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4 Dietary sources of unsaturated fatty acids for animals and their subsequent availability in milk, meat and eggs

A summary of research findings

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1 Summary

Meat has been criticised in the past for its high content of saturated fatty acids, which are damaging to health. Modification of diets offered to animals can allow beneficial unsaturated fatty acids to pass into meat and also milk and eggs. Unsaturated fatty acids are believed to have positive implications for human health by reducing the risk of cardiovascular disease and they may also reduce the incidence of some cancers, asthma, diabetes and a number of other conditions. The possibility of consuming a greater proportion of these fatty acids as part of an every day diet will appeal to the public, rather than consuming dietary supplements. This report encompasses a review of the literature in relation to dietary sources of beneficial fatty acids (linseed, hemp, chia seed, marine algae, fish oil/meal, lupins, oilseed rape, soya, grass and clover) available for feeding animals and their subsequent availability in milk, meat (beef, lamb, pork, poultry) and eggs. Overall, inclusion of these feeds in the diet improves the fatty acid profile of milk, meat and eggs by increasing conjugated linoleic acid levels and decreasing the ratio of *n*-6:*n*-3 fatty acids. Care must be taken when introducing these feeds into diets as some may reduce milk yield, fat and protein concentrations in dairy cows. Furthermore, increased levels of polyunsaturated fatty acids in meat can occasionally lead to a more rapid colour change from red to brown, due to oxidation. The effects of inclusion of polyunsaturated fatty acids on flavour parameters in meat are not clear cut, with some authors finding no effects and others reporting deleterious effects. Further research is required on the effect of fatty acid enrichment of animal diets on meat eating quality. Producing for niche markets will become increasingly essential in future farming systems as a consequence of the Common Agricultural Policy. The development of specialist animal diets rich in unsaturated fats could play a valuable role in creating niche markets for animal products. However, consumer perception and acceptance will be the driving force behind the success of niche market products.

2 Introduction

Fats are mainly triglycerides, formed from fatty acids and glycerol. Fatty acids modulate lipid metabolism and other physiological systems that shape risk factors for a number of chronic diseases. The effects on health can be beneficial or harmful, depending on the specific fatty acids and the mix of fatty acids in the diet and the body.

Omega-3 (*n*-3) fatty acids have been, and continue to be subject to extensive research, regarding their potential benefits for human health. Two important recent reviews are those of Ruxton *et al*. (2004), who provided a review of evidence for *n*-3 polyunsaturated fatty acids (PUFA) linked health benefits and Wang and Jones (2004), who examined the role of conjugated linoleic acid (CLA) for human and animal health. However, not all the evidence is wholly positive and a major review in America of research findings by the Agency for Healthcare Research and Quality (AHRQ), (2004) provides balanced reports on fatty acids and their efficacy or not, on an array of medical conditions. The British Nutrition Foundation (2000) has recommended a minimum daily intake level of long chain PUFA of between 1.5-3 g/day for adults and children.

This report provides a detailed review of the literature on dietary fatty acid sources for beef cattle, sheep, pigs, poultry and dairy cows and the subsequent availability of the beneficial fatty acids in meat, eggs or milk.

3 Fatty acid classification

Fatty acids are classified according to carbon chain length and degree of saturation (as defined by the number of double bonds in the molecule). Nutritionists also identify which "omega" family an unsaturated fatty acid belongs to, *n*-3, *n*-6 or *n*-9, by the position of the first double bond on the carbon chain counting from the methyl end of the molecule. Fatty acids can be classified as saturated (no double bonds), monounsaturated (one double bond) or polyunsaturated (PUFA, two or more double bonds).

3.1 Common fatty acids

A list of the more common fatty acids is shown in Table 1 and their chemical configuration is shown in Figures 1-8.

Table 1 Common fatty acids and their abbreviations

Common name	Abbreviation
Myristic acid*	C14:0
Palmitic acid*	C16:0
Stearic acid*	C18:0
Oleic acid	C18:1
Linoleic acid	C18:2
Linolenic acid	C18:3
Eicosapentaenoic acid (EPA)	C20:5
Docosapentaenoic acid (DPA)	C22:5
Docosahexaenoic acid (DHA)	C22:6

* Saturated fatty acid

Figure 1 Myristic acid (C14:0)

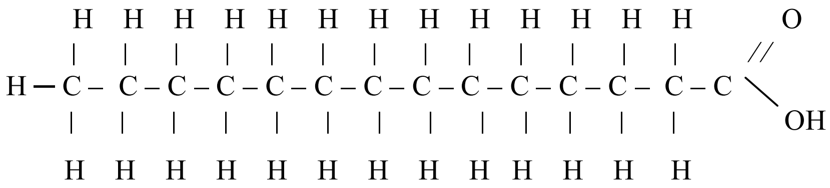


Figure 2 Palmitic acid (C16:0)

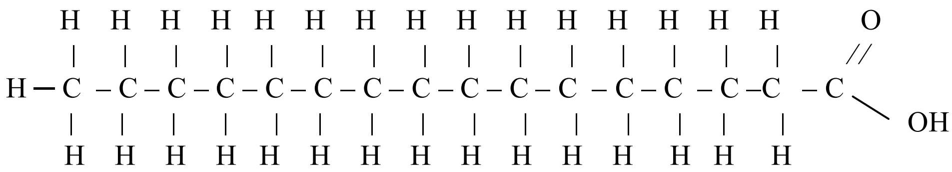


Figure 3 Stearic acid (C18:0)

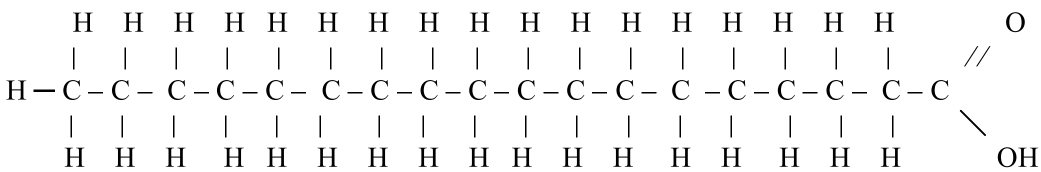


Figure 4 Oleic acid (C18:1)

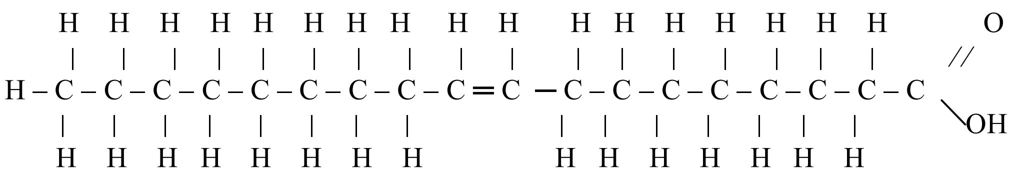


Figure 5 Linoleic acid (C18:2)

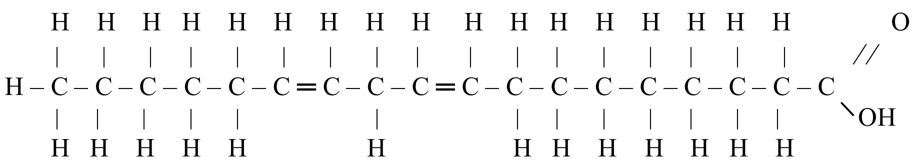


Figure 6 Linolenic acid (C18:3)

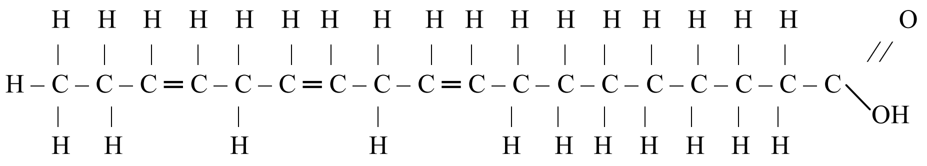


Figure 7 EPA (Eicosapentaenoic acid) (C20:5)

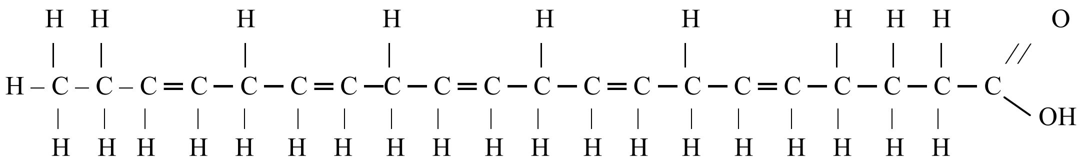
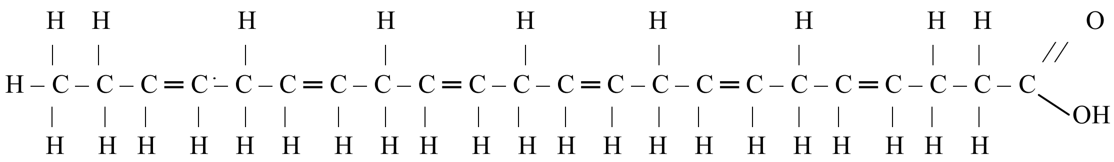


Figure 8 DHA (Docosapentaenoic acid) (C22:5)



3.2 Saturated fatty acids

Saturated fatty acids, C12:0–C16:0 are those that have no double bonds and are considered as the 'bad' fatty acids, in that they increase serum cholesterol in humans (Bruckner, 1992). Stearic acid (C18:0) is a saturated fatty acid that has different biological effects than other saturated fatty acids and is considered to have a neutral effect on cholesterol levels. Its main sources are in animal fats, vegetable oils and chocolate (Dietary Guidelines Advisory Committee Report, 2005).

3.3 Unsaturated fatty acids

Unsaturated fatty acids, with double bonds, are the more 'beneficial' fatty acids in terms of human health, and have many health benefits attributed to them. The main sources of unsaturated fatty acids are vegetable oils although there are also some in animal products, such as meat and dairy products. Unsaturated fatty acids can be further broken down into monounsaturated fatty acids (MUFA) or PUFA as follows.

3.3.1 Monounsaturated fatty acids

Monounsaturated fatty acids contain only one double bond in their chemical composition. Vegetable oils e.g. canola oil, olive oil, high oleic safflower and sunflower oils and nuts are rich in MUFA (Dietary Guidelines Advisory Committee Report, 2005).

3.3.2 Essential Fatty Acids

Essential fatty acids (EFA) are required for the formation of healthy cell membranes, for proper development and functioning of the brain and nervous systems, and for the production of hormone-like substances called eicosanoids. Eicosanoids regulate many body functions such as blood pressure, blood viscosity, vasoconstriction, immune and inflammatory responses. The *n*-3 and *n*-6 fatty acids are EFA, as they cannot be produced within the human body and must come from dietary sources. Research from a review by Williams (2000) suggests that a deficiency of *n*-3 PUFA may be linked with hypertension, inflammatory disorders, immune disorders, depression and neurological dysfunction.

3.3.3 Polyunsaturated fatty acids

Polyunsaturated fatty acids have two or more double bonds, and may be of two types, based on the position of the first double bond as follows:

3.3.3.1 n-6 PUFA linoleic acid

This is one of the omega-6, or *n*-6 fatty acids that is required by humans but cannot be made in the human body. It is therefore considered essential in the diet. Linoleic acid is an 18-carbon chain with 2 double bonds, and as the closest double bond to the methyl group in linoleic acid is 6 carbon atoms away from the methyl group, linoleic acid is called an *n*-6 fatty acid. A deficiency of dietary *n*-6 PUFA is characterised by rough, scaly skin and dermatitis (Dietary Guidelines Advisory Committee Report, 2005). The main sources of linoleic acid are liquid vegetable oils including soybean oil, corn oil and safflower oil.

3.3.3.2 n-3 PUFA α-linolenic acid

This is an omega-3, or *n*-3 fatty acid that has to be taken into the human body via dietary means because it is not synthesized by humans. It is therefore considered essential in the diet. α-linolenic acid is an 18-carbon chain with 3 double bonds. In α-linolenic acid, the double bond closest to the methyl group is only 3 carbons away, so it is an *n*-3 fatty acid. The number of double bonds in a fatty acid affects their function, in that the greater the number of double bonds present, the lower the melting point of the fat. The *n*-3 PUFA have positive implications for human health and have been described as the 'beneficial' fatty acids in the past (Chow, 1992; Sargent, 1996).

A deficiency of α-linolenic acid in the diet can result in symptoms including scaly and hemorrhagic dermatitis, hemorrhagic folliculitis of the scalp, impaired wound healing and growth retardation (Dietary Guidelines Advisory Committee Report, 2005). α-linolenic acid is obtained from plant sources including soybean oil, canola oil, walnuts and flaxseed. Polyunsaturated fatty acids are less oxidatively stable than MUFA. Relative oxidation rates of linolenic (C18:3):linoleic (C18:2):oleic (C18:1):stearic (C18:0) are described as 150:100:10:1 (Sonntag, 1979).

3.3.4 Docosahexaenoic acid (DHA) and Eicosapentaenoic acid (EPA)

Fish that naturally contain more oil e.g. salmon, tuna, trout are higher in EPA and DHA than lean fish e.g. cod, haddock, flounder (Dietary Guidelines Advisory Committee Report, 2005). Docosahexaenoic acid and EPA have been shown to assist in the control of cardiovascular disease (Simopoulos, 1999), as well as being therapeutic for arthritis (Kremer, 2000), autoimmune disease (Harbige and Fisher, 2001), inflammatory effects (Grimm *et al*., 2002) and depression (Puri *et al*., 2001).

Docosahexaenoic acid is an *n*-3 fatty acid that is found in fish such as tuna, salmon and mackerel. Docosahexaenoic acid is very important for human health. A hexaenoic acid has 6 double bonds. Eicosapentaenoic acid is an *n*-3 fatty acid that is commonly found in oily cold water fish, for example tuna, salmon and mackerel. It is seldom that plant foods contain EPA. Eicosapentaenoic acid is very important for human health and has 5 double bonds.

3.3.5 Ratio of *n*-3:*n*-6 fatty acids

In recent years, the view has developed that the ratio of *n*-3 and *n*-6 fatty acids is important for human health. Simopoulos (2000) stated that appropriate quantities of *n*-6 and *n*-3 fatty acids in the diet should be considered when making dietary recommendations for humans. This author also suggested that a balanced ratio of *n*-6 and *n*-3 fatty acids is required for normal growth and development in addition to possibly reducing cardiovascular disease and other chronic diseases along with improving mental health. Furthermore, Wijendran and Hayes (2004) stated that the balance between *n*-6 and *n*-3 fatty acids is important in influencing cardiovascular health.

In the Western world, where people consume large quantities of cereals, bread, fried foods and margarine, the diet consists mainly of *n*-6 fatty acids. Simopoulos (1999) reported an *n*-6:*n*-3 ratio of human diets in the Western world ranging from 20-30:1 and suggested that Western diets lack *n*-3 fatty acids in comparison to the diet with which humans evolved on. Hals *et al*. (2000) reported that the ideal *n*-6:*n*-3 ratio in children has yet to be determined. In the world review of nutrition and dietetics by Simopoulos (2003), the scientific evidence available regarding the importance of *n*-6:*n*-3 in the prevention and management of different diseases and conditions was considered. It was stated that increased quantities of *n*-3 fatty acids in the human diet exert suppressive effects on diseases such as cardiovascular disease, cancer, inflammatory and autoimmune diseases. It was stated that in the secondary prevention of cardiovascular disease, an *n*-6:*n*-3 ratio of 4:1 was linked with a 70% decrease in mortality and a ratio of 4:1 appears optimal for brain function. A ratio of *n*-6:*n*-3 of 2.5:1 reduced rectal cell proliferation in patients suffering from colorectal cancer. This review also stated that an *n*-6:*n*-3 ratio of 2-3:1 suppressed inflammation in patients with rheumatoid arthritis and a ratio of 5:1 was beneficial to asthma patients, whereas a ratio of 10:1 had adverse effects. It was concluded that based on these examples, the optimal ratio of *n*-6:*n*-3 may vary according to different diseases or conditions.

3.3.6 *Cis* and *trans* fatty acids

Fatty acid double bonds are of two configurations, namely *cis* (carbon chains on the same side of a double-bond) and *trans* (carbon chains on the opposite side of a double-bond). The majority of double bonds made by biological systems have the *cis* configuration (http://www.benbest.com/health/essfat.html#bdy).

There are two main categories of *trans* fatty acids, namely those that are man-made and those which are naturally occurring. Conjugated linoleic acid is a mixture of *cis* and *trans* fatty acids. Man-made *trans* fats have health concerns associated with them, as they raise low density lipoprotein (LDL) cholesterol levels (Mensink and Katan, 1990) and lower high density lipoprotein (HDL) cholesterol levels (Williams, 2000), therefore increasing the risk of coronary heart disease in humans.

Saturated fats (e.g. butter or lard) and foods having fatty acids with *trans* double bonds (e.g. margarine) tend to be solids at room temperature. However, natural fatty acids with *cis* double bonds (e.g. vegetable oils) tend to be liquids.

Trans fatty acids are unsaturated fatty acids that contain at least one double bond in the *trans* configuration. Elaidic acid (C18:1) is the predominant *trans* fatty acid in processed fats, such as margarine and is produced from the chemical hydrogenation of fats. The predominant naturally occurring *trans* fatty acid is *trans*-vaccenic acid (C18:1). Some studies have shown milk fat depression in cow's milk when *trans* fatty acids were offered to cows (Gaynor *et al*., 1994; Loor and Herbein, 1998). *Trans* vaccenic acid and CLA are intermediate products in the ruminal biohydrogenation of dietary unsaturated fatty acids, and as such, will be found in ruminant products such as beef, lamb and dairy products.

4 Cholesterol and Lipoproteins

Cholesterol and triglycerides are packaged into lipoprotein particles for transport in the circulation system. The composition and properties of the different lipoprotein fractions varies substantially. The predominant lipoprotein particles are chylomicrons, very-low density lipoproteins (VLDL), low density lipoprotein (LDL) and high density lipoprotein (HDL).

4.1 Lipoproteins

Low-density lipoprotein, or 'bad' cholesterol, is the main cholesterol carrier in the body. Too much LDL cholesterol in the blood stream of humans can accumulate and contribute to atherosclerosis. An overabundance of LDL can lead to the formation of plaque, the accumulation of which on arterial walls, contributes to a decreased arterial diameter. The American Heart Association describes an LDL cholesterol level of 160 mg/dL or more as high.

High-density lipoprotein, or 'good' cholesterol, carries one-third to one quarter of the cholesterol in the human blood. Experts think that HDL cholesterol may help remove excess cholesterol and carry it away from the artery. It appears that high levels of HDL prevent heart disease and the American Heart Association recommends a HDL level of >40 mg/dL.

Cholesterol is transported in the blood, mainly by the lipoproteins LDL, HDL, and VLDL, whilst chylomicrons transport dietary cholesterol absorbed from the intestine. A high total cholesterol concentration is known to be a major risk factor for coronary heart disease (Dietary Guidelines Advisory Committee Report, 2005). Patients with coronary heart disease have significantly elevated levels of oxidised LDL and the general belief is that LDL-cholesterol needs to be lowered to achieve a reduction in the incidence of coronary heart disease in humans (Hornung, 2002). A study by the American Heart Association found that cholesterol reduction is effective in reducing the incidence of coronary heart disease, but a reduction of 8-9% would be required to lower total mortality rates (Holme, 1990).

4.2 Triglycerides

Triglycerides are comprised of three fatty acids plus glycerol. They constitute the main constituents of fats and oils. Triglycerides circulate in the blood in the form of lipoproteins, mainly in chylomicrons and VLDL.

5 Evidence of the effects of fatty acids on human health

5.1 Coronary heart disease/Cardiovascular disease

There is a positive relationship between the serum triglyceride value and the incidence of coronary heart disease in humans (Dietary Guidelines Advisory Committee Report, 2005). High levels of triglycerides in the blood are positively correlated with metabolic syndrome, which is a condition that increases the risk of cardiovascular disease in humans (Dietary Guidelines Advisory Committee Report, 2005). Four years ago, Ford *et al*. (2002) recognised that the increasing prevalence of metabolic syndrome in the USA (1 in 4 individuals) had important public health implications.

McLeod *et al*. (2004) presented a review of research evidence supporting claims that CLA enriched diets provided protection against the risk of heart disease. The American Heart Association (2004) cite an observational study by Iso *et al.* (2001) with 76,865 nurses over a 20 year period, which suggested that nurses with the highest level of *n*-3 consumption had a reduced risk of cardiac arrest and heart disease. Calder (2004) described a number of references from epidemiological and case-control studies, which indicated that the consumption of fish or long chain *n*-3 fatty acids reduced the risk of death from cardiovascular disease in Western populations. However, Calder (2004) also noted that not all studies were in agreement with this finding.

A long term randomised clinical trial was conducted by the American Medical Association, to compare the effects of lean red meat (beef, veal and pork) vs. lean white meat (poultry and fish) on serum lipid levels among free-living persons with hypercholesterolemia (Davidson *et al*., 1999). In this study, 191 men and women were counselled to follow a National Cholesterol Education Program. Step one of this program involved the inclusion of 6 oz of lean meat in their diet per day, for 5-7 days per week. These people were randomly assigned to consume at least 80% of their meat in the form of lean white meat or lean red meat. Serum lipid levels were then measured 4, 12, 20, 28 and 36 weeks after randomisation. Results from the trial indicated that people offered lean white meat or lean red meat had similar reductions in the quantity of LDL cholesterol produced and a similar increase in the quantity of HDL cholesterol produced. These levels were maintained throughout the

36 weeks of the trial in people offered lean red or white meat. Davidson *et al*. (1999) also found that the leanness of meat was a more important factor over and above whether the meat was white or red, in relation to serum cholesterol levels in humans.

5.2 Cancer

The evidence of a relationship between total fat intake and certain cancers is suggestive but not conclusive. Data from both animal and clinical investigations have suggested that diets high in total fat, protein, calories, alcohol, and meat (red and white) and low in calcium and folate are associated with an increased incidence of colorectal cancer (http://www.cancer.gov/cancerinfo/pdq/prevention).

