewe stabiliteit, soos geëvalueer deur tiobarbituriese suur reaktiewe stowwe (TBARS) waardes, is bepaal na 30, 90, 120 en 150 dae van berging teen -20°C. Daar was geen statistiese verskille ($P>0.05$) in die gemiddelde daaglikse toename (1.85 ± 0.111 g) of voeromsetverhoudings (2.29 ± 0.397) tussen die rantsoengroepe nie. TBARS waardes het betekenisvol toegeneem ($P<0.05$) met die verhoging in bergingsperiode, maar het betekenisvol afgeneem ($P<0.05$) met verhoogde vitamine E vlakke. Eksperiment 2 is uitgevoer met dagoud braaikuikens ten einde die effek van vyf konsentrasies van vitamine E (0, 40, 80, 120 and 160 mg α-tokoferyl acetaat / kg voer) op hul prestasie en die oksidatiewe stabiliteit van hul verkoelde karkasse te evalueer. Die eksperimentele diëte is gevoer vanaf dagoud tot 42-dae-ouderdom. Die oksidatiewe stabiliteit, geëvalueer deur middel van TBARS waardes, kleur afname en mikrobiologiese stabiliteit is bepaal na 0, 4, 8, 10 en 12 dae van berging teen 4°C. Vetsuuranalises is gedoen op die monsters van dae 0 en 12. Daar was geen statistiese verskille ($P>0.05$) in die gemiddelde daaglikse toename (1.88 ± 0.117 g) of voeromsetverhoudings (2.37 ± 0.467) tussen die rantsoengroepe nie. TBARS waardes het betekenisvol verhoog ($P<0.05$) met die verlengde bergingsperiode, maar het betekenisvol afgeneem ($P<0.05$) met verhoogde viatmine E vlakke. Daar was geen statistiese verskille ($P>0.05$) in kleur metings vir L^* (44.97 ± 0.662), a^* (5.23 ± 0.315) of b^* (12.76 ± 0.321) waardes tussen behandelings nie. Mikrobiologiese tellings het betekenisvol verhoog ($P<0.05$) oor tyd met die vitamine E

konsentrasie wat geen effek getoon het nie. Daar was geen statisties betekenisvolle verskille ($P>0.05$) vir enige van die vetsuurgroepe tussen die behandelings nie. Soortgelyks het geen van die vetsure statisties betekenisvolle ($P>0.05$) konsentrasieveranderings oor tyd aangetoon nie. Daar was geen statistiese verskil ($P>0.05$) in die pH (6.01 ± 0.206) tussen die rantsoengroepe nie.

Acknowledgements

Work is not primarily a thing one does to live, but the thing one lives to do. It is, or should be, the full expression of the worker's faculties, the thing in which he finds spiritual, mental and bodily satisfaction, and the medium in which he offers himself to God – Dorothy Sayers.

I wish to express my sincere acknowledgement and appreciation to the following people:

Dr Hoffman and Prof Hayes for their support, guidance and exceptional knowledge.

Gerrit Ferreira and the staff of Mariendahl for support and assistance with the experimental animals throughout the trial period.

Dr Van Jaarsveld and the staff at the Medical Research Council for the use of their gas chromatograph and assistance with the interpretation of fatty acid analyses.

The Foundation for Research Development, the Harry Crossley Trust and the Protein Research Trust for financial assistance.

Albrecht for his support, understanding and love.

My parents for their encouragement and interest in my work.

Table of Contents

Part 1

Literature Study: Lipids, lipid metabolism and manipulation in poultry

1 Introduction: Importance of research on lipid metabolism in poultry

The importance of commercial broiler farming in South Africa has increased quite rapidly during the last decades partly because of the lowered production costs and health considerations associated with white meat, but also because of the increasing cost of red meat. Broiler production in South Africa for 1996/1997 was 829 ton and the consumption was 833 ton or 20.19 kg/year per capita. Conversely, the production for beef and veal was only 493 ton and the consumption 532 ton or 12.40 kg/year per capita (Abstract of Agricultural Statistics, 1998). Due to the rapid growthrate of broilers it is generally acknowledged that feed usually constitutes the major operating cost in intensive broiler production. Research on broiler nutrition and feed technology plays an increasingly important role in the rapid development of the global broiler industry. Advances in the field of basic and applied research on broiler nutrition have led to the formulation and manufacture of feeds, which are more nutritionally complete and more readily available, compared to that of a few years ago.

Lipids remain one of the most important nutrients required by broilers. Some of the major remaining practical and theoretical problems in broiler nutrition centre on lipids. As in human nutrition, controversies continue to exist over what constitutes a 'desirable' dietary intake of lipid for broilers, particularly in relation to the balance between saturated and unsaturated fatty acids in the diet and, above all, the balance between n-6 and n-3 polyunsaturated fatty acids (PUFA), i.e. the n-6 and n-3 essential fatty acids (EFA) (Leskanich and Noble, 1997).

Medical discoveries detailing the therapeutic effects of fish oils, characterised by high levels of n-3 PUFA, in the treatment and prevention of cardiovascular disease and other diseases in humans, have promoted research on non-fish foods containing elevated levels of n-3 polyunsaturates. The fatty acid composition of the lipids of broiler muscle tissues may be modified to match human nutritional guidelines better by appropriately manipulating the fatty acid composition of the diet (Leskanich and Noble, 1997). Any increase in polyunsaturated fatty acids depends wholly on the level in the feed, and poultry are at an advantage to best effect these changes, because dietary fatty acids are absorbed by monogastric animals and deposited in tissues without significant modification (Moran, 1996). The growing awareness that some Western societies have too high a dietary ratio of n-6/n-3 PUFA (Simopoulos, 1991) is of direct relevance to broiler nutrition and lipid metabolism. Meaningful concentrations of n-3 polyunsaturates have been incorporated into major poultry tissues, so that the production of broiler meat with a high concentration n-3 PUFA becomes advantageous for the broiler industry.

It is necessary for broiler nutritionists to solve the technical and theoretical problems (eg. right quantity to prevent dieases but not to increase oxidation of tissues) posed by n-3 PUFA in broiler production. Simultaneously, broiler nutritionists have opportunities to exploit broilers as model species to investigate the basic roles of n-3 PUFA in the nutrition of animals, including man.

2 Importance and composition of dietary lipids

Dietary lipids are required by poultry both for the provision of metabolic energy, generated as ATP through the oxidative metabolism of fatty acids, and for the production of polar lipids, i.e. phospholipids and sphinogolipids, which play a vital role in the structure of biological membranes at both cellular and subcellular levels. This dual role is reflected in the compartmentalisation of body lipid into adipose tissue, comprising mainly of triacylglycerols, and cell membrane lipid composed mainly of polar lipids and cholesterol (Reed, 1980). Of all dietary ingredients, lipids are the richest in energy (39.2 kJ/g versus 23.4 and 17.2 kJ/g for proteins and carbohydrates, respectively) (Mervyn and Leat, 1983). Besides, dietary lipids play important roles in the provision of essential fatty acids (EFA) and as carriers of fat-soluble vitamins and other compounds, such as sterols. Dietary long-chain polyunsaturated fatty acids are precursors for the synthesis of eicosanoids in poultry. Lipids are also important for the flavour and textural properties of the feed consumed by poultry as well as the poultry meat itself.

Fats and oils from natural sources are composed of a combination of mixed glycerides. The chemical and physical properties of food fats and oils are dependant on the properties of the component fatty acids (chain length, number and position of the double bonds) as well as on the nature of their incorporation into the acylglycerols (Reed, 1980).

2.1 Definition of lipids

The definition of lipids is often stated in terms of solubility. Lipids are a group of substances that are insoluble in water and soluble in fatty substances and organic solvents, such as ether, chloroform, and benzene. Lipids include waxes, oils, fats, steroids and related substances ranging from soaps to petrochemicals (Reed, 1980).

In household terms, simple lipids are often divided into the two groups, fats and oils, based on their melting points.

2.2 Chemical composition of fats and oils

The most common molecule in both dietary and body fats and oils is the triglyceride. Triglycerides are made up of three fatty acids attached to a molecule of glycerol, and they vary in their physical properties according to the chemical structures of fatty acids (Reed, 1980).

2.2.1 Fatty acids

The chemical structures of fatty acids share some common characteristics. Fatty acids consist of a straight carbon chain with a terminal carboxyl group. The chemical elements are the same as those in carbohydrates, but proportionately less oxygen is present in fatty acids. The vast majority of naturally occuring fatty acids have an even number of carbon atoms.

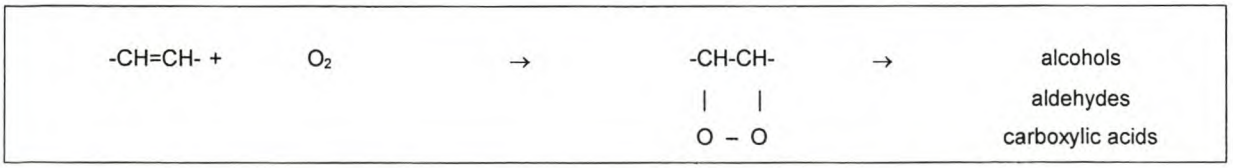
There are two determinants for the consistency of a lipid: (1) the length of the predominant fatty-acid chains and (2) the presence or the absence of double bonds (Reed, 1980).

Fatty acids with twelve or more carbon atoms are referred to as long-chain fatty acids and are typical of fats. The relative hardness of mutton and beef fats can be attributed to the prevalence of such long-chain fatty acids. A predominance of short-to-medium-chain fatty acids (4-6 or 8-12 carbons) contributes towards lower melting points and accounts for the relative softness of butterfat and oils.

The degree of saturation of a fatty acid refers to the presence or absence of double bonds. When a fatty acid is saturated, it means that the carbon atoms bond with all of the hydrogen atoms they can and are thus saturated with hydrogen. A fatty acid that contains double bonds is said to be unsaturated and contributes to the low melting points typical of oils. Oleic acid contains one double bond and is therefore termed monounsaturated. A fatty acid with more than one double bond is said to be polyunsaturated. The polyunsaturated fatty acids (PUFA) predominate in vegetable oils such as Canola oil.

2.2.2 Reaction at double bonds

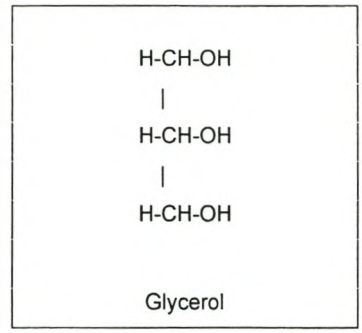
Double bonds are more reactive than single bonds, and several different reactions could occur at the double bonds of fatty acids, such as oxidation. Oxidation is the addition of oxygen at the double bonds.



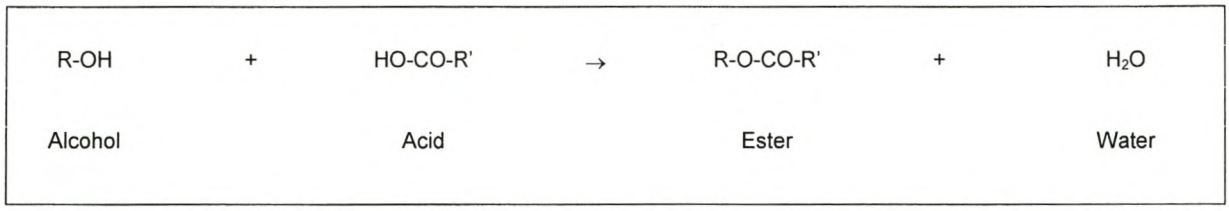
In the fatty acids of foods, oxidation occurs slowly and spontaneously in the presence of air and contributes to rancidity or spoilage. The aldehydes and carboxylic acids are largely responsible for the unpleasant odour and flavour of rancid lipids.

2.2.3 Glycerol

Glycerol itself is not a lipid but an alcohol that functions as a holder molecule for fatty acids. Due to its three -OH groups, glycerol is a trihydroxy alcohol and is uniquely suited to forming the backbone of triglycerides.

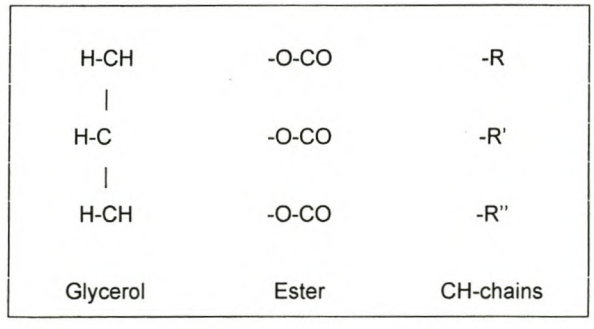


Each –OH group links with a carboxyl group of a fatty acid by esterification. An ester is formed when the removal of a molecule of water links an alcohol with an acid as follows:



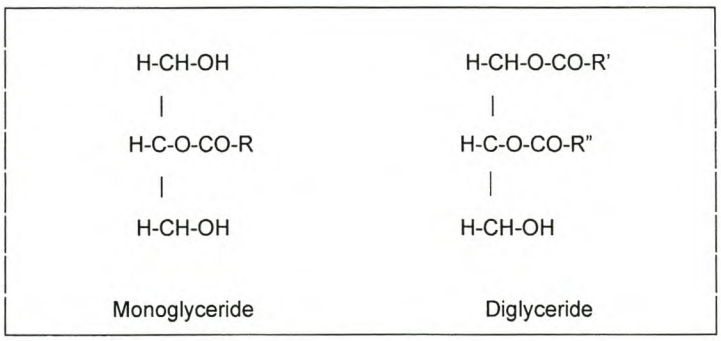
Glycerol can be loaded with as many as three fatty acids to form a large storage molecule for lipids in both feed and tissue.

The general term glyceride refers to an ester of glycerol, and the prefixes mono-, di- and tri- indicate the number of fatty acids attached. A triglyceride can be represented as follows:



A simple triglyceride has three molecules of the same fatty acid, but this form is far less common in nature than mixed triglycerides, which contain different fatty acids varying in carbon chains and double or single bonds.

The incompletely loaded mono- and diglycerides can be illustrated as follows:



2.3 Three classes of lipids

Lipids are divided into three classes: (1) simple lipids, (2) compound lipids, and (3) derived lipids.

2.3.1 Simple lipids

The simple lipids or true fats include the fatty acids, all of the mono-, di-, and triglycerides, and the waxes, which are fatty acid esters of long-chain alcohols or sterols. These waxes, including lanolin and beeswax, often serve to protect skins, furs, and leaves.

2.3.2 Compound lipids

The compound lipids differ from simple lipids by containing a non-lipid part in the molecule. Abounding in nature, the compound lipids are speciality molecules that can accomplish specific functions. For instance, as part of cellular membranes, the phosphorus-containing phospholipids help regulate cell permeability. The carbohydrate-containing glycolipids function in nerve tissue, and the protein-containing lipoproteins act as transport molecules for lipids (Reed, 1980).

2.3.3 Derived lipids

The derived lipids include other compounds that are actually or potentially related to active acetate. Since steroids are synthesized from active acetate, they and their variants are classed as derived lipids.

2.3.3.1 Cholesterol

Perhaps the best known steroid is cholesterol, a steroid alcohol with the hydroxy group, -OH, at the number 3 position, and a double bond at the number 5-carbon atom. The implication of cholesterol in cardiovascular disease (Grundy, 1991) has labeled it a dietary villain and somewhat obscured its normal physiological functions. Cholesterol is a necessary body substance that is found in all animal tissues, and is especially concentrated in the brain and nerve tissues. Cholesterol serves as the percursor for bile acids and for vitamin D. The cholesterol derivate 7-dehydrocholesterol is converted into vitamin D by irradiation with sunlight (Reed, 1980). Cholesterol is also the starting material for a number of hormones, notably the sex hormones – testosterone, the estrogens, and progesterone – and the adrenal cortex hormones – cortisone, cortisol and aldosterone.

The cholesterol pool in the body reflects both dietary consumption and internal synthesis. The only dietary sources are foods from animals, since only animals produce cholesterol. Liver, egg yolks, butterfat, meats, and some shellfish contain significant amounts. The liver and the intestinal mucosa synthesize the endogenous or internal source. Typically, about 2g is synthesized daily in an adult (Reed, 1980).

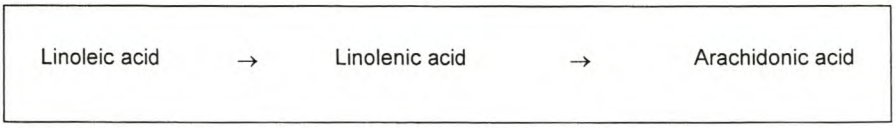
The only means of excreting cholesterol is through the formation of bile acids and their subsequent release into the small intestine. Bile secretions, which are close to 100 percent cholesterol, aid in both the digestion and absorption of lipids and are partially reabsorbed in the ileum and recirculated to the liver. Those that are not reabsorbed are excreted in the faeces. Any substance that binds with bile salts to prevent their resorption increases the excretion of cholesterol (Reed, 1980).

2.4 The essential fatty acids (EFA)

At least one lipid compound is a dietary essential for the chicken, which means that it cannot be produced adequately in the body and must be secured from the diet. Linoleic acid (C18:2n6) is an eighteen-carbon-atom, polyunsaturated fatty acid that cannot be synthesized by the chicken to meet bodily needs. Symtoms of deficiency is poor growth, eczema and watery discharge.

Historically, three PUFA were thought to be dietary essentials: linoleic, α-linolenic (C18:3n3), and arachidonic acid (C20:4n6). All showed EFA activity; they remedied deficiency symptoms in rats and

improved the dermatitis of infants whose diets were low in PUFA. However, more recent studies cast doubt on the essentiality of all but linoleate. Linolenic and arachidonic acids can be synthesized in the body if linoleic acid is in adequate supply (Gurr, 1971).



Although not all of the physiological functions of EFA are known, some have been identified. Linoleic acid is sometimes referred to as vitamin F even though it cannot correctly be classified as a vitamin because it provides energy. Linoleic acid and the two PUFA made from it are structurally necessary in cellular membranes and for the synthesis of tissue lipids, a function thought to be related to dermatitis in deficient infants. Along with other PUFA, linoleic acid helps reduce serum cholesterol. In addition, by making arachidonic acid available, EFA furnish the starting material for prostaglandin production. Often called 'local' hormones because they stimulate actions near their sites of synthesis, the prostaglandins lower blood pressure, reduce gastric secretion, stimulate release of pituitary hormones, inhibit fat catabolism and cause smooth muscles to contract (Reed, 1980).

Linoleic acid is plentiful in vegetable oils, especially safflower oil, which contains 74 % of the EFA. It is found to a lesser extent in poultry (Ratnayake et al., 1989). Although no RDA have been established, the Food and Nutrition Board of the NRC suggests that human consumption of linoleic acid should amount to 1-2 % of the total kilocalorie intake to prevent deficiency symptoms (Food and Nutrition Board, 1979).

3 Digestion, absorption and transport of lipids

Most of our knowledge of the processes of digestion, absorption and transport of fat has been derived from studies in the rat and human. The broad evidence available suggests that the basic physiochemical mechanisms concerned in fat assimilation are similar in monogastric species, such as the fowl.

In efficiently digesting, absorbing and transporting lipids, the body faces major problems due to the physical incompatibility of lipids in water. The water-insoluble lipids are potentially biological 'oil slicks' that can block both digestion and absorption of fatty acids and can handicap transport. The solubility problems are two-fold: (1) Greasy surfaces of globules of dietary fat must be changed so that hydrophilic (water-loving) enzymes can approach them for digestive purposes. (2) Fatty molecules must be altered so that they can be transported in a watery medium, both in the small intestine and later in the systemic blood.

3.1 Emulsification

The key to reducing the physical incompatibility between lipids and water is emulsification. Agents that dissolve in both lipids and water can combine lipids and water to form emulsions. One end of a molecule of an emulsifying agent is lipid-soluble, while the other end can be ionised and combines readily with water. The shield of water keeps the droplets from coalescing and makes them compatible with hydrophilic substances. The emulsifiers of digestion are the colic acids or bile acids produced from cholesterol in the liver. The key function of the salts of these acids is to reduce the physical incompatibility between lipids and water. By acting as emulsifying agents, bile salts make lipids digestible and facilitate their absorption. With the steroid or bile part dissolved in the dietary lipid and in water, a micelle is formed. The hydrophilic lipases can then gain access for digestion, and lipids can be delivered by the aqueous medium to the intestinal lining for absorption (Reed, 1980).

3.2 Lipid digestion

3.2.1 Digestion in the fowl

The digestive tract of the fowl differs in a number of respects from that of other monogastric animals such as pigs and humans. In the fowl the beak replaces the lips and cheeks, the teeth being absent. The crop or diverticulum of the oesophagus is a pear shaped sac whose main function is to act as a reservoir for holding food, although some microbial activity occurs here and results in the formation of lactic and acetic acids. The oesophagus terminates at the proventriculus or glandular stomach, which

leads into the gizzard, a muscular organ that undergoes rhythmic contractions and grinds the food with moisture into a smooth paste. The gizzard, which has no counterpart in the pig or human, although it is often compared to the pyloric part of the mammalian stomach leads into the duodenum, which encloses the pancreas as in mammals. The pancreatic and bile ducts open into the intestine at the termination of the duodenum. Where the small intestine joins the large intestine there are two large blind sacs known as the caeca. The large intestine is relatively short and terminates in the cloaca, from which urine and faeces are excreted together (McDonald et al., 1981).

The goal of digestion is to convert nonabsorbable molecules from the diet into absorbable structures. Lipid digestion relies on the physical changes of emulsification as well as on hydrolytic breakdown to accomplish this goal. The coefficient of digestibility for lipids is 0.95 (Reed, 1980).

Little or no chemical digestive changes occur in the beak or stomach of the chicken. No lipase is produced in the beak to hydrolyse lipids. In the stomach, gastric lipase initiates lipolysis or lipid breakdown of those lipids that are consumed in an emulsified form. However, this digestion is rather insignificant because relatively few of the lipids come in contact with gastric lipase and those that are hydrolysed often recombine to form new triglycerides in the tissue (McDonald et al., 1981).

The small intestine is the major site of lipid digestion. Here the acidity of the entering chyme (the thick semifluid mass of food) stimulates the duodenal mucosa to secrete the hormone cholecystokinin, which in turn triggers the release of bile salts from the gallbladder. The lipids are then emulsified, thereby making the ester linkages of the triglycerides accessible to the lipases. Pancreatic lipase and intestinal lipase attack the emulsified triglycerides. As a result, some triglycerides are hydrolysed completely to their glycerol and three fatty acid components (Figure 3.1). The digestive breakdown may also stop at any stage between intact triglycerides and complete breakdown; the most common digestive products at the intermediate stages are monoglycerides and two fatty acids (Reed, 1980).

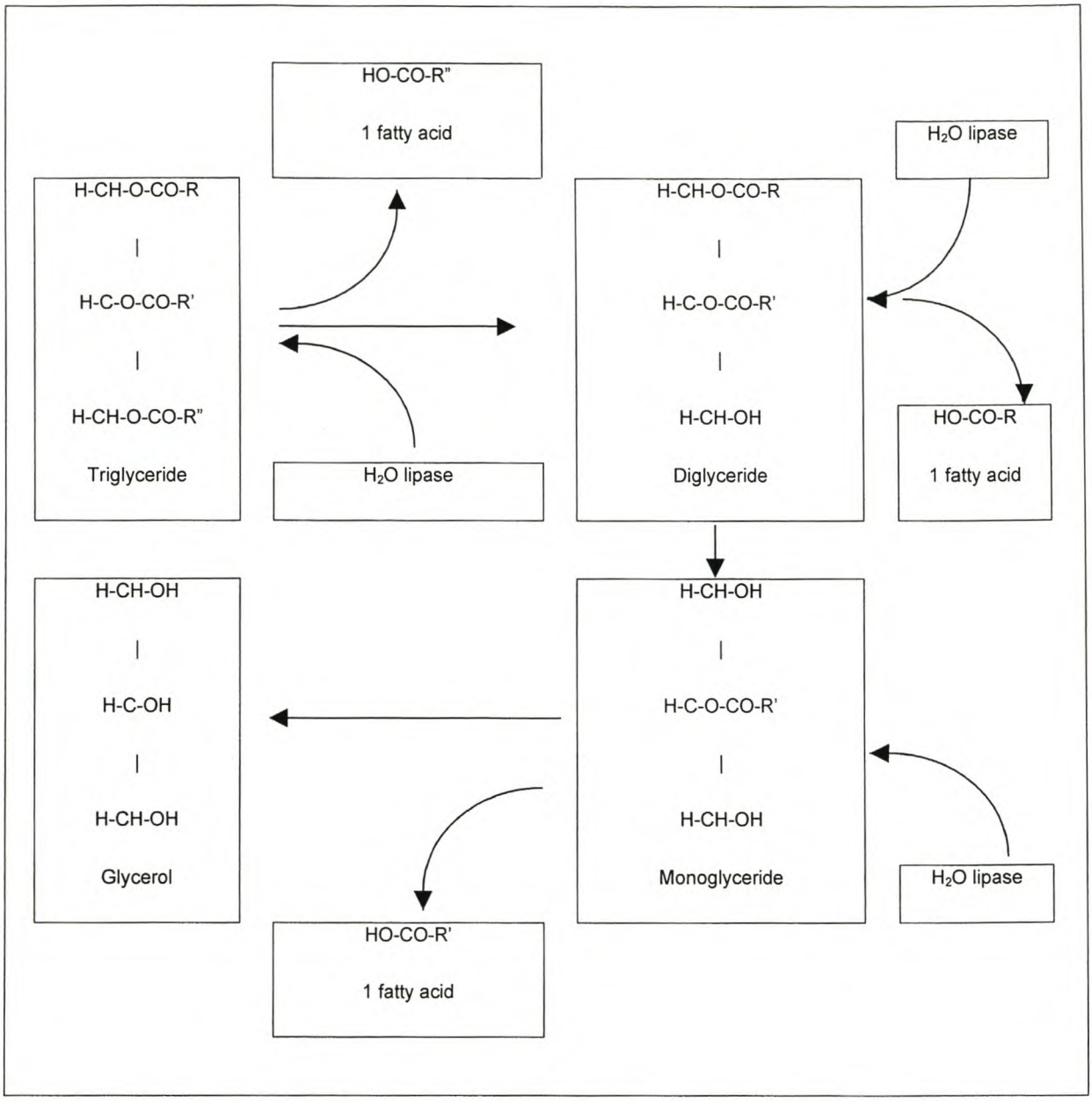


Figure 3.1: Catalysed by lipases, three successive hydrolytic cleavages of a triglyceride give one molecule glycerol and three molecules of fatty acids (Reed, 1980).

3.2.2 Digestion of dietary fatty acids

Fat absorption is a multistage metabolic process, the complexity of which is compounded by the insolubility of most lipids in an aqueous media. When fat is consumed a series of physiological events are begun that last for 16 – 24 h if no other food is consumed after the initial meal (Bisagier and Glickman, 1983). Most of the fatty acids in the human diet, whether of vegetable or animal origin, are of the C18 chain length, with lesser amounts of C16 chain length (almost invariably palmitic acid - C16:0) (Nelson, 1992).

In humans the orally consumed fatty food is mixed with lingual lipase, and hydrolysis of the triglycerides begins in the stomach (Muller et al., 1975). There is also a gastric lipase that, particularly in humans, may be more important than lingual lipase in the initial hydrolysis of fat. DeNigris et al.

(1988) reported that lingual lipase is not a major source of fat hydrolysis in humans but is important in other species. Jandacek et al. (1987) speculated that the gastric lipase is more specific for short- and medium-chain triglycerides or those with mixed chain lengths.

Very short chain (8 or 10 carbons) fatty acids are absorbed directly from the stomach into the circulation (Hyun et al., 1967). Fatty acids with chain lengths of 14 to 18 carbons, as free or esterified fatty acids, are mainly passed into the intestinal lumen, where pancreatic lipase, colipase, and bile are added to the digestive ferment and further lipolysis takes place. It is not clear if any significant portion of the free fatty acids liberated with chain lengths greater than 14 carbons are absorbed into the venous circulation from the stomach. The studies from Chen and co-workers (Chen et al., 1985) would suggest, however, that most of the n-3 fatty acids are largely passed from the stomach into the small intestine where their absorption takes place.

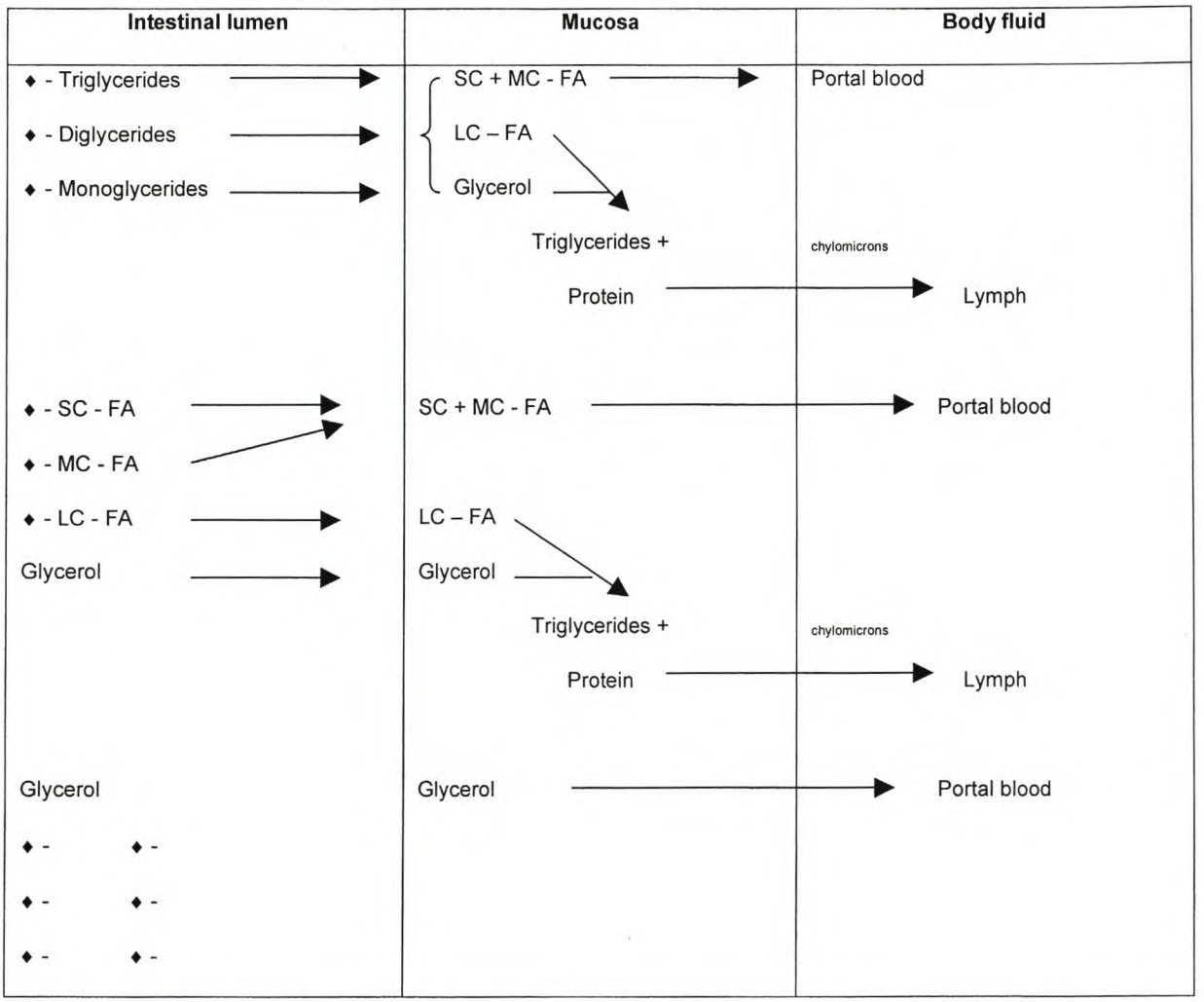
Lingual lipase and pancreatic lipase liberate fatty acids from the 1- and 3-positions of natural triglycerides, leaving the 2-monoglyceride intact (Borgstrom, 1974). The positional specificity of gastric lipase has not yet been determined. Phospholipids are also hydrolysed by pancreatic enzymes to free fatty acids and their derived lyso compounds (Tidwell et al., 1963). Pancreatic phospholipase A2 acts on the 2-position of phospholipids to produce 1-lyso compounds. Pancreatic lipase (1,3-acylglycerol ester hydrolase) will also act on the 1-position of phospholipids (Borgstrom, 1974). However, it is generally assumed that most phospholipids are absorbed by the enterocyte as the 1-lyso compound after being hydrolysed by phospholipid A2 (Nilsson, 1969). Cholesteryl esters, if present, are hydrolysed completely (Nilsson, 1969) to free cholesterol and free fatty acids. Sphinogomyelin, usually a minor component of the diet, may be absorbed intact (Nilsson, 1969).

It should also be noted that some long-chain free fatty acids are absorbed by the portal circulation, but the amount absorbed by this route may well vary with types and amounts of other nutrients being digested concurrently (Carey et al., 1983). There is still no evidence to suggest that significant quantities of the n-3 polyunsaturated fatty acids enter the circulation through the portal vein system (Muller et al., 1975), but experiments have not yet excluded entry by this route for fatty acids from ethyl or methyl esters.

Once the normally ingested fats have been hydrolysed to free fatty acids, monoglycerides, lysophospholipids, and free cholesterol, the hydrolysis products are absorbed by the enterocytes of the intestinal wall. The process of absorption from the intestinal lumen into the enterocyte is by positive diffusion (Carey et al., 1983). Free fatty acids, lysophospholipids, and 2-monoglycerides can be absorbed almost completely in the absence of bile salts (Borgstrom, 1977a), although the presence of bile is obligatory for the absorption of free cholesterol (Borgstrom, 1977b).

3.3 Absorption and destinations of digestion products

The passage of lipids from the intestinal lumen to systemic blood requires (1) travelling across the intestinal mucosa to the interior of the villi and (2) entering a body fluid for transfer to the systemic circulation. In both of these, the mucosal cells play major roles because they are capable of further lipolysis, reforming triglycerides, sorting out molecules for entrance into body fluids, and combining lipids with protein carriers (Figure 3.2).



♦ = bile salts

SC – FA = short-chain fatty acids

MC – FA = medium-chain fatty acids

LC – FA = long-chain fatty acids

Figure 3.2: Absorption of lipids and their entrance into either the portal blood or the lymphatic system. Note that lipids arrive at the mucosal cell in an emulsified form, but the bile salts remain in the intestinal lumen. In the mucosa, tri-, di-, and monoglycerides are hydrolysed to fatty acids and glycerol by mucosal lipase. Short- and medium-chain fatty acids can enter the portal blood with no further change. Long-chain fatty acids are reformed into new triglycerides, which then join with proteins to make the chylomicrons that can enter the lymphatic system (Reed, 1980).

Bile salts deliver the micelles to the mucosal cells of the jejunum where the lipids diffuse through the membrane. Most bile salts remain in the lumen to be reabsorbed and carried back to the liver by the portal blood for recycling. Once inside the mucosal membrane, lipids can undergo a number of changes depending on the form in which they enter and the body fluid that will ultimately carry them. Tri-, di- and monoglycerides undergo further lipolysis by mucosal lipase. The resulting short-chain fatty acids and glycerol are sufficiently water-soluble to enter the portal blood to be transported to the liver. Fatty acids with more than twelve carbon atoms are formed into new triglycerides, then wrapped in protein to counter solubility problems. These resulting chylomicrons enter the lacteals to be carried by the lymph system to the systemic blood via the thoracic duct. It is the size of the molecule and its solubility that determine whether it enters the portal blood or lymphatic system (Reed, 1980).

Additionally, both cholesterol and phospholipids can undergo digestive changes and be absorbed. Cholesterol is absorbed either free or esterified with a fatty acid. The amount absorbed is proportional to intake with about 50 % as a mean absorption. In general, phospholipids may be partially hydrolysed or absorbed intact. Lecithins, however, are digested and recombined in the mucosa.

Lipid digestion and absorption can be disrupted to produce a variety of abnormal consequences. For example, an insufficiency of bile salts or a blockage of the bile duct severely handicaps both processes. Moreover, this affects the digestion and absorption of other substances. An undisturbed film of fat on carbohydrates and proteins interferes with enzymatic contact, hence hinders digestion. Incomplete processing of lipids also leaves fat in the faeces, a condition called steatorrhea, which robs the body of EFA and fat-soluble vitamins.

3.4 Transport of lipids

The key to the blood transport of lipids is packing the lipids into aggregates with proteins. The proteins move freely in the watery medium taking with them their load of potentially incompatible lipids. The lipid-protein transport complexes, generally known as lipoproteins, are responsible for lipids being delivered to the cells for metabolism. These transport molecules all carry triglycerides, phospholipids, and cholesterol in conjunction with proteins, but the relative amounts of these components vary. Futhermore, the protein parts differ, and both the origins and destinations of transit also vary.

