The effect of long-chain polyunsaturated fatty acid and vitamin E supplementation of ewes on neonatal lamb behaviour and performance

Thesis

THE EFFECT OF LONG-CHAIN POLYUNSATURATED FATTY ACID AND VITAMIN E SUPPLEMENTATION OF EWES ON NEONATAL LAMB BEHAVIOUR AND PERFORMANCE

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ABSTRACT

Three experiments investigated the effect of long-chain polyunsaturated fatty acid (PUFA) and vitamin E supplementation of ewes upon lamb vigour and performance. In Experiment One, four diets were fed to 48 ewes in a two-by-two factorial design. Each diet contained either fish oil (high in C20:5*n*-3 and C22:6*n*-3) or Megalac® (control fat, C16:0) and a basal (50 mg/kg) or supranutritional (500 mg/kg) concentration of vitamin E. Fish oil supplementation significantly increased ewe gestation length, deposition of C22:6*n*-3 in lamb brain tissue and neonatal lamb vigour. It also had significant detrimental effects upon milk composition and lamb growth. Vitamin E supplementation of the ewe increased the concentration of the vitamin in lamb brain and muscle tissue, and improved lamb birthweight. Within Experiment Two, three treatment diets based on algae (high in C22:6*n*-3), linseed (high in C18:3*n*-3) or Megalac® were fed to sixty pregnant ewes. After parturition, thirty ewes were changed onto diets containing either linseed or Megalac®. Gestation length and brain C22:6*n*-3 content were unaffected by diet, although lamb vigour was improved by maternal PUFA supplementation. The use of strategic supplementation abrogated the effects of PUFA supplementation upon lamb growth rate. Nevertheless, significant effects of algal supplementation were observed upon milk composition after a 28-day change-over period. Experiment Three employed three diets, each containing either fish oil or Megalac® plus basal or supranutritional vitamin E, followed by Megalac® supplementation during lactation. Gestation length and lamb behaviour were unaffected by treatment diet. Differences in milk composition were observed 28 days after the diet change. Long-chain PUFA supplementation of the pregnant ewe appears to improve neonatal lamb vigour, although effects upon milk composition cannot be negated by changing the dietary fat source during lactation. The vitamin E status of the neonatal lamb may be manipulated by maternal supplementation.

AUTHOR'S DECLARATION

This thesis was composed by the author and is a record of work carried out by her on an original line of research. All sources of information are shown in the text and listed in the references; all help given by others is indicated in the acknowledgements.

None of this work has been presented in any previous application for a degree.

Judith Louise Capper

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LIST OF ABBREVIATIONS

A	algae diet
AA	algae diet fed during pregnancy and lactation
ACC	acetyl-CoA carboxylase
AL	algae diet fed in pregnancy, Linseed diet in lactation
ATP	adenosine triphosphate
α-TTP	α-tocopherol transfer protein
AM	algae diet fed in pregnancy, Megalac® diet fed in lactation
ANOVA	analysis of variance
BAT	brown adipose tissue
βHB	β-hydroxybutyrate
BHT	butylated hydroxytoluene
CCK	cholecystokinase
CK	creatine kinase
CLA	conjugated linoleic acid
CoA	co-enzyme A
CP	crude protein
DHA	docosahexaenoic acid
DM	dry matter
DUP	digestible undegradable protein
EDTA	ethylene diamine tetra-acetate
EFA	essential fatty acid
ERDP	effective rumen-degradable protein
FASt	fatty acid synthase
FB	fish oil/Incromega plus additional 50mg/kg vitamin E
FH	fish oil + supranutritional vitamin E (500mg/kg in premix) during pregnancy (FH), Megalac® + supranutritional vitamin E (500mg/kg in premix) during lactation (MH)
FL	fish oil + basal vitamin E (50mg/kg in premix) during pregnancy (FL), Megalac® + basal vitamin E (50mg/kg in premix) during lactation (ML)
FME	fermentable metabolisable energy
FS	fish oil/Incromega plus additional 500mg/kg vitamin E
GPx	glutathione peroxidase
HDL	high density lipoprotein
HSL	hormone-sensitive lipase
KOH	potassium hydroxide
L	linseed diet
LDL	low density lipoprotein
LL	linseed diet fed during pregnancy and lactation
LM	linseed diet fed in pregnancy, Megalac® diet fed in lactation
LPL	lipoprotein lipase
M	Megalac® diet
MB	Megalac® plus additional 50mg/kg vitamin E
ME	metabolisable energy
ML	Megalac® + basal vitamin E (50mg/kg in premix) during pregnancy and lactation
MM	Megalac® diet fed during pregnancy and lactation
MS	Megalac® plus additional 500mg/kg vitamin E
NEFA	non-esterified fatty acids
NDF	neutral detergent fibre
NST	non-shivering thermogenesis
PGE_2	prostaglandin E_2

PGFM	13,14-dihydro-15-keto-prostaglandin $F_{2\alpha}$
PGG_2	prostaglandin G_2
PGH_2	prostaglandin H_2
PLTP	plasma phosphatidylglycerol transfer protein
PMSG	pregnant mare serum gonadotrophin
PUFA	polyunsaturated fatty acid
SCD	stearoyl-CoA desaturase
T_3	3,3',5:triiodothyronine
T_4	thyroxine
TAP	tocopherol-associated protein
TH	thyronine
UCP-1	uncoupling protein-1
VFA	volatile fatty acid
VLDL	very low density lipoproteins
WCOT	wall coated open tubular

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1. LITERATURE REVIEW

1.1 INTRODUCTION

Four million lambs are estimated to die during the neonatal period on hill and upland farms each year in the UK, representing a substantial financial loss to the sheep industry (Merrell, 1998). A major factor contributing to this high mortality rate is hypothermia due to delayed suckling and exhaustion of brown fat reserves (Slee, 1981; Singer, 1998). Both neonatal birthweight and vigour are positively correlated to a reduced rate of neonatal mortality (Jean and Chiang, 1999; Tuchsherer *et al.*, 2000). Standing and suckling as soon as possible after birth is therefore imperative to facilitate the ingestion of colostrum and ensure maximal lamb survival (O'Connor and Lawrence, 1992).

Mammalian brain and nervous tissue contains high concentrations of the long-chain polyunsaturated fatty acids (PUFAs) docosahexaenoic acid (C22:6*n*-3) and arachidonic acid (C20:4*n*-6; Uauy *et al.*, 2000). An optimum supply of C22:6*n*-3 is essential for the correct formation and development of brain tissue in the foetal and neonatal animal, deficiencies being associated with impaired cognitive development and visual acuity (Koletzko, 1992). However, there is no preformed source of either C20:4*n*-6 or C22:6*n*-3 within normal commercial ewe diets, and these fatty acids must be endogenously synthesised from their precursors (linoleic acid: C18:2*n*-6; and α-linolenic acid: C18:3*n*-3). This process of elongation and desaturation has been demonstrated in several species (Arbuckle and Innis, 1992; Su *et al.*, 1999; Uauy *et al.*, 2000), although the efficiency of this mechanism in the ruminant has been debated by Voigt and Hagemeister (2001). The conversion of dietary C18:3*n*-3 to C22:6*n*-3 has been reported in ruminants by Wachira *et al.* (2002). However, given the increased demand for deposition into foetal tissues during pregnancy, it is not known whether this mechanism is effective in the pregnant ewe. It

may therefore be suggested that the diminished neonatal lamb vigour and increased levels of lamb mortality observed in extensive livestock systems may result from sub-clinical PUFA deficiency in the ewe.

Vitamin E (α-tocopherol) plays an essential role as a cellular antioxidant preventing the peroxidation of unsaturated fatty acids and scavenging free radicals within the cell (Wang and Quinn, 1999). Studies by Merrell (1998) and Kott *et al.* (1983) have indicated that feeding supranutritional dietary concentrations of vitamin E to ewes is associated with improved neonatal vigour or reduced lamb mortality. Work published by Mino and Nishino (1973), Njeru *et al.* (1994), and Léger *et al.* (1998) concentrated on the relationship between maternal and neonatal plasma concentrations as the principal indicator of vitamin E status and concluded that low plasma concentrations in the neonate are indicative of negligible placental transfer. Consequently it is suggested that pregnant ewes may be being fed sub-optimal levels of vitamin E and that neonatal ruminants are deficient in the vitamin as a result of low placental transfer from dam to offspring.

The objectives of the current study were to investigate the effects of long-chain PUFA and vitamin E supplementation of ewes on lamb vigour and performance.

1.2. FATTY ACIDS

1.2.1. Fatty acid nomenclature and classification

1.2.1.1. Definitions of lipids and fatty acids

Lipids are organic compounds that are insoluble in water but generally soluble in organic solvents (Fennema, 1996; Figure 1.1). This definition encompasses many compounds, including tocopherols, carotenoids and steroids; however, in this review it will serve to refer to fatty acids and their associated derivatives. The IUPAC-IUB Commission on Biochemical Nomenclature (1976) defines a fatty acid as an aliphatic monocarboxylic acid that can be liberated by hydrolysis from naturally occurring fats and oils. A fatty acid therefore consists of a hydrocarbon chain with a carboxylic acid group at one end. Fatty acids may also contain other functional groups, including hydroxy, keto, or epoxy groups or cyclopropane rings (Brondz, 2002).

1.2.1.2. Nomenclature of fatty acids

Fatty acids may be classified by four different systems: trivial names (e.g. linoleic acid), IUPAC names (e.g. 9,12-octadecanoic acid), carboxyl-reference names (e.g. C18:2$\Delta^{9,12}$) and omega-reference names (e.g. C18:2*n*-6; Mead *et al.*, 1986). The latter two classification methods make use of formulas differentiating fatty acids according to the number of carbon atoms in the acyl chain and the number and position of their double bonds (Koletzko, 1992). Within the omega-reference system, the number after the "C" and before the colon represents the number of carbon atoms in the acyl chain and the number after the colon indicates the number of double bonds (Calder, 2001; Broadhurst *et al.*, 2002). The position of the terminal double bond (the bond closest to the methyl end) in unsaturated fatty acids is denoted by the number after the "*n*-", the number of carbon atoms between the terminal double bond and the omega carbon atom (Koletzko, 1992). For

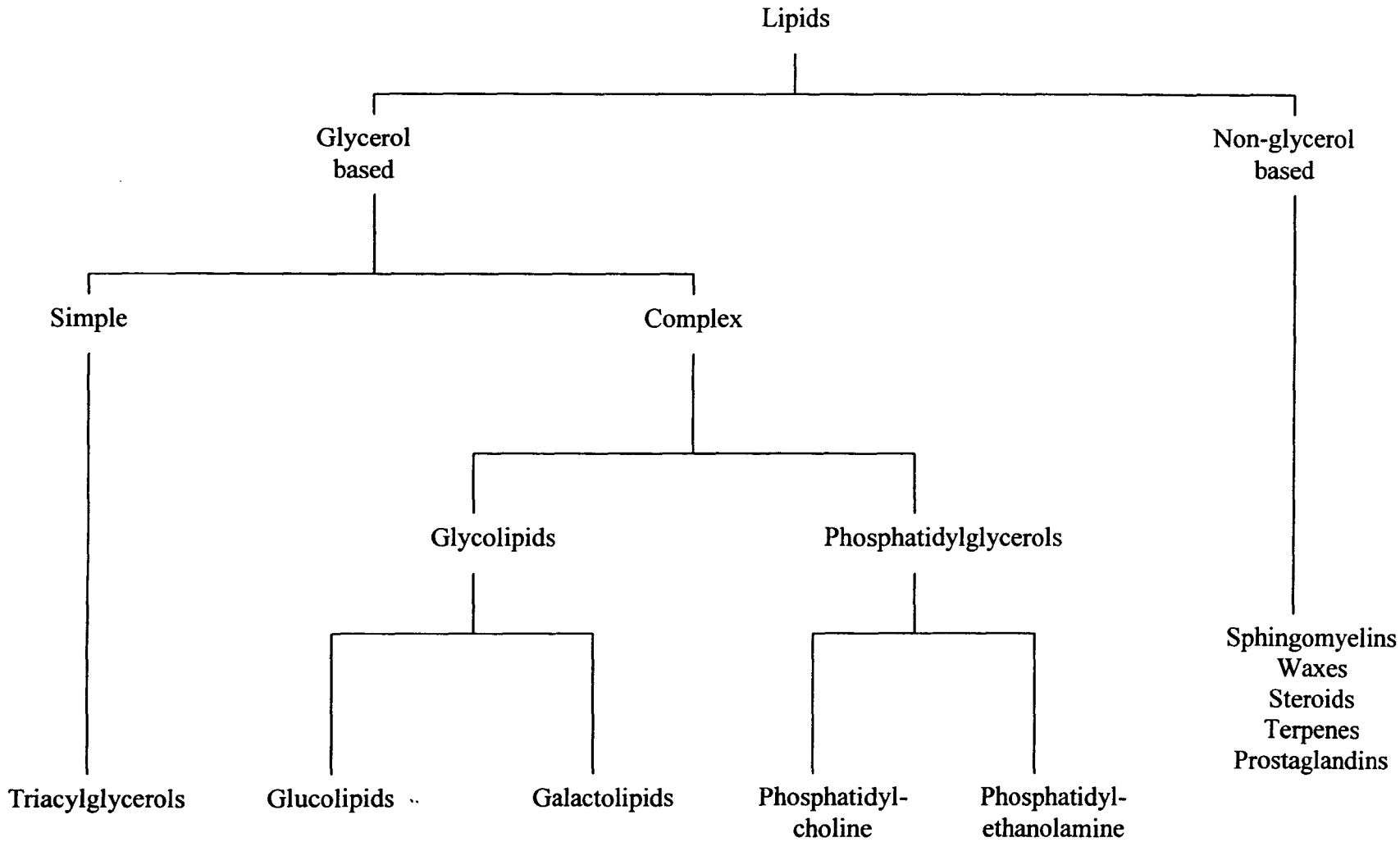


Figure 1.1. Lipid classification (McDonald et al., 1995; Chesworth et al., 1998)

example, C18:3*n*-3 has 18 carbon atoms in the acyl chain (C18), three double bonds (C18:3) and is an omega-3 fatty acid (*n*-3).

1.2.1.3. Fatty acid isomerism

Modifications in the structure of the hydrocarbon chain give rise to fatty acid isomers with distinctly different properties despite their identical shorthand classification. Positional isomerism occurs when two fatty acids have the same number of carbon atoms and double bonds, but the double bonds are located on different carbon atoms, e.g. the double bond of oleic acid (C18:1*n*-9) is located on a different carbon from that of *trans*-vaccenic acid (C18:1*n*-7; I.S.E.O, 2002). Geometric isomerism arises from a change in the configuration of the hydrogen atoms within double bonds (I.S.E.O., 2002). Most fatty acids found in humans and animals have *cis* double bonds: the *cis* configuration occurring when the hydrogen atoms at either side of the double bond are located on the same side of the molecule (Fennema, 1996). *Trans* double bonds occur when the hydrogen atoms are on opposite sides of the acyl chain; these fatty acids are commonly found in bacterial lipids (Chesworth *et al.*, 1998).

1.2.1.4. Fatty acid classification

Saturated fatty acids contain no double bonds in the carbon chain (e.g. C18:0, stearic acid), whereas unsaturated fatty acids have one or more double bonds (e.g. C18:3*n*-3; Calder, 2001). Fatty acids with one double bond (e.g. C18:1*n*-9) are termed monoenoic; fatty acids with more than one double bond are polyenoic (e.g. C20:5*n*-3, eicosapentaenoic acid; Berg Schmidt *et al.*, 2001; Calder, 2001; Table 1.1). Branched-chain, hydroxy- and cyclic fatty acids also occur in many microorganisms and some plant lipids (Chesworth *et al.*, 1998).

1.2.1.5. Saturated fatty acids

Saturated fatty acids have the chemical formula $H(CH_2)_n$-COOH, where *n* denotes the number of carbons in the acyl chain, ranging from 0-40 (Brondz, 2002). Most saturated fatty acids have an even number of carbon atoms in the acyl chain (Abayasekara and Wathes, 1999). A characteristic feature of saturated fatty acids is the absence of double bonds, all carbon-carbon bonds in the acyl chain are single bonds, therefore the molecule has a straight chain structure. Most saturated fatty acids are solid at room temperature (Calder, 2001), the melting point increasing with each increment of chain length (Enser, 1984). The most abundant saturated fatty acid in plant tissues is C16:0 (palmitic acid), this fatty acid being contained in many oils and fats as a glyceryl ester (Brondz, 2002). It is therefore the predominant fatty acid in ruminant diets (Christie, 2002) and is commonly found in animal tissues (Christie, 2003).

1.2.1.6. Unsaturated fatty acids

Unsaturated fatty acids may be further differentiated into three principal groups: *n*-3, *n*-6 and *n*-9, which differ in the position of their terminal double bond (Koletzko, 1992). Fatty acids termed *n*-7, *n*–11 and *n*-12 also occur in small quantities within plant and mammalian tissues. Monoenoic fatty acids contain a single double bond, often situated at the *n*-9 position, and between 10 and 30 carbon atoms (Christie, 2003). The most common monoenoic fatty acid is C18:1*n*-9, found in significant amounts in seed oils including sunflower, safflower, olive and canola oil (Leifert *et al.*, 1999) and in animal lipids (Chesworth *et al.*, 1998). A significant number of positional isomers of monoenoic fatty acids may be found within a single lipid source, for example, C18:1*n*-12 is found within seed oils whereas C18:1*n*-7 is ubiquitous within animal and plant lipids (Christie, 2003). Monoenoic fatty acids containing less than 18 carbon atoms are liquid at room temperature and are more vulnerable to oxidation than saturated fatty acids due to the presence of a double bond (Christie, 2003).

Table 1.1. Nomenclature and sources of fatty acids (Enser, 1984; Calder, 2001 and Brondz, 2002)

Systemic name	Trivial name and notation	Sources
Methanoic	Formic (C1:0)	-
Ethanoic	Acetic (C2:0)	De novo synthesis
Propanoic	Propionic (C3:0)	De novo synthesis
Butanoic	Butyric (C4:0)	De novo synthesis
Hexanoic	Caproic (C6:0)	De novo synthesis
Octanoic	Caprylic (C8:0)	De novo synthesis
Decanoic	Capric (C10:0)	De novo synthesis, coconut oil
Dodecanoic	Lauric (C12:0)	De novo synthesis, coconut oil
Tetradecanoic	Myristic (C14:0)	De novo synthesis, milk
Cis-9-tetradecenoic	Myristioleic (14:1)	De novo synthesis, milk
Hexadecanoic	Palmitic (C16:0)	De novo synthesis, milk, animal fats, palm oil, fish oil
9-Hexadecanoic	Palmitoleic (C16:1*n*-7)	Desaturation of palmitic acid, fish oil
Heptadecanoic	Margaric (C17:0)	De novo synthesis
Octadecanoic	Stearic (C18:0)	De novo synthesis, milk, animal fats
Cis-9-Octadecanoic	Oleic (C18:1*n*-9)	Desaturation of stearic acid, milk, animal fat, vegetable oils
Trans-9-Octadecanoic	Eladic (C18:1*n*-9)	-
Trans-11-Octadecanoic	Vaccenic (C18:1*n*-7)	Ruminal biohydrogenation
9,12-Octadecadienoic	Linoleic (C18:2*n*-6)	Milk, animal fats, vegetable and oilseed oils, green leaves
9,12,15-Octadecatrienoic	α-Linolenic (C18:3*n*-3)	Green leaves, vegetable and oilseed oils
6,9,12-Octadecatrienoic	γ-Linolenic (C18:3*n*-6)	Synthesised from linoleic acid, borage and evening primrose oils
Eicosanoic	Arachidic (C20:0)	-
5,8,11-Eicosatrienoic	Mead (C20:3*n*-9)	Synthesised from oleic acid
5,8,11,14-Eicosatetraenoic	Arachidonic (C20:4*n*-6)	Synthesised from linoleic acid, some fish oils
5,8,11,14,17-Eicosapentaenoic	Timnodonic (C20:5*n*-3)	Synthesised from α-linolenic acid, fish oils, marine algae
Docosanoic	Behenic (C22:0)	-
7,10,13,16,19-Docosapentaenoic	Clupanodonic (C22:5*n*-3)	Synthesised from α-linolenic acid, fish oils, marine algae
4,7,10,13,16,19-Docosahexaenoic	Cervonic (C22:6*n*-3)	Synthesised from α-linolenic acid, fish oils, marine algae

Polyenoic fatty acids usually contain between 18-24 carbon atoms in the acyl chain and are predominantly found in cell membrane phosphatidylglycerols, esterified onto a phosphatidylglycerol backbone (Leifert *et al.*, 1999).

1.2.1.7. Essential fatty acids

The two 18-carbon unsaturated fatty acids C18:2*n*-6 and C18:3*n*-3 were identified by Burr and Burr (1929) as being essential for growth and normal reproduction in the rat. These fatty acids are classified as essential fatty acids (EFAs): endogenous synthesis of these fatty acids requires not only an acyl carbon chain containing 18 carbon atoms but also a specific desaturase enzyme which is lacking in humans and animals (Knipp *et al.*, 1999; Innis, 2000). Consequently, a preformed source of these fatty acids must be included in the ruminant diet (Leifert *et al.*, 1999; Czauderna *et al.*, 2001; Ikemoto *et al.*, 2001). The EFAs C18:2*n*-6 and C18:3*n*-3 are precursors for conditionally essential long-chain PUFAs C20:4*n*-6, C20:5*n*-3 and C22:6*n*-3 (Koletzko, 1992).

Considerable quantities of C18:2*n*-6 are stored in adipose tissue and may be mobilised in times of restricted dietary supply in the ruminant (Hornstra *et al.*, 1995). Conversely, C18:3*n*-3 and its derived long-chain PUFAs are not stored in significant amounts in body fat under normal dietary conditions (Hornstra *et al.*, 1995), therefore it is not possible to supplement a sub-optimal dietary supply by mobilization of adipose tissue. However, it is possible to supplement the concentrations of C18:3*n*-3, C20:5*n*-3 and C22:6*n*-3 in adipose tissue of ruminants by feeding high concentrations of linseed or fish oil (Wachira *et al.*, 2002). Symptoms of C18:2*n*-6 deficiency include scaly dry skin lesions and growth retardation (Makrides *et al.*, 1996). A deficiency in C18:3*n*-3 is manifested in mammals as alopecia, loss of visual acuity and neurologic problems (Makrides *et al.*, 1996). Both syndromes are reversible given appropriate supplementation (Makrides *et al.*, 1996).

A sub-optimal EFA supply will eventually result in endogenous synthesis of surrogate fatty acids that are not present under normal conditions (Hornstra *et al.*, 1995). The biosynthesis of mead acid (C20:3*n*-9) from C18:1*n*-9 is inhibited by endogenously derived long-chain PUFAs (Hornstra *et al.*, 1995). Therefore increased concentrations of this fatty acid indicate that animals are deficient in both essential and derived long-chain PUFAs (Leifert *et al.*, 1999). Neonatal lambs often have relatively high concentrations of C20:3*n*-9 within plasma, thought to indicate a low transfer rate of EFAs across the placenta (Noble *et al.*, 1982).

1.2.1.8. Conjugated fatty acids

Possibly the most important conjugated fatty acid is conjugated linoleic acid (CLA), a term used to describe a mixture of positional and geometric isomers of C18:2*n*-6 (Chouinard *et al.*, 1999; Bessa *et al.*, 2000; Donovan *et al.*, 2000). These fatty acids were first identified by Kepler *et al.* (1966) as intermediates in the biohydrogenation of C18:2*n*-6 and are found mainly in ruminant body fluids and tissues (Chouinard *et al.*, 1999; Clegg *et al.*, 2001; Voigt and Hagemeister, 2001). In contrast to unsaturated fatty acids which have one or more methyl groups between each double bond (methylene-interrupted double bond system), conjugated fatty acids (e.g. *cis*-9, *trans*-11 C18:2) have double bonds separated by a single carbon-carbon bond (Bessa *et al.*, 2000). The predominant and most biologically active isomer of CLA is *cis*-9, *trans*-11 C18:2 (Bessa *et al.*, 2000; Sergeil *et al.*, 2001). A significant dietary role for CLA has been discovered in the prevention of human diseases including atherosclerosis, cancer, diabetes and cardiovascular conditions (Clegg *et al.*, 2001; Czauderna *et al.*, 2001; Sergeil *et al.*, 2001). Consequently, a significant amount of research has been directed at increasing the CLA content of ruminant products for human consumption (Voigt and Hagemeister, 2001).

1.2.1.9. Lipid Compounds

The predominant lipid compounds in animal and plant tissues are the triacylglycerols and phosphatidylglycerols (Enser, 1984). Triacylglycerol molecules consist of a glycerol molecule with fatty acids esterified onto each of the three hydroxyl groups (Christie, 2002). These are classified as simple lipids yielding two hydrolysis products: glycerol and fatty acids (Bruss, 1997). Within ruminant tissues, triacylglycerols account for up to 95 g/kg of the lipids present (Enser, 1984). Different fatty acids may be esterified onto each of the three hydroxyl groups in ruminant triacylglycerols (*sn*-1, -2 and -3), subject to alteration by the organ of synthesis and the enzyme catalysing the esterification (Enser, 1984).

Most phosphatidylglycerols comprise one glycerol molecule in conjunction with two esterified fatty acids and one phosphate group (Enser, 1984). A substituent group (e.g. choline, serine or ethanolamine) may be further linked to the phosphate of phosphatidylglycerols (Chesworth *et al.*, 1998). Consequently they are classified as complex lipids, producing three or more compounds under hydrolysis (Christie, 2002). Phosphatidylglycerols comprise approximately 0.5-3.0 % (w/w) of ruminant tissue (Enser, 1984). The predominant phosphatidylglycerol compounds within animal tissue are phosphatidylcholine (lecithin) and phosphatidylethanolamine (cephalin; Enser, 1984). These compounds are present as vital components of cell membranes (Chesworth *et al.*, 1998). Again, the fatty acid composition of phosphatidylglycerols varies and has significant effects on lipid function. For example, phosphatidylethanolamine is high in C20:4*n*-6 and is a principal fatty acid found within lipid bilayers in cell membranes, whereas C18:2*n*-6 predominates in phosphatidylcholine, which plays an essential role in nerve cell signal transduction (Enser, 1984). In contrast to triacylglycerols, specific fatty acid types are found at each *sn* position with principally saturated fatty acids in the *sn*-1

position and unsaturated fatty acids on *sn*-2 (Enser, 1984). Certain fatty acid pairs are also found in phosphatidylglycerols, e.g. C16:0/C18:2*n*-6 and C18:0/C20:4*n*-6 (Enser, 1984).

1.2.2. Fatty acid sources within the ruminant diet

The diet of domesticated ruminants usually contains approximately 30 g/kg lipid (Chilliard, 1993), the fatty acids supplied to animal tissues originating from either exogenous dietary sources or endogenous synthesis. Dietary fatty acid sources may be of plant or animal origin: many plants contain high concentrations of fatty acids within the seed, grain or leaves, and animal by-products provide both essential and non-essential fatty acids (Palmquist, 1984).

1.2.2.1. Forages

Grass is the most significant fatty acid source in the diet of the grazing ruminant. Fresh grass contains 3-4 % fatty acids (Ashes *et al.*, 1992), which are concentrated in the chloroplasts at 22 g lipid/100 g tissue (Sargent, 1997). The predominant fatty acid in grass is C18:3*n*-3 (Moore and Christie 1984). At 55-65 % of total fatty acids (w/w), this fatty acid is predominantly found in chloroplast membranes (Sinclair *et al.*, 2002) with highest concentrations being observed during spring and autumn (Dewhurst and Scollan, 1997; Chilliard *et al.*, 2001b). Relatively high quantities of C18:2*n*-6 are also found in grass, in conjunction with small amounts of C18:1*n*-9 (Ashes *et al.*, 1992; Hornstra *et al.*, 1995; Leifert *et al.*, 1999). There are small differences in fatty acid composition between grass species (Table 1.2), processing grasses to produce hay or silage may also have appreciable effects on their fatty acid composition (Dewhurst and Scollan, 1997).

1.2.2.2. Oilseeds

Plant seeds such as maize, canola, safflower, sunflower and cottonseed contain high concentrations of unsaturated fatty acids within triacylglycerols (Demeyer and Van Nevel,

1995). Indeed, plant seed oils are the principal fatty acid source in ruminants fed concentrate-based diets. This may be via the inclusion of whole or crushed oilseeds as an energy or protein source, or by the inclusion of plant oils specifically to increase the dietary fat or energy content. The majority of seed oils contain a significant proportion of C18:2*n*-6 (Ashes *et al.*, 1992; Leifert *et al.*, 1999; Calder, 2001; Table 1.3). By contrast, C18:3*n*-3 is only found in appreciable quantities in canola and rapeseed oils, where it is present in combination with C18:2*n*-6, and in linseed (Sargent, 1997). Linseed is the only oilseed in which C18:3*n*-3 predominates, with an oil content of 400 g/kg DM, of which up to 50 % of total fatty acids is C18:3*n*-3 (Calder, 2001; Voigt and Hagemeister, 2001).

Cereal grains are not significant sources of fatty acids at approximately 40 g lipid/kg DM (AFRC, 1993). However, C18:2*n*-6 is the main fatty acid found in cereal grain, and animals fed on grain-based diets tend to have increased deposition of *n*-6 fatty acids in body tissues (Enser *et al.*, 1998a). Long-chain PUFAs such as C22:6*n*-3, C20:5*n*-3 and C20:4*n*-6 are not found in noteworthy quantities within terrestrial plants, therefore they must either be supplied by endogenous synthesis from their EFA precursors, or from dietary sources such as marine plants or fish oils.

1.2.2.3. Marine plants

Marine algae contain between 10-700 g/kg DM lipid (Borowitzka, 1988), including high concentrations of *n*-3 fatty acids (Kitessa *et al.*, 2001d; Broadhurst *et al.*, 2002). Indeed, many of the long-chain *n*-3 PUFAs found in fish oils originate from *de novo* biosynthesis within phytoplankton (Sargent and Henderson, 1995). As in the terrestrial plants, lipids are stored within the chloroplasts, contributing 10-20 % of the cell weight and being influenced by species and season (Sargent and Henderson, 1995). Diatomic and eustigmatophytic phytoplankton have high C20:5*n*-3 contents and low C22:6*n*-3

Table 1.2. Fatty acid composition of grasses (g/kg fatty acids; Dewhurst and Scollan, 1997)

	C16:0	C18:0	C18:1*n*-9	C18:2*n*-6	C18:3*n*-3	Saturates	Unsaturates	Total fatty acids (g)
Lolium perenne	209	44	51	132	516	273	709	22.5
Lolium multiflorum	217	46	53	137	503	282	702	20.8
Lolium hybrids	212	43	51	143	509	273	710	23.2
Lolium-Fescue hybrids	200	39	51	135	532	258	725	23.0
Fescue	201	50	41	116	551	272	713	21.9
Cocksfoot	209	31	26	152	532	260	720	19.1
Timothy	190	43	43	164	513	251	726	21.8

Table 1.3. Fatty acid composition of vegetable oils (g/kg lipid; Kennelly, 1996; Chesworth et al., 1998)

	C16:0	C18:0	C18:1*n*-9	C18:2*n*-6	C18:3*n*-3	Saturates	Unsaturates
Coconut	89	44	56	22	NA	830	70
Cottonseed	250	30	170	540	NA	290	710
Linseed	50	30	200	160	550	90	910
Maize	126	18	300	543	5	NA	NA
Olive	143	20	653	164	NA	NA	NA
Palm Kernal	82	21	133	21	NA	NA	NA
Palm	480	40	380	90	NA	NA	NA
Rapeseed (high erucic)	30	10	160	140	100	NA	NA
Rapeseed (low erucic)	40	20	560	260	100	NA	NA
Safflower	70	20	90	800	<10	100	900
Soyabean	80	30	240	580	80	100	900
Sunflower	60	40	200	660	<10	120	880

NA = Data not available

concentrations; conversely, dinoflagellates are high in C22:6*n*-3 and contain negligible amounts of C20:5*n*-3 (Givens, 1997). The main species of interest with reference to animal nutrition, the microalgae *Schizochytrium sp.*, contains up to 250 g/kg C22:6*n*-3 and 250 g/kg docosapentaenoic acid (DPA; C22:5*n*-6), but only 10 g/kg C20:5*n*-3 (Chilliard *et al.*, 2001a). Algal oils used in animal feed manufacture derive from large-scale microalgae production, the algal biomass (whole algal cells which encapsulate long-chain PUFAs) containing high concentrations of both *n*-3 fatty acids and antioxidants (Hind, 1997). Research to date has concentrated on the use of algal biomass to enrich meat, milk and eggs produced for human consumption with long-chain *n*-3 PUFAs in an attempt to address their perceived health benefits (Hind, 1997).

1.2.2.4. Animal fats and fish oils

As a corollary of the ban on feeding both tallow and proteins of animal origin (DEFRA, 2002), fish oil is the principal animal fat source within ruminant diets. Consumer concerns exist regarding the potential effects of feeding animal fats to ruminants and the sustainability of fish oil production. It is therefore possible that this source will be banned from animal diets in the future (Hind, 1997).

Fish lipids differ from those found in animal tissues due to their high concentration of long-chain PUFAs, specifically those of the *n*-3 family, with a total lipid content varying from 1 % (cod) to 24 % (mackerel; Opstvedt, 1984). Fish are classified as "oily" or "non-oily" according to their fatty acid storage organ (Sargent, 1997). Oily fish have a high fatty acid content in the muscle tissues (up to 20 % of tissue weight; e.g. mackerel, herring) whereas non-oily fish (cod, founder) accumulate fatty acids in the liver (Calder, 2001). Furthermore, oily fish tend to feed on zooplankton, whilst larger, non-oily fish consume small, zooplanktonivorous fish (Sargent and Henderson, 1995).