In general, fat of animal origin seems to be associated with the highest risk of prostate cancer. Researchers in Australia have found that PUFA, including α-linolenic acid are effective in reducing the growth of human prostate cancer cell lines (Attar-Bashi *et al*., 2002). However, possible negative effects of CLA enrichment on animal models of human colon and prostate cancer cell lines were reported by Wahle *et al*. (2004). These authors suggested that more research was necessary to allow a critical evaluation of the effects of CLA on human health.

In an analysis of the link between nutrition and cancer, Bingham and Riboli (2004) noted a difficulty in judging whether fat intake in the diet is a risk factor for breast cancer in humans. Equally, in a review of the literature by Kushi and Giovannucci (2002), it seems that total fat intake is not linked with the risk of breast cancer. Holmes *et al*. (1999) also reported no link between total dietary fat intake and breast cancer in humans.

However, CLA has been shown to be a potent anti-carcinogen in various cancer models. Dietary intake of CLA has been implicated in a reduced risk of breast cancer. Researchers in Moscow and Finland have stated that enhancing the concentration of CLA in bovine milk would have positive effects on human health and improve the healthy image of milk (McGuire and Griinari, 2003).

Ip *et al.* (2003) reported that CLA had inhibitory effects on cancer cell proliferation using laboratory rats with induced mammary carcinogenesis and suggested that CLA could be considered important in breast cancer prevention. Cho *et al.* (2003) conducted *in vitro* studies and demonstrated CLA inhibition of a human colon cancer cell line. Other positive effects of *n*-3 fatty acids on human cancer cell lines have been reported by Maggiora *et al.* (2004) and Song *et al*. (2004).

5.3 Brain function

In a review of the health benefits of *n*-3 fatty acids by Ruxton *et al*. (2004), brain development and function were examined. These authors reported that the human nervous system contains a significant quantity of DHA. Animal studies have been conducted to examine the role of DHA in brain development where monkeys offered a diet that was deficient in *n*-3 PUFA had a poor visual acuity and an increased incidence of stereotypys, thus suggesting an impaired brain development (Ruxton *et al*., 2004). Yehuda *et al*. (1999) described how *n*-3 PUFA are vital for an optimal brain function. Work with premature babies demonstrated an accumulation of DHA in the brain tissue of the foetus during the last trimester of pregnancy (Clandinin *et al*., 1980). Otto *et al*. (1997) indicated that a further foetal requirement for *n*-3 PUFA is met by the placenta and if expectant mothers are deficient in *n*-3 PUFA, this deficiency can be passed onto their offspring. Connor *et al*. (1996) showed that pregnant mothers who consumed sardines or fish oil on a regular basis had an elevated plasma DHA that would transfer to the foetus. After birth, the mother plays a role in providing her child with DHA via breast milk. In animal models, it appears that a deficiency of DHA can result in an impaired brain development. It was also noted that human evidence for this statement is deficient. Jørgensen *et al*. (2001) reported a positive correlation between fish consumption of mothers and an improved visual acuity of their children. Daniels *et al*. (2004) reported that mothers who ate fish four times weekly during pregnancy had babies with greater developmental scores at 18 months of age, in comparison to children from mothers who ate no fish. In a clinical trial with pregnant women, Helland *et al*. (2003) reported a relationship between *n*-3 PUFA intake and infant IQ.

Dairy Crest launched 'Brainy milk' on 26 May 2005, under the name of St Ivel Advance, the first milk in the United Kingdom (UK) to be enriched with *n*-3 PUFA. This milk will be sold in Waitrose supermarkets at 79 pence/litre or £1.45/2 litres, after which it will be available on Tesco's shelves, among other supermarkets. It has been reported that suppliers of this milk will not receive any additional revenue as the milk is enriched with *n*-3 PUFA during the processing of the milk. In May 2005, a trial was conducted with children offered fish oil supplements by researchers at Oxford University. Data from this study indicated that children who consumed fish oil supplements for three months had a significantly improved behavior and significantly improved ability to read and spell. Marks and Spencer also launched an *n*-3 rich milk called 'Super whole milk', which contains 10 times more EPA/DHA (48 mg) than regular milk. This milk is produced by offering cows fish oil in their diet (www.nutraingredients.com/news/news-ng.asp?n=60248-first-omega-milkss).

5.4 Diabetes

Simopolous (2003) stated that many incidents of diabetes are associated with an increased production of different chemicals in the body, all of which are increased by a rise in *n*-6 fatty acid intake and decreased by increasing *n*-3 fatty acid intake (either α-linolenic acid or EPA and DHA). Ros (2003) reviewed the effect of dietary *cis* MUFA and their metabolic control of Type II diabetes and concluded that there is substantial evidence that offering diabetic patients a relatively high fat content based on MUFA rich foods, provides a greater degree of metabolic control that is similar to or perhaps even better than fats obtained from high carbohydrate diets.

5.5 Stroke

Research by Iso *et al*. (2001) was inconclusive regarding the possible benefits from increased *n*-3 fatty acid intake (from fish oil) in the diet on the incidence of stroke in humans. However, in subsequent studies by Iso *et al*. (2002), there was evidence that people with higher linoleic acid levels in their blood had a reduced risk of stroke. Simon *et al*. (1995) linked high serum levels of α-linolenic acid with a reduced risk of stroke in middle-aged men, who had a high-risk cardiovascular profile.

5.6 Arthritis

Kremer (2000) examined the effect of offering fish oil to people with rheumatoid arthritis and reported beneficial findings. Volker *et al*. (2000) reported a significant improvement in people suffering from arthritis when fish oil was offered in the diet. Furthermore, Darlington and Stone (2001) also recorded positive responses of offering fish oil to people suffering from arthritis. However, these authors cautioned against a daily fish oil intake exceeding 750 mg/day. The Arthritis Research Campaign (UK) web page features an article by Dr Gail Darlington, summarising some of the claimed benefits of fatty acids (fish oil in particular) on arthritis, but this states that further research is necessary to prove these claims (http://www.arc.org.uk/about_arth/booklets/ 6010/6010.htm#foods).

5.7 Infant development

Milner and Allison (1999) provided a summary of details from a workshop held by the American Society of Nutritional Sciences. They cite 138 scientific papers relating to infant nutrition and development, and also consider the importance of fatty acids for infants. Innis (2004) stated that *n*-3 and *n*-6 fatty acids are essential for embryo development, infant brain development and retina development.

5.8 Cold

A recent report suggests that CLA is an effective cold-fighter in humans (http://www.nutraingredients.com/news/news-ng.asp?n=51428-further-evidence-for). In a clinical trial performed by Loders Croklaan Lipid Nutrition, it was discovered that Safflorin, a CLA derived from safflower oil, had beneficial effects on the human immune system. Volunteers who took a daily dose of 2 g Safflorin had an overall reduced incidence of cold symptoms, such as a sore throat, coughing and sneezing, in comparison to volunteers offered a placebo.

5.9 Asthma

Eskimos have a low incidence of asthma, which is possibly due to their high intake of *n*-3 fatty acids (http://www.omega3sealoil.com/Chapter3_2.html).

6 Oxidative stability of fatty acids

6.1 Antioxidants

Antioxidants are ‘compounds that react with and neutralize the free radicals that could otherwise lead to cellular damage and off flavours, odours, tastes and appearances in food products’ (www.goldcirclefarms.com/glossary.html). A free radical is a highly reactive chemical that can contain oxygen and is produced when molecules are split to give products with unpaired electrons by a process called oxidation. Free radicals can damage important lipids. Antioxidants include Vitamin E and selenium. Lipids, especially PUFA are prone to oxidative damage (Douillet *et al*., 1993).

6.1.1 Vitamin E

Vitamin E is a powerful antioxidant that prevents the oxidation of lipids. The quantity of Vitamin E associated with the fat globule membrane is the main factor determining the oxidative stability in milk. Data from Belgium (Focant *et al*., 1998) have shown that supplementing a cow's diet of oilseeds (containing extruded rapeseed and linseed) with an oral supplement containing 9616 IU of Vitamin E increased the concentration of α-tocopherol in milk by 45% and was sufficient to prevent milk fat depression and oxidation. Canadian work has also shown that Vitamin E increases the oxidative stability of milk (Nicholson and St-Laurent, 1991). Danish researchers found that increasing the dietary level of Vitamin E in pig's diets increased the concentration of α-tocopherol, an antioxidant, in the plasma and liver of the pigs (Lauridsen *et al*., 1999).

Irish researchers examined the effect of dietary supplementation of Vitamin E on the oxidative stability of beef (O'Grady *et al*., 2001). Dietary Vitamin E supplementation resulted in an elevated plasma and muscle α-tocopherol and reduced the susceptibility of beef muscle tissue to lipid and oxymyoglobin oxidation.

6.1.2 Selenium

Selenium is one of the most important nutritional antioxidants and is an essential trace mineral. Selenium activates an antioxidant enzyme called glutathione peroxidase. Selenium as gluthathione peroxidase also appears to support the activity of Vitamin E (α-tocopherol) in limiting the oxidation of lipids. Animal studies indicate that Selenium and Vitamin E tend to spare one another and that Selenium can prevent some of the damage resulting from Vitamin E deficiency in models of oxidative stress (Burk and Levander, 1999). O'Grady *et al*. (2001) concluded that adjusting dietary Selenium has limited potential for reducing lipid oxidation in beef or heightening the stabilizing effect of Vitamin E, except in cases where dietary Selenium is limiting.

7 Biohydrogenation of fatty acids in the rumen of livestock

Food consumed by ruminants passes through the rumen, which acts as a fermentation vat where millions of bacteria, protozoa, and fungi in the rumen ferment the feed and release end products, which are used by the animal for maintenance and growth of body tissues. Bacteria present in the rumen are responsible for the extensive transformation of dietary lipid by lipolysis to release free fatty acids from plant lipids and biohydrogenation, to convert unsaturated fatty acids in plants to saturated end products. Hydrogenation is the process whereby hydrogen molecules are added directly to a MUFA or PUFA to make it more saturated. When this occurs in the rumen, it is known as biohydrogenation, which is the process whereby unsaturated fatty acids in plant matter are converted to a more saturated lipid (Jenkins, 2004) and the extent of rumen biohydrogenation mainly depends of the type of diet offered to the animal (Lawson *et al*., 2001).

Biohydrogenation of linoleic acid in the rumen begins with its conversion to CLA and one of the more common CLA isomers produced from biohydrogenation of linoleic acid is cis-9, *trans*-11 C18:2. As biohydrogenation progresses, double bonds in the CLA intermediates are then hydrogenated further to *trans* fatty acids, having only one double bond. A final hydrogenation step by the ruminal microbes eliminates the last double bond yielding stearic acid as the final end product. The hydrogens are shown on opposite sides of the double bond for *trans* fatty acids, but on the same side of the double bond for *cis* fatty acids. Although the difference in structure between *trans* and *cis* fatty acids appears small, it causes significant differences in their physical and metabolic properties as previously described.

8 Fatty acid absorption

The process of ruminal biohydrogenation of fatty acids results in a much smaller quantity of dietary unsaturated fatty acids reaching the small intestine of the cow. Jenkins (2004) stated that although the intake of linoleic acid in the dairy cow diet ranged from 70-200 g/day, only 10-50 g of this reached the small intestine. Conversely, up to 500 g/day of stearic acid reached the small intestine of dairy cows, even though a much smaller quantity of stearic acid was consumed. This suggests that stearic acid is the main fatty acid absorbed in cows, irrespective of the quantity consumed by the cow.

9 Protecting fatty acids in the rumen

A protected fat is one that is treated so that it is capable of resisting biohydrogenation by rumen microbes. This process allows for a greater proportion of the 'beneficial' fatty acids to reach the small intestine, where they are absorbed. When a protected fat is offered to dairy cows, the CLA content of their milk is reduced (Lawson *et al*., 2001) as the bacteria are unable to convert the linoleic acid into CLA. A range of processes are available to achieve protection of the fat.

9.1 Methods of Chemical protection

9.1.1 Formaldehyde

Formaldehyde treatment of fatty acids involves surrounding the unsaturated fatty acid by a protective capsule, composed of a cross-linked aldehyde treated protein, thereby protecting the internal fatty acid from hydrogenation. In a dairy cow, this would allow for a greater proportion of the PUFA to reach the milk. Research has shown that levels of PUFA reaching the tissues of cattle and sheep were greatly enhanced when formaldehyde treated lipid was offered (Cook *et al*., 1972; Faichney *et al*., 1973). In lactating dairy cows, milk linoleic acid content increased from 3% to 30% of total fatty acids when a formaldehyde-protected supplement was offered and this returned to normal when the supplement was withdrawn (Cook *et al*., 1972).

9.1.2 Calcium salts

When diets are supplemented with calcium salts, biohydrogenation of fatty acids is reduced as the calcium salt of the fatty acid is less soluble at the rumen pH. Wu *et al*. (1991) reported 49% biohydrogenation of fatty acids from calcium salts of palm oil, compared with 80% for animal-vegetable fat and 62% for control diet fatty acids.

Conversely, data by Chouinard *et al*. (1998) indicated that feeding calcium salts of soyabean oil which is rich in linoleic acid, or linseed oil which is rich in linolenic acid, to dairy cows, only had a small effect on the proportion of these fatty acids reaching the milk fat. The ability of calcium salts to prevent interactions between microbes and fatty acids has been shown with fatty acids in palm oil (Chilliard *et al*., 2000) and the technique is more effective with saturated than unsaturated fatty acids.

Canadian research has indicated that the conversion of dietary lipids to a calcium salt is an effective tool for manipulating milk fat content on commercial dairy farms (Gervais *et al*., 2003) as the conversion decreased the proportion of short and medium chain fatty acids in milk fat, and increased the proportion of long chain fatty acids in milk fat. These researchers reported that the concentration of *trans*-10, *cis*-12 CLA increased in milk fat and there was no change in the concentration of *cis*-9, *trans*-11 CLA.

9.2 Physical protection

9.2.1 Heat treatment

Some authors have reported that roasting cottonseed reduced ruminal biohydrogenation of fatty acids (Pires *et al*., 1997). Conversely, American studies have demonstrated that roasting soybeans does not protect fatty acids in the rumen (http://www.traill.uiuc.edu/dairynet/ paperDisplay.cfm?Type=paper&ContentID=232).

Extrusion is another commonly used method to treat feeds for ruminant diets. With this method, feeds are fed into an extruder barrel, where a central revolving shaft forces the feed through the extruder. The feed is treated by the heat generated through friction and/or steam, which is frequently injected during the process.

9.2.2 Prilled fats

Prilling, also known as melt spraying, spray congealing, spray chilling or melt atomisation, is a process whereby molten liquids or mixtures are atomised and the droplets formed are left to cool, forming a prill or bead (http://www.aveka.com/prilling_and_melt_spraying.htmm). Prilled fats mainly contain the saturated fats, palmitic and stearic acids (Coppock and Wilks, 1991).

9.3 Natural protection

9.3.1 Seed coat

Offering whole oilseeds such as soybean, cottonseed or sunflower seed to animals increases the proportion of unsaturated fatty acids reaching the milk and tissue of the animal. This is due to the nature of the hard outer seed coat. When the seed coat is broken, the oil is exposed to breakdown by rumen flora. DePeters *et al*. (1985) reported that when the proportion of whole cottonseed was increased from 0 to 20% in diets offered to cows, the proportion of oleic acid in milk increased from 23 to 32% of total fatty acids, but there was no change in the quantity of linoleic and linolenic acids reaching the milk. Further work by Murphy (2000) indicated that it is necessary to damage the seed coat, in order to release the oilseed lipid in the small intestine.

10 Fatty acid sources

A number of feeds with a desirable fatty acid profile are available for beef cattle, dairy cows, sheep, pigs and poultry as follows.

10.1 Linseed (*Linum usitatissimum L.)*

10.1.1 Background

Traditionally grown for industrial purposes, linseed is rich in essential fatty acids, particularly α-linolenic acid, but is highly unpalatable to both animals and humans, due to the high level of PUFA, which renders it prone to oxidation and rancidity. However, plant breeders have successfully produced new edible oil varieties, with variable and desirable fatty acid ratios (International European Network for Industrial Crops and their Application (IENICA), 2002a). Traditional uses of linseed oil are as a drying oil in paints, varnishes, printing ink and putty. Linseed cake is widely used in animal feeds. Under certain conditions such as weather, herbicide treatment or soil status, linseed can accumulate large quantities of cyanogenic glycosides that convert to prussic acid. Prussic acid is poisonous to livestock in that it inhibits oxygen utilisation by the cells and as a consequence, the animal dies from asphyxia.

10.1.2 Fatty acid profile

Linseed can produce seed with an oil content of approximately 40%, of which up to 95% is extractable. However, different processing methods can be employed to render different oils and extraction residues, therefore having variable oil contents. Ground unextracted linseed meal can have 35% oil, with ground linseed cake having 10% oil and extracted linseed meal having 3% oil (Animal Feed Resources Information Systems, 2004a).

The fatty acid composition of linseed is very consistent, regardless of the variety cultivated or growing location, climatic or soil influences (Bean and Leeson, 2002). New edible "Linola" linseed (often referred to as Solin) has been bred to have an altered *n*-3:*n*-6 ratio, in favour of the latter, to allow for use as an oxidatively stable ingredient in processed foods and Table 2 highlights these differences.

Table 2 Fatty acid profile (%) of industrial grade linseed compared with Linola (www.ienica.net/crops/linola.htm)

Linseed	C16:0* Palmitic	C18:0* Stearic	C18:1 *n*-9 Oleic	C18:2 *n*-6 Linoleic	C18:3 *n*-3 Linolenic
Industrial	6	2.5	19	24	47
Linola	6	4	16	72	2

*Saturated fatty acid

10.1.3 Oxidative Stability

Linseed oil and linseed meal from industrial varieties have a low oxidative stability, whereas linola oil, with a much higher *n*-6 content, has a greatly increased oxidative stability. Meal from Linola can be offered to ruminants and to monogastrics in the same proportion as ordinary linseed (www.ienica.net/crops/linola.htm).

10.1.4 Land use

In the UK, some 28,800 ha of linseed was grown in 2004, which includes estimates for linola but not including linseed grown on set-aside land. Most of the UK production is for industrial non-food use (Department of the Environment, Food and Rural affairs (DEFRA), 2005).

10.2 Hemp (*Cannabis sativa L.*)

10.2.1 Background

Hemp is capable of producing oil yields of 32 to 38% (Hemp Oil Canada Incorporated, 2005) (http://www.hempoilcan.com/compo.html). Hemp oil is of growing interest as it is rich in essential fatty acids, especially α-linolenic acid (C18:3 *n*-3).

10.2.2 Fatty acid profile

Approximately 90% of the oil from hemp can be extracted, with the oil containing 75-80% PUFA, having a full range of *n*-3, *n-6* and *n-9* fatty acids. Unsaturated fats comprise approximately 90% of total oil. Hemp oil contains approximately 20% α-linolenic acid (*n*-3) and 55% linoleic acid (*n*-6), which is close to the 'ideal' *n*-6:*n*-3 ratio of 3:1 for human intake (British Nutrition Foundation, 2004). Table 3 demonstrates the unsaturated fatty acids present in hemp oil.

Table 3 Fatty acid profile of hemp

Unsaturated fatty acid	% of Total fatty acids
Oleic acid (C18:1 *n*-9)	10.5
Linoleic acid (C18:2 *n*-6)	55.2
Gamma-Linoleic acid (C18:3 *n*-6)	3.1
α-Linolenic acid (C18:3 *n*-3)	20.0
Eicosaenoic acid (C20:1)	0.5

(Hemp Oil Canada Incorporated, 2005)

10.2.3 Oxidative stability

Hemp oil contains 100-150 mg/100 g of Vitamin E, with gamma-tocopherol dominant to α-tocopherol (13-20 IU/100 g), which may afford some degree of oxidative stability (Hemp Oil Canada Incorporated, 2005).

10.2.4 Land use

Hemp has only been grown as a fibre crop on an experimental basis in Northern Ireland in recent years, and less than 20 ha was grown in Northern Ireland in 2004, compared to the UK mainland, where over 2,000 ha were grown in 2004

(DEFRA, 2004). Seed crops utilise different varieties, are sown at lower seed rates and are managed differently from fibre crops. In 2004, the first seed hemp crop was grown in Northern Ireland and small areas have also been grown in England. The Food and Agriculture Organisation of the United Nations (2004) report some 26,993 ha of hemp seed harvested in the world in 2004.

10.3 Chia (*Salvia hispanica L.)*

10.3.1 Background

Chia is a plant native to South America and not yet cultivated in the European Union. Chia produces a seed with approximately 30% oil. The content of *n*-3 fatty acids in Chia can be in excess of 80%, making it the richest known plant source of essential fatty acids. A local company, R. Craig and Sons, Ballymoney have applied for a licence to import and sell Chia seed as an animal feedstock.

10.3.2 Fatty acid profile

Data for oil extraction from Chia seed suggest that approximately 90% is recoverable (Ayerza, 1995). Some 58% of the extracted oil from chia is *n*-3 α-linolenic acid (C18:3). This is the highest bearing source of α-linolenic acid known in plants. Ayerza (1995) reported that the oil yield of chia seed from different regions could vary by as much as 20% (32-39%) and that the fatty acid profile of the oil can also vary. Linolenic acid content, for example, was reported to range from 52 to 63% between locations.

Table 4 displays the fatty acid profile of chia seed oil as given by Craig and Sons (2004) and mean values from data by Ayerza (1995).

Table 4 Fatty acid profile of Chia oil as a percentage of total oil

Chia oil	Craig and Sons	Ayerza
Total oil content (% w/w)	32.2	36.9
C18:3 (Linolenic)	58.7	60.2
C18:2 (Linoleic)	18.8	20.3
C18:1 (Oleic)	6.9	7.6
C18:0* (Stearic)	3.0	3.3
C16:0* (Palmitic)	6.7	6.6

*Saturated fatty acid

10.3.3 Oxidative stability

Ayerza and Coates (2001a) reported a high number of antioxidant constituents of chia seed oil, which include chlorogenic acid, caffeic acid, myricetin acid, quercitin acid and kaempferol flavonoids. The Selenium content of chia seed has been reported as 1 mg/100 g. No values for Vitamin E content of chia were included from either source.

10.3.4 Land use

Chia is not currently grown in the UK or Northern Ireland. It is unlikely that the crop can be grown in the British Isles, as it requires climatic conditions similar to its native land.