It is the variation of the relative amounts of lipid and proteins that makes possible the classification of the transport lipoproteins. Those with greater proportions of protein are of greater density as determined by ultracentrifugation. The chylomicrons contain the least protein, about 2 % of the total aggregate. The other major serum lipoproteins are (1) very low-density lipoproteins (VLDL) with about 9 % protein, and (3) high-density lipoproteins (HDL) with 50 % protein (Figure 3.3) (Gurr, 1971).

The chylomicrons are responsible for carrying most dietary lipids (the low- and medium-chain fatty acids that are absorbed into the portal vein are transported in the blood bound to the albumin) from the intestinal mucosa to the target cells that can metabolise them, which mostly include all cells except those of the brain. At their destinations, a special enzyme called lipoprotein lipase hydrolyses the triglycerides, clearing the chylomicrons from the blood. Liver and adipose tissue are particularly active receivers of chylomicron-carried lipids (Gurr, 1971).

The VLDL performs a parallel function to the chylomicrons except that VLDL carries the triglycerides that are produced within the body. This means that triglycerides synthesised mainly in the liver and intestinal mucosa are packaged with the special proteins of VLDL for delivery to the cells. When VLDL reach appropriate targets, these lipoproteins too are cleared by lipoprotein lipase. The process of unloading VLDL involves a change in the protein component, as well as the removal of large amounts of triglycerides, and results in the formation of LDL (Gurr, 1971).

Lipoprotein (composition shown in percent of total aggregate)	**Probable Origin**	**Destination**
CHYLOMICRON 2% 83% 7% 8%	Intestinal mucosa (mostly exogenous triglycerides)	Most cells except brain, particularly adipose and liver tissues.
VLDL 9% 50% 18% 22%	Liver and intestinal mucosa (mostly endogenous triglycerides)	Most cells
LDL 21% 11% 22% 46%	Most cells	Most cells except liver
HDL 50% 8% 22% 20%	Cells of peripheral tissues	Liver cells

= proteins = triglycerides

= phospholipids = cholesterol and other lipids

Figure 3.3: The major serum lipoproteins, their compositions, general origins, and primary destinations (Gurr, 1971).

In both LDL and HDL, the triglyceride component is less predominant. In LDL, cholesterol is the major lipid and accounts for 40 - 46 % of the total aggregate. Protein is the major component of HDL, but HDL also carries proportionally large amounts of cholesterol, about 20 %. A significant difference between LDL and HDL is their destination. LDL target on the peripheral tissue (outside the liver). In contrast, HDL has been shown to pick up cholesterol from peripheral tissues and carry it to the liver

(Schwartz et al., 1978). Since the only route of cholesterol excretion is through the liver, the delivery service performed by HDL is thought to favour cholesterol excretion through bile synthesis.

3.4.1 Fatty acid transport

In the enterocyte, the lipolysis products are reesterified (Brown and Johnston, 1964). Cholesterol can be synthesised de novo if there are inadequate dietary quantities, but even if no cholesterol is supplied by the diet, the bile supplies appreciable quantities to the enterocyte during fat absorption (Borgstrom, 1977a). The newly synthesised triglycerides, phospholipids, and cholesterolesters are combined with the de novo synthesised apolipoproteins, mainly apo B-48, with some apo A-I, A-II, A-IV, and E to form nascent chylomicrons (Simmond, 1972). The chylomicrons are then secreted into the lymphatic system and carried out by the thoracic lymphatic duct to the vena cava, where they enter the circulation (Simmond, 1972). The chylomicrons interact with serum lipoproteins, absorbing additional apoproteins and albumin and exchanging phospholipids and free fatty acids. This process produces the mature chylomicron that interacts with circulating lipoprotein lipase that is released from the capillary endothelial cells (Fielding and Fielding, 1977). Lipoprotein lipase initially hydrolyses chylomicron triglycerides to free fatty acids and 2-monoglycerides.

The liver does not clear chylomicrons directly (Scow et al., 1977). Their core lipids are hydrolysed first by the lipoprotein lipase-colipase system to form chylomicron remnants that are then cleared from the circulation by the apo E receptor (Jones et al., 1984). Apolipoprotein B-48 does not bind to the low-density lipoprotein (LDL) receptor (Hui et al., 1981). The chylomicron remnant has apo B-48, apo E, phospholipids, cholesterol, cholesterol ester, and some triglycerides (Havel, 1982).

Lipoprotein lipase is activated by apo C-II (Fielding and Fielding, 1977). It has positional specificity for the primary ester bonds of acylglycerols (Nillson-Ehle et al.,1973) and hydrolyses the triglycerides to 1,2- and 2,3-diacylglycerols and free fatty acids. It also hydrolyses 1,2- and 2,3-diacylglycerols to 2-monoacylglycerols and 1- and 3-monoglycerides to free glycerol and fatty acids (Morley et al., 1975). Lipoprotein lipase has no effect on 2-monoglycerides (Scow et al., 1977); 2-monoglycerides are hydrolysed by lipase in the liver (Havel, 1985). This observation may have important implications for absorption, transport and metabolism of n-3 fatty acids, as the long chain n-3 fatty acids may be preferentially distributed in certain positions of the triglyceride molecules (Bottino et al, 1967). Lipoprotein lipase also hydrolyses the primary acyl ester bond of phosphatidylcholine to the 2-acyl lyso compound and a free fatty acid (Scow and Egelrud, 1976).

The lipoprotein lipase hydrolysis takes place in the peripheral tissues in contact between the chylomicron and the endothelial cells, and the free fatty acids are taken up by the proximal tissue (Kane et al., 1983). Some free fatty acids released by lipoprotein lipase undoubtedly become bound to albumin and are cleared by the liver (Bergman et al., 1971). As the size of the chylomicron is reduced through hydrolysis of its triglycerides, the apoprotein-phospholipid-cholesteryl coat forms 'buds' that apparently split to form nascent HDL particles (Nicoll et al., 1990). There is essentially no

triglyceride or cholesterol ester in these particles, which consist exclusively of apo E, apo A-I, apo A-II, and apo C with phospholipid and unesterified cholesterol. These nascent HDL particles then acquire cholesteryl esters through the action of the lecitin-cholesterol acyl transferase in blood (Glomset and Verdery, 1977). Some results suggest that HDL particles, probably HDL_2, are cleared by the liver although the evidence is not yet conclusive (Glomset and Verdery, 1977).

In the eucalorie or excess calories condition, the liver catabolises chylomicron remnants, synthesises triglycerides from carbohydrate, and forms very low density lipoproteins (VLDL), which are released into the circulation (Havel, 1985). VLDL are also substrates for endothelial lipoprotein lipase. They lose triglycerides by hydrolysis and are transformed to intermediate density lipoproteins (IDL) and LDL. LDL is taken up by the LDL receptor (Goldstein et al., 1983) of the peripheral tissue and the liver. LDL primarily transports cholesteryl esters to the peripheral tissues, where they are hydrolysed to free cholesterol and then reacylated (Sodhi et al., 1978).

4 Metabolism of dietary lipids

4.1 Triglyceride metabolism and storage

The major energy-yielding lipid is the triglyceride. Once the triglyceride arrives at a cell, most often a liver or adipose tissue cell, this molecule has two alternative fates. Triglycerides can be metabolised immediately, so that the 9 kcal of potential energy per gram can be put to immediate use, or they may be stored until the energy is needed.

4.1.1 Metabolising triglycerides for energy

The energy-rich components of triglycerides are the fatty acids. Once the fatty acids are freed, they are subject to the catabolic process called beta-oxidation (Figure 4.1). The metabolic goal of β-oxidation is the formation of active acetate, which in the form of acetyl CoA can enter the fundamental compound of lipid metabolism; it is not only the breakdown product of fatty acids but it is also the building block from which fatty acids are synthesised.

Acetyl CoA can enter the following metabolic pathways (Figure 4.2) (Reed, 1980):

1. citric acid cycle (CAC) to yield energy
2. fatty-acid anabolism to yield new fatty acids
3. ketone production to yield the ketones (acetoacetate, acetone, and betahydroxy butyrate or β-OH butyrate)
4. steroid production to yield cholesterol and others:

1. The complete processing of a molecule of acetyl CoA through the CAC and the subsequent electron transport system yields twelve molecules of ATP. The energy richness of fats can be appreciated when one considers the number of acetyl CoA molecules that can be derived from a triglyceride. For example, a simple triglyceride with three eighteen-carbon-atom fatty acids will yield nine molecules from each of the fatty acids and one from glycerol, a total of twenty-eight. In contrast, a molecule of glucose provides only two molecules of acetyl CoA.

2. Called lipogenesis, fatty-acid synthesis is accomplished in the liver and most tissues by a process that approximates the reverse of β-oxidation (Figure 4.3). Any substance that yields acetyl CoA can ultimately contribute to fatty acids.

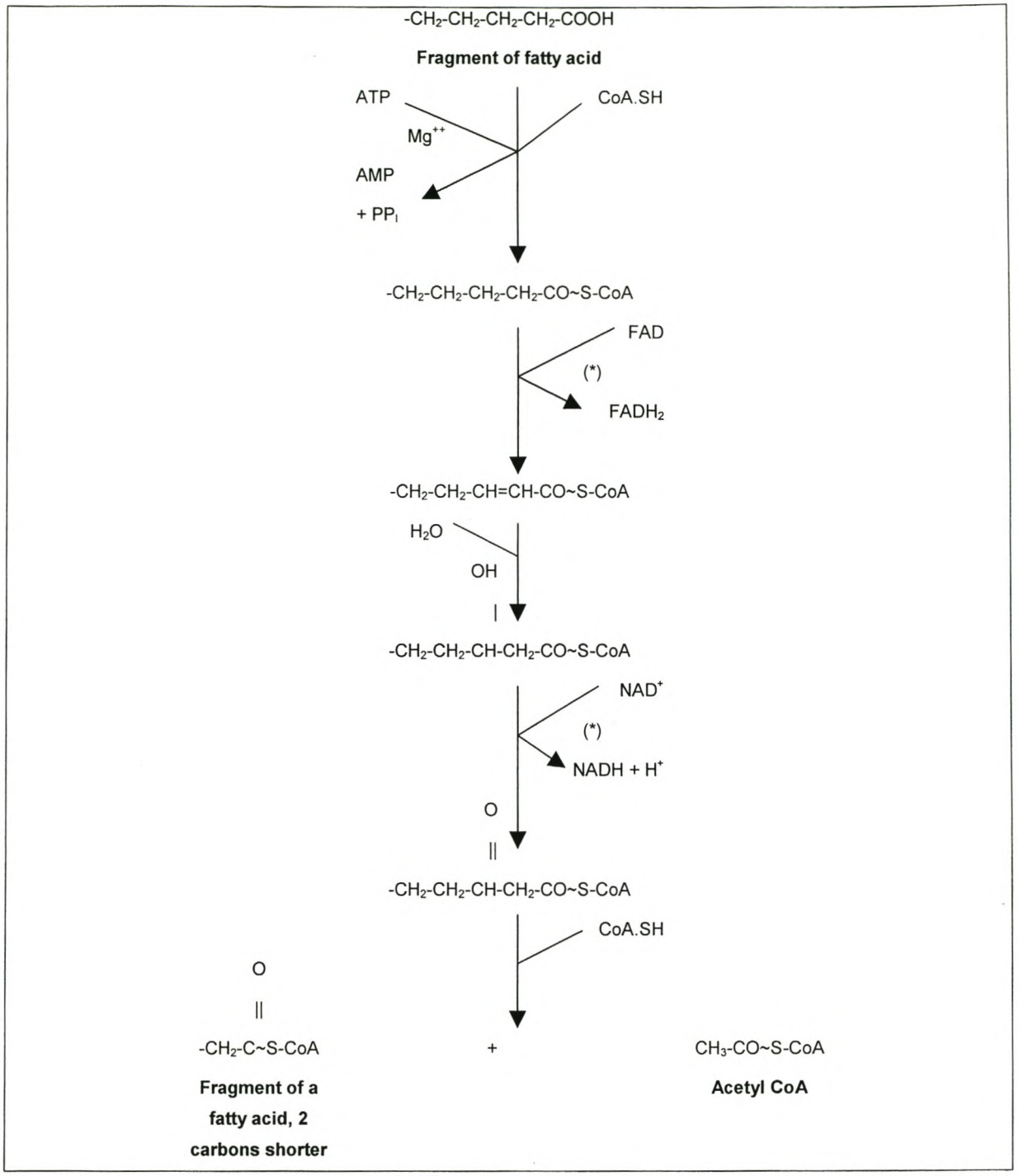


Figure 4.1: Beta-oxidation of fatty acids. The oxidative breakdown of fatty acids takes place in the mitochondria and yields acetyl CoA. Oxidation of $FADH_2$ (*) by the respiratory chain generates ATP (Reed, 1980).

3. Ketone production is a normal process that ordinarily uses relatively little of the acetyl CoA pool. However, several abnormal conditions can cause an accumulation of acetyl CoA and an increase in the amount entering this alternative metabolic pathway.

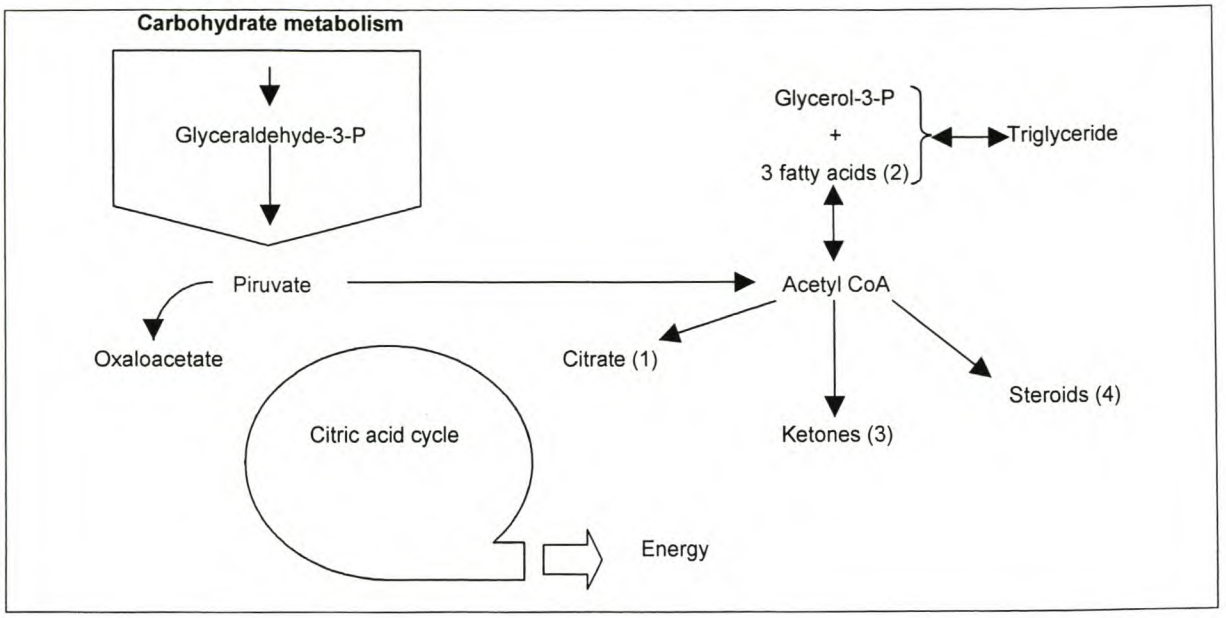


Figure 4.2: A schematic view of fat metabolism as it relates to carbohydrate metabolism. Note that hydrolysis of triglycerides frees glycerol so it can be catabolised as a carbohydrate. The fatty acids are oxidised to acetyl CoA. Acetyl CoA can then (1) enter the citric-acid cycle to be used for energy, or it can be made into (2) new fatty acids, (3) ketones, or (4) steroids (Reed, 1980).

4. Steroid synthesis occurs in all cells as a result of a complex series of steps by which acetate molecules are fashioned into a five-carbon unit (isoprene unit). These units are then enzymatically connected. The finished product is the characteristic multiple-ring structure of the steroid nucleus and its derivatives.

4.1.2 Triglyceride storage

Triglycerides are stored in adipose tissue or fat depots located subcutaneously and around some vital organs. Triglycerides travel freely between the various depots and the liver and are in a constant state of turnover but do not normally accumulate in the liver.

The character of adipose tissue is determined genetically although feeding practices also influences it. Each adipose cell has an enormous capacity for storage because fat droplets can replace much of the cytoplasm. Adipose tissue serves some useful functions in addition to holding reserve energy. Subcutaneous fat serves as insulation and as a shock absorber.

Since the liver is the main site of lipid metabolism, triglycerides continually enter it and undergo changes, but under normal conditions the triglycerides is removed to other storage sites.

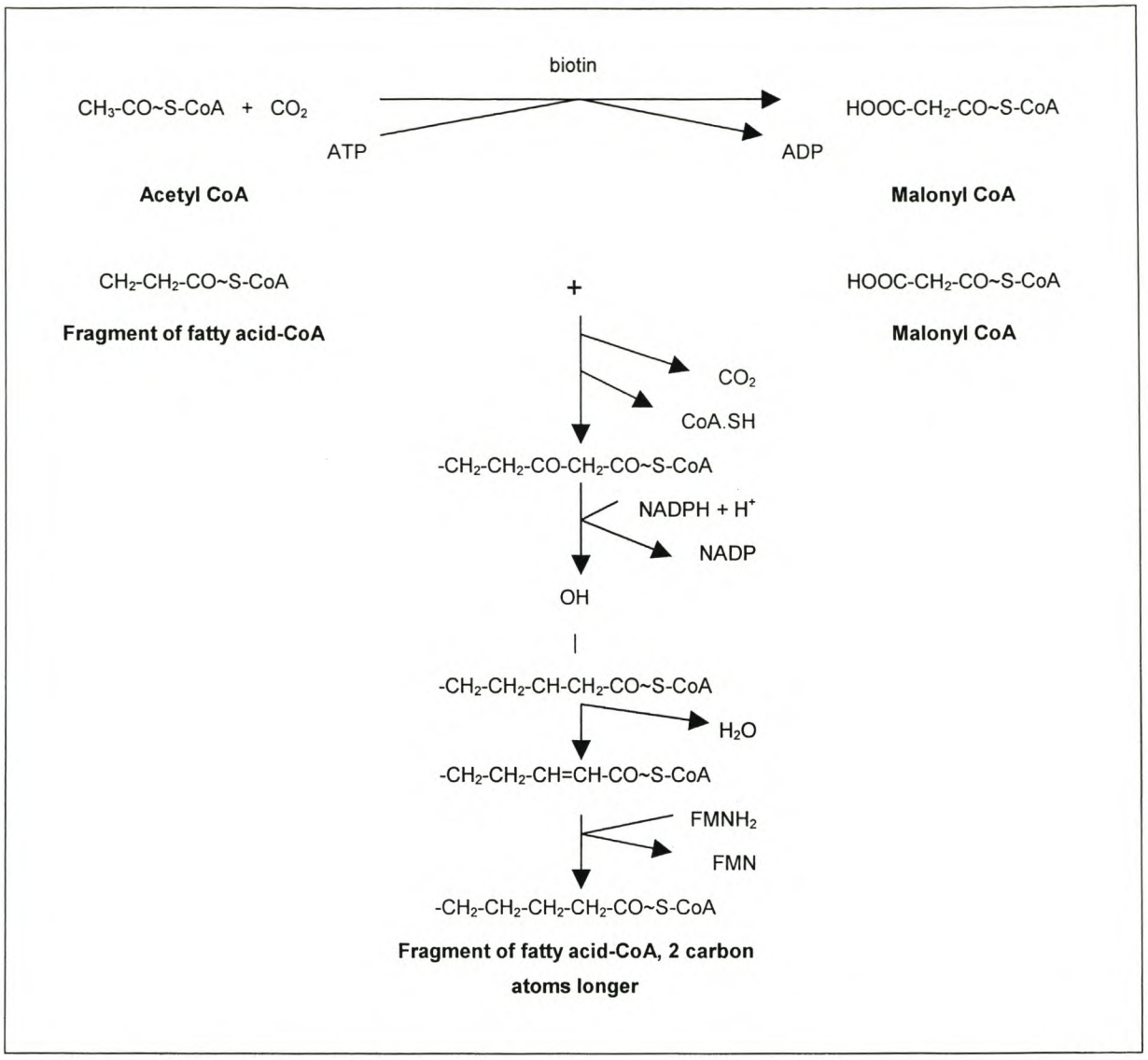


Figure 4.3: Biosynthesis of fatty acids, the building of fatty acids by adding two-carbon fragments from malonyl CoA, a product of acetyl CoA. This energy-requiring process occurs mainly in the cytosol (Reed, 1980).

4.2 Fatty acid metabolism

4.2.1 Synthesis

The liver is the primary site for lipid metabolism (Volpe and Vagelos, 1973) and is the organ most susceptible to dietary induced changes in metabolism. Figure 4.4 gives the sequence of the synthesis of fatty acids de novo from acetate in mammalian liver. Adipose tissue is also a major organ system in which fatty acid synthesis can occur when excess calories are consumed (Hollands and Cawthrone, 1981).

$$CH_3\text{-}CO\text{-}S\text{-}CoA + 7ATP + 14NADPH + 14H^+ \rightarrow CH_3\text{-}(CH_2)_{14}\text{-}COOH + 8CoA\text{-}SH + 7ADP + 7P_i + 14NADP^+ + 6H_2O$$

Figure 4.4: The general equation for the de novo synthesis of palmitic acid from acetyl CoA in most tissues (Lane et al., 1974).

There have been many studies of fatty acid synthesis from acetate (Lynen, 1967), and the complete sequence of steps in the reaction has been elucidated, primarily by Vagelos and Larrabee (1967). Acetate as acetyl CoA is condensed with bicarbonate to malonyl CoA by acetyl CoA carboxylase and then four malonyl CoA residues are combined by fatty acid synthetase to form lauric (C12:0) acid. Then two acetyl CoA residues are added to produce palmitic acid (Wakil et al., 1958). If only one acetyl CoA is added to lauric acid, myristic acid (C14:0) will be formed. The synthetic reactions up to this point all take place within the fatty acid synthetase complex (Wakil et al., 1958).

Biotin is a required co-factor for the conversion of acetyl CoA to malonyl CoA by acetyl CoA carboxylase (Lynen, 1967). Acetyl CoA carboxylase is a widely distributed enzyme found in most mammalian tissues (Waite and Wakil, 1962) and other species as well (Birnbaum, 1969). The enzyme is polymeric with protomers having a molecular weight of about 215 000 in rat liver (Tanabe et al., 1975) and polymers having molecular weights up to 8 million in arian liver (Gregolin et al., 1968). It is the rate limiting enzyme for fatty acid synthesis in most mammalian tissues (Vagelos and Larrabee, 1967), although in some circumstances fatty acid synthetase may be rate-limiting (Chang et al., 1967). Regulation of acetyl CoA carboxylase is usually achieved through the supply of acetyl CoA or citrate produced by the Krebs cycle (Lane et al., 1974). Palmitoyl CoA (and other medium-chain acyl CoA) is a strong inhibitor of acetyl CoA carboxylase in vitro (Taketa and Pogell, 1966). Regulation by dietary fatty acid may proceed through the formation of medium-chain acyl CoA derivatives (Leveille, 1967).

The sequence of reactions involved in the condensation of malonyl CoA to palmitic acid (C16:0) is shown in Figure 4.5. This reaction is catalysed by fatty acid synthetase and requires one molecule of Acetyl CoA, seven NADPH molecules, and 14 protons. Fatty acid synthetase is a multienzyme complex (Veech and Guynn, 1974) and has been isolated from many tissues, such as pigeon liver (Lornitzo et al., 1974), rat liver (Burton et al., 1968), mammary glands of several species (Nadedkar and Kumar, 1969), bacteria (Volpe and Vagelos, 1976), and yeast (Lynen, 1969). A central component of the fatty acid synthetase complexes is the acyl carrier protein ACP, which, although having no catalytic properties of its own, is essential to bind acyl CoA molecules as they are condensed into longer chain fatty acids within the confines of the fatty acid synthetase complex. The ACP can release an acyl CoA molecule when it contacts an active site in the complex. Although the mechanism of the reactions of fatty acid synthetase was largely investigated in bacterial systems and yeast, the general principles are similar in mammalian and avian tissues. Free palmitic acid (C16:0) is the primary product of fatty acid synthetase reactions (Volpe and Vagelos, 1973) in animal tissues.

Some myristic (C14:0) and lauric acid (C12:0) is also formed, and a trace of stearic acid (C18:0) may also be produced (Wakil et al., 1983).

Once free palmitic acid is released from the synthetase complex, it can be esterified into complex lipids, elongated to stearic acid (the usual synthetic pathway for de novo synthesis of stearic acid), or desaturated to palmitoleic acid (C16:1n7) (Beare-Rogers, 1977). Different enzymatic pathways are involved in each reaction. The regulation and control of these secondary pathways are poorly understood (Beare-Rogers, 1977). However, little or no fatty acid synthesis takes place in humans when adequate or excess calories are consumed on a high fat diet. Conversely, when carbohydrate is fed in excess of caloric requirements, the conversion of carbohydrate to fatty acids is rapid (Groener and Van Golde, 1977). The products are mainly palmitic, stearic, and oleic acids, which are esterified to glycerol to form triacylglycerols (Masoro et al., 1950) and then stored in adipocytes in the adipose tissue reservoirs. The fatty acids are transported as very low density lipoproteins (VLDL) which is produced in the liver.

Adipocytes do not absorb VLDL or intact triglycerides directly (Kane et al., 1983). The triglycerides must be hydrolysed by lipoprotein lipase localised in the capillary endothelial cells (Kane et al., 1983). The free fatty acids released by this reaction diffuse through the capillary wall and are then taken up by the adipocytes and resynthesized to triglycerides (Bergman et al., 1971). In monogastric animals the fatty acid composition of the adipose tissue will reflect that of the diet (Lands et al., 1990), but if a low fat, high carbohydrate diet is eaten consistently, the adipose will consist mainly of triglycerides containing palmitic, stearic, oleic, and linoleic acids. Individuals eating diets containing large amounts of linoleic acid will deposit this component readily in the adipose tissue (Thomas et al., 1987). Rats fed diets high in polyunsaturated 20- and 22-carbon n-3 fatty acids will store the excess in the adipose tissue (Nelson et al.,1987).

In individuals eating high fat diets with excess caloric intake, much of the exogenous fatty acids can be stored directly in the adipose tissue without going to the liver. The chylomicrons formed by the enterocytes in the gut can also be hydrolysed by lipoprotein lipase in the capillary endothelial cells (Scow et al., 1977). Uptake of the liberated free fatty acids can then proceed similarly to that described for free fatty acids liberated from the VLDL made in the liver.

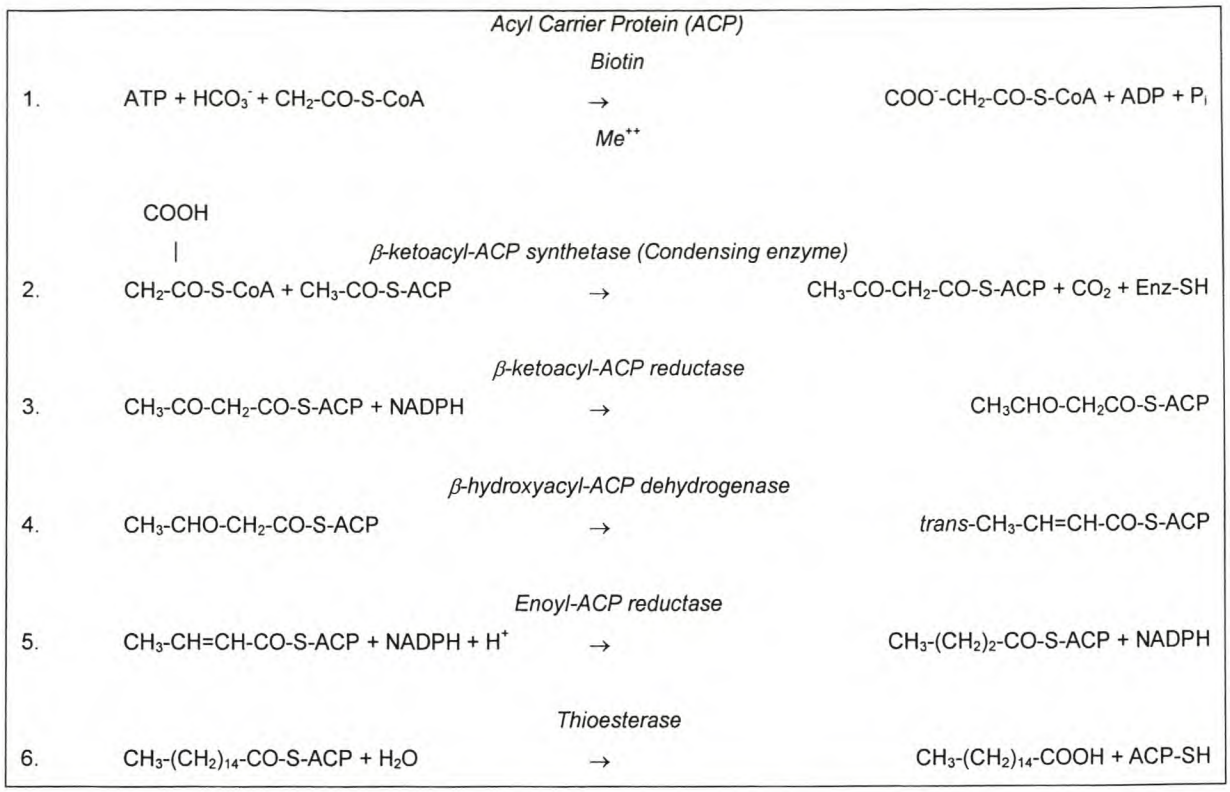


Figure 4.5: The sequential reactions and important enzymes of the fatty acid synthetase complex for the condensation of malonyl CoA to palmitic acid (Lane et al., 1974).

Fatty acid synthesis in mammalian systems produce only saturated acids and monounsaturated fatty acids of the n-9 series, usually oleic acid (Volpe and Vagelos, 1973). Animals lack the ability to desaturate fatty acids in the n-6 or n-3 position of the fatty acid chain. Only plants possess the enzyme systems necessary to effect those reactions (Pollard et al., 1979). Many land plants also lack the n-3 desaturase. Aquatic plants in colder water produce abundant amounts of the n-3 fatty acids (Lee and Loeblich, 1971). Conversely, animals have chain elongation systems and desaturases that act on the n-12, n-15, and n-18 positions that are lacking in plant tissues (Nugteren, 1965). Delta 9-10 dehydrogenases are ubiquitous in both the plant and animal kingdoms. The delta 9-10 dehydrogenase in mammalian tissue is the most active lipid enzyme (Elovson, 1965).

In animal tissues the desaturation of the de novo synthesized fatty acids stops with the production of the monounsaturated fatty acid with a double bond in the 9-10 position of the fatty acid chain. If palmitate is the substrate for the dehydrogenase, the double bond will appear in the n7-8 position of the chain as the 9-10 dehydrogenase desaturates nine carbons from the carboxyl end of the molecules. Palmitoleic (C16:1n7), oleic (C18:1n9), and *cis*-vaccenic acid (C18:1n11) are the main products of this reaction. Chain elongation by another enzymatic system, the fatty acid elongase system, will produce eicosenoic, erucic, and nervonic acids by elongation of oleic acid by this system (Seubert and Podack, 1973).

In the absence of dietary essential fatty acids (EFA), the same enzymes that desaturate linoleic and α-linolenic acids will desaturate eicosenoic acid to produce the eicosatrienoic acid (C20:3n5), or

"Mead acid", characteristic of essential fatty acid deficiency (Fulco and Mead, 1959). This long-chain polyunsaturated fatty acid of the n-9 series has double bonds at the n-9, n-12, and n-15 positions of the carbon chain. It is not an essential fatty acid and will not replace arachidonic acid or alleviate the symptoms of EFA deficiency. It will be incorporated into the same tissues and complex lipids as arachidonic acid. The Mead acid is not a substrate for the cyclooxygenase reaction, and no eicosanoids are produced from it.

The Δ4, Δ5, and Δ6 desaturates prefer substrate fatty acids with double bonds in the n-6 and, secondarily, the n-3 position of the carbon chain. The exception is the Δ4 desaturate, which may prefer a substrate with a double bond in the n-3 position. The fatty acid C22:5n6 does not accumulate appreciably when adequate n-3 fatty acids are in the diet; C22:6n3 is the dominant product. Dietary EFAs are the precursors of the biologically active eicosanoids (Dustig et al., 1981). Most people, with the exception of strict vegetarians, consume some arachidonic acid, but most of the body reserves come from the conversion of dietary linoleic acid to arachidonic acid by its desaturation and elongation as shown in Figure 4.6. This pathway is found in all omnivores (Lands, 1986). Obligatory carnivores, at least the domestic house cat, may require arachidonic acid in their diet. Cats have lost the ability to desaturate linoleic acid. As the same dehydrogenases desaturate α-linolenic acid to EPA and DHA, it could be speculated that cats should have a requirement for EPA or DHA as well as arachidonic acid. Of course, animal tissues contain significant quantities of DHA so this dependence will not be detected unless the animals are fed semisynthetic diets. If eicosanoid production from arachidonic acid is the main reason cats require arachidonic acid, then conceivably they may not have a requirement for EPA and DHA (Lands, 1986).

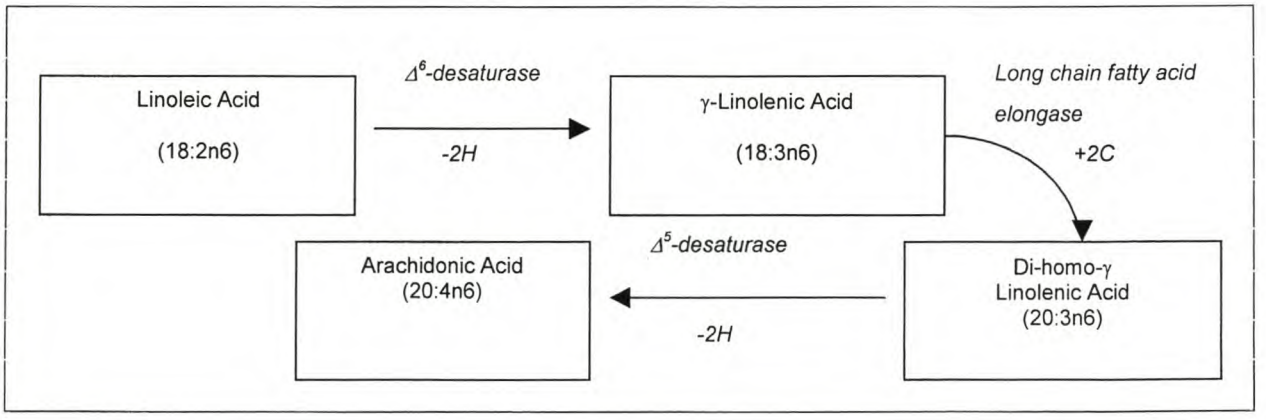


Figure 4.6: Metabolic pathway for the conversion of dietary linoleic acid to arachidonic acid in vivo by the Δ5,6 desaturates and long-chain fatty acid elongase reactions (Nugteren, 1965).

Sprecher and Lee (1975) have shown that dietary polyunsaturated fatty acids are not elongated and then desaturated, but are desaturated and then elongated. Figure 4.7 displays the main sequence of reactions that yield polyunsaturated fatty acids from their dietary precursors. The 22:2n6 and the 20:3n3 can be further elongated and desaturated, but the 5,11,14-20:3 and 5,11,14,17-20:4 are not

metabolised further (Sprecher and Lee, 1975). A methylene-interrupted double bond cannot be inserted into a long-chain fatty acid between two existing methylene-interrupted double bonds.