A marine food-chain hierarchy exists in which zooplanktonivorous fish consume zooplankton crustaceans, and in turn these feed upon zooplankton (Sargent, 1997). Given the monogastric nature of fish digestion, it is logical to conclude that the high PUFA concentrations found within fish lipids originate from marine algae and seaweeds. However, variation in fish fatty acid composition compared to that within marine plants arises as a result of the ability of zooplankton to synthesise long-chain PUFAs not supplied by the phytoplankton diet (Sargent, 1997). The distribution of fatty acids within zooplankton has a major impact on the PUFA composition of fish species: fish consuming zooplankton containing high concentrations of wax esters tend to have increased concentrations of C20:1*n*-9 and C22:1*n*-11, and reduced concentrations of *n*-3 fatty acids (Table 1.4). By contrast, C20:5*n*-3 and C22:6*n*-3 are increased and both C20:1*n*-9 and C22:1*n*-11 are reduced in zooplankton containing an appreciable concentration of triacylglycerols (Sargent, 1997). The high concentrations of long-chain PUFAs within fish oil are suggested by Sargent and Henderson (1995) to function to maintain the fluidity of cell membrane bilayers irrespective of fluctuations in environmental temperature and pressure.

The fatty acid composition of fish oil is further influenced by the concentration of oil within the body, dependent on sexual maturity and stage of development (Sargent and Henderson, 1995). Although fish can synthesise saturated and monounsaturated fatty acids (Ackman, 1982), marine fish appear to have lost the ability to elongate C18 fatty acids to C20 and C22 PUFAs although this biological mechanism remains active in freshwater fish (Sargent, 1997). Therefore, the catfish *Anarchichas lupus* has a high C20:5*n*-3 content compared to the cod *Gadus morhua* (Ackman, 1982).

Table 1.4. Fatty acid composition of fish oils (g/kg fatty acids)

	C18:0	C18:1*n*-9	C18:2*n*-6	C18:3*n*-3	C20:5*n*-3	C22:6*n*-3	Saturates	Monounsaturates	*n*-3 PUFA
Salmon flesh (wild)[a]	30	190	10	10	70	100	200	520	240
Salmon flesh (farmed)[a]	30	130	30	20	70	120	240	430	280
Tuna oil[b]	160	140	10	10	60	220	390	200	290
Herring oil[a]	10	100	10	10	60	60	210	530	160
Capelin oil[c]	10	130	10	10	90	50	170	530	190
Anchovy oil[a]	40	120	10	10	170	90	280	230	310
Pilchard oil[a]	-	-	-	-	170	90	-	80	160
Menhaden oil[d]	29	128	9	6	177	62	270	-	259
Atlantic Mackerel oil[d]	-	-	12	-	115	151	-	-	284
Commercial fish oil[e]	30	160	20	20	80	110	230	440	220

[a] Sargent (1997)
[b] Kitessa *et al.* (2001)
[c] Bryhni *et al.* (2002)
[d] Givens (1997)
[e] Keady and Mayne (1999b)

Functions of fatty acids

Saturated fatty acids have a significant role to play as an energy store within adipose tissue (Demeyer and Doreau, 1999; section 1.2.4.10.). By contrast, long-chain PUFAs have a number of other significant functions within ruminant tissues.

1.2.3.1. Role of fatty acids within cell membranes

The *n*-3 and *n*-6 fatty acids are vital for the maintenance and function of cell structural membranes (Craig-Schmidt *et al.*, 1996; Ikemoto *et al.*, 2001). The fatty acid composition of the phosphatidylglycerols in the lipid bilayer determines the physical and chemical properties of the membrane (Innis, 2000). There is a preferential incorporation of long-chain PUFAs into phosphatidylglycerols, the level of incorporation having a significant effect upon membrane fluidity, metabolite permeability and enzyme activity (Koletzko, 1992; Sawosz *et al.*, 2001). Membrane fluidity is increased by the degree of fatty acid unsaturation and the concentrations of short-chain and *cis*-fatty acids present (Conn *et al.* 1987). The incorporation of long-chain PUFAs into retinal and neural cell membranes increases membrane fluidity but predisposes the membranes to oxidative damage by reactive oxygen and nitrogen species (McMurray and Rice, 1982).

1.2.3.2. Role of fatty acids within nervous tissue

The promotion of optimal membrane fluidity conferred by long-chain PUFAs is essential in mammalian nervous and visual organs. Consequently, a significant proportion of ruminant neural tissue dry matter (DM) consists of endogenously elongated and desaturated or preformed dietary long-chain PUFAs (Goustard-Langelier *et al.*, 1999; Uauy *et al.*, 2000; Williard *et al.*, 2001). These fatty acids are concentrated in synaptosomal membranes and retinal photoreceptors (Koletzko, 1992). Indeed, the brain and retina are unique in their specificity for long-chain PUFA deposition in cell membranes (Crawford *et al.*, 1997). Brain development in the foetal ruminant consis

neuronal multiplication, formation of synaptosomal contacts between individual neurones and myelinisation of nerve cells (Salvati *et al.*, 1999). The two preliminary processes require significant amounts of long-chain PUFA for membrane deposition (Arbuckle and Innis, 1992). Long-chain PUFAs are also involved in the synthesis of myelin (Christie, 1978) required for optimal nerve and brain function, and with compounds such as gangliosides and sphingolipids which are related to cognitive function (Knipp *et al.*, 1999). The principal fatty acid contained in synaptic end sites is C22:6*n*-3, leading Rooke *et al.* (2001a; 2001b) to suggest that it may be essential for synaptosome formation. Long-chain PUFA deficiency originating from an absence of fatty acid precursors is manifested as hypomyelination, by contrast, increasing the fatty acid supply up-regulates genes responsible for myelin production (Salvati *et al.*, 1999). Consequently, fatty acid supply may have regulatory effects on cerebral and visual development, and deficiencies act as limiting factors in brain function (Koletzko, 1992).

Photoreceptor cells within the ruminant retina are responsible for transforming signals from absorbed light into electrical messages via stacks of flattened disks, each containing an excitable, fluid membrane (Koletzko, 1992). The fluidity of this membrane is maintained by high concentrations of phosphatidylglycerols (Koletzko, 1992), each rhodopsin molecule surrounded by 60 phosphatidylglycerols enriched with C22:6*n*-3 at up to 60 % of total fatty acids (Weisinger *et al.*, 1996; Crawford *et al.*, 1997). The high concentrations of C22:6*n*-3 in photoreceptors require specific mechanisms for C22:6*n*-3 deposition, which are significantly affected by deficiencies in long-chain PUFAs (Koletzko, 1992). Alterations in electroretinograms as a result of EFA deficiency have been observed before the onset of clinical deficiency symptoms in rats, and these symptoms rapidly reversed by fatty acid supplementation (Koletzko, 1992).

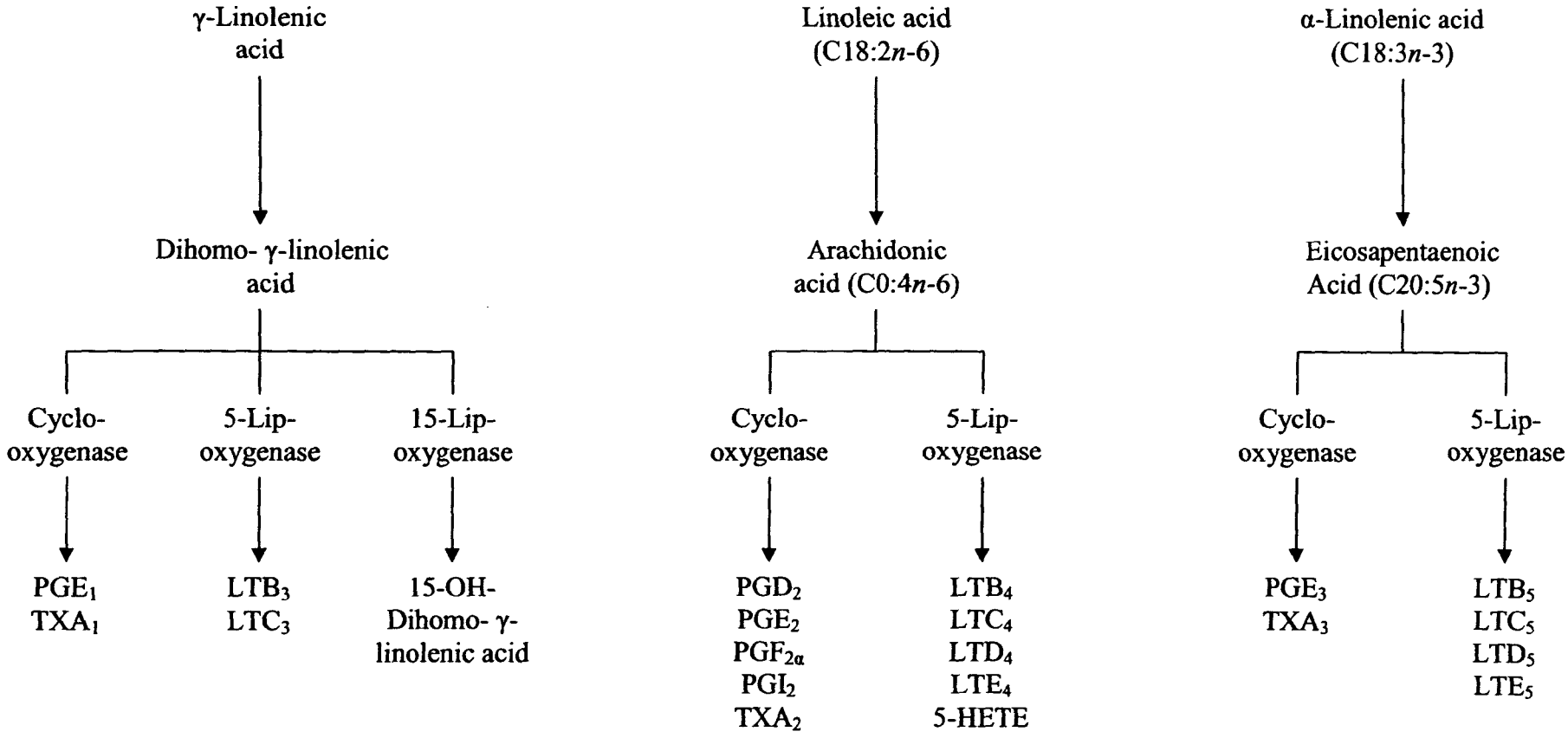
1.2.3.3. Fatty acids and prostaglandin synthesis

Within the ruminant, the long-chain PUFAs C18:2*n*-6, C18:3*n*-3, dihomo-γ-C18:2*n*-6, C20:4*n*-6 and C20:5*n*-3 are direct or indirect precursors for the bioactive eicosanoid molecules known as prostaglandins, thromboxanes and leukotrienes (Figure 1.2; Lands, 1982). Broadly similar to hormones, eicosanoids may exert effects upon any cell possessing the appropriate receptor (O'Neil, 1994). Although they are primarily autocrine or paracrine in action and primarily exert local effects, they may also elicit an endocrine effect via the circulation or lymph system (Mead *et al.*, 1986). Prostaglandins are responsible for the regulation of many cellular functions (Wainwright, 2002), including the duration and intensity of inflammatory and immune responses (Calder, 2001). They are also associated with pregnancy, gastrointestinal and kidney function and inflammation (James and Cleland, 2000). Structurally, they consist of fatty acid molecules containing a cyclopentane ring (prostaglandins) or tetrahydropyran system (thromboxanes) within the acyl chain (Brondz, 2002).

Bioactive dienoic prostaglandins, including thromboxane A_2, prostaglandin E_2 (PGE_2) and four different leukotrienes (leukotrienes B_4, C_4, D_4 and E_4) are synthesised from the principal eicosanoid precursor, C20:4*n*-6 (Abayasekara and Wathes, 1999). The rate-limiting release of C20:4*n*-6 from the *sn*-2 position of phosphatidyglycerols is regulated by an C20:4*n*-6-selective acyl-CoA synthase and achieved via cytosolic phospholipase A_2 (O'Neill, 1994) and specific lipases (Hansen *et al.*, 1999). Free C20:4*n*-6 is then converted to prostaglandin G_2 (PGG_2) by cyclooxygenase (also known as PGH synthase), which reacts with hydroperoxides to produce the endoperoxide intermediates PGG_2 and prostaglandin H_2 (PGH_2; Hansen *et al.*, 1997). Prostaglandin synthetase enzymes catalyse the formation of the *n*-6-derived prostaglandins and thromboxanes from PGH_2 (O'Neil, 1994). Leukotrienes are produced via the reaction of C20:4*n*-6 with lipoxygenase (O'Neill, 1994).

Prostaglandins derived from *n*-6 fatty acids act as cardio-agonists, promoting blood vessel constriction and cholesterol accumulation (Hind, 1997). Prostaglandin E_2 is pro-inflammatory in action, augmenting pain and oedema by promoting phagocytosis at the site of injury (Lands, 1982). Moreover, the stimulatory eicosanoids contained in amniotic fluid and associated with muscle contraction at parturition (prostaglandin $F_{2\alpha}$, PGE_2, 13,14-dihydro-15-ketoprostaglandin $F_{2\alpha}$ (PGFM) and thromboxane A_2) are produced, directly or indirectly, from C20:4*n*-6 (Hansen and Olsen, 1988; Olsen *et al.*, 1990; Hansen *et al.*, 1999).

Eicosanoids produced from the *n*-3 series fatty acids tend to be less biologically active than those produced by C20:4*n*-6 (Abayasekara and Wathes, 1999), and include the inhibitory and thrombolytic 3-series prostanoids prostaglandin E_3, thromboxane A_3 and leukotrienes B_5, C_5, D_5 and E_5 (Hornstra *et al.*, 1995; Calder, 2001). Prostacyclins, specifically prostaglandin I_2 and prostaglandin I_3 are also produced from C20:5*n*-3 (Olsen *et al.*, 1992). Dietary supplementation with C20:5*n*-3 reduces the concentration of C20:4*n*-6 within cellular membranes via competitive inhibition, therefore reducing the amount of substrate available for prostaglandin synthesis (Calder, 2001). Moreover, it is suggested that cyclooxygenase has an equal or greater affinity for *n*-3 than for *n*-6 series fatty acids; therefore, similar concentrations of C20:5*n*-3 and C20:4*n*-6 in the cell will effectively inhibit the synthesis of C20:4*n*-6-derived prostaglandins (Lands, 1982; Hansen and Olsen, 1988; Fahey *et al.*, 2002). Eicosanoids are not produced directly from C22:6*n*-3, however, Huang and Craig-Schmidt (1996) noted inhibition of *n*-6 derived prostaglandin synthesis with dietary C22:6*n*-3 supplementation of neonatal piglets. It is suggested that this may occur via retroconversion of C22:6*n*-3 to C20:5*n*-3, a mechanism which has been demonstrated in ruminants by Wachira (1999).



HETE = Hydroxyeicosatetraenoic acid, LT = Leukotriene, PG = Prostaglandin, TX = Thromboxane

Figure 1.2. Prostaglandin synthesis (Lands, 1982 and Calder, 2001)

Petit *et al.* (2002) demonstrated that the production of PGFM (13,14-dihydro-15-keto-$PGF_{2\alpha}$) in dairy cattle was reduced by feeding whole linseed or infusing linseed oil. This concurs with the theory that dienoic prostaglandin synthesis is inhibited by *n*-3 fatty acids. Nonetheless, it was hypothesised that this effect was caused by a reduction in the *n*-6:*n*-3 ratio rather than an effect of C20:5*n*-3 or C22:6*n*-3 *per se*. Hansen and Olsen (1988) also reported a decrease in C20:4*n*-6-derived prostaglandins and increase in prostaglandins derived from C20:5*n*-3 in humans fed a diet rich in *n*-3 fatty acids.

1.2.4. Digestion, absorption and metabolism of fatty acids in the ruminant

1.2.4.1. Fatty acid digestion within the rumen

Ruminal lipid digestion occurs via hydrolysis of lipid compounds followed by biohydrogenation of unsaturated fatty acids (Mackie *et al.*, 1991; Figure 1.3). Upon entering the rumen, ester bonds within dietary lipids are rapidly hydrolysed by lipase, galactosidase and phospholipase enzymes produced by ruminal microorganisms, to their constituent free fatty acids and associated compounds (Doreau and Chilliard, 1997a; Doreau *et al.*, 1997; Demeyer and Doreau, 1999). The extent of ruminal lipid hydrolysis is dependent on a combination of microfloral activity and rumen pH (Doreau and Ferlay, 1994). Ruminal bacteria are unable to hydrolyse all ester linkages, for example, the predominant hydrolysing bacteria *Anaerovibrio lipolytica* (Doreau *et al.*, 1997) specifically hydrolyses triacylglycerols containing medium or long-chain fatty acids via lipase and esterase enzymes (Moore and Christie, 1984). Furthermore, Jenkins (1993) suggest that bacteria with non-specific esterase activity may not be able to hydrolyse the ester linkages of long-chain PUFAs. It has also been suggested that dietary plant lipases may have a significant role to play in the hydrolysis of ester bonds (Moore and Christie, 1984), however, their activity may be negligible in ruminants (Doreau *et al.*, 1997). Doreau *et al.* (1997) reported that lipids are hydrolysed at an efficiency of 85-90 %, of which 30 % is executed by protozoa and the remainder via bacteria.

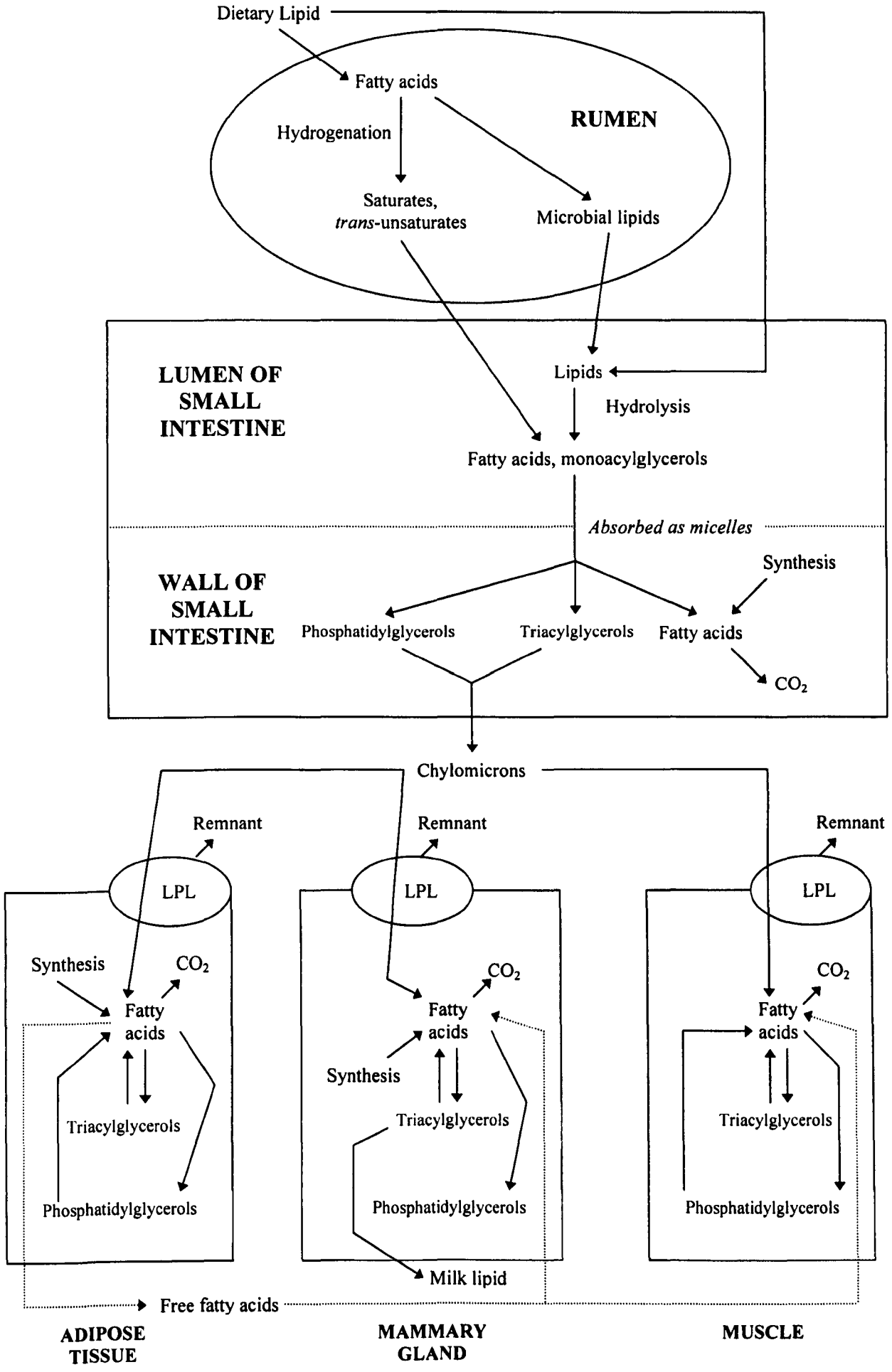


Figure 1.3. Lipid digestion and metabolism (Enser, 1984)

1.2.4.2. Biohydrogenation of unsaturated fatty acids

Following hydrolysis, unesterified fatty acids are adsorbed onto feed particles and unsaturated fatty acids biohydrogenated to form fatty acids that have a lower degree of unsaturation or are saturated (Kennelly, 1996; Demeyer and Doreau, 1999). Biohydrogenation may only occur if the fatty acid contains a free carboxyl group, therefore lipolysis is a prerequisite for this form of digestion (Jenkins, 1993). Biohydrogenation was first demonstrated by Reiser (1951) using a mixture of ruminal microorganisms and long-chain fatty acids. The metabolic reason for the conversion of unsaturated fatty acids to their saturated derivatives is unknown, but it is suggested to be a protective mechanism against the toxic effects of unsaturated fatty acids on rumen microflora (Palmquist, 1984).

During biohydrogenation, the *cis*-12 double bonds of C18:3*n*-3 and C18:2*n*-6 are converted to a *trans*-11 unsaturated bond by an isomerisation reaction. The *cis*-9 bond and *trans*-11 bonds are then hydrogenated via reductase enzymes to produce *trans*-11 C18:1 and finally, C18:0, the primary product of ruminal biohydrogenation (Figure 1.4; Kennelly, 1996; Doreau and Chilliard, 1997a; Gulati *et al.*, 1999). Two groups of hydrogenating bacteria exist in the rumen: group A act upon C18:2*n*-6 and C18:3*n*-3 to form *trans*-11 C18:1 and *cis*-vaccenic acid (*cis*-11 C18:1) as previously explained. Group B bacteria then hydrogenate C18:1 (*cis* and *trans*) to C18:0 (Demeyer and Doreau, 1999).

Biohydrogenation of unsaturated fatty acids is almost complete, with between 70-90 % of dietary PUFAs being reduced or saturated (Chilliard, 1993). Therefore, some unsaturated fatty acids escape hydrogenation and are presented to the duodenum in their original form (Jenkins, 1993; Enser *et al.*, 1998a; Kitessa *et al.*, 2001c). Incomplete hydrogenation of C18:3*n*-3 and C18:2*n*-6 results in the production of *cis* and *trans* isomers of the C18 fatty acids (Doreau and Chilliard, 1997a). *Trans*-11 C18:1 are the predominant isomers of C18:1 found in the rumen (Demeyer and Doreau, 1999). Conjugated fatty acids are also

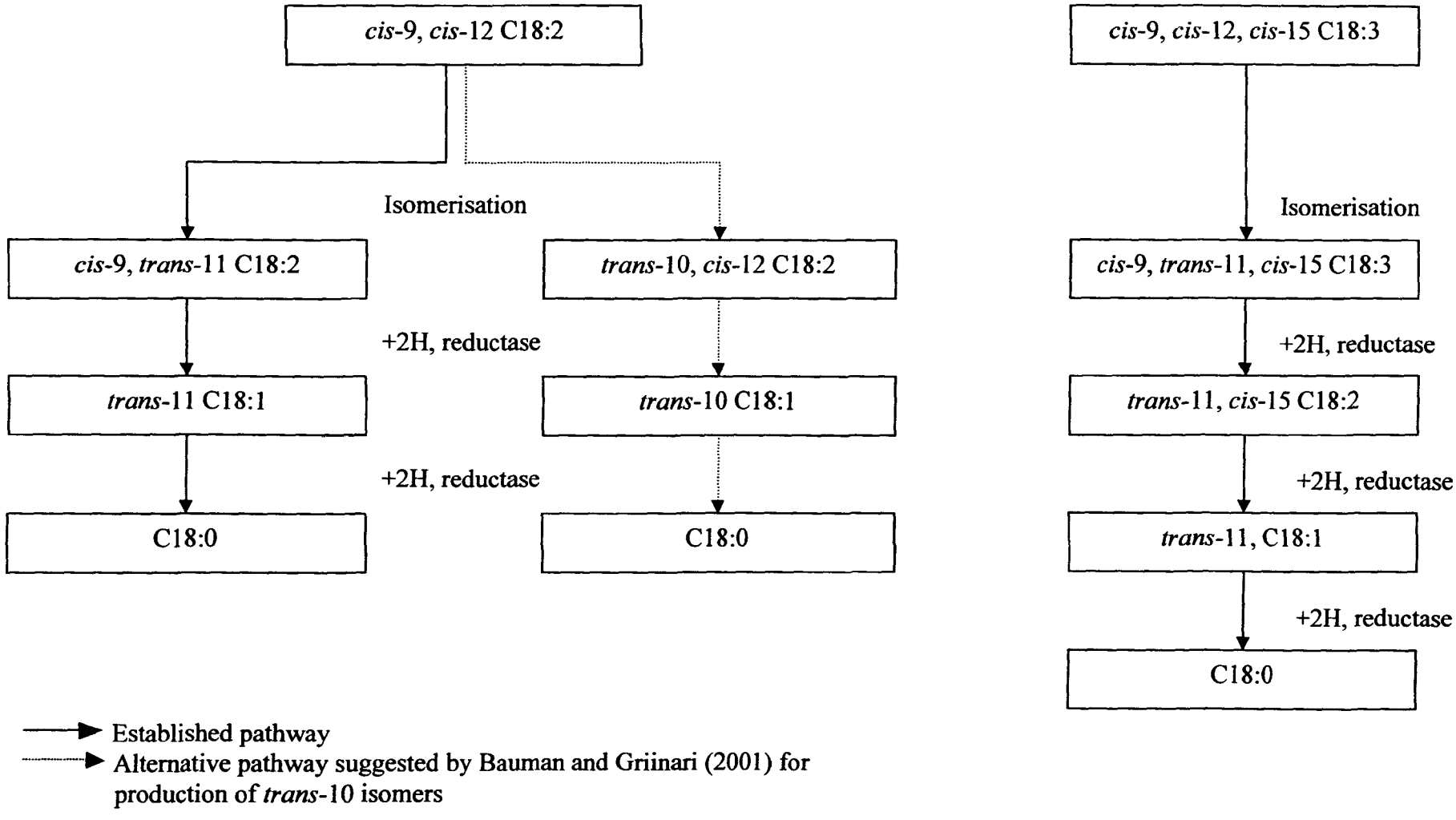


Figure 1.4. Biohydrogenation of linoleic acid and α-linolenic acid (Moore and Christie, 1984; Jenkins, 1993; Bauman and Griinari, 2001)

produced from incomplete biohydrogenation of PUFAs (Baumgard *et al.*, 2000). The two predominant CLA isomers produced by incomplete biohydrogenation of C18:2*n*-6 are *cis*-9,*trans*-11 C18:1 and *trans*-10,*cis*-12 C18:1 (Demeyer and Doreau, 1999).

Biohydrogenation of C18:2*n*-6 ranges from 70-95 % with values for C18:3*n*-3 ranging from 85-100 % (Chilliard *et al.*, 2000). Biohydrogenation of C18:2*n*-6 increases with dietary concentration (Cieślak *et al.*, 2001), therefore the proportion that escapes hydrogenation remains constant, however, there are no results to indicate that a similar relationship exists between C18:3*n*-3 intake and hydrogenation (Doreau and Ferlay, 1994). Conflicting evidence exists as to whether C20 and C22 fatty acids (specifically C20:5*n*-3 and C22:6*n*-3) are biohydrogenated within the rumen (Doreau *et al.*, 1997; Gulati *et al.*, 1999; Kitessa *et al.*, 2001c). Ashes *et al.* (1992) hypothesised that a lack of specific enzymes prevented the hydrogenation of C20 and C22 fatty acids. Furthermore, Gulati *et al.* (1999) proposed that C20:5*n*-3 and C22:6*n*-3 are hydrogenated to a lesser extent than C18:2*n*-6 and C18:3*n*-3, at approximately 10 % hydrogenation with the isomerisation and saturation of C20:5*n*-3 occurring at a greater rate than that of C22:6*n*-3. *In vivo* work by Cooper *et al.* (2002) appears to contradict this hypothesis: values ranging from 62-86 % and 61-80 % were reported for the biohydrogenation of C20:5*n*-3 and C22:6*n*-3 respectively. Furthermore, extensive biohydrogenation of C20:5*n*-3 and C22:6*n*-3 within dietary fish oil was reported in sheep by Chikunya *et al.* (2004) and in cattle by Scollan *et al.* (2001).

The work of Doreau and Chilliard (1997b) confirmed that long-chain unsaturated oils have a significant effect on rumen fermentation with a decrease in cellulolytic and methanogenic bacteria, decrease in acetate and increase in propionate production. However, it has been hypothesised that the hydrogenation of C20:5*n*-3 and C22:6*n*-3 may, in part, be affected by the duration of long-chain fatty acid supplementation (Chilliard *et al.*, 2001b). The

ruminal ecosystem is subject to long-term adaptive change which may result in an increased biohydrogenation of fatty acids derived from dietary fish oils or algae after microbial adaptation (Chilliard *et al.*, 2001b).

Although the diet of the grazing ruminant is relatively high in unsaturated fatty acids, ruminant milk and meat products have relatively low PUFA contents as a result of biohydrogenation (Gulati *et al.*, 1999; Kitessa *et al.*, 2001c). To significantly increase the long-chain PUFA composition of digesta at the duodenum and promote the absorption of the fatty acids in their original form, it is therefore necessary to protect them from ruminal biohydrogenation (Kennelly, 1996). The development of ruminally inert lipids makes the inclusion of high concentrations of unsaturated fatty acids in ruminant diets feasible without affecting either the microbial ecosystem or the process of ruminal fermentation (Wu *et al.*, 1991; Börsting *et al.*, 1992; Kitessa *et al.*, 2001c). Methods of lipid protection include encapsulating the lipid within a protein carrier protected from degradation (Ashes *et al.*, 1992); saponifying the fatty acids within a calcium soap (Kennelly, 1996); crystallisation of dietary lipids (Chilliard, 1993) or adsorbing fatty acids onto a carrier material such as vermiculite (Cooper *et al.*, 2002). Currently, the most effective method of protecting fatty acids against ruminal biohydrogenation is the encapsulation of oils within a formaldehyde-treated protein coat (Doreau and Chilliard, 1997a). The protein passes through the rumen unchanged, thereby protecting the lipid from biohydrogenation, but is digested in the abomasum and presents the unsaturated fatty acids for absorption within the small intestine (Doreau and Chilliard, 1997a).

1.2.4.3. Disappearance of fatty acids from the rumen

A proportion of ingested short-chain fatty acids have been shown to be lost between digesta entering the rumen and arriving at the small intestine (Wu and Palmquist, 1991). Some disappearance of long-chain PUFAs has also been reported by Wu *et al.* (1991), the

rate of this loss accelerating with increasing dietary fat intake (Doreau and Ferlay, 1994). Evidence suggests that short-chain fatty acids may be degraded to volatile fatty acids within the rumen, however Jenkins (1993) concluded that long-chain fatty acids were unaffected by this mechanism. It is interesting to note that protecting fatty acids from rumen degradation does not affect the rate of fatty acid disappearance in the rumen (Doreau and Ferley, 1994). Digesta leaving the rumen commonly contains approximately 100-200 mg lipid/g DM; including 2-20 mg PUFAs, 5-15 mg *trans*-18:1 fatty acids and 15-30 mg microbial lipids per 100 mg fatty acids (Doreau *et al.*, 1997; Demeyer and Doreau, 1999).

1.2.4.4. Abomasal fatty acid digestion

The fatty acid composition of digesta remains relatively constant after passage through the omasum and abomasum, the only notable change being the release of fatty acids from bacterial and protozoal cells (Moore and Christie, 1984). Fats saponified with calcium salts may also disassociate in the abomasum, releasing free fatty acids (Doreau and Ferlay, 1994). Consequently, a significant increase in the proportion of unsaturated fatty acids in digesta is exhibited when animals are fed saponified fat sources (Moore and Christie, 1984).

1.2.4.5. Intestinal fatty acid digestion

The lipid component of digesta entering the small intestine at the duodenum contains a high proportion of unesterified saturated fatty acids (Kennelly, 1996) with a variable proportion of unsaturated fatty acids originating from microbial cell contents and protected fats (Moore and Christie, 1984). These fatty acids are adsorbed onto feed particles (Bauchart, 1993), endothelial cells and microbial cells within digesta (Doreau and Ferlay, 1994). The small intestine is the principal site of fatty acid absorption in ruminants

(Jenkins, 1993). The following equation was derived by Doreau and Chilliard (1997a) predicting the relationship between duodenal fatty acid flow and fatty acid intake.

$$FAD = 0.801\ FAI + 9.29$$

FAD = Fatty acid flow at duodenum (g/kg DM intake) and FAI = Fatty acid intake (g/kg DM intake).