10.4 Marine Algae - Microalgae and Phytoplankton

10.4.1 Background

These primitive, but complex organisms, form the basis of the marine food chain and are the primary source of EPA and DHA in the biosphere. Algal oils are however, regarded as novel foods in the European Union and consequently, their use has yet to be accepted (Sanders, 2000).

10.4.2 Fatty acid profile

With an oil content of some marine algae in excess of 60% (Nichols *et al*., 2004), marine algae are potentially the best truly sustainable source of PUFA. Although some terrestrial plants may have significant quantities of fatty acids, they cannot produce the long chain *n*-3 PUFA, EPA (C20:5) or DHA (C22:6), both of which are essential for human embryo and infant neurological development and general health (Milner and Allison, 1999). The only sources of EPA and DHA are marine organisms, which include all fish, crustaceans, gastropods and microorganisms.

The percentage of oil extractable from marine algae varies greatly according to the species and strain used. Mansour *et al*. (2004) reported one strain of endemic Australian thraustochyrid algae, which contained >60% fatty acids in the oil, with DHA being the primary constituent, while other strains had varying amounts of DHA and EPA (32-54%). Similar differences in fatty acid components were recorded previously by Bowles *et al*. (1999), who reported isolates of thraustochyrid from cold temperate environments that contained >50% oil (mainly DHA), while some strains from sub-tropical waters contained ~37% oil. Table 5 displays the long chain *n*-3 (DHA and EPA) fatty acid profile of a range of marine microorganisms.

Table 5 Long chain fatty acid content of lipids of three marine microalgae (%)

	EPA	**DHA**
Thraustochytrium aureum	-	52
Schizochytrium sp.	6	30
Crypthecodinium cohnii	-	44

(Sijtsma and de Swaaf, 2003)

10.4.3 Oxidative stability

During a trial using algae as a feed supplement for lactating dairy cows, Franklin *et al*. (1999) reported having to store the feed in a cool dry place to prevent oxidation. Jimenez–Escrig *et al*. (2001) suggested that the harvesting and drying processes used for marine algae lipids contributed to a reduced antioxidant action.

10.4.4 Land use

In Northern Ireland and the UK, there are no commercial producers of marine algae as a feedstock for animals at present. Other uses of marine algae include agar, alginate and fertilisers. There are no commercially exploitable stocks of marine algae for producing agar in the UK. Ireland and Scotland supply seaweed as a raw material for the alginate industry and the market leader for fertiliser production from seaweed in the UK is Maxicrop International Ltd. (http://www.w-isles.gov.uk/minch/seaweed/seaweed-04.htm).

10.5 Fish Oil/Fish Meal

10.5.1 Background

Fish meal is normally used whole as an animal feedstock and contains approximately 10% oil. European Union regulations currently restrict fish meal as a feed for pigs and poultry only. Concerns regarding the decline in fish stocks could affect the availability of fish meal as an animal feedstuff by 2010 (International Fish meal and Fish Oil Organisation (IFFOO), 2001).

10.5.2 Fatty acid profile

The fatty acid profile of fish oil varies between fish species. Givens *et al*. (2000) list the long chain fatty acid content of Atlantic mackerel, Atlantic herring, salmon (farmed) and Bluefin tuna (as reported by Nettleton (1991)). These species contained 9, 7, 6 and 4 g/kg EPA (C20:5) and 16, 9, 12 and 12 g/kg DHA respectively. The content of linolenic acid (C18:3) in Bluefin tuna was zero, whereas for the other three species a value of 1 g/kg was reported. The fatty acid composition of a fish oil used by AbuGhazaleh *et al*. (2002) as a dietary supplement for dairy cows is detailed in Table 6.

Table 6 Major components of the fatty acid profile of a fish oil dietary supplement offered to dairy cows

Fish oil	**g/100 g fatty acids**
C16:0* (Palmitic)	17.71
C18:0* (Stearic)	3.26
C18:1*cis*9 (Oleic)	8.35
C18:2 *cis*9 *cis*12 (Linoleic)	1.14
C18:3 *n*-6 (γ-Linolenic)	0.30
C18:3 *n*-3 (α-Linolenic)	1.21
C20:5 *n*-3 (EPA)	11.64
C22:6 *n*-3 (DHA)	8.77
Unsaturated	48.92
Saturated	28.83

*Saturated fatty acid (Abughazaleh *et al*., 2002)

10.5.3 Oxidative stability

Fish oil has been reported to contain both Vitamins A and E (458 and 1.21 IU/g respectively), which act as antioxidants (Dobrzański *et al.*, 2002). Fish oils need to be stabilised with antioxidants to maintain their quality and prevent oxidation, the products of which can be unpalatable and toxic to animals (Animal Feed Resource Information System, 2004b).

10.5.4 Use

The quantity of fish meal used in the UK for animal feed during 2003 was 233,000 tonnes (Fish meal Information Network, 2004). Most of this was used for fish farming, but poultry and pigs accounted for 25,000 and 15,000 tonnes respectively (http://www.gafta.com/fin/finfacts5.html).

10.6 Lupins

10.6.1 Background

Forage lupins are of a growing interest in the UK as they have a protein content of 30-42% and could be a home grown protein feed for livestock. There are a number of different types of lupins, including white lupin (*Lupinus albus*) (36-40% crude protein), yellow lupin (*Lupinus luteus*) (34-42% crude protein) and blue or narrow-leafed lupin (*Lupinus angustifolius*) (30-34% crude protein). Lupins either have a determinate or indeterminate flowering. Determinate varieties of lupin will flower for a set period and ripen with an earlier maturity, whereas indeterminate flowering varieties will flower for long periods and ripening can be prolonged under cool and wet weather conditions. Yields of 4.5 and 5.18 tonne/ha were reported when lupins were sown at 20.9 and 28.6 plants/m^2 respectively (Crowley, 2001). Crowley (2001) reported that new varieties of lupin are required in Ireland with scope for wider sowing dates and earlier maturity as data showed that lupins could only be sown between 14 September and 29 September at Oak Park, Co Carlow. As lupins are approximately half the size of beans, they are easier to dry and handle. Premium Crops Ltd, Soya UK Ltd. and Saxon Agriculture Ltd. have put major investment into the development of lupins and interest exists among farmers, both for use as a grain and forage.

10.6.2 Fatty acid profile

The oil content of lupins can vary according to species, but the white lupin (*Lupinus albus)* has an oil content of 10% (http://www.soya-uk.com/lupin%20feed%20and%20UT.htm). The fatty acid profile of lupin seed is demonstrated in Table 7.

Table 7 Fatty acid profile of lupin seed

Fatty acid (% DM)	Lupin seed
C14:0 (Myristic acid)*	0.3
C16:0 (Palmitic acid)*	10.1
C18:0 (Stearic acid)*	1.8
C18:1 (Oleic acid)	57.2
C18:2 (Linoleic acid)	21.1
C18:3 (Linolenic acid)	8.5

*Saturated fatty acid (Froidmont and Bartiaux-Thill, 2004)

10.6.3 Land Use
Lupins have not been a major crop in Northern Ireland and the UK for a number of years, but they are becoming of increasing interest as a forage crop for animals.

10.7 Oilseed Rape (*Brassicus napus L.*)
10.7.1 Background
There are two main types of oilseed rape (OSR): (1) High Erucic Acid Rape (HEAR) which is classed as industrial as the oil contains high levels (>45%) of erucic acid and the meal is high in glucosinalates (sulphur compounds), which render it unpalatable to animals and humans and (2) "double" (OO) varieties, which have been bred to produce oils that are low in these constituents and can be digested by animals and humans (IENICA, 2002b).

A new type of oil crop, Canola, has been derived from OSR by irradiative mutation. It is not termed a "transgenic" plant as it was developed by induced mutation of natural *Brassicus napus* genetic material, with no foreign genes inserted. It is not a genetically modified plant. The advantage of canola is that it has altered levels of fatty acids with different ratios of *n*-6:*n*–3 PUFA (Raymer, 2002).

10.7.2. Fatty acid profile
Although OSR contains some 40% oil, the extraction process leaves less than 5% in rape meal, and of this, less than 10% are *n*-3 fatty acids. The fatty acid profile of OSR and canola are given in Table 8.

Table 8 Fatty acid composition (%) of rape and Canola oil

	C16:0* Palmitic	C18:0* Stearic	C18:1 Oleic	C18:2 Linoleic	C18:3 Linolenic	C22:1 Erucic
Oilseed rape			50-60	20-30	6-12	<1
Canola	5	2	66	19	8	

*Saturated fatty acid (IENICA, 2002 a,b)

10.7.3. Oxidative stability

Oxidative stability of rapeseed is low, due to the high levels of PUFA in the oil. Rape and canola meal have a high oxidative stability as they have a lower oil content after extraction and processing, which removes anti-nutritional factors, such as sinapine and glucosinalates.

10.7.4. Land use

Oilseed rape is widely grown throughout the UK, with over 500,000 ha grown in 2004 (DEFRA, 2004). In Northern Ireland however, the area of OSR has fallen to a very low level, with less than 200 ha grown in 2004, with the highest area planting being 1,200 ha in 1990/1991 (DARDNI, 2004). Crop yields are on a par with the mainland UK, but it is not financially attractive to local cereal growers, due to the absence of local processing facilities (Easson *et al*., 2004).

10.8 Soya

10.8.1 Background

Soya is the world's main protein and oil crop, being widely used for both human and animal foodstuffs. In 2004, 91 million ha of soyabean were harvested in the world, with a yield of 2.48 tonne/ha (Food and Agriculture Organisation of the United Nations, 2004). Soya bean contains approximately 38% protein and 18% oil. Japanese soyabean is reported to have a high fat content, while Chinese and Indian soyabean are known for their high protein content. A lot of genetic variation exists with the soyabean crop as there are thousands of varieties available, including red, black and green. The most popular variety is the yellow soyabean (*Glycine max*). The United States is the world's largest producer of soyabean.

10.8.2 Fatty acid profile

Soya yields between 16-20% oil, with 95% being extractable and has a fatty acid profile with low (2-12%) *n*-3, but high *n*-6 levels. Rarely offered as a pure oil, cheaper soya animal feed products (bean meal and hulls) contain 2-3% oil (Ewing, 1997). The fatty acid profile of soya oil is shown in Table 9.

Table 9 Fatty acid profile of soya oil (%)

C18:0* Stearic	C16:0* Palmitic	C18:1 Oleic	C18:2 Linoleic	C18:3 Linolenic
2-6	2-10	23-32	48-52	2-12

*Saturated fatty acid (http://www.ienica.net/crops/soya.htm)

10.8.3 Land use

Soya is not grown in Northern Ireland as it requires higher temperatures for growth and development but it is grown in the South of England. The total UK area is approximately 250 acres at the moment and soya can be grown successfully as far as North of York. Yields are typically 2.5 tonne/ha (Personal communication, Soya

UK Ltd). Although considerable quantities of meal and bean are imported into Northern Ireland as feedstuff for sheep, cattle and pigs, the oil content in these is low (<3%) and the *n*-3 fraction would be negligible.

10.9 Conjugated Linoleic Acid Precursors

10.9.1 Grass and Clover

Although CLA itself is not found in plants, it is produced after microbial fermentation in the rumen of animals offered forage-type diets. Clover and grass are therefore important sources of linolenic acid, but the content varies with species and variety of the plant (Mandell, 2004). Both linolenic acid and CLA are found in higher quantities in the milk and meat of animals that have consumed forage-type diets. Canadian research has shown that beef and dairy producers have the opportunity to produce milk and meat with a desirable fatty acid profile, by simply resorting to the extensive use of pasture for feeding their animals (Mandell, 2004). However, the downside to extensive forage feeding is that there is a smaller quantity of available energy in forages compared to grains, so the cost of production could increase and producers would need to get a higher price for their meat/milk.

The potential of forage breeding and management to enhance fatty acid levels in ruminant meat was discussed by Dewhurst *et al*. (2003a). These authors suggested herbage breeding to increase beneficial fatty acid levels in grasses and clovers as likely to be the most cost effective method of enriching ruminants with PUFA. Improvements in the understanding of oxidative loss during the wilting of silage and ruminal biohydrogenation of fatty acids can improve fatty acid enhancement strategies.

In an American study by Clapham *et al*. (2004), the fatty acid profile of a number of forages was examined on a monthly basis, in order to determine the effect of season. The forages examined were (1) alfalfa (*Medicago sativa* L.) (2) bluegrass (*Poa spp*.) (3) tall fescue (*Festuca arundinacea Schreb*) (4) orchardgrass (*Dactylis glomerata L*.) (5) white clover (*Trifolium repens L*.) and (6) triticale (X *Triticosecale Wittmack*). These researchers concluded that the concentration of linolenic acid increased by 50% or more in late summer/early autumn for all forages except triticale. If one was producing fatty acid-enriched meat/milk by including any of the above forages (except triticale) in the diet, seasonal variation would need to be considered.

10.10 Grass

10.10.1 Background

Grass is still the principal food source of Northern Ireland farm animals (excluding poultry and pigs). Different types of grasses are used for grazing or cutting for silage and hay and approximately one fifth of the grass area in Northern Ireland is under 5 years old, with old grass areas ploughed up and re-seeded, as productivity declines over time.

10.10.2 Fatty acid profile

Grass contains a relatively low lipid fraction, being approximately 4%, but there are small variations in the lipid content between different grass types. A high proportion (50-57%) of the lipid in grass is *n*-3 α-linolenic acid. The fatty acid profile of grass and grass silage is given in Table 10.

Table 10 Fatty acid profile of grass and grass silage

Composition	Grass	Grass silage
Oil (g/kg DM)	29	28
Fatty acids (g/100 g Fatty acid methyl esters)		
C14:0 (Myristic acid)*	4.6	5.4
C16:0 (Palmitic acid)*	20.8	24.0
C16:1 (Palmitoleic acid)	2.4	0.6
C18:0 (Stearic acid)*	3.3	2.9
C18:1 (Oleic acid)	5.7	6.3
C18:2 (Linoleic acid)	14.0	14.5
C18:3 (Linolenic acid)	49.2	46.2

*Saturated fatty acid (French *et al.*, 2000)

10.10.3 Land use

Over 17 million ha of land in the UK was under grass in 2004, with 848,200 ha of this in Northern Ireland (DARDNI, 2004).

10.11 Clover

10.11.1 Background

Clover is usually grown as an integral mixture with grass seed. There are two main clovers grown in Northern Ireland, white and red clover, the former normally sown as 1 kg/14 kg of grass, with red clover as a smaller percentage, as it can dominate grass swards very quickly. Clover is very important for organic farming systems.

White clover varieties used in Northern Ireland include AberAce, AberDai, AberHerald, AberVantage, Alice, Aran (S), Avoca, Chieftain, Crusader (P), Barblanca, etc. It has been reported that a grass/white clover sward that is well managed can produce a similar dry matter (DM) yield to that of a pure ryegrass sward that was fertilised with 200 kg N/ha, but white clover is sometimes difficult to establish and maintain in pasture. Ledgard *et al.* (1995) reported that white clover production declined due to fertiliser N application. However, clover can be classified by leaf size as small, medium or large. Large leafed varieties can tolerate N use and compete better with its companion grass for silage production. Medium leafed varieties are more suited to grazing situations, but they can also be used for silage.

Finally, small leafed white clover varieties can tolerate close and frequent grazing. Clover is also more nutritious than grass, in that it contains more minerals and protein (http://www.ruralni.gov.uk/livestock/grass/grass_land_technical_projects/effect/dpreport.htm).

Red clover (*Trifolium pratense*) can be sown in pure swards as a specialist hay or silage crop or for grazing. Most cultivars of *Trifolium pratense* do not persist past 2-4 years. The benefits of red clover include the ability to convert atmospheric N into a form that can be used by plants, therefore reducing the requirement for fertiliser. Red clover has a reported yield of 10-15 tonnes DM/ha, but it must be remembered that some cultivars contain phyto-oestrogens, which may reduce the fertility of animals (Jones *et al*., 2003). Furthermore, red clover may also cause bloat in sheep but the risk is negligible when offering red clover silage (Jones *et al*., 2003).

A project is underway at Greenmount College, called the Clover E.F.F.E.C.T. (European Farms For Effective Clover Technology), with the aim of increasing the uptake and utilisation of white clover in European grassland systems, by demonstrating new ways to improve the performance and reliability of grass/white clover pastures on farm. Twenty-four demonstration farms were established within areas in Europe that favour the growth of white clover, representing a wide range of soil and climatic conditions, where clover is used in conventional farming systems and organic farming systems.

Doyle and Topp (2004) reported that the economic advantage to organic systems, by using forage legumes, will depend on new advances and management techniques that decrease the risk associated with a more difficult crop establishment and a higher nitrate leaching of forage legumes compared to grasses. These authors also reported that from an economic perspective, forage legumes are probably best grown in a mixture with grass. Rutter *et al*. (2004) examined dietary preferences of dairy cows grazing ryegrass and white clover and reported that dairy cows showed a mean preference for white clover, being 74% and there was a diurnal pattern to preferences, in that the proportion of time cows spent eating perennial ryegrass was higher in the evening than in the morning. These authors concluded that further research is required to determine why sheep and cattle choose to eat mixed diets when offered a choice of grass and clover.

10.11.2 Fatty acid profile

The fatty acid profile of red and white clover is given in Tables 11 and 12 respectively.

Table 11 Fatty acid profile of red clover in Autumn and Spring

Fatty acid (mg/g total fatty acids)	Autumn	Spring
C14:0 (Myristic acid)*	11.0	13.6
C16:0 (Palmitic acid)*	310.5	241.6
C18:0 (Stearic acid)*	48.1	43.5
C18:1 *cis*9 (Oleic acid)	80.0	52.1
C18:2*n*-6 (Linoleic acid)	214.0	190.6
C18:3*n*-3 (α-Linolenic acid)	336.3	458.7

*Saturated fatty acid (Loor *et al.*, 2003)

Table 12 Fatty acid profile of stolon tissue in white clover Aber Hearld

Fatty acid (mg/g DM)	Base of white clover
C16:0 (Palmitic acid)*	3.99
C16:1 (Palmitoleic acid)	0.04
C18:0 (Stearic acid)*	0.50
C18:1 (Oleic acid)	0.34
C18:2 (Linoleic acid)	3.41
C18:3 (Linolenic acid)	4.21
Unsaturated fatty acids	9.58
Saturated fatty acids	4.50
% Unsaturated fatty acids	66.8

*Saturated fatty acid (Collins *et al.*, 2002)

10.11.3 Oxidative stability

Recent data have suggested that the oxidative stability of milk from cows offered red clover silage is less than that of cows offered grass silage (Al-Mabruk *et al.*, 2004). This was due to a more rapid loss of α-tocopherol and an increased production of thiobarbituric acid reactive substances, during the storage of the milk from red clover silage-fed cows in comparison to grass silage-fed cows. However, these authors concluded that this oxidative deterioration of milk could be combated by Vitamin E supplementation in the diet.

10.12 Other Novel Plant Sources of *n*-3 PUFA

Researchers in Melbourne have examined the *n*-3 PUFA content of the leaves or heads of spinach, watercress, parsley, Chinese cabbage, brussel sprouts, bok choy, cos lettuce, broccoli, Chinese broccoli and baby bok choy. Three PUFA were present in all of these vegetables, namely C16:3 *n*-3, C18:2 *n*-6 and C18:3 *n*-3. It was found that watercress and mint contained a greater concentration of C16:3 *n*-3 and C18:3 *n*-3, while parsley had the highest concentration of C18:2 *n*-6. Mint had 195 mg/100 g C18:3 *n*-3 and watercress had the highest concentration of C16:3 *n*-3, being 45 mg/100 g. However, there was no EPA and DHA in any of these vegetables (Li *et al*., 2001).

10.12.1 Stock

10.12.1.1 Background

Stock (*Matthiola incana)* is a member of the *Brassicaceae* family. It grows in ten weeks and is also known as 'ten week stock'. This is a common plant in many gardens in Northern Ireland. Stock is unique, in that approximately 65% of its total fatty acids are *n*–3 α-linolenic acid. (http://www.newcrops.uq.edu.au/newslett/ncnl1117.htm).

10.12.1.2.Fatty acid profile

The oil present in stock has been found to be rich in *n*-3 linolenic acid (Yaniv *et al*., 1996). Grown in Jerusalem, a yield of 750 kg seeds/ha was recorded, with 20-25% oil in the seed, being equivalent to 150 litres of oil/ha.

The fatty acid profile of stock grown in Australia is given in Table 13.

Table 13 Fatty acid profile of stock (% oil content)

Saturated	C18:1 Oleic	C18:2 Linoleic	C18:3 *n*-3 α-Linolenic	Oil content
11	14	13	60	22

(Francis and Campbell, 2003)

10.12.1.3 Land use

Stock is not grown as of yet in Northern Ireland for its oil, but the Horticulture Unit at Greenmount College, Co. Antrim are interested in growing stock.

10.12.2 Chickpea (*Cicer arietinum L.*)

Much of the world's chickpea supply (80 to 90%) comes from India. Australian researchers examined the effect of offering a chickpea versus wheat diet on human serum lipids and lipoproteins, with at least one coronary heart disease risk factor or a family history of coronary heart disease (Pittaway *et al*., 2004). Results showed a reduction in the concentration of serum total cholesterol and LDL cholesterol of 3.9% and 4.7% respectively, upon consuming the chickpea diet.

Italian researchers investigated changes in intramuscular fatty acid levels of Barbaresca lambs, offered a change in diet from soyabean and maize meal to chickpeas. Maize and soyabean supplement offered with a commercial pelletised meal was replaced with a diet consisting of 42% chickpea, until slaughter at 132 days. Total PUFA and *n*-3 levels in the chickpea-fed lambs were higher than lambs offered the maize/soyabean diet (Priolo *et al*., 2003).

11 Fatty acids in milk, meat and eggs

Australian work has shown that under normal feeding conditions, the *cis*-9 *trans*-11 CLA profile of monogastric food products (pork and chicken) is less than that of ruminant products (lamb, beef, and dairy), when expressed as a fraction of the fat (Fogerty *et al*., 1988). The *cis*-9 *trans*-11 isomer is important, as it has been shown to reduce mammary tumour activity in rats when added to their diet or consumed as a natural component of butter (Ip *et al*., 1999).