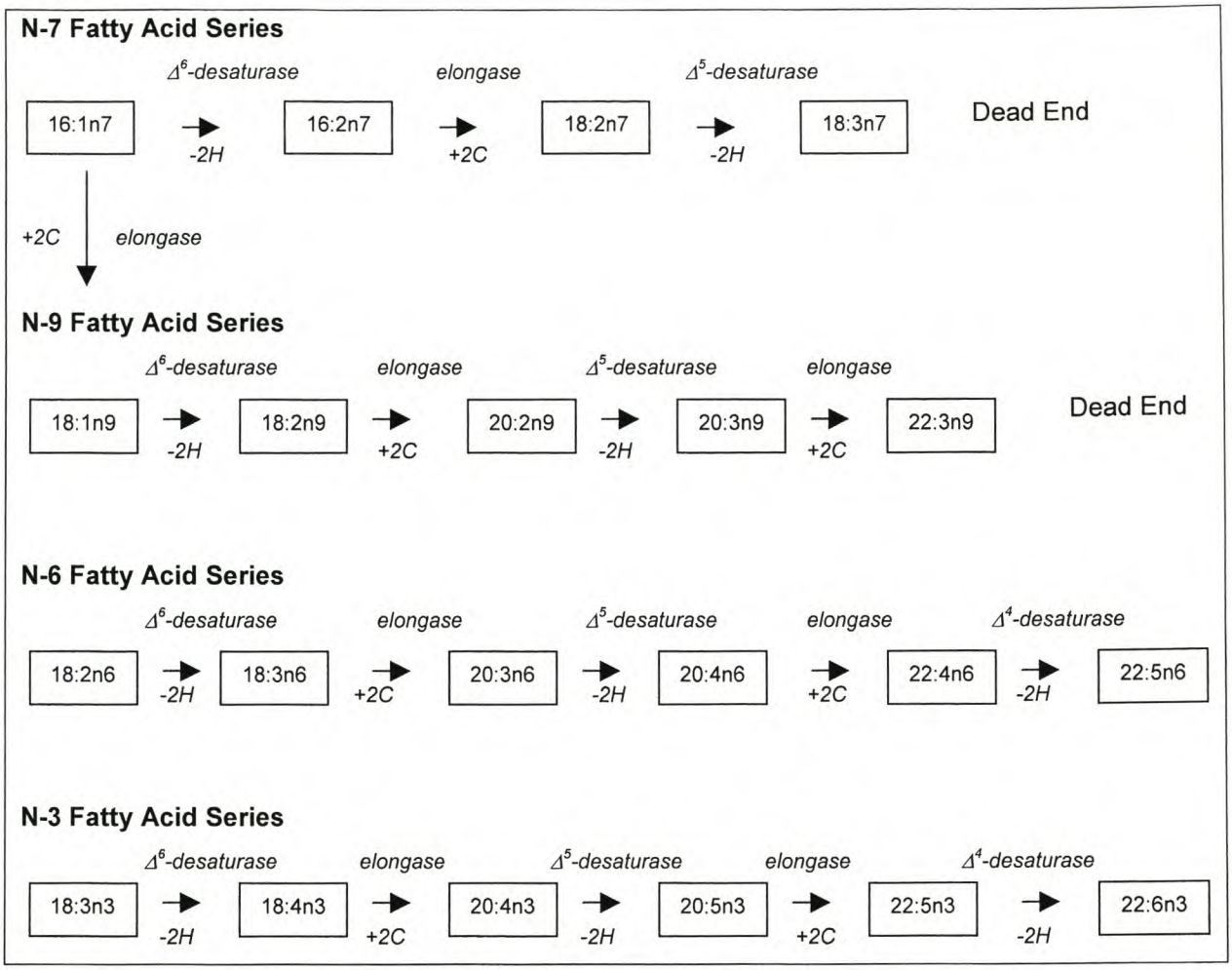


Figure 4.7: The metabolic pathways for the conversion of dietary unsaturated fatty acids to their long-chain polyunsaturated metabolites (Sprecher and Lee, 1975).

4.2.2 Catabolism

The enzymatic pathways for the catabolism of fatty acids are essentially the same for dietary and endogenously synthesised fatty acids. There may be significant functional differences between the utilisation of dietary fatty acids and those synthesised endogenously. Dietary α-linolenic acid is a good example of a fatty acid not readily incorporated into tissue lipids. It is mainly oxidised to CO_2 and water (Aeberhard et al., 1978). Some is converted to EPA and DHA, but only a small portion of the α-linolenic acid enters this pathway (De Gomez-Dumm and Brenner, 1975). If all other fatty acids are removed from the diet and α-linolenic acid is fed as the only source of dietary fat, then a higher percentage is incorporated into tissue lipids (Mohrhauer and Holman, 1963). It is a competitive discrimination that prevents α-linolenic acid from being stored in tissues, not an intrinsic property of this compound.

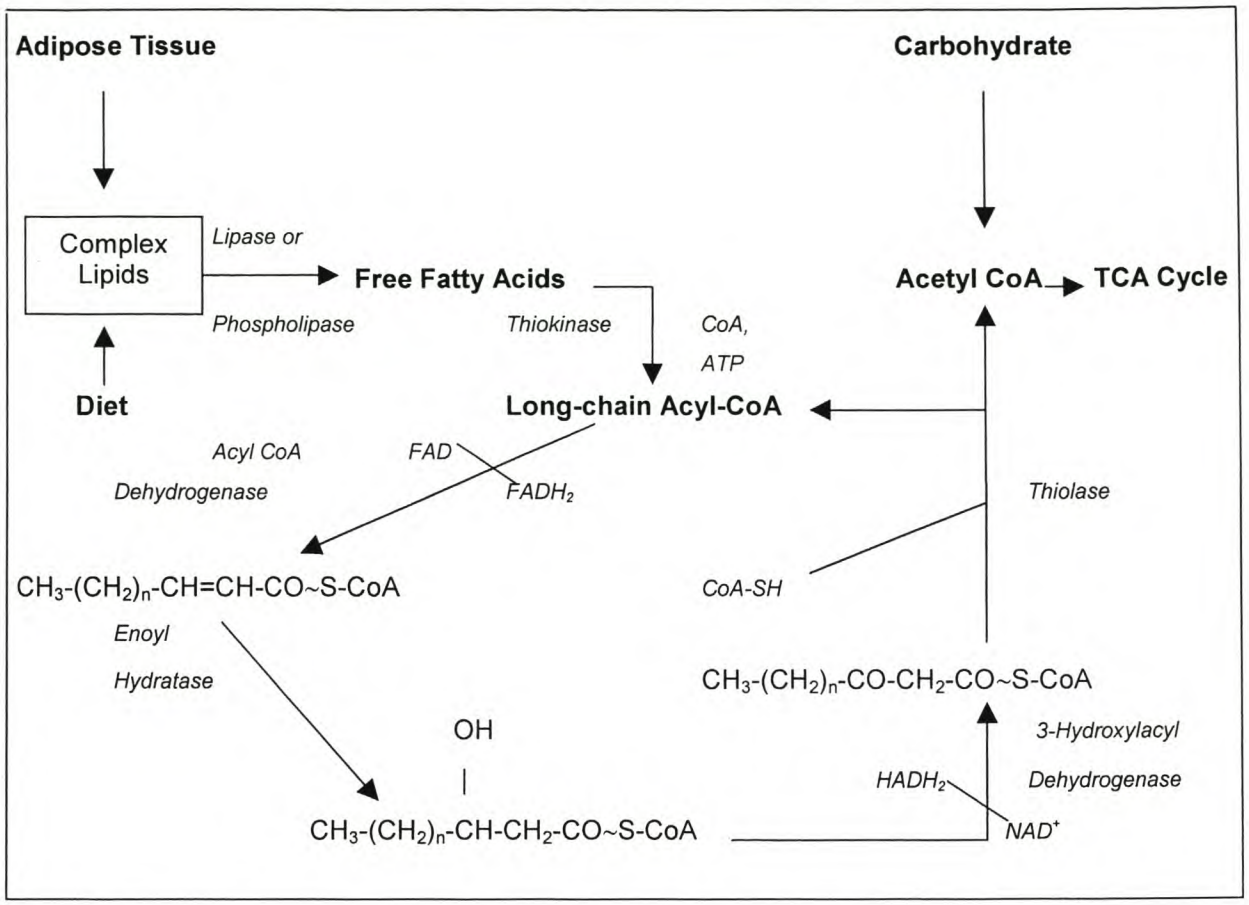


Figure 4.8: The classic β-oxidation cycle for the metabolic degradation of fatty acids in mitochondria. This cycle degrades fatty acids of 18 or fewer carbon atoms. See text for further information on degradation of 20-carbon and longer fatty acids (Bronfman et al., 1979).

Fatty acids are oxidised to CO_2 and water by enzyme systems in both mitochondria and peroxisomes (Bronfman et al., 1979). Mitochondria, which produce metabolic energy via ATP and the Krebs cycle, use the classical β-oxidation (Figure 4.8), whereas peroxisomes use a modified β-oxidation system with a fatty acyl CoA oxidase in the first step of the reaction. This step involves hydrogen peroxide and is the rate-limiting step of the oxidation (Foerster et al., 1981). Both pathways use fatty acyl CoA as substrates, but the peroxisomes have a very different preference for fatty acid chains (Osumi et al., 1980). Fatty acids shorter than 16 carbons are oxidised at the same rate by both systems (Christiansen et al., 1977). Peroxisomes can oxidise fatty acids with chains longer than 18 carbons that mitochondria will not normally degrade. Peroxisomes will oxidise monounsaturated fatty acids such as 20:1n9, 22:1n11, 22:1n9 and 24:1n9 that are not substrates for the mitochondrial β-oxidation system (Christiansen et al., 1977). It has been speculated that the peroxisome's oxidation pathway is primarily a chain-shortening mechanism for these long-chain fatty acids so they can enter the mitochondrial β-oxidation system (Osmundsen, 1987). In this manner peroxisomes can be involved in the regulation of the fatty acid composition of the tissues.

Docosahexaenoic acid (C22:6n3) can be retroconverted to EPA (20:5n3). Figure 4.9 shows this mechanism. EPA is normally present only in trace amounts in the tissue lipids of animals eating a diet derived largely from terrestrial plants and animals. EPA is not a major fatty acid in the tissue of aquatic mammals (Nelson, 1971). Conversely, DHA is the major n-3 fatty acid in most tissues in mammals. It is possible, as EPA appears to have several unique pharmacological and biochemical actions when consumed in large amounts (Leaf and Weber, 1988), that DHA, through retroconversion, supplies EPA on demand if there is an inadequate dietary supply of α-linolenic acid.

Consequently, the variable activity of each fatty acid's metabolic pathway acts to produce the fatty acid composition of the tissues. The fatty acid composition of any particular tissue is determined by a complex interplay among synthesis, degradation, and diet. Yet there are limits within which some variation is possible and beyond which it is impossible to alter the composition without interfering with the metabolic function of the tissue. The species have evolved over a long period, presumably eating a constant diet. Tissues and organs evolve to have specific functional roles. Presumably the fatty acid composition of any particular tissue has been optimised by evolutionary forces to optimise function. One would naturally expect that deviations from normal fatty acid composition in an organ would impair its function. Thus, the fatty acid composition of a tissue is important to its function and not simply the reflection of yesterday's diet. Even human adipose tissue is not simply a repository of dietary fatty acids. The bulk of adipose tissue reflects the excess calories taken in over a lifetime, but its fatty acid composition is determined only partially by diet. A significant portion of its fatty acid composition is affected by metabolic determinants under genetic control.

The β-oxidation system depends on carnitine as a co-factor (Bieber et al., 1982). In both the mitochondria and the peroxisomes, the initial substrate is a medium- or long-chain acyl CoA derivative of the fatty acid. Conversely, the cyclo-oxygenase and lipo-oxygenase systems that produce eicosanoids will accept only the free fatty acid as substrate (Nelson, 1992). These free fatty acids are released from phospholipids in cellular membranes by the action mainly of phospholipids A_2 (Lands and Crawford, 1976). As free fatty acids do not normally exist in tissues, this is one method by which the production of eicosanoids is regulated. Trauma and disease produce free fatty acid (FFA) in tissues by liberating acid hydrolases (lipases and phospholipases) from lysosomes in damaged cells (Pitt, 1975). The liberated FFAs generate eicosanoids, and a chain reaction is then initiated through the eicosanoid cascade (Vane, 1964).

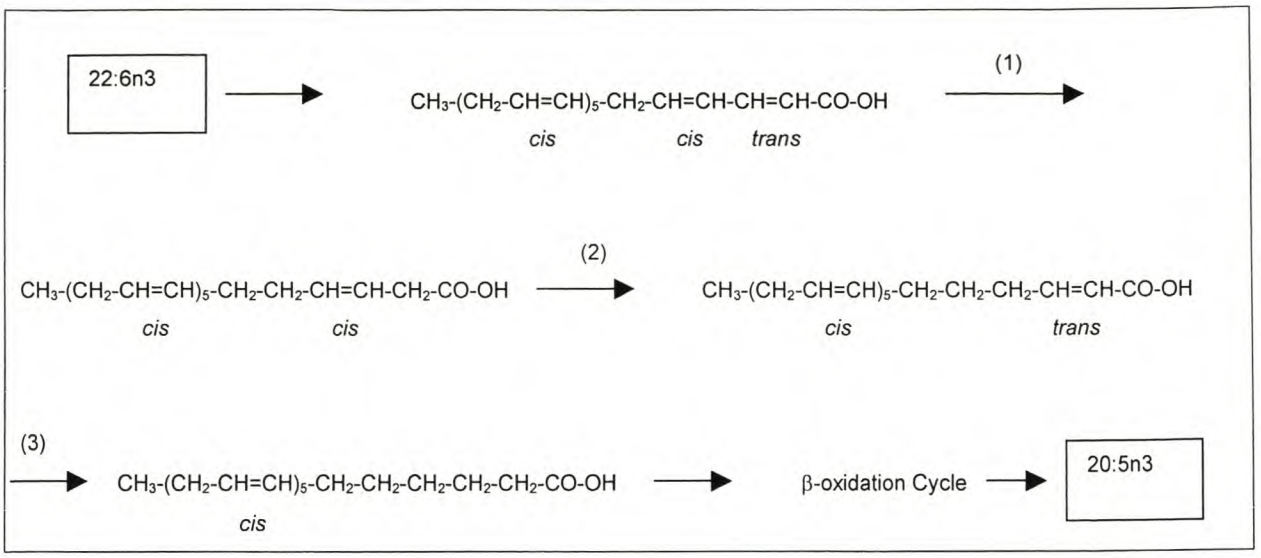


Figure 4.9: The probable metabolic pathway for the retroconversion of docosahexaenoic acid to eicosapentaenoic acid in vivo. The enzymes are (1) 2,4-dienoyl CoA reductase; (2) Δ3-cis-Δ2-trans-enoyl CoA isomerase; and (3) intramitochondrial nucleotide-dependant reductive enzymes (Sprecher, 1991).

4.3 Metabolic Regulators

Factors that exert regulatory influences on lipid metabolism include a number of vitamins, hormones, and enzyme systems. Both the breakdown of fatty acids and their synthesis are vulnerable to vitamin deficiencies because of their key roles as coenzymes. Beta-oxidation requires riboflavin, niacin, and pantothenic acid. Lipogenesis requires biotin in addition to riboflavin, niacin, and panthothenic acid (Osmundsen,1987).

Insulin favours fatty-acid synthesis, and the hormones that trigger energy-consuming processes increase β-oxidation. By permitting glucose to enter glycolysis in muscle and fat cells, insulin increases the pool of glycerol and acetyl CoA. Consequently, insulin has a secondary effect of increasing fat production when glucose is in excess. Insulin also favours the storage of fat by directly enhancing the uptake of fatty acids by adipose tissue (Cahill, 1971).

The hormones that stimulate processes requiring energy include the growth hormone, ACTH or cortisone and hydrocortisone, the thyroid-stimulating hormone TSH or thyroid hormones, epinephrine, and glucagon. These hormones increase the removal of triglycerides from storage, the freeing of fatty acids, and the oxidation of the fatty acids for energy (Reed, 1980).

As with all metabolic processes, enzymes are key mediators of lipid metabolism. In some cases, the capabilities of enzyme systems directly affect the concentrations of circulating lipids. For example, insufficient activity of lipoprotein lipase results in a type of hyperlipoproteinemia, an elevated level of circulating lipoproteins (Nichaman et al., 1967). A number of enzymes respond to the relative

amounts of substrates and products to shift the direction of anabolism and catabolism. The enzyme acetyl CoA carboxylase is a particularly important regulator of fatty-acid synthesis. Acetyl CoA carboxylase catalyses the first step in anabolism and responds to several influences (Lane et al., 1974). An influx of free fatty acids inhibits the enzyme's activity, which probably explains why fatty acid synthesis is reduced during times of caloric deficiency. The availability of large amounts of acetyl CoA from carbohydrates, on the other hand, activates acetyl CoA carboxylase and leads to fat storage.

4.3.1 Regulation of fatty acid metabolism

The effect of dietary fatty acids on lipid metabolism can be separated into three major categories: (1) the influence of dietary fat on de novo fatty acid synthesis and fatty acid oxidation; (2) the influence of dietary fatty acids on circulating lipoproteins, blood lipids, and cholesterol; and (3) the influence of dietary fatty acids on eicosanoid production and maintenance of physiological homeostasis. Each topic is discussed separately below.

4.3.1.3 De novo synthesis

The de novo synthesis is well understood, and many of the biochemical mechanisms have been described (Volpe and Vagelos, 1973). The regulatory processes are generally known and predictable. Dietary fatty acids suppress de novo synthesis, as does starvation. Although the two conditions are physiologically quite different, the regulatory processes are very similar. Both acetyl CoA carboxylase and fatty acid synthetase are inhibited by excess acyl CoA, particularly palmitoyl CoA (Volpe and Vagelos, 1973). As described earlier, when fatty acids are consumed, they are transported to the liver, where they are converted to acyl CoA derivatives for further metabolic utilisation (Jelsema and Morre, 1978). The excess acyl CoA produced in the liver suppresses de novo fatty acid synthesis by limiting both acetyl CoA carboxylase and fatty acid synthetase. It was previously thought that only polyunsaturated fatty acids suppressed fatty acid synthesis in vivo, but Kelley and co-workers (Kelley et al., 1986) showed that saturated fatty acid also suppressed fatty acid synthesis in cultured rat hepatocytes. Hence, it is likely that all dietary fatty acids, except short-chain fatty acids absorbed through the portal vein, suppress de novo synthesis. Thus, there is no need to synthesize fatty acids de novo if dietary sources are abundant.

Starvation mobilises fatty acids from adipose and peripheral tissues and transports them to the liver as free fatty acids (FFA) bound to albumin (Groener and Van Golde, 1977). The FFAs are converted to their acyl CoA derivatives in the liver, and there they act to suppress de novo fatty acid synthesis identically as dietary derived acyl CoA derivatives. The activation of the free fatty acids from adipose tissue is a complex phenomenon, produced by the drop in blood glucose when fasting depletes liver glycogen stores. The drop in blood glucose levels causes insulin and glucagon levels to change (Cahill, 1971). Insulin receptors on the adipocytes stimulate these cells to hydrolyse their

triacylglycerol stores while simultaneously turning off their synthetic capacity to produce triglycerides de novo (Cahill, 1971).

4.3.1.4 Circulating lipoproteins, lipids, and cholesterol

Dietary fatty acids have a profound effect on the lipids and lipoprotein levels in the circulation. As circulating cholesterol levels and lipoproteins are intimately related to the pathology of cardiovascular disease (Lindgren et al., 1951), there is great interest in this topic both in the scientific community and on the part of the general public.

As the gut and liver are the major sites of lipoprotein synthesis and catabolism, the transport system for delivery and removal of fatty acids to and from peripheral tissues responds to dietary fatty acids. It has been known for many years (Anitschkow and Chalatov, 1983) that diets high in fat and cholesterol tend to raise blood cholesterol. It was soon observed (Insull et al., 1959) that isocaloric substitution of n-6 polyunsaturated fatty acid for saturated fatty acids caused a reduction in the plasma cholesterol level even when cholesterol was present in the diet. Keys et al. (1965) had shown in the 1960s that stearic acid was an exception to the rule that saturated fatty acids raise blood cholesterol levels. Monounsaturated fat did not raise or lower the plasma cholesterol level. Monounsaturated fatty acids have beneficial effects as a substitution for lauric, myristic, or palmitic acid because the substitution has the same effect as removing the saturated fat from the diet (Ackman, 1983). Thus, substituting monounsaturated fatty acids or simply removing the saturated fat from the diet would result in cholesterol lowering (Grundy, 1991). Substituting polyunsaturated fatty acids will lower the plasma cholesterol level beyond the value observed when saturated fat is simply removed from the diet. Mattson and Grundy (1985) showed that oleic acid lowers plasma cholesterol to a similar level as linoleic acid, although Hegsted (1991) has questioned this claim.

The role of dietary n-3 long-chain fatty acids in raising or lowering blood cholesterol levels is complex. Previous reports (Ahrens et al., 1959) suggested that these compounds were similar to their n-6 counterparts in lowering plasma cholesterol. Other studies did not confirm that observation, particularly when the effect of substituting polyunsaturated for saturated fatty acids was considered (Phillipson et al.,1985). Harris (1989) reviewed this subject in some detail and developed a clearer picture of the effect of long-chain, polyunsaturated n-3 fatty acids on plasma cholesterol and lipoprotein levels in normolipemic and hyperlipemic individuals. Long-chain n-3 polyunsaturated fatty acids may raise LDL levels in normolipemic individuals. Connor and co-workers (1981) first reported that the main effect of these compounds is not on the plasma cholesterol levels but on the triglyceride and VLDL levels. The reduction in VLDL levels, particularly in hyperlipemic individuals, explains why investigators first thought that n-3 long-chain polyunsaturated fatty acids lowered plasma cholesterol levels. VLDL carries mainly triglycerides but does have about one-fifth as much cholesterol as LDL. Thus, if an individual's VLDL is elevated, both the cholesterol and triglyceride may be elevated as well. Normalising one's VLDL could lower one's cholesterol level and raise one's LDL levels, but the

results may be a reduction in one's total plasma cholesterol level with no reduction in one's cardiovascular risk (Harris, 1989).

This subject is confounded also by the influence of caloric level and percent fat in the diet. Reducing total fat calories in the diet will usually lower plasma cholesterol regardless of type of fatty acids reduced. Replacing saturated with monounsaturated fat or polyunsaturated fat, either n-6 or n-3, will also lower plasma cholesterol levels, with the magnitude of the lowering being n-6 > monounsaturated > n-3. Similarly, removing all saturated fat from the diet will usually produce maximal lowering (Harris et al., 1983). Individuals eating a low fat diet over a long period of time will invariably have lower plasma cholesterol levels than individuals eating a high fat diet regardless of the type of fatty acid in the diet (Liebman and Bazzarre, 1983).

The mechanism by which dietary fatty acids raise or lower blood cholesterol levels is still poorly understood (Grundy, 1991). It would appear that regulation is partly achieved through the stimulation or suppression of LDL receptors (Spady and Dietschy, 1985); however, how dietary fatty acids control the expression of LDL receptors is not known.

The mechanism by which n-3 long-chain, polyunsaturated fatty acids reduce VLDL is now partially understood (Nestel et al., 1984). It appears that these compounds suppress both triglyceride synthesis in the liver and gut and the assembly of nascent VLDL (Nestel et al., 1984). Wong and Nestel (1987) have investigated this phenomenon in considerable detail. It is not known how these long-chain, polyunsaturated fatty acids interfere with triglyceride and VLDL synthesis, but it is assumed that they may specifically inhibit one or more of the enzymes that lead to the formation of acyl CoAs that are needed to activate fatty acids for further metabolic utilisation.

Mensink and Katan (1990) showed interest in the role of *trans* fatty acids in lipid metabolism, and specifically their influence on the plasma cholesterol levels. Previously, Vergroesen (1972) found some elevation of plasma cholesterol by dietary fat containing 35% *trans* fatty acids. Conversely, Mattson and co-workers (1975) did not observe an elevation of plasma cholesterol in subjects fed diets containing fat with 34% *trans* fatty acids. Gottenbos (1983) reviewed this subject and concluded that *trans* fatty acids can raise plasma cholesterol levels and may be mildly atherogenic but have no effect on EFA metabolism. Additionally, dietary linoleic acid eliminated the hypercholesterolemic influence of dietary *trans* fatty acids consumed concurrently. Kritchevsky (1983) also conclude that *trans* fatty acids act metabolically much like saturated fatty acids and have no other significant adverse metabolic consequences.

Mensink and Katan (1990), in a carefully controlled human feeding study, compared diets containing either saturated (19%), monounsaturated (23%), or *trans* (11%) fatty acids. Polyunsaturated fat and other dietary constituents were held constant. They found that *trans* fatty acids raised plasma cholesterol levels but less than the equivalent amount of saturated fat. They also reported that *trans* fatty acids lowered plasma HDL cholesterol levels, which saturated fatty acids do not, and raised plasma LDL levels an amount equivalent to saturated fatty acids. These findings would have an

adverse effect on one's cardiovascular risk profile (Grundy, 1990) beyond that suggested by the change in total plasma cholesterol levels. Thus, dietary *trans* fatty acids may not be as harmless as previously supposed, but, as Grundy (1990) pointed out, *trans* fatty acids constitute only 3 or 4% of the average Western diet and therefore are unlikely to present a major health hazard to the population. Individuals, particularly those with existing adverse cardiovascular risk factors, may want to avoid these compounds in their diets.

4.3.1.5 Conversion to eicosanoids

Lands (1986) speculated that excess dietary n-6 fatty acids are the major causes of many chronic diseases in the Western world. There has been a great increase in n-6 polyunsaturated fatty acids in the diets of the Western world in the last hundred years, corresponding to the rise in debilitating chronic diseases, such as heart disease, cancer, and autoimmune diseases. Conversely, Galli and co-workers (1981) showed that excess linoleic acid suppresses eicosanoid production by inhibiting the cyclo-oxygenase reaction. Land's (1986) view is that current levels of linoleate in the Western diet are much higher than those that would be obtained on a diet not strongly influenced by agricultural oilseed production. Our current Western diet evolved quite recently with the development of efficient seed processing methods in the late 19^{th} and early 20^{th} centuries. Prior to that time the Western diet generally had a lower fat content, lower linoleate content, and perhaps a higher n-3 level, much like the diet found in third world countries today. The increase in linoleate levels will lead to a concurrent increase in arachidonic acid levels. This presumably would make more arachidonic acid available for eicosanoid production. Thus, the population would develop more inflammation, thrombosis and cancer if Land's conclusions are correct.

For each eicosanoid that promotes inflammation and thrombosis there is an eicosanoid whose production should go up correspondingly. The regulation and control processes for eicosanoid production are poorly understood, thus it is only speculation that eicosanoid production is related directly to arachidonic acid levels in tissues. Essential fatty acid deficiency is a state of malnutrition. It leads to dermal lesion, excessive skin water loss, and sterility (Burr and Burr, 1929). No great improvement in health is apparent from lack of eicosanoids; in fact, just the opposite is true.

Ferretti and co-workers (1989) evaluated the effects of moderate dietary change on eicosanoid production. They found that less eicosanoid was excreted in the urine by subjects who consumed a diet low in polyunsaturated (n-6) fatty acids as compared to subjects eating a diet high in linoleic acid. Previously Zollner et al. (1979) had suggested that the diet could alter eicosanoid production rates. Also, Adam et al. (1982) used two different levels of linoleate (0 and 20%) in the diet of human volunteers to show a difference in the excretion of tetranorprostanedioic acid, which is derived from both the E and F series of prostaglandins. Ferretti and co-workers (1987) measured PGE-M, which is a measure of the production of only the E series of eicosanoids. Ferretti and co-workers (1991) reported results from a study in which the total eicosanoid production as measured by PGE-M in the urine was reduced in a group of subjects consuming a salmon diet. The salmon group showed a

significant reduction in PGE-M levels in their urine compared to a group of subjects eating a diet without salmon. The level of linoleate in the diet was held constant in both groups. The n-3 fatty acids in the salmon group were replaced by oleic acid so that the percent of calories from fat was the same in both diets. Thus, it would appear that dietary long-chain, polyunsaturated n-3 fatty acids could inhibit eicosanoid production in humans. The n-3 and n-6 long-chain polyunsaturated fatty acids compete for the same enzymes. Generally, eicosanoids derived from the n-3 series of fatty acids have less biological activity than the corresponding eicosanoids from arachidonic acid.

4.4 Metabolic differences between exogenous and endogenous acids

Much is known about the fundamental metabolism and enzymology of fatty acids. The mechanisms of absorption and transport are also well characterised, but the details of the mechanisms by which dietary fatty acids control the production of endogenous fatty acids, eicosanoids and blood lipid levels are not understood as well. There may be a significant difference between the metabolism of exogenous and endogenous fatty acids.

This conclusion is shown by the action of various fatty acids on selected physiological parameters. For instance, lauric, myristic, and palmitic acids in the diet raise blood cholesterol levels whereas stearic acid does not (Grundy, 1991). Yet when excess carbohydrate is fed in a low fat diet, there is a rapid synthesis of palmitic, stearic, and oleic acids but no corresponding rise in the blood cholesterol levels (Hegsted, 1991). Palmitic acid must be synthesised before stearic or oleic, and lauric and myristic acids must be synthesised before palmitic acid (Emken et al., 1988). The question that can be asked is why then do the exogenous fatty acids raise the plasma cholesterol but the endogenous fatty acids do not. Lane et al. (1974) reported that the fatty acid synthetase complex binds the malonyl CoA and acetyl CoA condensation products, decanoic, lauric, and myristic acids, until the palmitoyl CoA is released. This process is highly compartmentalised. Neither free lauric nor myristic acid is likely to be produced by endogenous synthesis. Palmitoyl CoA is elongated to stearoyl CoA or esterified to glycerol phosphate or dihydroxyacetone phosphate to be converted to triglycerides or other complex lipids (Seubert and Podack, 1973). High tissue concentrations of these compounds will be found in vivo only when they are derived from dietary sources.

The entrance of dietary (preformed) fatty acids into the pathways of lipid metabolism must be different from that of fatty acids synthesised de novo. Stearic acid from dietary sources is not easily converted to oleic acid (Bonanome and Grundy, 1988). It is oxidised to CO_2 and water; yet stearic acid synthesised de novo is largely converted to oleic acid. It may be difficult for preformed dietary fatty acids to enter the typical synthetic pathways of lipid metabolism. The compartementalisation of the process may be rigid, with little exchange between oxidation and synthesis.

The essential fatty acids obviously have different metabolic pathways from those of the saturated and monounsaturated fatty acids. Also n-3 and n-6 fatty acids have different reaction rates with most enzyme systems that have been studied (Sprecher, 1991). Linoleic acid is rapidly incorporated into tissue and complex lipids and elongated and desaturated to arachidonic acid (Lands et al., 1990). α-Linolenic acid is excluded from most tissues and complex lipids (Tinoco, 1982). If large amounts of α-linolenic acid are fed, it appears in the blood and liver and to some extent adipose tissue in a lower proportion than that fed in the diet. Conversely, linoleic acid will usually be incorporated into tissues and complex lipids in proportionally larger amounts than is fed in the diet. Thus, linoleic acid is markedly conserved whereas α-linolenic acid is even more strikingly eliminated from the tissues. α-Linolenic acid is converted to EPA and DHA, but only slowly (Adam et al., 1986). Eicosapentaenoic acid is another fatty acid that does not persist in most mammalian tissues (Nelson et al., 1991). It is either rapidly oxidised or converted to DHA, a fatty acid that is strongly conserved.

The metabolism of EPA is different from that of α-linolenic acid in several important aspects. Unlike α-linolenic acid, when EPA is fed it is rapidly incorporated into various tissues and complex lipids. Dietary EPA will produce comparable levels in the tissue within 2 weeks that approximate the level in the diet (Nelson et al., 1991). Tissue levels of α-linolenic acid rarely reach the level fed in the diet even after prolonged feeding periods. It would be very interesting to determine why tissues discriminate against α-linolenic acid but not EPA. Perhaps α-linolenic acid is simply incompatible with the massive body stores of linoleic acid. No experimental study can be done to test whether α-linolenic acid would be readily incorporated into tissue stores if linoleic acid were not present in normal quantities. One would have to induce EFA deficiency in the test animals, which would then be so ill that the results would be difficult to interpret. Linolenic acid is a good source of calories. It is eaten world-wide. Enough α-linolenic acid is converted to EPA and DHA to promote growth and development in the foetal and neonatal offspring (Crawford et al., 1989). The enzyme systems that convert α-linolenic acid to EPA and DHA are repressed in the adolescent and adult once growth slows and the development of the central nervous system is largely completed. DHA then provides a source of EPA by retroversion (Nelson, 1992). In omnivores there is always some DHA in the diet. Herbivores have abundant sources of α-linolenic acid in their diets. The requirements for conversion of α-linolenic acid to EPA and DHA must be very low in adults. It appears that the n-3 series is discriminated against in favour of the n-6 series in mammalian tissues. As Western diets contain abundant amounts of linoleic acid, the conversion rate of α-linolenic acid to EPA and DHA will be lowered even further.

Conversely, arachidonic acid may be metabolised at a much faster rate than DHA. Lands estimated that the approximate turnover of arachidonic acid is 0.12 g/day for the adult rat (Lands et al., 1990). Extrapolating to humans, this would be about 3 g/day, which would require the conversion of an equal number of grams of linoleic acid to arachidonic acid each day to maintain homeostatic equilibrium. The normal Western diet cannot supply this much preformed arachidonic acid. The only rich sources of dietary arachidonic acid are certain organ meats and some species of South Pacific ocean fish (Sinclair et al., 1983).

5 The essential fatty acids and their role in human health

The essentiality of fat in the diet has been known since 1929 (Burr and Burr, 1929), when it was first recognised that linoleic acid (Δ9,12-octadecadienoic acid, 18:2n6) and possibly other acids are essential fatty acids. Since this initial observation there have been numerous published studies documenting the fact that certain polyunsaturated fatty acids cannot be synthesised de novo from endogenous precursors and are therefore essential dietary elements (Alfin-Slater and Aftergood, 1968). Indeed, it is now recognised that there are two essential fatty acids (EFA), 18:2N6 and α-linolenic acid (Δ9,12,15-octadecatrienoic acid, 18:3n3). Although the volume of literature associated with the biological significance of polyunsaturated fatty acids (PUFA) in laboratory animals is immense, relatively few studies have focused on the essentiality of 18:2n6 and 18:3n3 with respect to humans. In fact, the critical issues related to EFA physiological functions and requirements in humans are still being investigated. The results of some of these recent investigations are discussed in this chapter.

5.1 Linoleic acid requirement

The physiological effects of linoleic acid (C18:2n6) deficiency has been very well characterised, particularly in rodent species (Burr and Burr, 1929). The various deficiency symptoms include depressed growth, scaly dermatoses, increased permeability of skin, fatty liver, kidney damage, and impaired reproduction (Burr and Burr, 1929). The symptoms of 18:2n6 deficiency in mammals is summarised in Table 5.1. A biochemical method of establishing EFA deficiency is to calculate the ratio of Δ5,8,11-eicosatrienoic acid (C20:3n9) to arachidonic acid (Δ5,8,11,14-eicosatetraenoic acid, C20:4n6). Although the validity of this index has been questioned (Horrobin and Cunnane, 1981), ratios above 0.2 and 0.4 are considered the upper limit of normalcy in the rat (Alfin-Slater and Aftergood, 1968) and human (Holman et al., 1979), respectively.

Table 5.1: Symptoms of linoleic acid deficiency in mammals (Yamanaka et al., 1981).

Diminished growth	Alopecia
Scaly dermatoses	Fatty liver
Inflamed epidermis	Kidney degeneration
Diminished skin pigmentation	Impaired reproduction and sterility
Increased transepidermal water loss	Increased basal metabolic rate
Increased water consumption	Impaired protein utilisation
Impaired wound healing	Electrocardiographic aberrations
Increased susceptibility to infection	Increased fragility and permeability of cellular membranes
Loss of muscle tone	
Caudal necrosis	

Ingestion of approximately 1-2% of daily calories as 18:2n6 is widely accepted as the amount needed to meet the EFA requirement of rodent species and humans. However, it is important to note that the 1-2% figure is only an approximation of 18:2n6 dietary requirement for humans. Indeed, this estimate is considered so nebulous that the highly promulgated National Research Council Recommended Dietary Allowances (RDAs) do not even list an 18:2n6 requirement (National Research Council, 1989). Although many attempts have been made to determine the human 18:2n6 requirement, most studies have met with a plethora of problems regarding what physiological criteria to use for normality. Human infants and children are generally thought to require 1-2% of total dietary energy as 18:2n6 to prevent EFA deficiency (Rivers and Frankel, 1981). However, it has been argued that the minimum requirement has been set "far too high" for infants and should be changed to less than 0.5% of calories (Cuthbertson, 1976). The scientific response to this data remains mixed primarily because it is believed that children require more 18:2n6 as a percentage of total daily calories than adults because growth increases the demand for cell membrane constituents (Holman et al., 1979). In a more recent study on premature infants (Farrell et al., 1988), the average amount of 18:2n6 required achieving normal fatty acid nutrition was 1.19 g/kg per day, or approximately 10% of total caloric intake. The prodigious requirement for 18:2n6 was attributed to the premature infants' small amount of adipose tissue and the great need for energy production from fat (Farrell et al., 1988). A higher requirement for 182n6 during pregnancy (4.5% of calories) has also been suggested. In addition, an FAO/WHO expert committee has advised increased intakes of 18:2n6 (5-7% of calories) during lactation. Other examples of physiological conditions that may alter the 18:2n6 requirement are protein malnutrition (Hill and Holman, 1980), genetic disorders that affect the relative activity of unsaturated fatty acid metabolic pathways, intestinal malabsorption (Gourley et al., 1982), and n-3 fatty acid intake (Bourre et al., 1989). Since the metabolic pathways for the biosynthesis of long-chain PUFA consist of a series of desaturation and elongation enzymatic steps (Brenner, 1981), dietary 18:3n6 and 20:4n6 may have superior biopotency with respect to 18:2n6 (Rivers and Frankel, 1981). Attempts to determine the 18:2n6 requirement of isolated mammalian cells in culture have created an interesting paradox. Many studies (Bailey, 1980) indicate that most normal human cells do not require 18:2n6 or any PUFA for normal growth or metabolism. The reason for this is unknown.