When fatty acid intakes exceed 47 g/kg DM intake, the duodenal flow of fatty acids is lower than the dietary fatty acid intake. Low-fat diets therefore enhance duodenal fatty acid flow relative to dietary intake (Demeyer and Doreau, 1999). The proportion of unsaturated fatty acids in duodenal digesta may further be increased by the activity of intestinal desaturase enzymes (Enser, 1984). This was demonstrated by Kennelly (1996), who reported that the ratio of saturated to unsaturated C18 fatty acids was lower in intestinal digesta than in rumen liquor. Moreover, pancreatic lipase enzymes and bile salts confer a degree of hydrolytic activity within the small intestine (Börgstrom, 1977; Moore and Christie, 1984). Phosphatidylglycerols may also be degraded in the jejunum (Börgstrom, 1977), with specific enzymes hydrolysing the ester bonds at position one to release saturated fatty acids and at position two to release unsaturated fatty acids (Moore and Christie, 1984).

As fatty acids pass through the small intestine, they are transformed from an insoluble particulate phase to a soluble micellar form (Bauchart, 1993; Doreau and Ferlay, 1994). The presence of bile and pancreatic acids are essential for optimal fatty acid absorption (Moore and Christie, 1984). Bile salts facilitate the interaction between fatty acids, bile phosphatidylglycerols and water, forming a crystalline lipid solution (Bauchart, 1993). Moreover, they inhibit further lipid hydrolysis by preventing lipase enzymes from bin

to their substrate (Börgstrom, 1977). Fatty acids are converted into type III amphiphiles, polar molecules containing a hydrophilic and hydrophobic component (Brindley, 1984). The hydrophobic sections of the amphiphiles cluster together, forming a circular or oblong molecule with a hydrophilic surface: a micelle (Brindley, 1984). Fatty acids are thus absorbed within a micellar emulsification throughout the jejunum (Doreau and Chilliard, 1997a; Demeyer and Doreau, 1999).

1.2.4.6. Intestinal fatty acid absorption

Approximately 20 % of total fatty acids are absorbed in the upper jejunum into muscosal cells (Bauchart, 1993). Lipid compounds absorbed here principally consist of dietary unesterified saturated fatty acids and phosphatidylcholine (Moore and Christie, 1984). A mixture of dietary unesterified saturated fatty acids, bile acids, phosphatidylcholine, lysophosphatidylcholine and endogenous unsaturated fatty acids comprising 60 % of total lipid digesta is then absorbed in the middle and lower jejunum, and the remainder absorbed before the digesta reaches the ileum (Moore and Christie, 1984; Bauchart, 1993).

Ruminants fed significant quantities of unsaturated fatty acids via "protected" lipid supplements exhibit intestinal lipid digestion similar to that of the monogastric (Enser, 1984). Moore and Christie (1984) report that the digestive disperal of fatty acids among feed particles in the ruminant leads to a more efficient lipid absorption when compared to the monogastric. This may also confer increased solubility of fatty acids in conjunction with bile salts and the promotion of micelle formation by the acidic environment of the duodenum and jejunum (Bauchart, 1993).

1.2.4.7. Fatty acid digestibility

Digestibility is influenced by the fatty acid composition (Börsting *et al.*, 1992) and physical characteristics of the diet (Bauchart, 1993) and also by the efficiency of ruminal

biohydrogenation (Chilliard, 1993). Wu *et al.* (1991) suggested that true fatty acid digestibility may increase with moderate fat intake, but decrease with further augmentation of the dietary lipid content. Furthermore, Bauchart (1993) hypothesised that a ceiling might exist for the efficiency of pancreatic lipase and bile salts in the ruminant, which would limit digestion in animals fed fat-supplemented diets.

Fatty acid digestibility has been shown to decrease with increasing chain length (Doreau and Chilliard, 1997a). This may have a significant impact on the efficacy of diets that increase the dietary long-chain PUFA supply in an attempt to increase their concentrations in tissues (Börsting *et al.*, 1992). Börsting *et al.* (1992) stated that C16:0 has a higher digestibility than C18:0, by contrast, experimental work by Doreau and Chilliard (1997a) suggested that no differences in saturated fatty acid digestibility exist with increasing chain length or intake. Fatty acid digestibility has been suggested by Wu *et al.* (1991) to increase with unsaturation, an unsaturated octadecanoic acid being more digestible than a saturated acid. This is partly due to the greater hydrophobicity of unsaturated fatty acids which interact at a higher level with bile salts and enhance micelle formation with subsequent increases in absorption efficiency (Wu *et al.*, 1991).

1.2.4.8. Fatty acid absorption within the large intestine

Doreau and Ferlay (1994) report that the extent of fatty acid absorption in the ruminant large intestine is relatively insignificant although a degree of microbial fatty acid synthesis occurs and therefore the faecal fatty acid content is higher than that of the digesta. If the ruminal digestion of fibrous feedstuffs is disrupted by long-chain PUFA supplementation of the ruminant, digestion is shifted to the large intestine thus increasing the proportion of ingested fatty acids eliminated in faeces (Doreau and Ferlay, 1994).

1.2.4.9. Cellular fatty acid metabolism

Unesterified fatty acids and lysophosphatidylglycerols penetrate the rumen enterocyte via the microvilli (Bauchart, 1993) and are transported from the cell surface to the smooth endoplasmic reticulum by a fatty acid binding protein (Campbell *et al.*, 1998). Acyl-co-synthetase then enzymatically converts the fatty acids to their CoA derivatives, the reaction being catalysed by CoA and ATP (Brindley, 1984; Moore and Christie, 1984):

A desaturase enzyme is also present within intestinal mucosa which converts approximately 10 % of total C18:0 to C18:1 (Enser, 1984; Moore and Christie, 1984; Chilliard, 1993). The fatty acid CoA derivatives are esterified and react with mono- and diacylglycerol molecules to form di- and triacylglycerols via transferase enzymes (Conn *et al.*, 1987). Phosphatidylglycerols may also be formed by acylation of lysophospholipids (Moore and Christie, 1984). These lipid compounds are deposited as lipoproteins into lipid vesicles by smooth endoplasmic reticulum (Brindley, 1984). Within each lipoprotein molecule, amphiphilic molecules surround the lipid component thus permitting transport within the aqueous medium (Brindley, 1984). Lipoproteins consist of a central hydrophobic core containing high proportions of triacylglycerols and cholesterol esters and a hydrophilic surface layer containing phosphatidylglycerols, apoproteins and cholesterol (Moore and Christie, 1984; Bauchart, 1993).

Five types of lipoproteins are synthesised, classified according to their lipid:protein ratio: chylomicrons, very low density lipoproteins (VLDL), intermediate density lipoproteins (IDL), low density lipoproteins (LDL) and high density lipoproteins (HDL; Bauchart, 1993). The two predominant lipoproteins synthesised post-absorption are the chylomicrons and VLDL (Christie, 1978). A continuous range of lipoprotein sizes from chylomicrons to VLDL exists in the sheep, their principal role being to transport fatty acids from plasma to tissues (Clegg *et al.*, 2001). The size and type of lipoprotein produced is

dependent on the rate of synthesis and the fatty acid composition of digesta (Brindley, 1984).

Lipoproteins are transported in golgi vesicles to the cell surface and exocytosed into the intercellular space (Brindley, 1984; Moore and Christie, 1984). Chylomicrons and VLDL migrate to the lymphatic lacteals and into the blood via the thoracic and intestinal lymph ducts (Conn *et al.*, 1987; Doreau and Chilliard, 1997a). This is the principal route for the entry of long-chain esterified fatty acids, however, short-chain non-esterified fatty acids and some long-chain fatty acids bind to albumin in the portal blood (Brindley, 1984; Chilliard, 1993; Enjalbert, 1995).

Within plasma, lipoproteins acquire the apoproteins apo-E and apo-C from HDL in the liver (Moore and Christie, 1984). These molecules divert fatty acid catabolism away from the liver towards metabolism sites including muscle, adipose tissue and the mammary gland (Moore and Christie, 1984). As the lipoproteins are transported through these sites they are bound onto capillary endothelium by lipoprotein lipase (LPL; Brindley, 1984). This, the rate-limiting step of core triacylglycerol hydrolysis, produces fatty acids, IDL and acylglycerols (Bauchart, 1993; Clegg *et al.*, 2001). Fatty acids in the *sn*-1 position are preferentially hydrolysed by LPL, hence the high concentration of C16 and C18 fatty acids (palmitate, stearate, oleate) in adipose and mammary lipids (Clegg *et al.*, 2001). Fatty acids then diffuse through the cells underlying the capillary wall and are metabolised within the tissue (Brinkley, 1984; Moore and Christie, 1984). If the rate of hydrolysis exceeds the cellular capacity for diffusion, LPL is inhibited and the chylomicron is released, this may happen several times until the chylomicron remnant can no longer be hydrolysed efficiently and is metabolised by the liver (Brindley, 1984).

The site at which chylomicrons and VLDL are metabolised is dependent on the nutritional status of the animal. Under normal isocalorific conditions, fatty acids are diverted to adipose tissue for storage, by contrast, during starvation they are utilised by skeletal muscles, cardiac muscle and liver as an energy source (Conn *et al.*, 1987). Under the control of the hormone prolactin, the mammary gland is the primary site of fatty acid metabolism for milk lipid synthesis (Conn *et al.*, 1987). The activity of LPL in adipose and mammary tissues is regulated by plasma insulin; high concentrations of insulin stimulate LPL activity and *vice versa* (Enser, 1984; Barber *et al.*, 1997).

1.2.4.10. Fatty acid metabolism within adipose tissue

Fatty acids are principally stored as triacylglycerols within subcutaneous, intramuscular, intermuscular and abdominal adipose tissue in the ruminant (Demeyer and Doreau, 1999). A small proportion of fatty acids are also stored as phosphatidylglycerols within intramuscular lipids (Demeyer and Doreau, 1999). The potential lipid storage capacity is dependent upon the age and breed of the sheep (Demeyer and Doreau, 1999). For example, the fat content of the carcass increases with age, and is lower in breeds selected for lean, muscular carcasses (e.g. Texel sheep) compared to those selected for hardiness (e.g. Welsh Mountain sheep; Carson *et al.*, 1999). Furthermore, individual muscles differ in their fatty acid composition. Enser *et al.* (1998a) reported increased concentrations of unsaturated fatty acids in muscle high in phosphatidylglycerols, i.e., red oxidative muscle fibres compared to white muscle fibres. Despite having significant effects on liveweight gain and carcass composition (Nürnberg *et al.*, 1998), sex appears to have little effect on intramuscular fatty acid composition (Enser *et al.*, 1998a).

Fatty acid storage in adipose tissue is controlled by a balance of uptake, synthesis, esterification, lipolysis and re-esterification (Chilliard, 1993). Post-hydrolysis by LPL, fatty acids are absorbed into adipose cells, converted to triacylglycerols (Demeyer and

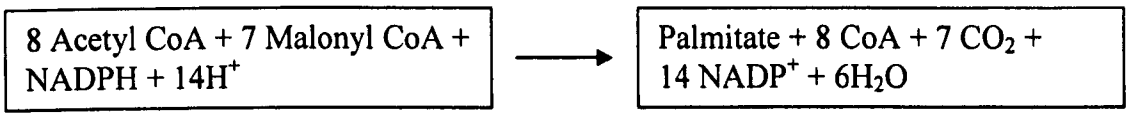
Doreau, 1999) and stored within large triacylglycerol droplets surrounded by cytoplasm (Conn *et al.*, 1987). Saturated fatty acids account for a major proportion of stored lipid in adipose tissue, the predominant fatty acids being C16:0, C18:0 and *cis* C18:1, with smaller quantities of C14:0, *cis* C16:1, C17:0 and *trans* C18:1 (Chilliard *et al.*, 2001a). Adipose and muscle lipids are predominantly saturated due to biohydrogenation (Demeyer and Doreau, 1999). However, as the fat content of the carcass increases, the concentration of monoenoic fatty acids in tissues is augmented as a consequence of Δ-9 desaturase activity producing C18:1*n*-9 from C18:0 (Enser *et al.*, 1998b; Velasco *et al.*, 2001).

Under normal dietary conditions, ruminant adipose tissues do not contain considerable quantities of C22:6*n*-3, although C18:3*n*-3 is found in relatively high concentrations (Poumes-Ballihaut *et al.*, 2001). Instead, PUFAs are found in high quantities in the phosphatidylglycerols, the fatty acid composition reflecting their role in the maintenance of cell membrane fluidity (Curtis-Prior, 1988). The minor amounts of long-chain *n*-3 PUFAs stored in adipose tissue are suggested to act as a reservoir for release during sub-optimal supply (Poumes-Ballihaut *et al.*, 2001). Nevertheless, the extent to which *n*-3 fatty acids may be mobilised from adipose tissue is yet to be confirmed.

Catabolism of adipose tissue involves the hydrolysis of triacylglycerols by hormone-sensitive lipase (HSL) to produce free fatty acids and glycerol (Conn *et al.*, 1987; Demeyer and Doreau, 1999). Regulated by adenaline and glucagon (stimulatory) or insulin (inhibitory; Demeyer and Doreau, 1999; Raclot *et al.*, 2001), the release of fatty acids by HSL is thought to be the rate-limiting step. This mobilisation is selective for fatty acid chain length, being reduced by increasing chain length at a specific level of unsaturation (Raclot *et al.*, 2001). Non-esterified fatty acids combine with albumin within the blood, forming stable, soluble fatty acid-albumin complexes (Conn *et al.*, 1987). These complexes are metabolised in the liver (Conn *et al.*, 1987) or transported to tissues as a

source of oxidative energy (Demeyer and Doreau, 1999). Short-term feed deprivation leads to the non-specific mobilisation of fatty acids from triacylglycerols; however, after prolonged starvation, fatty acids may be selectively mobilised according to cellular requirements (Enser, 1984).

Adipose tissue is the primary site of *de novo* fatty acid synthesis in non-lactating ruminants (Christie, 1978; Demeyer and Doreau, 1999) as hepatic synthesis is negligible due to low acetyl-CoA carboxylase activity (Kennelly and Glimm, 1998). Fatty acids are produced within the adipose cell via the following reaction (Enser, 1984):



Acetate is the predominant fatty acid precursor via *de novo* synthesis in the ruminant (Conn *et al.*, 1987; Clegg *et al.*, 2001). Malonyl CoA undergoes a condensation reaction catalysed by the enzyme β-ketoacyl synthetase to form fatty acids and carbon dioxide, (Enser, 1984). The cellular enzyme system reduces both the ketoacyl group and double bond, and dehydrogenates the hydroxyacyl group to produce a saturated fatty acid, two carbons longer than the original precursor (Enser, 1984). This sequence is repeated using malonyl CoA until the required fatty acid is synthesised. Odd-numbered fatty acids may be produced when a three-carbon propionate precursor is substituted for acetate (Enser, 1984).

De novo fatty acid synthesis within adipose tissue is regulated by metabolic, hormonal and dietary effects on acetyl-CoA carboxylase activity (Enser, 1984). Synthesis is inhibited during lipolysis and starvation and increased during replenishment of dietary precursors

(Enser, 1984). Acetyl CoA activity in adipose tissue is reduced during lactation with fatty acids being partitioned towards mammary tissues (Clegg *et al.*, 2001).

1.2.4.11. Metabolism of fatty acids within mammary tissue

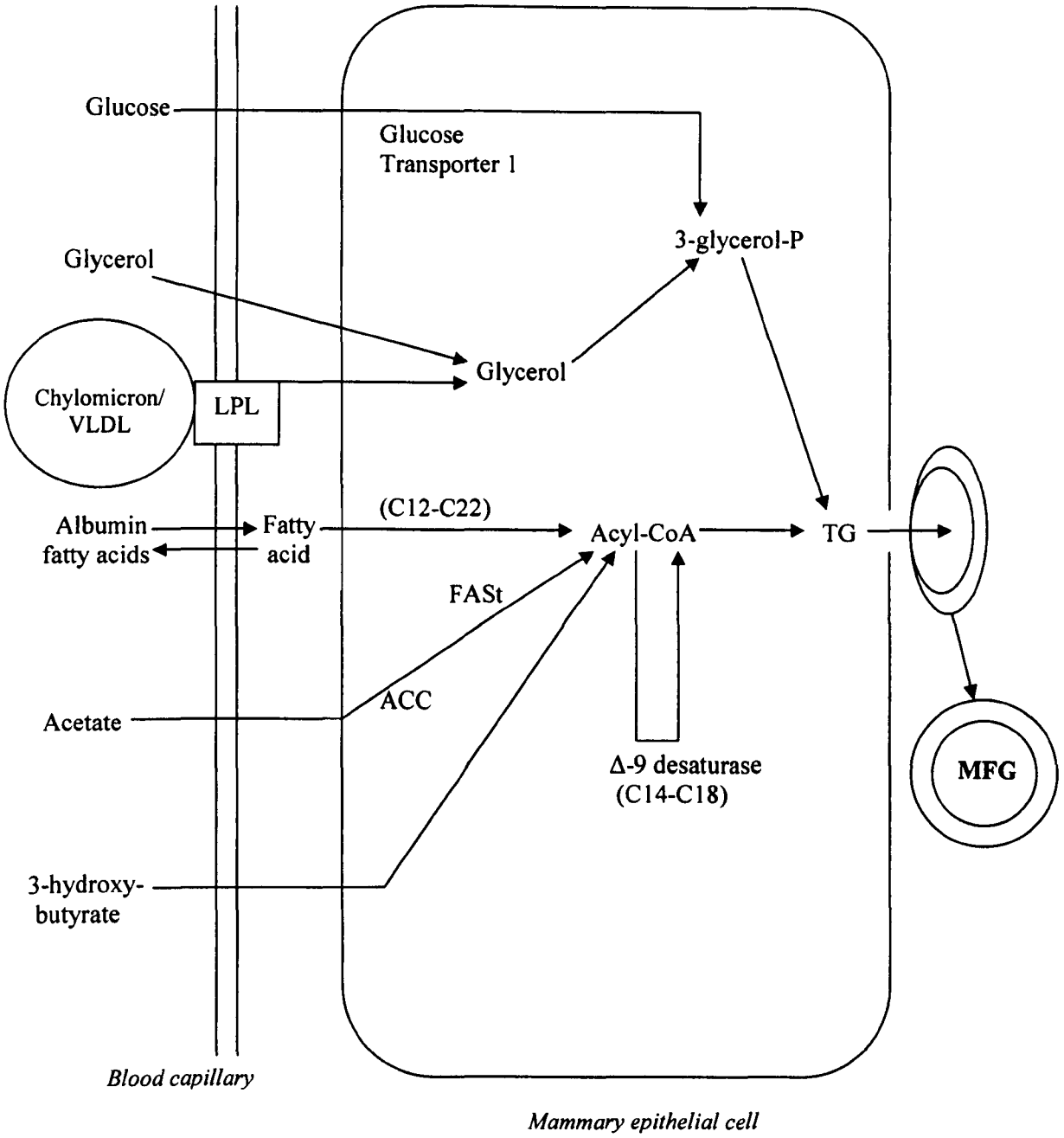
Triacylglycerols are the principal lipid compounds contained within milk fat, the remainder consisting of diacylglycerols, phosphatidylglycerols, unesterified fatty acids, cholesterol and cholesterol esters (Barber *et al.*, 1997; Bauman and Griinari, 2001). The fatty acid composition of milk lipids is dependent on the energy status of the animal and the balance of fatty acids supplied from endogenous and exogenous sources (Kennelly, 1996; Wijesundera *et al.*, 2001). Short and medium-chain fatty acids make up approximately 500 g/kg of total milk fatty acids, with long-chain fatty acids accounting for 450 g/kg (predominantly C18:0 and C18:1) and PUFAs for 20-30 g/kg (Demeyer and Doreau, 1999). *De novo* synthesis accounts for almost all short-chain, and half of medium-chain fatty acids secreted into milk lipids (Demeyer and Doreau, 1999).

During early lactation, ruminants are habitually in a negative energy balance (Potański *et al.*, 2001) and milk fat production is maintained by the lipolysis of adipose tissue from body reserves (Barber *et al.*, 1997). Consequently, the activity of LPL and fatty acid synthase within adipose tissue are significantly reduced during early lactation. As lactation progresses, the reliance on stored fatty acids declines and endogenous fatty acid synthesis in adipose tissue increases, restoring lipid reserves (Barber *et al.*, 1997).

Short and medium-chain fatty acids (C4-C16) are synthesised in the mammary epithelial cells via a modification of the fatty acid synthase system (Barber *et al.*, 1997; Figure 1.5). Acetyl and butyryl CoA are converted to malonyl CoA by acetyl-CoA carboxylase (Chilliard *et al.*, 2000; Wright *et al.*, 2002) via a condensation reaction with the elimination of carbon dioxide as in adipose tissue (Enser, 1984; Barber *et al.*, 1997). This is then

reduced via β-carbon to produce a saturated acyl chain, extended by 2 carbons (Barber *et al.*, 1997). A further six condensation and reduction reactions result in the formation of C16:0 (Barber *et al.*, 1997). In lactating animals, the acyl chain may be terminated at 8-10 carbons by a CoA ester removal system and these fatty acids added to the developing milk fat globule (Barber *et al.*, 1997). *De novo* synthesis is terminated in a significant proportion of the fatty acids produced in mammary tissue, hence the high short and medium-chain fatty acid concentration of milk fat (Enser, 1984).

Most endogenously synthesised fatty acids are saturated as the Δ-9 desaturase enzyme has little specificity for fatty acids with less than 18 carbons in the acyl chain (Chilliard *et al.*, 2000). A significant proportion of the saturated fats supplied to the mammary gland are desaturated to monoenoic fatty acids via Δ-9 desaturase (Palmquist, 1984), particularly when *de novo* synthesis is inhibited (Agenas *et al.*, 2002). This enzyme is particularly active in the conversion of C18:0 to C18:1*n*-9 and of *trans*-vaccenic acid to *cis*-9, *trans*-11 CLA (Chilliard *et al.*, 2001a). The mechanisms which regulate Δ-9 desaturase activity are, as yet unknown. Agenas *et al.* (2002) suggested that the activity of this enzyme may be a means by which the fluidity of milk fat is maintained, hence its inhibition by long-chain PUFAs which directly contribute to membrane fluidity. *De novo* synthesis of fatty acids within the mammary gland is most significantly inhibited by dietary PUFAs, followed by monounsaturated and saturated fatty acids (Barber *et al.*, 1997). Consequently, the proportions of various fatty acids in milk fat reflect the varying contributions of endogenous *de novo* synthesis and exogenous dietary supply (Barber *et al.*, 1997; Agenas *et al.*, 2002). The deposition of exogenous fatty acids into milk fat is dependent upon their supply from lipoproteins and consequent hydrolysis by LPL.



ACC = Acetyl-CoA carboxylase
FASt = Fatty acid synthase
LPL = Lipoprotein lipase
MFG = Milk fat globule
TG = Triacylglycerol
VLDL = Very low density lipoprotein

Figure 1.5. Milk fat synthesis and secretion (Chilliard et al., 2000)

Triacylglycerols are formed within mammary tissue by the action of transacylase enzymes upon glycerol and fatty acids (Enser, 1984) with specific fatty acids are esterified onto the three different *sn* positions of glycerol. Short and medium-chain fatty acids found in milk are esterified at positions *sn*-1 and *sn*-3 (Christie, 1978). *Cis* and *trans* isomers of individual fatty acids are also found at positions *sn*-2 and *sn*-3 respectively on the triacylglycerol (Demeyer and Doreau, 1999). Thus the rate of triacylglycerol synthesis is partly regulated by the ability of the exogenous fatty acid supply to provide long-chain fatty acids suitable for esterification at position *sn*-1 (Demeyer and Doreau, 1999). Triacylglycerols and phosphatidylglycerols formed within the mammary gland are contained within a membrane and exocytosed from the surface of alveolar epithelial cells to the minor and major ducts leading to the teat cistern (Christie, 1978). Milk lipid contains small droplets ranging in size from 0.1-20μm which consist of a triacylglycerol core surrounded by a plasma membrane (Briard *et al.*, 2003). The small quantities of phosphatidylglycerols found in milk are situated within this membrane (Christie, 1978).

1.2.5. Fatty acid synthesis via desaturation and elongation

The EFAs C18:2*n*-6 and C18:3*n*-3 are the precursors for endogenous synthesis of the long-chain fatty acids C20:4*n*-6, C20:5*n*-3 and C22:6*n*-3 (Hornstra *et al.*, 1995; Marin and Alaniz, 1998; Hempenius *et al.*, 2000). Synthesis of long-chain PUFAs ensures that their neural and visual functions are maintained in the animal, even under conditions of suboptimal dietary supply (Enser, 1984). It has been postulated that this mechanism evolved in humans due to a change from a diet rich in *n*-3 and *n*-6 PUFAs in which there was no need for endogenous synthesis; to one rich in saturated fats with negligible *n*-3 and *n*-6 fatty acid intakes (Hornstra *et al.*, 1995). However, this hypothesis does not fully explain the existence of the system in other animals, specifically ruminants.

1.2.5.1. Mechanisms of desaturation and elongation of fatty acids

The mechanisms by which EFAs are desaturated and elongated into C20:4*n*-6, C20:5*n*-3 and C22:6*n*-3 within cells are presented in Figures 1.6 and 1.7. In *n*-6 fatty acids, C18:2*n*-6 is desaturated to form C18:3*n*-6, then elongated to form C20:3*n*-6; before further desaturation to produce C20:4*n*-6 (Sprecher, 2000). Desaturation is achieved by single desaturase enzymes specific to a position on the acyl chain, however, the elongase system is more complex and involves a group of four enzymes (β-ketoacyl CoA synthase, β-ketoacyl CoA reductase, β-hydroxyacyl CoA dehydrase and *trans*-2-enoyl CoA reductase; Leonard *et al.* 2004).

Fatty acids of the *n*-3 series have a similar progression from C18:3*n*-3 to C20:5*n*-3 and an extra elongation and desaturation step to form C22:6*n*-3. Although it was originally suggested that C22:6*n*-3 was formed from C20:5*n*-3 via a Δ-4 desaturase, the existence of the Δ-4 desaturase enzyme has not been verified (Ferdinandusse *et al.*, 2001). Sprecher (2000) proposed pathways for two enzyme steps and a β-oxidation reduction within the peroxisome by which C22:6*n*-3 may be formed from C20:5*n*-3. Further supporting evidence for this pathway was found in humans suffering from Zellweger syndrome who have impaired peroxisome function (Ferdinandusse *et al.*, 2001). These humans metabolised C18:3*n*-3 to C24:5*n*-3 and C24:6*n*-3 but were unable to perform successful retroconversion of C24:6*n*-3 to C22:6*n*-3. By contrast, healthy humans were able to produce C22:6*n*-3 from labelled C24:5*n*-3 and C24:6*n*-3. During the β-oxidation step proposed by Sprecher (2000), long-chain PUFAs are oxidised by straight-chain acyl-CoA oxidase. They are then hydrated and dehydrogenated by D-bifunctional protein and thiolytically cleaved by 3-ketoacyl-CoA thiolase and sterol carrier protein X (Ferdinandusse *et al.*, 2001).

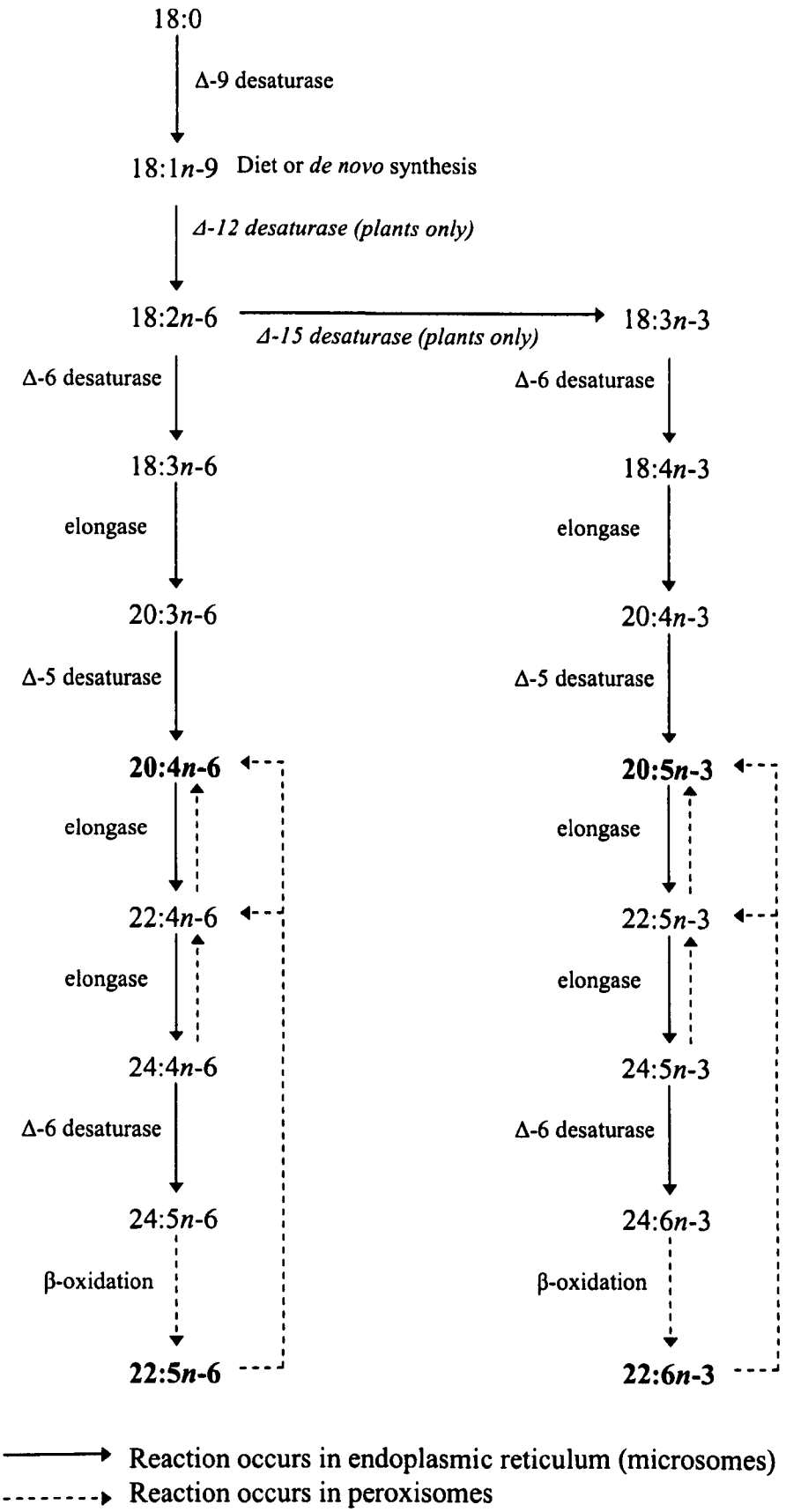


Figure 1.6. Mechanisms of fatty acid desaturation and elongation (Sprecher, 2000; Broadhurst et al., 2002; Leonard et al., 2004)

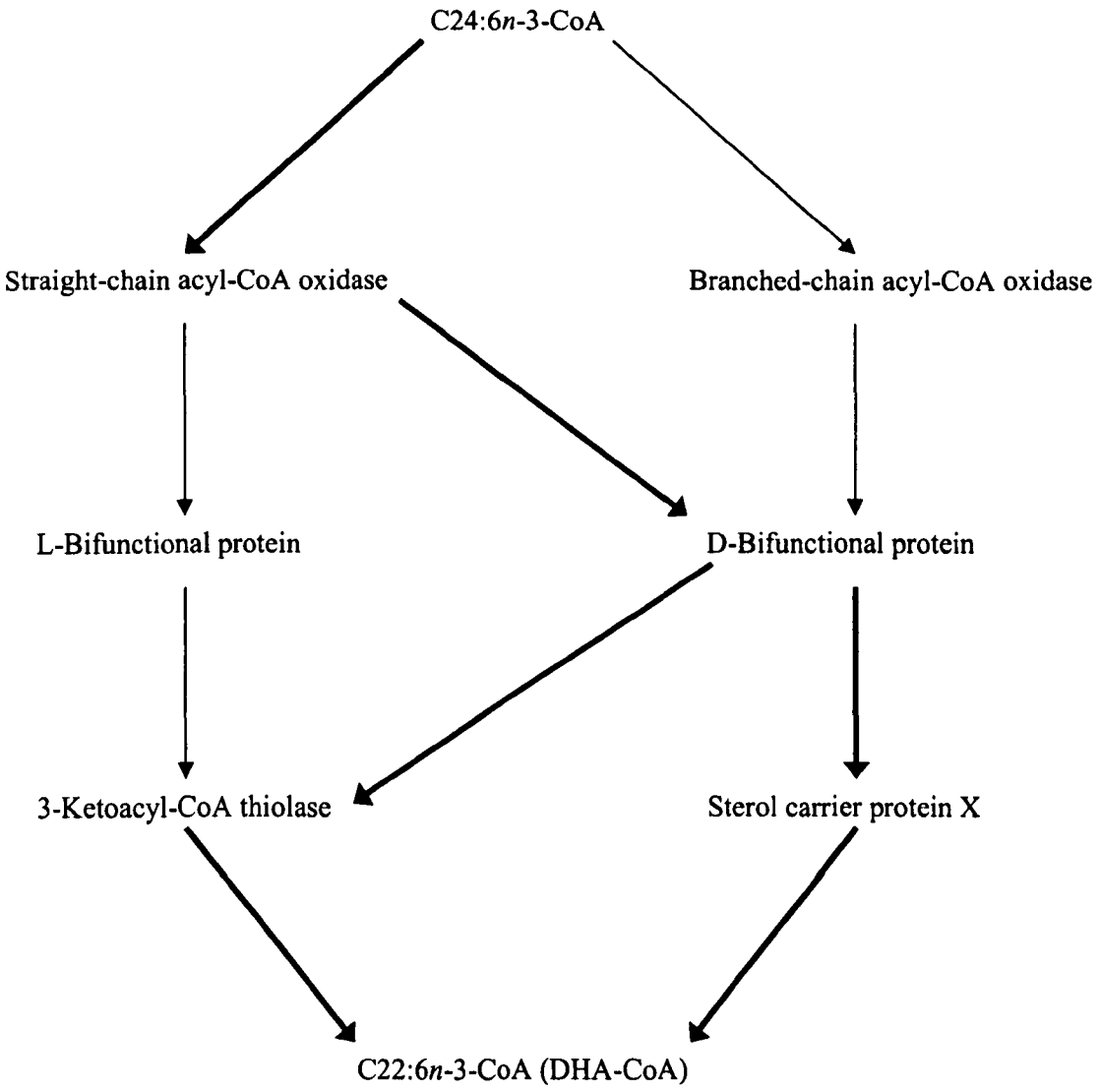


Figure 1.7. Beta-oxidation of fatty acids (Ferdinandusse et al., 2001)

1.2.5.2. Factors affecting the efficiency of desaturation and elongation

The synthesis of long-chain PUFAs from precursor EFAs is dependent on the relative specificities of the desaturase enzymes (Δ-4, Δ-5, and Δ-6) and upon competition between individual fatty acids for these enzymes (Enser, 1984). The mechanisms for elongation of C18:3*n*-3 to C22:6*n*-3 and C20:5*n*-3 and C18:2*n*-6 to C20:4*n*-6 involve two common steps: desaturation by Δ-6 desaturase and Δ-5 desaturase enzymes.