11.1 Fatty acids in Milk

Over 450 different fatty acids are present in milk fat, but only a relatively small number affect the physical and chemical properties or the nutritional value of the fat (Walstra and Jenness, 1984) (Table 14). Ruminant milk fat is characterised by the presence of short and medium chain fatty acids produced by *de novo* synthesis of fatty acids from dietary digestive products (acetate and butyrate). The longer chain C18 fatty acids and approximately 50% of the C16 fatty acids are obtained directly from the cow's diet, but are subject to ruminal hydrolysis and hydrogenation unless they are protected. The proportion of palmitic acid (C16:0) and oleic acid (C18:1) in milk fat strongly reflect the cow's diet, with palmitic acid being highest in the winter when large amounts of concentrates and silage are fed, and lowest in the summer, when the cows are grazing fresh grass.

In an American study by Kelsey *et al*. (2003), to examine the effect of breed, parity and stage of lactation on the CLA content of milk fat from dairy cows, it was concluded that the milk fat of Holstein cows had a higher content of CLA than Brown Swiss cows. However, the breed differences were minor and furthermore, parity and days in milk also had little or no effect on the CLA content of milk. These authors also concluded that CLA content of milk was independent of milk yield, milk fat percentage and milk fat yield.

Table 14 Fatty acid profile of milk fat

Fatty acid	Proportion (g/100 g fatty acids)
C4:0 (Butyric*)	3
C6:0 (Caproic*)	2
C8:0 (Caprylic*)	1
C10:0 (Capric*)	3
C12:0 (Lauric*)	4
C14:0 (Myristic*)	12
C16:0 (Palmitic*)	26
C18:0 (Stearic*)	11
C18:1*cis*	28
C18:1*trans*	0.4
C18:2 (Linoleic)	2
C18:3 (Linolenic)	1

*Saturated fatty acid (Walstra and Jenness, 1984)

11.2 Fatty acids in Beef

Approximately 80% of the fatty acids in beef are composed of saturated palmitic acid (C16:0) (27%), saturated stearic acid (C18:0) (18%), and monounsaturated oleic acid (C18:1) (33%). The remaining 22% is distributed among 30 different fatty acids (Whetsell *et al*., 2003). Beef is also a source of two other saturated fatty acids, lauric acid (C12:0) (less than 1%) and myristic acid (C14:0) (2-3%). Lauric and myristic fatty acids have been reported to be responsible for increasing 'bad' cholesterol levels in blood serum (Grundy, 1994) and have also been shown to be strongly linked with the incidence of early heart attack in humans (Kromhout *et al*., 1995). However, as these two fatty acids are present in very small quantities in beef, they are likely to have little negative impact on human health.

Research data suggests that lean beef does not need be eliminated from cholesterol-lowering diets, as it is considered to be like fish and chicken, and does not promote cholesterol build up in humans (Denke, 1994). The major important PUFAs found in beef are linoleic acid (C18:2) (3.5%), α-linolenic acid (C18:3) (1.5%), arachidonic acid (C20:4) (1%), EPA (C20:5) (<1%), DPA (C22:5) (<1%) and DHA (C22:6) (<1%) (Enser *et al*., 1998). Both EPA and DHA are important fatty acids as they have positive implications for human health, as discussed earlier.

Cholesterol levels in humans are directly related to cardiovascular disease. However, the predominant fatty acids in beef, such as oleic (C18:1 *cis*-9) and stearic (C18:0) appear to be essentially neutral in their effects on cholesterol levels (Whetsell *et al*., 2003).

11.3 Fatty acids in Lamb

Lean cooked lamb has a reported monounsaturated fatty acid and PUFA content of 43.8% and 6.5% respectively, of total fat (http://www.beefnutrition.org/uDocs/ACF32.pdf). Research is underway to examine the fatty acid profile of lamb raised with ewes, compared with weaned lambs. Initial results from this trial indicate that lamb meat had a very different fatty acid profile, depending on rearing regime (http://www.wrightson.co.nz/knowhow/cw_details.asp?article_id=2499). Enser *et al*. (1996) reported that the *n*-6:*n*-3 ratio of lamb at retail was lower (and hence more favourable) than that of beef or pork (1.32, 2.11 and 7.22 respectively). This is due to the fact that lamb contains a higher proportion of *n*-3 α-linolenic acid than that of beef or pork.

11.4 Fatty acids in Pork

It has been shown that CLA can be easily incorporated into the tissue of pigs and therefore suggested that CLA-fortified pork could be a source of CLA for the human diet (Ostrowska *et al*., 2002). Linoleic acid (C18:2) is present at approximately 15 mg/100 mg total fatty acids in the backfat and muscle of the average UK pig (Wood *et al*., 1999). Data in Table 15 demonstrates the comparative fatty acid profile of beef, lamb and pork loin steaks purchased from four supermarkets (Enser *et al*., 1996). These data show that pork is much richer in linoleic acid that either beef or lamb (14.2, 2.4 and 2.7% of total fatty acids respectively). The high linoleic acid content of pork has been attributed to originate from cereal-based diets, which are rich in *n*-6 fatty acids, giving a much higher *n*-6:*n*-3 ratio, which is undesirable (Wood *et al*., 2003). However, supplementing the diet of pigs with feeds rich in C18:3 (such as canola and linseed) can help reduce the unfavourable *n*-6:*n*-3 ratio (Wood *et al*., 2003).

Table 15 Fatty acid profile of beef, lamb and pork loin steaks purchased from four supermarkets

	Beef	**Lamb**	**Pork**
Muscle[b]			
C16:0* (Palmitic)	25.0	22.2	23.2
C18:0* (Stearic)	13.4	18.1	12.2
C18:1 *n*-9 (Oleic)	36.1	32.5	32.8
C18:2 *n*-6 (Linoleic)	2.4	2.7	14.2
C18:3 *n*-3 (α-Linolenic)	0.70	1.37	0.95
C20:4 *n*-6 (Arachidonic)	0.63	0.64	2.21
C20:5 *n*-3 (EPA)	0.28	0.45	0.31
C22:6 *n*-3 (DHA)	0.05	0.15	0.39
C22:5 *n*-3 (DPA)	0.45	0.52	0.62
Total fatty acids[c]	3.8	4.9	2.2
n-6:*n*-3	2.11	1.32	7.22
Fat [b]			
C16:0* (Palmitic)	26.1	21.9	23.9
C18:0* (Stearic)	12.2	22.6	12.8
C18:1 *n*-9 (Oleic)	35.3	28.7	35.8
C18:2 *n*-6 (Linoleic)	1.1	1.3	14.3
C18:3 *n*-3 (α-Linolenic)	0.48	0.97	1.43

[b] % of Total Fatty Acids; [c] % of Muscle (Enser *et al*., 1996)

11.5 Fatty acids in Poultry

The fatty acid profile of poultry meat can be modified by altering the quantity and type of fat administered to the bird. Unique to poultry, fatty acids can be absorbed directly into the portal blood system, followed by transport to the liver. Previous reports have indicated that by humans eating an average meal of 100 g of *n*-3 PUFA-enriched chicken, this would contribute approximately 140 mg of *n*-3 PUFA to the human diet (http://www.poultryindustrycouncil.ca/Factsheets/Factsheets/fact58.htm).

Previous research has shown that offering *n*-3 and *n*-6 PUFA in the diet of broilers reduced their abdominal fat pad mass, plasma triglycerides and cholesterol levels when compared to that of broilers that were offered saturated fatty acids in the diet (Newman *et al*., 1998).

11.6 Fatty acids in Eggs

Yolk lipids comprise approximately 20% PUFA, 45% MUFA and 35% saturated fatty acids, with approximately 5% of the total lipid being free cholesterol. Recent research by Maria Luz Fernandez, at the University of Connecticut demonstrated that the dietary cholesterol in eggs does raise the LDL-1 and LDL-2 cholesterol fractions in humans upon consumption, but it does not have an impact on the small, dense LDL-3 - LDL-7 particles, the lipoproteins associated with the greatest threat for cardiovascular disease in humans.

12 Research - past and current

Research is ongoing around the world as scientists and plant breeders attempt to increase the oil yield and improve the oil composition of oil seed crops. The following information details trials from around the world, where animals were offered various dietary sources of beneficial fatty acids and the subsequent availability of these fatty acids in meat, milk and eggs was measured.

12.1 Linseed

Linseed was traditionally grown for its oil in the past, which is used in the manufacture of paints, varnishes and linoleum. Breeders are now producing linseed varieties with a desirable fatty acid profile. The Bleu Blanc Coeur research programme in France involved an examination of the health benefits for humans who eat foods (meat, dairy and bakery products) originating from cattle and poultry offered a diet rich in linseed. The results demonstrated that diabetic people and people suffering from high cholesterol levels all showed an improvement in their condition when consuming a diet that was rich in linseed-fed meat, dairy and poultry products and bread enriched with extruded linseed. Furthermore, these linseed rich foods were reported to have a better flavour than other similar products (http://www.prowse.co.uk/ AUDELOR/A260204.htm).

12.1.1 Linseed - Milk

The effect of offering dairy cows a linseed supplement (550 g/day), with added Vitamin E (9616 International units), with one of two basal diets consisting of grass silage or corn silage plus sugar beet pulp and soybean meal, on milk fat oxidation was studied by Belgian researchers (Focant *et al.,* 1998). Milk fatty acid levels increased, while the fat and protein content of the milk was reduced when linseed was offered. The α-tocopherol concentration increased with the addition of Vitamin E to the diet, providing a greater resistance to oxidation of the milk.

Ward *et al.* (2003) conducted a study to examine the effect of supplementing fresh forage with solin (an edible linola type linseed), on CLA levels in plasma and milk. The authors reported that offering fresh forage, compared with conserved hay increased milk fat vaccenic acid (C18:1 *n*-7) and CLA proportions by 22% and 15% respectively. Furthermore, it was concluded that feed supplementation with solin is a superior method of increasing CLA levels in milk fat, compared to a fresh forage only diet as the inclusion of crushed solin seed further increased milk fat vaccenic acid and CLA proportions by 41% and 25% respectively.

Offering whole linseed plus Vitamin E to dairy cows to increase levels of *n*-3 α-linolenic acid in milk was examined by Deaville *et al*. (2004). Cows were offered a total mixed ration, based on grass silage supplemented with 6 or 8 g Vitamin E/day and 78, 142 or 209 g/kg of xylose-treated (rumen protected) whole cracked linseed. Results showed that milk fat from linseed-fed cows had a reduced saturated fatty acid content and a substantially increased CLA concentration. No significant increase in milk Vitamin E concentration was recorded at either 6 or 8 g/day inclusion in the diet.

Gonthier *et al*. (2004) reported on ruminal fermentation and nutrient utilisation in a dairy cow feeding trial, comparing a control diet of silage with a supplemented diet, by offering three types of flaxseed (linseed) (1) raw (2) unprocessed and (3) micronised and extruded flaxseed, as 12.6% of ration. It was concluded that flaxseed addition to the diet at this level had adverse effects on ruminal fermentation but improved total tract nutrient utilisation. Further work by Gonthier *et al*. (2005) reported that feeding micronised or extruded flaxseed to dairy cows increased long chain PUFA and decreased saturated fat levels in milk with blood plasma levels displaying similar characteristics. However, offering flaxseed resulted in a reduction in milk yield and energy corrected milk, by 1.8 and 1.4 kg/day respectively. Loor *et al*. (2005) examined the fat yield and fatty acid profile of milk from cows offered high (65%) or low (35%) concentrate diets consisting of long cut grass hay forage and a concentrate mix of ground wheat, soybean meal and rapeseed meal, with or without linseed oil, included at 3% DM. Milk fat yield and *n*-3 fatty acid levels were lower in the cows supplemented with concentrate diets, whereas linseed oil addition significantly increased CLA concentration in milk, although milk fat and milk yield was reduced. Dhiman *et al*. (2000) reported that dairy cows offered diets rich in linoleic (*n*-6) and linolenic (*n*-3) fatty acids produced milk with higher CLA levels. Selected groups of cows were offered a basal forage diet of alfalfa-corn silage (3:1) and a supplement of (1) roast cracked soya (2) soybean oil or (3) linseed oil at 1%-4.4% DM. The *n*-3 α-linolenic acid levels were highest in milk from linseed-fed cows, although this still represented a poor transfer of fatty acids from linseed into milk.

12.1.2 Linseed - Meat

12.1.2.1 Linseed - Beef

Campo *et al*. (2003) reported that meat from steers grazing pasture for 60-90 days pre-slaughter, along with 125 g/kg (DM) whole linseed as a supplement, did not have adverse flavour traits and meat had elevated *n*-3 fatty acid levels. Offering steers linseed or linseed oil as a supplement to a grass/clover forage also improved the *n*-6:*n*-3 ratio, from 4.2 to 2 and the shelf life of meat was unaffected.

Researchers at Institute of Grassland and Environmental Research (IGER) in Wales have investigated the possibility of manipulating the fatty acid composition of beef, by offering animals diets rich in *n*-3 fatty acids. Animals were offered grass silage and one of four concentrates for 120 days; (1) megalac (experimental control, rich in saturated palmitic acid, C16:0), (2) whole linseed (α-linolenic acid, C18:3 *n*-3), (3) fish oil (EPA, C20:5 *n*-3 and DHA, C22:6 *n*-3) and (4) linseed plus fish oil in equal amounts. Data showed that in comparison to the control, offering linseed to the beef cattle doubled the concentration of linolenic acid and significantly increased the level of EPA in the meat (http://www.seedsofhealth.co.uk/articles/healthy_beef.shtml).

The effect of offering rumen-protected linseed to calves that were deficient in Vitamin E and Selenium was examined by Kennedy and Rice (1992). Calves were offered a low Vitamin, low Selenium diet, supplemented with a rumen-protected linseed. Euthanatised after 6 to 11 days feeding (two died at 6 and 8 days), five out of eight calves developed severe macroscopic myocardial alterations, compared to calves not offered the fatty acid supplement. Control calves offered a diet supplemented with Selenium and Vitamin E did not show any alterations to heart tissue.

12.1.2.2 Linseed - Lamb

Linseed meal can be offered to lambs and ewes at inclusion rates of 7.5% and 20% respectively, while whole linseed is offered to ewes at an inclusion rate of 5% of the diet (Ewing, 1997). Demirel *et al*. (2004) reported that lambs supplemented with linseed while at grass had an elevated PUFA:saturated fatty acid ratio in the liver and adipose tissue (but not muscle) and the *n*-6:*n*-3 ratio was improved. Surprisingly, Chikunya *et al.* (2004) reported that the beneficial PUFA in a formaldehyde-treated linseed plus fish oil diet for sheep was subject to intensive biohydrogenation in the rumen. Blood plasma levels of C18:3 *n*-3 fatty acids were increased in sheep, when a linseed supplement (formulated to provide fatty acids at 50 g/kg DM) was offered with dried grass, but a linseed plus fish oil mix (fatty acids at 50 g/kg DM) increased the concentration of long chain PUFA C22:6 *n*-3 (DHA) and C20:5 *n*-3 (EPA) fatty acids.

Data from an earlier study, which examined the effects of linseed addition to a shelled corn, alfalfa hay and corn silage diet of lambs, demonstrated that while linseed was the most efficient feed converter in lambs, with larger weight gains, the lambs finished condition was poor and they were sold at a loss (Patterson, 1919).

Wachira *et al*. (2002) reported that offering whole linseed or fish oil to lambs, along with a basal diet of dried grass lowered the *n*-6:*n*-3 ratio in the meat. These researchers also concluded that linseed feeding doubled the proportion of linolenic acid in the *longissimus dorsi* of the lambs (1.4-3.1) and in the subcutaneous adipose tissue (1.2-2.6). Furthermore, offering fish oil significantly increased the muscle proportion of DHA in the lambs (0.3-0.8).

Cooper *et al*. (2004) offered lambs (29 ±2.1 kg) one of five concentrate-based diets, which were formulated to have a similar fatty acid content (60 g/kg DM). The diets contained either (1) linseed oil (2) fish oil (3) protected linseed and soyabean (4) fish oil and marine algae or (5) protected linseed and soyabean plus marine algae. Lambs were slaughtered at 40 kg weight and data demonstrated that lambs offered the protected linseed and soyabean diet had twice the percentage of *n*-3 linolenic acid in the muscle (3.8%) than lambs offered the linseed oil diet. Furthermore, lambs offered the protected linseed and soyabean diet had 5.5 times more *n*-3 linolenic acid in the muscle than lambs offered the fish oil and marine algae diet. Similar findings were reported for the adipose tissue of the lambs.

12.1.2.3 Linseed - Pork

Pork can be enriched with *n*-3 fatty acids, by offering pigs flaxseed (Specht-Overholt *et al*., 1997). Linseed meal is generally offered to sows and finishing pigs at inclusion rates of 5% and 10% respectively. However, linseed has had a softening effect on carcass fat. Linseed meal is deficient in essential amino acids, it is rich in protein and is best used together with other feeds (Ewing, 1997). Matthews *et al*. (2000) examined the effect of including linseed at 0, 50 and 100 g/kg (DM) in the diet of finishing pigs on the subsequent fatty acid levels. With linseed supplementation, it was demonstrated that *n*-3 levels increased significantly in the muscle, liver and kidney of pigs. Increasing linseed levels in the diet of pigs had no negative impact on oxidative stability and sensory evaluation of taste, while odour and colour of meat were normal. Sheard *et al*. (2000) concluded that dietary enrichment of pig meat with linseed improved its nutritional value, without affecting the quality and taste parameters.

12.1.2.4 Linseed - Poultry

Linseed is primarily used for *n*-3 enriched egg production with poultry, a practice that is already established in the food industry. Zanini *et al*. (2004) examined the effect of offering dietary oils plus Vitamin E on fatty acid levels in the thigh and chest meat of roosters. Offering linseed oil reduced the *n*-6:*n*-3 ratio in thigh meat and with the inclusion of Vitamin E, levels of *n*-3 C18:3, C20:5 and C22:6 all increased. A similar *n*-3 enrichment of breast and thigh meat was recorded by Nam *et al*. (1997), after dietary supplementation with 10% linseed and 100 µg/kg Vitamin E. Levels of α-tocopherol and oxidative stability of meat also increased significantly. Linseed is toxic to poultry at inclusion levels above 3% in the diet, due to the adverse effect on B Vitamins and metabolism (Ewing, 1997).

12.1.3 Linseed - Level of inclusion in the diet

Linseed meal or whole linseed can be included in the diet of different animals at different levels, as demonstrated in Table 16.

Table 16 Recommended levels of linseed inclusion in the diet of different animals (%)

	Linseed meal	**Whole linseed**
Milk	7.5	5
Beef	20	20
Poultry	2.5	0
Pork	5-10	0
Lamb	7.5	0
Ewe	20	5

(Ewing, 1997)

12.1.4 Linseed - Availability/Protection

If whole linseed is coated with sodium bicarbonate, it can escape biohydrogenation in the rumen. Equally, xylose-treatment of whole or cracked linseed can offer protection against biohydrogenation in the rumen (Ewing, 1997).

12.1.5 Linseed - Advantages/Disadvantages

Linseed is a valuable source of digestible protein, but it is not rich in calcium or lysine. Excess feeding of linseed to dairy cows can cause a reduction in milk fat content and souring can cause “bloom” in cattle. In pork and lamb, overfeeding with linseed can cause the carcass fat to become soft and meat flavour to taint. Rations containing more than 5% linseed will retard growth and can cause death in poultry (Ewing, 1997).

The effect of including linseed in the standard diet of livestock and the subsequent fatty acid profile of humans consuming these animals as part of their diet, has been studied by Weill *et al.* (2002). Extruded linseed (5% of diet) fed animal products contained more *n*-3 fatty acids and more CLA than control diet animals and the *n*-6:*n*-3 ratio was reduced by 54%, 60% and 86% in butter, meat and eggs respectively. Consumption of these products significantly modified the plasma and erythrocyte fatty acid composition of the human subjects examined, with a sharp increase in CLA.

Farmers often offer their animals linseed so that they have healthier skin and shiny coats.

12.2 Hemp

The use of hemp seed or hemp oil as an animal feed is not yet widely exploited.

12.2.1 Hemp - Eggs

Silversides *et al*. (2002) investigated the effect of offering hemp seed meal (*n*-6:*n*-3 of 5:1) at 0, 5, 10, and 20% in a cereal diet to laying hens. Results indicated that PUFA levels in eggs were increased and saturated fatty acid levels were reduced. Increasing the quantity of hemp in the diet increased the percentage of α-linolenic and linoleic acid in eggs. No other uses of hemp as an animal feedstock have been found to date.

12.3 Chia

12.3.1 Chia - Milk

In an ongoing study by Ayerza and Coates (2001b), lactating cows offered a basal diet of grazed pasture were supplemented with 10% chia seed. After sensory evaluation of milk from the chia-supplemented and unsupplemented cows, it was concluded that milk odour and taste were not influenced by chia seed inclusion in the diet.

12.3.2 Chia - Meat

No information is available with regard to chia as a feed source for beef, pork or lamb production.

12.3.2.1 Chia - Poultry

Meat from chickens offered a standard diet plus 10% or 20% chia supplement did not affect the preference of a sensory panel in terms of flavour or aroma of brown or white meat. The proportion of *n*-3 linolenic acid was enhanced significantly in both meats when chia was included in the diet. However, body weight of the birds was reduced by up to 6.2% and feed conversion was reduced with the 20% chia supplement (Ayerza and Coates, 2002).

Offering 14% (16.8 g in 120 g/day) chia seed as a dietary supplement to a standard diet for laying hens increased that the *n*-3 fatty acid content of eggs to 16%, without adversely affecting egg odour, flavour or acceptability. The *n*-3:*n*-6 was reported to be 0.99. A further trial comparing the effects of offering chia seed (15% of diet), with whole linseed and linseed oil, supported the previous findings and indicated that chia was the more effective supplement for enhancing the *n*-3 fatty acid content of eggs (Craig and Sons, 2004).