5.2 Linolenic acid requirement

The essentiality of α-linolenic acid (C18:3n3) in higher animals and humans has been debated for many years. Although, similar to C18:2n6, it cannot be synthesised in vivo (Brenner, 1981), initial attempts to elicit C18:3n3 deficiency in mammals produced ambiguous results (Tinoco et al., 1979). During the early 1970s (Fiennes et al., 1973) and again in the 1980s, convincing evidence in support of the role of C18:3n3 in the brain and retina were presented (Holman et al., 1982). Estimates for the C18:3n3 requirement of humans has been listed at 0.4% (Bjerve et al., 1989), 0.54% (Holman et al., 1982), and 0.2-0.3% of calories (Bjerve et al., 1987). In addition, it has been recommended that the diet provide an adequate amount of n-3 fatty acids and a PUFA n-6/n-3 ration of 4:1-10:1 (Neuringer

and Conner, 1986), 6:1 (Bourre et al., 1989), and 4:1 (Simopoulos, 1989a), particularly during infancy, pregnancy, and lactation. The current estimate of the PUFA n-6/n-3 ration in the Western diet is 10-11:1 (Simopoulos, 1989b).

The very limited ability of C18:3n3 to substitute for 18:2n6 (or its metabolic derivatives) and reverse classic EFA deficiency signs, that is, growth, reproduction, or dermal symptoms (Tinoco, 1982), is a major reason the essentiality of 18:3n3 has remained controversial and why the requirement remains ill defined. However, substantial evidence now indicates that 18:3n3 and its metabolic fatty acid derivatives [Δ5,8,11,14,17-eicosapentaenoic acid (EPA) (C20:5n3) and Δ4,7,10,13,16,19-docosahexaenoic acid (DHA) (C22:6n3)] have their own specialised and distinct functions in the retina and central nervous system (Wheeler et al., 1975). Recent dietary estimates to prevent n-3 depletion of liver and brain phospholipids are 800-1100 mg/day of C18:3n3 and 300-400 mg/day of C20:5n3 and C22:6n3 combined (Simopoulos, 1989b). A summary of recommendations for dietary PUFA is listed in Table 5.2.

Table 5.2: Recommendations for dietary PUFA intake[a] (Simopoulos, 1989a).

Polyunsaturated fatty acids
18:2n6 = 14 g/day (4.8% of calories)
18:3n3 = 3 g/day (1.0% of calories)
(EPA) 20:5n3 plus 22:6n3 (DHA) = 0.8 g/day (0.27% of calories)
Total PUFA = 18 g/day (6-7% of calories)
n-6/n-3 ration = 4:1
(n-3 as 18:3)/(n-3 as EPA plus DHA ration) = 4:1

[a] For normal healthy adults free of genetic disorders, medical disorders, etc.

5.3 Metabolic destinations of linoleic and linolenic acid

Once ingested, C18:2n6 and C18:3n3 can be desaturated and elongated, primarily in the liver (Brenner, 1981) in a manner such that the methylene-interrupted pattern of unsaturated double bonds is maintained (Brenner, 1981). The microsomal enzymatic reactions do not permit crossover between fatty acid metabolites from the C18:2n6 and C18:3n3 sequences (Brenner, 1981). The desaturation and elongation steps are influenced by numerous nutritional and hormonal factors (Brenner, 1981). A competitive interaction between C18:2n6 and C18:3n3 exists, such that n-3 PUFA suppress the metabolism of n-6 PUFA and n-6 PUFA suppress the metabolism of n-3 PUFA, although less strongly (Rahm and Holman, 1964). It is generally believed that the capacity to convert C18:2n6 and C18:3n3 into 20- and 22-carbon fatty acids in humans is very limited (Nichman et al., 1967). Interestingly, in an investigation using deuterated C18:2n6 (Emken et al., 1987), desaturation/elongation products were not detected in plasma lipids. The authors concluded that 18:2-$^{2}H_4$ to 20:4-$^{2}H_4$ conversion is extremely low in normal subjects. In a separate series of studies using mixtures of triglycerides containing deuterium-labelled fatty acids, Emken et al. (1988) demonstrated the

conversion of C18:3n3 to C20:5n3 and C22:6n3 but were again unable to detect conversion of C18:2n6 to C20:4n6. The exclusive conversion of C18:3n3, but not C18:2n6, in these studies is most intriguing. It is possible that the C18:2n6 conversion products were sequestered in tissues following ingestion and therefore could not be released into the plasma lipid pool, which was subsequently analysed. In comparative studies, Siguel and Maclure (1987), using derivative precursor ratios, and El Boustani et al. (1989), using 20:3n6-$^{2}H_{4}$, demonstrated that desaturase activity operates in humans and that the desaturation/elongation enzymes have a preference for n-3 versus n-6 fatty acids. The "very active conversion" (Siguel and Maclure, 1987) of C18:3n3 suggests that vegetarians can accumulate C20:5n3 and C22:6n3 and need not alter their diets to include fish products (which are rich in C20:5n3 and C22:6n3).

5.4 Physiological functions

The broad array of deficiency symptoms suggests that essential fatty acids play a prominent physiological role in different organs. For example, C18:2n6 and C18:3n3 and their desaturation/elongation products (C20:4n6, C20:5n3 and C22:6n3) are involved in the homoviscous control of the membrane lipid bilayers of most cells (Burr and Burr, 1930). In this capacity, they can regulate the conformational freedom of proteins and thereby influence membrane signal transduction events. The identification of C18:2n6 as a constituent of epidermal sphingolipids is an example of the regulation of membrane barrier function by an EFA (Hansen, 1986). The emerging role of n-3 PUFA in nerve tissue is supported by studies showing an altered electroretinogram, decreased visual activity, and impaired learning ability in animals deprived of C18:3n3 (Neuringer and Conner, 1986). Deprivation of C18:3n3 and its major fatty acid metabolite, C22:6n3, eventually results in decreased brain and retina C22:6n3 content. It is generally believed that C22:6n3 is synthesised from C18:3n3 by the liver (Emken et al., 1988), secreted into the bloodstream in lipoprotein form, and taken up by the brain and retina, possibly by a C22:6n3 receptor (Scott and Bazan, 1989).

The essential fatty acids are also the precursors for prostaglandins, hydroxy fatty acids, sulfidopeptide-leukotrienes, and lipoxins, collectively referred to as eicosanoids (Lands, 1979). These oxygenated metabolites are formed rapidly upon stimulus and, acting as autacoids, exert a profound influence on many cellular reactions. A flurry of interest focusing on the dietary modulation of eicosanoid formation in humans have surfaced in the last decade (von Schacky et al., 1985). These dietary PUFA manipulations are highly significant because many diseases are associated with an overproduction of eicosanoids from 20:4n6 (derived from 18:2n6), and the formation of n-6 eicosanoids can be antagonised by dietary n-3 PUFA (Lands, 1989). Interestingly, the 20:4n6 pool(s) for prostaglandins in humans is not quickly influenced by dietary 18:2n6 and 20:4n6 because of a large pool size of 20:4n6 and a low conversion of 18:2n6 to 20:4n6, whereas the n-3 PUFA pool(s), which is considerably smaller, is immediately influenced by n-3 PUFA supplementation (Hamazaki et al., 1989).

6 Sources of dietary fatty acids

Fatty acids are rarely consumed as the free acid. Most free acids are toxic (Constantinides and Kiser, 1981) to mammalian cells and are an irritant to the stomach if consumed in sufficient quantity without the buffering effect of other nutrients (Nelson and Ackman, 1988). In the presence of other nutrients, acylglycerols are hydrolysed rapidly in the stomach by the action of lingual lipase (Hyun et al., 1967) and gastric lipase (Vallot et al., 1985). Most of the conversions of dietary acyl esters to free fatty acids take place in the intestines by the action of pancreatic lipase.

It is also apparent that dietary fatty acids are not entirely equivalent to endogenously synthesised fatty acids. Eicosapentanoic acid (EPA) has pharmacological actions when consumed in significant quantities (Kremer, 1991), yet the metabolic precursor of EPA, α-linolenic acid, when consumed in large quantities appears to have little influence on most physiological processes (Tinoco, 1982). This could be a dose effect, but EPA cannot be synthesised in vivo in quantities sufficient to initiate its pharmacological actions. Also the consumption of excessive quantities of carbohydrate, beyond that necessary to satisfy daily metabolic energy needs and in the absence of dietary fat, induces the synthesis of palmitic, stearic, and oleic acids. Yet plasma cholesterol and low-density lipoprotein (LDL) levels do not rise significantly (McNamara, 1987). Thus, one should always remember that there is a compartmentalisation of fatty acid metabolism. Generally, and although there are numerous anastomoses between the compartments, dietary fatty acids are metabolised along a different pathway than endogenously synthesised fatty acids.

Approximately 99% of the fatty acids consumed are acyl esters, usually triacylglycerols, occasionally mono- or diglycerides and, to a lesser extent, phospholipids and other complex lipids found in plant and animal tissues (Norris, 1983). Plants, particularly oilseeds, provide rich sources of triglycerides containing polyunsaturated fatty acids, usually of the n-6 variety. Animal fats, at least from the domestic animals consumed in the Western diet, are a rich source of saturated medium-chain fatty acids and long-chain monounsaturated fatty acids, primarily oleic acid. Leafy vegetables and some fruits and oilseeds contribute long-chain, polyunsaturated fatty acids of the n-3 variety, mainly α-linolenic acid (C18:3n3) (Hunter, 1987). Canola is an example of an oilseed containing a high level of n-3 polyunsaturates, especially α-linolenic acid. Fresh fish and processed fish oils are the only significant source of 20-carbon and longer n-3 polyunsaturated fatty acids in the Western diet (Ackman, 1986). The Western diet is considered n-3 deficient by some investigators (Lands, 1986). Other populations, particularly Eskimos, maintaining a traditional life-style, being a prime example, but including the Japanese and other populations consuming a large amount of fish, obtain a significant percentage of their calories from long-chain, polyunsaturated n-3 fatty acids (Dyerberg and Jorgensen, 1982). The health status of these groups has been the subject of intense scrutiny over the past two decades (Hirai et al., 1987), and consequently fish oil has been touted to have great nutritional and health benefits (Molgaard et al., 1990).

7 Manipulation of the n-3 fatty acid composition of avian meat

An imbalance in the human dietary intake of various types of fatty acids has become apparent (Simopoulos, 1991). Results of fundamental studies have shown specific and beneficial effects on human health and well-being through the consumption of long-chain n-3 polyunsaturated fatty acids, in particular eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), which play an important role in rectifying this imbalance (Kromhout et al., 1985). As a result of these observations, recent recommendations pertaining to the consumption of dietary fat have stressed the importance of consuming higher levels of these fatty acids. In addition to increasing oily fish consumption, intake of the n-3 polyunsaturates can be enhanced through the consumption of commonly occuring non-fish foods containing elevated levels of these fatty acids. The fatty acid composition of the lipids of avian egg yolk and muscle tissues may be modified to match human nutritional guidelines better by appropriately manipulating the fatty acid composition of the diet; for example, modifications of the avian diet have raised total n-3 fatty acid content in egg yolk to over 200 mg (Cherian and Sim, 1992). Beneficial effects on blood lipoprotein characteristics in humans who consumed eggs with such modified fatty acids contents have been recorded (Jiang and Sim, 1993).

As in the case of eggs, meaningful concentrations of n-3 polyunsaturates have been incorporated into major poultry muscle tissues (Hargis and Van Elswyk, 1993). Concerns have been expressed over the development of unacceptable odours in eggs and meat from birds fed diets with increased polyunsaturated lipids. However, by using only high quality materials, limiting the amount of fish oil or fishmeal within the diet and optimising the provision of supplemental dietary antioxidants, the production of eggs and meat with increased n-3 polyunsaturates is practicable.

7.1 Lipid composition of conventional broiler meat

The total lipid content and lipid class compositions of light and dark broiler muscle and skin are shown in Table 7.1. The total lipid content of white meat is approximately half of that of dark meat, and skin contains the highest proportion of lipid. The total fat content of light muscle with skin has therefore been quoted as approximately 10 times higher (11.1 g / 100 g muscle) than muscle without skin (Decker and Cantor, 1992). The triacylglycerol (TG) content of white meat was virtually half that of dark meat. In contrast, phospholipids (PL) and cholesterol (CH) were appreciably higher in white than in dark meat. In both muscle types, free fatty acids (FFA) and diacylglycerols (DG) were present in only trace amounts. In contrast to the muscle tissues, the skin showed an almost total preponderance of triacylglycerol with traces of diacylglycerol, free fatty acid and phospholipid.

Table 7.1: Total lipid content and lipid class composition of white and dark meats and skin from chickens fed a standard diet (Ratnayake et al., 1989).

	Total lipid (% wt fresh tissue)	Lipid class (% wt total lipid)				
		FFA	TG	CH	DG	PL
White meat (breast)	0.9	tr	43	2	tr	55
Dark meat (thigh)	2.2	tr	83	1	tr	16
Skin	30.3	tr	100	-	tr	tr

(-) not detected

FFA = free fatty acids; TG = triacylglycerols; CH = cholesterol; DG = diacylglycerols; PL = phospholipids

Table 7.2 shows the fatty acid composition of the total lipids extractable from white and dark muscles and skin from broilers fed standard diets. As well as showing similarities in fatty acid composition, a number of differences are evident. In all tissues the principal fatty acid was oleic acid, followed by palmitic and linoleic acids. The levels of total saturates, total monounsaturates and total polyunsaturates in the white muscle tissues assumed an approximate 33% distribution of each. Monounsaturated fatty acids were higher in dark meat than in light meat. In contrast, total polyunsaturated fatty acids were higher in white meat. This difference is a reflection of the higher levels of both the total n-6 and total n-3 polyunsaturates in the white muscle. Among the long chain polyunsaturated fatty acids (both n-6 and n-3) arachidonic acid was most prominent. In comparison with the muscle tissues, the skin contained much higher levels of palmitoleic and oleic acids, which substituted for stearic acid and long chain polyunsaturated fatty acids.

Table 7.2: Fatty acid composition (major fatty acids, percentage of total present) of the total lipids extractable from white and dark meats and skin from chickens fed a standard diet (Ratnayake et al., 1989).

Fatty acids	**White (breast)**	**Dark (thigh)**	**Skin**
C16:0	23.8	22.6	24.0
C18:0	7.5	7.6	5.1
Total SFA	**33.5**	**32.2**	**30.7**
C16:1n7	4.5	6.3	7.8
C18:1n9	29.1	32.0	39.4
C20:1n9	0.5	0.5	0.6
C22:1n9	0.4	0.6	0.4
Total MUFA	**34.5**	**39.4**	**47.8**
C18:2n6	17.8	18.3	18.2
C18:3n3	0.5	0.7	1.0
C20:4n6	5.0	3.7	0.6
C20:5n3	0.7	0.6	0.4
C22:5n3	0.9	0.5	0.1
C22:6n3	1.8	1.0	0.1
Total PUFA	**32.0**	**28.5**	**21.4**
Total n-6 PUFA	27.4	25.1	19.7
Total n-3 PUFA	4.5	3.4	1.8

The lipid and fatty acid compositions of muscles from other avian species have been described by Decker and Cantor (1992). Turkey light muscle has a lower total lipid content (1.6%) than any of the other meats with duck and goose having the highest contents (6% and 7%, respectively) (Table7.3). Compared with chicken muscle, turkey muscle is composed of a slightly higher content of saturates, a lower level of monounsaturates and slightly higher content of polyunsaturates. The content of the long chain polyunsaturated fatty acids (n-6 and n-3) are similar. As observed for the broiler chicken, the light and dark muscles of the turkey including skin (data not shown) contain lower levels of saturates and polyunsaturates, but a higher content of monounsaturates than those muscles with skin (Decker and Cantor, 1992). Duck and goose muscles are predominanly of the dark type throughout the carcass and contain 6.0% and 7.1% of lipid without skin, respectively. With skin, the total fat contents increase to 39.3% and 33.6% for the duck and goose, respectively (Decker and Cantor, 1992). The fatty acid contents of duck and goose muscles are very similar and contain approximately 50% saturated, 33% monounsaturated and 16% polyunsaturated fatty acids. The long chain polyunsaturated fatty acids in duck and goose fat are notable in their apparent absence, although this may simply be a reflection of their absence in the respective diets rather than due to the type of species. The fatty acid content of pheasant muscle is characterised by fewer saturates and more monounsaturates and polyunsaturates than duck or goose muscle. However, no long chain polyunsaturates are present. The fatty acid profile of quail is similar to that of light muscle of the broiler except for the absence of DHA and the presence of higher levels of linoleic acid. As the authors did not mention whether the species in Table 7.3 were fed the same diets, the question can be asked whether the results of the fatty acid composition is species specific or potentially nutritional or a combination of both.

Table 7.3: Fatty acid composition (major fatty acids, percentage of total present) of the total lipid extractable from the raw muscles of various avian species (no skin) (Decker and Cantor, 1992).

Fatty acids	Turkey (light)	Duck	Goose	Pheasant	Quail
C16:0	21.3	28.3	28.3	25.3	22.5
C18:0	13.9	17.9	17.7	21.1	13.3
Total SFA	**42.0**	**50.3**	**50.4**	**40.9**	**35.0**
C16:1n7	3.7	5.1	5.2	6.7	1.2
C18:1n9	21.3	30.6	30.4	32.3	29.4
C20:1n9	0.9	-	-	-	0.3
C22:1n9	0.9	-	-	-	-
Total MUFA	**22.7**	**33.4**	**33.4**	**38.6**	**34.0**
C18:2n6	25.0	15.1	15.4	18.2	26.8
C18:3n3	0.9	1.9	1.9	2.7	0.6
C20:4n6	7.4	-	-	-	3.5
C20:5n3	-	-	-	-	0.3
C22:5n3	0.9	-	-	-	0.5
C22:6n3	1.9	-	-	-	-
Total PUFA	**35.3**	**16.3**	**16.3**	**20.5**	**31.0**
Total fat (g / 100 g)	1.6	6.0	7.1	3.6	4.5

(-) not detected

7.2 Manipulation of the n-3 fatty acid content of poultry meat

The fatty acid composition of the muscle and adipose tissue can be altered considerably by changes in the fatty acid composition of the diet (Hargis and VanElswyk, 1993). Thus, in the chicken, the content of saturated fatty acids can be increased by feeding coconut oil (Yau et al., 1991), the content of oleic acid can be increased by feeding olive oil (Yau et al., 1991) and the content of linoleic acid can be increased by feeding maize oil (Marion and Woodroof, 1963), safflower oil (Miller and Robisch, 1969) or soybean oil (Scaife et al., 1990). Marked alterations in muscle and adipose tissue fatty acid compositions have been achieved in the absence of effects on the total lipid content of the tissues or the relative proportions of major lipids, including cholesterol (Yau et al., 1991). Yau et al. (1991) observed differences in moisture and cholesterol contents of breast muscle due to changes in dietary oil, but these changes were said not to be of any practical relevance. In addition, no changes in total protein contents of the breast muscle were observed. The adipose tissue has been shown to be more affected by dietary lipid composition than breast muscle, presumably due to the lipid storage function of the adipose tissue (Yau et al., 1991). No sex or genotype differences in total lipid content, lipid composition or fatty acid composition of muscles have been observed when feeding either standard diets or diets with modified fatty acid compositions (Ratnayake et al., 1989).

A number of studies have examined the effects of dietary long chain polyunsaturated fatty acids, as contained in fish oil or fishmeal, on the fatty acid composition of the broiler carcass (Nash et al., 1995). Many of these studies were conducted with the aim of enhancing the human dietary intake of long chain n-3 polyunsaturates, and the specific aim of conferring beneficial effects to human health and resistance to disease. These studies have shown that the deposition of n-3 polyunsaturates in muscle and adipose tissues increases with the amount of fish oil or fishmeal supplied by the diet and the duration of feeding (Ratnayake et al., 1989). Table 7.4 shows the fatty acid composition of white and dark muscle from broilers fed 4%, 8% and 12% redfish meal diets, and is illustrative of the marked changes in fatty acid composition that occur in general as a result of including fish oil in the broiler diet. The redfish meal contained 10% by weight of total lipid (Ratnayake et al., 1989), a level which is similar to that recorded by others (Nash et al., 1995). Increasing the level of the dietary fishmeal caused an increase in the accumulation of n-3 polyunsaturates, particularly EPA, DPA and DHA, at the expense of decreasing levels of n-6 polyunsaturates. The deposition of EPA, DPA and DHA was higher in the white muscle than in the dark muscle, with the total concentration of these fatty acids reaching almost 11% of total fatty acids in the group receiving the 12% redfish meal. The increased deposition of n-3 polyunsaturates in breast (white) muscle compared with thigh (dark) muscle has been observed (Hulan et al., 1988). In both muscle tissues the deposition of DHA was higher than that of EPA and DPA; EPA and DPA accured in similar quantities. In the skin (results not shown), Ratnayake et al. (1989) observed levels of EPA and DHA of approximately 0.6% and 0.3%, respectively, in all three experimental diets shown in Table 7.4; although these concentrations were much lower than those observed in the muscle tissues, it was said that an appreciable human dietary contribution could be made due to the high total lipid content of the skin.

Table 7.4: Fatty acid composition (major fatty acids, percentage of total present) of the total lipid extractable from white and dark muscle from chickens fed varying levels of redfish meal (RFM) (Ratnayake et al., 1989).

	4% RFM		8% RFM		12% RFM	
	White	Dark	White	Dark	White	Dark
C16:0	24.5	23.8	25.8	24.9	25.2	25.3
C18:0	7.9	7.9	7.7	7.5	8.0	8.1
Total SFA	**34.5**	**33.7**	**35.9**	**34.4**	**35.6**	**35.6**
C16:1n7	4.5	6.7	4.9	7.3	4.9	7.6
C18:1n9	29.4	33.3	31.4	35.4	30.2	34.0
C20:1n9	0.7	0.8	0.9	1.1	1.0	1.3
C22:1n9	0.6	0.6	0.4	0.4	1.0	0.5
Total MUFA	**35.2**	**41.3**	**37.6**	**44.2**	**37.0**	**43.3**
C18:2n6	15.9	16.1	14.2	13.8	12.0	12.6
C18:3n3	0.4	0.6	0.5	0.7	0.3	0.6
C20:4n6	3.3	2.6	2.3	1.7	2.2	1.7
C20:5n3	1.4	0.7	1.6	0.9	2.3	1.2
C22:5n3	1.3	0.7	1.0	0.8	2.3	1.1
C22:6n3	4.0	1.9	4.6	1.9	6.0	2.5
Total PUFA	**30.3**	**25.0**	**26.5**	**21.3**	**27.5**	**21.0**
Total n-6 PUFA	22.7	20.8	18.4	16.8	16.3	15.4
Total n-3 PUFA	7.5	4.2	8.1	4.5	11.2	5.6

Increases in the long chain polyunsaturated fatty acids have been shown to occur primarily in the phospholipid fraction of the muscle (Hulan et al., 1988). Neudoerffer and Lea (1967) investigated the changes in turkey muscle phospholipid and found significant changes in several phospholipid molecular species as a result of feeding 2.5% beef fat or anchovy oil at levels of 2.5% and 5%. Phosphatidylcholine was the most abundant phospholipid in the muscle. However, EPA and DHA were found in highest concentrations in the phosphatidylethanolamine fraction in which DHA attained the level of approximately 25% of total fatty acids and was substituted for arachidonic acid. The long chain n-3 polyunsaturates were also found at relatively high levels in phosphatidylcholine and phosphatidylserine. Arachidonic acid was found at the highest level in the phosphatidylinositol fraction (approximately 20%).

Hulan et al. (1988) calculated the amount of long chain n-3 polyunsaturates which could be supplied to the human as a result of consuming the fatty acid-modified meat. It was stated that a 100 g portion of fat-modified chicken meat equally divided between 50 g breast meat and 50 g thigh meat would provide approximately 142 mg of EPA + DPA + DHA (61 mg from breast + 81 mg from thigh). This provided slightly more than the 138 mg of these fatty acids found in 100 g of cod flesh. In a subsequent study, Hulan et al. (1989) observed that feeding a 12% redfish meal diet would provide about 197 mg of EPA + DPA + DHA per 100 g of chicken.

Based on a wide range of clinical investigations, recommendations have been put forward which emphasize the need for alterations in the fatty acid composition of the human diet. One of the recommendations (Simopoulos, 1989b) pertains to the amount of polyunsaturated fatty acids consumed, in particular the relative amounts of the so-called n-6 and n-3 polyunsaturated fatty acids. The perceived present-day dietary imbalance between these classes of fatty acids should be rectified by an increased consumption of the long chain n-3 fatty acids, particularly EPA and DHA, which are found in relative high concentrations in fish oil. These fatty acids can make important contributions, not only in the context of various diseases (most notably coronary heart disease), but also to critical aspects of neonatal growth and development.

The enhancement of the levels of EPA and DHA in commonly occuring non-fish foods can be beneficial to the population. Such foods, in which there has been successful incorporation of these fatty acids, include poultry meat and table eggs. Problems with flavour and oxidative deterioration have been encountered in feeding diets containing fishmeal or fish oil. However, it is likely that a compromise exists in which the inclusion levels can be closely monitored, while the health benefits to the consumer of poultry products with a modified fatty acid composition can be realized.

8 Lipid Oxidation

There are numerous papers dealing with vitamin E and oxidation that one has difficulty in finding something new to say. However, interest for the problem of oxidation, particularly lipid oxidation, should remain high, simply because it continues to be a problem.

It is a fact that oxidation is essential for life, and it must be accepted that oxidation goes on in all animal-derived products. Sometimes, in order to obtain the desired final product quality, oxidative processes may be essential. One example is the blooming of beef, the oxygenation of myoglobin, to obtain the desired red colour. However, when lipid peroxides are formed, the deterioration of quality starts immediately. It is well documented that these oxidative changes can directly affect characteristics such as colour, flavour, texture, nutritive value and safety (Buckley and Morrisey, 1992).

Although much information already exists, continued efforts in research are still justified. On the one hand, technology for the development of meat products may result in a new or higher ranking for quality criteria (e.g. requirements in shelf life, restructured products, speciality products, and safety). On the other hand, scientific technology can offer new opportunities to assess quality, such as more sensitive methods and higher capacities to run samples in automated systems.

The following basic principles should be considered:

1. Oxidation is hard to avoid. In some cases it proceeds at a very slow rate, in others more rapidly.
2. Measures taken to reduce the propagation of radicals are stochastic processes. This means that antioxidants have a certain chance or probability to trap a radical according to its chemical characteristics, or because of its specific distribution pattern. Therefore, a chemically suitable antioxidant can only act efficiently if it is not too distant from the place of appearance of a radical. Otherwise, the radical could cause severe damage and propagate in a chain-reaction-like manner before being trapped.
3. The antioxidant has to be present in an adequate amount and in the right tissue structure. This can be summarised in the slogan 'right place right quantity'.

8.1 Reactivity of saturated and unsaturated fatty acids

Saturated fatty acids, being composed of paraffinic hydrocarbon chains, are generally considered to have a very low reactivity. Introduction of one or more double bonds into a fatty acid provides an active centre that can be the site of a variety of reactions – undesirable, as in the case of oxidation, or desirable for industrial purposes, such as hydrogenation, or for analytical purposes, such as reactions with halogens or mercuric compounds.

The relative stability of oxidation at 100°C has been given by DeMan (1992) as follows: saturated fatty acids 0.8, oleic acid 1.1, linoleic acid 13.7, and linolenic acid 25.5. Since fatty acids occur in food fats and oils as mixtures of mixed glycerides, the interactions between these different fatty acids must be taken into account. Raghuveer and Hammond (1967) studied the rate of autoxidation of mixtures of triunsaturated glycerides and tridecanoin and found that randomisation with sodium methoxide decreased the rate of autoxidation. They suggested that concentration of the unsaturated fatty acids in the 2-position of the glycerides stabilise fat towards autoxidation.

Autoxidation of unsaturated fatty acids involves attacks on the α-methylene group, resulting initially in a number of unsaturated hydroperoxides. Saturated fatty acid oxidation occurs at high temperatures (100-120°C) and is mainly β-oxidation. Stearic acid yields lower fatty acids with an even number of carbon atoms and in addition, small amounts of oxalic acid and methyl ketones with odd carbon numbers. The latter result from the decarboxylation of β-keto acids.

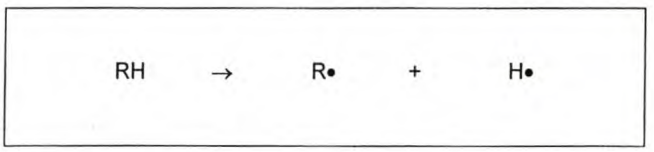
8.2 Autoxidation

The unsaturated bonds present in all fats and oils represent active sites that can react with oxygen. This reaction leads to the formation of primary, secondary, and tertiary oxidation products that may make the fat or fat-containing food unsuitable for consumption.

The process of autoxidation and the resulting deterioration in the flavour of fats and fatty foods are often described as *rancidity*. DeMan (1992) distinguishes several types of rancidity. Common oxidative rancidity can be seen in fats such as lard and is characterised by sweet but undesirable odour and flavour that progressively become more intense and unpleasant as the reaction progresses. *Flavour reversion* is the term used for the objectionable flavours that develop in oils containing linolenic acid such as soybean oil. This type of oxidation is produced with considerably less oxygen than common oxidation.

Among the factors that affect the rate of autoxidation are the amount of oxygen present, degree of unsaturation, presence of antioxidants, presence of pro-oxidants (especially copper and some organic compounds such as heme-containing molecules and lipoxidase), storage temperature, and exposure to light.

The autoxidation reaction can be divided into three stages: initiation, propagation, and termination. In the initiation part, hydrogen is subtracted from an olefinic compound to yield a free radical:



The removal of hydrogen takes place at the carbon atom next to the double bond and can be brought about by the action of, for example, light or metals. The dissociation energy of hydrogen in various olefinic compounds is listed in Table 8.1 (DeMan, 1992). Once a free radical has been formed, it will combine with oxygen to form a peroxy free radical, which can in turn abstract hydrogen from another unsaturated molecule to yield a peroxide and a new free radical, thus starting the propagation reaction. This reaction may be repeated up to several thousand times and has the nature of a chain reaction.

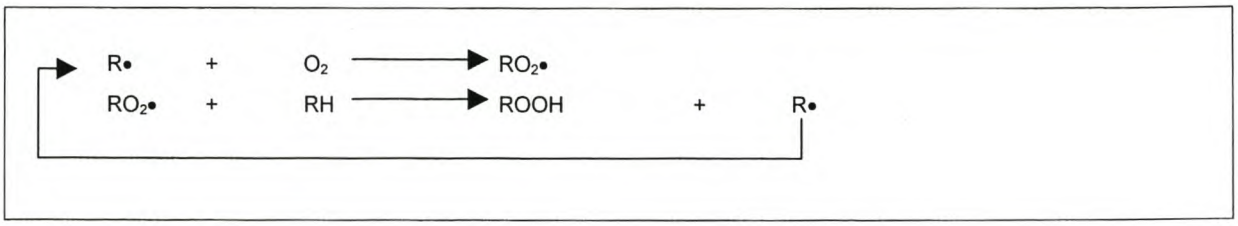
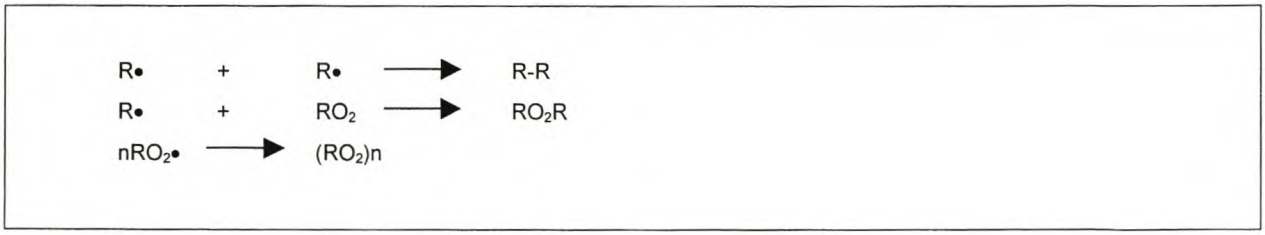


Table 8.1: Dissociation energy for the subtraction of hydrogen from olefinic compounds and peroxides.

Compound	**ΔE (kcal/mol)**
CH2=CH2	103
CH3-CH2-CH3	100
CH3-CH=CH2	85
CH3-CH2-CH=CH-CH2-	77
-CH=CH-CH2-CH=CH-	65
ROOH	90

The propagation can be followed by termination if the free radicals react with themselves to yield non active products, as shown here:



The hydroperoxides formed in the propagation part of the reaction are the primary oxidation products. These oxidation products comprise a variety of compounds including carbonyls, which are the most important. The peroxides have no importance to flavour deterioration, which is wholly caused by the secondary oxidation products. The nature of the process can be represented by the curves of Figure 8.1. In the initial stages of the reaction, the amount of hydroperoxides increases slowly; this stage is

termed the *induction period*. At the end of the induction period, there is a sudden increase in peroxide content. Because peroxides are easily determined in fats, the peroxide value is frequently used to measure the progress of oxidation. Organoleptic changes are more closely related to the secondary oxidation products, which can be measured by various means, including the benzidine value, which is related to aldehyde decomposition products. As the aldehydes are themselves oxidised, fatty acids are formed; these free fatty acids can be considered tertiary oxidation products. The length of the induction period, therefore, depends on the method used to determine oxidation products.

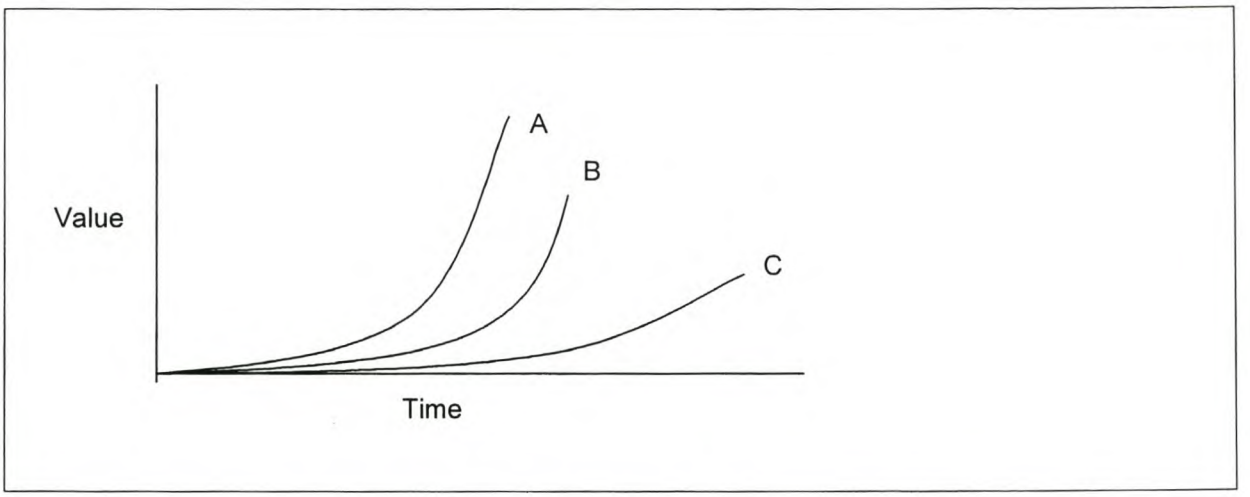
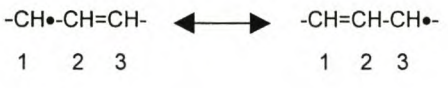
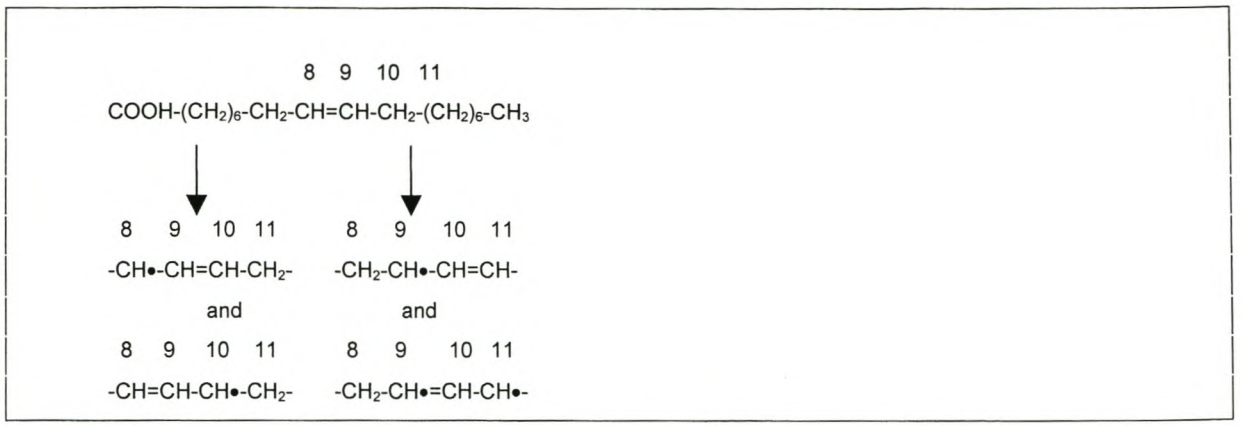


Figure 8.1: Autoxidation of lard. A, peroxide value; B, benzidine value; C, acid value.