Desaturation by Δ-6 desaturase is thought to be the rate-limiting step where C18:1*n*-9, C18:2*n*-6 and C18:3*n*-3 compete for the binding site in the microsomal enzyme system (Williard *et al.*, 2001). This enzyme has the highest specificity for C18:3*n*-3, followed by C18:2*n*-6 and C18:1*n*-9 (Koletzko, 1992). Consequently, increased cellular concentrations of long-chain *n*-3 compared to *n*-6 fatty acids are exhibited after desaturation and elongation of equivalent concentrations of their precursor fatty acids (Calder, 2001). This specificity also explains the high plasma concentrations of C20:3*n*-9 produced from desaturation of C18:1*n*-9 in the absence of C18:2*n*-6 and C18:3*n*-3 (Koletzko, 1992; Leifert *et al*, 1999).

Sprecher (2000) hypothesised that separate Δ-6 desaturase enzymes exist which are specific for fatty acids of different chain lengths, but their existence has not yet been proven. If the Δ-6 desaturase is non-specific for chain-length, it has important consequences for the elongation and desaturation of both *n*-3 and *n*-6 fatty acids, as five fatty acids (C18:0, C18:2*n*-6, C18:3*n*-3, C24:5*n*-6 and C24:6*n*-3) compete for the enzyme's binding site (Sprecher, 2000).

1.2.5.3. Inhibition of the desaturation and elongation of fatty acids

High concentrations of C18:2*n*-6, C18:3*n*-3 and their long-chain PUFA derivatives within tissues inhibit the synthesis of long-chain PUFAs by Δ-desaturase enzymes (Sargent, 1997;

Bougle *et al.*, 1999; Poumes-Ballihaut *et al.*, 2001). Consequently, desaturation and elongation of C18:3*n*-3 to C22:6*n*-3 is relatively low in humans, as the high C18:2*n*-6 intake negates the specificity of the Δ-6 desaturase enzyme for C18:3*n*-3 (Voigt and Hagemeister, 2001). The ratios of C20:3*n*-6:C18:2*n*-6 and C20:4*n*-6:C20:3*n*-6 provide indications of the efficiency of Δ-5 and Δ-6 desaturase activity. A decrease in either ratio suggests inhibition of the respective enzyme (Poumes-Ballihaut *et al.*, 2001). Within the *n*-6 pathway, the formation of C20:3*n*-6 is limited by Δ-6 desaturase, however, once the mechanism has progressed past this stage, a significant proportion of C20:3*n*-6 is converted to C20:4*n*-6 (Uauy *et al.*, 2000). Desaturation and elongation of γ-linolenic acid (C18:3*n*-6) from borage oils may also produce C20:4*n*-6, a mechanism that is not as susceptible to inhibition via Δ-6 desaturase. However, Makrides *et al.* (1995) demonstrated that supplementing infants with preformed C18:3*n*-6 and C22:6*n*-3 did not result in an appreciable increase in C20:4*n*-6 concentrations.

1.2.5.4. Desaturation and elongation of fatty acids within neural tissue

The liver, brain and placenta have been suggested to be significant areas of endogenous synthesis of C22:6*n*-3 in humans, rats and ruminants (Noble *et al.*, 1985; Koletzko, 1992; Williard *et al.*, 2001). Hepatic desaturation and elongation is beyond the scope of this review and placental desaturation is discussed in section 1.2.6.3.2., therefore, this section concentrates on neural synthesis.

Williard *et al.* (2001) reported that C22:6*n*-3 may be synthesised in the brain from C18:3*n*-3. The synthesis of C22:6*n*-3 demonstrably increases during long-chain PUFA deficiency, suggesting that a mechanism exists to maintain the concentration of this fatty acid in neural tissue and that this mechanism is regulated by dietary supply. This synthesis occurs within astrocytes, which incorporate C22:6*n*-3 into cell lipids and release it as a free fatty acid (Williard *et al.*, 2001). The amount of C22:6*n*-3 produced within astrocytes is dependent

on preformed dietary supply (Williard *et al.*, 2001) as the brain and retina appear to have a preferential uptake mechanism for preformed C22:6*n*-3.

1.2.5.4.1. Evidence of *de novo* synthesis of long-chain PUFAs in humans

Uauy *et al.* (2000) demonstrated that human infants are able to desaturate and elongate EFAs to their long-chain PUFA derivatives. However, Koletzo *et al.* (1996) reported that infants were unable to synthesise C20:4*n*-6 or C22:6*n*-3 in quantities similar to those supplied preformed by maternal milk. Several studies have demonstrated that feeding dietary formulae enriched with EFAs or with a high C18:3*n*-3:C18:2*n*-6 ratio do not increase the concentrations of C22:6*n*-3 in liver and brain phosphatidylglycerols to those found in breast-fed infants (Poumes-Ballihaut *et al.*, 2001). Furthermore, research in bottle-fed human infants has shown that a preformed C22:6*n*-3 dietary supply is significantly more effective at maintaining plasma phosphatidylglycerol C22:6*n*-3 concentrations than increasing the concentration of C18:3*n*-3 in the diet (Goustard-Langelier *et al.*, 1999). Similar findings have led to the labelling of long-chain PUFAs as conditionally essential, in that they can be synthesised endogenously from their precursor fatty acids, but the synthesis is ineffective at meeting requirements (Uauy *et al.*, 2000).

1.2.5.4.2. Evidence of *de novo* synthesis of long-chain PUFAs in animals

Arbuckle and Innis (1992) fed neonatal piglets with sows' milk or one of two fatty acid supplemented formulae: a low-C18:2*n*-6, high-C18:3*n*-3 or high-C18:2*n*-6, low-18:3*n*-3 formula, and reported similar concentrations of C22:6*n*-3 in brains and retinas from the milk and high-C18:3*n*-3 formula-fed animals. However, the synthesis of C22:6*n*-3 from C18:3*n*-3 within brain tissue was reported by Arbuckle and Innis (1992) to be only 24 % as effective as supplying preformed dietary C22:6*n*-3 to pigs. A significant amount of C22:5*n*-6 was found in nervous tissues from piglets fed the low-C18:3*n*-3 diet whilst low C22:6*n*-3 concentrations were also reported, suggesting that this level of dietary C18:3*n*-3

was insufficient for C22:6*n*-3 deposition. The low ratio of C22:6*n*-3 to C22:5*n*-3 in these animals also suggested *n*-3 fatty acid deficiency (Arbuckle and Innis, 1992).

Furthermore, Su *et al.* (1999) reported a bioequivalence of 7:1 for C18:3*n*-3 compared to preformed C22:6*n*-3 for brain, and 12:1 for retinal tissue. Inhibition of C22:6*n*-3 synthesis via competition for Δ-desaturase enzymes may also have significant effects on long-chain PUFA accretion in the brain: Craig-Schmidt *et al.* (1996) suggested that a dietary ratio of 9:1 C18:3*n*-3:C18:2*n*-6 plus 1 % preformed C22:6*n*-3 may be sufficient for optimal deposition of C22:6*n*-3 in piglet nervous tissue.

1.2.6. Ruminant requirements for long-chain PUFAs

The ruminant dietary requirements for EFAs and long-chain PUFAs have not been investigated in any significant depth. It was previously assumed that the long-chain PUFA requirements of humans and animals were met by synthesis from EFA precursors. However, research into the requirements of neonatal animals, specifically humans, has shown that they may require a source of exogenous long-chain fatty acids for optimal brain and nervous system development (Su *et al.*, 1999; Ikemoto *et al.*, 2001; Broadhurst *et al.*, 2002).

These findings may be extrapolated to other neonatal animals that have similar characteristics, i.e. insufficient production of long-chain PUFAs from precursors and an absence of preformed PUFAs in the diet (Craig-Schmidt, 1996; Bondia-Martinez *et al.*, 1998; Poumes-Ballihaut *et al.*, 2001). Adequate synthesis from precursor EFAs has been demonstrated in adult animals (Craig-Schmidt *et al.*, 1996). Given that the major period of brain and nervous system development occurs during the pre and postnatal period, the increased requirement for preformed long-chain fatty acids by pregnant and lactating mammals may exceed the endogenous supply (Hornstra *et al.*, 1995).

1.2.6.3. Fatty acid supply to the foetal ruminant

There is a high positive correlation between maternal and neonatal long-chain PUFA status in humans, suggesting that PUFA status of offspring may be manipulated successfully by supplementing pregnant or lactating women (Hornstra *et al.*, 1995). However, the epitheliochorial placenta of ruminants is less permeable than the haemochorial placenta of humans, leading to low fat accumulation in the neonate (Père, 2003). Indeed, Noble *et al.* (1985) reported that the concentrations of non-esterified fatty acids are low in the foetus and suggested that there is a low transfer rate of unesterified fatty acids across the placenta. Rooke *et al.* (1998) also postulated that the relatively minor increases observed in C22:6*n*-3 deposition in the foetal pig compared to those supplied by the maternal diet were due to limited placental transfer of long-chain PUFAs.

Hempenius *et al.* (2000) suggested that preformed long-chain PUFAs may be transferred across the rodent placenta from the dam to the foetus. Furthermore, an *in vitro* study by Campbell *et al.* (1998) described a human placental fatty acid-binding protein which preferentially bound C20:4*n*-6 and C22:6*n*-3 compared to C18:2*n*-6 and C18:0. This selective transfer of long-chain PUFAs from maternal plasma has been termed "biomagnification" by Broadhurst *et al.* (2002), a group of researchers who also reported that desaturation and elongation do not occur in the placenta. Zakhariv and Yanovich (1992) also suggested that C20:4*n*-6 present in the foetal ruminant is derived from maternal circulation and that low concentrations in the foetus are a result of negligible placental transfer. By contrast, Noble *et al.* (1985) reported that the high concentrations of C20 and C22 fatty acids found in foetal ruminants resulted from elongation and desaturation of saturated fatty acids due to a low maternal EFA and PUFA supply. Consequently, Noble *et al.* (1985) suggested that the placenta has a significant role in the provision of long-chain PUFAs to the foetus via desaturation and elongation of EFAs.

Shand *et al.* (1978) observed that the desaturase activity of neonatal ruminant liver is stimulated by nutrient ingestion and is not active in foetal lambs. Neonatal plasma contains low concentrations of EFAs (Shand *et al.*, 1978), with C18:1*n*-9 accounting for a significant proportion of the fatty acids available for desaturation and elongation (Noble *et al.*, 1985). This gives rise to an increase in the concentration of C20:3*n*-9 in neonatal plasma compared to maternal plasma (Noble *et al.*, 1985). Essentially, the foetal and neonatal ruminant is dependent on a supply of long-chain PUFAs from the dam, whether by an adequate EFA supply at the placenta (Hornstra *et al.*, 1995) or preformed supply from dam plasma and colostrum.

1.2.7. Effects of long-chain PUFA supplementation upon performance parameters

1.2.7.1. Effects of long-chain PUFAs upon dry matter intake

Diets containing high total fat or long-chain PUFA concentrations are known to disrupt mechanisms of ruminal fermentation (Chilliard, 1993; Velasco *et al.*, 2001) thereby inhibiting forage digestion and reducing DM intake (Doreau and Chilliard, 1997b; Donovan *et al.*, 2000). Both Palmquist (1984) and Cant *et al.* (1997) suggested that the observed reductions in forage digestion are related to dietary fats coating fibre particles and protecting fibrous material from microbial degradation. By contrast, Szumacher-Strabel *et al.* (2001a; 2001b) proposed that dietary fatty acids have an antimicrobial effect within the rumen, reducing fibre digestion by retarding the metabolism and growth of protozoal and cellulolytic bacterial species.

Donovan *et al.* (2000) suggested that the reduction in DM intake of dairy cattle observed with increasing fish oil intake resulted from inhibition of microbial respiration, and consequent lysis of bacterial cells, by PUFAs. These results concur with those of Chilliard and Doreau (1997) who demonstrated that adding fish oil to the diets of lactating cattle reduced DM intake by 1.6 kg/day. Similarly, Lacasse and Ahnadi (1998) induced a 25 %

reduction in feed intake of lactating cattle via supplementation with unprotected fish oils. Infusing fish oil directly into the duodenum increased DM intake in the study published by Doreau and Chilliard (1997b), this adds weight to the hypothesis that reductions in DM intake occur as a result of ruminal disruption. Alterations in the microbial population would also clarify the reduction in acetate and increase in propionate production exhibited by ruminants supplemented with PUFAs (Chilliard, 1993; Doreau and Chilliard, 1997a; Doreau and Chilliard, 1997b).

1.2.7.2. Effects of long-chain PUFAs on milk yield

Postnatal lamb growth is affected by myriad factors, including breed, genetic potential and environmental parameters, but the major contributing factor is maternal milk yield and composition (Louveau *et al.*, 2000). Colostrum and milk provide essential nutrients for the neonatal and growing lamb in addition to compounds such as immunoglobulins, hormones, enzymes and growth factors (Louveau *et al.*, 2000). Predictably, a high positive correlation between milk intake and pre-weaning growth has been demonstrated in ruminants (Penning *et al.*, 1980).

The principal effects of PUFA supplementation upon milk yield and composition are summarised in Table 1.5. Regarding yield, lactose is the primary determinant of milk osmotic potential and is positively correlated to milk yield (Ploumi *et al.*, 1998; Agenäs *et al.*, 2002). An increase in milk lactose production results in a homeostatic increase in water uptake by the mammary gland in an attempt to maintain osmotic pressure (Kennelly and Glimm, 1998; Agenäs *et al.*, 2003). A reduction in fibre digestion in the rumen as a consequence of long-chain PUFA supplementation has been demonstrated to increase the ruminal production of propionate (Chilliard, 1993). As propionate is the precursor of lactose, it is logical to suggest that long-chain PUFA supplementation may increase milk yield. This theory concurs with the results of Chilliard and Doreau (1997) and Keady *et al.*

Table 1.5. Effects of different dietary fat sources upon ruminant milk yield and composition

Species	Treatment	Effect of fat source upon milk composition				Reference
		Yield (kg/day)	Fat (g/kg)	Protein (g/kg)	Lactose (g/kg)	
Cattle	Control (grass silage, concentrate)	17.3*	46.0	39.4	48.4	Shingfield *et al.* (2003)
	Control + 250 g fish oil	14.1*	42.8	39.6	46.8	
Cattle	Control (maize silage, alfalfa silage, concentrate)	31.7**	29.7**	31.7	49.7	Donovan *et al.* (2000)
	Control + fish oil at 1 % of DMI	34.2**	27.9**	31.9	49.9	
	Control + fish oil at 2 % of DMI	29.2**	23.7**	32.1	49.5	
	Control + fish oil at 3 % of DMI	30.1**	23.0**	31.7	48.9	
Cattle	Control (grass silage, concentrate)	22.5***	42.3***	32.7***	49.3***	Keady *et al.* (2000)
	Control + 150 g fish oil	25.0***	40.4***	32.0***	49.9***	
	Control + 300 g fish oil	25.2***	36.6***	30.1***	50.5***	
	Control + 300 g fish oil premix	25.7***	32.5***	28.5***	50.6***	
	Control + 450 g fish oil	25.7***	27.3***	28.9***	50.4***	
Goats	Control (Lucerne, grain pellets)	1.88	41.5	32.0	-	Kitessa *et al.* (2001b)
	Control + protected tuna oil	1.35	43.3	35.5	-	
	Control + unprotected tuna oil	1.77	40.7	33.3	-	
Cattle	Control (maize silage, concentrate)	26.5**	38.6**	28.8**	48.1	Chilliard and Doreau (1997)
	Control + 300 ml fish oil	28.0**	25.3**	27.9**	47.6	
	Control + 20 g methionine	25.8**	38.5**	30.7**	47.6	
	Control + 300 ml fish oil + 20 g methionine	28.2**	25.5**	29.1**	47.4	
Cattle	Control (grass silage, alfalfa hay, concentrate)	22.3	39.0**	30.6	45.1	Cant *et al.* (1997)
	Control + fish oil at 2 % of DMI	21.1	27.4**	28.3	44.6	
	Control + monensin (14.5 mg/kg DM)	21.7	36.1**	29.3	45.5	
	Control + fish oil + monensin	20.2	25.3**	28.4	45.5	
Cattle	Control (alfalfa hay, maize silage, concentrate)	23.9	37.0*	31.4	-	Franklin *et al.* (1999)
	Control + protected marine algae	23.2	29.5*	30.2	-	
	Control + unprotected marine algae	24.0	29.5*	29.8	-	

DMI = dry matter intake

* = treatment means are significantly different at the $p<0.05$ level; ** = treatment means are significantly different at the $p<0.01$ level; *** = treatment means are significantly different at the $p<0.001$ level

Table 1.5. Effects of different dietary fat sources upon ruminant milk yield and composition (continued)

Species	Treatment	Effect of fat source upon milk composition				Reference
		Yield (kg/day)	Fat (g/kg)	Protein (g/kg)	Lactose (g/kg)	
Sheep	Control (alfalfa hay, concentrate)	1.15***	43.3***	52.4**	54.8***	Papadopoulos *et al.* (2002)
	Control + 23.5 g marine algae	1.00***	43.2***	54.6**	55.1***	
	Control + 47 g marine algae	0.93***	43.9***	55.9**	54.0***	
	Control + 94 g marine algae	1.11***	49.0***	55.8**	51.0***	
Cattle	Control (grass silage, concentrate) + Megalac	22.1	41.2*	32.5*	45.9*	Petit *et al.* (2002a)
	Control + formaldehyde-treated whole linseed	21.7	39.7*	32.8*	46.3*	
	Control + fish oil + formaldehyde-treated whole linseed	22.2	31.1*	30.8*	46.1*	
Cattle	Control (grass and maize silage, concentrate) + Megalac	33.5	41.4*	28.6*	45.7*	Petit (2002)
	Control + whole linseed	35.7	38.1*	29.8*	47.1*	
	Control + micronised soyabeans	34.4	37.0*	28.7*	47.0*	
Cattle	Control (grass silage, concentrate) + sunflower seed	23.0*	44.4	32.5*	44.6	Petit (2003)
	Control + formaldehyde-treated sunflower seed	25.8*	43.9	31.7*	45.6	
	Control + whole linseed	22.4*	42.3	34.1*	43.6	
	Control + formaldehyde-treated whole linseed	24.9*	43.3	33.4*	44.9	
Cattle	Control (grass silage, concentrates)	25.0*	38.9	32.8*	50.6	Brzóska *et al.* (1999)
	Control + linseed oil calcium salts at 3 % of DMI	23.7*	41.7	31.9*	51.9	
	Control + linseed oil calcium salts at 6 % of DMI	22.9*	42.3	30.8*	51.7	
	Control + linseed oil calcium salts at 9 % of DMI	23.6*	40.6	31.2*	51.3	
Cattle	Control (maize silage, alfalfa hay, concentrate)	32.1	35.1**	33.8	49.0	Whitlock *et al.* (2002)
	Control + fishoil at 2 % of DMI	29.1	27.9**	33.8	48.2	
	Control + extruded soyabeans at 2 % of DMI	34.6	32.7**	33.0	49.0	
	Control + fish oil (1 %) + extruded soyabeans (1 %)	31.1	31.4**	32.8	48.7	

DMI = dry matter intake

* = treatment means are significantly different at the $p<0.05$ level; ** = treatment means are significantly different at the $p<0.01$ level; *** = treatment means are significantly different at the $p<0.001$ level

(2000). However, the conflicting effects of fatty acids on milk yield between the aforementioned studies and those of Cant *et al.* (1997), Donovan *et al.* (2000) and Shingfield *et al.* (2003) suggest that effects of PUFAs on lactose production are not consistent. Furthermore, supplementation with protected fatty acids should eliminate the effect of long-chain PUFAs on milk yield (Kitessa *et al.*, 2001d). However, this suggestion is challenged by the results of Brzóska *et al.* (1999), Kitessa *et al.* (2001b) and Petit (2003).

1.2.7.3. Effects of long-chain PUFAs on milk fat concentration

The majority of data in Table 1.5. imply that ruminant milk fat concentration is depressed by long-chain PUFA supplementation of the lactating animal. Modifications to rumen function that alter the balance of ruminal VFA production have a significant effect on the level of milk fat production as they reduce the production of acetate (Komprda *et al.*, 2001). This fatty acid is the precursor for *de novo* synthesis of short and medium-chain fatty acids within the mammary gland (Chilliard *et al.*, 2000).

The production of *trans* octadecanoic fatty acids via incomplete ruminal biohydrogenation of PUFAs has also been suggested to directly inhibit milk fat synthesis (Romo *et al.*, 1996; Brzóska *et al.*, 1999; Bauman and Griinari, 2001). Increasing PUFA intake is positively correlated with increased *trans* fatty acid production (Davis and Brown, 1970; Bauman and Griinari, 2001; Precht *et al.*, 2001), the highest *trans* C18:1 concentrations being found in milk from ruminants fed fish oils (Demeyer and Doreau, 1999). Both Hagemeister *et al.* (1991) and Bauman and Griinari (2001) reported that milk fat concentrations were maintained when long-chain fatty acids were infused directly into the duodenum although the same fatty acids depressed milk fat when fed in the diet. This supports the theory that milk fat synthesis is inhibited by *trans* fatty acids produced during biohydrogenation of PUFAs. *Trans*-10, C18:1 and the *trans*-10,*cis*-12 isomer of CLA appear to have the most

significant inhibiting effect upon *de novo* fatty acid synthesis (Baumgard *et al.*, 2000; Chilliard *et al.*, 2000; Chilliard *et al.*, 2001b).

Chilliard *et al.* (2001b) suggested that extensive ruminal biohydrogenation of dietary C20:5*n*-3 may occur, leading to the production of metabolites that inhibit *de novo* fatty acid synthesis. Indeed, addition of fish oils to the diet has been shown to increase the concentrations of *trans* octadecanoic fatty acids in milk, despite the low C18:2*n*-6 and C18:3*n*-3 concentrations in marine oils (Bessa *et al.*, 2000). However, supplementation with marine algae has been shown to have similar effects to fish oil on CLA and *trans* 18:1 production and milk fatty acid concentrations, regardless of its negligible C20:5*n*-3 content (Chilliard *et al.*, 2001b). Therefore the biohydrogenation of C20:5*n*-3 alone is unlikely to confer these effects.

Chilliard *et al.* (2001b) reported that long-chain PUFAs may prevent the last step of biohydrogenation, reducing the amount of C18:0 in the rumen via the inhibition of group B bacteria and increasing concentrations of *trans* C18:1 fatty acids. The high C20:5*n*-3 content of fish oils may inhibit the gene expression of Δ-desaturase enzymes as exhibited in rodent models (Chilliard *et al.*, 2001b). Alternatively, PUFAs may affect the thioesterase (Chilliard *et al.*, 2001b) or LPL enzymes involved in fatty acid synthesis and metabolism (Cant *et al.*, 1997). Direct inhibition of mammary acetyl CoA carboxylase (ACC) has also been suggested as a mechanism involved in the inhibition of *de novo* fatty acid synthesis by PUFAs (Brzoska *et al.*, 1999; Baumgard *et al.*, 2000; Wright *et al.*, 2002).

In an attempt to identify the enzymes inhibited by long-chain PUFAs, Ahnadi *et al.* (1998) examined gene expression in the mammary gland. Dietary supplementation of lactating cattle with protected fish oil reduced ACC, fatty acid synthase (FASt), LPL and stearoyl-

CoA desaturase (SCD) whereas unprotected fish oil only significantly reduced LPL. Reductions in short and medium-chain fatty acid synthesis were highly correlated with ACC and FASt activity (Ahnadi *et al.*, 1998). Moreover, Barber *et al.* (1997) described the inhibition of SCD and FASt, and a reduction in the total amount of ACC within the mammary gland conferred by dietary PUFA supplementation. The effect of fish oils on LPL activity have also been recounted by other researchers (Chilliard and Doreau, 1997; Keady and Mayne, 1999b; Chilliard *et al.*, 2001b). It is clear that unsaturated fatty acids have inhibitory effects on a range of enzymes involved in *de novo* fatty acid synthesis.

Unsaturated fatty acids successfully protected from ruminal biohydrogenation do not affect milk fat secretion, as confirmed by Brzóska *et al.* (1999). However, the extent to which protection is successful is extremely variable between methods and studies. Some studies showed similar effects of "protected" and "unprotected" fish oils on milk production and composition (Chilliard *et al.*, 2001b; Lacasse and Ahnadi, 1998).

1.2.7.4. Effects of long-chain PUFAs on milk fat composition

Changes in milk fat concentration as a result of dietary manipulation notably affect the fatty acid composition of milk lipids. *De novo* fatty acid synthesis is reduced by unsaturated fatty acid supplementation with a shift in milk triacylglycerol composition away from short and medium-chain fatty acids towards long-chain fatty acids (Barber *et al.*, 1997; Donovan *et al.*, 2000; Bauman and Griinari, 2001).

The addition of oilseeds to the diet increases the C18:2*n*-6 and C18:3*n*-3 concentration of milk fat (Petit, 2002; 2003; Table 1.6). Gulati *et al.* (1997 ; 2002) and Petit *et al.* (2002) described increased transfers of C18:3*n*-3 into milk by feeding protected oilseeds or infusing seed oils into the duodenum. By contrast, Hagemeister *et al.* (1991) reported a transfer efficiency for C18:3*n*-3 of only 1 % when linseed oil was fed to lactating cattle.

Table 1.6. Effects of different dietary fat sources compared to control fats upon ruminant milk fatty acid composition

Species	Type of fat supplement	Effect of fat source upon milk composition						Reference
		Saturated fatty acids	C18:1 *trans*	C18:3*n*-3	C20:4*n*-6	C20:5*n*-3	C22:6*n*-3	
Cattle	Fish oil	Variable	Increased	Unchanged	Increased	Increased	Increased	Shingfield *et al.* (2003)
Cattle	Fish oil	Reduced	Increased	Unchanged	Increased	Increased	Increased	Donovan *et al.* (2000)
Cattle	Fish oil	Reduced	Increased	Unchanged	Increased	Increased	Unchanged	Keady *et al.* (2000)
Cattle	Protected fish oil	Reduced	Increased	Unchanged	-	Increased	Increased	Lacasse *et al.* (2002)
	Unprotected fish oil	Reduced	Increased	Increased	-	Increased	Increased	
Cattle	Fish oil	-	-	Unchanged	Unchanged	Increased	Increased	Cant *et al.* (1997)
Cattle	Fish oil	Reduced	Increased	Unchanged	-	Increased	Increased	Whitlock *et al.* (2002)
	Fish oil + extruded soyabeans	Reduced	Increased	Unchanged	-	Unchanged	Increased	
Cattle	Protected marine algae	Reduced	Increased	Unchanged	Unchanged	-	Increased	Franklin *et al.* (1999)
	Unprotected marine algae	Reduced	Increased	Reduced	Unchanged	-	Increased	
Sheep	Marine algae	Variable	Increased	Reduced	Increased	Increased	Increased	Papadopoulos *et al.* (2002)
Cattle	Whole linseed	Increased	Unchanged	Increased	Unchanged	-	Unchanged	Petit (2002)
	Micronised soyabeans	Increased	Unchanged	Increased	Unchanged	-	Unchanged	
Cattle	Whole linseed	Increased	Reduced	Increased	Unchanged	Increased	-	Petit (2003)
	Formaldehyde-treated whole linseed	Increased	Reduced	Increased	Unchanged	Increased	-	
Cattle	Calcium salts of linseed oil	Reduced	-	Increased	Unchanged	Unchanged	Unchanged	Brzóska *et al.* (1999)
Goats	Canola oil	Variable	-	Unchanged	-	-	-	Mir *et al.* (1999)

Minor increases in C20:5*n*-3 have also been observed when linseed oil was fed as a result of endogenous fatty acid desaturation and elongation (Hagemeister *et al.*, 1991). However, the study of Brzóska *et al.* (1999) showed that feeding protected linseed did not increase the proportions of C20:5*n*-3 or C22:6*n*-3 in milk.

The proportions of C16:0, C18:0 and C18:1*n*-9 within milk lipid are significantly reduced and increases in CLA (specifically *cis*-9, *trans*-11 CLA), *trans* C18:1 fatty acids, long-chain PUFAs (C22:6*n*-3, C20:5*n*-3, C20:4*n*-6) have been observed by Lacasse *et al.* (1998), Keady *et al.* (2000) and Chilliard *et al.* (2001b) as a result of fish oil supplementation. Most studies have reported slight increases in C22:6*n*-3 and C20:5*n*-3 content of milk as a consequence of fish oil supplementation, but this often amounts to a difference of less than 0.15 % when data are expressed as a proportion of total fatty acids (Chilliard *et al.*, 2001b; Ramaswamy *et al.*, 2001). This low concentration may be attributed to the presence of C22:6*n*-3 and C20:5*n*-3 in cholesterol esters and phosphatidylglycerols rather than triacylglycerols (Chilliard *et al.*, 2000).

Table 1.7. Transfer efficiencies for C20:5n-3 and C22:6n-3 from fish oil to ruminant milk fat

	Efficiency of transfer into milk fat		
Species	**C20:5*n*-3**	**C22:6*n*-3**	**Reference**
Goats	5-6 %	7-8 %	Gulati *et al.* (1999)
Cattle	24 %	14 %	Gulati *et al.* (2002)
Cattle	20 %	8 %	Donovan *et al.* (2000)
Cattle	61 %	19 %	Keady *et al.* (2000)
Cattle	9 %	16 %	Cant *et al.* (1997)

Transfer efficiencies from dietary fish oil to milk vary considerably between studies, from 5-6 % for C20:5*n*-3 and 7-8 % for C22:6*n*-3 (Gulati *et al.*, 1999) to 9 % and 16 % for C20:5*n*-3 and C22:6*n*-3 respectively (Cant *et al.*, 1997; Table 1.7). Marine algae significantly increases the proportions of C20:4*n*-6, C22:5*n*-6 and C22:6*n*-3 in milk lipids, but does not tend to considerably increase C20:5*n*-3 concentrations (Chilliard *et al.*, 2001b).

1.2.7.5. Effects of long-chain PUFAs on milk protein concentration

Long-chain PUFA supplementation of lactating ruminants tends to reduce milk protein concentrations (Donovan *et al.*, 2000; Cant *et al.*, 1997 and Petit *et al.*, 2002). Gulati *et al.* (2002) reported that increasing the unsaturated fatty acid intake reduces microbial protein synthesis in ruminants, which may reduce milk protein concentrations. It has also been postulated that protein concentration may be concurrently depressed by a dilution effect where fish oil increases milk yield without a concurrent increase in protein synthesis, or by a reduction in casein synthesis (Chilliard and Doreau, 1997; Doreau and Chilliard, 1997a; Keady *et al.*, 2000).

Protein yield may also be reduced by a combination of effects of PUFAs on milk yield and protein concentration (Kennelly, 1996). Lacasse and Ahnadi (1998) reported a reduction in milk protein yield with both unprotected and protected fish oils. These observations are in agreement with the work by Keady *et al.* (2000), which described declining protein concentration with increasing dietary fish oil intake. However, reductions in milk protein yield between studies may be due to a decline in milk yield conferred by PUFA supplementation without differences in constituent composition, as reported by Donovan *et al.* (2000).

1.2.7.6. Effect of long-chain PUFAs on neural tissue development

The majority of long-chain PUFA deposition in nervous tissues occurs during pre- and postnatal brain growth (Ward *et al.*, 1996; Poumes-Ballihaut *et al.*, 2001), the extent of development within each period varying between species. Foetal fatty acid deposition is significantly increased during late pregnancy (Campbell *et al.*, 1998; Morley, 1998), C22:6*n*-3 being preferentially incorporated and retained within the phosphatidylglycerols of brain and retinal tissues (Ward *et al.*, 1996; Rooke *et al.*, 1998; Broadhurst *et al.*, 2002).