Researchers examined the effect of supplementing a standard diet with 10% chia seed on the fatty acid composition of the breast and thigh muscle of broilers (Craig and Sons, 2004). These authors reported that *n*-3 α-linolenic acid concentrations were significantly higher in the meat of broilers offered 10% chia, compared to broilers offered a control diet. Table 17 shows the difference in the *n*-3 content between the chia-supplemented and the control diet birds. No adverse effects on texture, appearance, flavour or odour were detected during sensory evaluation of the meat. In a further sensory evaluation, no oxidation was detected when the taste and smell of eggs were examined. These studies were performed on behalf of Craig and Sons (2004).

Table 17 Mean *n*-3 fatty acid content of broiler breast meat

***n*-3 fatty acids**	**Control diet**	**10% Chia diet**
C18:3 (Linolenic acid)	6.32	12.67
C22:5 (DPA)	0.42	0.35
C22:6 (DHA)	1.28	1.11

(Craig and Sons, 2004)

12.3.3 Chia - Dietary Inclusion

Limited data are available regarding the effect of rate of inclusion of chia seed in the diet of animals on the subsequent quality attributes. However, the work of Ayerza (1995) and Craig and Sons (2004) indicate that offering chia seed at rates of up to 10% in the diet of lactating cows and 10-20% in the diet of broilers, does not have any adverse effect on the sensory quality of the produce.

12.3.4 Chia - Advantages/Disadvantages

The beneficial effects of using chia as a feed supplement for cows and poultry have been highlighted in the aforementioned trials. Researchers have reported that the high PUFA content of the seed is a means of introducing the beneficial *n*-3 fatty acids into the food chain.

12.4 Marine Algae

Research into the large-scale production of marine algae (principally by large scale fermentation processes) as a source of fatty acids, has been ongoing for several years (e.g. Apt and Behrens, 1999). The fact that plant oils contain virtually none of the long chain essential fatty acids, EPA and DHA, which can only be sourced form marine organisms (fish and algae), has encouraged research into the possibility of developing plants which can produce oils bearing these fatty acids.

Abbadi *et al*. (2004) described the biosynthesis of fatty acids by transgenic oilseeds and the limitations of natural systems, where the genetic PUFA synthesising code was transferred from other organisms, such as fungi and microalgae, into plants. Green and Singh (2004) also reported on research in this area. A project was initiated by the Commonwealth Scientific and Industrial Research Organisation (CSIRO), in 2004, entitled 'The Food Futures Flagship Program', which is aimed at investigating the potential and scientific feasibility of this new technology (http://www.csiro.au/index.asp?type=blank&id=FlagshipPrograms).

Current research is aimed at the eventual commercial production of microbial oils on an industrial scale, for both human and animal consumption. Apt and Behrens (1999) reported on advances in the production of PUFA rich oil from microalgae and concluded that controlled fermentation systems and developments in cryopreservation could enhance large-scale production techniques of heterotrophic algae. Ni Faolain and Fitzpatrick (1999) conducted a study to define the fatty acid content of three marine microalgae, namely, *Skeletonema cotatum* (a diatom) and two flagellates, *Sochrysis galbana* and *Tetra-selsius suecica.* Sijtsma and de Swaaf (2003) reviewed recent advances in the production of DHA from marine organisms and reported that controlled large-scale systems of production could produce a nutritionally rich marine algae feedstuff of consistent quality. de Swaaf *et al*. (2003) studied strains of the algae *Crypthecodinuim* and reported that these strains had oil with *n*-3 levels (DHA) of approximately 30%. Scientists at the CSIRO are investigating the potential of transferring algal genes, which code for oil production into oilseed plants. The rationale behind this is that the fatty acid profile of the oil can be altered to produce long chain PUFA-rich oilseeds (Nichols *et al*., 2004). The possibility of transferring the entire genomic sequence coding for microalgal long chain PUFA biosynthesis to other organisms to create more efficient and economical production pathways was explored by Green and Singh (2004).

Very little work has been undertaken regarding the usefulness of marine algae as a ruminant feed, due to the low efficiency of DHA transfer to meat in ruminant species. The majority of animal nutrition studies with marine algae have been related to poultry and aquatic (farmed fish) production systems (Personal communication, R. Abril, Martek Biosciences).

The situation for pigs, cattle and sheep is not well researched and doubts as to the practicalities and cost effectiveness are prescient. It has been reported that high *n*-3 pork can be produced by offering pigs diets containing marine or vegetable sources of *n*-3 fatty acids (http://www.gov.mb.ca/agriculture/livestock/nutrition/bza04s02.html).

12.4.1 Marine algae - Milk

Franklin *et al*. (1999) reported no incidence of rancidity in milk from cows offered a diet containing rumen/intestinal-protected and unprotected marine algae. In the study of Franklin *et al*. (1999), cows were offered 910 g/day xylose-coated marine algae or 910 g/day of unprotected marine algae in a basal diet consisting of alfalfa hay, corn silage, corn grain and soybean meal, with vitamins and trace elements. Results showed that the addition of marine algae to the diet of cows reduced their feed intake and milk fat concentration, increased the CLA levels (and DHA in particular) in milk and reduced intestinal fat content. Sensory evaluation tests did not detect oxidative effects on flavour or odour in the milk. The marine algae feedstuff used by Franklin *et al*. (1999) was DHA Gold, which is a proprietary brand of an animal feed product from an American company, Martek Biosciences, USA (http://www.martekbio.com/Nutritional_Products/Introduction.asp). The rumen protection xylose coating was applied to the DHA Gold marine algae by LignoTech USA, Rothschild, WI, using a patented process.

12.4.2 Meat

12.4.2.1 Marine algae - Poultry/Eggs

Herber and van Elswyk (1996) reported on findings from two studies, comparing the effects of offering a range of dietary treatments on the production of *n*-3 enriched eggs from 24 and 56 week old hens. The effect of offering marine algae feed (DHA Gold) at 2.4% (200 mg DHA/day) and 4.8% (400 mg DHA/day) of the basal corn and soybean diet was compared to offering EPA-rich fish (menhaden) oil at 1.5% of the diet. Hens offered the marine algae diet produced eggs with a high DHA content, especially at 4.8% inclusion in diet, and the *n*-6 content of the eggs was reduced, compared to eggs from hens offered a basal control diet. Abril *et al.* (2000) reported no adverse effects of supplementing the diet of hens with 165, 495 and 825 mg DHA/hen/day, in terms of egg quality. These authors also concluded that marine algae (DHA Gold) is safe to use as an additional feed source for the DHA enrichment of eggs.

At Gold Circle farms in America, hens are offered a high quality feed that includes a marine microalgae, which is naturally rich in DHA. This special algae exists in nature and is the same as that which provides cold water fatty fish like salmon, with its DHA. Research also shows that the DHA is not destroyed by heat whilst cooking the egg. The DHA is incorporated into the lipoprotein of the yolk of the egg, which protects it from the heat (http://www.goldcirclefarms.com/faq.html). Gold Circle Farms have a number of egg products for sale, including DHA *n*-3 cage-free eggs and DHA *n*-3 cage-free liquid eggs, which were available in early 2005. There is approximately 150 mg DHA in each egg produced and 3-4 Gold Circle Farms eggs are equivalent to 3 oz salmon, in terms of DHA content. The positive implications of ‘designer eggs’ on human health were recently displayed in Health Magazine (October, 2004).

In a review of the potential of marine algae as an alternative *n*-3 fatty acid source to fish oil (Givens *et al*., 2000), it was concluded that while there is some evidence that offering marine algae to laying hens is effective in enhancing *n*-3 levels in eggs, the situation for pigs, cattle and sheep is not well researched and doubts as to the practicalities and cost effectiveness are prescient.

12.5 Fish Oil/Fish meal

12.5.1 Fish oil/Fish meal - Milk

Shingfield *et al*. (2003) examined the effect of offering fish oil to lactating cows that were offered a basal diet of silage and concentrate (ratio 60:40). The milk fatty acid profile and milk yield of the cows were compared after a 14-day period on the basal diet, and after a further 14 days with the fish oil supplement (250 g fish oil/day). Data showed that the addition of fish oil to the cow's diet decreased DM intake and milk yield, but the CLA content of the milk increased.

In a trial assessing the effects of supplementing dairy cows offered pasture and soya bean diets, with rumen protected fish (tuna) oil, Kitessa *et al.* (2004) reported that milk from cows offered the rumen protected tuna oil had total *n*-3 fatty acid levels that were three to four fold greater than milk from the pasture plus soybean fed cows. Concentrations of EPA and DHA were 6.9 and 10.1 g/kg milk fat respectively in cows offered the tuna oil-supplement, whereas these fatty acids were undetectable in the milk fat of cows that were not offered the fish oil supplement. The taste and smell of the milk from the tuna oil supplemented and unsupplemented cows was similar, and these authors concluded that it is possible to increase milk *n*-3 PUFA levels, without adversely affecting sensory characteristics or yield.

Equally, Wright *et al*. (2003) reached similar conclusions after offering dairy cows a combination of fish meal and feather meal pellets, at 4:1 and 1:4 combinations, supplemented at 3.75%, 11.75% and 27%, with a basal diet of corn silage. Wright *et al*. (2003) reported that DHA levels in the milk were greater for cows offered the fish oil diets. However, as DHA concentration the in diet increased, the concentration decreased in milk, therefore suggesting that the efficiency of transfer of DHA is related to diet composition and quantity.

In another study by AbuGhazaleh *et al*. (2002), the fatty acid profile of milk and rumen digesta from cows offered a supplement consisting of fish oil or soya beans in various combinations, with a basal diet of 25% corn silage, 25% alfalfa hay and 50% concentrate was examined. The supplemented diets offered were (1) a control (basal) diet (2) a diet with 2% added fat from menhaden fish oil (3) a diet with added fat from 2% extruded soybeans and (4) a diet with 1% added fat from extruded soybeans and 1% added fat from menhaden fish oil. Results indicated that CLA concentration of milk increased with all the fat supplemented diets, with no difference in milk CLA content between the fat supplements. Rumen fatty acid salts and free fatty acids in milk increased in all fat supplemented diets. Dry matter intake of the cows offered the fish oil diet decreased slightly.

Lynch *et al*. (2005) studied the effects of offering elevated CLA levels in the diet of cows on the flavour and stability of pasteurised milk. Cows were offered a control diet or a control diet supplemented with 2% soya oil and 1% fish oil (CLA diet). Milk from the cows offered the CLA diet had significantly higher CLA concentrations than cows offered the control diet, with no adverse effect on flavour. Furthermore, no difference in the preference for milk from cows on both diets was reported.

12.5.2 Fish oil/Fish meal - Meat

12.5.2.1 Fish oil/Fish meal - Beef

Noci *et al*. (2004) examined the fatty acid composition of muscle from steers offered a diet consisting of wilted or unwilted grass silage and a concentrate mixed with fish oil. Four concentrate rations were enriched with 0, 10, 20 or 40 g/kg of fish oil and offered to steers with wilted silage. Analysis of the *M. longissimus dorsi* muscle and intramuscular fat indicated that an increase in fish oil intake in the diet of steers resulted in a concomitant linear increase in the CLA concentration of muscle and a linear decrease in *n*-6:*n*-3 in muscle. Eicosapentaenoic acid and DHA concentrations of muscle also increased linearly as the quantity of fish oil in the diet increased. It was concluded that supplementation of beef cattle diets with fish oil enhanced the concentration of beneficial fatty acids in the meat.

Researchers at IGER in Wales offered steers grass silage and one of four concentrates for 120 days (1) Megalac (experimental control, rich in saturated palmitic acid, C16:0), (2) whole linseed (α-linolenic acid, C18:3 *n*-3), (3) fish oil (EPA, C20:5 *n*-3 and DHA, C22:6 *n*-3) and (4) linseed plus fish oil, in equal amounts. Offering fish oil doubled the levels of EPA and DHA, whilst the mix gave results that were intermediate between offering linseed and fish oil (http://www.iger.bbsrc.ac.uk/Practice/Publications_&_Leaflets/informationsheets/HealthyBeef.htm).

12.5.2.2 Fish oil/Fish meal - Lamb

Demirel *et al*. (2004) supplemented the standard diet of a lamb with fish oil and Vitamin E and examined fatty acid profile of muscle, liver and adipose tissue. Three fat sources were included in the diets (1) Megalac (2) formaldehyde-treated linseed or (3) formaldehyde-treated linseed plus fish oil at a 1:1 (w/w). Including linseed and fish oil in the diet increased long chain *n*-3 levels in lamb liver and adipose tissue only. Chikunya *et al*. (2004) offered similar diets to sheep and reported that linseed addition to the diet only increased C18:3 *n*-3 levels in plasma, but that the addition of a fish oil and linseed mix to the diet of sheep increased EPA and DHA levels in lamb blood plasma.

Australian researchers offered a protected tuna oil supplement or tallow for 42 days (3% DM) to lambs on a basal diet of lucerne hay and examined the fatty acid composition of the meat (Kitessa *et al*., 2001). Data demonstrated that both the EPA and DHA levels in the muscle tissue of the lambs offered the tuna oil were three times more prevalent than in the lambs offered tallow (1.8% and 1.5% EPA and DHA respectively for lambs offered tuna oil). It was also noted that the level of linoleic acid in the muscle and adipose tissue of the lambs was twice as much in tuna oil-supplemented lambs than in tallow-supplemented lambs.

Other research has indicated that the fatty acid composition of lamb offered a low or medium quality pasture or roughage diet can be altered significantly by offering fish meal or soy meal for six or seven weeks prior to slaughter (Ponnampalam *et al*., 2000).

12.5.2.3 Fish oil/Fish meal - Pork
Fish meal has been described as 'a feed with a very healthy future' for pigs by the Fish meal Information Network (http://www.gafta.com/ fin/FINpigs.pdf). Pork can be enriched with *n*-3 fatty acids by offering pigs fish meal/fish oil (Specht-Overholt *et al*., 1997). Currently, BASF have the international marketing license to include synthetic CLA in animal feed (Dugan *et al*., 2004). It has been reported that PUFA levels over 14% (this level would rarely be achieved in commercial diets) can cause pigs to have a soft carcass fat and oxidation of PUFA, which can lead to off-odours or flavours (http://www.gafta.com/fin/FINpigs.pdf).

Ruiter *et al*. (1978) examined the effect of offering piglets a standard grain diet with 100 g/day of either fish oil (mackerel) or olive oil. Findings showed the fatty acid composition of blood serum, breast muscle and liver of piglets supplemented with fish oil had a significantly increased *n*-3 fatty acid content and a reduced *n*-6 and *n*-9 fatty acid content. Some indications of "yellow fat" disease, associated with Vitamin E deficiency, were noticed in the carcass of pigs offered the fish oil diet. The fat characteristics of pigs offered a control diet consisting of corn (22%), milo (22%), barley (22%), wheat bran (12%), soybean meal (9%), de-fatted rice bran (4%), fish meal (2.5%), vitamins and trace elements and the control diet plus three levels of fish oil (2, 4 and 6%), for four weeks prior to slaughter, was studied by Irie and Sakimoto (1992). Biopsy samples of fat indicated that DHA and EPA levels increased from the first week of supplementation and increased linearly with higher inclusion levels of fish oil in the diet. Fat colour was not significantly different between the control and fish oil-supplemented group, but fat hardness decreased with increasing fish oil intake.

Furthermore, Fritsche *et al*. (1993) studied the effect of substituting lard with fish oil in a practical sow diet, on the enrichment of *n*-3 fatty acids in suckling pigs. At 120 days gestation, fish oil was added at 0, 3.5 and 7% to the sow diet, which consisted of ground corn (59%), oats (10%), and soybean meal (20%), in place of the same quantities of lard. Offering fish oil in the diet increased the sow serum *n*-3 constituents to six-fold that of sows offered the lard diet. This maternal gain was also transferred to newborn piglets, where a five to ten-fold increase in serum *n*-3 PUFA levels was recorded. It was concluded that offering fish oil supplemented diets to sows during late gestation and lactation enriched their piglets with *n*-3 PUFA.

12.5.3 Dietary Inclusion

The suggested rates of inclusion of fish meal in dairy cow diets for milk production are given as 500 to 750 g/day (Fish meal Information Network, 2004) up to 5% maximum as a supplement for cow diets (Ewing, 1997).

12.6 Lupins

12.6.1 Lupins - Milk

When Froidmont and Bartiaux-Thill (2004) compared the effects of offering dairy cows soya bean meal, lupins or a lupin/pea mix on the fatty acid profile, they reported that lupin seed in the diet tended to reduce medium chain fatty acids ($P<0.1$) and increase long chain fatty acids ($P<0.01$) in the cow's milk. Furthermore, C18:2 and *n*-6:*n*-3 were significantly reduced in the lupin-based diet, compared to the soya bean meal diet. A high level of C18:0 was also recorded in the milk from cows offered the lupin feed, which was attributed to rumen hydrogenation of C18:1, the main fatty acid present in lupin seeds. Other research has also shown that the incorporation of lupin seed into dairy cow diets reduced *n*-6:*n*-3 fatty acids, which is beneficial for human health (Henderickx, 2002).

12.7 Oilseed Rape

Canadian plant breeders were successful in breeding a new OSR strain called Canola, which has a very desirable fatty acid profile (Raymer, 2002). Monsanto have currently applied to the European Union, for a licence to grow genetically modified OSR, with an altered fatty acid profile.

Previous research at Harper Adams University has demonstrated that calves offered an essential oil (OreGin) (from OSR), as part of an early weaning ration, had greater live weight gains and a reduced incidence of scouring (Farmers Weekly, 2005).

12.7.1 Oilseed rape - Milk

Givens *et al*. (2003) offered whole cracked rapeseed in three experimental diets, based on different ratios of grass silage, maize silage, wheat, rapeseed meal and soya bean meal to dairy cows and examined the subsequent effect on milk fatty acid levels and constituents. Data indicated that offering a rapeseed feed supplement at 2 kg/day significantly increased the milk fatty acid content, especially C18:1. In an experiment with fattening bulls, White *et al*. (2003) found that substituting lupins with 2.15 kg/day rumen-protected canola meal in a diet consisting of grass silage plus crushed lupins (5.4 kg DM) and barley (4:1), had no effect on milk fat concentration. When extruded rapeseed and linseed supplemented with Vitamin E was offered to dairy cows, the proportion of all C18 fatty acids increased, in particular, the *n*-9 oleic acid and *n*-6 fatty acids, for both the rapeseed and linseed diets (Focant *et al.*, 1998). Furthermore, it was also noted that without supplementing the oilseed diets with 9616IU Vitamin E, the oilseeds had a 30-40% reduction in the resistance to oxidation.

Research by McNamee *et al*. (2002) demonstrated that by including both ground or unground rapeseed in a cow's diet for 2 weeks (620-640 g fatty acids/day), the proportion of C18:1 in milk increased to approximately 300 g/kg of total fatty acids, compared to 214 g/kg for a barley-based control diet. Furthermore, there was a reduced proportion of the saturated C16:0 in milk fat when rapeseed was fed. Not only does this have health implications for humans, in terms of a PUFA-rich milk, but also for butter making, in that offering rapeseed to cows decreased the solid fat content of the milk, giving a softer and more spreadable butter. Stanton (1999) also concluded that rapeseed is very effective in increasing CLA levels in milk, by over

50%, when offered as a supplement to cows at pasture, without affecting milk yield and composition. More importantly, Siedel *et al*. (2005) reported that fat modified milk (from offering cows rapeseed cake) showed positive effects on LDL:HDL and lipoprotein concentrations in humans, both of which are linked with the incidence of coronary heart disease.

12.7.2 Oilseed rape - Meat

12.7.2.1 Oilseed rape - Beef

Australian researchers examined the fatty acid profile of beef from feedlot steers offered protected canola seed and sunflower seed meal supplements (Ashes *et al*., 1993). Fifty Hereford cross steers were offered barley-based concentrate rations containing (1) control (containing 15% untreated sunflower meal) (2) 15% protected sunflower seed meal (3) 10% protected canola seed (4) 15% protected sunflower seed meal plus 10% protected canola seed or (5) 15% protected sunflower seed meal plus 15% protected canola seed, for 133 days. Data showed that including protected canola seed with or without protected sunflower seed meal in the diet of steers increased the proportion of C18 PUFA in the subcutaneous, perirenal and omental fat. The proportion of linoleic (C18:2) and linolenic (C18:3) fatty acids increased by three and five fold respectively, and the proportion of saturated palmitic acid was reduced by 20-25%, with the greatest decline being recorded in steers offered 15% protected canola seed.

It appears that rapeseed needs to be rumen protected, in order to achieve benefits in terms of an increased PUFA profile in the meat of steers offered this feed. Ekeren *et al*. (1992) reported no difference in the fatty acid profile of meat from steers offered rapeseed in an unprotected form compared to meat from animals offered rumen-protected rapeseed. Similar findings were reported by St John *et al*. (1987) and Huerta-Leidenz *et al.* (1991).

12.7.2.2 Oilseed rape – Lamb

Preliminary data from a study at The Agricultural Research Institute of Northern Ireland, Hillsborough indicates that there is a lack of a major effect of offering an oilseed rape-based concentrate on the fatty acid profile of lamb meat, relative to lambs offered a soyabean-based concentrate (Dawson, personal communication). Equally, Australian researchers (Ponnampalam *et al*., 1997) compared the effect of offering lambs canola, soy and fish meal, with a basal diet of oaten chaff and lucerne *ad libitum* (80:20). The lambs were 32.1 kg and were allocated to one of the four diets for seven weeks. Data obtained were similar to that from the study of Dawson (personal communication), in that offering fish meal and soyabean meal to lambs significantly increased the total EPA and DHA in the muscle (32.8 and 29.2 mg/100 g meat respectively), relative to the basal diet alone or basal diet plus canola meal (23.6 and 20.9 mg/100 g meat respectively).

12.7.2.3 Oilseed rape – Pork

Leskanich *et al.* (1997) offered pigs diets containing 2% rapeseed oil and 1% fish oil and a minimum of 100 mg α-tocopherol/kg diet, during the finishing period. These authors reported a significantly increased *n*-3 content in the pig fat, without any significant effect on the sensory attributes of the meat.