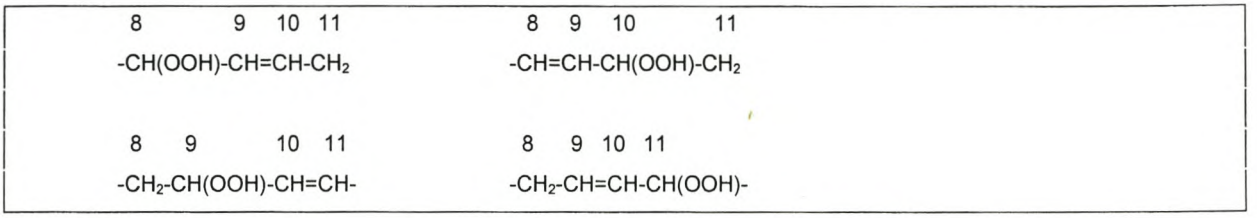
Although even saturated fatty acids can be oxidised, the rate of oxidation greatly depends on the degree of unsaturation. In the series of 18-carbon-atom fatty acids – 18:0, 18:1, 18:2, 18:3 – the relative rate of oxidation has been reported to be in the ratio of 1:100:1200:2500. The reaction of an unsaturated compound proceeds by the abstraction of hydrogen from the α-carbon, and the resulting free radical is stabilised by resonance as follows:



If oleic acid is taken as an example of a monoethenoid compound (*cis*-9-octadecenoic acid), the reaction will proceed by abstraction of hydrogen from carbons 8 or 11, resulting in two pairs of resonance hybrids.

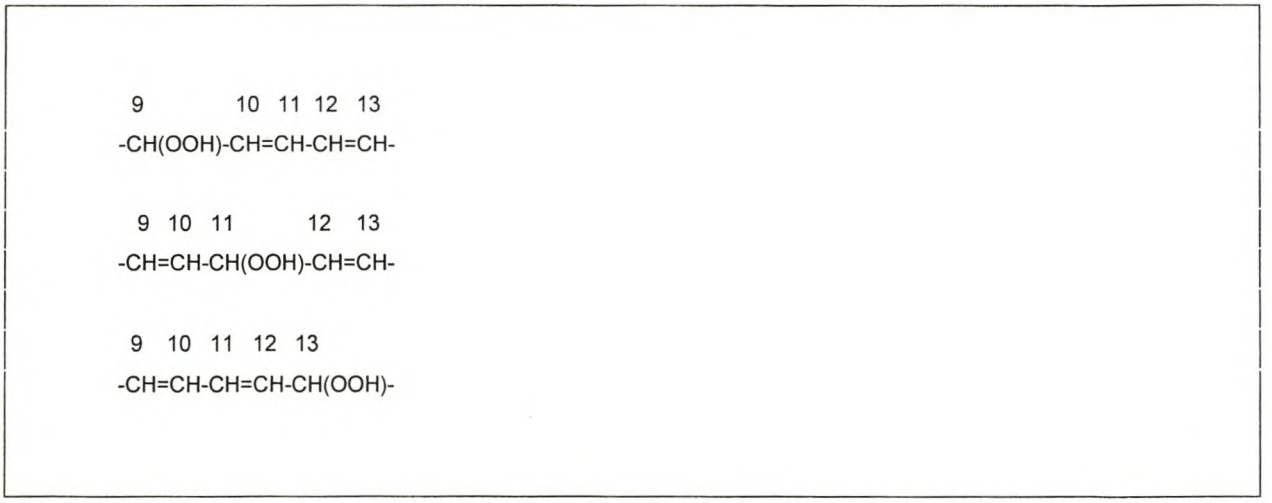


This leads to the formation of four isomeric hydroperoxides:



In addition to the changes in double-bond position, there is isomerization from cis to trans, and 90% of the peroxides formed may be in the trans configuration.

From linoleic acid (*cis-cis*-9,12-octadecadienoic acid), three isomeric hydroperoxides can be formed:



In this mixture of 9-, 11-, and 13- hydroperoxides, the conjugated ones occur in greatest quantity because they are the most stable forms. The hydroperoxides occur in the cis-trans and trans-trans configurations, the content of the latter being greater with higher temperature and greater extent of

oxidation. From the oxidation of linolenic acid (*cis-cis-cis*-9,12,15-octadecatrienoic acid), six isomeric hydroperoxides can be expected according to theory (DeMan, 1992):

9 10 11 12 13 14 15 16
-CH(OOH)-CH=CH-CH=CH(OOH)-CH_2-CH=CH-

11
-CH=CH-CH(OOH)-CH=CH-CH_2-CH=CH-

12
-CH=CH-CH_2-CH(OOH)-CH=CH-CH=CH-

13
-CH=CH-CH=CH-CH(OOH)-CH_2-CH=CH

14
-CH=CH-CH_2-CH=CH-CH(OOH)-CH=CH-

16
-CH=CH-CH_2-CH=CH-CH=CH-CH(OOH)-

Hydroperoxides of linolenate decompose more readily than those of oleate and linoleate because active methylene groups are present. The active methylene groups are the ones located between a single double bond and a conjugated diene group. The hydrogen at this methylene group could readily be abstracted to form dihydroperoxides. The possibilities here for decomposition products are obviously more abundant than with oleate oxidation.

The decomposition of hydroperoxides has been outlined by Keeney (1962). The first step involves decomposition to the alkoxy and hydroxy free radicals:

R-CH(OOH)-R → R-CO•H-R + •OH

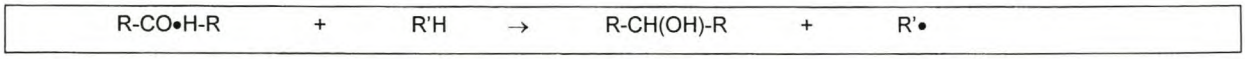
The alkoxy radical can react to form aldehydes:

R-CO•H-R → R• + RCHO

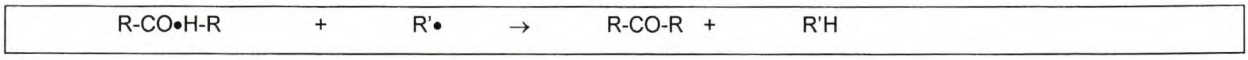
This reaction involves fission of the chain and can occur on either side of the free radical. The aldehyde that is formed can be a short-chain volatile compound, or it can be attached to the glyceride

part of the molecule; in the latter case the compound is non-volatile. The volatile aldehydes are predominantly responsible for the oxidised flavour of fats.

The alkoxy radical may also abstract a hydrogen atom from another molecule to yield an alcohol and a new free radical:



The new free radicals formed may participate in propagation of the chain reaction. Some of the free radicals may interact with themselves to terminate the chain, and this could lead to the formation of ketones as follows:



As indicated, a variety of aldehydes have been demonstrated in oxidised fats. Alcohols have also been identified, but the presence of ketones is not so certain. Keeney (1962) listed the aldehydes that may be formed from breakdown of hydroperoxides of oxidised oleic, linoleic, linolenic, and arachidonic acids (Table 8.2).

The aldehydes are powerful flavour compounds and have very low flavour thresholds; for example, 2,4-decadienal has a flavour threshold of less than 1 ppb. The presence of a double bond in an aldehyde generally lowers the flavour threshold considerably. The aldehydes can be further oxidised to carboxylic acids or other tertiary oxidation products.

When chain fission of the alkoxy radical occurs on the other side of the free radical group, the reaction will not yield volatile aldehydes but will instead form non-volatile aldehydoglycerides. Volatile oxidation products can be removed in the refining process during deodorization, but the non-volatile products remain, and this could result in lower oxidative stability of oils that were already oxidised before refining.

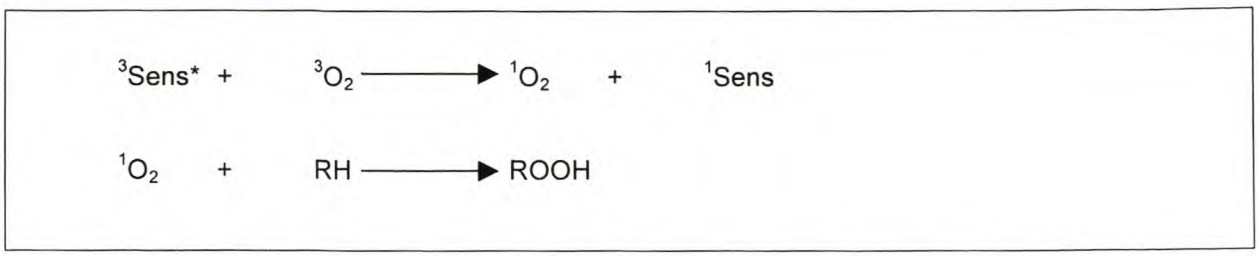
Table 8.2: Hydroperoxides and aldehydes (with single oxygen function) that may be formed in autoxidation of some unsaturated fatty acids (Keeney, 1962).

Fatty acid	Methylene group involved	Isomeric hydroperoxides formed from the structures contributing to the intermediate free radical resonance hybrid	Aldehydes formed by decomposition of the hydroperoxides
Oleic	11	11-Hydroperoxy-9-ene	Octanal
		9-Hydroperoxy-10-ene	2-Decenal
	8	8-Hydroperoxy-9-ene	2-Undecenal
		10-Hydroperoxy-8-ene	Nonanal
Linoleic	11	13-Hydroperoxy-9,11-diene	Hexanal
		11-Hydroperoxy-9,12-diene	2-Octenal
		9-Hydroperoxy-10,12-diene	2,4-Decadienal
Linolenic	14	16-Hydroperoxy-9,12,14-triene	Propanal
		14-Hydroperoxy-9,12,15-triene	2-Pentenal
		12-Hydroperoxy-9,13,15-triene	2,4-Heptadienal
	11	13-Hydroperoxy-9,11,15-triene	3-Hexenal
		11-Hydroperoxy-9,12,15-triene	2,5-Octadienal
		9-Hydroperoxy-10,12,15-triene	2,4,7-Decatrienal
Arachidonic	13	15-Hydroperoxy-5,8,11,13-tetraene	Hexanal
		13-Hydroperoxy-5,8,11,14-tetraene	2-Octenal
		11-Hydroperoxy-5,8,12,14-tetraene	2,4-Decadienal
	10	12-Hydroperoxy-5,8,10,14-tetraene	3-Nonenal
		10-Hydroperoxy-5,8,11,14-tetraene	2,5-Undecadienal
		8-Hydroperoxy-5,9,11,14-tetraene	2,4,7-Tridecatrienal
	7	9-Hydroperoxy-5,7,11,14-tetraene	3,6-Dodecadienal
		7-Hydroperoxy-5,8,11,14-tetraene	2,5,8-Tetradecatrienal
		5-Hydroperoxy-6,8,11,14-tetraene	2,4,7,10-Hexadecatetraenal

8.3 Photo-oxidation

Photo-oxidation is an oxidation process of unsaturated fatty acids resulting from exposure to light in the presence of oxygen and a sensitizer. Photo-oxidation involves singlet oxygen, an exited state of oxygen. Singlet oxygen, $^1O_2$, forms monohydroperoxides from unsaturated fatty acids by a mechanism that does not involve free radicals. Secondary oxidation products can then be formed by free-radical side reactions. Photosensitisers can be chlorophyll, heme compounds, dyes, and, in the case of milk products, riboflavin (Sattar and DeMan, 1975).

The singlet oxygen reaction involves energy transfer from a singlet sensitiser to ground state triplet oxygen ($^3O_2$), which is subsequently activated to the singlet state ($^1O_2$). The reaction of singlet oxygen with an acceptor then yields a hydroperoxide according to the following equations:



The sensitisers can accept the energy from light in the visible part of the spectrum.

Substances known as quenchers, that are able to deactivate the singlet oxygen, can inhibit this type of reaction. Examples of effective quenchers are β-carotene and α-tocopherol (DeMan, 1992).

8.4 Thermal oxidation

The fatty acids in food lipids are exposed to heat during processing and also during cooking, baking, frying, and other treatments of foods. Great care is taken to minimise thermal oxidation of fats and oils during processing, for example, by removing oxygen by vacuum processing. However, even the most careful techniques can result in some thermal decomposition. Breakdown products can be detected after heating at 180°C in vacuum for 1 hr by using very sensitive analytical methods. Commercial and household frying operations may result in greatly accelerated autoxidation reactions. The presence of water may result in hydrolysis and the release of free fatty acids. One of the most important changes is the formation of polymeric compounds. The polymerisation reaction may take place by conversion of part of the *cis-cis*-1,4-diene system of linoleates to the *trans-trans* conjugated 1,3-diene. The 1,4- and 1,3-dienes can combine in a Diels-Alder type addition reaction to produce a dimer. Larger structures can be formed by addition reactions with other glycerides. Polymer formation results in increased viscosity, which in turn may greatly increase foaming of the frying fat. This eventually limits the usefulness of the fat (DeMan, 1992).

8.5 Enzymatic oxidation

Enzymatic oxidation reactions are important in both animal and plant systems. In animal systems they involve mainly the oxidative transformation of arachidonic acid to prostaglandins, thromboxanes, and leukotrienes. These compounds are formed in all mammalian tissues and have a broad range of biological activities. The first product formed from arachidonic acid is prostaglandin G_2 (PGG_2), which contains hydroperoxide and cyclic peroxide functions. The enzyme involved in this reaction is cyclo-oxygenase. The 5-hydroperoxide reacts further to form leukotrienes. PGG_2 is the precursor of prostaglandins and thromboxanes. The 5-hydroperoxide and the 5,6-epoxide are precursors of the leukotrienes (Gunstone, 1986).

Enzymatic oxidation in plant systems is mediated by a widely occurring group of enzymes that use molecular oxygen to catalyse the oxidation of lipids containing a *cis-cis*-1,4-pentadiene group. This reaction leads to the formation of conjugated hydroperoxides. The best known of these enzymes is soy lipoxygenase (linoleate:oxygen oxidoreductase, E.C. 1.13.11.12). The reaction is highly specific for the cis-cis-methylene-interrupted group and forms the basis for an analytical method for measuring essential fatty acids. The reaction is shown in Figure 8.2.

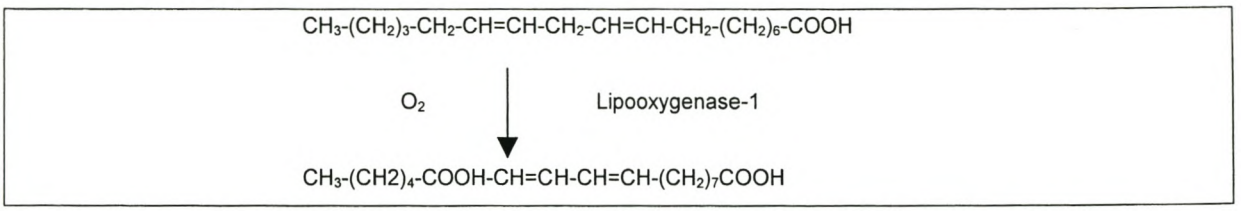


Figure 8.2: Lipoxygenase-catalysed reaction of cis-cis methylene-interrupted compounds.

9 Vitamin E and lipid oxidation

Meat is well recognised as a highly nutritious food, contributing quality protein, essential minerals and trace elements, and a range of B vitamins. In addition to its nutritive value, meat has other prestige values. The taste of meat is particularly attractive. The three sensory properties by which customers judge meat quality are appearance, texture, and flavour (Liu et al., 1995). The most important of these properties is the appearance because this strongly influences the initial decision of the customer to purchase or reject the product. One of the main factors which limits the acceptability of meat and meat products is the process of lipid oxidation. In raw meat, these result in the formation of brown pigments (especially in beef), increased drip losses and the development of unacceptable odours, while in cooked and stored meats it causes off-flavours, such as 'warmed-over' flavour. As well as having a negative impact on the acceptability of meats and meat products, lipid oxidation may also have food safety implications. Concerns have been expressed over the possible atherogenic effects of lipid oxidation products (e.g. malondialdehyde and cholesterol oxides) in the body (Addis and Warner, 1991). Preventing lipid oxidation during processing, storage and retail display is therefore essential in order to maintain the quality, wholesomeness and safety of meats, and to ensure that customers will make repeat purchases. However, the oxidative stability of meat is low and difficult for retailers to predict accurately (especially in the case of poultry or fish, which are rich in polyunsaturated fatty acids – PUFA), and oxidation often results in meat being discounted or even removed from retail display before the end of its normal bacteriological shelf-life.

Oxidative changes in muscle foods are generally quantified by the measurement of secondary degradation products. Results are expressed as Thiobarbituric Acid Reactive Substances (TBARS) numbers. It is accepted that TBARS numbers correlate well with sensory scores of oxidised and warmed-over flavour in muscle food. TBARS numbers greater than 1 correlate significantly with oxidised scores obtained by trained panellists for meats stored under frozen conditions (Buckley and Morrisey, 1992). The possibility that products of lipid oxidation may be toxic to humans is also a real threat to the 'healthy' image of meats.

It is well recognised that lipid oxidation in meat and meat products can be effectively controlled by antioxidants, and many of the studies to date have focused on the use of synthetic antioxidants. However, as today's consumers continue to become more health conscious, resistance to the use of synthetic antioxidants in foods has also increased. At the present time, there is considerable interest in the potential antioxidant activity of a range of naturally occurring substances, such as extracts from vegetables, fruits and grains, and spices and herbs. Vitamin E, especially when incorporated in the diet of animals, has proved to be an efficient antioxidant, and is acceptable to the consumer.

9.1 Chemistry of vitamin E

At least eight compounds have been isolated from a number of plant oils that have vitamin E activity. All have a 6-chromanol ring, but two chemically distinct series of compounds exist which differ from one another in the degree of side-chain saturation: tocols and trienols. The tocols possess a phytol side chain, and the trienols have a similar structure with double bonds at the 3', 7' and 11' positions. Isomeric forms of tocols and treinols exist which differ in the number and location of methyl groups on the chromanol ring.

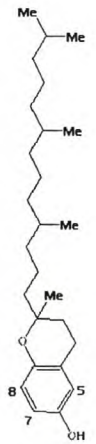


Figure 9.1: Basic structure of tocols.

The term tocopherols is used as the generic description for all mono-, di- and trimethyl tocols, and the term vitamin E is used as the generic term for all tocols and tocotrienol derivatives qualitatively exhibiting the biological activity of α-tocopherol. α-Tocopherol has been shown to be the most active form of vitamin E, and in addition accounts for almost all the vitamin E activity in living tissue.

9.2 Absorption and tissue distribution

The absorption of dietary tocopherols takes place principally through the lymphatic system, where they are transported within triglyceride-rich chylomicrons (Traber et al., 1988) and carried to the liver. Vitamin E is then re-secreted by the liver, incorporated into very-low-density lipoproteins (VLDL), and eventually transported into cells within low-density lipoproteins (LDL) following recognition and uptake from the plasma by specific LDL receptors (Traber and Kayden, 1984).

In addition to its presence in plasma, vitamin E is also found in erythrocytes and platelets, and is in fact widely distributed throughout the body. It is most concentrated in cell fractions rich in membranes, such as microsomes and mitochondria (Machlin, 1984).

9.3 Function of vitamin E

Dietary vitamin E deficiency is associated with the development of lesions in the reproductive, nervous and cardiovascular systems, and in skeletal muscle and liver. Vitamin E plays a role in the regulation of haem biosynthesis (Nair, 1972), acts as a modulator in the immune response (Bendich et al., 1986), and is also involved in gene regulation. However, the most important function of vitamin E is as an *in vivo* antioxidant that protects tissue lipids from free-radical attack (Tappel, 1972). Vitamin E is now well accepted as nature's most effective lipid-soluble, chain-breaking antioxidant protecting cell membranes from oxidative damage.

9.4 Membrane antioxidant hypothesis

Diplock and Lucy (1973) proposed that α-tocopherol is located within biological membranes, and that the system is stabilised by physico-chemical forces involving lipid-lipid interactions between α-tocopherol and polyunsaturated phospholipds.

The chromanol ring of α-tocopherol is located on the polar surface of the phospholipids, with the phytol side chain interacting with the unsaturated fatty acyl chain of the phospholipids (particularly those derived from arachidonic acid) through London-van der Waal's dispersion-attraction forces in the interior of the membrane. In this location, α-tocopherol protects the highly oxidisable polyunsaturated fatty acids from peroxidation with free radicals produced by the adjacent membrane-bound enzyme, NADPH oxidase (Diplock, 1985).

Although the membrane hypothesis has not been accepted by all investigators, many of the biochemical and pathological effects of vitamin E inadequacy may be explained by cell structure alterations caused by oxidative attack on membrane lipids (Machlin, 1984). The specific localisation of α-tocopherol in the membrane allows it to function very efficiently compared to other antioxidants. α-Tocopherol added to post-mortem muscle does not become an integral part of the membrane, and consequently is not as effective as α-tocopherol incorporated into the membranes by dietary means.

9.5 Generation of free radicals

The free-radical mechanism of lipid oxidation involves an initiation process, resulting in the removal of a proton from an unsaturated fatty acid. There is still considerable confusion as to the nature of the initiators or catalysts. In living systems, the normal utilisation of oxygen by cells results in the production of highly reactive free-radical products such as superoxide anions ($O_2^{\bullet -}$).

The sites of free-radical generation encompass all cellular constituents including mitochondria, lysosomes and plasma membranes. In normal biological systems, superoxide anions, which are not particularly reactive, are readily removed by the action of superoxide dismutase. The product of this reaction is hydrogen peroxide (H_2O_2), which is actually metabolised to the harmless end products of water and oxygen.

Neither $O_2^{\bullet-}$ nor H_2O_2 are very reactive. However, they may be transformed into highly reactive species in the presence of a suitable transition-metal catalyst such as the ferrous (Fe^{2+}) or cuprous (Cu^{2+}) cations (Machlin 1984).

The reactive species is most likely to be the hydroxyl radical ($OH^{\bullet}$). Prime targets for the hydroxyl radical are the unsaturated double bonds in membrane lipids. The breaking of these double bonds results in lipid damage and the production of lipid hydroperoxides. The latter can decompose to yield a variety of alkoxy and peroxy radicals, saturated and unsaturated aldehydes, ketones and alkenes. The effects of lipid oxidation in living systems are to some extent reversible, but evidence is now emerging that oxidation of lipids may have long-term consequences for health in humans and animals (Halliwell and Gutteridge, 1985).

9.6 Antioxidant defence system

Fortunately, living organisms have an array of protective mechanisms both for preventing formation of oxidants and lipid peroxides and for repairing oxidative damage.

The antioxidant substances are of two types:

1. preventive antioxidants, which form a well-developed and essentially endogenously-controlled defence system, and
2. the chain-breaking antioxidants, which include vitamins E and C (Table 7.1).

In health tissue, the preventive antioxidants include the enzymes superoxide dismutase, glutathione peroxidase, glutathione reductase and possibly catalase.

Table 7.1: Antioxidants in living organisms (Thurnham, 1990).

Preventive antioxidants	Superoxide dismutase Glutathione peroxidase Catalase
Chain-breaking antioxidants	**α-Tocopherol** Ascorbate β-Carotene

The selenium-containing glutathione peroxidase destroys hydrogen peroxide and peroxides generated in the aqueous phase by dismutation of the superoxide ion by superoxide dismutase.

Catalase hydrolyses hydrogen peroxide, thus preventing its accumulation in the biological system. On the other hand, α-tocopherol, which constitutes the second line of defence in biological systems, is the major lipid-soluble antioxidant, breaking the chain of lipid peroxidation in cell membranes and preventing the formation of lipid hydroperoxides (Halliwell, 1987). It can also scavenge the superoxide ion, the hydoxyl radical and other free radicals generated during the reaction of hydrogen peroxide with metmyoglobin.

9.7 Lipid oxidation in muscle systems

The following points were also discussed in Chapter 6, but due to the investigations following, it is necessary to revise and emphasize the role vitamin E plays in preventing lipid oxidation.

Fat or lipid oxidation is one of the primary causes of loss of quality in meat and meat products during storage. Oxidative rancidity begins shortly after death, and involves the formation of a complex mixture of aldehydes, ketones, alcohols etc. from the breakdown of lipid hydroperoxides (Reindl and Stan, 1982). The flavour and odour of this mixture is unacceptable to consumers. Oxidation of unsaturated fatty acids also adversely affect the colour, texture, nutritive value and safety of meats (Addis, 1986).

Cooked muscle systems are particularly susceptible to lipid oxidation, and its control is a major challenge to meat processors and food retailers, since the demand for 'ready-to-eat, ready to heat' meals is predicted to increase steadily over the next decade.

The term 'warmed-over flavour' was first introduced by Tims and Watts (1958) to describe the form of oxidative rancidity which develops rapidly in cooked meats during storage. Warmed-over flavour (WOF) differs from the normal rancidity encountered in raw meats, fatty tissues, rendered fat or lard, in that it is not apparent until the systems have been stored. Most of the work on WOF has been done on red meats, but the problem occurs equally, if not more seriously, in white meat such as poultry (Igene and Pearson, 1979) and fish (Tichivangana and Morrisey, 1985).

The rate and extent of lipid oxidation are dependent on a number of factors, the most important being the level of polyunsaturates present in the particular muscle system (Allen and Foegeding, 1981). Earlier studies concluded that triacylglycerols and phospholipids are important in the development of rancidity in chicken (Igene et al., 1980) and fish (Tichivangana and Morrissey, 1982).

However it is now generally accepted that the phospholipids present in the subcellular membranes (microsomes, mitochondria), rather than triacylglycerols, are responsible for the initial development of oxidised flavours in raw and cooked meat products during storage. The phospholipid fraction is highly unsaturated, and contains fatty acids with more than two double bonds (Gray and Pearson, 1987). It is therefore not surprising that the phospholipid fraction contributes approximately 90% of the thiobarbituric-acid-reactive substances (TBARS) in chicken fat (Pikul et al., 1984). Disruption of the

integrity of the muscle membrane by mincing or restructuring alters the compartmentalised cellular system, and facilitates the formation of free radicals for propagation of the oxidative reaction (Buckley and Morrissey, 1992). This provides further evidence that the intracellular phospholipid fraction is the primary lipid component involved in oxidative changes.

Lipid oxidation in meats is catalysed by myoglobin, haemoglobin, cytochromes, non-haem iron and other heavy transition metals (Tichivangana and Morrissey, 1985), and by sodium chloride added during processing (Rhee et al., 1983). Lipid oxidation is inhibited by nitrites, chelating agents such as phosphates, EDTA and citrates (Sato and Hegarty, 1971) and synthetic phenolic antioxidants (Chastain et al., 1982).

Considerable interest has been expressed in the use of antioxidants incorporated in the diet and their effects on meat quality (Gray and Pearson, 1984). Dietary supplementation with vitamin E has been shown to improve the oxidative stability of chicken meat (Brekke et al., 1975), turkey meat (Webb et al., 1972), pork (Buckley and Connolly, 1980), beef (Faustman et al., 1989), veal (Shorland et al., 1981).

Ascorbic acid, is addition to scavenging free radicals directly in the cytoplasm, may also contribute to the regeneration of α-tocopherol and the antioxidant peptide glutathione (Wilson, 1987). The α-tocopherol may enter into a coupled sequence of reactions with ascorbic acid, glutathione and NADPH to regenerate α-tocopherol (Machlin, 1984). β-Carotene has also been recently suggested to have an antioxidant function (Burton, 1989).

Peptides such as carnosine, homocarnosine and anserine may also be important antioxidants in muscle (Kohen et al., 1988). There are several nutritionally essential minerals incorporated into protective antioxidant enzymes. Zinc, copper and manganese are required for the activity of the two types of superoxide dismutase, and selenium is an essential component of glutathione peroxidase.

9.8 Other functions of vitamin E

There is now considerable interest in the possible toxicological effects of lipid oxidation products in relation to a variety of pathological processes, including inflammation and rheumatiod arthritis (Halliwell and Gutteridge, 1985), artherosclerosis (Quinn et al., 1987), mutagenesis and carsinogenesis. Recent analysis of epidemiological evidence by Gey et al. (1991) suggests that low plasma levels of vitamin E and C and β-carotene are associated with an increased risk of heart disease. Overall, these researchers concluded that vitamin E is the most important factor explaining cross-cultural differences in ischaemic heart disease.

10 Fatty acid nomenclature

Table 10: The common or systematic names of fatty acids and their associated shorthand notations.

Common or systematic name	Shorthand notation
Myristic acid	C14:0
Palmitic acid	C16:0
Palmitoleic acid	C16:1n7
Heptadecanoic acid	C17:0
Heptadecenoic acid	C17:1n7
Stearic acid	C18:0
Oleic acid	C18:1n9
Vaccenic acid	C18:1n7
Linoleic acid	C18:2n6
γ-Linolenic acid	C18:3n6
α-Linolenic acid	C18:3n3
Eicosanoic acid	C20:0
Octadecatetraenoic acid	C18:4n3
Eicosadienoic acid	C20:2n6
Eicosenoic acid	C20:1n9
Dihomo-γ-linolenic acid	C20:3n6
Eicosatrienoic acid	C20:3n9
Arachidonic acid	C20:4n6
Eicosapentaenoic acid	C20:5n3, EPA
Erucic acid	C22:1n9
Docosatrienoic acid	C22:3n9
Docosatetraenoic acid	C22:4n6
Docosapentaenoic acid (n-6)	C22:5n6
Docosapentaenoic acid	C22:5n3, DPA
Docosahexaenoic acid	C22:5n3, DHA

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Part 2

Metabolisable energy of Canola acid oil and Famarol acid oil for broiler chickens

Metabolisable energy of Canola acid oil and Famarol acid oil for broiler chickens

Abstract In trials with 21-day-old male broilers the true metabolisable energy value, corrected for nitrogen retention (TMEn) was determined by the balance method for Canola acid oil (CAO) and Famarol acid oil (FAO). The trials were duplicated, each time using different samples of the two oils from the same source (experiment 1 and 2). Each of the two oils were blended in two ratios with a basal diet to form the test diets, viz. 100% Basal; 96% Basal: 4% Oil; 92% Basal: 8% Oil. In experiment 3, 50 % bran was added to the maize to form the basal diet. The balance trials lasted for 3 days after an adaptation period of 4 days. The TMEn values determined by regression for the broilers of CAO did not differ significantly ($P>0.05$) between experiments 1 and 2. However, the value for experiment 3 was significantly ($P<0.05$) higher than those for experiments 1 and 2. The TMEn values of FAO also did not differ significantly ($P>0.05$) between experiments 1 and 2, although the value for experiment 3 was significantly higher than that of experiment 1. The addition of 50 % bran to the basal diet in experiment 3 could have stimulated the digestive breakdown process and hence increase the secretion of digestive enzymes. This could lead to an increase in the utilisation of the test lipid and therefore an increase in the TMEn value. The TMEn values of CAO differed significantly ($P<0.05$) from those of FAO for all three the experiments (exp. 1:30.6 ± 0.399 MJ/kg for CAO vs. 25.9 ± 0.441 MJ/kg for FAO; exp. 2: 31.0 ± 0.633 MJ/kg for CAO vs. 26.1 ± 0.668 MJ/kg for FAO: exp. 3: 32.1 ± 0.867 MJ/kg for CAO vs. 27.1 MJ/kg for FAO).

Introduction

Fats and oils are now commonly added to poultry diets as an economic means of producing energy-rich formulations. In some feeds, added fats can contribute 20% of overall dietary energy. The increasing use of fats in poultry diets of high nutrient density has made their evaluation of particular relevance to the efficiency of least cost diet formulation.

Although oils and fats are particularly important energy-yielding ingredients in diets for poultry, they are however of variable nutritive value due to their chemical composition (Wiseman et al., 1992). The degree of saturation of the constituent fatty acids, their chain length and the proportion of free fatty acids present within a blend all have a pronounced effect upon the dietary energy value of fats and oils (Wiseman et al., 1991).

Heat treatment employed during processing and refining, and processing conditions that may favour oxidation are known to have undesirable effects upon the subsequent nutritive value of fats and oils. Molecular changes taking place during processing are also detrimental (Wiseman et al, 1992). These range from 'oxidised fats' produced at comparatively low temperatures (Frankel et al., 1977) through to dimeric and polymeric structures at higher temperatures (Ohfuji and Kaneda, 1973). Many of the fats and oils used in animal feeds are by-products derived from

refining crude oils for use in human foodstuffs. These by-products contain compounds such as free fatty acids that are prone to oxidation. Iwaoka and Perkins (1978) found that the presence of compounds arising from heat processing of fats and oils had an adverse biological effect on the animal.

Acid oils are one of the by-products of the chemical refining of crude oils. During the process, the crude oil is first treated with water to remove gums and other soluble components. The free fatty acids are then removed by the addition of a weak alkaline solution that converts to water soluble soaps. The resultant soap-stock is then treated with an acid to convert the soap back to the free fatty acids. Acid oils have a high free fatty acid content (Wiseman et al., 1992) and contain other impurities such as water, which could lead to depression of feed efficiency by 4% to 6% (Lipstein and Bornstien, 1968). In a comparison of sunflower acid oil and sunflower oil Wiseman et al. (1992) showed that the acid oil had a much lower apparent metabolisable energy (AME) value than the refined oil (30.6 MJ/kg versus 37.5 MJ/kg).

It was the objective of the current study to determine the metabolisable energy value of Canola acid oil and a commercial feed acid oil (Famarol).

Materials and methods

Oil samples

Three trials were conducted to estimate the metabolisable energy values of Canola acid oil (CAO) and Famarol acid oil (FAO). Both the CAO and FAO were obtained from commercial oil refineries in Cape Town. The CAO was imported from Canada, as there was no South African CAO commercially available at the start of the trials. The FAO is a commercial feed oil used by the South African poultry industry. It is a mixture of different vegetable and marine oils. In each trial new samples of oil were taken from the refineries.

Oil samples were analysed for fatty acid composition by gas chromatography according to standard procedures as described by Hoffman et al. (2000). The fatty acid profiles of the oils are summarised in Table 1. The calculation of the ratio of unsaturated to saturated fatty acids (U: S) using conventional procedures ([all unsaturated fatty acids] : [all saturated fatty acids]) indicated that CAO contained a higher percentage of unsaturated fatty acids than FAO oil.

Table 1: Fatty acid profiles of canola oil and famarol oil (% recoverable fatty acids).

Fatty acids	Famarol oil	Canola oil
C12:0	0.29	-
C14:0	0.46	0.07
C16:0	13.72	5.34
C18:0	4.43	2.40
C20:0	0.75	0.35
C22:0	0.65	-
Total saturated fatty acids	**20.3**	**8.16**
C18:1n9	38.72	58.82
C18:2n6	31.70	21.08
C18:3n3	8.08	10.81
C20:1n9	0.81	0.41
Total unsaturated fatty acids	**79.31**	**91.12**
U:S	**3.9**	**11.2**
Unknowns	0.39	0.72

Diets and treatments

In experiments 1 and 2 maize was used as the basal diet and for experiment 3 the basal diet consisted of a 50:50 mixture of maize and bran. Each of the two oils were blended with the basal diet to form the test diets, viz. 100% Basal; 96% Basal: 4% Oil; 92% Basal: 8% Oil. To ensure homogenous samples, the oils were mildly heated before being mixed into the basal diet. There were thus 5 dietary treatments in each experiment – 4 diets containing the two oil samples at different inclusion levels plus the basal diet fed as a control. Each of the diets was fed at two intake levels, namely *ad libitum* intake and 40% of *ad libitum* intake (Du Preez et al., 1986).

Birds and management

For each of the three experiments one hundred and twenty 21-day-old male broiler chickens were allocated to 40 wire cages (3 broilers per cage) in an environmentally controlled room where an artificial lighting pattern of 23¾ hours of light alternating with ¼ hour of darkness was maintained, approximating a commercial lighting cycle. Room temperature was controlled by electrical fans for the duration of the experiment. Each cage was equipped with a feed trough designed to limit wastage, a suspended automatic waterer, and a metal tray to collect the faeces.

Trial procedure

The experimental diets were fed for an acclimatisation period of 4 days, followed by a collection period of 3 days during which feed intake and excreta production was recorded. Excreta was dried in a forced-draught oven at 80°C to constant weight, then allowed to reach equilibration with atmospheric moisture for 24h. Excreta samples were ground through a laboratory mill fitted with a 1mm screen and stored in airtight containers until analysis. Gross energies (GE) of the finely ground excreta and experimental diets were determined in a solid state bomb calorimeter (Model

CP 500, Digital Data Systems, PO Box 35872, Northcliff, Johannesburg). Table 2 lists the gross energies for the basal diet and two oils for experiment 1, 2 and 3. Nitrogen (N) was measured according to the macro-Kjeldahl procedure (AOAC, 1995).