Supplementation of pregnant humans with long-chain *n*-3 PUFAs has been reported to increase concentrations of C20:5*n*-3 and C22:6*n*-3 in neonatal tissues (Matorras *et al.*, 1999); with similar results in pigs as reported by Rooke *et al.* (1998; 1999; 2000; 2001a; 2001b). It has been hypothesised that C20:5*n*-3 and C22:6*n*-3 may be essential for optimal visual and cognitive function in neonatal animals and human infants (Weisinger *et al.*, 1996; Campbell *et al.*, 1998; Goustard-Langelier *et al.*, 1999) and optimal brain and retinal function in adults (Williard *et al.*, 2001). Indeed, Dijck-Brouwer *et al.* (2005) reported that the classification of infants as neurologically abnormal at birth was negatively correlated with the EFA and C22:6*n*-3 status of the child. Moreover, Suzuki *et al.* (1998) investigated maze-learning ability in second-generation *n*-3 PUFA supplemented mice and observed that fish oil-supplemented mice were significantly less likely to stray from the path of a maze than those fed palm oil. Brain phosphatidylglycerols from fish oil-supplemented mice contained significantly more C22:6*n*-3 and less C20:4*n*-6 when compared to palm-oil supplemented mice. These increases were highly positively correlated with the enhancement of synaptic membrane fluidity. As the synaptic membrane has a significant role to play in learning and memory, Suzuki *et al.* (1998) concluded that these functions might be improved by increased membrane fluidity.

These findings agree with the work of Ikemoto *et al.* (2001) who supplemented rats with perilla oil (*n*-3 sufficient) or safflower oil (*n*-3 deficient) during the principal period of brain growth and found that perilla-fed rats produced more correct answers in a brightness-discrimination learning test. Moreover, changing from the safflower to the perilla diet post-weaning restored brain C22:6*n*-3 concentrations and, to a certain extent, learning behaviours, indicating that the effects of this deficiency on the CNS are reversible. It was suggested that mechanisms by which learning behaviour is altered by *n*-3 fatty acid deficiency may be a result of changes in neurotransmission, brain protein synthesis,

reduced brain phosphatidylglycerol synthesis or changes in melatonin concentrations (Ikemoto *et al.*, 2001).

Infants fed maternal milk tend to have higher long-chain PUFA concentrations in neural tissues (Bougle *et al.*, 1999), long-chain PUFA supplementation of formulae also results in an increase in the C22:6*n*-3 content of brain tissues (Bondia-Martinez *et al.*, 1998). This may further be manifested as improvements of scores on tests of visual acuity (Morale *et al.*, 2005). In humans, the effects of *n*-3 fatty acids on the CNS and retina are thought to be quantitatively more important in pre-term infants given the exponential rate of brain development and PUFA accretion during late pregnancy. This finding may be extrapolated to all mammalian species. In full-term infants, Bougle *et al.* (1999) reported few differences in CNS maturation, fatty acid composition or visual acuity, regardless of the long-chain PUFA supply of the diet. This is in contrast to the results of Hornstra *et al.* (1995) who reported that C22:6*n*-3 supplementation of infant formulae improved indices of motor and mental development (Bayleys test) attributed to increased brain C22:6*n*-3 concentrations. Makrides *et al.* (1995) also reported that C22:6*n*-3 accretion in brain was augmented by breast-feeding with concurrent improvements in visual evoked potentials at 4-5 months of age. Changing from a fatty acid-deficient formula to one supplemented with 0.36 % C22:6*n*-3 also enhanced indices of development, although there was no compensational deposition of C22:6*n*-3 into tissues (Makrides *et al.*, 1995). Indices of mental and motor development appeared to increase with C22:6*n*-3 supplementation in a dose-dependent manner (Morley, 1998), but many of these differences disappeared later in the experiment. By contrast, Hoffman *et al.* (2004) reported that supplementing infants with dietary C22:6*n*-3 during the first year of life improved visual acuity. The long-term effects of *n*-3 fatty acid supplementation during the postnatal period still remain unclear, nevertheless, it is assumed that neonatal nutrition may have long-term effects on the neural pathways responsible for visual acuity (Carlson and Werkman, 1996).

Goustard-Langelier *et al.* (1999) demonstrated that piglets fed formulae supplemented with fish oil had augmented C22:6*n*-3 concentrations in specific areas of the brain when compared to a standard formula, deficient in *n*-3 fatty acids. The deposition of C22:6*n*-3 was particularly high in the temporal lobe, known to be involved in both sensory output and visual and auditory information processing (Goustard-Langelier *et al.*, 1999). Rooke *et al.* (1998) reported increased C22:6*n*-3 concentrations of brain and other tissues conferred by tuna oil supplementation of pregnant sows compared to soyabean oil supplementation. Similar results were also described in experiments using targeted tuna oil supplementation of pregnant sows (Rooke *et al.*, 2001a) and in piglets born to sows fed salmon oil during pregnancy (Rooke *et al.*, 2001b). However, the results of the various studies published by Rooke *et al.* (1998; 2000; 2001a; 2001b) suggested that retinal C22:6*n*-3 concentrations were relatively inflexible and not altered by dietary supplementation.

Dietary supplementation with *n*-3 fatty acids may have undesirable effects on the growing animal. Arbuckle and Innis (1992); Koletzko (1992) and Su *et al.* (1999) reported that the relatively high concentrations of C20:5*n*-3 in fish oils compete with C20:4*n*-6 within mammalian tissue. The concentrations of C20:4*n*-6 in phosphatidylglycerols are reduced, thus inhibiting eicosanoid production, affecting membrane fluidity, reducing growth rate and inducing immunosuppression. Neural tissues contain high concentrations of C20:4*n*-6, which should be regarded as a conditionally essential fatty acid, especially given its role as an eicosanoid precursor (Huang and Craig-Schmidt, 1996).

1.2.7.7. Effects of long-chain PUFAs upon behaviour

Further to the work of Hornstra *et al.* (1995), Makrides *et al.* (1995) and Bougle *et al.* (1999) in human infants, research has begun into the possibility of improving neonatal

animal vigour by the supplementation of diets with long-chain PUFAs during pregnancy. Rooke *et al.* (2001a) reported that piglets from sows supplemented with tuna oil from day 91 of pregnancy made contact with the udder and found a teat significantly quicker than piglets from sows fed a control diet. These behavioural measurements are thought to indicate enhanced neonatal vigour and improved visual acuity. Piglets born to sows fed tuna oil in late pregnancy were also heavier at birth (Rooke *et al.*, 2001a). Piglet birthweight has been implicated as a possible indicator of vigour and is a reliable predictor of mortality in that low birthweight piglets are less likely to survive than moderate or heavy-weight offspring (Jean and Chiang, 1999). Behavioural parameters or measures of viability were not reported for the study of Rooke *et al.* (2001b). However, piglet liveweight was increased by the addition of salmon oil to maternal diets, which could be suggested to enhance vigour. By contrast, a study by Rooke *et al.* (1998) reported that vigour scores of piglets from sows fed different fat sources were significantly higher when sows were supplemented with soyabean oil compared to tuna oil, despite increases in C20:5*n*-3 and C22:6*n*-3 in tissues of piglets on the tuna oil treatment.

Supplementing the diets of piglets from birth to between 18 and 30 days of age with high dietary concentrations of long-chain *n*-3 PUFAs resulted in significantly more correct maze entries when compared to supplementation with a low-*n*-3-PUFA diet, and a lesser tendency to perform stereotypic behaviours in the study of Ng and Innis (2003). Furthermore, these alterations in behaviour were associated with increased phosphatidylglycerol C22:6*n*-3 within the frontal cortex. However, the effect of long-chain *n*-3 PUFA supplementation upon ruminant behaviour is yet to be explored.

1.2.7.8. Effects of long-chain PUFAs upon neonatal mortality

Approximately four million neonatal lambs die each year in the UK, a significant proportion of these deaths occurring in extensive farming systems on hill and upland

farms, at a potential loss of £120 million to the UK sheep industry (Fitt and Packington, 1998; Merrell, 1998). The principal cause of this high mortality rate is hypothermia and starvation caused by a combination of placental insufficiency, a lack of colostrum, hypoxia during birth and failure of the interaction between ewe and lamb behaviours (Dwyer and Lawrence, 1998; Fitt and Packington, 1998).

Colostrum intake is positively correlated with lamb survival (Nowak, 1996) as lambs with negligible or sub-optimal intakes during the first twelve hours of life have an increased mortality rate. The main functions of colostrum are to provide an energy source; generate the production of heat from non-shivering thermogenesis, enhance the immune status by immunoglobulin ingestion and facilitate expulsion of the meconium (Tuchsherer *et al.*, 2000). Neonatal lambs that are able to maintain their body temperature via a combination of ewe grooming behaviour and endogenous heat production, despite the heat loss resulting from evaporation of placental fluids, are significantly more likely to survive (O'Connor and Lawrence, 1992; Tuchsherer *et al.*, 2000). This assumption is supported by the results of Tuchsherer *et al.* (2000) who demonstrated that piglets which survived had higher birthweights, suckled earlier and had a lower drop in body temperature at one hour *post partum* compared to piglets subsequently that died during the first 10 days of life.

It is suggested that neonatal mortality may be reduced by maternal long-chain PUFA supplementation, due to improved nervous tissue development impacting upon vigour, colostrum intake and enhanced thermogenesis. Jean and Chiang (1999) concluded that supplementing pregnant sows with medium-chain triacylglycerols would improve piglet survival. However, this conclusion was based on the assumption that increased energy in the form of fat would improve vigour and reduce mortality resulting from energy insufficiency. Studies using pre-term human infants have also reported an improved prognosis as a consequence of long-chain PUFA supplementation (Hornstra *et al.*, 1995).

Neonatal animals with a high surface area:volume ratio are particularly vulnerable to heat loss, therefore, thermogenesis is essential to improve maintain body temperature (Herpin *et al.*, 2002). The influence of long-chain PUFAs on enhancement of neonatal lamb vigour may be augmented by their role in the facilitation of non-shivering thermogenesis (NST), a mechanism by which heat is generated without muscular contraction (Herpin *et al.*, 2002). Heat conservation is promoted by piloerection, shivering and constriction of blood vessels close to the skin in response to an acute drop in environmental temperature before NST is stimulated by the sympathetic nervous system (Nedergaard *et al.*, 2001; Subramanian and Vollmer, 2001). This mechanism occurs exclusively in brown adipose tissue (BAT; Takahsahi *et al.*, 2000; Margareto *et al.*, 2001) under the control of iodothyronine 5'-deiodinase (Trayhurn *et al.*, 1993), uncoupling protein-1 (UCP-1) (Takahsahi *et al.*, 2000) and the thyroid hormones thyronine (TH), thyroxine (T_4) and 3,3',5:triiodothyronine (T_3; Louveau *et al.*, 2000).

The inert T_4 is converted to the biologically active T_3 by iodothyronine 5'-deiodinase within BAT (Trayhurn *et al.*, 1993). This is required for the optimum function of UCP-1 (Liu *et al.*, 1998), so-called due to its ability to uncouple oxidative phosphorylation by allowing protons to re-enter the mitochondrial matrix with a concurrent release of heat energy (Nedergaard *et al.*, 2001; Figure 1.8). During thermogenesis, a cAMP and protein kinase cascade is activated which in turn, stimulates HSL to hydrolyse stored triacylglycerols and release free fatty acids (Klaus, 2001). Fatty acids are oxidised, and the resulting proton enters the respiratory chain. Synthesis of ATP is bypassed via the uncoupling of this process by UCP-1 and the resulting energy is released as heat (Klaus, 2001). The total amount of UCP in the neonatal animal determines its potential for NST (Nedergaard *et al.*, 2001). However, the exact mechanism by which T_3 is essential for UCP-1 function is as yet, undetermined (Nedergaard *et al.*, 2001).

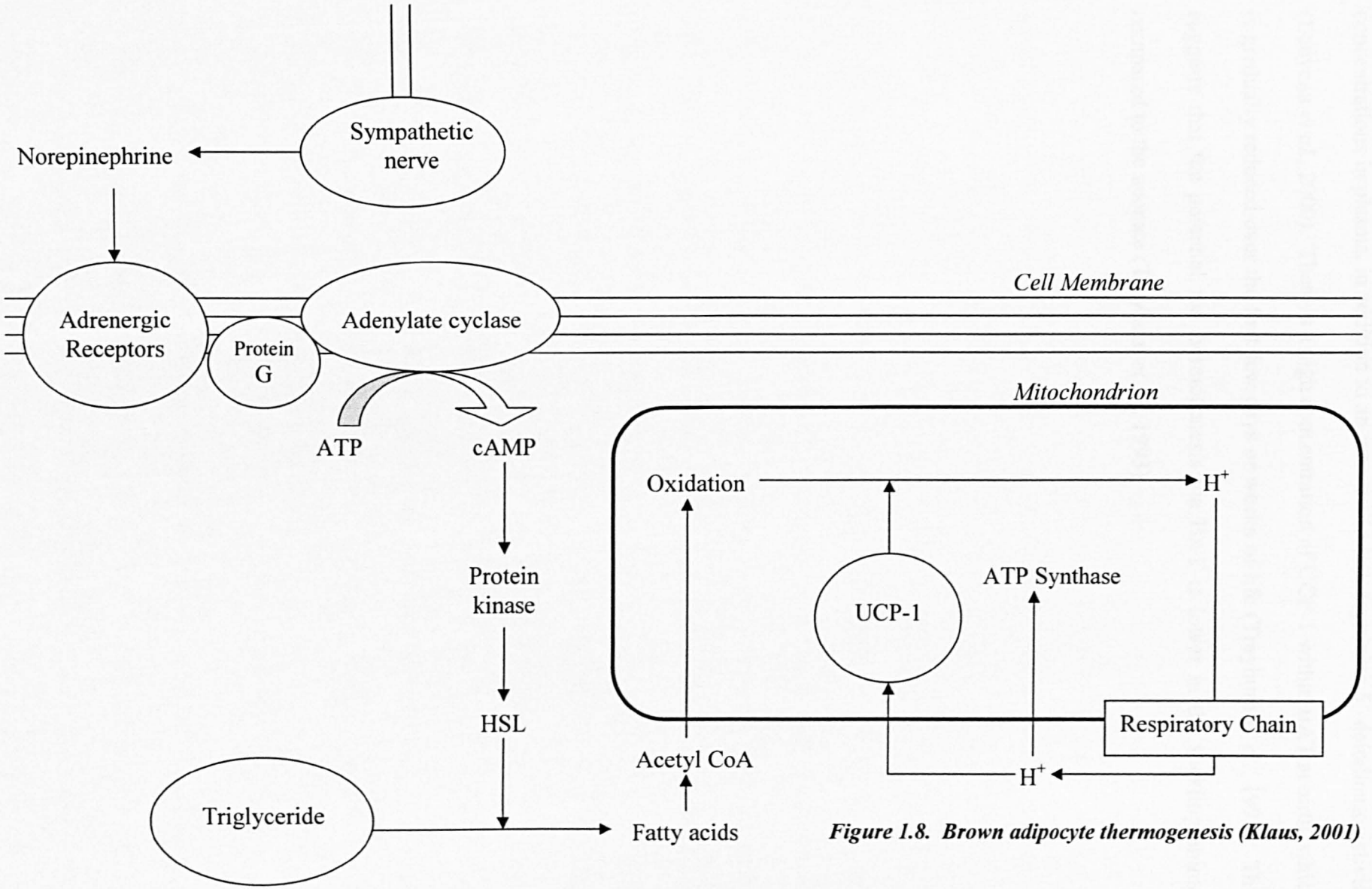


Figure 1.8. Brown adipocyte thermogenesis (Klaus, 2001)

In the immediate post-parturient period, the neonate experiences a surge in T_3 and T_4 concentrations in plasma, in addition to an increase in iodothyronine 5'-deiodinase activity (Louveau *et al.*, 2000). There is a high concentration of UCP-1 within BAT at birth, which is gradually reduced over the first few days or weeks of life (Trayhurn *et al.*, 1993). This suggests that the potential for thermogenesis via BAT is lower in the suckling animal compared to the neonate (Trayhurn *et al.*, 1993).

1.3. VITAMIN E

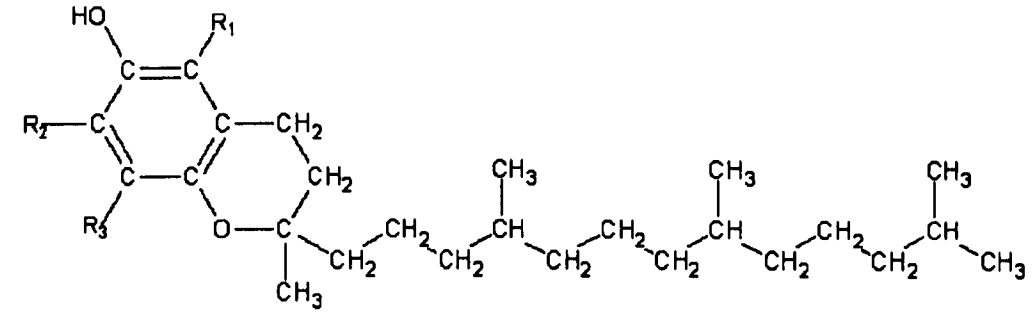
1.3.1. Chemical classification of vitamin E

1.3.1.1. Background and chemical structure of vitamin E

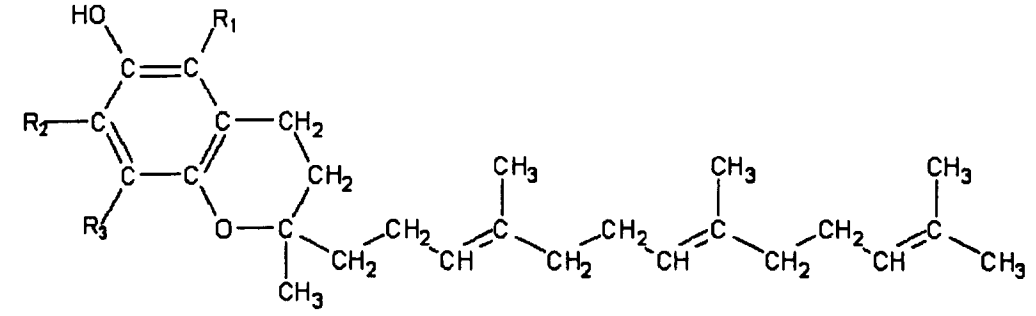
In 1922, Evans and Bishop induced a deficiency syndrome in a pregnant rat by supplementing the diet with rancid fat. This condition led to reabsorption of the foetus. Adding fresh salad leaves to the diet revoked this symptom and it was concluded that a specific compound within the plants was responsible for this reversal (Evans and Bishop, 1922). In 1924, Sure suggested that this compound be named "vitamin E".

Two compounds isolated by Evans *et al.* (1936) from wheat germ oil were shown to have the properties ascribed to the vitamin and were characterised as α- and β-tocopherols, tocopherol from the Greek "tokos" meaning "childbirth" and "phorein", "to bring forth". Subsequently, two additional tocopherols were identified and classified as γ- and δ-tocopherol, and the tocotrienols were discovered in plant oils (Azzi and Stocker, 2000). Therefore, vitamin E is the collective name for a group of fat-soluble tocol molecules classified into two groups: the tocopherols and the tocotrienols (Rice and McMurray, 1982). Both tocopherols and tocotrienols consist of a 16-carbon isoprenoid side chain attached to a 2-methyl, 6-chromanol aromatic ring (Putnam and Comben, 1987; Figure 1.9.). The carbon chain is wholly saturated in the tocopherols, and contains three double bonds in the tocotrienols (McMurray and Rice, 1982).

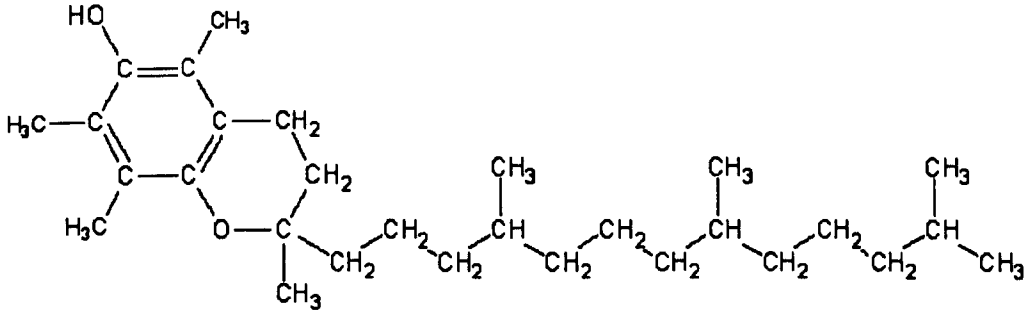
A methyl group may be substituted for a carbon atom on one or more of the three positions of the chromanol ring (carbons five, seven and eight) of tocopherols and tocotrienols, resulting in a possible eight different compounds in each tocol family (Azzi and Stocker, 2000). However, only the four tocopherol compounds, designated α, β, γ and δ have been isolated in nature, of which α and γ predominate (Dutta-Roy, 1999).



tocopherol



tocotrienol



alpha-tocopherol

Figure 1.9. Structure of tocopherol, tocotrienol and alpha-tocopherol (Putnam and Comben, 1987 and Azzi and Stocker, 2000)

Chiral centres exist at each methyl branch point and a further eight optical isomers of each tocopherol and two of each tocotrienol are possible (Putnam and Comben, 1987). Consequently, a significant number of compounds exist with activity similar to that of vitamin E. Nonetheless, they are not all of equal biological strength to the most potent vitamin E compound, α-tocopherol (Morrissey *et al.*, 1993; Table 1.8).

Table 1.8. Chemical structure of the tocol group of compounds (Putnam and Comben, 1987)

-Tocopherol	-Tocotrienol	R_1 atom	R_2 atom	R_3 atom
α	5,7,8-trimethyl-	CH_3	CH_3	CH_3
β	5,8-dimethyl	CH_3	H	CH_3
γ	7,8-dimethyl-	H	CH_3	CH_3
δ	8-methyl-	H	H	CH_3

1.3.1.2. Biological activity of vitamin E

Putnam and Comben (1987) postulated that tocol molecules are inserted into the membranes of mitochondria, microsomes and lysosomes of individual cells leaving the isoprenoid chains unbound. A different methylation structure on the chromanol ring results in a molecule that does not correspond to the space into which the molecule must fit for maximum activity (Putnam and Comben, 1987). Consequently, changes in ring methyl groups, stereochemistry of the chiral centres or unsaturation of the isoprenoid chain have significant effects on the biological activity of vitamin E isomers (Azzi and Stocker, 2000). Studies in pigs and cattle have demonstrated that only α-tocopherol is absorbed and utilised (McMurray and Rice, 1982). Other studies employing the rat foetal gestation-resorption assay have indicated that β-tocopherol has up to 57 %, γ-tocopherol up to 31 % and δ-tocopherol up to 1.4 % of the activity of α-tocopherol (Yang, 2003).

Naturally occurring vitamin E found in grains and forages has the stereo-conformation *RRR* (Yang, 2003) whereas synthetic (*all-rac*) vitamin E used for feed supplementation is a racemic mixture of the eight stereo-isomers (*RRR, RRS, RSR, RSS, SSS, SRS, SSR* and *SRR*; Yang, 2003). It has been concluded that, in humans, *RRR*-α-tocopherol has up to three-fold higher biological activity than *all-rac*-α-tocopherol (Azzi and Stocker, 2000). By contrast, Putnam and Comben (1987) stated that a synergism between the stereoisomers in *all-rac*-α-tocopherol results in an vitamin E source more potent than the form occurring naturally in forages. However, a selection pressure against *all-rac*-α-tocopherol in favour of *RRR*-α-tocopherol appears to exist post-absorption (Traber *et al.*, 1996). It is assumed

that the tissue distribution of the tocols is related to the relative requirements of individual tissues for specific molecules. For example, the human brain contains a substantial amount of α-tocopherol and no tocotrienols (Azzi and Stocker, 2000), by contrast, γ-tocopherol comprises over 50 % of total tocopherols in human skin (Yang, 2003).

1.3.2. Sources of vitamin E within the ruminant diet

1.3.2.1. Forages

Ruminants are unable to synthesise α-tocopherol or convert other tocopherol isomers into the α-configuration and are therefore dependent on an adequate dietary supply (Putnam and Comben, 1987). Green forage is the main vitamin E source in the diet of grazing ruminants: cattle grazing fresh pasture may ingest up to 1,675 mg vitamin E/day (Herdt and Stowe, 1991). Plants produce appreciable quantities of tocols, with α-tocopherol being found within the chloroplast and the β-, γ- and δ- isomers found in other cellular locations within seeds and leaves (Yang, 2003). The mixture of the different tocol isomers and species changes according to the specific tissue and maturity of the plant (Putnam and Comben, 1987). Evidence also suggests that the proportions of different tocols may change after harvest, especially if plants or grains are processed before feeding (Putnam and Comben, 1987). Dried forages such as hay are relatively high in tocopherols, although vitamin E content declines with maturity and is affected by harvest time and technique (Maas *et al.*, 1984; Herdt and Stowe, 1991). Green forages and grass silages contain approximately 50 mg α-tocopherol/kg DM, whereas dried forages such as hay may contain less than 10 mg/kg if exposed to UV light and heat (Putnam and Comben, 1987).

1.3.2.2. Oilseeds and cereals

Vitamin E is found in the highest concentrations in plant latex lipids, followed by edible plant oils from oilseeds such as sunflower, linseed and rapeseed (Visioli and Galli, 2001; Table 1.9). Predictably, given vitamin E's antioxidant properties, oilseeds containing high

concentrations of unsaturated fatty acids have concurrent concentrations of vitamin E (Azzi and Stocker, 2000). Cereal grains and protein sources such as soyabean meal (after oil removal) contain approximately 8 mg vitamin E/kg DM (McMurray and Rice, 1982). However, preservation of "moist" cereals by the addition of propionic acid or caustic soda results in significant α-tocopherol loss and concentrations of <1 mg/kg have been reported due to the additive effect of moisture and acid (McMurray and Rice, 1982; Putnam and Comben, 1987).

Table 1.9. Vitamin E contents of selected feedstuffs

Feedstuff	α-tocopherol (mg/kg)	β-tocopherol (mg/kg)	γ-tocopherol (mg/kg)	δ-tocopherol (mg/kg)
Wheatgerm oil	1210[b]	650	240	250
Sunflower oil	600[b]	20	10	10
Safflower oil	390[b]	10	170	240
Palm oil	280[b]	ND	320	70
Olive oil	200[b]	ND	10	ND
Rapeseed oil	170[b]	170	350	10
Peanut oil	130[b]	ND	220	20
Maize oil	110[b]	110	600	20
Soya bean oil	100[b]	100	800	300
Sesame oil	10[b]	10	240	30

NA = data not available
ND = not detectable (<0.5mg/100g)
[a]Azzi and Stocker, 2000
[b]Yang, 2003

1.3.3. Antioxidant functions of vitamin E

Vitamin E has a vital role as a lipid-soluble antioxidant within animal cells (Morrissey *et al.*, 1993; Asadian and Mezes, 1996; Gonzalez-Corbella *et al.*, 1998). An antioxidant is a substance that prevents the formation and propagation of free radicals, reducing the adverse effects of reactive oxygen and nitrogen species on mammalian physiological function (Wiseman, 1996; Leifert *et al.*, 1999). Vitamin E is the most potent chain-breaking antioxidant acting against the peroxidation of lipids and lipoproteins in cell membranes (Skomial *et al.*, 2001). This is of particular importance in neural cells where, as a consequence of the high PUFA concentrations, membranes are inherently unstable and vulnerable to oxidation (Putnam and Comben, 1987).

1.3.3.1. Free radicals

Free radicals are highly reactive molecules containing one or more unpaired electrons (Wiseman, 1996; Edwards *et al.*, 2001). Whilst some free radicals are essential for normal cell function (e.g. the free radicals produced by the metabolism/elongation of C20:4*n*-6), most are highly damaging to cell lipids and proteins (McMurray and Rice, 1982; Vatassery, 1998; Edwards *et al.*, 2001). Conventional cell metabolism leads to the reduction of oxygen and formation of peroxides and superoxides, which are a significant source of free radicals (Vatassery, 1998).

1.3.3.2. Lipid peroxidation

Lipid peroxidation is the inevitable consequence of a chain reaction initiated by the action of hydroxyl radicals ($OH^{\cdot}$) on polyunsaturated fatty acids, leading to oxidative damage to membrane lipoproteins and fatty acids (Figure 1.10.). Peroxidation occurs when a polyunsaturated fatty acid is attacked by a free radical, with the consequent removal of an electron from the fatty acid and the formation of a lipid radical ($L^{\cdot}$; Conn *et al.*, 1987). With the addition of oxygen, the reaction continues to produce a highly potent lipid peroxyl radical ($LOO^{\cdot}$) (Herdt and Stowe, 1991). The lipid peroxyl radical attacks a further PUFA molecule and continues the chain reaction (Herdt and Stowe, 1991).

1.3.3.3. Vitamin E's mode of action against peroxidation

Vitamin E may prevent the peroxidation of unsaturated fatty acids by donating a hydrogen atom from its chromanol ring to the lipid radical and reforming the reduced lipid molecule (LH; Ehrenkranz, 1980).

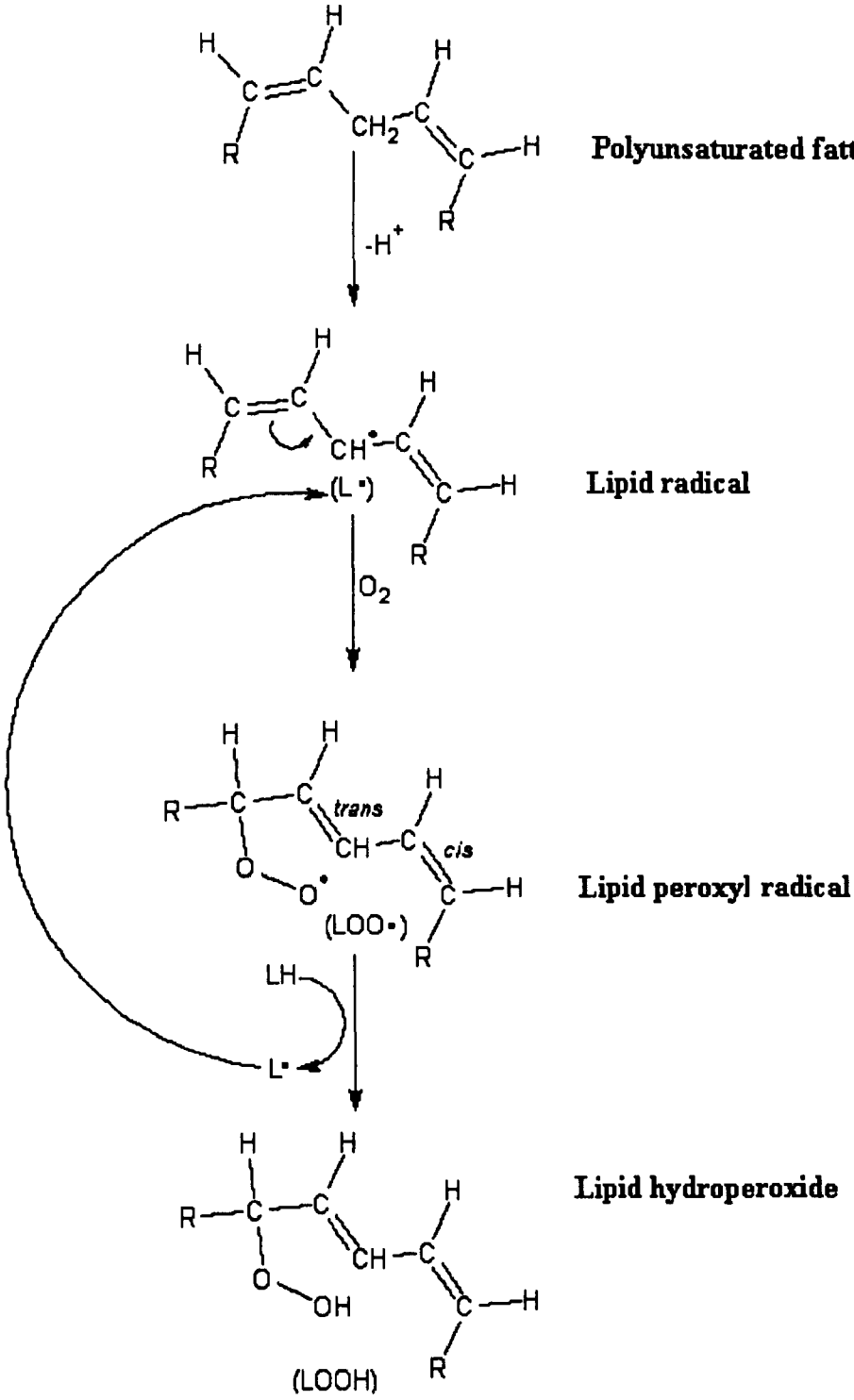


Figure 1.10. Lipid peroxidation (Conn et al., 1987)

Alternatively it may donate a hydrogen atom to the lipid peroxyl, resulting in the formation of a lipid hydroperoxide (LOOH) (Wiseman, 1996; Chaudiere and Ferrari-Iliou, 1999). Although an α-tocopherol radical is formed, this radical is non-reactive and does not further propagate the chain reaction (Herdt and Stowe, 1991). Furthermore, α-tocopherol

is able to scavenge the peroxyl radical approximately 10 times faster than the lipids react with PUFAs (Herdt and Stowe, 1991). The antioxidant potency of vitamin E is dependent upon the differing rates of the reactions that form lipid peroxyl radicals, and those which scavenge the peroxyl radical (Niki, 1996).

The resonance-stabilised α-tocopherol radical may then be "recycled" by the donation of an electron from ascorbic acid (Halpner *et al.*, 1998). Putnam and Comben (1987) therefore postulated that an adequate ascorbic acid supply may reduce the basal vitamin E requirement and that symptoms of vitamin E deficiency may be exacerbated by low cellular concentrations of ascorbic acid. It is also suggested that the coenzyme ubiquinol may perform a similar role to ascorbic acid (Wiseman, 1996; Chaudiere and Ferrari-Iliou, 1999). Alternatively, the α-tocopherol radical may scavenge a second lipid-peroxyl radical and decompose to α-tocopherol-quinone (Halpner *et al.*, 1998; Figure 1.11).