Research at the Federal Research Farm Königshof, in Austria, was conducted to determine the effect of substituting sorghum, triticale and barley for rapeseed, in the diets of 210 growing pigs (Frickh *et al*., 1998). Tests began at 30 kg live mass at the start of the study and ended at 100 kg. Rapeseed was included at levels of 0, 3, 6 and 9% in the diet. In another component of the study, for two groups of pigs, rapeseed was reduced during the second fattening period, from 9% to 4.5% and 0%, respectively. Average daily feed intake, average daily gain and feed conversions were unaffected by the levels of rapeseed included in the diet. Pigs offered diets containing 6% rapeseed had a higher lean meat content and slaughtering percentage, but a concomitant lower fat content than pigs offered 0, 3, 4.5 and 9% rapeseed in the diet. Sensory evaluations of the meat indicated that meat colour and pH was not affected by the level of rapeseed offered in the diet. External fat PUFA increased as the proportion of rapeseed in the diet increased (from 12.4 % (0% rape), 14.7 % (3% rape), 16.9% (6% rape) to 18.7 (9% rape)). Reducing the quantity of rape from 9% to 4.5% to 0% in the second study decreased PUFA by 16.7% and 12.8% respectively. The authors recommended offering 9% rapeseed in the pigs diet to begin with, and to reduce this to 2-3% during the fattening period due to the presence of soft fat at higher levels of inclusion.

12.7.2.4 Oilseed rape - Poultry

Researchers in Croatia and the Czech Republic examined the effect of offering rapeseed or rape oil on the fatty acid profile of chicken meat (Kralik *et al*., 2003). These researchers compared the fatty acid profile of chickens that were offered swine lard, rapeseed or rape oil in three treatments as follows:

		Balance of diet
Treatment 1:	7.5% swine lard	Corn Soyabean meal Sunflower meal Limestone Phosphonal Salt Premix
Treatment 2:	6.2% rape oil	
Treatment 3:	13.5% rapeseed plus 2% swine lard	

Data from the study demonstrated that the addition of rapeseed and rape oil to the diet of the chickens lowered the saturated fatty acid content and increased the proportion of MUFA and α-linolenic acid in the muscle and abdominal fat of the chickens. Furthermore, the *n*-6:*n*-3 decreased from 21.3 to 6.6 and 7.0 in the breast muscle of the chickens and from 16.3 to 7.6 and 8.2 in the abdominal fat from chickens offered treatments 1, 2 and 3 respectively. A reduction in *n*-6:*n*-3 has significant implications for human health. However, the addition of rapeseed and rape oil to the broiler's diet led to a significant decrease in carcass weight.

12.7.3 Oilseed rape - Dietary inclusion

Dietary inclusion of full fat rapeseed is recommended only for poultry. Rapeseed meal can be offered in the diet of ruminants and monogastrics. Table 18 displays an example of some dietary recommendations for rape meal.

Table 18 Feed concentration inclusion of rape meal (% per species)

Cattle		Swine		Sheep	
Calf	5	Grower	2.5	Ewe	20
Dairy	25	Finisher	5	Lamb	5
Beef	25	Sow	2.5		

(Ewing, 1997)

Other researchers in the Czech Republic have stated that heat-treated rapeseed cake can be offered at more than 3% of the total daily ration, without influencing animal health (Komprda *et al.*, 2002).

12.8 Soya

12.8.1 Soya - Milk

In a study by Ure *et al*. (2005), where dairy cows were offered treated extruded soyabean meal along with a basal diet of 440 g/kg forage and 560 g/kg grain, it was reported that the proportion of C18:2 in milk fat was greater for cows offered calcium oxide plus lignosulfonate-treated and twice extruded soyabean meal, relative to cows offered twice extruded soyabean meal or lignosulfonate-treated and twice extruded soyabean meal. These authors concluded that calcium salts of fatty acids are useful for protecting the lipid fraction of extruded soyabean meal. AbuGhazaleh *et al*. (2004) also concluded that the concentration of *cis*-9, *trans*-11 CLA and vaccenic acid can be increased in milk fat within a week, by offering dairy cows a blend of fish meal and extruded soyabean. These authors also concluded that such an increase remained relatively constant after supplementation for a period of five weeks. Previous research has also shown that supplementation of a cow's diet with full fat soybean significantly elevated CLA levels in milk (Stanton, 1999). Doherty and Mayne (1996) reported that including 410 g/day soya oil in the diet of dairy cows resulted in a significantly reduced milk fat and protein concentration.

12.8.2 Soya – Meat

12.8.2.1 Soya - Beef

Researchers in Israel performed a study to ascertain the effect of soyabean supplementation on the fatty acid profile in lipid depots of fattening bull calves (Aharoni *et al*., 2005). In this study, bull calves were offered a total mixed ration (ground maize, wheat silage, safflower silage plus vitamins and minerals) plus one of three dietary treatments as follows (1) ground maize plus soyabean meal (2) soyabean meal plus soyabean oil or (3) extruded full fat soyabean. The concentration of CLA in the intramuscular fat of the calves was 3.4, 13.0 and 15.4 mg/g respectively, for the three diets. Respective CLA concentrations in the subcutaneous fat were 5.2, 20.3 and 26.6 mg/g. Vaccenic acid concentrations were three fold greater in calves offered treatments 2 and 3, relative to treatment 1. These researchers also reported that blood Vitamin E concentration increased with oil supplementation, thereby improving the shelf life of the meat. The conclusion drawn from this study was that by supplementing a high forage fattening diet with soyabean oil or extruded full fat soyabean, at a level of 33 g added oil/kg diet DM, the concentration of the *cis*-9 *trans*-11 isomer of CLA increased by 281-410%, in both the intramuscular and subcutaneous fat.

Madron *et al*. (2002) also concluded that offering soyabean in the diet of steers increased CLA concentrations in meat. Madron *et al*. (2002) finished crossbred Angus steers for 111 days, with extruded full fat soybean. Dietary fat levels were 3.9%, 5.8% and 7.8% for the control, 12% extruded full fat soyabean and 25.6% extruded full fat soyabean diets respectively. The mean *cis*-9, *trans*-11 CLA concentration in the *longissimus*, round, and chuck combined, was 0.66%, 0.69% and 0.77% for the control, 12% extruded full fat soyabean, and 25.6% extruded full fat soyabean diets respectively. The *cis*-9, *trans*-11 CLA concentration was significantly higher ($P<0.05$) in steers offered the 25.6% extruded full fat soyabean diet. Conversely, Beaulieu *et al*. (2002) offered steers 5% soybean oil in a finishing study, for 102 days, and reported that the *cis*-9, *trans*-11 CLA concentration of the fatty acids in the meat represented 0.32-0.35% of fat, and were not significantly different from the control treatment.

12.8.2.2 Soya – Lamb

Initial data from a study at Hillsborough indicates a lack of a major effect of an oilseed rape-based concentrate on the fatty acid profile of lamb meat, relative to lambs offered a soyabean-based concentrate (Personal communication, Dr Lynne Dawson) as shown in Table 19.

Table 19 Effect of diet on fatty acid composition of lamb meat (preliminary results)

% total fatty acids	Linoleic acid (C18:2*n*-6)	α Linolenic acid (C18:3*n*-3)	EPA (C20:5*n*-3)	DHA (C22:6*n*-3)	*n*-6:*n*-3
Soyabean	7.01	2.04	0.64	0.68	2.71
Oilseed rape	7.23	2.40	0.78	0.72	2.31

(Dawson, personal communication)

12.8.2.3 Soya - Pork

Lemke (2005) demonstrated that the meat from pigs offered a full fat extruded soyabean diet was 250% richer in *n*-3 fatty acids and Vitamin E, compared to pigs offered conventional diets. However, this effect could be negated because soya is also rich in *n*-6 fatty acids, and as such, this would also alter the *n*-6:*n*-3 to a more unfavourable value.

12.8.2.4 Soya – Poultry

It has been reported that poultry consume approximately 49% of the soybeans offered to farm animals (http://www.agbios.com/cstudies.php?book=FSA&ev=GTS&chapter=Nutrition). Koci *et al*. (1996) examined the effect of offering iso-nutritious diets containing 0 or 14% extruded soybeans on the carcass characteristics of 42-day old chickens. Data showed that although there were no differences between treatments in terms of carcass yield, chickens offered soybeans had more abdominal fat (3.1 and 2.2% respectively, P<0.05), which was also more unsaturated (28.5 and 15.7% for linoleic acid respectively).

Other studies by Crespo and Esteve-García (2001) and Sanz *et al*. (1999) have shown that offering PUFA in the diet of broilers reduces the level of fat deposition. Consequently, the inclusion of soybean oil may reduce the percentage of abdominal fat in chickens at the expense of a greater degree of unsaturation of the fatty acids (Crespo and Esteve-García, 2002). Furthermore, Zollitsch *et al*. (1992) noted that the flavour of chicken improved with the introduction of soybean oil into the diet as a replacement for rapeseed oil.

12.9 Grass

Offering extensive quantities of pasture to animals may have an impact on the taste of meat or its shelf life. However, consumers may have a preference for this unique 'grassy' taste or indeed, the taste may change upon preparation at cooking (Mandell, 2004).

New Zealand researchers have examined the endogenous synthesis of *cis*-9*, trans*-11 CLA in dairy cows offered fresh pasture. Data showed that endogenous synthesis accounted for over 91% of the *cis*-9*, trans*-11 CLA secreted in the milk fat of cows offered fresh pasture (Kay *et al*., 2004). Researchers at IGER, in Wales investigated the potential to exploit sources of *n*-3 fatty acids in grass. The proportion of beneficial fatty acids in fresh grass varies with plant factors, such as stage of maturity and light treatments. These researchers have found that there are approximately 10 major fatty acids in grasses and that the fatty acid composition of grass is under considerable genetic control. These researchers also believe that there is potential to select for grasses with higher levels or altered types of fatty acids (http://www.seedsofhealth.co.uk/articles/healthy_beef.shtml).

12.9.1 Grass/Grass silage - Milk

It is well established that dairy products are the main source of CLA (Kelsey *et al*., 2003). Research in Portugal has shown that the concentration of CLA in milk fat is further enhanced by the dietary intake of pasture by cows (Rego *et al*., 2004). Lawson *et al*. (2001) reviewed the role of dairy products in supplying CLA to the human diet. These authors concluded that the CLA content of milk increases when cows are offered grazed grass, although many studies recorded a considerable variation in the concentration of CLA in raw milk. The effect of pasture intake on

CLA levels in milk fat was also studied by Stockdale *et al*. (2001). These authors reported that offering fresh pasture alone gave relatively high concentrations of CLA in milk (1.0-1.9 g/100 g milk fat). Some variation in the CLA concentration of milk has been attributed to varying intake of feed, which ranged from <10 kg to >20 kg DM/cow/day. In New Zealand, MacGibbon *et al*. (2001) sampled milk from 44 herds of cows supplying a single dairy and recorded seasonal variation in milk fat CLA levels. It was concluded that milk fat from cows grazing at pasture tended to have higher levels of CLA than those reported from other countries where cows were offered supplemented diets.

Equally, Gulati *et al*. (2001) concluded that when grazing dairy cows were supplemented with an oilseed-based protected fat at 2 kg/day, they had increased levels of *n*-3 fatty acids in their milk. Ekern *et al*. (2003) studied CLA levels in milk from dairy cows offered a grass silage-based diet, supplemented with barley-based concentrates and oat concentrates and normal versus high fat oats. Both the normal and high fat oat concentrate increased CLA levels in milk and reduced milk fat content. Overall, milk yield was increased with both diets, as was the PUFA:saturated fat. Ward *et al*. (2003) conducted two studies concurrently, to determine the effect of offering fresh forage versus a hay diet and fresh forage with a barley-based concentrate on CLA levels in the plasma and milk of cows. In one study, it was reported that as fresh forage intake increased in the diet of cows, from 65% to 85% of the total diet, and the quantity of concentrate was reduced, CLA, vaccenic acid and linolenic acid levels increased by 26%, 18% and 27% respectively. In the second study, when compared to conserved hay, fresh forage increased plasma CLA by 71%, but had no effect on plasma linolenic acid levels. Fresh forage compared to conserved hay increased milk fat vaccenic acid and CLA contents by 22% and 15% respectively.

12.9.2 Grass/Grass silage – Meat

12.9.2.1 Grass/Grass silage – Beef

The fatty acid composition of the intramuscular fat from steers offered grazed grass, grass silage and grass silage with a concentrate additive was investigated by French *et al*. (2000). Ten groups of five steers were offered one of five rations for 85 days prior to slaughter. The five diets offered were: (1) grass silage *ad libitum* plus 4 kg concentrate (2) 8 kg concentrate plus 1 kg hay (3) 6 kg grazed grass DM plus 5 kg concentrate (4) 12 kg grazed grass DM plus 2.5 kg concentrate and (5) 20 kg grazed grass DM. Concentrations of PUFA were greater in meat from steers offered the grass diet (diet 5) than with any other diet. A linear decrease in saturated fatty acid levels was also recorded.

Steen and Porter (2003) also examined the effect of offering pasture and high concentrate diets on the concentration of CLA in beef muscle and subcutaneous fat. Steers were offered a range of either (1) high concentrate and grass silage diets or (2) low concentrate, high silage diets, followed by grazing on perennial ryegrass pasture for 23 weeks prior to slaughter, for both groups. It was concluded that muscle and subcutaneous fat from grass-fed steers contained three times more CLA than steers that did not have access to pasture. Steen *et al*. (2003) also reported that the muscle of pasture-fed beef contained higher concentrations of *n*-3 PUFA

(141 and 49 ±8.2 mg/100 g muscle) and long chain *n*-3 PUFA (58 and 27 ±3.8 mg/100 g muscle) than that of concentrate-fed beef.

Furthermore, Poulson *et al*. (2004) examined the CLA content of beef from Angus crossbred steers offered high grain diets, with or without protected CLA polymers as a supplement, or a forage only diet consisting of grass plus clover. During an adaptive feeding period, all animals except those offered forage only were housed together and offered a basal ration containing 520 g/kg corn silage, 213 g/kg alfalfa hay, 250 g/kg rolled barley and 17 g/kg vitamin/mineral pre-mix. Forage only steers were offered alfalfa hay and a free choice vitamin/mineral pre-mix during the housing period. After 195 days on these diets, all groups were switched to finishing diets, which consisted of a high grain diet of 123 g/kg corn silage, 67 g/kg alfalfa hay, 764 g/kg rolled-barley and 17 g/kg vitamin/mineral pre-mix, with one group receiving a synthetic CLA isomer as a calcium salt addition to the diet (84 g/animal). Forage only animals were pasture grazed, with only minerals and vitamins available. Examination of the subcutaneous adipose tissue samples showed that enrichment of the high grain diet with CLA (using partially rumen protected CLA isomers) increased the CLA (*trans*-10, *cis*-12 CLA) content of beef by 380%, compared to the high grain only diet. Subcutaneous adipose tissue from the steers raised on forage and pasture without grain or CLA supplementation, had an enhanced CLA and Vitamin E content (466% and 300% respectively), compared to the high grain-fed beef animals.

Warren *et al*. (2002) investigated the effect of breed and diet on the lipid composition and quality of bovine muscle. Aberdeen Angus and Holstein-Friesian cattle were grazed and offered identical diets of grass silage with sugar beet shreds or a barley-based concentrate plus chopped straw. Results indicated that grazing beef cattle on grass pasture increased the levels of *n*-3 fatty acids from 0.8 to 3.7% in the phospholipid fraction of the *longissimus dorsi* and increased EPA and DHA in beef muscle in comparison to beef animals offered the barley/straw diet.

The effect of grazing duration on the fatty acid profile of the *M. Longissimus dorsi* from beef heifers was studied by Noci *et al*. (2003). Heifers were assigned one of four dietary treatments: (1) perennial ryegrass grazing (2) grass silage/concentrate diet (3) silage and concentrate plus 40 days grazing and (4) silage and concentrate plus 90 days grazing. Data showed a linear increase in CLA levels and a linear decrease in *n*-6:*n*-3 with extended grazing treatments and grazing duration played a significant role in influencing these ratios. In a study by Scollan *et al*. (2003), the fatty acid profile of the muscle of beef cattle offered a diet consisting of grass silage *ad libitum* supplemented with one of three concentrate treatments was examined. The three concentrate treatments were (1) Megalac 100 g/kg/day (2) soyabean, linseed and sunflower seed oils 1000 g/day/kg (3) soyabean, linseed, sunflower seed (500 g/day) plus Megalac (54 kg/day). Data showed that beef animals offered the silage and seed oil diets had muscle tissue with a lower total fat content, a reduced saturated fatty acid content and a higher proportion of *n*-3 fatty acids, in comparison to the muscle tissue from steers offered the proprietary supplemented diet.

In a study investigating the effect of the method of grass silage preservation, combined with fish oil supplementation, on the fatty acid composition of muscle of steers, Noci *et al*. (2003) reported that wilting of grass prior to ensiling increased the CLA concentration of neutral lipids in beef, without fish oil addition to the diet.

12.9.2.2 Grass - Lamb

Nuernberg *et al*. (2004) examined the effect of grazing lambs on grass pasture in comparison to the intensive feeding of indoor lambs with concentrate, to enhance the concentration of *n*-3 fatty acids in lamb muscle. Pasture-fed lambs had a significantly higher proportion of CLA in the muscle and total *n*-3 in muscle lipids and a reduced *n*-6:*n*-3, compared to concentrate-fed lambs. Consistent with these findings, Aurousseau *et al*. (2004), whilst comparing the fatty acid profile of the muscle of grass-fed lambs with that of concentrate and hay-fed lambs, reported that grass-fed lambs produced meat with a higher CLA content and greater *n*-3:*n*-6.

Fisher *et al*. (2000) examined the fatty acid profile of meat from pure Welsh Mountain lambs on upland flora, pure Soay lambs on lowland grass, Suffolk cross lambs on lowland grass and Suffolk cross lambs offered concentrates. Forage-fed Suffolk lambs had higher *n*-3 concentrations in the meat, whereas the concentrate-fed lambs had higher meat *n*-6 levels. Sensory evaluations by UK taste panellists of grilled chops clearly favoured the pasture-fed lamb. Pasture grazed Soay lambs also had an improved *n*-3:*n*-6.

Spanish researchers investigated sensory characteristics and the fatty acid composition of British and Spanish lamb carcasses. Two concentrate-fed Spanish breeds, Rasa Aragonesa and Merino and grazed grass-fed British Welsh Mountain early lambs were examined. British lamb carcasses had higher *n*-3 and lower *n*-6 levels than the Spanish lamb and it was suggested that these differences were due to diet/production systems. Spanish tasters preferred the Spanish lamb meat for taste and texture (Sañudo *et al*., 2000).

German studies have indicated that the concentration of *n*-3 fatty acids in the muscle of lambs increased significantly when grass was offered in the diet as opposed to concentrate, and the *n*-6:*n*-3 was 2:1 with grazing lambs in comparison to 6:1 for concentrate-fed lambs (http://www.healthybeef.iger.bbsrc.ac.uk/posters/Poster%2004.pdf).

12.10 Clover

12.10.1 Clover - Milk

Dewhurst (2004) reported that cows offered red clover silage had increased levels of linoleic and linolenic acid in their milk, over and above that of cows offered grass silage or grass silage plus red clover silage. In agreement with this, by comparing CLA levels in milk from all grass paddocks (predominantly Kentucky bluegrass), to grass and red clover paddocks, Wu *et al*. (1997) reported that cows grazed on red clover pasture produced milk with higher CLA concentrations than milk from cows on grass only paddocks. The ratio of red clover to grass was 1:4. Dewhurst *et al*. (2003b) also conducted two feeding studies with lactating cows offered silages from pure ryegrass, alfalfa, white clover and red clover, with a standard concentrate at a flat rate (8 kg/day in Year 1 and 4 kg/day in Year 2). Data showed that clover silages and red clover in particular, increased *n*-3 α-linolenic acid levels in milk.

12.10.2 Clover – Meat

12.10.2.1 Clover - Beef

While animals that are offered grass benefit from the high α-linolenic and α-tocopherol levels it contains, mixed swards of grass and clover are very important forage sources for beef production. Scollan *et al*. (2002) examined the effect of offering different forages on the fatty acid profile of beef from purebred Welsh Black and Simmental steers. Steers were offered a (1) grass sward (2) grass/white clover sward or (3) grass/red clover sward. Steers were grazed from May to October, with silage from the same sward offered during the winter housing period. Cattle were grazed on the same sward from the following May until slaughter, at around 465 days. Clover grazing increased carcass weight, conformation and fatness and oxidative stability of meat from steers. Beef flavour and colour were unaffected by breed or diet. Offering grass and grass plus red clover produced beef with higher Vitamin E levels than grass plus white clover-fed beef. Polyunsaturated fatty acid levels from legume grazed beef increased and a significant improvement in PUFA:saturated fatty acids was noted. No adverse effects on beef quality were detected.

12.10.2.2 Clover – Lamb

Research at IGER in Wales by Fraser *et al*. (2004) compared the fatty acid profile of lambs finished on red clover, lucerne or perennial ryegrass. The data generated from the study indicated that grazing forage legumes significantly increased the proportion of linoleic and linolenic acid in lamb muscle tissue and the concomitant proportion of unsaturated to saturated fatty acids (0.19, 0.16 and 0.12 for lambs offered red clover, lucerne and perennial ryegrass respectively). The *n*-6:*n*-3 was 1.13, 1.08 and 0.98 for lambs grazing red clover, lucerne and perennial ryegrass respectively.

13 Fatty acids and meat quality

It is well established that the fatty acid composition of muscle lipids has an important impact on meat flavour, because lipid degradation can produce aldehydes, which influence the flavour of meat at cooking. Meat from different animals may be prone to different rates of oxidation, due to differing quantities of fats and fatty acid composition.

13.1 Shelf Life and quality of *n*-3 enriched meat

13.1.1 Beef

Ruminant meat can have significant increases in PUFA levels without deleterious effects, after grazing on grass or grass/clover, which are rich in *n*-3 fatty acids and Vitamin E. Researchers at IGER examined the impact of feeding PUFA-rich diets on meat quality because increased levels of PUFA in meat may sometimes lead to colour changes in meat from red to brown, due to oxidation. Muscle samples from animals offered a fish oil diet underwent more oxidative changes during their retail display and also had a more rapid colour deterioration. Animals offered a grass silage diet supplemented with linseed-produced beef with a shelf life that was as long as the control treatment (www.seedsofhealth.co.uk/articles /healthy_beef.shtml).