Table 2: Laboratory analyses of basal diet and oil samples for gross energy.

Experiment	GE (MJ/kg)		
	Basal diet	**Canola oil**	**Famarol oil**
1	16.358 (100% maize)	39.174	39.518
2	16.270 (100% maize)	39.108	39.226
3	16.892 (50:50 maize:bran)	39.648	39.482

Calculation of TMEn values

The nitrogen corrected gross energy (GE) excreta were regressed on the gross energy intake on each of the different components of the diet in the following model:

$$GE(excreta) = a + b_1GE(basal) + b_2GE(canola\ oil) + b_3GE(famarol\ oil) \pm Standard\ error.$$

Simple linear models were used to describe the metabolisability of the oil samples. The Dual Semi-quick (DSQ) method of Du Preez et al. (1986) allowing different levels of feed intake was used to calculate TMEn values. This was done by relating the nitrogen corrected excreted energy to the energy consumed from either the oil or the basal diet by means of a multiple regression analysis. The complement of the regression coefficients multiplied with the gross energy value of the oil gave the TMEn. The central assumption made in all assays for TMEn is that energy voided as excreta is linearly related to the energy input (McNab, 1990). The following multiple regression model was used to relate nitrogen-corrected excreted energy to partitioned energy intake from the basal diet or the individual oils:

Results

The results for the regression equations obtained in the three experiments are presented in Table 3. The TMEn-values for the different oils were calculated by multiplying the complement of the slopes of the lines obtained for each of the oils in the different experiments with their respective gross energy values (McNab, 1990):

$$TMEn = (1-b) \times GE$$

Table 3: Regression equations obtained in experiments 1, 2 and 3 relating nitrogen-corrected energy excreted to energy intake for broilers consuming a basal diet and two oils mixed in various ratios.

Exp.	a		b_1 (Basal)		b_2 (Canola)		b_3 (Famarol)	R-sq	Std dev (Basal)	Std dev (Canola)	Std dev (Famarol)
1	-1023*	+	0.169	+	0.219	+	0.345	95.6	0.05004	0.01019	0.01115
2	-1077*	+	0.164	+	0.207	+	0.335	97.6	0.03951	0.01619	0.01702
3	-288*	+	0.370	+	0.190	+	0.314	98.3	0.01184	0.02188	0.02065

*Intercepts not significantly different from zero (P>0.05).

The resultant TMEn (MJ/kg) values of the basal diet and oils for experiment 1, 2 and 3 are given in Table 4.

Table 4: The TMEn (MJ/kg) of the basal diet and the two oils.

Experiment	Basal diet	Canola oil	Famarol oil
1	$13.6^{a} \pm 0.804$	$30.6^{c} \pm 0.374$	$25.9^{e} \pm 0.454$
2	$13.6^{a} \pm 0.635$	$31.0^{c} \pm 0.653$	$26.1^{ef} \pm 0.663$
3	$10.6^{b} \pm 0.216$	$32.1^{d} \pm 0.862$	$27.1^{f} \pm 0.825$

Values with different superscripts are significantly different (P<0.05).

Discussion

There has been relatively little work carried out on the nature of so called non-saponifiables in fats. Impurities are most often referred to collectively as M.I.U. (moisture, impurities and un-saponifiables). Acid oils contain most of these compounds which will have little nutritional value, hence the lower ME values obtained for acid oils compared to refined oils (Wiseman et al., 1992).

As mentioned, conditions such as heating and oxidation procedures employed during the processing of acid oils are likely to have a pronounced effect upon the chemical structures and therefore the metabolisable energy values of the oils. Table 4 indicates that the TMEn values of CAO obtained in experiments 1 and 2 did not differ significantly, and neither did the TMEn values of FAO obtained in experiment 1 and 2 differ significantly. In the case of CAO, experiment 3 had a significantly ($P<0.05$) higher value than those of experiments 1 and 2, and in the case of FAO the value of experiment 3 was significantly higher than that of experiment 1. This result could be explained by the 'extra caloric effect' of lipids (Golian and Polin, 1984). When calculating the energy value of the lipid under experimental conditions, it is assumed that the differences in dietary energy values are due solely to the test lipid, since other dietary factors are constant. However, if the basal nutrients affect the digestibility of the lipid, then there could be a corresponding increase in the energy value of the lipid as a result of this enhanced energy absorption in the diet. The addition of 50% bran to the basal diet in experiment 3 could have stimulated the digestive breakdown process and hence increase the secretion of digestive enzymes. This could lead to an increase in the utilisation of the test lipid and therefore an increase in the ME value. There was also a significant difference ($P<0.05$) between the TMEn values of the basal diets for the experiments. This is expected as the contribution of the 50% bran (ME of bran = 7.8 MJ/kg) in experiment 3 will result in a lower TMEn value for the basal diet in experiment 3.

Both the Canola and Famarol oils are acid oils, therefore they are products of the oil industry produced during the refining of crude oils and will contain free fatty acids, impurities and denatured fatty matter together with a quantity of the original crude oil. Further denaturation of the oil will occur during the process of saponification, acidulation and recovery. Thus denatured fatty acids in the acid oil will be derived partly from the original crude oil and from products denatured during processing (Wiseman et al., 1992).

Wiseman (1995) reported the ratio of unsaturated to saturated fatty acids as the major determinant of the AME of lipids. Long-chain saturated fatty acids are considered to be poorly absorbed in comparison with long-chain unsaturated fatty acids (Renner and Hill, 1961). The TMEn values of CAO and FAO differed significantly ($P<0.05$) for all three experiments (Table 4). The lower TMEn value observed for FAO (a marine based oil) in comparison with the more unsaturated CAO is in agreement with previous research, showing the superiority of vegetable oils over marine oils as an energy source for broilers (Renner and Hill, 1960; Vila and Esteve-Garcia, 1996; Wiseman et al., 1986). This improvement is therefore probably a reflection of the higher percentage of long-chain unsaturated fatty acids in CAO compared to FAO (Table 1). Lipid digestion and absorption in poultry require the formation of bile salt micelles before the absorption

of the final products of lipolysis (2-monoglycerides and free fatty acids) can take place (Freeman, 1984). Long-chain polyunsaturated fatty acids (polar solutes) are absorbed more readily than long-chain saturated fatty acids (non-polar solutes) because of the differential solubility of the products in the bile salt solution (Wiseman et al., 1986).

It was found in this experiment that CAO has a higher TMEn value than FAO and yields more energy available for the broiler chicken per kg of feed. CAO would thus be a more "nutritious" substitute for FAO in commercial broiler diets.

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Part 3

Effects of various n-6/n-3 fatty acid ratios on the performance and body composition of broilers

Effects of various dietary n-6/n-3 fatty acid ratios on the performance and body composition of broilers.

Abstract The dietary effects of various combinations of Canola acid oil (CAO, a high level of C18:3n-3 and MUFA) and Famarol acid oil (FAO, a high level of 18:2n-6 and SFA) on tissue fatty acid composition were studied in broiler carcasses and abdominal fat pads. From day-old to six weeks, chicks were fed one of six diets containing 100% FAO, 80% FAO-20% CAO, 60% FAO-40% CAO, 40% FAO-60% CAO, 20% FAO-80% CAO, 100% CAO. There were no statistical differences ($P>0.05$) in average daily gain (1.71 ± 0.059 g) or feed conversion ratios (1.97 ± 0.051) among dietary groups. No statistical differences ($P>0.05$) were found in the chemical proximate composition of the carcasses for the moisture (66.20 ± 0.112 %), protein (17.63 ± 0.484 %), lipid (15.92 ± 1.507 %) and ash (0.95 ± 0.115 %) content among dietary groups. No statistical differences ($P>0.05$) were found in the chemical proximate composition of the abdominal fat pads for the moisture (28.77 ± 0.112 %), protein (3.03 ± 0.484 %), lipid (63.32 ± 9.789 %) and ash (0.45 ± 0.135 %) content among dietary groups. With the increase in dietary CAO levels, the percentages of C18:2n-6 and C20:4n-6 in the carcasses decreased respectively with 1.78 % from 20.88 % and 0.35 % from 1.05 %, whilst C18:3n-3 and longer chain n-3 fatty acids such as C20:5n-3 and C22:6n-3 increased respectively with 2.25 % from 1 %, 0.1 % from 0.1 % and 0.67 % from 0.2 %. The same tendency was seen in the abdominal fat pads where C18:2n-6 and C20:4n-6 decreased respectively with 1.55 % from 20.75 % and 0.98 % from 1.2 % with an increase in dietary CAO, whilst C18:3n-3, C20:5n-3 and C22:6n-3 increased respectively with 2.13 % from 1.15 %, 0.45 % from 0.03 % and 0.95 % from 0.05 %. The n-3/n-6 ratio in the carcasses and abdominal fat pads increased respectively with 0.16 % from 0.06 % and 0.19 % from 0.06 % with an increase in dietary CAO. These results clearly indicate that dietary CAO enriched with α-linolenic acid lower saturated fatty acids respectively in broiler carcasses and abdominal fat pads with 4.88 % and 10.63%, whilst increasing monounsaturated fatty acids with 3.87 % and 7.25 % respectively and polyunsaturated fatty acids with 1.02 % and 2.38 % respectively.

Introduction

One of the potentially most important sections of the branch of animal science dealing with animal nutrition is the study of the effect of diet on tissue fatty acid composition. This information is mainly of value because an imbalance in the human dietary intake of various types of fatty acids has become apparent (Simopoulus, 1991). There is much interest in the relative merits of monounsaturated, n-6 polyunsaturated and n-3 polyunsaturated fatty acids (PUFA) in the human diet and the role they play in the lessening of cardiovascular related diseases (Wiseman, 1997). Several sources of information suggest that man evolved on a diet with a ratio of n-6 to n-3 fatty acids of ~1 whereas today this ratio is ~10:1 to 20-25:1, indicating that Western diets are deficient in n-3 fatty acids compared with the diet on which humans evolved and their genetic parameters were established. It is thus important for human health to increase the consumption of n-3 fatty acids.

Dietary fatty acids are absorbed by monogastric animals and deposited in tissues without significant modification. There is therefore considerable potential for the manipulation of the fatty acid profiles of poultry tissue by dietary means so as to increase the supply of n-3 PUFA suitable for human consumption. Ajuyah and co-workers (1991) showed that the use of linseed oil or whole linseed in poultry diets resulted in tissue enrichment of n-3 PUFA (C20:5n-3 and C22:6n-3) derived from C18:3n-3 by desaturation and elongation. Hulan and co-workers (1989) indicated that marine oils and fishmeal containing residual lipid increased the C20 and C22 n-3 PUFA concentration of poultry tissue.

In the present study, various levels of two commercial acid oil blends, Canola and Famarol, were fed to broiler chickens and the influence of the diets on their performance and body composition was examined.

Materials and methods

Birds

At the start of the experimental period 216 one-day-old, vent sexed male and female broilers were reared to 42 days of age in an environmentally controlled rearing house. A 24-hour light period was followed. The starting temperature at day-old was 33°C and was gradually decreased to 21°C at 21 days of age. A standard commercial health and inoculation program was followed. Six birds per cage were allocated to 36 cages, 18 cages containing males and 18 females. All the birds in each cage were allocated to one of six dietary treatments. A randomised block design was used.

Diet composition

The nutrient composition of the experimental diets is given in Table 1. The basic formulation of the experimental diets (Table 2) contained supplemental ratios of Famarol acid oil (FAO): Canola acid oil (CAO) namely 100FAO: 0CAO, 80FAO: 20CAO, 60FAO: 40CAO, 40FAO: 60CAO, 20FAO: 80CAO and 0FAO: 100CAO. Both the Famarol and Canola oil are acid oils. Acid oils are a by-product of the chemical refining of crude oils. During the process, the crude oil is first treated with water to remove gums and other water-soluble components. The free fatty acids are then removed by the addition of a weak alkali solution converting it to water soluble soap. The resultant soap-stock is then treated with acid to convert the soap back to free fatty acids. Acid oils may derive from a single source, such as the CAO, or it may be derived from a mixture of acid oils. FAO is mainly a mixture of different vegetable and marine oils. Both oils were obtained from commercial oil refineries in Cape Town.

Table 1: Nutrient composition of experimental diets for broilers.

Nutrient	Percentage of diet
Metabolisable Energy (MJ/kg)	13.20
Protein	22.89
Arginine	1.45
Isoleucine	0.98
Leucine	2.38
Lysine	1.15
Methionine	0.57
Methionine + Cystine	0.95
Threonine	0.77
Tryptophan	0.23
Calcium	0.89
Sodium	0.16
Phosphor	0.40
Water	9.20

Bird weights per cage and food consumption per cage were recorded weekly. Average daily gain (ADG) and feed conversion ratios (FCR) were calculated with the following equations:

$$ADG = \frac{\text{Body weight gain}}{\text{Number of days}}$$

where

$$\text{Body weight gain} = \text{Live weight} - \text{Day-old weight}$$

$$FCR = \frac{\text{Total feed intake}}{\text{Total weight of birds}}$$

Table 2: The formulation of the basal diet for broilers.

Ingredient	Percentage of diet
Gluten	8.2
Lime	1.3
Coccidiostat	0.2
Lysine	0.5
Maize	51.2
Monocalciumphosphate	1.3
Methionine	0.2
Soybean meal	17.6
Salt	0.4
Sunflower meal	10.9
Vitamin/Mineral Mix[1]	0.4
Oil mixture	8.0

[1]Vitamin/Mineral mix: Vitamin A (10 000 000 IU), Vitamin D (2 000 000 IU), Vitamin E (20 000 IU), Vitamin K (2 g), Vitamin B1 (2 g), Vitamin B2 (5 g), Vitamin B6 (3 g), Vitamin B12 (0.01 g), Niacin (20 g), Calpan DL (10 g), Folic acid (0.5 g), Biotin (0.02 g), Antioxidant (125 g), Choline (300 g), Cobalt (0.5 g), Copper (6 g), Iron (20 g), Manganese (70 g), Iodine (1 g), Selenium (0.15 g), Zinc (50 g).

Chemical analysis

At 42 days of age, six birds from each cage were slaughtered. The weight of the plucked birds was recorded and the abdominal fat pad removed and weighed. The six carcasses and abdominal fat pads from each cage were minced and pooled and stored at -20°C for chemical analysis.

Proximate analysis was done on the carcass tissue, abdominal fat pads and feed of each cage. The samples were analysed for moisture content (105°C, 24h), nitrogen (protein = N x 6.25) and ash (A.O.A.C., 1995). The samples were also analysed for total lipid (using chloroform / methanol 2:1 as solvent) according to the method of Lee et al. (1996).

The lipids in the tissue, abdominal fat pads and feed were analysed for fatty acid content. Fatty acid methyl esters (FAME) were prepared according to Morrison and Smith (1964). The FAME were analysed with a GLC: Varian Model 3300, equipped with flame ionisation detection and two 30 m fused silica megabore DB-225 columns of 0.53 mm internal diameter (J&W Scientific, Folsom, CA). Gas flow rates were: hydrogen, 25 ml/min; air, 250 ml/min; and nitrogen (carrier gas), 5-8 ml/min. Temperature programming was linear at 4°C/min; initial temperature, 160°C; final temperature, 220°C held for 10 min; injector temperature, 240°C; and detector temperature, 250°C. The FAME were identified by comparison of the retention times to those of a standard FAME mixture (Nu-Chek-Prep Inc., Elysian, Minnesota).

Statistical analysis

A linear regression model was fitted to the data with diet (lipid variations) as predictor. Analyses of variance were performed on all the variables measured using the General Linear Models (GLM) procedure of SAS (Statistical Analysis System, 1985). The following model was fitted for main effects (diet, sex) and the interaction between them:

$$Y_{ij} = \mu + D_i + S_j + DS_{ij} + e_{ij}$$

where Y_{ij} is the dependent variable, μ = the overall mean, D_i = the i^{th} diet effect, S_j = the j^{th} sex effect, DS_{ij} = the interaction between diet and sex and e_{ij} = the residual error. The differences between diets and sexes were tested separately by means of the null hypothesis (H_0), with H_0:$\mu = \mu_0$ and the alternate hypothesis (H_a) being H_a:$\mu \neq \mu_0$. Mean and standard errors were calculated for each diet and sex and a matrix of excedence probabilities were calculated to test for differences with Student's t test. Differences between the variables were accepted as being significant if the probability of rejection of H_0 was less than 5% ($P<0.05$) for diets and sex.

Results

The average daily gain (ADG) and feed conversion ratio (FCR) of broilers fed the different experimental diets are shown in Table 3. There were no significant differences in ADG or FCR among the dietary and sex groups (P>0.05). No significant statistical interaction was found between diet and sex for ADG and FCR.

Table 3: Effects of various FAO/CAO ratios in the diets on average daily gain and feed conversion ratio of broilers.

	Ratio of FAO:CAO						
	100:0	**80:20**	**60:40**	**40:60**	**20:80**	**0:100**	**s.e.**
ADG	1.82	1.67	1.66	1.71	1.68	1.70	0.047
FCR	1.91	1.94	2.00	2.05	1.99	1.94	0.041

In Table 4 the proximate composition of the carcasses and abdominal fat pads of the broilers are depicted. There were no significant differences (P>0.05) in the protein, ash, moisture or lipid content of the carcasses and fat pads among the different dietary groups. The values of the experimental diets are given in parenthesis.

Table 4: Effects of various FAO/CAO ratios in the diets on the proximate composition of carcasses and *abdominal fat pads* of broilers.

	Ratio of FAO:CAO						
	100:0	**80:20**	**60:40**	**40:60**	**20:80**	**0:100**	**s.e.**
Moisture %	66.34	66.14	66.10	66.12	66.34	66.14	0.031
	28.91	*28.71*	*28.67*	*28.69*	*28.91*	*28.71*	*0.043*
	(10.44)	(10.46)	(10.20)	(10.24)	(10.27)	(10.21)	
Protein %	18.19	17.41	17.62	16.96	18.19	17.41	0.424
	3.59	*2.81*	*3.02*	*2.36*	*3.59*	*2.81*	*0.241*
	(19.51)	(19.19)	(19.52)	(19.05)	(18.86)	(18.79)	
Lipid %	18.09	16.40	16.45	15.29	15.77	13.53	1.443
	61.51	*57.84*	*54.12*	*81.12*	*58.01*	*67.34*	*5.777*
	(9.85)	(8.21)	(14.92)	(9.62)	(10.18)	(9.12)	
Ash %	0.85	1.05	1.07	0.84	0.85	1.05	0.132
	0.33	*0.53*	*0.65*	*0.35*	*0.33*	*0.53*	*0.108*
	(5.96)	(6.10)	(6.36)	(6.17)	(6.10)	(6.17)	

The fatty acid profiles of the two oils (Table 5) added to the experimental diet (Table 1) differed greatly. Canola acid oil contains a high level of α-linolenic acid (C18:3n-3) and monounsaturated fatty acids, while Famarol oil contains a high level of linoleic acid (C18:2n-6) and saturated fatty acids. This resulted in the various treatments containing different mixtures of CAO and FAO (Table 6) having a large variation in lipid composition, particularly the level of saturation. The 100%FAO:0%CAO diet contained 31.32% saturated fatty acids (SFA), which were mainly palmitic acid (C16:0) (23.38%) and stearic acid (C18:0) (6.52%), 45.78% monounsaturated fatty acids (MUFA), which were predominantly oleic acid (C18:1n9) (38.80%) and palmitoleic acid (C16:1n7) (6.18%). The percentage of polyunsaturated fatty acids (PUFA, 22.90%) consisted mainly of linoleic acid (C18:2n-6) (20.73%) and α-linolenic acid (C18:3n-3) (1.10%). The 100%FAO:0%CAO diet showed only trace concentrations of longer chained acids. At the other extreme, the 0%FAO:100%CAO diet had a saturated fatty acids concentration of 23.62%. The main components of the SFA were palmitic acid (C16:0) (17.70%) and stearic acid (C18:0) (4.60%). Oleic acid (C18:1n9) (45.90%) was the main monounsaturated fatty acid at a level of 51.28%. Palmitoleic acid (C16:1n7) (4.43%) also formed a major component of the MUFA with lower concentrations of gadoleic acid (C20:1n9) also being present. The 0%FAO:100%CAO diet had 25.12% PUFA, with C18:2n-6 (19.17%) and C18:3n-3 (3.30%) being major constituents. Only trace concentrations of the longer-chained PUFA were detected in this diet.

Table 5: Fatty acid profiles of Canola oil and Famarol oil used in the diets (% recoverable fatty acids).

Fatty acids	Famarol oil	Canola oil
C12:0	0.29	-
C14:0	0.46	0.07
C16:0	13.72	5.34
C18:0	4.43	2.40
C20:0	0.75	0.35
C22:0	0.65	-
Total saturated fatty acids	**20.3**	**8.16**
C18:1n9	38.72	58.82
C18:2n-6	31.70	21.08
C18:3n-3	8.08	10.81
C20:1n9	0.81	0.41
Total unsaturated fatty acids	**79.31**	**91.12**
U:S	**3.9**	**11.2**
n-3/n-6	0.25	0.51
Unknowns	0.39	0.72

The means of the fatty acid profiles of the total lipids of carcass and abdominal fat pads of the broilers on the different diets are presented in Table 6. The fatty acid profiles of the broiler carcasses and abdominal fat pads were significantly influenced by the content of lipid in the diet. Generally, the most common fatty acid in the diet was most abundant in the tissue and fat pads. The converse was true of the least abundant fatty acids. The levels of linoleic acid (C18:2n-6) and arachidonic acid (C20:4n-6) in the carcasses of chicks fed 100% and 80% CAO were significantly lower ($P<0.05$) than those in chicks fed 100% and 80% FAO. The differences increased with rising dietary CAO levels, whereas the levels of α-linolenic acid (C18:3n-3), eisosapentanoic acid (EPA) (C20:5n-3) and docosahexaenoic acid (DHA) (C22:6n-3) increased linearly as seen in Table 7. The linear regression equations in Table 7 plots the actual percentage of fatty acids in the feed over those in the tissue. The increase in dietary CAO levels also resulted in decreases in the rates of linoleic acid (C18:2n-6) and arachidonic acid (C20:4n-6) in the abdominal fat pads, but it increased the rates of α-linolenic acid (C18:3n-3), EPA (C20:5n-3) and DHA (C22:6n-3). Therefore, the ratios of n-3/n-6 fatty acids was significantly increased by rising the level of dietary CAO (Table 6).

With rising levels of CAO in the diet, the SFA decreased linearly by 4.88% and 10.63% for the carcasses and abdominal fat pads respectively (Table 6). This was mainly caused by decreases in the two principal saturated fatty acids namely palmitic acid (C16:0) and stearic acid (C18:0) in both tissues. Conversely, the MUFA increased with 3.87% in the carcasses and 7.25% in the abdominal fat pads, caused by increases in oleic acid (C18:1n9) and palmitoleic acid (C16:1n7). Table 6 shows a slight increase in PUFA (1.02% for carcasses and 2.38% for abdominal fat pads) with increased levels of CAO. This may be caused by a balancing effect of the n-3 and n-6 fatty acids. There was no significant difference ($P>0.05$) between the two sexes for any of the fatty acids, nor did any of the fatty acids show significant interaction between diet and sex.

Table 6: The fatty acid composition of the carcass (average for sexes, then male = M and female = F values) and *abdominal fat pad* total lipid from broilers receiving different dietary lipid variations of Canola and Famarol fatty acids. Diet lipid fatty acid concentrations are given in parenthesis.

	Ratio of FAO:CAO						
Fatty acids*	**100:0****	**80:20**	**60:40**	**40:60**	**20:80**	**0:100**	**s.e.**
C14:0	1.05^{a}	1.22^{a}	2.07^{b}	1.38^{ac}	1.45^{ad}	1.67^{bcd}	0.170
	M1.07	M1.17	M2.33	M1.60	M1.53	M1.57	M0.241
	F1.03	F1.27	F1.80	F1.17	F1.37	F1.77	F0.241
	1.75^{a}	*1.58^{a}*	*1.35^{ab}*	*0.80^{c}*	*0.98^{bc}*	*0.98^{bc}*	*0.156*
	M1.93	*M1.67*	*M1.43*	*M0.80*	*M0.90*	*M0.87*	*M0.220*
	F1.57	*F1.50*	*F1.26*	*F0.80*	*F1.07*	*F1.10*	*F0.220*
	(1.42)	(1.42)	(1.72)	(1.10)	(1.27)	(1.35)	
C16:0	23.98^{a}	23.38^{b}	22.57^{c}	21.73^{d}	20.65^{e}	20.08^{f}	0.116
	M23.90	M23.33	M22.50	M21.77	M20.77	M20.17	M0.163
	F24.07	F23.43	F22.63	F21.70	F20.53	F20.00	F0.163
	22.95^{a}	*21.25^{b}*	*19.72^{c}*	*17.75^{d}*	*16.78^{e}*	*15.28^{f}*	*0.125*
	M22.96	*M21.13*	*M19.73*	*M17.80*	*M16.87*	*M15.37*	*M0.171*
	F22.94	*F21.37*	*F19.74*	*F17.85*	*F16.70*	*F15.20*	*F0.171*
	(23.38)	(22.30)	(21.08)	(19.68)	(18.68)	(17.70)	
C18:0	6.57^{a}	6.15^{b}	5.68^{c}	5.37^{d}	5.15^{e}	4.97^{f}	0.061
	M6.53	M6.17	M5.70	M5.33	M5.10	M5.00	M0.087
	F6.60	F6.13	F5.67	F5.40	F5.20	F4.93	F0.087
	6.40^{a}	*5.95^{b}*	*5.40^{c}*	*4.95^{d}*	*4.53^{e}*	*4.20^{f}*	*0.061*
	M6.40	*M6.00*	*M5.43*	*M4.93*	*M4.53*	*M4.20*	*M0.086*
	F6.40	*F5.90*	*F5.37*	*F4.97*	*F4.53*	*F4.20*	*F0.086*
	(6.52)	(6.07)	(5.57)	(5.17)	(4.87)	(4.60)	
SFA	31.60^{a}	30.75^{b}	30.32^{c}	28.48^{d}	27.25^{e}	26.72^{f}	0.133
	M31.50	M30.67	M30.50	M28.70	M27.40	M26.73	M0.188
	F31.70	F30.83	F30.13	F28.27	F27.10	F26.70	F0.188
	31.10^{a}	*28.78^{b}*	*26.47^{c}*	*23.50^{d}*	*22.30^{e}*	*20.47^{f}*	*0.100*
	M31.30	*M28.80*	*M26.60*	*M23.53*	*M22.30*	*M20.43*	*M0.141*
	F30.90	*F28.77*	*F26.33*	*F23.47*	*F22.30*	*F20.50*	*F0.141*
	(31.32)	(29.78)	(28.37)	(25.95)	(24.82)	(23.62)	
C16:1n7	3.20^{a}	3.70^{b}	4.37^{c}	4.80^{d}	5.62^{e}	6.12^{f}	0.052
	M3.20	M3.70	M4.43	M4.73	M5.57	M6.10	M0.073
	F3.20	F3.70	F4.30	F4.87	F5.67	F6.13	F0.073
	5.60^{a}	*5.78^{b}*	*5.87^{c}*	*5.90^{c}*	*6.03^{d}*	*6.17^{e}*	*0.018*
	M5.60	*M5.80*	*M5.87*	*M5.90*	*M6.03*	*M6.17*	*M0.026*
	F5.60	*F5.77*	*F5.87*	*F5.90*	*F6.03*	*F6.17*	*F0.026*
	(4.43)	(4.77)	(5.15)	(5.37)	(5.85)	(6.18)	
C18:1n9	37.88^{a}	38.55^{b}	40.23^{c}	42.37^{d}	43.40^{e}	44.50^{f}	0.119
	M37.90	M38.57	M40.23	M42.27	M43.43	M44.47	M0.169
	F37.87	F38.53	F40.23	F42.47	F43.37	F44.53	F0.169
	39.95^{a}	*41.90^{b}*	*42.80^{c}*	*44.75^{d}*	*45.73^{e}*	*47.23^{f}*	*0.112*
	M40.03	*M41.80*	*M42.70*	*M44.80*	*M45.73*	*M47.20*	*M0.158*
	F39.87	*F42.00*	*F42.90*	*F44.70*	*F45.73*	*F47.27*	*F0.158*
	(38.80)	(40.17)	(41.42)	(43.47)	(44.58)	(45.90)	
C20:1n9	0.95^{ab}	0.47^{a}	1.08^{b}	0.95^{ab}	1.23^{b}	1.12^{b}	0.187
	M1.10	M0.47	M0.67	M0.97	M0.57	M0.87	M0.265
	F0.80	F0.47	F1.50	F0.93	F1.90	F1.37	F0.265
	0.58^{a}	*0.70^{a}*	*0.77^{a}*	*0.53^{a}*	*0.63^{a}*	*1.12^{b}*	*0.127*
	M0.60	*M0.63*	*M0.87*	*M0.53*	*M0.73*	*M1.10*	*M0.179*
	F0.57	*F0.77*	*F0.67*	*F0.53*	*F0.53*	*F1.13*	*F0.179*
	(0.80)	(0.60)	(0.93)	(0.77)	(0.97)	(1.15)	

MUFA	44.95[a]	44.63[a]	46.12[b]	47.68[c]	48.33[cd]	48.82[d]	0.148
	M45.10	M44.60	M45.63	M47.67	M47.70	M48.53	M0.209
	F44.80	F44.67	F46.60	F47.70	F48.97	F49.10	F0.209
	46.70[a]	*48.63[b]*	*49.47[c]*	*51.15[d]*	*52.15[e]*	*53.95[f]*	*0.103*
	M46.80	*M48.47*	*M49.47*	*M51.20*	*M52.27*	*M53.90*	*M0.145*
	F46.60	*F48.80*	*F49.47*	*F51.10*	*F52.03*	*F54.00*	*F0.145*
	(45.78)	(46.61)	(47.72)	(49.37)	(50.03)	(51.28)	
C18:2n-6	20.88[a]	20.42[b]	20.13[bc]	19.78[cd]	19.50[d]	19.10[e]	0.068
	M20.90	M20.47	M20.13	M19.80	M19.53	M19.13	M0.096
	F20.87	F20.37	F20.13	F19.77	F19.47	F19.07	F0.096
	20.75[a]	*20.50[b]*	*20.25[c]*	*19.88[d]*	*19.60[e]*	*19.20[f]*	*0.044*
	M20.80	*M20.60*	*M20.30*	*M19.88*	*M19.70*	*M19.30*	*M0.054*
	F20.70	*F20.40*	*F20.20*	*F19.88*	*F19.50*	*F19.10*	*F0.054*
	(20.73)	(20.45)	(20.15)	(19.83)	(19.58)	(19.17)	
C18:3n-3	1.00[a]	1.15[a]	1.77[b]	2.20[c]	2.60[d]	3.25[e]	0.061
	M1.00	M1.13	M1.80	M2.20	M2.60	M3.27	M0.087
	F1.00	F1.17	F1.73	F2.20	F2.60	F3.23	F0.087
	1.15[a]	*1.37[b]*	*1.80[c]*	*2.58[d]*	*2.85[e]*	*3.28[f]*	*0.056*
	M1.20	*M1.38*	*M1.90*	*M2.60*	*M2.85*	*M3.29*	*M0.034*
	F1.10	*F1.36*	*F1.70*	*F2.56*	*F2.85*	*F3.27*	*F0.034*
	(1.10)	(1.28)	(1.80)	(2.38)	(2.75)	(3.30)	
C20:4n-6	1.05[a]	1.00[a]	0.88[b]	0.87[b]	0.80[b]	0.70[c]	0.031
	M1.03	M1.00	M0.83	M0.90	M0.80	M0.70	M0.044
	F1.07	F1.00	F0.93	F0.83	F0.80	F0.70	F0.044
	1.20[a]	*1.10[ab]*	*1.00[bc]*	*0.90[c]*	*0.42[d]*	*0.22[e]*	*0.038*
	M1.20	*M1.10*	*M1.00*	*M0.90*	*M0.50*	*M0.10*	*M0.054*
	F1.20	*F1.10*	*F1.00*	*F0.90*	*F0.33*	*F0.33*	*F0.054*
	(1.75)	(1.92)	(1.65)	(1.32)	(1.17)	(0.68)	
C20:5n-3	0.10[a]	0.10[a]	0.15[b]	0.18[bc]	0.18[bc]	0.20[c]	0.017
	M0.10	M0.10	M0.13	M0.27	0.20	M0.20	M0.024
	F0.10	F0.10	F0.17	F0.10	F0.17	F0.20	F0.024
	0.03[a]	*0.08[a]*	*0.25[b]*	*0.35[c]*	*0.38[c]*	*0.48[d]*	*0.021*
	M0.00	*M0.10*	*M0.23*	*M0.40*	*M0.37*	*M0.47*	*M0.029*
	F0.07	*F0.07*	*F0.27*	*F0.30*	*F0.40*	*F0.50*	*F0.029*
	(0.10)	(0.17)	(0.20)	(0.22)	(0.23)	(0.25)	
C22:6n-3	0.20[a]	0.50[ab]	0.48[ab]	0.72[bc]	0.92[c]	0.87[c]	0.103
	M0.20	M0.50	M0.60	M0.57	M1.30	M0.97	M0.145
	F0.20	F0.50	F0.37	F0.87	F0.53	F0.77	F0.145
	0.05[a]	*0.18[a]*	*0.60[b]*	*0.80[bc]*	*0.90[c]*	*1.00[c]*	*0.050*
	M0.00	*M0.20*	*M0.57*	*M0.80*	*M0.90*	*M1.00*	*M0.070*
	F0.10	*F0.17*	*F0.63*	*F0.80*	*F0.90*	*F1.00*	*F0.070*
	(0.18)	(0.33)	(0.32)	(0.43)	(0.43)	(0.42)	
PUFA	23.45[a]	23.62[a]	23.57[a]	23.83[a]	24.42[b]	24.47[b]	0.175
	M23.40	M23.62	M23.87	M23.63	M24.90	M24.73	M0.235
	F23.50	F23.62	F23.27	F24.03	F23.93	F24.20	F0.235
	23.20[a]	*23.58[b]*	*24.07[c]*	*25.35[d]*	*25.55[d]*	*25.58[d]*	*0.151*
	M23.20	*M23.58*	*M23.93*	*M25.27*	*M25.43*	*M25.67*	*M0.177*
	F23.20	*F23.58*	*F24.20*	*F25.43*	*F25.67*	*F25.50*	*F0.177*
	(22.90)	(23.60)	(23.92)	(24.73)	(25.15)	(25.12)	
n-3/n-6	0.06[a]	0.08[a]	0.11[b]	0.15[c]	0.18[d]	0.22[e]	0.008
	M0.06	M0.10	M0.11	M0.14	M0.18	M0.23	M0.012
	F0.06	F0.06	F0.11	F0.16	F0.18	F0.21	F0.012
	0.06[a]	*0.08[a]*	*0.12[ab]*	*0.18[bc]*	*0.21[cd]*	*0.25[d]*	*0.022*
	M0.06	*M0.10*	*M0.11*	*M0.14*	*M0.18*	*M0.23*	*M0.031*
	F0.06	*F0.06*	*F0.13*	*F0.22*	*F0.24*	*F0.27*	*F0.031*
	(0.06)	(0.08)	(0.11)	(0.14)	(0.16)	(0.20)	

*Fatty acids identified as % of total fatty acids measured. Values in the same row with different superscripts differ significantly ($P<0.05$).

Table 7: Linear regression equations plotting the percentage fatty acids in the feed over the percentage in the tissue.

Equation: y = a + bx				
where x = % of fatty acid in feed		**b = slope**		
y = % of fatty acid in tissue		**a = intercept**		
Fatty acids	**Carcass**		**Abdominal fat**	
	b ± s.e.	**a ± s.e.**	**b ± s.e.**	**a ± s.e.**
C14:0	0.935 ± 0.422	0.182 ± 0.587	1.097 ± 0.388	-0.273 ± 0.540
C16:0	0.700 ± 0.024	7.736 ± 0.489	1.321 ± 0.025	-8.090 ± 0.515
C18:0	0.838 ± 0.033	1.066 ± 0.179	0.022 ± 0.001	4.117 ± 0.040
SFA	0.659 ± 0.025	11.192 ± 0.698	1.353 ± 0.019	-11.518 ± 0.533
C16:1n7	1.699 ± 0.040	-4.359 ± 0.215	0.297 ± 0.013	4.322 ± 0.070
C18:1n9	0.982 ± 0.022	-0.489 ± 0.955	0.981 ± 0.021	2.161 ± 0.893
C20:1n9	1.187 ± 0.509	-0.066 ± 0.451	0.772 ± 0.298	0.050 ± 0.264
MUFA	0.821 ± 0.051	6.982 ± 2.462	1.221 ± 0.034	-8.832 ± 1.662
C18:2n-6	1.117 ± 0.047	-1.231 ± 0.893	1.008 ± 0.033	0.886 ± 0.625
C18:3n-3	0.980 ± 0.035	-1.016 ± 0.111	0.978 ± 0.030	-0.834 ± 0.096
C20:4n-6	0.271 ± 0.030	0.499 ± 0.045	0.836 ± 0.058	-0.378 ± 0.086
C20:5n-3	0.739 ± 0.185	0.009 ± 0.037	3.076 ± 0.258	-0.336 ± 0.052
C22:6n-3	0.906 ± 0.208	0.002 ± 0.150	1.331 ± 0.105	-0.310 ± 0.076
PUFA	0.440 ± 0.097	13.235 ± 2.359	1.156 ± 0.064	-3.453 ± 1.561

Discussion

There were no significant differences (P>0.05) in average daily gain or feed conversion ratios among dietary and sex groups throughout the experimental period. This result was expected as the different diets were balanced for the energy:protein ratio. There were also no interactions between diet and sex for ADG and FCR. No significant differences (P>0.05) were found in the proximate analysis of the carcasses and abdominal fat pads among dietary and sex groups. There was also no statistical difference in the interaction between diet and sex for these parameters.