1.3.3.4. The vulnerability of cellular membranes to lipid peroxidation

The prevention of lipid peroxidation by α-tocopherol depends on the relative quantities of α-tocopherol, polyunsaturated fatty acids and other reactive oxidative species within the cell.

Deposition of α-tocopherol primarily occurs at cellular sites where free radicals are produced in high quantities, i.e. within the membranes of the mitochondria and endoplasmic reticulum (Dutta-Roy, 1999), and in membranes with high PUFA contents (Herdt and Stowe, 1991). The susceptibility of individual cell membranes to lipid peroxidation increases with the concentration of unsaturated fatty acids within the membrane, and the level of unsaturation of those molecules (McMurray and Rice, 1982). Therefore, a membrane containing a high proportion of unsaturated fatty acids is more susceptible to peroxidation than one with a low unsaturated fatty acid content.

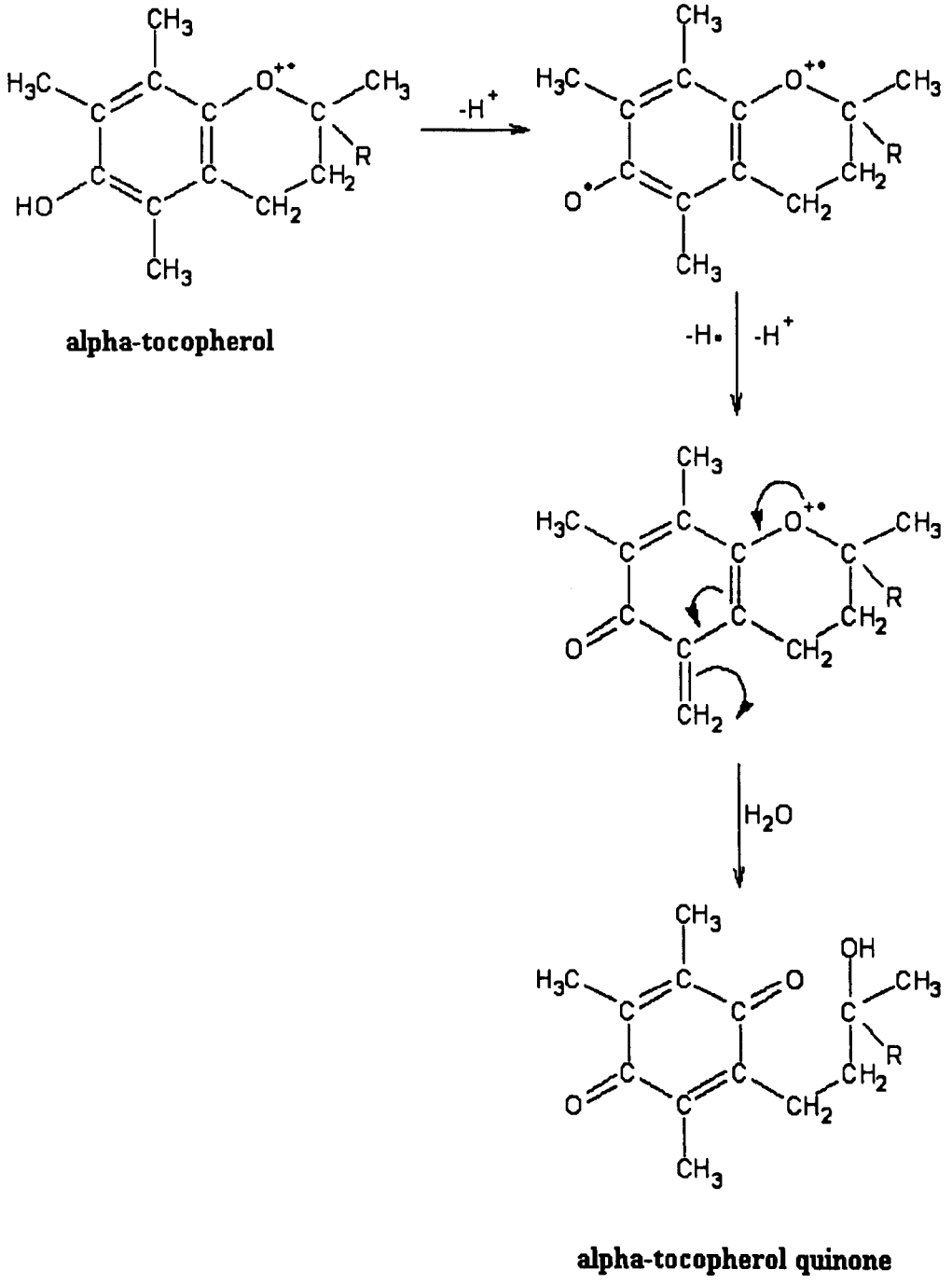


Figure 1.11. Conversion of alpha-tocopherol to alpha-tocopherol quinone (Chaudiere and Ferrari-Iliou, 1999)

Vitamin E acts as a structural component within cell membranes by introducing methyl groups of the chromanol ring within non-aqueous compartments of the membrane (Putnam and Comben, 1987; Wiseman, 1996). The structure of vitamin E resembles that of the membrane lipids, thus the molecule binds within a chemical complex of PUFAs and

membrane proteins, improving membrane stability via a reduction in membrane fluidity (Ehrenkranz, 1980).

1.3.3.5. Vitamin E and its interaction with glutathione peroxidase

Vitamin E acts synergistically with other biochemical compounds to prevent lipid peroxidation and specifically with the selenoprotein enzyme glutathione peroxidase (GPx). This enzyme interacts with α-tocopherol, removing peroxides within the cell by reducing them to their alcohol forms (Maas *et al.*, 1984; Putnam and Comben, 1987; Bourre *et al.*, 2000; Daun *et al.*, 2001). Increased concentrations of GPx in tissues are often correlated with low α-tocopherol concentrations, suggesting that GPx and vitamin E may have a complex, possibly compensational, relationship in the defence against lipid peroxidation (Daun, 2001). Nonetheless, although the actions of these two antioxidants complement each other, an excess of one does not compensate for a deficiency of the other. Furthermore, tissue GPx activity is a reliable indication of the selenium status of the animal.

1.3.4. Vitamin E and selenium deficiency

Vitamin E and selenium deficiency diseases may occur in all domesticated farm animals, but are manifested as various conditions and non-specific syndromes (McDowell *et al.*, 1996). In ruminants, deficiencies in vitamin E and selenium relative to the dietary peroxidation challenge leads to nutritional myopathy, also called nutritional muscular dystrophy, stiff-lamb disease or white muscle disease (Saez *et al.*, 1996; El-Neweehy *et al.*, 2000). The disease may be a result of metabolic factors triggering intracellular damage which is exacerbated by vitamin E or selenium deficiency, or may be a secondary disease resulting from a primary deficiency syndrome (McMurray and Rice, 1982).

1.3.4.1. Underlying causes of vitamin E and selenium deficiency

Nutritional myopathy was first identified in the UK in 1953 in young beef calves (Rice and McMurray, 1982). It mainly occurs in growing animals at turnout to pasture, especially after a prolonged period of housing on a diet low in vitamin E and selenium (<10 mg/kg of α-tocopherol and 0.2 mg/kg of selenium; Pehrson *et al.*, 1986; Hakkarainen *et al.*, 1987). It may also occur in animals supplemented with feedstuffs grown in selenium-deficient areas (Walsh *et al.*, 1993). Alternatively, it may be identified at birth as congenital nutritional myopathy in neonates born to dams deficient in selenium or vitamin E (Bostedt and Schramel, 1990). It has been demonstrated that cattle on low-fat diets do not develop nutritional myopathy, even when fed very low levels of vitamin E (Herdt and Stowe, 1991). Vitamin E and selenium deficiency does not inevitably lead to disease (Putnam and Comben, 1987) and mild or subclinical deficiencies are relatively common (Rice and McMurray, 1982; Topps and Thompson, 1984).

1.3.4.2. Symptoms of vitamin E and selenium deficiency

Symptoms of vitamin E and selenium deficiency vary according to species and severity of the disease (Table 1.10). The acute form of nutritional myopathy is manifested as sudden death from cardiac failure in young animals, with few preceding symptoms as a result of lesions within the cardiac muscle (Kennedy and Rice, 1992). By contrast, the first symptom of the moderately acute syndrome is gait stiffness, followed by an unwillingness or inability to stand with muscle tremors, laboured abdominal-type respiration and finally death due to cardiac arrest (Steele *et al.*, 1980). Sub-clinical or chronic nutritional myopathy may present itself as infertility in ewes and retarded growth in young animals (Saez *et al.*, 1996). Blood samples taken from diseased lambs show low levels of plasma α-tocopherol (<1.0 μg/ml), plasma selenium (<0.04 mg/kg) and erythrocyte GPx (<35 IU/g Hb) in conjunction with high levels of serum enzymes creatine kinase (CK), lactic dehydrogenase, aspartate transaminase and glutamic oxalacetic transaminase (Maas *et al.*,

Table 1.10. Symptoms of vitamin E and selenium deficiency

Species	Symptoms	Reference
Sheep	Weakness, staggering gait, frothing at mucous membranes, inability to stand	(Steele *et al.*, 1980)
Sheep	Low blood selenium concentrations, inability to walk or extend limbs fully, rigid skeletal muscles, mottled pale muscles at necropsy, swollen muscle fibres, blood and fluid accumulation in liver and kidney	(Maas *et al.*, 1984)
Sheep	Reduced GSHPx activity, reduced selenoproteins, suppressed immunity	(Rock *et al.*, 2001)
Sheep	Locomotive disorders, joint stiffness, ataxic movement, muscle tremors, dyspnea	(El-Neweehy *et al.*, 2000)
Sheep	Cardiac and skeletal muscle lesions, respiratory muscle lesions, pneumonia, inability to stand or suckle, still-birth	(Fitt and Packington, 1998)
Sheep	Reduced levels of α-tocopherol and GSHPx, increased level of serum creatine kinase	(Rice and McMurray, 1982)
Sheep	Infertility in ewes (subclinical) and reduced growth in lambs	(Saez *et al.*, 1996)
Cattle	Skeletal and cardiac myonecrosis, lameness, sudden death	(Walsh *et al.*, 1993)
Cattle	Inability to stand, stiff gait, increased serum aspartame aminotransferase and creatine kinase	(Pehrson *et al.*, 1986)
Cattle	Cardiac lesions, reduced α-tocopherol, GSHPx and haemoglobin levels, muscular dystrophy	(McMurray and Rice, 1982)

1984; Pehrson *et al.*, 1986; El-Neweehy *et al.*, 2000). At necropsy, bilateral lesions may be seen in skeletal and cardiac muscles and tissues appear pale and swollen due to both deposition of calcium in necrosed areas, and oedema (McMurray and Rice, 1982).

1.3.4.3. Prevention and cure of vitamin E and selenium deficiencies

Nutritional myopathy may be prevented by the provision of adequate dietary vitamin E and selenium relative to the dietary PUFA intake. Indeed, El-Neweehy *et al.* (2000) reported that the provision of vitamin E and selenium to affected Nadji lambs successfully reduced clinical symptoms. Oral vitamin E supplementation has also been reported to prevent nutritional myopathy induced by selenium deficiency (Steele *et al.*, 1980). Moreover, Walsh *et al.* (1993) successfully induced nutritional myopathy in calves by the provision of dietary PUFAs, but were unable to stimulate similar symptoms in lambs supplemented with vitamin E and selenium.

An injection of 1-2 mg selenium and 46 mg of α-tocopherol was confirmed by Maas *et al.* (1984) to return blood parameters of clinically affected lambs to normal levels within 21 days, though the response was relatively slow and was dependent on the severity of the initial symptoms. In view of their complementary, yet separate roles within the cell, supplementation with vitamin E may not resolve disease symptoms caused by selenium deficiency and *vice versa* (Maas *et al.*, 1984). It is difficult to determine whether an individual case of nutritional myopathy will respond to α-tocopherol or selenium, therefore, treatment with a combination of the two nutrients is advised (El-Neweehy *et al.*, 2000).

1.3.5. Vitamin E and its role in the immune system

Although the primary role of vitamin E is as a cellular antioxidant, it has a number of other functions within the animal (Putnam and Comben, 1987). Arguably the most significant

non-antioxidant function of vitamin E is the essential role it plays in immune system function. It has been demonstrated that vitamin E supplementation stimulates T-helper cells, thereby enhancing the animal's immune response (Putnam and Comben, 1987; Azzi and Stocker, 2000). Furthermore, in neonatal animals, supplementation of the dam with vitamin E increases the transfer of immunoglobulins from the dam to offspring via colostrum (Sikka *et al.*, 2002). Vitamin E also appears to reduce the production of both immunosuppressive glucocorticoids, and the inflammatory products of C20:4*n*-6 metabolism (Hidiroglou *et al.*, 1992).

1.3.6. Ruminant digestion of vitamin E

1.3.6.1. Degradation of vitamin E within the rumen

There is conflicting evidence as to whether, in ruminants, the majority of ingested vitamin E is absorbed through the small intestine, or if it is subject to ruminal degradation. Dietary vitamin E loss before the duodenum has been shown to increase from 8-42 % in sheep as the concentrate proportion of the diet increases from 20-80 % (Leedle *et al.*, 1993). Therefore, Leedle *et al.* (1993) suggested that only a proportion of dietary vitamin E is absorbed at the small intestine and that the amount degraded in the rumen has a high positive correlation with the concentrate content of the diet. Nonetheless, Chikunya *et al.* (2004) reported a flow of vitamin E equal to 90 % of dietary intake at the small intestine in supplemented sheep, a flow which was unaffected by the concentration of PUFAs within the diet. The characteristics of the rumen microorganism population change significantly when diet high in concentrates is fed, therefore it is possible that this change may favour a microbial strain that has a higher propensity for α-tocopherol degradation.

Leedle *et al.* (1993) investigated the supposition that vitamin E was degraded by rumen microorganisms using fistulated cattle fed a diet with a high concentrate:forage ratio. Concentrations of α-tocopherol acetate equivalent to 450 mg/animal/day were added to

rumen contents and the amount remaining after a 24 hour incubation period was recorded. Results suggested that rumen microorganisms do not degrade vitamin E and ruminal esterase enzymes are not biologically active upon α-tocopherol acetate. The double bonds of α-tocopherol are located within the aromatic ring and consequently are not easily biohydrogenated or anaerobically degraded. It is therefore logical to hypothesise that the potential for ruminal degradation is negligible. This is in contrast to the results published by Chikunya *et al.* (2004) who reported ruminal losses of 9 % for high vitamin E and 21 % for low vitamin E diets fed to sheep. It was suggested that α-tocopherol acetate is more stable in the rumen than *RRR*-α-tocopherol due to inherent biochemical differences; however, this hypothesis was disproved by Han and Owens (1999).

1.3.6.2. Small intestinal digestion and absorption of vitamin E

During lipid digestion, vitamin E is emulsified with fat globules within the small intestine and is incorporated into a water-soluble micellular solution of fatty acids and bile salts (Hollander *et al.*, 1975; Hollander, 1981; Borel *et al.*, 2001). Micelles are then transported through the unstirred water layer towards the membrane of the microvilli (the rate-limiting step in fat-soluble vitamin absorption) and vitamin E passively diffuses across the brush border of the upper small intestine (Figure 1.12; Hollander *et al.*, 1975; Hollander, 1981). As vitamin E is absorbed with the fatty acid component of the diet, any disruption or inefficiency in lipid digestion affects the absorption of vitamin E (Herdt and Stowe, 1991).

Hollander (1981) reported that, although medium-chain fatty acids promote vitamin E absorption in the small intestine, PUFAs inhibit the rate of absorption. A higher rate of inhibition was also observed with increasing PUFA concentration within the diet (Hollander, 1981). This may result from increased micelle size or the enhanced negative surface charge of micelles containing concentrations of high vitamin E (Hollander, 1981). Apparent losses of vitamin E between ingestion and incorporation into plasma may

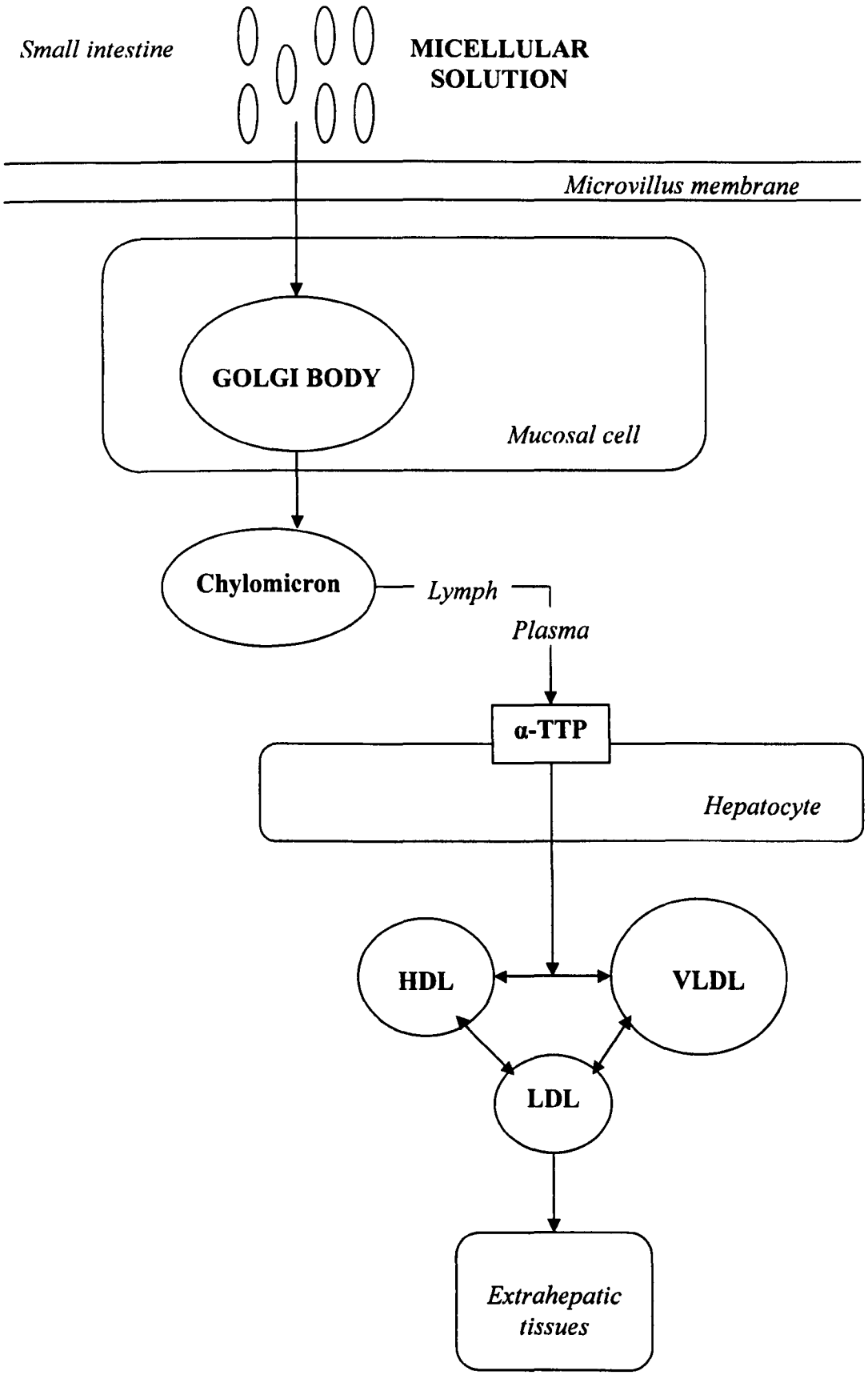


Figure 1.12. Vitamin E absorption and transport (Azzi and Stocker, 2000)

therefore be attributed to reduced intestinal absorption. Indeed, the study of Wachira (1999) indicated that the absorption of vitamin E in growing lambs fed on concentrate-based diets was particularly low. This was attributed to an interaction between dietary vitamin E and PUFA supplementation.

1.3.6.3. Post absorption metabolism of vitamin E

Following absorption, chylomicrons are exocytosed to the lymphatic system and released into the blood (Ehrenkranz, 1980). The liver then assimilates Vitamin E during chylomicron metabolism (Herdt and Stowe, 1991; Azzi and Stocker, 2000). A transfer protein specific to α-tocopherol (α-TTP) is responsible for the uptake of α-tocopherol from hepatic cells into very low-density lipoproteins (Schelling *et al.*, 1995). The specificity of this protein may explain the preponderance of α-tocopherol in plasma and tissues compared to the other tocopherol isomers (Schelling *et al.*, 1995). The plasma phosphatidylglycerol transfer protein (PLTP) facilitates the exchange of α-tocopherol between HDL and LDL fractions, and these lipoproteins deliver the vitamin to target cells (Azzi and Stocker, 2000). Azzi and Stocker (2000) also described a tocopherol-binding protein (TAP: tocopherol-associated protein) found in liver, prostate and brain, which is hypothesised to be responsible for the regulation of α-tocopherol concentrations in these tissues. Some discrimination has also been observed between *RRR*-α-tocopherol and *all-rac*-α-tocopherol within human and animal tissues, suggested to be due to a specific transfer protein (Hidiroglou *et al.*, 1992; Traber, 1996). At high supplemental doses, this discrimination is not apparent, possibly due to saturation of receptor sites (Behrens and Madère, 1991).

1.3.6.4. Vitamin E storage within animal tissue

The majority of absorbed vitamin E is stored within fat droplets of adipose tissue and muscle with a small amount present in liver (Herdt and Stowe, 1991; Traber, 1996).

Adipose tissue vitamin E turnover is extremely slow and analysis may give an indication of long-term vitamin E status. By contrast, liver and muscle provide both a labile source of vitamin E and a site for prolonged storage (Morrissey *et al.*, 1993).

1.3.7. The ruminant vitamin E requirement

The Agricultural Research Council (1980) do not prescribe an official vitamin E requirement for ewes or lambs save for a suggestion that 10-15 mg/kg DM is the minimal concentration required in the diet. However, it is logical to suggest that the dietary supply should be related to the minimum basal requirement for antioxidant vitamins. Furthermore, Thakur and Srivastava (1996) proposed that the additional vitamin E requirement conferred by dietary nutrients that increase the peroxide challenge to the animal (e.g. PUFAs) should also be considered when proposing a dietary allowance for vitamin E. Moreover, the status of the animal in terms of other antioxidant compounds and possible compensational interrelationships should be considered.

1.3.7.1. Quantification of the ruminant vitamin E requirement

To define the ruminant's requirement for a specific nutrient, it is essential to quantify the threshold of dietary supply below which clinically-detectable deficiency symptoms occur (Hyldgaard-Jensen, 1977; Putnam and Comben, 1987), and to manipulate tissue concentrations of that nutrient until appropriate end-points are reached (Ward *et al.*, 1996). However, the vitamin E requirement of ewes may be further confounded by the composition of the diet (Putnam and Comben, 1987; Farnworth *et al.*, 1995). It has been postulated that the vitamin E requirement should be correlated with the PUFA intake, assuming that an adequate dietary supply of selenium is provided (Herdt and Stowe, 1991; Farnworth *et al.*, 1995). Putnam and Comben (1987) suggested that optimum ruminant allowances might be better expressed as requirements according to liveweight and production level, for example, 1 mg vitamin E/day/kg liveweight plus 5 mg vitamin E/kg

milk production and 3 mg vitamin E/g dietary PUFAs. Furthermore, Pehrson *et al.* (1986) recommend a supplementary level of 1 mg α-tocopherol per 0.6 g supplementary dietary PUFAs. Plasma vitamin E concentrations are correlated both with the amount stored in liver and dietary intake, and may be a suitable indicator of vitamin E status in mature animals (Hidiroglou *et al.*, 1992). The index proposed by Hidiroglou *et al.* (1992) for use in cattle may therefore be used to evaluate the adequacy of nutritional supplementation upon plasma vitamin E concentrations (Table 1.11).

Table 1.11. Adequacy of plasma vitamin E concentrations in ruminants (Hidiroglou et al., 1992)

Vitamin E status	Plasma α-tocopherol (mg/kg)	Plasma α-tocopherol (μmol/l)
Deficient	< 2.0	< 4.65
Marginal	2.0-3.0	4.65-6.97
Minimal	3.0-4.0	6.97-9.30
Adequate	> 4.0	> 9.30

1.3.7.2. Vitamin E requirements of the pregnant ewe and neonatal lamb

The vitamin E requirements of pregnant ruminants are further complicated in that the foetal requirement must be estimated (Farnworth *et al.*, 1995). Plasma vitamin E concentrations are negligible in neonatal animals (Martin and Hurley, 1977; Agricultural Research Council, 1980; Van Saun *et al.*, 1989) and this has led to the supposition that neonatal lambs are deficient in vitamin E as a result of low placental transfer (Mahan and Vallet, 1997; Fitt and Packington, 1998). Cuesta *et al.* (1995) suggested that this correlation between maternal and foetal bovine serum vitamin E indicates that placental transfer is insufficient, despite increased plasma vitamin E concentrations being observed in lambs born to supplemented ewes. The placenta also appears to be able to discriminate between transfer of *RRR*-α-tocopherol and *all-rac*-α-tocopherol to the foetal lamb, resulting in lower levels of the latter isomer within foetal plasma (Acuff *et al.*, 1998). Merrell (1998) reported that vitamin E supplementation of pregnant ewes increased plasma

concentrations of this vitamin in neonatal lambs. However, as plasma samples were taken at 24-36 hours of age they would have been confounded by colostrum consumption. It is not clear whether plasma vitamin E is a reliable indicator of vitamin E status, especially in neonatal animals at risk of deficiency (Vatassery *et al.*, 1988).

Vitamin E is efficiently transported across mammary tissue, resulting in relatively high concentrations in colostrum and suckling lamb plasma (Kott *et al.*, 1998; Merrell, 1998). Consequently, some researchers consider that the foetal vitamin E requirement is impossible to satisfy via maternal supplementation during gestation, and that providing a high vitamin E supply via colostrum is sufficient to meet neonatal lamb requirements (Mahan and Vallet, 1997; Fitt and Packington, 1999). An adequate vitamin E supply is vital for the formation and maintenance of cell membranes during rapid postnatal growth in the neonatal lamb. As the diet of the neonate consists solely of milk for the first few weeks of life, the only mechanism by which the neonatal lamb vitamin E supply may be manipulated is via the diet of the lactating ewe (Maas *et al.*, 1984). A specific requirement, other than the increase in requirements with an increase in unsaturated fatty acids, is therefore yet to be established.

1.3.8. Effect of supplemental vitamin E upon neonatal lamb vigour and mortality

Both Merrell (1998) and Kott *et al.* (1998) demonstrated improvements in lamb vigour when ewes were supplemented with vitamin E during pregnancy. Consequently, the hypothesis that vitamin E does not cross the placenta in any appreciable amount may be appropriate for further investigation. It has been postulated that vitamin E has an effect on the vigour and survival of neonatal lambs, especially in adverse environmental conditions, due to the correction of sub-clinical maternal deficiencies by supplementation (Merrell, 1998). Kott *et al.* (1998) supplemented pregnant ewes with either basal (30 mg/day) or high (250 mg/day) levels of vitamin E and detected a significant reduction in lamb

mortality during the early lambing season. Consequently, the total weight of lambs weaned per ewe was significantly greater for ewes supplemented with high levels of vitamin E. Kott *et al.* (1983) also reported reduced pre-weaning mortality of lambs produced by ewes supplemented with 122 mg α-tocopherol acetate compared to control ewes. In a similar study by Merrell (1998), ewes were supplemented with 33 IU or 133 IU/day of vitamin E during pregnancy, but supranutritional vitamin E supplementation had no effect on lamb mortality. This result may have been confounded by favourable weather conditions that reduced the typical lamb mortality figure by 50 %. Nevertheless, Merrell (1998) observed that lambs from ewes supplemented with high levels of vitamin E were more vigorous immediately after birth and ingested colostrum approximately three minutes earlier than unsupplemented lambs. Williamson *et al.* (1995) also reported that maternal vitamin E supplementation (804 mg IU at two weeks *pre-partum*) improved lamb vigour score but that lamb supplementation (404 mg injection at birth) did not affect lamb behaviour. The biochemical mechanism by which vitamin E crosses the placenta and affects lamb behaviour has yet to be defined.

1.3.9. Effect of supplemental vitamin E upon birthweight and growth rate

Kott *et al.* (1998) observed that birthweights and growth rates of lambs from ewes supplemented with vitamin E were similar to those of unsupplemented ewes. Furthermore, Martin and Hurley (1977) fed up to 500 mg vitamin E/day to pregnant rats and reported no effect of supplementation on litterweight. The latter study is in contrast to the results of Gentry *et al.* (1992) who reported an increase in the birthweight of lambs produced by ewes injected with 1005 mg of α-tocopherol at 21 days *pre-partum*.

Gentry *et al.* (1992) also reported that lamb liveweight gain was increased by vitamin E supplementation of the ewe and lamb. This study agrees with the report of Norton and McCarthy (1986) who recorded significantly higher liveweight gains in ram lambs from

ewes supplemented with vitamin E. Williamson *et al.* (1995) reported an increase in lamb liveweight gain resulting from maternal vitamin E supplementation, but no significant effect of direct supplementation of the lamb. This suggests that the observed effect may have been due either to differences in milk production or composition; or a difference in vitamin E utilisation between intramuscular injections and dietary sources. Macit *et al.* (2003a) found that food conversion efficiency was significantly improved by the vitamin E supplementation of growing lambs, and in a similar study, showed that lamb supplementation significantly increased daily liveweight gain (Macit *et al.*, 2003b). Gentry *et al.* (1992) suggest that these improvements in liveweight gain and FCR in growing animals may be attributed to an enhanced immune status. It is not possible to conclude with any certainty whether the performance response to supplementation is due simply to an improvement in vitamin E status, or to the mitigation of reduced performance resulting from sub-clinical deficiency, especially when significant responses are seen at relatively low supplementation levels.

1.4. CONCLUSION

Research in human infants and pigs suggests that supplementation of pregnant ruminants with long-chain PUFAs may improve neonatal lamb vigour and performance. This may results from increased deposition of DHA into neural tissue. However, given the hydrogenating effect of the rumen upon PUFAs, further research is needed to elucidate whether it is possible to improve lamb vigour via maternal dietary supplementation. Supplementation of ruminants with vitamin E appears to have significant effects upon neonatal lamb behaviour. Nevertheless, the majority of evidence suggests that vitamin E cannot cross the placenta from the dam to the foetus. Consequently, the mechanisms by which the effects on vigour are mediated warrant further investigation.

2. GENERAL MATERIALS AND METHODS

2.1. Routine Experimental Procedures

2.1.1. Animals

Ewes belonging to the Harper Adams University College early-lambing flock (Edgmond, Newport, Shropshire, UK), containing 160 Suffolk x North of England Mule, Friesland x Lleyn and Charollais x Lleyn ewes, were oestrus synchronized in July of 2000 and August of 2001 and 2002. Progestagen-impregnated sponges (Chronogest; Intervet UK Ltd, Cambridge, UK) were inserted into the vagina of ewes, removed after 14 days and the ewes injected with the appropriate amount of pregnant mare serum gonadotrophin (PMSG; Table 2.1) via the intramuscular route. Within Experiments One and Three, ewes were served by Charollais rams during the first oestrus after sponge removal. In Experiment Two, laparoscopic artificial insemination was performed upon ewes using semen from Charollais rams as a consequence of the foot and mouth outbreak in the UK. Rams were subsequently grazed with the ewes for a 30 day period. Raddle crayons were attached to rams 21 days after they were first allowed access to ewes and crayon marks used to identify ewes that had not conceived to the first service. At 84 days of gestation, ewes were ultrasonically scanned to determine the number of foetuses carried.

Table 2.1. Dosage of pregnant mare serum gonadotrophin (PMSG) given to ewes in Experiments One, Two, and Three

Experiment	Breed	PMSG dosage (IU)
1	Charollais X Lleyn	350
	Lleyn X Friesland	350
	Suffolk X North of England Mule	500
2	Lleyn X Friesland	400
	Suffolk X North of England Mule	400
3	Suffolk X North of England Mule	400

2.1.2. Straw intake

Daily straw intake for ewes in Experiments One and Two was calculated by feeding a set amount of straw at the start of the experiment, weighing back the refused straw three times per week (Monday, Wednesday and Friday) at 07.30 and increasing the amount of straw fed the day after each weigh-back by a factor of 1.25. Daily intakes were then calculated from the weekly data. During Experiment Three, straw intake was fed at a flat rate during pregnancy, calculated according to data collected in Experiments One and Two, and the refused material weighed back once per week (Tuesdays at 07.30). The straw intake was then stepped from weeks +1 to +4 of lactation and the same method of intake calculation applied.

2.1.3. Ewe liveweight and condition score

The liveweight and condition score of ewes in all three experiments was measured at 14.00 on Wednesday of each week during the trial. Ewes were weighed using an EziWeigh apparatus in Experiments One and Two (Tru-Test Ltd, Auckland, New Zealand) and an I.A.E scales system (Industrial and Agricultural Engineers, Riverside Works, Macclesfield Road, Leek, UK) in Experiment Three. All scales were calibrated using standard weights. Immediately post-weighing, ewe condition score was assessed, by the same technician over the three experiments, using the method described by Russel *et al.* (1969).

2.1.4. Lamb liveweight

Lambs were weighed at +12 hours post-lambing in Experiments One and Two and at three hours *post partum* in Experiment Three to give an indication of birthweight without confounding maternal and neonatal behavioural measurements. Subsequently, lambs were weighed at 10.00 at 7, 14, 21 and 28 days of age using FG-60K scales (A and D Co., Japan) calibrated using standard weights.