Scollan *et al*. (2005) also reported that the sensory attributes of beef, in terms of a fishy taste and greasy taste score higher and the shelf life may be reduced as the quantity of *n*-3 PUFA in the animal's diet increases. Earlier research at the University of Bristol and IGER was undertaken to examine the shelf life of fatty acid enriched beef (Richardson *et al.,* 2003). Meat, particularly long chain *n*-3 rich meat, from beef steers offered a variety of diets with fatty acid rich supplements, had a reduced shelf life. Mincing of meat released pro-oxidants, against which high levels of dietary Vitamin E were ineffective. Grass grazed animals produced meat with high *n*-3 PUFA concentrations, which had a good shelf life. Table 20 lists the fatty acid content of the meat and the effect of the different treatments on shelf life. The TBARS value is a measure of the extent of oxidation, in that the higher the TBARS value, the greater the degree of oxidation. The mechanism by which this occurs is that 2-thiobarbituric acid reacts with malonaldehyde to form a red pigment. Malonaldehyde is an end product of oxidative deterioration and the amount of red pigment formed in the test increases as oxidative rancidity increases.

Table 20 Fatty acid composition and shelf life parameters (g/100 g total fatty acids)

	C18:2 (Linoleic)	C18:3 (Linolenic)	EPA	TBARS[A]	Sat[B]	Vitamin E (mg/kg)
Megalac	2.6	0.6[a]	0.3[a]	0.6[a]	20[a]	6.9[a]
Linseed	2.3	1.2[c]	0.5[a]	1.1[a]	18[a]	6.5[a]
Fish oil	1.6	0.6[a]	0.6[b]	3.6[b]	16[b]	5.7[b]
Linseed/fish	2.0	0.9[b]	0.5[a]	1.3[a]	18[a]	6.4[a]
Megalac	3.0	0.7[a]	0.3	0.5[a]	20[a]	4.5[a]
Megalac+PLS	6.3	1.4[b]	0.3	1.9[b]	18[b]	3.7[b]
PLS	9.3	2.0	0.4	3.0	16[a]	3.8[b]
Grass	2.6	2.0	1.1	0.7	18	4.0
Red clover	3.4	2.5	1.0	0.9	18	4.1
White clover	3.1	2.2	1.0	0.6	18	3.5

[A] mg/MDA/kg; [B] Colour Saturation Units
[abc] Values in columns for a given trial with different superscripts are significantly different ($P<0.05$)
PLS = Rumen protected supplement

(Richardson *et al*., 2003)

Widayaka *et al*. (2001) examined the effect of storage and cooking on lipid oxidation of raw and cooked beef. At an oven temperature of 200°C, the beef was cooked until it reached a temperature of 85°C. Raw and cooked samples of meat were subsequently stored in oxygen impermeable bags and frozen for 4, 8 and 12 weeks. Lipid oxidation and a sensory evaluation of the meat were assessed at weeks 0, 4, 8 and 12. Data showed that lipid oxidation occurred in both raw and cooked meat and as storage time increased, so did lipid oxidation. It was also reported that lipids in cooked meat were more readily oxidised than lipids in raw meat. In terms of sensory evaluation characteristics of the meat, the panel of tasters were unable to detect rancidity of the beef upon storage for 12 weeks.

Elmore *et al*. (1999) examined the effect of diet offered to beef cattle on the composition of volatiles produced during cooking. Higher levels of lipid oxidation volatiles were present in all steaks upon cooking, from animals offered dietary PUFA.

13.1.2 Lamb

During an evaluation of the eating quality attributes of lamb offered UK grass grazed and Spanish concentrate-fed lamb, UK tasters preferred the grass-fed lamb (which had a greater odour and flavour intensity), while Spanish tasters favoured the concentrate-fed lamb. These preferences in taste were attributed to olfactory experience and familiarity (Sañudo *et al*., 2000).

The effect of offering a protected sunflower seed supplement to lambs for varying periods of time, on the fatty acid profile of meat and meat flavour was summarised by Park *et al*. (1975) as shown in Table 21. These data demonstrate that increasing the period of feeding sunflower seed supplement to lambs from 0 to 6 weeks led to a substantial increase in C18:2. The members of the taste panel described the meat with the high C18:2 content as having a 'sweet' and 'oily' flavour.

Table 21 Effect of feeding a protected sunflower seed supplement to lambs on the fatty acid profile of meat and meat flavour

Period of feeding (weeks)	**0**	**2**	**4**	**6**
C18:2 in carcass fat (mg/g)	20	95	162	205
Meat aroma	3.6^{b}	3.5^{b}	3.2^{a}	3.2^{a}
Meat flavour	3.9^{b}	3.8^{b}	3.2^{a}	3.0^{a}
Different aroma	1.4^{a}	1.6^{a}	2.5^{b}	3.0^{b}
Different flavour	1.3^{a}	1.6^{a}	2.8^{b}	3.6^{b}

^{a,b} Mean values with unlike superscript letters were significantly different (P<0.05)

Research in New Zealand by Knight *et al*. (2004) has shown that raising the cooking temperature from medium to well done increased the cooking loss and DM of lamb, but it did not affect the fatty acid content or fatty acid composition and the proportion of CLA in the meat was unaffected by cooking. Furthermore, these authors concluded that the fatty acid profile of raw lamb was an underestimation of the fatty acid profile of cooked lamb, when the lamb was cooked with bone and fat intact, in particular.

13.1.3 Pork

Wood *et al*. (2003) conducted a review of the effects of ruminant and monogastric diet enrichment on meat quality. The addition of linseed to the feed ration of pigs reduced the *n*-6:*n*-3, but when concentrations of α-linolenic acid approached 3%, shelf life and flavour were affected. A few years earlier, Wood *et al*. (1999) reported that a concentration of 3 mg C18:3 fatty acids in muscle and fat tissue could be achieved by offering pigs whole linseed, having no effect on meat odour or flavour in some studies, but affecting odour and flavour in others. It was concluded that as the concentration of PUFA is increased, and the concentration of saturated fatty acids in the diet of pigs is decreased, the flavour intensity of pork is reduced, with an increase in the incidence of abnormal flavours (Cameron and Enser, 1991). Conversely, West and Myer (1987) demonstrated no deleterious effect of offering high levels of C18:2 in the diet on the flavour of pork. Sheard *et al*. (2000) assessed the sensory quality and oxidative stability of *n*-3 enriched pork and concluded that the controlled addition of high PUFA dietary supplements did not adversely affect the eating quality parameters.

Mourot and Hermier (2001) questioned the feasibility of dietary manipulation of modern pig (and chicken) diets to increase PUFA levels in pork and chicken meat. Both meat types have a low intramuscular fat content, a trait enhanced by many years of selective breeding. It is described as ironic that consumers want leaner meats and at the same time request a higher *n*-3 content, which can only be attained by having a greater fat content within the meat. This in turn can result in a "yellow" fat and early oxidation of the meat, which the consumer will not buy! Findings by Ostrowska *et al*. (2003) reported that supplementing pig diets with CLA increased the carcass lipid CLA, but the resultant changes in the fatty acid profile of pig meat can have adverse affects on consumer acceptance.

The influence of fatness and genetic factors on fatty acid composition of meat was reviewed by De Smet *et al*. (2004). Findings indicated that genetics/heritability had only a minor effect compared to diet. The authors suggested that attempting to develop new breeds of animals with fatty acid proportions desirable for new consumer tastes is not realistically worthwhile. Rather, they encourage further biochemical and molecular studies of fatty acid metabolism and genetics.

Lemke (2005) described a study in Minnesota where pigs were offered full fat extruded soyabean meal and had 250% more *n*-3 fatty acids and Vitamin E than pigs offered a conventional diet. An evaluation of such soyabean-fed meat by a sensory panel indicated that the taste and texture of the meat compared favourably to pork produced by traditional diets.

13.1.4 Poultry

Myers and Harris (1975) examined the effect of microwave energy and conventional heating on the fatty acid profile of meat and poultry. These authors reported no significant difference in the fatty acid profile as a result of heat treatment, but found that decreases in fatty acids were mainly due to a change in oleic acid. Poultry appeared to be most affected by heat treatment.

The fatty acid content of raw and cooked chicken thighs was examined by Cortinas *et al*. (2004). Data showed that when broiler thighs were cooked at 106°C for 77 minutes in a conventional oven, the reduction in fatty acids in the meat was 7.0%, 5.3% and 6.6% for saturated fatty acids, MUFA and PUFA respectively. Conversely, Grau *et al*. (2001) did not record any difference in the fatty acid profile of raw and cooked broiler thighs, when cooked at a lower temperature (80 to 90°C). Other researchers who cooked broiler thigh meat at an even higher temperature (200°C) reported that the proportion of PUFA in the meat declined by 12.4% (Lopez-Ferrer *et al*., 1999).

Canadian researchers examined the effect of offering flaxseed and menhaden fish oil in broiler diets, on sensory attributes and the fatty acid profile of cooked meat (Gonzalez-Esquerra and Leeson, 2000). In this study, 330 one-day old male broiler chicks were offered one of 11 dietary treatments, comprising combinations of flaxseed (100 g/kg) and menhaden fish oil (7·5 or 15 g/kg). The broilers were offered these diets for 7 or 14 days prior to slaughter. Data showed that linolenic acid was deposited in the dark meat of the chicken, while the long chain *n*-3 fatty acids were deposited in the white meat. Offering flaxseed and menhaden oil to broilers for just 7 days prior to slaughter resulted in a significant increase in *n*-3 fatty acids in the meat. In a sensory evaluation of the meat, breast meat was unaffected by offering the broilers 100 g/kg flaxseed for 14 days prior to slaughter but the sensory attributes of the thigh meat were reduced.

14 Discussion

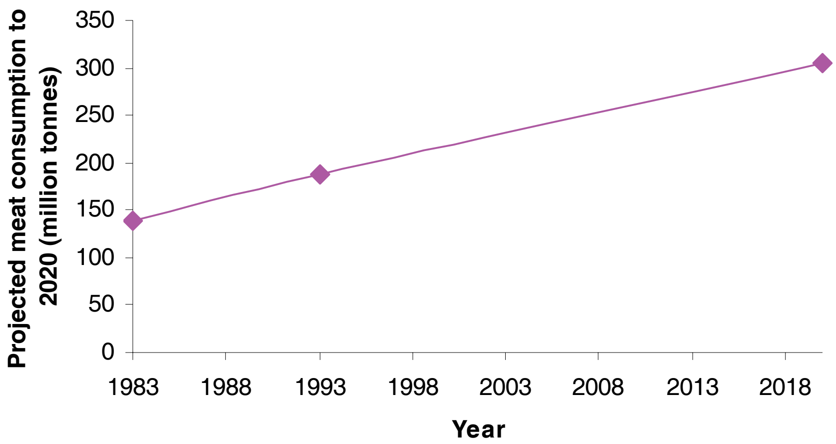
14.1 Meat consumption trends

In the past, meat, and in particular red meat, has been criticised for its high content of saturated fatty acids. The beneficial PUFA are present in meat at low concentrations, but can be further enhanced by dietary manipulation. A substantial body of research now exists with regard to the effect of dietary sources of PUFA on the fatty acid profile of milk, meat and eggs and the subsequent effect on oxidative stability and some aspects of meat quality. Consequently, meat, including red meat has been regarded as a healthy food over the past number of years and as such, is favourable to humans for consumption.

In a recent review of future needs for global meat production, Thomas Elam, an economist at the University of Indiana/Purdue University, Indianapolis, projected that world demand for meat will increase by 55%, during the period from 2001 to 2025 (Elam, 2004). This prediction was based upon projections that (1) the global economy will increase by 30% and when people earn a greater income, they will eat more meat and (2) world population will increase by 25%, from 6.2 to 7.8 billion. Wood *et al.* (1998) described how sales of meat in many countries had remained static or even declined in the past few years. These authors quoted that total meat sales in the UK dropped by 3.5% between 1985 and 1995, with a 19% reduction in consumption recorded for beef and lamb, but an 8% increase for poultry. Such a decline has since placed a major emphasis on meat quality and its concomitant health attributes for humans.

Delgado *et al.* (1998) presented data demonstrating the past and projected meat consumption trends up to 2020 as shown in Figure 9.

Figure 9 Meat consumption trends 1983-2020



(Delgado *et al.*, 1998)

14.2 Polyunsaturated fatty acid uptake: Ruminant vs. Monogastric

There are certain difficulties associated with the uptake of PUFA in ruminants, in comparison to monogastrics. In monogastric animals, dietary fatty acids are absorbed unchanged into the small intestine and are further incorporated into the tissue lipids. Within the rumen, a large proportion of unsaturated fatty acids are hydrogenated, leaving a large proportion of saturated muscle fatty acids and a smaller proportion of unsaturated fatty acids in cattle and sheep, relative to pigs (Wood *et al.*, 1999). Hence, the PUFA:saturated fatty acids is altered in an unfavourable manner. Conversely, the *n*-6:*n*-3 PUFA value is more favourable in cattle and sheep than it is in pigs, mainly due to C18:3, which cattle and sheep can ingest from grass. As the large fermentation vat, or the rumen, is the site for biohydrogenation of fatty acids, some of these C18:3 fatty acids are broken down into C18:0 and some pass through unchanged, into the small intestine, where they are absorbed. There is further difficulty in incorporating the *n*-3 PUFA, including EPA and DHA, into triaglycerols in ruminant species, in comparison to pigs. Consequently, these *n*-3 PUFA are usually found in the muscle tissue of ruminants and not in the fat tissue.

14.3 Polyunsaturated fatty acids and oxidative stability

It is well established that the uptake of PUFA by animals in large quantities can have a negative impact on meat quality, in terms of oxidative stability and shelf life. O'Grady *et al*. (2001) reported that dietary Vitamin E supplementation resulted in an elevated plasma and muscle α-tocopherol in beef cattle and reduced the susceptibility of beef muscle tissue to lipid and oxymyoglobin oxidation. Equally, Focant *et al*. (1998) demonstrated that by supplementing a cow's diet of oilseeds with Vitamin E, the concentration of α-tocopherol increased in milk by 45% and this was sufficient to prevent milk fat depression and oxidation. It would therefore appear that including Vitamin E in a cow's diet at very high levels could maintain milk fat. Selenium also appears to support the activity of Vitamin E (α-tocopherol) in reducing the oxidation of lipids. Animal studies indicate that Selenium and Vitamin E spare one another and that Selenium can prevent some of the oxidative damage resulting from Vitamin E deficiency (Burk and Levander, 1999).

14.4 Polyunsaturated fatty acid intake and meat quality attributes

The review of the literature conducted for this study highlights the gap in knowledge that exists, with regard to data on the meat eating quality of beef, lamb, pork or poultry supplemented with PUFA-rich feeds. Wood *et al*. (1999) provided some data on the technological aspects of meat quality, i.e. tenderness, juiciness and flavour. These authors reported that most speculation regarding the role of intramuscular or marbling fat and tenderness comes from Europe and the UK. It is thought that as the degree of fatness in an animal increases, it increases in several locations in the body and therefore affects tenderness of the meat. In terms of flavour, lipids play a role as when they are broken down, they produce aldehydes, alcohols and ketones, which can influence the composition of the meat (Wood *et al*., 1999). Wood *et al*. (1999) concluded that offering cattle and sheep whole linseed could also increase the proportion of C18:3 from 0.7 mg/100 g to >1 mg/100 g, but this increase resulted in greater lipid oxidation during storage of the meat and the production of volatile compounds during the cooking of meat, but, surprisingly, this did not have any negative impact on the odour or flavour of the meat. Furthermore Wood *et al*. (1999) reported that very high levels of C18:2 in beef, achieved by offering protected oil supplements caused beef to be 'oily, bland or pork-like' upon cooking. These authors concluded that altering the fatty acid profile of ruminant meat (which is normally relatively saturated) affects meat quality more so than that of altering the fatty acid profile of pig meat (which is generally more unsaturated than ruminant meat). In summation, from the limited data that are available, the effects of offering PUFA-rich feeds to animals on the subsequent meat quality attributes are variable, and are also limited to only a small number of feeds, such as linseed, fish oil, oilseed rape, soyabean and sunflower oil. The effect of offering hemp, chia, marine algae and lupins to animals on the concomitant meat quality attributes warrants investigation.

14.5 Polyunsaturated fatty acids - Beneficial or not?

Sanders (2000) gave a summary of PUFA in the European food chain. In this review, it was stated that the intake of linoleic acid (C18:2) has increased in many Northern European countries. Figures for the UK were estimated as approximately 10 g/day in the late 1970s to approximately 15 g/day in the 1990s. The author emphasised that there are few reliable estimates of long chain *n*-3 fatty acid intake, but these are generally 0.1-0.5 g/day in humans.

However, a recent report makes the subject of PUFA and their alleged health benefits a little more controversial. In a report by the French Food authority, the safety of CLA was questioned (http://www.nutraingredients.com/news/news-ng.asp?n=59280-french-food-body). There are currently no foods with added CLA available in France but the country is Europe's biggest market for CLA supplements, with more than 20 firms offering the fatty acid in capsule form. However, Spain is so far the only European market to offer CLA-enriched foods in the form of a dairy range and CLA cookies. The adverse effects of CLA underlined in the report, focus mainly on the *trans*-10, *cis*-12 isomer, which has been linked to an increase in oxidative stress markers, which are associated with inflammation.

Equally, in a review published in 2003, researchers said that CLA had been found to raise levels of *trans* fats in the adipose tissue and muscle tissue and it was stated that the *trans*-10, *cis*-12 isomer could increase the risk of diabetes, by increasing insulin resistance. The authors concluded that while there is insufficient evidence, the studies suggest that there could be a serious public health risk from some of the CLA isomers, which should not be authorised in supplement form. It is important to remember that it is merely CLA supplements that are subject to criticism in such reports and not natural sources of CLA. Over 90% of the CLA isomers found in ruminant products are in the other major form, *cis*-9, *trans*–11 C18:2, commonly known as rumenic acid.

14.6 How much meat or milk would one need to consume in order to meet the recommended daily intake of *n*-3 fatty acids in the human diet?

The Scientific Advisory Committee on Nutrition (2004) produced a document detailing advice on fish consumption and the associated benefits and risks. In this report, recommended daily intakes of long chain *n*-3 PUFA were given, where long chain PUFA were defined as fatty acids that comprise 20 or 22 carbon atoms, such as C20:5 *n*-3 (EPA), C22:5 *n*-3 (DPA) and C22:6 *n*-3 (DHA). In this report, it was recommended that the average consumption of long chain *n*-3 PUFA in the UK population should increase from approximately 0.1 g/day to 0.2 g/day. Although C18:3 *n*-3 is acknowledged as the parent molecule for the *n*-3 family of fatty acids, it was not included as a long chain *n*-3 PUFA by the Scientific Advisory Committee on Nutrition (2004). This is due to the reduced effectiveness of the elongation and desaturation of C18:3 that occur in mammals in the production of EPA and DHA *in vivo* (Personal communication, Dr. Joanne Lunn, British Nutrition Foundation). However, there is evidence to suggest that women may possess a greater ability to convert C18:3 *n*-3 to EPA, DPA and DHA than men (Burdge and Wootton, 2002) and for this reason, calculations were also performed using C18:3 *n*-3 as a long chain *n*-3 PUFA.

In a study by Enser *et al*. (1998), the fatty acid content and composition of UK beef animals (Hereford X Friesian, Angus X Friesian) and lambs (Suffolk X Clun) offered grass and bull beef animals offered concentrates (containing barley and soyabean meal) plus barley straw *ad libitum* was examined. The muscles examined in this study were *M. triceps brachii, M. longissimus dorsi, M. gluteobiceps* and *M. gluteus medius*. Using these data to calculate the required dietary intake of grass-fed beef muscle, concentrate-fed bull muscle and grass-fed lamb muscle to meet the 0.2 g/day long chain *n*-3 PUFA/day target, it was calculated that 110 g grass-fed beef muscle/day or 134 g grass-fed lamb muscle/day or 318 g concentrate fed bull beef muscle would have to be consumed per day (using EPA, DPA and DHA only as the long chain *n*-3 PUFA). If we include C18:3 *n*-3 as a long chain *n*-3 PUFA, then the respective requirements would be reduced substantially, being 60 g, 63 g or 195 g per day. This demonstrates that a smaller quantity of grass-fed beef and lamb than concentrate-fed beef would have to be eaten to meet the 0.2 g long chain *n*-3 PUFA/day target.

In an earlier study by Enser *et al*. (1996), the fatty acid profile of beef, lamb and pork muscle originating from fifty samples of loin steaks from each species, purchased from four supermarkets on separate occasions during February and March 1994, were given. However, in this study the source of the meat was unidentified, the animal production systems were unknown and the muscle type(s) examined were not given. Based on these data, calculations demonstrated that one would need to consume 675 g of beef/day or 364 g of lamb/day or 689 g of pork/day to meet the recommended daily intake of 0.2 g long chain *n*-3 PUFA/day. If we also classified C18:3 *n*-3 as a long chain *n*-3 PUFA, then one would need to consume 356 g/day, 164 g/day or 400 g/day of beef, lamb or pork respectively to meet the 0.2 g/day target. These beef and lamb figures are substantially greater than those calculated from the data of Enser *et al*. (1998), but it is impossible to explain why, due to the unknown source and production system. There is a deficit of information regarding the *n*-3 long chain PUFA profile of pork.

In a study by Nuernberg *et al*. (2005), the fatty acid profile of the *longissimus* muscle of German Holstein and German Simmental bulls raised on a concentrate or pasture-based system was compared. Bulls on the pasture-based system were offered a concentrate containing 46% C18:3 *n*-3 (pelleted concentrate consisted of 12% barley and 10% coarsely cracked linseed), while the concentrate offered to the bulls in the indoor concentrate system only had 6.1% C18:3 *n*-3 (concentrate consisted of winter barley, molasses and soyabean meal). Using these data to calculate the daily intake of beef required to meet the recommended 0.2 g long chain *n*-3 PUFA per day, gave the results shown in Table 22.