In monogastric animals the fatty acid composition of the tissues will reflect that of the diet (Lands et al., 1990). Analysis of the fatty acid composition of the carcasses and abdominal fat pads showed that palmitic acid (C16:0) was the most predominant saturated fatty acid (Table 6). Free palmitic acid (C16:0) is the primary product of fatty acid synthetase reactions within the tissues (Volpe and Vagelos, 1973). Some myristic acid (C14:0) and lauric acid (C12:0) is also formed, and a trace of stearic acid (C18:0) may also be produced (Wakil et al., 1983). Once free palmitic acid (C16:0) is released from the synthetase complex, it can be esterified into complex lipids,

elongated to stearic acid (C18:0) (the usual synthetic pathway for the *de novo* synthesis of stearic acid), or desaturated to palmitoleic acid (C16:1n7) (Beare-Rogers, 1977). Different enzymatic pathways are involved in each reaction.

Oleic acid (C18:1n9) was the most predominant monounsaturated fatty acid in both the carcass and the abdominal fat pads (Table 6). This was expected as fatty acid synthesis in animal systems produce only saturated acids and monounsaturated fatty acids of the n9 series, usually oleic acid (Volpe and Vagelos, 1973). Animals lack the ability to desaturate monounsaturated fatty acids in the n-6 or n-3 position of the fatty acid chain. In animal tissues the desaturation of the *de novo* synthesised fatty acids stops with the production of the monounsaturated fatty acid with a double bond in the 9-10 position of the fatty acid chain. (If palmitate is the substrate for the dehydrogenase, the double bond will appear in the 7-8 position of the chain as the 9-10 dehydrogenase desaturates nine carbons from the carboxyl end of the molecules.) Thus, palmitoleic acid (C16:1n7), oleic acid (C18:1n9) and *cis*-vaccenic acid (C18:1n11) are the main products of this reaction. Chain elongation by another enzymatic system, the fatty acid elongase system, will produce eicosanoic acid (C20:1n9), erucic acid (C22:1n13) and nervonic acid (C24:1n9) by elongation of oleic acid (Seubert and Podack, 1973).

Linoleic acid (C18:2n-6) was the most predominant polyunsaturated fatty acid in the tissues (Table 6). Thomas and co-workers (1987) stated that individuals eating diets containing large amounts of linoleic acid will deposit this component readily in the tissue and complex lipids where it will be elongated and desaturated to arachidonic acid (C20:4n-6) (Lands et al., 1990). Sprecher and Lee (1975) have shown that dietary polyunsaturated fatty acids are not elongated and then desaturated, but are desaturated and then elongated. The results of the present study suggest that dietary CAO enriched with α-linolenic acid (C18:3n-3) exerts a different effect on the lipid metabolism and fatty acid composition in growing chicks from that of FAO enriched with linoleic acid. Once ingested, linolenic acid and α-linolenic acid can be desaturated and elongated, primarily in the liver (Brenner, 1981) in a manner such that the methylene-interrupted pattern of unsaturated double bonds is maintained. The microsomal enzymatic reactions do not permit crossover between fatty acids metabolites from the C18:2n-6 and C18:3n-3 sequences (Brenner, 1981). The desaturation and elongation steps are influenced by numerous nutritional and hormonal factors (Brenner, 1981). A competitive interaction between linoleic acid and α-linolenic acid exists, such that n-3 PUFA suppress the metabolism of n-6 PUFA and n-6 PUFA suppress the metabolism of n-3 PUFA, although less strongly (Rahm and Holman, 1964).

Analysis of the fatty acid composition of the carcasses and abdominal fat pads showed that the enrichment of n-3 fatty acids in those tissues is due to an increase of n-3 fatty acids as a whole in

the diet; i.e., the increase of the CAO level in the diets resulted in the increased accumulation of α-linolenic acid (C18:3n-3) in those tissues (Table 6). Dietary α-linolenic acid (C18:3n-3) is not readily incorporated into tissue lipids. It is mainly oxidised to CO_2 and water (Aeberhard et al., 1978). Some is converted to EPA (C20:5n-3) and DHA (C22:6n-3), but only a small portion of the α-linolenic acid (C18:3n-3) enters this pathway (De Gomez-Dumm and Brenner, 1975). However, if all other fatty acids are removed from the diet and α-linolenic acid (C18:3n-3) is fed as the only source of dietary fat, then a higher percentage is incorporated into tissue lipids (Mohrhauer and Holman, 1963). Thus, it is a competitive discrimination that prevents α-linolenic acid (C18:3n-3) from being stored in tissues, not an intrinsic property of this compound.

The longer chain n-3 fatty acids were also deposited into the tissues in proportion to their concentration in the diet (Table 7). This result is consistent with other reports for broiler chicks (Olomu and Baracos, 1991a; Phetteplace and Watkins, 1989). Studies using whole flaxseed for poultry diets have shown that whole eggs and various tissues are enriched with n-3 PUFAs derived from dietary α-linolenic acid (C18:3n-3) (Caston and Leeson, 1990; Cherian and Sim, 1991; Jiang et al., 1991). Docosahexaenoic acid (DHA) (C22:6n-3) can be retroconverted to eicosapentanoic acid (EPA) (C20:5n-3). EPA is normally present only in trace amounts in the tissue lipids. Conversely, DHA is the major long-chain n-3 fatty acid in most tissues. A few tissues contain 22:5n-3 in specific organs, but this fatty acid was not detected in this experiment. EPA appears to have several unique pharmacological and biochemical actions when consumed in large amounts (Leaf and Weber, 1988). In such cases, DHA, through retroconversion, supplies EPA on demand if there is an inadequate dietary supply of α-linolenic acid.

In this investigation, there was a significant increase in n-3/n-6 ratios in various tissue lipids with up to 80% or 100% CAO feeding. It is well known that there is a competitive inhibition between linoleic acid and α-linolenic acid on desaturation reactions responsible for the synthesis of tissue PUFAs (Kinsella, 1988). This competitive inhibition depends on the ratio of the two fatty acids in a diet. Thus, an increase in dietary α-linolenic acid (C18:3n-3) results in the reduction of γ-linolenic acid (C18:3n-6) and arachidonic acid (C20:4n-6) by inhibiting the conversion reaction from linoleic acid (C18:2n-6) to these fatty acids (Garg et al., 1989; 1990; Olomu and Baracos, 1991b). Also n-3 and n-6 fatty acids have different reaction rates with most enzyme systems (Sprecher, 1991). Linoleic acid is rapidly incorporated into tissue and complex lipids and elongated and desaturated to arachidonic acid (Lands, et al., 1990). α-Linolenic acid is excluded from most tissues and complex lipids (Tinoco, 1982). If large amounts of α-linolenic acid are fed, it appears in the blood and liver in a lower proportion than fed in the diet. This is consistent with the results for carcass tissue in Table 6. Conversely, linoleic acid will usually be incorporated into complex lipids in proportionally larger amounts than is fed in the diet, as can be seen in the results for abdominal

fat in Table 6. Thus, linoleic acid is conserved whereas α-linolenic acid is eliminated in tissues of broilers fed high levels of linoleic acid. α-Linolenic acid is converted to EPA and DHA, but only slowly (Adam et al., 1986), whereas linoleic acid was rapidly converted to arachidonic acid. This can be seen in the 100%FAO diet (Table 6), resulting in a high level of C20:4n-6 (1.05% for carcass and 1.20% for abdominal fat) and low levels of C20:5n-3 (0.10% for carcass and 0.03% for abdominal fat) and C22:6n-3 (0.20% for carcass and 0.05% for abdominal fat). EPA does not persist in tissue (Nelson et al., 1991). It is either rapidly oxidised or converted to DHA, a fatty acid that is strongly conserved. This is consistent with the results found in Table 6, showing C22:6n-3 at a constantly higher percentage in the tissues than C20:5n-3.

In conclusion, an increase in the human dietary n-3/n-6 fatty acid ratio is essential in today's Western diet to help prevent coronary heart disease by reducing plasma lipids (Kinsella et al., 1990). The results of this experiment showed that substitution of Famarol oil with Canola oil in broiler diets can increase the ratio of n-6 to n-3 fatty acids in broiler carcasses and abdominal fat pads to 5:1, a ratio more suitable for human health. Increasing the level of n-3 fatty acids in the diets was also effective in reducing the level of saturated fatty acids in the carcasses and abdominal fat pads of broiler chickens resulting in "healthier" chickens. The increased n-3 fatty acids also suppressed the formation of long-chain n-6 PUFAs derived from linoleic acid ensuring a more balanced eicosanoid metabolism (Harris, 1989). Overall, in view of the prevalence of human coronary heart disease, consumption of n-3 PUFA enriched broilers could be considered as a useful complementary option for the amelioration of coronary vascular disease.

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Part 4

Effect of dietary vitamin E on the performance of broilers and oxidative stability, colour, microbiological stability, fatty acid composition and pH of broiler meat during refrigerated and frozen storage

Effect of dietary vitamin E on the performance of broilers and oxidative stability, colour, microbiological stability, fatty acid composition and pH of broiler meat during refrigerated and frozen storage.

Abstract Experiment 1 was carried out with 220 one-day-old broiler chicks to evaluate the effect of eleven concentrations of vitamin E (0, 20, 40, 60, 80, 100, 120, 140, 160, 180 and 200 mg α-tocopheryl acetate / kg diet) on their production performance and the oxidative stability of their frozen broiler carcasses. The diets with vitamin E levels 0 to 100 mg were given from day-old to 42 days of age while the diets with vitamin E levels 120 to 200 mg were given from 21 to 42 days of age. The oxidative stability, evaluated by thiobarbituric acid reactive substances (TBARS) values, was determined after 30, 90, 120 and 150 days of storage at -20°C. There were no statistical differences (P>0.05) in average daily gain (1.85 ± 0.111 g) or feed conversion ratios (2.29 ± 0.397) among dietary groups. TBARS values increased significantly (P<0.05) with increasing time of storage (basal diet: day 30 = 1.71 ± 0.51; day 150 = 4.89 ± 0.51), but decreased significantly (P<0.05) with increasing vitamin E levels (day 150: basal = 4.89 ± 0.51; 100 mg / kg = 1.09 ± 0.27). Experiment 2 was carried out with day-old broiler chicks to evaluate the effect of five concentrations of vitamin E (0, 40, 80, 120 and 160 mg α-tocopheryl acetate / diet) on their performance and the oxidative stability of their refrigerated carcasses. The experimental diets were fed from day-old to 42 days of age. The oxidative stability, evaluated by TBARS values, colour deterioration and microbiological stability were determined after 0, 4, 8, 10 and 12 days of storage at 4°C. Fatty acid analysis was done on the samples of days 0 and 12. There were no statistical differences (P>0.05) in average daily gain (1.88 ± 0.117 g) or feed conversion ratios (2.37 ± 0.467) among dietary groups. TBARS values increased significantly (P<0.05) with increasing time of storage, but decreased significantly (P<0.05) with increasing vitamin E levels. There were no statistical differences (P>0.05) in colour measurements for L* (44.97 ± 0.662), a* (5.23 ± 0.315) or b* (12.76 ± 0.321) values between treatments. Microbiological counts increased significantly (P<0.05) over time with vitamin E concentration showing no effect. There were no statistical differences (P>0.05) for any of the fatty acid groups measured (SFA: Day 0 = 26.1 ± 1.13%, Day 12 = 26.1 ± 1.17%; MUFA: Day 0 = 41.4 ± 1.46%, Day 12 = 40.2 ± 2.28%; PUFA: Day 0 = 32.4 ± 1.95%, Day 12 = 33.8 ± 2.52%) among dietary groups. Similarly, none of the fatty acids showed statistical significant (P>0.05) proportion changes over time. There were no statistical differences (P>0.05) in pH (6.01 ± 0.206) among dietary groups.

Introduction

Lipid oxidation is a major cause of meat quality deterioration as products of autoxidation of unsaturated fatty acids affect wholesomeness and nutritional value (Pearson et al., 1983). Lipid oxidation is an important determinant of shelf life of meats and meat products. Post-slaughter biochemical changes involved in the conversion of muscle to meat are accompanied by a loss of cellular antioxidant defences and an increased propensity of meat lipids to undergo oxidation

(Morrisey et al., 1994). This contributes to undesirable changes in a number of quality parameters, including loss of water-holding capacity, texture and flavour.

The lipids in poultry exhibit a higher degree of unsaturation than red meats due to a relatively high content of phospholipids (Igene and Pearson, 1979). The degree of unsaturation of the phospholipids of the membrane is an important factor in determining the oxidative stability of meats, with the oxidative potential increasing as the degree of unsaturation of the lipids in the meat increases. The phospholipids are located in membrane structures, and the initiation of lipid peroxidation in tissues has been tracked to the membranal level (Machlin, 1984). The production of meat, particularly chicken, with a more highly unsaturated profile has been the focus of some attention, as such meats are perceived as having a 'healthier' image. Accordingly, poultry meat and meat products are rather susceptible to oxidative deterioration, and oxidation often determines shelf life of poultry meat products.

Membranal lipids are protected against oxidative attack by a number of natural occurring antioxidants, including the chain-breaking antioxidants ascorbic acid and α-tocopherol. α-Tocopherol (vitamin E) is deposited in subcellular membranes in positions adjacent to the phospholipid chains and is a highly efficient scavenger of neighbouring fatty acyl radicals (Buettner, 1993). The addition of α-tocopherol to animal diets is an effective means of improving the oxidative stability of meats, in addition to improving flavour (Sheldon et al., 1997). Elevated α-tocopherol levels in poultry feeds increase tissue concentrations thereof resulting in improved stability of membranal structures which may be expected to increase the oxidative stability of meat and meat products (Sheehy et al., 1993).

Feeding poultry a higher level of natural dietary antioxidants provides the poultry industry with a simple method for improving oxidative stability, sensory quality, shelf life, and acceptability of poultry meats (Buckley and Morrissey, 1992). In addition to the stabilising effect on meat and meat products, a raised α-tocopherol level significantly enhances feed conversion efficiency, average weight, and net income per bird (Kennedy et al., 1992).

The purpose of the present study was to investigate the effect of vitamin E supplementation on quality attributes in chicken meat following refrigerated and frozen storage.

Materials and Methods

Birds

At the start of experiment 1, 220 one-day-old broilers were reared to 42 days of age in an environmentally controlled rearing house. A 24-hour light period was followed. The initial environmental temperature at day-old was 33°C and was gradually decreased to 21°C at 21 days of age. A standard commercial health and inoculation program was followed. Five birds per cage were allocated to 44 cages. All the birds in each cage were allocated to one of eleven dietary treatments. In experiment 2, 100 one-day-old broilers were used. All rearing procedures were the same as in experiment one. Five birds per cage were allocated to 20 cages. All the birds in each cage were allocated to one of five dietary treatments. A randomised block design was used in both experiments.

Diet composition

The nutrient composition of the experimental diets is given in Table 1. In experiment 1, ten levels of vitamin E (supplied by Hoffmann La-Roche, Basel, Switzerland) were supplemented to the basal diet (Table 2), resulting in 11 treatments: 1) basal diet (control); 2) basal + 20 mg/kg feed; 3) basal + 40 mg/kg feed; 4) basal + 60 mg/kg feed; 5) basal + 80 mg/kg feed; 6) basal + 100 mg/kg feed; 7) basal + 120 mg/kg feed; 8) basal + 140 mg/kg feed; 9) basal + 160 mg/kg feed; 10) basal + 180 mg/kg feed; 11) basal + 200 mg/kg feed. Treatments 2 to 6 were fed from day-old to 42 days of age (throughout the trial), while treatments 7 to 11 were fed from 21 to 42 days of age (last three weeks of the trial). In experiment 2, four levels of vitamin E were supplemented to the basal diet (Table 2), resulting in 5 treatments: 1) basal diet (control); 2) basal + 40 mg/kg feed; 3) basal + 80 mg/kg feed; 4) basal + 120 mg/kg feed; 5) basal + 160 mg/kg feed. All treatments were fed from day-old to 42 days of age.

Bird weights per cage and feed consumption per cage were recorded weekly. Average daily gain (ADG) and feed conversion ratios (FCR) were calculated with the following equations:

$$\text{ADG} = \frac{\text{Body weight gain}}{\text{Number of days}}$$

where

$$\text{Body weight gain} = \text{Live weight} - \text{Day-old weight}$$

$$\text{FCR} = \frac{\text{Total feed intake}}{\text{Total weight of birds}}$$

Table 1: Nutrient composition of experimental diets.

Nutrient	Percentage of diet
Metabolisable Energy (MJ/kg)	13.20
Protein	22.89
Arginine	1.45
Isoleucine	0.98
Leucine	2.38
Lysine	1.15
Methionine	0.57
Methionine + Cystine	0.95
Threonine	0.77
Tryptophan	0.23
Calcium	0.89
Sodium	0.16
Phosphorus	0.40
Moisture	9.20

Table 2: The formulation of the basal diet.

Ingredient	Percentage of diet
Gluten	8.2
Lime	1.3
Coccidiostat	0.2
Lysine	0.5
Maize	51.2
Monocalciumphosphate	1.3
Methionine	0.2
Soybean meal	17.6
Salt	0.4
Sunflower meal	10.9
Vitamin/Mineral Mix[1]	0.4
Oil mixture	8.0

[1]Vitamin/Mineral mix: Vitamin A (10 000 000 IU), Vitamin D (2 000 000 IU), Vitamin E (20 000 IU), Vitamin K (2 g), Vitamin B1 (2 g), Vitamin B2 (5 g), Vitamin B6 (3 g), Vitamin B12 (0.01 g), Niacin (20 g), Calpan DL (10 g), Folic acid (0.5 g), Biotin (0.02 g), Antioxidant (125 g), Choline (300 g), Cobalt (0.5 g), Copper (6 g), Iron (20 g), Manganese (70 g), Iodine (1 g), Selenium (0.15 g), Zinc (50 g).

Chemical analysis

At 42 days of age the five birds from each cage were slaughtered. The weight of the plucked birds was recorded and the abdominal fat pads removed. The five carcasses from each cage were stored in separate oxygen permeable plastic bags at -20°C (experiment 1) or 4°C (experiment 2) until required for chemical analysis. In experiment 1 (frozen storage) one carcass from each cage was taken from storage on days 30, 90, 120 and 150, minced and measured for oxidative stability by means of TBARS values (Uchiyama and Mihara, 1978). In experiment 2

(refrigerated storage) pH measurements were taken 45 min, 2, 4 and 24 hrs post-mortem. One carcass from each cage was taken from storage on days 0, 4, 8, 10 and 12, measured for colour degradation, minced and analysed for microbial spoilage and oxidative stability by means of TBARS values. Fresh meat colour of the muscle was evaluated using a Colorgard System 2000 colorimeter (Pacific Scientific, Silver Spring, MD, USA) to determine L*, a* and b* values (Commission International de l' Eclairage, 1976), with L* indicating brightness, a* the red-green range and b* the blue-yellow range. Fatty acid analysis was done on the samples from days 0 and 12. Fatty acid methyl esters (FAME) were prepared according to Morrison and Smith (1964). The FAME were analysed with a GLC: Varian Model 3300, equipped with flame ionisation detection and two 30 m fused silica megabore DB-225 columns of 0.53 mm internal diameter (J&W Scientific, Folsom, CA). Gas flow rates were: hydrogen, 25 ml/min; air, 250 ml/min; and nitrogen (carrier gas), 5-8 ml/min. Temperature programming was linear at 4°C/min; initial temperature, 160°C; final temperature, 220°C held for 10 min; injector temperature, 240°C; and detector temperature, 250°C. The FAME were identified by comparison of the retention times to those of a standard FAME mixture (Nu-Chek-Prep Inc., Elysian, Minnesota). Microbial stability was measured on the samples of days 0, 4, 8 and 10 by means of aerobic plate counts (APC). Total aerobic microbial counts were conducted by using Plate Count Agar (Merck). Ten grams of the meat were homogenised in 100 ml of sterile distilled water, serially diluted and plated onto the surface of the plates. All plates were incubated at 37°C for 48 to 72h. In a separate experiment, 100 gram fresh meat samples with vitamin E levels of 0, 80 and 160 mg vitamin E / kg feed were inoculated with active growing cells of *Listeria innocua*, *Staphylococcus aureus* and *Salmonella enteritidis* (10^6 CFU per gram). The growth of these bacteria were monitored by plating onto Trypticase Soy Agar (Merck), Baird-Parker Agar (Merck) and Salmonella-Shigella Agar (Merck), respectively. Serial dilutions were made as described previously. All plates were incubated at 37°C for 48 to 72 h.

Statistical analysis

The GENSTAT package (GENSTAT 5 Release 3.2, 1997) was used to fit regression models to the data with storage time as predictor. Analysis of variance was performed on all variables measured. The following model was fitted for main effects (diet, storage time) and the interaction between them:

$$Y_{ij} = \mu + D_i + T_j + DT_{ij} + e_{ij}$$

where Y_{ij} is the dependant variable, μ = the overall mean, D_i = the i^{th} diet effect, T_j = the j^{th} time effect, DT_{ij} = the interaction between diet and time and e_{ij} = the residual error. The differences between diets and storage times where tested separately by means of the null hypothesis (H_0), with H_0: $\mu = \mu_0$ and the alternate hypothesis (H_a) being H_a: $\mu \neq \mu_0$. Mean and standard errors were

calculated for each diet and time and the main effects were compared by use of least significant differences. Differences between the variables were accepted as being significant if the probability of rejection of H_0 was less than 5% ($P<0.05$) for diets and time.

Results

Average daily gain and feed conversion ratios

The average daily gain and food conversion ratio (FCR) of chicks' fed the different experimental diets for experiment 1 and 2 are shown in Table 3. There were no significant differences in ADG or FCR among the dietary groups ($P>0.05$) for either of the experiments.

Table 3: Effects of various vitamin E levels in the diets on average daily gain and food conversion ratios of broilers.

	ADG	FCR
Experiment 1 (-20°C)		
1) Basal	1.79 ± 0.173	2.32 ± 0.434
2) 20 mg vitamin E / kg feed	1.84 ± 0.137	2.24 ± 0.223
3) 40 mg vitamin E / kg feed	1.91 ± 0.067	2.28 ± 0.230
4) 60 mg vitamin E / kg feed	1.90 ± 0.024	2.05 ± 0.130
5) 80 mg vitamin E / kg feed	1.83 ± 0.056	2.11 ± 0.041
6) 100 mg vitamin E / kg feed	1.82 ± 0.063	2.19 ± 0.335
7) 120 mg vitamin E / kg feed	1.84 ± 0.107	2.56 ± 0.684
8) 140 mg vitamin E / kg feed	1.81 ± 0.083	2.16 ± 0.086
9) 160 mg vitamin E / kg feed	1.94 ± 0.197	2.53 ± 0.618
10) 180 mg vitamin E / kg feed	1.75 ± 0.107	2.44 ± 0.631
11) 200 mg vitamin E / kg feed	1.87 ± 0.163	2.33 ± 0.449
Experiment 2 (4°C)		
1) Basal	1.90 ± 0.072	2.33 ± 0.173
2) 40 mg vitamin E / kg feed	1.89 ± 0.073	2.34 ± 0.240
3) 80 mg vitamin E / kg feed	1.85 ± 0.062	2.12 ± 0.035
4) 120 mg vitamin E / kg feed	1.84 ± 0.131	2.70 ± 0.756
5) 160 mg vitamin E / kg feed	1.98 ± 0.223	2.66 ± 0.680

Oxidative stability: TBARS values

The TBARS values for experiment 1 and 2 are given in Table 4. TBARS values increased significantly ($P<0.05$) for both experiments with increasing time of storage (Figures 1 and 2).

Regression equations were fitted to the data and both experiments showed significant linear equations for oxidation over time (Table 5).

Table 4: The effect of storage on the oxidative stability (TBARS values – mg malondialdehyde / kg tissue) of broiler carcasses fed different levels of vitamin E.

	TBA absorbance				
Experiment 1 (-20°C)	**Day 30**	**Day90**	**Day 120**	**Day 150**	
Supplemented throughout trial					
1) Basal	$1.71_a^{e} \pm 0.51$	$3.27_b^{f} \pm 0.51$	$4.73_c^{i} \pm 0.51$	$4.89_c^{f} \pm 0.51$	
2) 20 mg vitamin E / kg feed	$1.07_a^{bd} \pm 0.07$	$2.50_b^{b} \pm 0.07$	$2.72_c^{d} \pm 0.07$	$3.33_d^{b} \pm 0.07$	
3) 40 mg vitamin E / kg feed	$1.00_a^{bcd} \pm 0.10$	$2.29_b^{bc} \pm 0.10$	$2.54_c^{cdh} \pm 0.10$	$2.84_d^{c} \pm 0.10$	
4) 60 mg vitamin E / kg feed	$0.81_a^{ab} \pm 0.10$	$2.06_b^{cd} \pm 0.10$	$2.30_c^{efh} \pm 0.10$	$2.68_d^{c} \pm 0.10$	
5) 80 mg vitamin E / kg feed	$0.76_a^{ac} \pm 0.05$	$1.96_b^{d} \pm 0.05$	$2.06_c^{f} \pm 0.05$	$2.10_c^{d} \pm 0.05$	
6) 100 mg vitamin E / kg feed	$0.62_a^{a} \pm 0.27$	$1.00_{ab}^{d} \pm 0.27$	$1.05_b^{a} \pm 0.27$	$1.09_b^{a} \pm 0.27$	
Supplemented last 3 weeks					
7) 120 mg vitamin E / kg feed	$1.10_a^{b} \pm 0.03$	$2.54_b^{b} \pm 0.03$	$3.18_c^{b} \pm 0.03$	$3.54_d^{b} \pm 0.03$	
8) 140 mg vitamin E / kg feed	$1.03_a^{bc} \pm 0.04$	$2.50_b^{b} \pm 0.04$	$2.65_c^{cd} \pm 0.04$	$2.94_d^{c} \pm 0.04$	
9) 160 mg vitamin E / kg feed	$0.82_a^{ab} \pm 0.15$	$2.20_b^{cd} \pm 0.15$	$2.41_b^{ce} \pm 0.15$	$2.72_c^{c} \pm 0.15$	
10) 180 mg vitamin E / kg feed	$0.79_a^{acd} \pm 0.09$	$2.14_b^{cd} \pm 0.09$	$2.24_{bc}^{ef} \pm 0.09$	$2.28_c^{d} \pm 0.09$	
11) 200 mg vitamin E / kg feed	$0.67_a^{a} \pm 0.26$	$1.61_b^{e} \pm 0.26$	$1.66_b^{g} \pm 0.26$	$1.70_b^{e} \pm 0.26$	
Experiment 2 (4°C)	**Day 0**	**Day 4**	**Day 8**	**Day 10**	**Day 12**
1) Basal	$0.98_a^{a} \pm 0.12$	$1.53_b^{d} \pm 0.23$	$3.09_c^{d} \pm 0.23$	$4.56_d^{e} \pm 0.24$	$4.72_d^{e} \pm 0.24$
2) 40 mg vitamin E / kg feed	$0.89_a^{a} \pm 0.08$	$1.10_b^{a} \pm 0.02$	$2.53_c^{a} \pm 0.02$	$2.75_d^{a} \pm 0.02$	$3.36_e^{a} \pm 0.02$
3) 80 mg vitamin E / kg feed	$0.67_a^{b} \pm 0.09$	$0.98_b^{ab} \pm 0.07$	$2.27_c^{a} \pm 0.07$	$2.52_d^{b} \pm 0.07$	$2.82_e^{b} \pm 0.07$
4) 120 mg vitamin E / kg feed	$0.59_a^{b} \pm 0.16$	$0.79_b^{bc} \pm 0.10$	$1.99_c^{b} \pm 0.02$	$2.09_{cd}^{c} \pm 0.02$	$2.13_d^{c} \pm 0.02$
5) 160 mg vitamin E / kg feed	$0.31_a^{c} \pm 0.08$	$0.59_a^{c} \pm 0.22$	$0.97_b^{c} \pm 0.32$	$1.02_b^{d} \pm 0.22$	$1.06_b^{d} \pm 0.32$

[a]Values in the same column within each experiment with different superscripts differ significantly ($P<0.05$).

[a]Values in the same row within each experiment with different subscripts differ significantly ($P<0.05$).

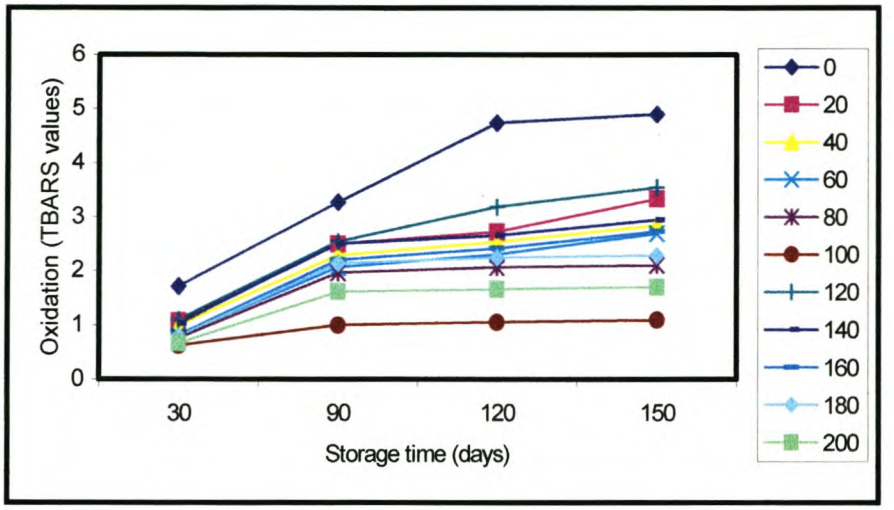

Figure 1: The effect of different levels of vitamin E (mg vitamin E / kg feed) on the oxidative stability (TBARS) of broiler carcasses during storage at -20°C for up to 5 months.

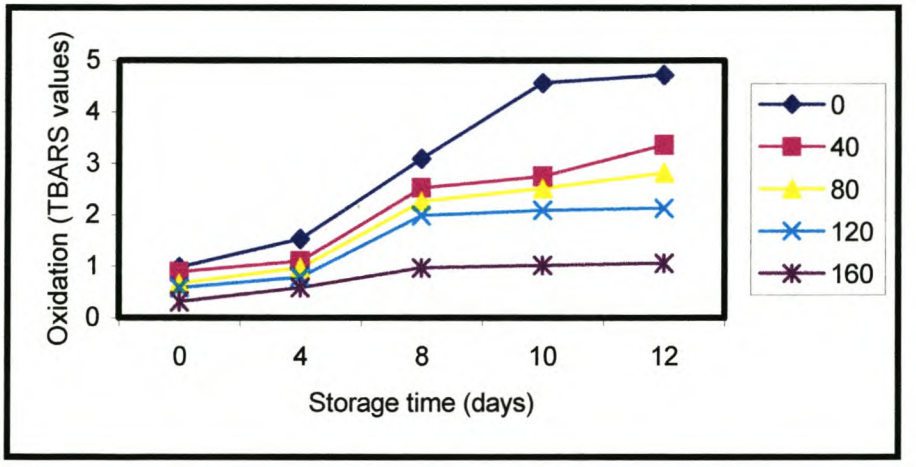

Figure 2: The effect of different levels of vitamin E (mg vitamin E / kg feed) on the oxidative stability (TBARS) of broiler carcasses during storage at 4°C for up to 12 days.

In experiment 1 (frozen storage), the TBARS values of the basal diet was significantly higher than any of the other diets receiving vitamin E. The two diets receiving the highest concentrations of vitamin E (diet 6 – 100 mg vitamin E / kg feed continuously and diet 11 – 200 mg vitamin E / kg feed last 3 weeks) had the lowest TBARS values which did not differ statistically from each other on day 30. By 90 days storage the only TBARS values that had not increased statistically

significantly was that of diet 6. At 150 days storage the TBARS values of this diet had increased, the values differing statistically from that at 30 days storage, but not from 90 days (Table 4). According to the linear regression equation fitted to the data (Table 5), diet 6 showed the slowest rate of increase (0.004 / day) in TBARS values. Generally, the TBARS decreased significantly ($P<0.05$) with an increase in dietary vitamin E levels (Table 4) for both experiments.

In the refrigerated investigation (experiment 2), both the basal diet and diet 2 (40 mg vitamin E / kg feed) had significantly higher TBARS values than the other 3 diets. Diet 5 (160 mg vitamin E / kg feed) had the lowest TBARS values on day 0 as well as for the rest of the experimental period. The TBARS values of diet 5 on day 4 did not differ statistically from that of day 0. On day 8, diet 5 had a TBARS value (0.97 ± 0.32) similar to that of the basal diet on day 0 (0.98 ± 0.12). Diets 3 and 4 had 4-day TBARS values similar and lower (respectively) than that of the basal diet on day 0 (Table 4). Diet 5 also had the slowest rate of increase (Table 5) of TBARS values. As expected, the refrigerated (experiment 2) broiler carcasses had a much higher rate of increase of TBARS values compared to that of the frozen (experiment 1) carcasses (Table 5).

Table 5: Linear regression equations plotting storage time over TBARS values for broilers fed different dietary levels of vitamin E.

Equation: y = a + bx		
where x = storage time (days)	**a = intercept**	
y = TBARS values	**b = slope**	
Experiment 1 (-20°C)	**a ± s.e.**	**b ± s.e.**
1) Basal	0.885 ± 0.331	0.028 ± 0.003
2) 20 mg vitamin E / kg feed	0.609 ± 0.095	0.018 ± 0.001
3) 40 mg vitamin E / kg feed	0.663 ± 0.113	0.015 ± 0.001
4) 60 mg vitamin E / kg feed	0.448 ± 0.101	0.016 ± 0.001
5) 80 mg vitamin E / kg feed	0.598 ± 0.146	0.012 ± 0.001
6) 100 mg vitamin E / kg feed	0.566 ± 0.165	0.004 ± 0.002
7) 120 mg vitamin E / kg feed	0.558 ± 0.077	0.021 ± 0.001
8) 140 mg vitamin E / kg feed	0.724 ± 0.138	0.016 ± 0.001
9) 160 mg vitamin E / kg feed	0.488 ± 0.139	0.016 ± 0.001
10) 180 mg vitamin E / kg feed	0.616 ± 0.170	0.013 ± 0.002
11) 200 mg vitamin E / kg feed	0.552 ± 0.184	0.009 ± 0.002
Experiment 2 (4°C)		
1) Basal	0.639 ± 0.182	0.344 ± 0.023
2) 40 mg vitamin E / kg feed	0.648 ± 0.103	0.217 ± 0.013
3) 80 mg vitamin E / kg feed	0.522 ± 0.085	0.196 ± 0.011
4) 120 mg vitamin E / kg feed	0.497 ± 0.099	0.150 ± 0.012
5) 160 mg vitamin E / kg feed	0.337 ± 0.097	0.067 ± 0.012

Colour deterioration

The CIELAB (Commission International de l' Eclairage, 1976) colour measurements for experiment 2 are given in Table 6. There were no statistical differences (P>0.05) in colour measurements for colours L*, a* or b* among dietary groups. Different regression equations were fitted for each colour over time and all three colours showed statistically significant (P<0.05) cubic regressions over time. However, only 10% of the variation were explained by this regression and it was concluded that the cubic tendency could not be deemed biologically significant.

Table 6: The effect of refrigerated (4°C) storage on the colour deterioration of broiler carcasses fed different levels of vitamin E.