2.1.5. Colostrum and milk

Milk yield was measured and colostrum and milk samples obtained using a method adapted from Doney *et al.* (1979). At +12 hours *post partum* lambs were confined behind a wire mesh barrier within the pen, providing visual, olfactory and limited tactile contact with the ewe but preventing suckling. A 1 ml intramuscular injection of oxytocin (Oxytocin Leo, LEO Animal Health, Buckinghamshire, UK) was then administered to the ewe and the ewe hand milked until the udder was empty. Colostrum was fed to the lambs via a stomach tube if required. At +4 hours from the end of the first milking, another 1 ml intramuscular injection of oxytocin was administered and the ewe fully hand milked before the lambs were returned to the ewe. The time interval between the end of the first and second milkings and the volume of colostrum produced during this interval was recorded and 4 x 50 ml sub-samples were taken and stored at -20 °C until analysis. The same method was repeated at 21 (Experiment One) or 28 (Experiments Two and Three) days *post partum* to obtain milk samples and a measure of yield. Milk and colostrum secretion rate (ml/hour) were calculated according to the following equation:

Yield (ml)/[Time interval between the end of the first and second milkings (hours)]

2.2. Parturient measurements

2.2.1. Lambing routine

The duration of lambing was recorded as the time from the appearance of the first lamb at the vulva to the expulsion of the final (second or third) lamb. To avoid confounding maternal and behavioural observations, assistance was only provided to ewes during parturition if the lamb was not seen one hour after fluids appeared at the vulva; if a lamb was present at the vulva for two hours without further progress or if contractions occurred for two hours without the appearance of parturient fluids or a lamb. Assistance given was minimal and consisted of correcting lamb presentation unless the ewe was considered to be

unable to continue without further intervention. Assistance was recorded by the codes shown in Table 2.2. Assistance beyond the correction of presentation was used as a co-variate when analysing behavioural measurements as this was reported by Cagnetta *et al.* (1995) to influence neonatal behaviour. In Experiments One and Two, stillborn lambs were replaced by lambs that were fostered on from a group pen of surplus ewes to balance *post partum* data.

Table 2.2. Codings used for assisted lambing

Assistance (1)		Assistance (2)		Code
Corrected Presentation	1 leg back			C1
	2 legs back			C2
	Head back			C3
	1 leg + head back			C4
	Breech			B
Pulled	< 2 min			P1
	> 2 and < 5 min			P2
	> 5 min			P3
Corrected Presentation	1 leg back	**Pulled**	< 2 min	C1 P1/2/3
	2 legs back		> 2 and < 5 min	C2 P1/2/3
	Head back		> 5 min	C3 P1/2/3
	1 leg + head back			C4 P1/2/3
	Breech			B P1/2/3
Caesarian				CAE

2.2.2. Maternal behaviour

Ewes were focal sampled after birth until both lambs had successfully suckled, and the performance of specific behaviours recorded. Two maternal behaviour scores were calculated for each ewe (one per lamb) and combined to give a total maternal behaviour score according to the method described in Table 2.3. In addition, in Experiments Two and Three, the latencies of three specific maternal behaviours were recorded (time taken from expulsion of the lamb to the ewe standing; making contact with the lamb and vocalising). Definitions of maternal behaviours were adapted from Dwyer and Lawence (1999) and are shown in Table 2.4.

Table 2.3. Maternal behaviour scoring system

Behaviour	Score
Ewe gets up within 1 minute of lamb expulsion	15
Ewe gets up within 3 minutes of lamb expulsion	10
Ewe gets up within 5 minutes of lamb expulsion	5
Ewe does not get up	0
Ewe initiates close contact with lamb	25
Ewe approaches lamb but does not initiate physical contact	15
Ewe does not approach lamb	5
Ewe withdraws from lamb (desertion)	0
Ewe vigorously grooms lamb	50
Ewe grooms lamb but is easily distracted/loses interest	30
Ewe makes sniffs/noses lamb	10
Ewe does not groom lamb	0
Frequent low-pitched vocalisation (1 bleats/min) by ewe	20
Occasional vocalisation by ewe (0.3 bleats/min)	10
Ewe does not vocalize	0
Ewe stands and facilitates suckling	40
Ewe stands allowing lamb to suck	30
Ewe circles when lamb attempts to suck	20
Ewe moves forwards/backwards when lamb attempts to suck	10
Ewe kicks/butts lamb and will not allow lamb to suck	0

Table 2.4. Definitions of maternal and neonatal behaviours[a]

Animal	Behaviour	Definition
Ewe	Grooming	Licking and nibbling movements directed towards the lamb
Ewe	Sniffing/nosing	Ewes touches the lamb with her nose with no evidence of grooming
Ewe	Withdrawing	Ewe moves back directly from away from the lamb at her head (2+ steps)
Ewe	Facilitates sucking	Ewe crouches and turns one hindleg out to aid lamb sucking
Ewe	Circling (in response to sucking)	Ewe moves hindquarters away from lamb and attempts to maintain contact at head
Ewe	Moving backwards	Ewe moves backwards away from lamb
Ewe	Moving forwards	Ewe steps forwards over the top of lamb
Ewe	Butting	Ewe pushes lamb down or away with downwards, sideways or forwards movements of her head
Ewe	Abandonment/rejection	Ewe does not lick lamb, butts lamb if it approaches
Lamb	Standing successfully	Lamb supports itself on all four feet for at least 5 seconds
Lamb	Seeking the udder	Lamb has head under ewe in udder region
Lamb	Successful suck	Lamb has teat in its mouth, appears to suck for > 5 seconds

[a] adapted from Dwyer and Lawrence, 1999

2.2.3. Neonatal behaviour

Neonatal lambs were focal-sampled from expulsion until successful suckling and the latencies of standing, searching for the udder and successful suckling were recorded according to the definitions in Table 2.4.

2.2.4. Slaughter procedure

The second-born triplet lamb from each triplet-bearing ewe was removed from the ewe immediately after expulsion and weighed using FG-60K scales (A and D Co., Japan) calibrated with standard weights. The lamb was then removed to a separate room and euthanased by an intravenous injection of sodium pentobarbitone (200mg/ml, Pentoject, Animalcare Ltd, Common Road, Dunnington, York, UK) into the jugular vein at a dosage of 0.8 ml/kg bodyweight. Cessation of the heartbeat was then confirmed by stethoscope. Blood samples were obtained by cardiac puncture and collected in evacuated tubes containing lithium heparin as an anticoagulant. Plasma was produced from the sample as described in section 2.3.4.1. The brain was then removed according to the procedure documented by the USDA Plant and Animal Health Inspection Service (2001) and the *semimembranosis* muscle was dissected out from the right hind leg. Tissue and plasma samples were stored at –20 °C prior to analysis for fatty acids and vitamin E.

2.3 Analytical procedures

2.3.1. Feed analysis

2.3.1.1. Dry matter

Samples were analysed for DM according to the method described in MAFF (1986). A sub-sample of feed was weighed into a foil tray, dried in an oven at 80 °C for 24 hours and then re-weighed at hourly intervals until a constant weight was achieved. The dry matter content of the sample was calculated according to the following equation:

DM (g/kg) = [Dry sample weight (g)/Fresh sample weight (g)] x 1000

Following dry matter determination, feed samples were ground using a 1 mm mill (Retsch zm- 1000) and further analysis was performed on the ground sample.

2.3.1.2. Ash

Dried, ground sample (2 g) was accurately weighed into a porcelain crucible and the total weight recorded. The sample was then ashed at 550 °C in a muffle furnace (Gallenkamp Muffle Furnace size 3,SanyoGallenkamp PLC, Monarch Way, Loughborough, UK) and cooled within a dessicator vessel. The residual weight of feedstuff remaining within the crucible after cooling was considered to be the ash content and was calculated via the equation below:

Ash (g/kg DM) = [Ashed sample weight (g)/Original sample weight (g)] x 1000

2.3.1.3. Crude protein (CP)

The crude protein content of feedstuffs was calculated via the kjeldahl digestion method. Dried, ground feed (0.5 g) was weighed in duplicate into nitrogen-free filter papers folded to form an envelope and added to digestion tubes containing two kjeldahl tablets (catalyst). Concentrated sulphuric acid (16 ml) was added to each tube, which were then digested at 450 °C for 45 minutes, allowed to cool and 75 ml deionised water added. Samples were then analysed using a Tecator 1035 autoanalyser (Foss UK Ltd., Parkway House, Station Road, Didcot, Oxon, UK) using hydrochloric acid (0.2 M) within a titration reaction.

2.3.1.4. Neutral detergent fibre (NDF)

The neutral detergent fibre content of feedstuffs was determined according to according to the method detailed by Van Soest *et al.* (1991). Dried, ground feed (0.5 g) was weighed into a crucible and digested within a fibretec apparatus (Tecator 1020, Foss UK Ltd., Parkway House, Station Road, Didcot, Oxon, UK) with 25 ml of neutral detergent solution (93 g disodium ethylene diamine tetra-acetate dihydrate (EDTA), 34 g sodium borate, 150 g sodium lauryl sulphate, 50 ml 2-ethoxy ethanol and 22.8 g anhydrous disodium

phosphate made up to 5 litres with distilled water) plus 0.5 ml octanol acting as an anti-foaming agent. After 30 minutes digestion, the sample was washed and filtered three times with 20 ml of deionised water at 80 °C. Exactly 2 ml of α-amylase solution (2.2 g of α-amylase E.C.3.2.1.1. from *bacillus sbtilis*) was dissolved in 99 ml of distilled water, filtered, and 11 ml of 2-ethoxy ethanol added. The resulting solution was mixed with the samples and boiled for 30 minutes, before washing three times with deionised water at 80 °C and once with 20 ml of 100 % acetone. Crucibles and digested samples were removed to an oven and dried at 100 °C overnight. They were then cooled in a dessicator, weighed and ashed at 550 °C for 4 hours. The resulting residue was cooled and weighed, the NDF content of the feedstuff being calculated as:

NDF (g/kg DM) =

[(Residue weight (g) - Ash content (g))/Original sample weight (g)] x 1000

2.3.2. Colostrum analysis

2.3.2.1. Colostrum protein

Colostrum protein content was determined by the kjeldahl digestion method. Samples were defrosted and heated to 40 °C in a circulating water bath before 2 ml colostrum was weighed in duplicate into digestion tubes containing two kjeldahl tablets (catalyst). Concentrated sulphuric acid (16 ml) was added to the tubes, which were then digested at 450 °C for 45 minutes, allowed to cool and 75 ml deionised water added. Samples were then analysed using a Tecator 1035 autoanalyser (Foss UK Ltd., Parkway House, Station Road, Didcot, Oxon, UK) using hydrochloric acid (0.2 M) within a titration reaction.

2.3.2.2. Colostrum fat

The concentration of fat within colostrum samples was determined by the Gerber method described in MAFF (1986). Samples were defrosted and heated to 40 °C in a circulating

water bath, a 10 ml colostrum sample was then diluted to 1 in 3 with distilled water. Sulphuric acid (10 ml) was added to Gerber butyrometer tubes followed by 10.94 ml of diluted colostrum, repeated in duplicate. Mixing of sulphuric acid and colostrum was achieved by the use of a milk pipette for colostrum addition, tilted at an angle of approximately 30° to the top of the tube. Amyl alcohol (1 ml) was added to the tube, resulting in a third distinct layer, a lock stopper was then inserted into the neck and each tube was shaken in a protected stand. Samples were shaken until all white particles within the mixture had disappeared, the butyrometer was then centrifuged at 311 *g* for 5 minutes prior to immersion in a water bath at 65 °C for between three and ten minutes. The fat content of the colostrum was read from the graduated scale on the butyrometer until a constant reading was achieved and the difference between duplicate readings was 0.05 %. The total fat content (g/kg) was calculated by multiplication of the mean of the two duplicate readings by a dilution factor of 30.

2.3.3. Milk analysis

The protein, fat, lactose and solids-not-fat content of milk samples were determined using a Dairylab 2 infrared milk analyser. Samples were defrosted and heated to 40 °C in a circulating water bath before thorough mixing and a 1 ml subsample introduced into the analyser via a metal probe. The analysis was then repeated and the mean composition of the two duplicates recorded. The analyser was calibrated for the analysis of ewe milk using standards of known concentrations of fat (Quality Management, Trenslo House, Tile Street, Bury, Lancashire, UK), ranging from 30-260 g/kg.

2.3.4. Blood analysis

Samples were collected in evacuated "Vacutainer" (BD Vacutainer Systems, Preanalytical solutions, Belliver Industrial Estate, Plymouth, UK) tubes containing the anticoagulants

lithium heparin or potassium oxalate for the production of plasma or whole blood; or into plain tubes containing no additive to produce serum.

2.3.4.1. Initial preparation

Samples for plasma production were immediately centrifuged at 2290 *g* in a Beckman Avanti 30 centrifuge (Beckman Coulter UK Ltd, Kingsmead Business Park, London Road, High Wycombe, Buckinghamshire, UK), the plasma removed and stored in 1.5 ml micro-centrifuge tubes at –20 °C before analysis. Plasma samples from tubes containing potassium oxalate were analysed for urea, β-hydroxybutyrate (βHB) and non-esterified fatty acids (NEFA; Experiment One only). Heparinised plasma was analysed for vitamin E and fatty acids. Blood samples for serum production were stored at 4 °C for four hours after sampling before centrifugation at 2290 *g* in a Beckman Avanti 30 centrifuge. The serum was then removed and stored in 1.5 ml micro-centrifuge tubes at –20 °C before analysis for CK.

Whole blood samples from tubes containing lithium heparin were aliquotted into micro-centrifuge tubes and stored at –20 °C before analysis for GPx. Prior to storage, a sub-sample of whole blood was taken and packed cell volume calculation applied according to the method described in Kerr (2002). Briefly, blood was mixed thoroughly, a capillary tube was filled to approximately three-quarters of its length with the sample and the end sealed with a clay sealant (Cristaseal, Biochrom Ltd, Cambridge Science Park, Milton Road, Cambridge, UK). The tube was then centrifuged for 5 minutes (1000 *g*), removed from the apparatus and the packed cell volume determined using a microhaematocrit reader.

2.3.4.2. Analysis

Urea, βHB and NEFA were determined in plasma samples by photometric analysis using Bayer (Urea; kit number TO1-1819-85, Bayer Diagnostics, Newbury, UK) Randox (βHB; kit number RB1007, Randox, County Antrim, UK) and Wako (NEFA; NEFA C Test, Wako chemicals, USA) methods and reagents. Concentrations of CK in serum were analysed by a enzymatic method using the TO1-1882-85 kit from Bayer; GPx concentrations were analysed by an enzymatic procedure using the RS504 kit manufactured by Randox. All analyses were performed on a Bayer Technicon Ra-1000 autoanalyser (Bayer Plc, Strawberry Hill, Newbury, Berkshire, UK).

2.3.5. Fatty acid analysis

2.3.5.1. Feed samples

Feed fatty acid extraction was performed according to the method described by Wachira *et al.* (2002), derived from Folch *et al.* (1957). Approximately 300 mg of ground, thawed feed was weighed into a 30 ml Pyrex screw-top tube and the exact sample weight recorded. To this sample was added 6 ml saponification mixture (140.3 g potassium hydroxide dissolved in 250 ml distilled water plus 0.5 g quinol dissolved in 260 ml methanol) and 100 µl internal C21 standard (150 mg heneicosanoic acid dissolved in 10 ml chloroform). This mixture was then saponified in a circulating water bath at 60 °C for three hours, shaking every 15 minutes. The mixture was then cooled, 3 ml 10N H_2SO_4 was added and the tube returned to the water bath for a further hour of saponification at 60 °C. The mixture was then refrigerated overnight. The following day, 12 ml distilled water and 5 ml of 40-60 °C petroleum ether was added and the sample shaken vigorously for one minute. The water and petrol layers in the tube were separated by centrifugation at 220 *g* for five minutes and the top (petrol) layer removed into a clean tube. This was repeated twice more until there were three petrol layers in the tube (approximately 15 ml petrol). A small spatula of sodium hydrogen carbonate was then added to neutralise any acidity and a large

spatula of anhydrous sodium sulphate to remove any remaining water and the sample was shaken for approximately one minute. After centrifugation at 220 *g* for five minutes, the petrol was decanted into a clean tube, flushed with nitrogen and stored at –20 °C until methylation.

2.3.5.2. Brain samples

Brain fatty acid extraction was performed according a method modified from that described by Wachira *et al.* (2002), derived from Folch *et al.* (1957). Approximately 0.50 g of thawed, homogenised brain tissue was weighed into a 30 ml Pyrex screw-top tube and the exact weight recorded. To this samples was added 6 ml saponification mixture (140.3 g potassium hydroxide dissolved in 250 ml distilled water plus 0.5 g quinol dissolved in 260 ml methanol) and 100 µl internal C21 standard (150 mg heneicosanoic acid dissolved in 10 ml chloroform). This mixture was saponified in a circulating water bath at 60 °C for three hours, shaking every 15 minutes. The mixture was then cooled, 3 ml 10N H_2SO_4 was added and the tube returned to the water bath for a further hour of saponification at 60 °C. The mixture was then refrigerated overnight. The following day, 12 ml distilled water and 5 ml of 40-60 °C petroleum ether was added and the sample shaken vigorously for one minute. The water and petrol layers in the tube were separated by centrifugation at 220 *g* for five minutes, 5 drops of absolute ethanol added and the mixture again centrifuged at 400 *g* for 10 minutes. The top (petrol) layer was then removed into a clean tube. This was repeated twice more until there were three petrol layers in the tube (approximately 15 ml in total). A small spatula of sodium hydrogen carbonate was then added to neutralise any acidity and a large spatula of anhydrous sodium sulphate to remove any remaining water and the sample was shaken for approximately one minute. After centrifugation at 220 *g* for five minutes, the petrol was decanted into a clean tube, flushed with nitrogen and stored at –20 °C until methylation.

2.3.5.3. Plasma

Plasma fatty acids were analysed according to the method described in Enser *et al.* (1996), derived from Folch *et al.* (1957). Thawed plasma samples were briefly mixed using a vortex mixer, and 1 ml of plasma transferred into a 15 ml Pyrex screw-top tube using a calibrated pipette. Exactly 2.5 ml quinol in methanol (0.5 g quinol dissolved in 500 ml methanol), 0.8 ml 10 M KOH (140.3 g potassium hydroxide dissolved in 250 ml distilled water) and 40 µl internal C21 standard (75 mg heneicosanoic acid dissolved in 10 ml chloroform) were added and the mixture was saponified in a circulating water bath at 60 °C for two hours, shaking every 15 minutes. The mixture was then cooled and refrigerated overnight. The following day, 5 ml distilled water and 3 ml of 40-60 °C petroleum ether was added and the sample shaken vigorously for one minute. The water and petrol layers in the tube were separated by centrifugation at 220 *g* for five minutes, two drops of absolute ethanol were added to removed the gel formed by centrifugation and the top (petrol) layer was removed into a clean tube. This was repeated twice more until there were three petrol layers in the tube. A small spatula of sodium hydrogen carbonate was then added to neutralise any acidity and a large spatula of anhydrous sodium sulphate to remove any remaining water and the sample was shaken for approximately one minute. After centrifugation at 220 *g* for five minutes, the petrol was decanted into a clean tube, flushed with nitrogen and stored at –20 °C until methylation.

2.3.5.4. Methylation of feed, tissues and plasma

Feed, plasma and tissue sample fatty acids extracted into petroleum ether were dried under nitrogen in a 60 °C circulating water bath and 0.5 ml petroleum ether added to the residue. Approximately 12 drops of diazomethane were then added to the samples which were left in a fume cupboard for any residual diazomethane to evaporate. Subsequently, samples were dried under nitrogen and a suitable amount of petroleum ether added (0.7 ml for

plasma samples, 1.5 ml for feed samples) before the methylated samples were transferred into vials and run on a gas chromatograph.

2.3.5.5. Colostrum and milk

Fatty acid analysis of colostrum and milk samples was carried out according to a method modified from Folch *et al.* (1957) and Christie (1982). Exactly 1 ml of colostrum or milk was weighed into a 30 ml pyrex screw-top tube, to which 100 µl of internal (C21) standard (200 mg heneicosanoic acid (C21:0) dissolved in 10 ml chloroform) 10 ml methanol and 5 ml chloroform were added. The mixture was then homogenised at the highest speed for approximately 20 seconds and filtered through an 11 cm Whatman No. 54 filter paper into a clean 30 ml pyrex screw-top tube. Exactly 10 ml of chloroform was added to the original sample tube and this was again homogenised for 20 seconds before being used to rinse the filter paper. The homogenised probe was rinsed with a chloroform wash between samples. 5 ml of 0.88 % KCl (8.8 g KCl in 1 litre distilled water) was added, the sample capped and shaken vigorously for 2 minutes before overnight storage in the dark.

The following day, the top layer was aspirated using a water vacuum pump. Precisely 5 ml of 2:1 chloroform:methanol mixture and 5 ml methanol were then added to the sample, which was transferred to a 100 ml flask and dried via rotary evaporation. The extracted lipid was removed from 100 ml flasks using three 2.5 ml chloroform washes into a 10 ml volumetric flask and the sample made up to 10 ml with chloroform. Sample were then transferred to 10 ml Pyrex screw-topped tubes and flushed with nitrogen before storage at –20 °C until methylation.

2.3.5.6. Methylation of colostrum and milk samples

Samples were allowed to reach room temperature before 4 ml of extracted lipid in chloroform was transferred to a 15 ml Pyrex screw-top tube and dried under nitrogen in a

50 °C circulating water bath. 2.5 ml hexane was added to the resulting lipid and the sample vortex mixed before the addition of 100 µl of sodium methoxide. The tubes were capped tightly and shaken gently for 5 minutes before the addition of 5 µl acetic acid. One gram of anhydrous calcium chloride was added, the mixture shaken gently for five minutes and allowed to stand for one hour before centrifugation at 2000 *g* for five minutes. A vial of the supernatant was then taken for analysis via gas chromatography.

2.3.5.7. Gas chromatography of methylated samples

Fatty acids were analysed on a PerkinElmer 8500 gas chromatograph (GC; PerkinElmer Life and Analytical Sciences Ltd, Boston, MA, USA). The GC was fitted with a 50 m x 0.22 mm i.d x 0.25 µm film thickness wall coated open tubular (WCOT) fused silica capillary column manufactured for fatty acid methyl ester analysis, with Bis-cyanopropylsiloxane as the stationary phase (SGE International Pty Ltd). It also had attached an PerkinElmer AS 8300 autosampler (SGE International Pty Ltd, Ringwood, VIC. 3134, Australia), a flame ionisation detector and utilised helium as the carrier gas, split at 70:1. Samples were injected at 250 °C with a detector temperature of 280 °C. The oven temperature was increased from 70 °C initially to 175 °C at a ramp rate of 25 °C/minute followed by increases of 1.5 °C/minute to 200 °C; 1 °C/minute to 210 °C and a final increase at 30 °C/minute to 240 °C which was maintained until the end of the analysis. Individual peaks were identified by the retention times of a fatty acid methyl ester standard (Sigma-Aldrich, Poole, UK) and the linearity of response confirmed using a two reference fatty acid mixtures; a standard manufactured by Restek (Bellefonte, PA 16823-8812) for all samples plus an additional standard from Larodan Fine Chemicals AB (S-216 16 Malmo, Sweden) for colostrum and milk. Total and individual fatty acid concentrations were quantified according to the internal standard added (heneicosanoic acid, Greyhound Chromatography and Allied Chemicals, 88 Grange Road West, Birkenhead, Wirral, Cheshire, CH43 4XF) according to the equation overleaf.

Total fatty acid concentration (mg/sample weight) =

{[(100/std %) x (C21 area)] – (C21 area)} x (C21 added/C21 area)

Samples were adjusted for the amount of sample used; the concentrations of individual fatty acids calculated according to a similar equation:

Individual fatty acid concentration (mg/sample weight) =

Individual fatty acid area x (C21 added/C21 area)

2.3.6. Vitamin E analysis

2.3.6.1. Feed

Feed samples were analysed for vitamin E using a modification of the method described by Manz and Philipp (1981). Samples and solvents were protected from sunlight using amber-stained glassware and all solvents used were analytical or HPLC grade. Exactly 10 g of thawed, ground feed sample was mixed thoroughly and transferred into a 250 ml round bottomed flask containing 50 ml of methanol-ascorbic acid solution (0.5 g crystallised ascorbic acid dissolved in 4 ml warm distilled water mixed with 20 ml ethanol and made up to 100 ml with methanol). The mixture was then put in a water bath and brought up to boiling point under nitrogen gas. Precisely 5 ml of potassium hydroxide solution (1 kg KOH pellets dissolved in 1 litre distilled water) was added and the mixture saponified under reflux for twenty minutes, shaking periodically. The flask was then rinsed three times with 30 ml water and the rinse placed in a 500 ml separating funnel. Exactly 120 ml of ether was used to further rinse the flask, and the process repeated twice. The combined ether phases were washed with distilled water until a neutral pH was reached before transferring to a 500 ml Erlenmyer flask containing 30 g sodium sulphate. The mixture was allowed to dry for 30 minutes before filtering through cotton wool into a

500 ml flask and washing the sodium sulphate three times with 20 ml ether. An aliquot containing 0.1 mg alpha-tocopherol (calculated according to the predicted vitamin E concentration of the sample) was then evaporated to dryness on a rotary evaporator under partial vacuum at 50 °C and the residue dissolved in 10 ml petroleum ether for HPLC analysis.

The HPLC system was fitted with a normal phase Prodigy ODS-2 analytical column of 4.6 mm i.d. x 150 mm length packed with silica (Phenomenex, UK). The mobile phase consisted of 98 % methanol and 2 % deionised water. Fluorescence detection was at an excitation of 293 nm and emission of 326 nm.

2.3.6.2. Plasma

Plasma samples were analysed for vitamin E using a modification of the method described by McMurray and Blanchflower (1979). Samples and solvents were protected from sunlight using amber-stained glassware and all solvents used were analytical or HPLC grade. Plasma samples were thawed and mixed thoroughly before a 0.5 ml sample was transferred into a 15 ml polypropylene tube screw-capped tube. Exactly 1 ml ethanol was added and the sample immediately vortex mixed to precipitate the plasma protein component. During mixing, 2.0 ml n-hexane was added and the sample shaken for three minutes at a speed of 1400 on a Vibrax shaker (Ika-Works Inc, USA). Following centrifugation at 1500 *g* for 10 minutes at 4°C, 1.0 ml of the supernatant hexane layer was extracted and transferred to HPLC vials.

The HPLC was calibrated using three standard solutions containing known concentrations of α-tocopherol at 2.0, 4.0 and 8.0 µg/ml. The HPLC system comprised a Krontron HPLC with autosampler, a programmable fluorescence detector and an integrator. The system was fitted with an injector valve consisting of a 50µl loop and a normal phase Bondapak

C18 analytical column of 3.9 mm i.d. x 150 mm length packed with Bondapak (Waters, USA). The mobile phase consisted of 97 % methanol and 3 % deionised water. Fluorescence detection was at an excitation of 295 nm and emission of 330 nm; α - tocopherol being detected at approximately 5.5 minutes.

The concentration of α-tocopherol in the sample was calculated according to the following formula:

Integrated value (μg/ml) x 9.2 = μmol/l vitamin E

2.3.6.3. Colostrum and milk

A modification of the method described by Burton *et al.* (1985) was used to determine the vitamin E concentrations of colostrum and milk samples. All reagents used were analytical grade. Colostrum and milk samples were thawed and allowed to reach room temperature. Samples were mixed thoroughly and 1 ml of sample was transferred into a 15 ml Pyrex test tube. Exactly 2 ml of ethanol was added to precipitate the protein contained within the sample before vortex mixing. Precisely 1 ml of sodium dodecyl sulphate (0.08 M for milk and 0.12 M for colostrum, dissolved in distilled water) solution was then added and the mixture was vortex mixed for a second time. Subsequently, 1 ml of hexane containing 0.05 % butylated hydroxytoluene (BHT) as an antioxidant and 60 μl of *rac* dimethyltocol (5 % w/v distilled in n-hexane) as an internal standard was added and the resulting mixture vortex mixed for one minute before centrifugation at 1000 *g* for two minutes to separate the organic and aqueous phases. The organic layer containing tocopherol in hexane was removed to a clean Pyrex 15 ml test tube and the extraction procedure repeated twice more upon the residue. The three extracts were then combined and dried under nitrogen in a 60 °C water bath. Immediately after drying, the residue was re-suspended in 1 ml of HPLC

grade n-hexane and transferred into an amber HPLC vial and stored at –20 °C until analysis. Reagent blanks and standards of *rac* 5,7-dimethyltocol diluted in n-hexane were used to quantify results and were prepared using the procedure outlined above.

2.3.6.4. Tissues

Entire hind legs were allowed to thaw overnight at 4 °C before samples were allowed to reach room temperature and the *m. semimemebranosus* muscle dissected out. Visible adipose and connective tissue were removed, the muscle chopped into small cubes and minced using a domestic food processor. The muscle was then thoroughly mixed and 1 g ± 0.05 g weighed into a Pyrex 30 ml test tube with the exact weight recorded. Exactly 2 ml of ethanolic BHT solution (0.1% w/v in ethanol) was added to denature the protein component and to act as an antioxidant, and the mixture homogenised via vortex mixing. Subsequently, 2.8 ml of ascorbic acid solution (8.8% w/v in distilled water) was added and the mixture again vortexed. Hydrolysis of the sample lipids was then achieved by the addition of 2.5 ml KOH (14.52% w/v in 4:1 mixture of ethanol and distilled water) and the mixture was vortexed before placing in a shaking water bath at 80 °C for 15 minutes. Tubes were then cooled in ice and 4 ml of hexane plus 60 µl internal standard (5% w/v distilled in n-hexane) added before vortex mixing. The organic and aqueous phases were then separated by centrifugation for two minutes at 2000 *g*. The organic phase containing alpha-tocopherol in hexane was transferred to a clean 15 ml Pyrex tube and dried under nitrogen in a 60 °C water bath. Immediately after drying, the residue was re-suspended in 1 ml of HPLC grade n-hexane and transferred into an amber HPLC vial and stored at –20 °C until analysis. Reagent blanks (containing approximately 0.75 ml distilled water to approximate the water content of the muscle samples) and standards of *rac* 5,7-dimethyltocol were used to quantify results and were prepared using the procedure outlined above.

Brain samples were thawed at 4 °C before removal of the brain stem and cerebellum and performance of the analysis as outlined above, the only difference being saponification of the brain lipids for 30 minutes at 70 °C.

The HPLC employed for the analysis of colostrum, milk, muscle and brain samples was calibrated using nine standard solutions containing known concentrations of α-tocopherol ranging from 0.01-5.0 μg. The HPLC system comprised a Gilson isocratic HPLC with autosampler, an ABI 980 programmable fluorescence detector and a Kontron data system integrator. The system was fitted with an injector valve consisting of a 100 μl loop and a HPLC Technology Techsphere column of 4.6 mm i.d. x 250 mm length packed with Techsphere silica 5μ (HPLC Technology). The mobile phase consisted of 96% n-hexane methanol and 4% 1,4-dioxane. Fluorescence detection was at an excitation of 297 nm and emission of 330 nm; α-tocopherol being detected at approximately 4.95 minutes with a runtime of eight minutes.

The concentration of α-tocopherol in the sample was calculated according to the peak height for α-tocopherol compared to known concentrations of internal and external standards. Vitamin E concentrations were adjusted to account for percentage recovery (94 % for colostrum, 93 % for milk, 86 % for muscle, 95 % for brain). Recoveries were calculated by spiking samples with α-tocopherol before subjecting them to the vitamin E analysis procedure. Concentrations of α-tocopherol were calculated as shown below:

Colostrum/Milk α-tocopherol (μg/ml) =

[(Peak height of sample x μg/ml standard)/Peak height of standard]

Muscle/Brain α-tocopherol (μg/g) =

[(Peak height of sample x μg/ml standard)/Peak height of standard]/[sample weight (g)]

3. FISH OIL AND VITAMIN E SUPPLEMENTATION OF PREGNANT AND LACTATING EWES: EFFECTS UPON EWE AND LAMB PERFORMANCE

3.1. Introduction

The long-chain PUFAs C20:4*n*-6 and C22:6*n*-3 have a crucial role to play in the development of mammalian foetal brain and nervous tissue (Koletzko, 1992) and therefore may influence foetal development and neonatal vigour. Studies conducted in pigs have reported that increasing the dietary supply of long-chain PUFAs during pregnancy and lactation improves neonatal vigour and viability (Rooke *et al.*, 1998). Furthermore, *n*-3 PUFA supplementation has been shown to have a significant impact on cognitive development and learning ability in humans (Morley, 1998). There is no preformed source of C22:6*n*-3 in the commercial ruminant diet and requirements must be met by endogenous synthesis from C18:3*n*-3 (Voigt and Hagemeister, 2001). However, the extent of this synthesis is thought to be negligible (Voigt and Hagemeister, 2001), particularly during pregnancy when PUFAs must be synthesised for deposition into the foetal nervous tissue. Fish oils may be included within the diet of the pregnant ewe to increase the dietary long-chain PUFA supply, thus increasing the supply to the foetal lamb. Nevertheless, studies by Wachira *et al.* (2000) and Chikunya *et al.* (2004) suggested that ruminal biohydrogenation of unprotected PUFAs may reduce the amount available for absorption and metabolism by the animal. Therefore, for long-chain PUFAs to be absorbed in their original form, a mechanism must be employed to protect the fatty acids from biohydrogenation.

Research published by McDowell *et al.* (1998) and Merrell (1998) reported that feeding supranutritional concentrations of vitamin E to ruminants confers improvements in immune competence and neonatal vigour. Nonetheless, studies in both animals (Njeru *et al.*, 1994) and humans (Mino and Nishino, 1973; Léger *et al.*, 1998) have concluded that the negligible concentrations of vitamin E in the plasma of neonatal animals result from

low placental transfer from dam to offspring. Consequently, the mechanism by which neonatal vigour may be improved by maternal supplementation warrants further investigation.