Table 22 Quantity of beef (g/day) that one would have to eat daily to achieve the recommended intake of 0.2 g long chain *n*-3 PUFA per day

Long chain *n*-3 PUFA	**Breed of animal**	**Concentrate system**	**Grass system**
C18:3 *n*-3, EPA, DPA, DHA	German Holstein	805	272
C18:3 *n*-3, EPA, DPA, DHA	German Simmental	871	517
EPA, DPA, DHA	German Holstein	1271	568
EPA, DPA, DHA	German Simmental	1824	545

(Nuernberg *et al.*, 2005)

These data also demonstrate that the quantity of beef required to be eaten to meet the recommended 0.2 g/day is much lower on the grass versus concentrate-based production system, which is most likely due to the grass being rich in these fatty acids and due to the concentrate offered in this system, which had 46% C18:3 *n*-3 and contained coarsely cracked linseed. Furthermore, there appeared to be an effect of breed in this study as the grass-fed German Holstein had a greater proportion of C18:3 *n*-3 in the muscle (1.67%) than the German Simmental (0.13%), but this vast difference was not seen in the concentrate-based system. Some of the data in this study are similar to those calculated from the study of Enser *et al.* (1996).

The only conclusions we can draw from comparing these three studies is that a smaller quantity of grass-fed beef than concentrate-fed beef will be required to meet the recommended human dietary intake of 0.2 g long chain *n*-3 PUFA/day and it appears that the quantity required may vary with breed of animal used.

Jahan *et al.* (2004) examined the fatty acid composition of retail chicken breasts from nine products with different production regimes, namely conventional (chilled and frozen), organic and free range. These data were used to calculate the quantity of chicken that a human would need to eat on a daily basis to consume the recommended 0.2 g *n*-3 long chain PUFA per day as shown in Table 23. However, DPA was not analysed for in this study and calculations were based on C18:3 *n*-3, EPA and DHA followed by EPA plus DHA.

Table 23 Quantity of chicken (g/day) that one would have to eat daily to achieve the recommended intake of 0.2 g long chain *n*-3 PUFA per day

Chicken type	Treatment	Quantity chicken required (g) C18:3 *n*-3, EPA, DHA	Quantity chicken required (g) EPA, DHA
Conventional	Chilled	428	728
Conventional	Frozen	358	521
Conventional	Chilled	257	523
Corn-fed conventional	Chilled	381	593
Conventional	Frozen	663	1793
Organic	Chilled	817	1654
Organic	Chilled	713	1753
Farmer's market-Free Range	Chilled	659	1115
Rare Breed-Free Range	Chilled	852	1928

(Jahan *et al*., 2004)

It is evident that there is a substantial degree of variation in the results, which is most likely attributable to the diet of the bird. These factors would need to be considered when providing recommended daily intakes of long chain *n*-3 fatty acids through eating chicken. Jahan *et al*. (2004) concluded that breed of chicken and diet type have an impact on the fatty acid composition and that further studies are required with larger samples as the two organic products examined were significantly different and had a fatty acid profile that was less desirable than the two standard chickens. Hence, it seems that it is difficult to give a definitive figure on chicken consumption to meet the recommended long chain *n*-3 PUFA intake per day. Supporting this conclusion are the data of Schreiner *et al*. (2005), who concluded that levels of long chain *n*-3 PUFA in the portal blood and tissues of broiler chickens reflects the concentrations of long chain *n*-3 PUFA in the diet (Schreiner *et al*., 2005).

The North/South Ireland food consumption survey by the Irish Universities Nutrition Alliance (2001) gave mean figures for the consumption of meats (g/day) by consumers only, where daily intakes of 31 g/day (beef and veal), 27 g/day (lamb), 29 g/day (pork) and 31 g/day (chicken, turkey and game) were given. Meat consumption is also predicted to increase by approximately 63% between 1993 and 2020, as indicated previously in this report. The possibility of offering *n*-3 rich feeds in the animal's diet to raise the proportion of long chain *n*-3 PUFA in the human diet via eating meat, combined with the prediction that meat consumption will increase, will make a significant contribution to meeting human dietary long chain *n*-3 PUFA requirements.

Although milk does not contain any EPA or DHA and has only 1% C18:3 *n*-3 in the fat, the sheer volumes of milk consumed by people each day will assist in helping to meet the recommended daily consumption of 0.2 g long chain *n*-3 PUFA/day. The North/South Ireland food consumption survey by the Irish Universities Nutrition Alliance (2001) gave a mean consumption of whole milk and low fat, skimmed and fortified milks by consumers of 205 g/day and 194 g/day respectively. If one considers that whole milk is approximately 4% fat, of which 1% is C18:3 *n*-3 (Walstra and Jenness, 1984), then one would need to drink 0.5 litres of whole milk on a daily basis to fully meet the recommended 0.2 g long chain *n*-3 PUFA/day.

These calculations of the required daily intake of beef, pork, lamb, poultry and milk to meet the recommended dietary intake of long chain *n*-3 PUFA daily, demonstrate that in order for a consumer to gauge the contribution these foods make, it would be necessary to have information on the diet and breed of animal used displayed on the packaging. While aiming to increase the dietary intake of long chain *n*-3 PUFA, it must be remembered that as one increases the fatty acid intake, one will also consume a greater proportion of fat, which also has a daily recommended intake, being <35% of daily food energy intake (Personal communication, Dr. Joanne Lunn, British Nutrition Foundation). Furthermore, one must also remember that milk, and to a lesser extent meat, also supply the hypercholesterolaemic fatty acids C12:0, C14:0 and C16:0 saturated fatty acids.

14.7 Polyunsaturated fatty acids sources and their availability in Northern Ireland

Data detailing the dietary sources of PUFA examined in this review and their related costs and ease of availability are recorded in Table 24.

There are currently no commercial producers of Marine Algae in Northern Ireland. The cost and ease of availability of the different dietary sources of PUFA is an important consideration when aiming at producing PUFA-rich products. Of all the PUFA sources examined in this review, chia is the most expensive feed, costing between £900-£1200/tonne. Chia could not be grown in Northern Ireland at present, as the climatic conditions are unsuited to its requirements. It would not be feasible to enhance a product with PUFA by offering this ingredient at its current price. The majority of the other feeds examined in this report are readily available and cost much less than chia. Grazed grass is the cheapest feed available for animals and is very beneficial in terms of CLA enrichment of meat and milk. Clover is currently important in organic farming systems and therefore, could contribute to the production of organically produced, CLA-fortified products, which is appealing from a human consumption perspective.

Table 24 Cost and availability of PUFA-rich feeds in Northern Ireland

Feed	Cost (£/tonne)	Availability	Growing opportunities
Linseed	160	Readily	YES
Hemp	N/A	Readily	YES
Chia	900-1200	*	NO
Marine Algae	N/A	**	NO
Fish replacer	230	Readily	N/A
Lupins	785	Readily	YES
Rapeseed meal	94	Readily	YES
Soya	150	Readily	NO
Grazed grass (DM)	72	Readily	YES
Clover	N/A	Readily	YES

*A local company, R. Craig and Sons, Ballymoney import and sell Chia seed
** Ireland and Scotland supply seaweed as a raw material for the alginate industry
N/A = Not available

14.8 Polyunsaturated fatty acids - Gaps in knowledge

In a review by Raes *et al.* (2004), regarding the effect of dietary fatty acids on the incorporation of long chain PUFA and CLA in lamb, beef and pork meat, these authors concluded that much more research into the mechanism of CLA formation is required, to fully understand how nutrition can influence CLA incorporation into the intramuscular fat of cattle and lamb. Data from this review have also indicated that there is a deficit of information regarding the effect of dietary PUFA on meat eating quality attributes.

14.9 Polyunsaturated fatty acids and Biotechnology

Nutraceuticals and functional foods are increasingly more important, once again due to the associated health benefits and indeed, due to promising marketing opportunities for niche market produce. A new functional food research centre was launched in Co. Cork at the end of 2003 and is funded by the Government to promote biotechnology.

15 Conclusions

The conclusions drawn from this report are presented in Tables 25 and 26.

Table 25 Effect of diet on the fatty acid profile of milk and ruminant meat

Feed	Main fatty acid	Milk	Beef	Lamb
Linseed	α-linolenic acid	↑PUFA, ↓fat + protein	↑*n*-3	↓*n*-6:*n*-3,↑PUFA:SFA
Hemp oil	α-linolenic acid			
Chia seed	α-linolenic acid			
Marine Algae	*n*-3	↑CLA, ↑DHA, ↓intake, ↓milk fat		
Fish oil/meal (unprotected)	*n*-3, EPA, DHA	↑CLA, ↓intake, ↓milk yield ↑DHA, ↑*n*-3	↑CLA,EPA,DHA ↓*n*-6:*n*-3	Can alter fatty acid profile significantly
Lupins	C18:1	↓*n*-6:*n*-3		
Canola/rape meal/seed	*n*-6, *n*-3	OSR ↑C18:1, ↓C16:0 ↑CLA	Protected canola ↑ C18:2, C18:3 ↓ C16:0	No major effect of OSR compared to soya
Soya	*n*-6	↑CLA, ↑C18:2, ↓SFA	↑*cis*-9, *trans*-11 CLA *	
Grass	α-linolenic acid	↑CLA, Small↑α-linolenic acid	↑CLA, ↓SFA	↑CLA, ↑total *n*-3 muscle lipids, ↓*n*-6:*n*-3
Red clover	α-linolenic acid	↑α-linolenic acid	Grass+red clover fed beef ↑Vitamin E than grass + white clover fed beef	↑α-linolenic acid, ↑C18:2, ↑PUFA:SFA

PUFA=Polyunsaturated fatty acid; CLA=Conjugated linoleic acid; EPA=Eicosapentaenoic acid; DHA=Docosahexaenoic acid; SFA=Saturated fatty acid;
* = Some studies only

Table 26 Effect of diet on the fatty acid profile of monogastric meat and eggs

Feed	Main fatty acid	Pork	Poultry	Eggs
Linseed	α-linolenic acid	*n*-3	*n*-6:*n*-3	*n*-3
Hemp oil	α-linolenic acid			α-linolenic acid, C18:2
Chia seed	α-linolenic acid		α-linolenic acid, body weight, FCR	*n*-3
Marine Algae	*n*-3	*n*-3		DHA
Fish oil/meal	*n*-3, EPA, DHA	*n*-3, *n*-6, *n*-9		
Lupins	C18:1			
Canola/rape meal/seed	*n*-6, *n*-3	PUFA	α-linolenic acid, *n*-6:*n*-3	
Soya	*n*-6	Few studies	PUFA	
Grass	α-linolenic acid			
Red clover	α-linolenic acid			

PUFA=Polyunsaturated fatty acid; EPA=Eicosapentanoic acid; DHA=Docasahexaenoic acid; FCR=Feed conversion ratio

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17 Appendices

Appendix 1 - Point summary of findings

1. Omega-6, or *n*-6 fatty acids and omega-3, or *n*-3 fatty acids are required to be ingested by humans as they cannot be made in the human body. α-linolenic acid, Docosahexaenoic acid (DHA) and Eicosapentaenoic acid (EPA) are *n*-3 fatty acids.
2. Balancing the intake ratio of *n*-3 and *n*-6 fatty acids in human diets has become increasingly important over the past number of years and it is important that the quantity of *n*-3 fatty acids increases and the quantity of *n*-6 fatty acids decreases to get the balance right. The desired ratio of *n*-3:*n*-6 fatty acids is 2:1 or optimally 1:1.
3. Conjugated linoleic acid (CLA) is a mixture of *cis* and *trans* fatty acids. It is found naturally in ruminant meat and dairy products where one particular isomer predominates.
4. Vitamin E is a powerful antioxidant that prevents the oxidation of lipids. Selenium is one of the most important nutritional antioxidants and is an essential trace mineral.
5. A protected fat is a fat that is treated so that it is capable of resisting rumen biohydrogenation by rumen microbes. Treatments include formaldehyde, calcium salts, heat, prilled fats and natural protection of the seed coat.
6. The different dietary sources of PUFA examined in this report were linseed, hemp, chia, marine algae, fish oil/fish meal, lupins, oilseed rape, soya, grass and clover. The potential recovery of PUFA in milk, meat (beef, lamb, pork and poultry) and eggs from these dietary sources were examined.
7. Linseed is rich in essential fatty acids, particularly α-linolenic acid. Linseed oil and linseed meal have a low oxidative stability, whereas linola oil, with a much higher *n*-6 content, has a greatly increased oxidative stability.
8. Hemp oil is of growing interest as it is rich in essential fatty acids, especially α-linolenic acid. Hemp oil contains Vitamin E and gamma-tocopherol, which may afford some degree of oxidative stability.
9. Chia contains over 80% *n*-3 fatty acids, making it the richest known plant source of fatty acids. It is however very expensive (£900-£1200/tonne) and is not suited to growing conditions in Northern Ireland. A high number of antioxidant properties have been reported in chia seed oil.
10. The percentage of oil extractable from marine algae varies greatly according to species and strain used. Harvesting and drying processes used for marine algae lipids contribute to a reduced antioxidant action. There are no commercial producers of marine algae as feedstock for animals in Northern Ireland and the UK at present.

11. European Union regulations currently restrict fish meal as a feed for pigs and poultry only. Fish oil offers the widest range of *n*-3 fatty acids in the most desirable ratios, with high proportions of long chain PUFA, especially EPA and DHA. Both Vitamins A (as carotene) and E act as antioxidants in fish meal.
12. Forage lupins are of a growing interest in Northern Ireland and their oil can vary according to species, but they are rich in C18:1.
13. Canola has altered levels of fatty acids with different *n*-6:*n*–3 PUFA and rape meal (and canola meal) have a high oxidative stability.
14. Soya has a fatty acid profile with low *n*-3, but high *n*-6 levels.
15. A high proportion (50-57%) of the lipid in grass is *n*-3 α-linolenic acid.
16. There are two main clovers grown in Northern Ireland, white and red clover and these are also rich in *n*-3 α-linolenic acid. The oxidative stability of milk from cows offered red clover silage has been reported to be less than that of cows offered grass silage.
17. Other novel sources of *n*-3 PUFA include spinach, watercress, parsley, chinese cabbage, brussel sprouts, bok choy, cos lettuce, broccoli, chinese broccoli, stock and chickpea.
18. Under normal feeding conditions, the *cis*-9 *trans*-11 CLA profile of monogastric food products (pork and chicken) has been reported to be less than that of ruminant products (lamb, beef, and dairy), when expressed as a fraction of the fat.
19. The longer chain C18 fatty acids and about 50% of the C16 fatty acids are obtained direct from the cow's diet, but are subject to ruminal hydrolysis and hydrogenation unless they are protected.
20. The proportion of C16:0 and C18:1 in milk fat strongly reflect the cow's diet, with C16:0 being highest in the winter when large amounts of concentrates and silage are offered, and lowest in the summer, when the cows are grazing fresh grass.
21. Milk fatty acid levels increase with the addition of linseed to the diet, while the fat and protein content of milk are reduced.
22. The addition of marine algae to the diet of cows reduces feed intake and milk fat concentration and increases CLA levels.
23. Fish oil addition to the diet decreases intake and milk yield, but the CLA content of the milk increases.
24. The *n*-6:*n*-3 of milk fat is reduced significantly by incorporating lupin seed in the diet of dairy cows.
25. The addition of oilseed rape to a cow's diet increases the proportion of C18:1 and reduces the proportion of the saturated C16:0 in milk fat. Modified milk fat achieved by feeding cows rapeseed cake, showed positive effects on the LDL/HDL ratio and lipoprotein concentrations in humans.

26. Supplementation of a cow's diet with full fat soybean significantly elevates CLA levels in milk.
27. The concentration of CLA in milk fat is further enhanced by the dietary intake of pasture by cows.
28. Clover silages and red clover in particular, increase *n*-3 α-linolenic acid levels in milk.
29. Offering whole linseed as a supplement to beef animals elevates *n*-3 fatty acid levels in meat.
30. An increase in fish oil intake in the diet of steers results in a linear increase in the CLA, EPA and DHA concentration of muscle, and a linear decrease in the *n*-6:*n*-3 of muscle.
31. Oilseed rape needs to be protected, in order to achieve benefits in terms of an increased PUFA profile in the meat of steers. Offering protected canola seed to steers increases C18:2 and C18:3 in meat and decreases saturated C16:0.
32. Supplementing a high forage fattening diet of steers with soyabean oil or extruded full fat soyabean increases the concentration of the *cis*-9 *trans*-11 isomer of CLA in both the intramuscular and subcutaneous fat of meat in some studies but not in others.
33. Offering a grass diet to steers increases meat CLA and decreases saturated fatty acids.
34. Offering grass and grass plus red clover produces beef with higher Vitamin E levels than grass plus white clover-fed beef.
35. Lambs supplemented with linseed, while at grass, have an elevated PUFA:saturated fatty acid in the liver and adipose tissue (but not muscle) and an improved *n*-6:*n*-3.
36. The fatty acid composition of lambs offered a low or medium quality pasture or roughage diet can be altered significantly by offering fish meal.
37. Preliminary data from a study at Hillsborough indicate that there is a lack of a major effect of offering an oilseed rape-based concentrate on the fatty acid profile of lamb meat, relative to lambs offered a soyabean-based concentrate.
38. Pasture feeding of lambs increases CLA in the muscle, decreases *n*-6:*n*-3 and increases total *n*-3 in muscle lipids.
39. Including red clover in the diet of lambs increases the proportion of linoleic and linolenic acid in lamb muscle tissue and increases the proportion of unsaturated to saturated fatty acids.
40. Linseed supplementation of pigs increases *n*-3 levels in the muscle, liver and kidneys.
41. High *n*-3 pork can be produced by offering pigs diets containing marine sources of *n*-3 fatty acids.

42. Fish oil addition to the diet of pigs increases the *n*-3 fatty acid content, and reduces *n*-6 and *n*-9 fatty acids in meat.
43. PUFA levels over 14% (this level would rarely be achieved in commercial diets) can cause pigs to have a soft carcass fat, and oxidation of PUFA.
44. External fat PUFA in pigs increases as the proportion of rapeseed in the diet increases.
45. Some authors recommend offering 9% rapeseed in the diet of pigs to begin with, and to reduce this to 2-3% during the fattening period, due to the presence of soft fat at higher levels of inclusion.
46. There are few reports of pigs offered soyabean, but in one report, meat from pigs offered a full fat extruded soyabean diet was 250% richer in *n*-3 fatty acids and Vitamin E, compared to pigs offered a conventional diet. However, caution must be taken in this study as soya is also rich in *n*-6 fatty acids and this could negate the beneficial increase in *n*-3 reported in this study.
47. Linseed oil reduces *n*-6:*n*-3 in thigh meat of broilers.
48. Chia seed increases *n*-3 linolenic acid in the brown and white meat of poultry, but with the bird having a reduced body weight by up to 6.2% and a reduced feed conversion.
49. The addition of rapeseed and rape oil to the diet of the chickens lowers the saturated fatty acid content and increases the proportion of monounsaturated fatty acids and α-linolenic acid in the muscle and abdominal fat of the chickens. Furthermore, *n*-6:*n*-3 also decreases.
50. However, the addition of rapeseed and oil to the broiler diets decreases carcass weight and there are few studies available detailing the effects of oilseed rape on poultry meat PUFA.
51. Offering soyabean to broilers reduced the level of fat deposition in some studies and increased the abdominal fat pad mass in others, but also increased the proportion of unsaturated fatty acids in the fat in both studies.
52. Linseed can be used as a dietary source of PUFA for poultry, primarily for *n*-3 enriched egg production.
53. Increasing the quantity of hemp in the diet increases the percentage of α-linolenic and linoleic acid in eggs.
54. Offering 14% chia seed as a dietary supplement to a standard diet for laying hens increases the *n*-3 fatty acid content of eggs, without adverse effects on egg odour, flavour or acceptability.
55. Hens offered a marine algae diet produce eggs with a higher DHA content.
56. Increased levels of PUFA in meat can sometimes lead to colour changes from red to brown, due to oxidation of the red muscle pigment.
57. Grass grazed animals produce meat with high *n*-3 PUFA concentrations, which have a good shelf life.

58. Lipids in cooked meat are more readily oxidised than lipids in raw meat.
59. Researchers in New Zealand have found that the proportion of CLA in lamb meat is unaffected by cooking.
60. Different views exist in the literature with regard to the effect of PUFA rich meat on flavour parameters, with some authors finding no deleterious effects and others reporting some.
61. Mixed views also appear in the literature with regard to the effect of cooking on the fatty acid profile of poultry, with some authors reporting a reduction in the beneficial fatty acids upon cooking and others reporting no effect of cooking. Differences may even exist between the effect of cooking on thigh and breast meat.
62. Additional research is required regarding the effect of fatty acid enrichment of animal diets on meat eating quality.
63. Meat consumption is expected to increase, in line with an increasing world population and greater income potential.
64. It has been reported that much more research into the mechanism of CLA formation is required, to fully understand how nutrition can influence CLA incorporation into the intramuscular fat of cattle and lamb.
65. All of the dietary sources of PUFA examined in this study are readily available in Northern Ireland, with the exception of chia seed and marine algae.
66. Producing niche products for niche markets will become increasingly more popular in Agriculture as a consequence the Common Agricultural Policy.
67. Consumer perception and acceptance will be the driving force behind the success of niche market products.
68. Consuming the beneficial PUFA as part of every day meals will appeal to the public.

Appendix 2 - Fatty acid concentration of dairy cows feeds

Fatty acid concentration (g/kg fatty acid)

Feed	C14:0	C16:0	C18:0	C16:1	C18:1	C18:2	C18:3	C20:5/C22:6 *n*-3
Cereal grain	<5	247	63	<5	252	**398**	40	-
Canola seed oil	-	38	25	-	**806**	102	28	-
Cottonseed oil	14	234	11	20	229	**478**	-	-
Maize silage	<5	190	22	<5	214	**503**	71	-
Perennial ryegrass	<5	204	28	<5	37	171	**560**	-
Pasture, Persian clover	<5	175	16	<5	13	146	**652**	-
Pasture, sub. Clover	<5	160	25	<5	18	147	**651**	-
Sunflower seed oil	-	56	22	-	251	**662**	-	-
Tallow, beef	63	274	141	-	**496**	25	-	-
Tuna oil	30	220	80	50	160	<5	<5	**440**

(Walker *et al.*, 2004)

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