			L*		
Experiment2 (4°C)	**Day 0**	**Day 4**	**Day 8**	**Day 10**	**Day 12**
1) Basal	$46.22_a^a \pm 1.40$	$46.14_a^a \pm 2.42$	$42.48_b^a \pm 1.33$	$43.31_{ab}^a \pm 2.67$	$43.75_{ab}^a \pm 3.10$
2) 40 mg vitamin E / kg feed	$45.70_{ab}^a \pm 2.43$	$44.74_{ab}^a \pm 1.05$	$42.67_a^a \pm 2.74$	$45.58_{ab}^a \pm 1.80$	$46.41_b^a \pm 2.51$
3) 80 mg vitamin E / kg feed	$43.72_a^a \pm 1.45$	$45.21_a^a \pm 3.16$	$43.98_a^a \pm 3.96$	$45.07_a^a \pm 1.23$	$47.59_a^a \pm 2.23$
4) 120 mg vitamin E / kg feed	$47.02_a^a \pm 3.02$	$45.34_a^a \pm 1.39$	$44.22_a^a \pm 3.88$	$43.97_a^a \pm 4.37$	$46.30_a^a \pm 2.58$
5) 160 mg vitamin E / kg feed	$45.61_{ab}^a \pm 2.66$	$42.95_a^a \pm 1.48$	$44.90_{ab}^a \pm 2.06$	$43.39_a^a \pm 1.46$	$47.54_b^a \pm 2.23$
			a*		
	Day 0	**Day 4**	**Day 8**	**Day 10**	**Day12**
1) Basal	$6.07_a^a \pm 2.38$	$3.90_a^a \pm 1.05$	$5.97_a^a \pm 1.43$	$5.61_a^{ab} \pm 0.81$	$4.72_a^a \pm 1.22$
2) 40 mg vitamin E / kg feed	$6.20_a^a \pm 1.38$	$4.10_a^a \pm 0.86$	$6.33_a^a \pm 2.50$	$6.62_a^a \pm 0.84$	$5.13_a^a \pm 2.47$
3) 80 mg vitamin E / kg feed	$5.54_{ab}^a \pm 2.23$	$3.86_a^a \pm 2.16$	$7.00_b^a \pm 2.15$	$4.15_{ab}^b \pm 0.93$	$5.41_{ab}^a \pm 1.71$
4) 120 mg vitamin E / kg feed	$5.12_{ab}^a \pm 0.31$	$3.76_a^a \pm 1.70$	$4.75_{ab}^a \pm 1.69$	$5.95_b^a \pm 1.45$	$4.41_{ab}^a \pm 0.73$
5) 160 mg vitamin E / kg feed	$5.79_a^a \pm 2.42$	$4.47_a^a \pm 0.80$	$5.42_a^a \pm 1.05$	$5.34_a^{ab} \pm 1.16$	$4.79_a^a \pm 0.46$
			b*		
	Day 0	**Day 4**	**Day 8**	**Day 10**	**Day 12**
1) Basal	$13.91_a^a \pm 0.54$	$13.24_{ab}^a \pm 0.99$	$11.81_b^a \pm 1.38$	$13.08_{ab}^a \pm 2.18$	$11.73_b^a \pm 1.17$
2) 40 mg vitamin E / kg feed	$13.23_a^a \pm 1.31$	$13.47_a^a \pm 1.03$	$13.91_a^b \pm 1.27$	$13.31_a^a \pm 1.68$	$10.74_b^a \pm 1.62$
3) 80 mg vitamin E / kg feed	$12.94_a^a \pm 1.42$	$11.96_a^a \pm 1.13$	$13.06_a^{ab} \pm 1.96$	$12.78_a^a \pm 1.16$	$11.61_a^a \pm 1.91$
4) 120 mg vitamin E / kg feed	$13.93_a^a \pm 0.95$	$11.61_a^a \pm 1.89$	$12.82_a^{ab} \pm 0.84$	$12.61_a^a \pm 2.25$	$12.86_a^a \pm 1.63$
5) 160 mg vitamin E / kg feed	$14.33_a^a \pm 1.62$	$11.85_a^a \pm 0.39$	$14.01_a^b \pm 1.08$	$11.67_a^a \pm 2.06$	$12.32_a^a \pm 2.52$

[a]Values in the same column with different superscripts differ significantly (P<0.05).

[a]Values in the same row with different subscripts differ significantly (P<0.05).

Microbiological stability

The aerobic plate counts (APC) of experiment 2 are listed in Table 7. The number of cells increased significantly (P<0.05) over time, regardless of the level of vitamin E (Figure 3; Table 8).

Although some of the APC showed statistical differences with vitamin E inclusion levels at specific storage periods, no trends were noted that could be attributed to dietary factors.

Table 7: The effect of storage on the microbiological spoilage (aerobic plate counts = log 10) of broiler carcasses fed different levels of vitamin E.

Experiment 2 (4°C)	Day 0	Day 4	Day 8	Day 10
1) Basal	$4.49_a^b \pm 0.37$	$7.16_b^a \pm 0.13$	$8.19_b^b \pm 0.17$	$8.90_c^{bc} \pm 0.23$
2) 40 mg vitamin E / kg feed	$5.59_a^a \pm 0.37$	$7.12_b^a \pm 0.39$	$7.89_c^a \pm 0.29$	$8.80_d^a \pm 0.19$
3) 80 mg vitamin E / kg feed	$5.10_a^b \pm 0.28$	$7.15_b^a \pm 0.32$	$7.96_c^{ab} \pm 0.28$	$8.91_d^{ac} \pm 0.21$
4) 120 mg vitamin E / kg feed	$5.05_a^{ab} \pm 0.23$	$7.37_b^a \pm 0.06$	$8.15_c^{ab} \pm 0.11$	$8.40_d^a \pm 0.16$
5) 160 mg vitamin E / kg feed	$5.33_a^{ab} \pm 0.77$	$7.37_b^a \pm 0.15$	$7.60_c^{ab} \pm 0.73$	$8.58_d^b \pm 0.11$

[a]Values in the same column with different superscripts differ significantly ($P<0.05$).

[a]Values in the same row with different subscripts differ significantly ($P<0.05$).

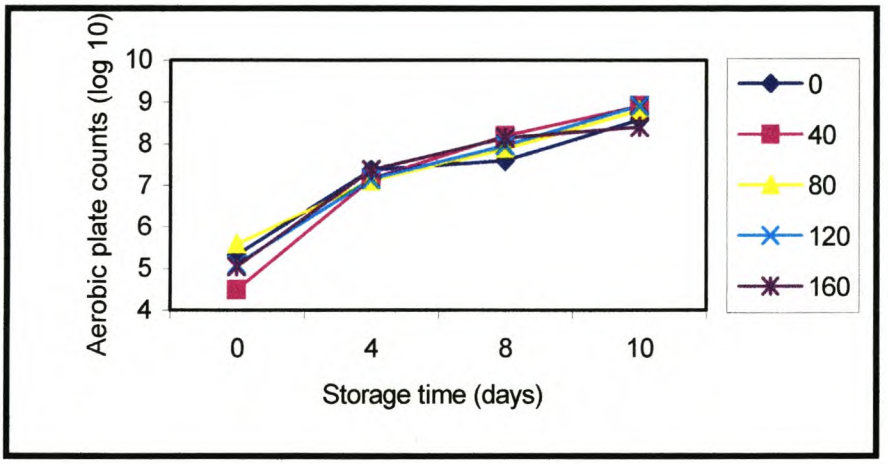

Figure 3: The effect of different levels of vitamin E (mg vitamin E / kg feed) on the microbiological spoilage (aerobic plate counts) of broiler carcasses during storage at 4°C for up to 10 days.

Table 8: Linear equations plotting refrigerated (4°C) storage time over APC for broilers fed different dietary levels of vitamin E.

Equation: y = a + bx		
where x = storage time (days)	**a = intercept**	
y = aerobic plate counts (log 10)	**b = slope**	
Experiment 2 (4°C)	**a ± s.e.**	**b ± s.e.**
1) Basal	5.612 ± 0.279	0.292 ± 0.042
2) 40 mg vitamin E / kg feed	4.844 ± 0.199	0.426 ± 0.030
3) 80 mg vitamin E / kg feed	5.677 ± 0.151	0.304 ± 0.023
4) 120 mg vitamin E / kg feed	5.301 ± 0.163	0.360 ± 0.024
5) 160 mg vitamin E / kg feed	5.441 ± 0.197	0.327 ± 0.029

The samples of day 0 for experiment 2 were also spiked with the following bacteria: *Staphylococcus aureus*, *Salmonella enteritidis* and *Listeria innocua*. Although the data were not statistically analysed, an increase in bacterial counts was seen over time (Table 9). No statistical differences in bacterial counts could be noted among dietary groups for the different periods.

Table 9: The effect of storage on the microbiological spoilage (aerobic plate counts = log 10) of broiler meat samples spiked with *Staphylococcus*, *Salmonella* and *Listeria* spp.

Experiment 2 (4°C)	**Day 1**	**Day 3**	**Day 6**
Staphylococcus			
1) Basal	5.44 ± 0.143	5.75 ± 0.213	5.87 ± 0.810
2) 80 mg vitamin E / kg feed	5.54 ± 0.063	5.86 ± 0.362	5.48 ± 0.675
3) 160 mg vitamin E / kg feed	5.73 ± 0.151	5.50 ± 0.281	6.21 ± 0.435
Salmonella			
1) Basal	7.55 ± 0.103	8.65 ± 0.337	10.24 ± 0.053
2) 80 mg vitamin E / kg feed	7.60 ± 0.175	8.74 ± 0.090	9.56 ± 0.788
3) 160 mg vitamin E / kg feed	7.48 ± 0.000	8.90 ± 0.137	9.85 ± 0.026
Listeria			
1) Basal	7.48 ± 0.000	9.42 ± 0.344	10.38 ± 0.077
2) 80 mg vitamin E / kg feed	7.58 ± 0.081	9.52 ± 0.083	10.54 ± 0.088
3) 160 mg vitamin E / kg feed	7.50 ± 0.117	9.09 ± 0.383	10.32 ± 0.130

Fatty acid analysis

Fatty acid analysis was done for experiment 2 on the samples of days 0 and 12. The aim was to monitor any changes in the fatty acid profile (a potential decrease in unsaturated fatty acids and increase in saturated fatty acids) over time. There were no statistical differences ($P>0.05$) for the fatty acid groups measured between treatments. On day 0 treatment 4 differed significantly from treatments 3 and 5 for both SFA and PUFA, but given the overall tendency of no statistical significance with time, this result may be due to experimental error. None of the individual fatty acids showed statistical significant ($P>0.05$) tendencies to change over time (Appendix 1). Table 10 gives the effect of refrigerated (4°C) storage on the fatty acid profile of the main fatty acid groups: saturated fatty acids (SFA), monounsaturated fatty acids (MUFA) and polyunsaturated fatty acids (PUFA).

Table 10: The effect of refrigerated (4°C) storage on the fatty acid profile of broiler carcasses fed different levels of vitamin E.

Fatty acids*	SFA		MUFA		PUFA	
	Day 0	Day 12	Day 0	Day 12	Day 0	Day 12
Experiment 2 (4°C)						
1) Basal	$26_a^{ab} \pm 0.93$	$26_a^{a} \pm 0.54$	$42_b^{a} \pm 1.49$	$41_b^{a} \pm 2.08$	$32_c^{ac} \pm 1.13$	$34_c^{a} \pm 2.43$
2) 40 mg vitamin E / kg feed	$26_a^{ab} \pm 1.21$	$25_a^{a} \pm 1.68$	$42_b^{a} \pm 2.08$	$41_b^{a} \pm 3.46$	$32_c^{ab} \pm 1.80$	$33_c^{a} \pm 3.67$
3) 80 mg vitamin E / kg feed	$26_a^{b} \pm 0.77$	$26_a^{a} \pm 1.23$	$41_b^{a} \pm 0.96$	$39_b^{a} \pm 2.02$	$34_c^{bc} \pm 1.36$	$35_c^{a} \pm 1.62$
4) 120 mg vitamin E / kg feed	$27_a^{a} \pm 1.14$	$26_a^{a} \pm 1.50$	$42_b^{a} \pm 1.47$	$41_b^{a} \pm 2.12$	$30_c^{a} \pm 1.81$	$33_c^{a} \pm 3.28$
5) 160 mg vitamin E / kg feed	$25_a^{b} \pm 0.91$	$27_a^{a} \pm 0.75$	$40_b^{a} \pm 0.50$	$39_b^{a} \pm 1.71$	$34_c^{b} \pm 1.32$	$34_c^{a} \pm 2.28$

* Fatty acids identified as % of total fatty acids measured.

[a]Values in the same column with different superscripts differ significantly ($P<0.05$).

$_a$Values in the same row with different subscripts differ significantly ($P<0.05$).

pH measurements

In experiment 2, pH measurements were taken 45 min, 2, 4 and 24 hrs post-mortem. There were no statistical differences ($P>0.05$) in pH among dietary groups (Table 11). Different regression equations were fitted over time and the data showed statistically significant ($P<0.05$) cubic regressions over time. However, only 44% of the variation were explained by this regression. Generally, all dietary treatments had a higher final pH24 than that at 45 min post-mortem.

Table 11: The effect of refrigerated (4°C) storage on the pH values of broiler carcasses fed different levels of vitamin E.

	pH values			
	45 min	**2 hrs**	**4 hrs**	**24 hrs**
Experiment 2 (4°C)				
1) Basal	$5.76_c^a \pm 0.225$	$6.07_{ab}^a \pm 0.311$	$6.03_b^a \pm 0.234$	$6.22_a^a \pm 0.236$
2) 40 mg vitamin E / kg feed	$5.71_c^a \pm 0.173$	$6.01_a^a \pm 0.133$	$6.01_a^a \pm 0.176$	$6.23_b^a \pm 0.209$
3) 80 mg vitamin E / kg feed	$5.74_c^a \pm 0.178$	$6.20_a^b \pm 0.144$	$5.98_b^a \pm 0.225$	$6.29_a^a \pm 0.175$
4) 120 mg vitamin E / kg feed	$5.74_d^a \pm 0.211$	$6.09_a^{ab} \pm 0.174$	$5.95_b^a \pm 0.119$	$6.21_c^a \pm 0.199$
5) 160 mg vitamin E / kg feed	$5.68_c^a \pm 0.234$	$6.03_a^a \pm 0.215$	$5.97_a^a \pm 0.286$	$6.23_b^a \pm 0.173$

[a]Values in the same column with different superscripts differ significantly ($P<0.05$).

[a]Values in the same row with different subscripts differ significantly ($P<0.05$).

Discussion

Average daily gain and feed conversion ratios

Table 3 showed that there was no statistical difference ($P>0.05$) in weight gain or food conversion ratios between the different inclusion levels of dietary vitamin E. This result differs from those of other authors. Kennedy et al. (1991) reported the results of an extensive study on the effects of increased vitamin E supplementation on profitable commercial broiler production. A total of 168 broiler flocks containing over 3 million birds were studied. The flocks were fed throughout their lives either normal diets or normal diets supplemented with vitamin E to contain a total concentration of 180 IU/kg feed. In the broiler flocks receiving the supplemented diets, the feed conversion ratio was improved by 0.8% ($P<0.05$) and the average weight per bird was increased by 1.4% ($P<0.05$), compared with those receiving the normal diets. When cost of the additional vitamin E was taken into consideration, the net income per 100 birds was increased by 2.82%. An explanation for the result in Table 3 may be that there was no increase in protein or energy utilisation among birds with an increase in dietary vitamin E. All dietary treatments were balanced for an energy: protein ratio. McIlroy et al. (1993) suggested that high dietary vitamin E may be most beneficial where there is a challenge to the defence system of the host and that significantly improved performance would occur more predictably under such conditions. This investigation was performed in an experimental facility where strict hygienic principles are applied (total mortality was 4.2% and 4.04% for experiment 1 and 2 respectively) and it is doubtful that the birds were challenged immunologically.

Oxidative stability: TBARS values

It is generally believed that lipid oxidation in muscle foods is initiated in the highly unsaturated phospholipid fraction in subcellular membranes (Gray and Pearson, 1987). The autocatalytic

peroxidation process probably begins immediately after slaughter. The biochemical changes that accompany post-slaughter metabolism and post-mortem ageing in the conversion of muscle to meat give rise to conditions where the process of lipid oxidation is no longer tightly controlled and the balance of pro-oxidative factors / antioxidative capacity favours oxidation. The conversion of muscle to meat is a direct result of the cessation of blood flow and the triggering or arresting of many metabolic processes. Orderly metabolic activity continues during the early post-slaughter period, but because of the cessation of blood flow the product of glycogen breakdown becomes lactic acid. This accumulates in the tissue, gradually lowering the pH from near neutrality to a more or less mildly acid (approximately pH 5.5) value. In the post-slaughter phase, it is highly unlikely that the armoury of antioxidant defensive systems available to the cell in the live animal still function because of quantitative changes in several metabolites and physical properties. In many instances, the defensive system may be weakened by nutritional deficiency and the peroxidation process may be greatly accelerated. The rate and extent of oxidation of muscle foods are also likely to be influenced by pre-slaughter events such as stress, and post-slaughter events such as: pH, carcass temperature and cold shortening. In addition, any disruption of the integrity of muscle membranes by mechanical deboning, mincing, restructuring or cooking alters cellular compartmentalisation. This facilitates the interaction of pro-oxidants with unsaturated fatty acids, resulting in the generation of free radicals and propagation of the oxidative reaction (Asghar et al., 1988).

The susceptibility of muscle tissue to lipid oxidation depends on a number of factors, the most important being the level of polyunsaturated fatty acids present in the particular muscle system (Allen and Foegeding, 1981). The phospholipids present in subcellular membranes (mitochondria and microsomes) are high in polyunsaturated fatty acids (Gray and Pearson, 1987) and the vulnerability of membranes to peroxidation is increased because of the close proximity of a range of pro-oxidants.

There is now considerable interest in the antioxidant properties of naturally occurring substances such as vitamin E (Loliger, 1991). Vitamin E, which is usually incorporated in the diet as α-tocopheryl acetate, constitutes the second line of antioxidant defence in biological systems, and is the major lipid-soluble antioxidant, breaking the chain of lipid peroxidation in cell membranes and preventing the formation of lipid hydroperoxides (Halliwell, 1987).

Figures 1 and 2 showed that TBARS values increased significantly ($P<0.05$) with increased time of storage. Oxidative changes in muscle foods are generally quantified by the measurement of secondary degradation products. Data are expressed as Thiobarbituric Acid Reactive Substances (TBARS) numbers. It is accepted that TBARS numbers correlate well with sensory scores of

oxidised and warmed-over flavour in muscle food. TBARS numbers greater than 1 correlate significantly with oxidised scores obtained by trained panellists for meats stored under frozen conditions (Buckley and Morrissey, 1992).

Table 12: Approximate scale for interpretation of TBARS values in meat and meat products (Frigg, undated).

Interpretation of TBARS values	
TBARS values	**Interpretation**
≤ 0.2	Good quality
0.2 – 0.5	Limited, tolerable
0.5 – 1.5	Somewhat oxidised
1.5 – 5.0	Oxidised
> 5	Rancid, non-edible

If a TBARS value cut-off point of 1.5 (Table 12) is used in the linear equations in Table 5, storage times for each of the dietary treatments can be estimated (Table 13).

Table 13: The effect of different dietary vitamin E levels on the storage time of broiler carcasses.

	Storage time (days)
Experiment 1 (-20°C)	
1) Basal	21
2) 20 mg vitamin E / kg feed	49
3) 40 mg vitamin E / kg feed	56
4) 60 mg vitamin E / kg feed	66
5) 80 mg vitamin E / kg feed	75
6) 100 mg vitamin E / kg feed	234
7) 120 mg vitamin E / kg feed	45
8) 140 mg vitamin E / kg feed	49
9) 160 mg vitamin E / kg feed	63
10) 180 mg vitamin E / kg feed	68
11) 200 mg vitamin E / kg feed	105
Experiment 2 (4°C)	
1) Basal	3
2) 40 mg vitamin E / kg feed	4
3) 80 mg vitamin E / kg feed	5
4) 120 mg vitamin E / kg feed	7
5) 160 mg vitamin E / kg feed	17

Table 4 shows that none of the treatments' meat samples were rancid (TBARS value > 5) before the end of the storage time. The samples from diet 1 (experiment 1) was oxidised from the first day of sampling (1 month), showing that broiler carcasses fed no supplemented vitamin E cannot be stored at -20°C for even up to 1 month (Table 13). The samples from treatment 1 (experiment 2) became oxidised at day 3. It is thus preferable to refrigerate broiler carcasses fed no supplemented vitamin E for less than 3 days before use. Only diet 6 from experiment 1 and diet 5 from experiment 2 was sufficiently stable against oxidation for the length of the storage times. The values given for diet 6 of experiment 1 and diet 5 of experiment 2 in Table 13 are only extrapolations of the regression equations and are not to be interpreted as actual storage times. It can however be speculated that by supplementing only 20 mg vitamin E / kg of feed, the storage time of broiler carcasses can be doubled under frozen (-20°C) conditions. In the case of refrigerated carcasses, the storage time can be extended with one day by supplementing only 40 mg vitamin E / kg of feed. However, before firm recommendations on optimum dietary levels can be made, further studies of this type must be carried out in which the chemical measurements of lipid oxidation are related to sensory evaluation using trained sensory panels and untrained consumers.

Table 4 shows that the samples from diets 7 to 10 (fed in last 3 weeks) in experiment 1 became oxidised at a faster rate than those from diets 2 to 5 (fed throughout the trial), although this difference was not significant. However, diet 6 differed significantly from diet 11. It can be speculated that the inclusion time of vitamin E in the feed only becomes valuable when high levels of vitamin E are fed continuously. Bartov and Frigg (1992) showed that oxidative stability was best in tissues from broilers which had received the vitamin E continuously during the trial and presumably had higher α-tocopherol contents in the meat. This further demonstrates the importance of the equilibrium of vitamin E in the tissue, particularly in membrane structures. Measures taken to reduce the propagation of radicals are stochastic processes. This means that antioxidants have a certain chance or probability to trap a radical according to its chemical characteristics, or because of its specific distribution pattern. Therefore, a chemically suitable antioxidant can only act efficiently if it is not too distant from the place of appearance of a radical. Otherwise, the radical could cause severe damage and propagate in a chain-reaction-like manner before being trapped. The antioxidant, for example α-tocopherol, has to be present in an adequate amount and in the right tissue structure. It can thus be speculated that diets 2 to 5, although given throughout the trial, contributed the same amount of vitamin E to the tissues than did diets 7 to 10 which was given only at the end of the trial.

Short-term feeding of broilers with 160 IU α-tocopherol/kg for the last 5 days prior to slaughter was effective in retarding the onset of rancidity in raw whole breast muscle (Marusich *et al.*, 1975) suggesting that a high vitamin E supplement could be given in the finishing diet as an alternative to continuous supplementation. However, considering the relative slow uptake of α-tocopherol into chicken muscle compared with other tissues (Sheehy *et al.*, 1991) and the apparent requirement to lay down the vitamin in specific locations within the muscle membranes for optimum protection, some authors have questioned whether short-term supplementation would guarantee adequate stability in processed muscle. Bartov and Frigg (1992) fed broilers a basal diet containing no added vitamin E for 7 weeks (treatment 1), the basal diet supplemented with 100 mg vitamin E/kg for 7 weeks (treatment 2), the basal diet supplemented with 150 mg/kg to 3 weeks and basal diet alone to 7 weeks (treatment 3), basal diet with 150 mg/kg, 0 and 100 mg/kg to week 3, from weeks 3 to 6 and from 6 to 7 weeks (treatment 4) and the basal diet alone to 5 weeks and then basal diet plus 100 mg/kg to 7 weeks (treatment 5). The stability of meat from treatments 3, 4 and 5 was significantly greater than that from treatment 1 (no vitamin E) but it was significantly lower than that from treatment 2 (vitamin E given continuously). Brandon *et al.* (1993) gave one group of broilers a basal diet containing 30 mg α-tocopheryl acetate/kg feed continuously up to slaughter at 6 weeks, while other groups were given a supplemented diet containing 200 mg α-tocopheryl acetate/kg for 1, 2, 3, 4 or 5 weeks immediately prior to slaughter. The α-tocopherol content of leg and breast muscle increased as the pre-slaughter supplementation period increased from 0 to 5 weeks. Supplementation of broiler diets with 200 mg α-tocopheryl acetate/kg for 5 weeks prior to slaughter improved the oxidative stability of ground muscle during refrigerated and frozen storage and protected against the pro-oxidant effect of salt. These results suggest that a pre-slaughter supplementation period of at least 4-5 weeks, feeding 200 mg α-tocopheryl acetate/kg (i.e. some 20 times higher than the NRC requirement), is necessary to attain the optimum protective benefit of α- tocopherol in processed meat. Short-term supplementation before slaughter can therefore always give a relative improvement, but more can be achieved with steady state conditions.

Colour deterioration

The rate of discolouration of meat is believed to be related to the effectiveness of oxidation processes and enzymic reducing systems in controlling metmyoglobin levels in meat (Chan et al., 1995). There were no statistical differences ($P>0.05$) in colour measurements for L*, a* or b* values (Table 5). This result corresponds with those of other authors. Santé and Lacourt (1994) found that lightness (L*) did not change significantly throughout seven days of storage for turkey meat. Dirinck et al. (1996) found that although supplemented pork samples (200 ppm vitamin E) were redder than control ones the differences were low and non significant. Cannon et al. (1996) reported that vitamin E supplementation reduced lipid peroxidation in fresh pork but did not

influence pork colour. Lanari et al. (1995) reported that, in pork, supplementation had a positive effect only during illuminated storage. The results of this investigation may be due to the fact that colour measurements were taken on the breast muscle (a white muscle) which contains low levels of myoglobin and therefore colour degradation could not be detected.

Microbiological stability

Extended storage accelerated bacterial growth in broilers (Figure 3). In experiment 2, no difference in bacterial load resulted from vitamin E supplementation. These results correspond with data from Chan et al. (1995) and Zerby et al. (1999). Table 7 shows that the microbial counts of the samples of day 0 were already above legal limits ($>10^3$ cfu g^{-1}). This may be the result of slaughter conditions, as the broilers were not slaughtered in a commercial abattoir with strict health regulations.

Fatty acid analysis

There is now considerable emphasis on modification of the fatty acid composition of animal tissues in an attempt to produce new 'designer' or 'functional' foods. However, increasing the degree of unsaturation of the muscle membranes by dietary manipulation reduces oxidative stability (Monahan et al., 1992). Saturated fatty acids, being composed of paraffinic hydrocarbon chains, are generally considered to have very low reactivity. Introduction of one or more double bonds into a fatty acid provides an active centre that can be the site of a variety of reactions – undesirable, as in the case of oxidation. Although even saturated fatty acids can be oxidised, the rate of oxidation greatly depends on the degree of unsaturation. The relative stability of oxidation at 100°C has been given as follows: saturated fatty acids 0.8, oleic acid 1.1, linoleic acid 13.7 and linolenic acid 25.5. In the series of 18-carbon-atom fatty acids – 18:0, 18:1, 18:2, 18:3 – the relative rate of oxidation has been reported to be in the ratio of 1:100:1200:2500 (DeMan, 1992).

There were no statistical differences ($P>0.05$) for any of the fatty acids measured among dietary groups, therefore Table 10 shows only the main fatty acid groups: saturated fatty acids (SFA), monounsaturated fatty acids (MUFA) and polyunsaturated fatty acids (PUFA). As in the case of colour deterioration, with a higher content of PUFA, broiler phospholipids could be more susceptible to oxidation and it is possible that vitamin E may be needed in greater quantities to affect fatty acid stability. None of the fatty acids showed statistical significant ($P>0.05$) tendencies to change over time. It was expected that the longer chain PUFA would have been broken down first into short chain SFA, resulting in fewer PUFA and more SFA on day 12. This was not detected by the fatty acid analysis of day 12. However, it can be postulated that there was breakdown of PUFA, MUFA and SFA (in that order of occurrence), but that the breakdown was at its end by day 12, resulting only in secondary oxidation products. Because the fatty acids were

identified as a % of the total fatty acids measured, no difference could be detected between the three main groups. The results would have been more accurate if the fatty acids were measured as mg fatty acid / g of fat. Inclusion of the samples of days 4, 8 and 10 may also have given a better pattern of fatty acid breakdown.

pH measurements

There were no statistical differences ($P>0.05$) in pH among dietary groups (Table 11). The data showed a statistically significant ($P<0.05$) cubic tendency over time. At 45 min post-slaughter the pH values were approximately 5.5 (Table 11). This is the result of irreversible anaerobic glycolysis which occurs when oxygen is permanently removed from the muscle at death (Lawrie, 1991). The resultant lactic acid production causes the pH to fall to a low of 5.5. From 45 min to 2 hrs post-mortem there was a slight increase in pH values, which was related to deamination reactions, as reported by Gill (1986). Deamination of amino acids, with liberation of ammonia from ammoniac, results in the increase in pH. However, this reaction is reversible and the release of the hydrogen atom from ammoniac causes the pH to fall from 2 to 4 hrs post-mortem (Table 11). At 24 hrs post-mortem lactic acid degradation stops while deamination continues, resulting in an increase of pH (Table 11).

Conclusion

The present investigation focussed primarily on the effect of vitamin E on the oxidative stability and quality of broiler meat. It was found that dietary supplements of vitamin E to broiler feed increases oxidative stability of broiler carcasses under frozen and refrigerated storage. Non-supplemented carcasses could not even be stored under frozen conditions for up to one month, whereas non-supplemented refrigerated carcasses could only be stored for three days. Under frozen conditions supplementation of as little as 20 mg vitamin E / kg of feed doubled the storage time, whereas supplementation of 40 mg vitamin E / kg feed extended storage time by one day in refrigerated broiler carcasses. If this concentration is increased to 160 mg vitamin E / kg feed, a TBARS value similar to that of broilers receiving no vitamin E will only be reached after 8 days of storage at 4°C. This has huge economic implications for the retail industry. This investigation further showed that vitamin E supplementation under these conditions had no significant effect on broiler performance, microbial spoilage, colour deterioration, fatty acid composition or post-mortem pH changes.

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Appendix 1

Table 1: The effect of refrigerated (4°C) storage on the fatty acid profile of broiler carcasses fed different levels of vitamin E.

SFA	C14:0		C16:0		C18:0	
	Day 0	Day 12	Day 0	Day 12	Day 0	Day 12
1) Basal	0.98 ± 0.14	0.95 ± 0.20	19.2 ± 0.45	18.9 ± 0.52	5.73 ± 0.61	5.80 ± 0.18
2) 40 mg vitamin E / kg feed	0.86 ± 0.16	0.95 ± 0.30	18.8 ± 0.79	18.2 ± 1.42	6.24 ± 0.43	5.95 ± 0.71
3) 80 mg vitamin E / kg feed	0.93 ± 0.07	0.99 ± 0.14	18.6 ± 0.95	18.7 ± 0.88	5.98 ± 0.44	6.39 ± 0.80
4) 120 mg vitamin E / kg feed	0.86 ± 0.14	0.94 ±0.24	20.2 ± 0.87	19.3 ± 1.34	6.07 ± 0.67	5.99 ± 0.41
5) 160 mg vitamin E / kg feed	0.96 ± 0.11	0.98 ± 0.17	18.2 ± 0.99	18.8 ± 1.57	5.97 ± 0.63	6.48 ± 0.86

MUFA	C16:1n7		C18:1n9		C20:1n9	
	Day 0	Day 12	Day 0	Day 12	Day 0	Day 12
1) Basal	5.19 ± 0.86	5.31 ± 0.25	36.2 ± 1.11	34.7 ± 2.01	0.53 ± 0.08	0.51 ± 0.04
2) 40 mg vitamin E / kg feed	4.61 ± 0.38	5.51 ± 1.21	36.4 ± 1.95	35.4 ± 2.53	0.58 ± 0.07	0.52 ± 0.03
3) 80 mg vitamin E / kg feed	5.26 ± 0.30	5.00 ± 0.52	35.0 ± 1.01	33.6 ± 1.55	0.52 ± 0.06	0.52 ± 0.04
4) 120 mg vitamin E / kg feed	5.37 ± 0.87	4.57 ± 0.64	36.5 ± 0.77	35.5 ± 1.58	0.56 ± 0.10	0.49 ± 0.03
5) 160 mg vitamin E / kg feed	4.99 ± 0.59	5.12 ± 1.32	34.9 ± 0.81	33.6 ± 1.40	0.52 ± 0.04	0.56 ± 0.11

PUFA	C18:2n6		C18:3n3		C20:4n6		C20:5n3		C22:6n3	
	Day 0	Day 12	Day 0	Day 12	Day 0	Day 12	Day 0	Day 12	Day 0	Day 12
1) Basal	27.1 ± 0.73	29.1 ± 1.71	1.61 ± 0.13	1.48 ± 0.07	0.96 ± 0.15	0.89 ± 0.31	0.45 ± 0.07	0.43 ± 0.09	0.65 ± 0.09	0.62 ± 0.22
2) 40 mg vitamin E / kg feed	27.6 ± 1.57	28.2 ± 2.93	1.56 ± 0.11	1.50 ± 0.29	0.90 ± 0.25	1.04 ± 0.31	0.42 ± 0.04	0.41 ± 0.12	0.65 ± 0.05	0.77 ± 0.21
3) 80 mg vitamin E / kg feed	29.2 ± 1.09	30.1 ± 1.41	1.49 ± 0.07	1.56 ± 0.06	0.75 ± 0.14	0.84 ± 0.13	0.38 ± 0.08	0.43 ± 0.06	0.67 ± 0.14	0.61 ± 0.09
4) 120 mg vitamin E / kg feed	25.5 ± 1.45	28.5 ± 2.64	1.46 ± 0.21	1.53 ± 0.15	1.06 ± 0.32	0.89 ± 0.27	0.41 ± 0.08	0.43 ± 0.14	0.70 ± 0.09	0.64 ± 0.12
5) 160 mg vitamin E / kg feed	29.5 ± 1.11	29.5 ± 1.79	1.47 ± 0.13	1.52 ± 0.14	0.90 ± 0.15	1.00 ± 0.46	0.41 ± 0.10	0.40 ± 0.06	0.70 ± 0.08	0.61 ± 0.05

General conclusions

Lipids remain one of the most important nutrients required by broilers. The growing awareness that some Western societies have too high a dietary ratio of n-6/n-3 polyunsaturated fatty acids is of direct relevance to broiler nutrition and lipid metabolism. Meaningful quantities of n-3 polyunsaturates have been incorporated into major poultry tissues, so that the production of broiler meat with high n-3 polyunsaturates becomes advantageous for the broiler industry as they are perceived as having a 'healthier' lipid profile.

In this investigation Canola acid oil (high n-3 polyunsaturated content) and Famarol acid oil (high n-6 polyunsaturated content) were selected as the experimental oils. In part two of the investigation it was shown that Canola acid oil, having a higher unsaturated:saturated fatty acid ratio than Famarol acid oil, also has a higher TMEn value than Famarol acid oil and yields therefore more energy available for the broiler per kg of feed. Canola acid oil would thus be a more "nutritious" substitute for Famarol acid oil in commercial broiler diets.

In part 3 of this investigation it was shown that dietary Canola acid oil could elevate the level of n-3 polyunsaturated fatty acids in broiler carcasses and abdominal fat pads. An increase in the human dietary n-3/n-6 fatty acid ratio is essential in today's Western diet to help prevent coronary heart disease by reducing plasma lipids (Kinsella et al., 1990). The results of this experiment showed that substitution of Famarol oil with Canola oil in broiler diets can increase the ratio of n-6 to n-3 fatty acids in broiler carcasses and abdominal fat pads to 5:1, a ratio more suitable for human health. Increasing the level of n-3 fatty acids in the diets was also effective in reducing the level of saturated fatty acids in the carcasses and abdominal fat pads of broiler chickens resulting in "healthier" chickens. The increased n-3 fatty acids also suppressed the formation of long-chain n-6 PUFAs derived from linoleic acid ensuring a more balanced eicosanoid metabolism (Harris, 1989). Overall, in view of the prevalence of human coronary heart disease, consumption of n-3 PUFA enriched broilers could be considered as a useful complementary option for the amelioration of coronary vascular disease. Unfortunately, such broiler meat is rather susceptible to oxidative deterioration, and oxidation often determines shelf life of poultry meat products. The addition of α-tocopherol (vitamin E) to broiler diets is an effective means of improving the oxidative stability of broiler meat. Elevated α-tocopherol levels in broiler feeds increase tissue concentrations thereof resulting in improved stability of membranal structures which may be expected to increase the oxidative stability of broiler meat and meat products. Part four of this investigation focussed primarily on the effect of vitamin E on the oxidative stability and quality of broiler meat. It was found that dietary supplements of vitamin E to broiler feed increases oxidative stability of broiler

carcasses under frozen and refrigerated storage. Non-supplemented carcasses could not even be stored under frozen conditions for up to one month, whereas non-supplemented refrigerated carcasses could only be stored for three days. Under frozen conditions supplementation of as little as 20 mg vitamin E / kg of feed doubled the storage time, whereas supplementation of 40 mg vitamin E / kg feed extended storage time by one day in refrigerated broiler carcasses. If this concentration is increased to 160 mg vitamin E / kg feed, a TBARS value similar to that of broilers receiving no vitamin E will only be reached after 8 days of storage at 4°C. This has huge economic implications for the retail industry. This investigation further showed that vitamin E supplementation under these conditions had no significant effect on broiler performance, microbial spoilage, colour deterioration, fatty acid composition or post-mortem pH changes.