3.2. Objectives

1) To investigate the effects of supplementing pregnant and lactating ewes with long-chain *n*-3 PUFAs in the form of fish oil upon ewe and lamb behaviour and performance.

2) To investigate the effects of supplementing pregnant and lactating ewes with supranutritional dietary concentrations of vitamin E upon ewe and lamb behaviour and performance.

3.3. Materials and methods

3.3.1. Experimental animals and housing

Thirty six twin-bearing and twelve triplet-bearing ewes with a mean age of 3.2 years (s.d. 1.86), a mean liveweight of 76.6 kg (s.d. 6.14) and a mean condition score of 3.3 units (s.d. 0.51) were selected from the Harper Adams University College early lambing flock (Edgmond, Newport, Shropshire, UK). Breeds used included the Suffolk x North of England Mule (n = 24), Friesland x Lleyn (n = 20) and Charollais x Lleyn (n = 4). Ewes were housed, individually penned and bedded on sawdust from week 15 of pregnancy (designated week -6) until week 4 (week +4) of lactation. Ewes were blocked according to litter size, breed, age, condition score and liveweight and randomly allocated to one of four treatment diets within a two x two factorial design. Ewes were fed the allocated diet throughout the duration of the experiment. An additional six ewes were housed in a group-pen, fed the control diet (MB) and bedded on straw to provide foster lambs for any ewes that did not bear two or three live lambs. The building was continually lit and all ewes had free access to fresh water supplies.

3.3.2. Experimental diets

A basal ration was formulated containing barley, sugar beet pulp, soyabean meal, sopralin™ (Trouw UK Ltd, Northwich, UK), rapeseed meal, urea and molasses (Table 3.1). To this diet was added 120 g/kg of a long-chain PUFA or control fat premix and 30 g/kg of vitamin/mineral supplement containing a basal (50 mg/kg) or supranutritional (500 mg/kg) concentration of vitamin E (Roche UK Ltd, Heanor, Derbyshire, UK). The PUFA premix comprised a mixture of crude unrefined Scandinavian fish oil mixed at a ratio of 0.75:0.25 with Incromega®, a by-product of omega-3 fatty acid production for the human market, high in C22:6*n*-3 (Trouw Nutrition UK, Northwich, UK). Butylated hydroxytoluene was added at a rate of 500 mg/kg to the fish oil to prevent oxidation of the component fatty acids. Both the fish oil and Incromega® were combined with a vermiculite carrier (Trouw Nutrition UK, Northwich, UK). Vermiculite was selected as a carrier material due to its highly adsorbent nature; it was also shown by Cooper *et al.* (2002) to provide a degree of protection against the ruminal biohydrogenation of unsaturated fatty acids. The control fat source consisted of a commercial saturated fat source (Megalac®, a calcium soap of C16:0) mixed with straw pellets. The two premixes were formulated to provide equal concentrations of fat (60 g/kg freshweight) within the concentrates. The resulting concentrates were isoenergetic and isonitrogenous with a metabolisable energy content of 13.1 MJ/kg DM, crude protein content of 212 g/kg DM and fatty acid content of 80.6 g/kg DM.

The treatment diets were therefore:

MB: Megalac® plus basal vitamin E

MS: Megalac® plus supranutritional vitamin E

FB: Fish oil/Incromega® plus basal vitamin E

FS: Fish oil/Incromega® plus supranutritional vitamin E

Ewes were fed a stepped concentrate ration (Table 3.2) in two equal meals at 08.00 and 16.00 daily during pregnancy, and at a flat-rate of 1.7 kg/day in three meals (at 08:00, 12:00 and 16:00) during lactation. Barley straw was initially offered at 0.80 kg/day and subsequently fed at intake levels calculated according to the method described in Chapter Two. The complete diet was formulated to fulfil the requirements of pregnant and lactating ewes as detailed by AFRC (1993).

Table 3.1. Raw material and chemical composition of the four treatment concentrates

	MB	MS	FB	FS
Raw material composition (g/kg)				
Barley	457	457	523	523
Sugar beet pulp	100	100	100	100
Soyabean meal	100	100	100	100
Rapeseed meal	50	50	50	50
Sopralin	8	8	12	12
Megalac®	52	52	-	-
Straw	68	68	-	-
Straw pellets	70	70	-	-
Fish oil	-	-	45	45
Incromega	-	-	15	15
Vermiculite	-	-	60	60
Molasses	50	50	50	50
Urea	15	15	15	15
Vitamins/Minerals	30	30	30	30
Predicted¶ chemical composition (g/kg DM)				
DM (g/kg)	864	864	871	871
CP	206	206	210	210
ERDP*	131	131	135	135
DUP*	78	78	79	79
EE (ether extract)	81	81	81	81
NDF	226	226	183	183
Ash	86	86	71	71
Vitamin E (mg/kg)	50	500	50	500
ME (metabolisable energy; MJ/kg DM)	13.2	13.2	13.1	13.1
FME (fermentable ME; MJ/kg DM)	9.9	9.9	10.2	10.2
ERDP:FME Ratio	13.2	13.2	13.2	13.2

Vitamin/Mineral supplement (Hac Ewe 25, Roche Products Limited, Heanor, Derbyshire) supplied per kg of diet: Calcium 7.06 g; Sodium 2.67 g; Phosphorus 1.65 g; Selenium 0.36 mg; Vitamin A 14,400 IU; Vitamin D 30,000 IU; Vitamin E 50 mg (basal); or 500 mg (supranutritional).

¶ (AFRC, 1993)

* calculated according to AFRC (1993) at a rumen outflow rate of 0.08 ml/hour

Table 3.2. Daily concentrate allowance for twin- and triplet-bearing ewes

Day of gestation	110	117	124	131	138	145	Lactation
Concentrate allowance (kg/day):							
Twin-bearing ewes	0.7	0.8	0.9	1.0	1.0	1.2	1.7
Triplet-bearing ewes	0.8	0.9	1.0	1.1	1.1	1.3	1.7

3.3.3. Experimental procedure

Concentrate and straw samples were taken weekly and stored in airtight bags at –20 °C until analysis. Ewe liveweight, body condition score, straw intake, maternal and neonatal behaviour, colostrum and milk production, lamb birthweight and liveweight were measured as described in Chapter Two.

3.3.3.1. Blood sampling

Blood samples were obtained from all ewes by jugular venepuncture at 11.00 at six weeks (day 103 of gestation, before the experimental concentrates were fed), four weeks (day 117) and two weeks (day 131) *pre-partum*; at 12 hours *post partum* and at two and four weeks into lactation. Blood and tissue samples were taken from neonatal lambs immediately after cessation of the heartbeat as described in section 2.2.4., and blood samples taken by jugular venepuncture from growing lambs at 11.00 at 14 and 28 days of age. Plasma and tissue samples were prepared as described in Chapter Two.

3.3.4. Sample analysis

Concentrate and straw samples were analysed for DM, ash, CP and NDF. In addition, concentrate samples were analysed for vitamin E and fatty acid composition. Ewe blood samples were analysed for urea, βHB, NEFA, CK and GPx at all time points and for vitamin E and fatty acids at 103 and 131 days of gestation and at 14 days *post partum*. Neonatal lamb blood and brain samples were analysed for vitamin E and fatty acids, neonatal lamb muscle samples were analysed for vitamin E. Plasma samples taken from growing lambs were analysed for CK and GPx at all time points and for vitamin E and fatty acids at 14 days of age. All analyses are described in Chapter Two.

3.3.5. Statistical analysis

Data were analysed as a two x two factorial design with main effects of fat source (fat) and vitamin E (vit E) concentration within the treatment concentrate and their interaction (F x V). Plotting lamb liveweight against time revealed linear growth rates, therefore overall growth rates were calculated using linear regression. The analysis of variance (ANOVA) function within Genstat 6 version 6.2 (Lawes Agricultural Trust, 2002) was used for all statistical analyses.

3.4. Results

Data from eight ewes were excluded from the analysis. Two ewes aborted at 131 days of gestation (treatments FB and MS); a further five ewes reared single lambs (one from treatments MB, MS and FS, two from treatment FB) and one ewe (treatment MB) suffered from chronic mastitis. Two ewes (treatments MB and MS) bore one live and one stillborn lamb and adopted a lamb of the same breed from the six group-housed ewes. The replacement lambs were introduced immediately after expulsion of the dead lamb. Data for these ewes was included, however, records relating to the adopted lambs were not included in the statistical analysis. One bottle-fed lamb and five lambs reared singly were also excluded from the analysis. Consequently, data from 46 ewes was utilised *pre-partum* and from 40 ewes and 86 lambs *post partum*.

Table 3.3. Chemical composition of the four treatment concentrates plus the straw

	Concentrate				Straw
	MB	**MS**	**FB**	**FS**	
Dry matter (g/kg)	859	865	865	863	881
Crude protein (g/kg DM)	186	181	172	176	37.3
Organic matter (g/kg DM)	923	931	895	884	954
Ash (g/kg DM)	77.0	68.9	105	116	46.2
Neutral detergent fibre (g/kg DM)	199	223	128	145	807
Vitamin E (mg/kg DM)	57.3	503	64.4	541	-
Total fatty acids (g/kg DM)	102	97.4	93.0	81.0	-

MB = Megalac + 50 mg/kg vitamin E; MS = Megalac + 500 mg/kg vitamin E; FB = Fish oil + 50 mg/kg vitamin E; FS = Fish oil + 500 mg/kg vitamin E

3.4.1. Diet composition

The chemical composition of the four treatment concentrates and straw is presented in Table 3.3. The DM and CP content were similar between treatments, although the fatty acid content of concentrate FS was lower than the other three treatment diets. The ash content of concentrates containing fish oil was augmented by the inclusion of vermiculite, the addition of straw pellets to the Megalac concentrates increased the NDF fraction. Vitamin E concentrations were similar to those predicted when formulating the diets.

Table 3.4. Fatty acid composition of the four treatment concentrates

	Concentrate			
Fatty acid (g/kg DM)	**MB**	**MS**	**FB**	**FS**
C16:0	41.0	39.0	18.2	17.3
C16:1*n*-7	0.29	0.31	3.58	3.02
C18:0	3.39	3.13	3.00	2.82
C18:1 *trans*	1.09	0.83	2.31	2.01
C18:1*n*-9 *cis*	30.9	28.8	11.1	10.0
C18:2*n*-6 *cis*	19.9	19.7	15.1	13.1
C18:3*n*-3 *cis*	1.75	1.83	2.44	1.90
C20:4*n*-6	0.15	0.14	0.74	0.76
C20:5*n*-3	ND	ND	4.21	2.54
C22:6*n*-3	ND	ND	4.45	2.62
RFA[a]	3.85	3.77	36.6	29.3

MB = Megalac + 50 mg/kg vitamin E; MS = Megalac + 500 mg/kg vitamin E; FB = Fish oil + 50 mg/kg vitamin E; FS = Fish oil + 500 mg/kg vitamin E

[a] RFA = All remaining fatty acids; ND = not detected

Concentrates containing Megalac (MB, MS) had the highest concentrations of C16:0, C18:1*n*-9 and C18:2*n*-6, but the long-chain PUFAs C20:5*n*-3 and C22:6*n*-3 were not detectable (Table 3.4). By contrast, the inclusion of fish oil plus Incromega as the principal fatty acid sources in concentrates FB and FS resulted in increased concentrations of C20:4*n*-6, C20:5*n*-3 and C22:6*n*-3.

3.4.2. Ewe performance parameters

3.4.2.1. Straw intake

Daily straw intakes declined from six weeks *pre-partum* to one week *pre-partum* for all treatments with average daily intakes of 0.68 kg DM at week –6 and 0.55 kg DM at week –1 (Figure 3.1). The mean daily straw intake increased steadily during lactation with average intakes of 0.55 kg DM at week 0 and 1.07 kg DM at week +3. There was no significant effect of dietary treatment on straw intakes, except at week –6 when ewes fed diet MB had higher intakes than those offered diets MS or FB (P=0.025; Table 3.5).

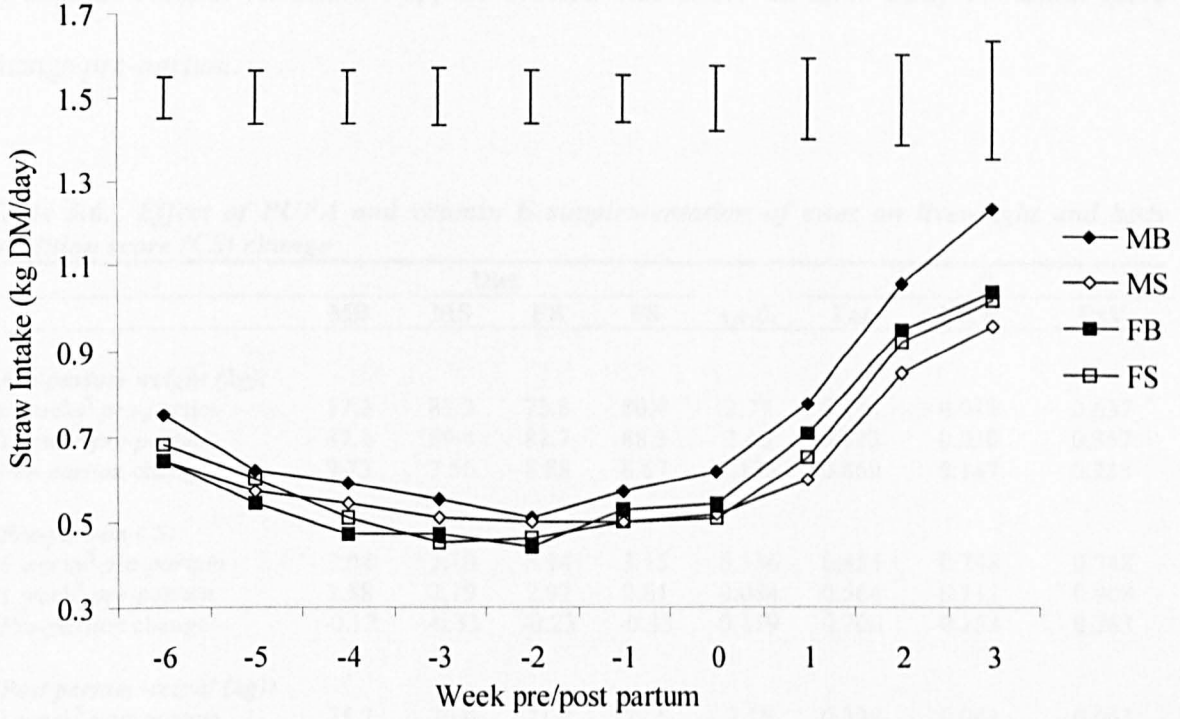


Figure 3.1. Effect of PUFA and vitamin E supplementation of ewes on daily straw intakes

Table 3.5. Effect of PUFA and vitamin E supplementation of ewes on daily straw intake

	Diet					P		
	MB	MS	FB	FS	s.e.d.	Fat	Vit E	FxV
Mean daily intake (kg DM):								
6 weeks[¶] *pre-partum*	0.75[b]	0.64[a]	0.64[a]	0.68[ab]	0.047	0.306	0.268	0.025
1 week[¶] *pre-partum*	0.57	0.50	0.53	0.50	0.055	0.505	0.216	0.586
Pre-partum intake (kg/day)	0.60	0.54	0.51	0.53	0.050	0.180	0.685	0.215
0 weeks[¶] *post partum*	0.62	0.52	0.54	0.51	0.076	0.346	0.235	0.540
3 weeks[¶] *post partum*	1.24	0.96	1.04	1.02	0.140	0.480	0.145	0.214
Post partum intake (kg/day)	0.92	0.73	0.81	0.77	0.101	0.598	0.122	0.291

MB = Megalac + 50 mg/kg vitamin E; MS = Megalac + 500 mg/kg vitamin E; FB = Fish oil + 50 mg/kg vitamin E; FS = Fish oil + 500 mg/kg vitamin E

Means without common superscripts are significantly different at the P<0.05 level

[¶] 6 weeks *pre-partum* = mean straw intake on days 103-110 of gestation; 1 week *pre-partum* = mean straw intake on days 138-145 of gestation; 0 weeks *post partum* = mean straw intake on days 0 – 7 of lactation, 3 weeks *post partum* = mean straw intake on days 21 – 28 of lactation

3.4.2.2. Liveweight and condition score

A bias existed at the start of the experiment in that ewes fed supranutritional concentrations of vitamin E were heavier than ewes offered basal vitamin E concentrations (80.4 kg vs. 75.6 kg respectively, P=0.019); however, the *pre-partum* liveweight change was not significantly affected by dietary treatment (Table 3.6). No significant effect of fat source

or supranutritional vitamin E supplementation was observed upon body condition score change *pre-partum*.

Table 3.6. Effect of PUFA and vitamin E supplementation of ewes on liveweight and body condition score (CS) change

	Diet					P		
	MB	MS	FB	FS	s.e.d.	Fat	Vit E	FxV
Pre-partum weight (kg):								
6 weeks[¶] *pre-partum*	77.3	81.3	73.8	80.4	2.77	0.194	0.019	0.637
1 week[¶] *pre-partum*	87.1	89.4	82.7	88.3	2.46	0.123	0.030	0.357
Pre-partum change	9.73	7.56	8.88	8.67	1.135	0.869	0.147	0.233
Pre-partum CS:								
6 weeks[¶] *pre-partum*	3.04	3.10	3.14	3.15	0.136	0.455	0.748	0.748
1 week[¶] *pre-partum*	2.88	2.79	2.92	2.81	0.084	0.564	0.112	0.908
Pre-partum change	-0.17	-0.33	-0.23	-0.33	0.129	0.706	0.158	0.763
Post partum weight (kg):								
1 week[¶] *post partum*	75.7	76.0	71.2	77.5	2.16	0.339	0.041	0.063
4 weeks[¶] *post partum*	72.1	71.6	69.3	75.6	2.48	0.737	0.106	0.062
Post partum change	-3.57	-4.37	-1.94	-1.83	1.059	0.010	0.647	0.553
Post partum CS:								
1 week[¶] *post partum*	2.82	2.71	2.90	2.75	0.083	0.305	0.032	0.772
4 weeks[¶] *post partum*	2.55	2.24	2.64	2.36	0.103	0.155	<0.001	0.837
Post partum change	-0.28	-0.46	-0.26	-0.39	0.074	0.391	0.006	0.536

MB = Megalac + 50 mg/kg vitamin E; MS = Megalac + 500 mg/kg vitamin E; FB = Fish oil + 50 mg/kg vitamin E; FS = Fish oil + 500 mg/kg vitamin E

[¶] 6 weeks *pre-partum* = day 103 of gestation; 1 week *pre-partum* = day 138 of gestation; 1 week *post partum* = day 7 of lactation; 4 weeks *post partum* = day 28 of lactation

Mean individual ewe liveweight *post-partum* was not significantly affected by dietary treatment; however, liveweight loss during lactation was reduced by the addition of fish oil to the experimental diets (1.88 kg vs. 3.97 kg for diets containing fish oil and Megalac respectively, P=0.010). Main effects of vitamin E concentration on individual body condition score were observed at one week *post partum* (2.86 units for ewes fed diets containing basal concentrations of vitamin E compared to 2.73 units in those offered supranutritional concentrates, P=0.032) and four weeks *post partum* (2.60 units for diets MB + FB, 2.30 units for diets MS + FS, P<0.001). Furthermore, more condition was lost by ewes offered supranutritional dietary concentrations of vitamin E during pregnancy and lactation with mean losses of 0.43 units compared to a loss of 0.27 units in ewes fed diets supplemented with basal vitamin E concentrations (P=0.006).

3.4.2.3. Metabolic profiles

Feeding fish oil compared to Megalac reduced mean plasma βHB concentrations both *pre-* (0.59 mmol/l vs. 0.74 mmol/l respectively, P=0.034) and *post partum* (0.53 mmol/l vs. 0.85 mmol/l respectively, P<0.001; Table 3.7). There was no significant effect of vitamin E supplementation level on mean *pre-* and *post partum* βHB concentrations.

Table 3.7. Effect of PUFA and vitamin E supplementation of ewes on plasma β-hydroxybutyrate, urea and non-esterified fatty acid concentrations

	Diet					P		
	MB	**MS**	**FB**	**FS**	**s.e.d.**	**Fat**	**Vit E**	**FxV**
Pre-partum concentration (mmol/l):								
Mean* plasma βHB	0.70	0.78	0.51	0.66	0.096	0.034	0.103	0.574
Mean* plasma urea	7.13[a]	7.58[ab]	7.85[b]	7.25[ab]	0.336	0.419	0.759	0.033
Post partum concentration (mmol/l):								
Mean¶ plasma βHB	0.77	0.94	0.53	0.53	0.096	<0.001	0.207	0.218
Mean¶ plasma urea	7.69	7.68	7.58	8.13	0.383	0.529	0.334	0.307
NEFA concentration (mmol/l):								
6 weeks§ *pre-partum*	0.90	0.97	0.90	0.94	0.103	0.876	0.412	0.855
12 hours *post partum*	0.65	0.73	0.39	0.53	0.081	<0.001	0.069	0.610
4 weeks§ *post partum*	0.60	0.63	0.51	0.52	0.085	0.127	0.768	0.864

MB = Megalac + 50 mg/kg vitamin E; MS = Megalac + 500 mg/kg vitamin E; FB = Fish oil + 50 mg/kg vitamin E; FS = Fish oil + 500 mg/kg vitamin E

Means without common superscripts are significantly different at the P<0.05 level

* Mean value = average of all measured values *pre-partum*

¶ Mean value = average of all measured values *post partum*

§ 6 weeks *pre-partum* = day 103 of gestation; 4 weeks *post partum* = day 28 of lactation

No significant main effect of ewe diet was recorded upon mean *pre-partum* plasma urea concentrations. However, a significant interaction effect between fat source and vitamin E concentration was observed, with basal dietary concentrations of vitamin E increasing plasma urea levels in ewes fed diets containing fish oil but reducing them when fed in combination with Megalac (P=0.033).

At 12 hours *post partum*, NEFA concentrations in ewes supplemented with long-chain PUFAs were lower than those exhibited by ewes fed diets containing Megalac (0.46 mmol/l compared to 0.69 mmol/l respectively, P<0.001). By contrast, PUFA

supplementation of the ewe had no significant effect upon plasma NEFA concentrations at four weeks *post partum*. Furthermore, no significant effect of dietary vitamin E concentration nor any significant interaction effect between fat source and dietary vitamin E concentration was observed upon plasma NEFA concentrations.

3.4.2.4. Antioxidant status

Ewes allocated to the fish oil diets had lower plasma vitamin E concentrations at the start of the experiment compared to those allocated to the Megalac treatments (4.76 μmol/l vs. 5.32 μmol/l respectively, P=0.016; Table 3.8). Maternal plasma vitamin E concentrations at two weeks *pre-partum* were increased by supranutritional dietary vitamin E supply (6.61 μmol/l) compared to basal dietary concentrations (3.57 μmol/l; P<0.001). Long-chain PUFA supplementation had an abrogating effect on maternal plasma vitamin E concentrations at two weeks *pre-partum* with means of 3.75 μmol/l in ewes supplemented with fish oil and 6.42 μmol/l in ewes offered diets containing Megalac (P<0.001). Plasma vitamin E concentrations declined in lactation for all treatments apart from FS, with main effects of dietary vitamin E concentration (6.75 μmol/l in ewes given supranutritional vitamin E supplementation compared to 2.35 μmol/l in those offered basal supplementation ewes, P<0.001) and fatty acid source (means of 3.75 μmol/l for ewes fed fish oil compared to 5.34 μmol/l in ewes offered Megalac, P<0.001) being observed.

Table 3.8. Effect of PUFA and vitamin E supplementation of ewes on plasma vitamin E concentrations

	Diet					P		
	MB	**MS**	**FB**	**FS**	**s.e.d.**	**Fat**	**Vit E**	**FxV**
Plasma vitamin E concentration (μmol/l):								
6 weeks[¶] *pre-partum*	5.61	5.04	4.64	4.88	0.302	0.016	0.463	0.073
2 weeks[¶] *pre-partum*	4.58	8.27	2.55	4.96	0.509	<0.001	<0.001	0.092
2 weeks[¶] *post partum*	2.87	7.81	1.82	5.69	0.527	<0.001	<0.001	0.166

MB = Megalac + 50 mg/kg vitamin E; MS = Megalac + 500 mg/kg vitamin E; FB = Fish oil + 50 mg/kg vitamin E; FS = Fish oil + 500 mg/kg vitamin E

[¶] 6 weeks *pre-partum* = day 103 of gestation; 2 weeks *pre-partum* = day 131 of gestation; 2 weeks *post partum* = day 14 of lactation

Table 3.9. Effect of PUFA and vitamin E supplementation of ewes on indicators of selenium status and cellular damage

	Diet					P		
	MB	MS	FB	FS	s.e.d.	Fat	Vit E	FxV
Pre-partum:								
Mean* erythrocyte GPx activity (U/ml PCV)	171	164	109	130	13.8	<0.001	0.485	0.166
Mean* serum CK activity (U/l)	259	209	393	179	143.1	0.612	0.202	0.425
Post partum:								
Mean¶ erythrocyte GPx activity (U/ml PCV)	185	185	160	147	14.1	0.006	0.510	0.492
Mean¶ serum CK activity (U/l)	135	165	188	261	55.0	0.066	0.196	0.585

MB = Megalac + 50 mg/kg vitamin E; MS = Megalac + 500 mg/kg vitamin E; FB = Fish oil + 50 mg/kg vitamin E; FS = Fish oil + 500 mg/kg vitamin E

* Mean value = average of all measured values *pre-partum*

¶ Mean value = average of all measured values *post partum*

Supplementing ewes with long-chain PUFAs reduced mean *pre-partum* GPx activities when compared to ewes fed diets containing Megalac (120 U/ml PCV vs. 168 U/ml PCV respectively, P<0.001; Table 3.9). A similar pattern was observed in mean GPx activities during lactation with lowest values recorded in ewes offered diets containing fish oil (154 U/ml PCV) in contrast to ewes fed Megalac (185 U/ml PCV; P=0.006). No significant main or interaction effects of dietary treatment were observed on ewe serum CK concentrations at any time point, although ewes supplemented with long-chain PUFAs tended to have higher concentrations *post partum* (P=0.06) compared to ewes fed Megalac.

3.4.2.5. Plasma fatty acids (six weeks *pre-partum*)

No significant differences in the proportions of individual fatty acids within plasma were observed at six weeks *pre-partum* (Table 3.10).

Table 3.10. Effect of PUFA and vitamin E supplementation of ewes on the proportions of fatty acids in ewe plasma samples collected at six weeks[¶] pre-partum (pre-treatment)

	Diet					P		
Fatty acid (g/100 g fatty acid)	**MB**	**MS**	**FB**	**FS**	**s.e.d.**	**Fat**	**Vit E**	**FxV**
C16:0	15.9	15.2	16.3	15.5	1.07	0.640	0.307	0.969
C16:1*n*-7	0.85	0.83	1.08	0.82	0.136	0.268	0.176	0.218
C18:0	25.2	23.3	23.8	23.9	1.80	0.740	0.493	0.449
C18:1 *trans*	5.46	5.24	5.76	5.16	0.600	0.795	0.346	0.659
C18:1*n*-9 *cis*	20.3	20.2	21.4	19.6	1.45	0.800	0.365	0.441
C18:2*n*-6 *cis*	5.57	4.95	5.72	5.38	0.497	0.414	0.190	0.691
CLA (*cis*-9,*trans*-11)	0.61	0.50	0.59	0.32	0.151	0.359	0.089	0.487
C18:3*n*-3 *cis*	3.82	3.20	3.46	3.18	0.346	0.447	0.080	0.501
C20:4*n*-6	2.12	1.82	2.28	2.14	0.303	0.274	0.319	0.726
C20:5*n*-3	2.52	2.21	2.58	2.33	0.281	0.664	0.172	0.890
C22:6*n*-3	1.71	1.45	1.32	1.55	0.217	0.362	0.908	0.131
RFA[§]	15.9	21.2	15.7	20.1	5.53	0.879	0.232	0.916
Total fatty acids (mg/ml)	1.19	1.20	1.05	1.14	0.073	0.072	0.394	0.442

MB = Megalac + 50 mg/kg vitamin E; MS = Megalac + 500 mg/kg vitamin E; FB = Fish oil + 50 mg/kg vitamin E; FS = Fish oil + 500 mg/kg vitamin E

[§] RFA = All remaining fatty acids

[¶] six weeks *pre-partum* = day 103 of gestation

Table 3.11. Effect of PUFA and vitamin E supplementation of ewes on the proportions of fatty acids in ewe plasma samples collected at two weeks[¶] pre-partum

	Diet					P		
Fatty acid (g/100 g fatty acid)	**MB**	**MS**	**FB**	**FS**	**s.e.d.**	**Fat**	**Vit E**	**FxV**
C16:0	23.9^{c}	22.6^{b}	16.2^{a}	16.7^{a}	0.47	<0.001	0.231	0.017
C16:1*n*-7	0.52	0.75	1.69	1.35	0.223	<0.001	0.742	0.083
C18:0	20.6	21.2	14.1	15.4	0.67	<0.001	0.065	0.456
C18:1 *trans*	2.15	2.45	9.14	8.36	0.401	<0.001	0.405	0.070
C18:1*n*-9 *cis*	15.8	17.0	8.07	9.25	0.461	<0.001	0.001	0.920
C18:2*n*-6 *cis*	20.0	18.8	10.2	9.66	0.62	<0.001	0.055	0.454
CLA (*cis*-9,*trans*-11)	0.22	0.36	2.00	2.18	0.348	<0.001	0.526	0.948
C18:3*n*-3 *cis*	1.30	1.23	2.38	2.32	0.273	<0.001	0.739	0.986
C20:4*n*-6	2.59	2.85	2.90	2.93	0.194	0.176	0.303	0.430
C20:5*n*-3	1.40	1.86	8.04	7.71	0.285	<0.001	0.751	0.062
C22:6*n*-3	1.32	1.72	5.17	5.16	0.178	<0.001	0.131	0.121
RFA[§]	10.2	9.18	20.1	19.0	0.640	<0.001	0.033	0.999
Total fatty acids (mg/ml)	1.27^{c}	1.10^{b}	0.649^{a}	0.716^{a}	0.0547	<0.001	0.235	0.008

MB = Megalac + 50 mg/kg vitamin E; MS = Megalac + 500 mg/kg vitamin E; FB = Fish oil + 50 mg/kg vitamin E; FS = Fish oil + 500 mg/kg vitamin E

[¶] two weeks *pre-partum* = day 131 of gestation

[§] RFA = All remaining fatty acids

Means without common superscripts are significantly different at the P<0.05 level

3.4.2.6. Plasma fatty acids (two weeks *pre-partum*)

With regard to the total concentration of fatty acids within plasma, there existed a significant main effect of fat source with means of 0.68 mg/ml for ewes fed fish oil

compared to 1.19 mg/ml for ewes fed Megalac (Table 3.11). Offering diets containing fish oil compared to Megalac reduced the proportions of both C16:0 (16.5 g/100 g fatty acids vs. 23.2 g/100 g fatty acids respectively, P<0.001) and C18:0 (14.7 g/100 g fatty acids vs. 20.9 g/100 g fatty acids respectively, P<0.001) within ewe plasma lipids.

Supplementing ewes with fish oil compared to Megalac increased the proportions of C16:1*n*-7 (1.52 g/100 g fatty acids in contrast to 0.64 g/100 g fatty acids respectively, P<0.001) within plasma. In addition, feeding fish oil during pregnancy reduced the proportions of C18:1*n*-9 *cis* within plasma lipids, with mean values of 8.66 g/100 g fatty acids in ewes fed fish oil compared to 16.4 g/100 g fatty acids in those fed Megalac (P<0.001). Supranutritional vitamin E supplementation also increased the proportion of C18:1*n*-9 *cis* within plasma, with mean values of 13.1 g/100 g fatty acids compared to 11.9 g/100 g fatty acids in ewes fed basal concentrations of vitamin E (P=0.001). Ewes fed fish oil also had significantly higher proportions of C18:1 *trans* within plasma lipids when compared to those fed Megalac (8.75 g/100 g fatty acids vs. 2.30 g/100 g fatty acids).

The proportion of CLA within ewe plasma was increased by the addition of long-chain PUFAs to the diet with means of 2.09 g/100 g fatty acids for those fed fish oil in contrast to 0.29 g/100 g fatty acids for those offered Megalac (P<0.001). There was no effect of vitamin E supplementation upon the proportional contribution of CLA to plasma fatty acids.

The most important contributor to the decrease in total *n*-6 fatty acids seen with fish oil supplementation was C18:2*n*-6 *cis*, found at half the concentration in ewes fed fish oil (9.94 g/100 g fatty acids) compared to those fed Megalac (19.4 g/100 g fatty acids, P<0.001). However, the proportion of C20:4*n*-6 within plasma was unaffected by either dietary fat source or vitamin E concentration.

Feeding diets containing fish oil to pregnant ewes increased the proportion of C18:3*n*-3 within plasma (2.35 g/100 g fatty acids for ewes supplemented with fish oil compared to 1.26 g/100 g fatty acid for ewes fed Megalac, P<0.001). Moreover, C20:5*n*-3 was increased in plasma samples by fish oil supplementation, at 7.87 g/100 g fatty acids compared with 1.63 g/100 g fatty acids for ewes fed Megalac (P<0.001). The proportion of C22:6*n*-3 within plasma was increased three-fold in ewes fed diets containing fish oil with means of 5.16 g/100 g fatty acids and 1.52 g/100 g fatty acids for those ewes offered fish oil and Megalac diets respectively (P<0.001). There was no significant effect of vitamin E supplementation on plasma *n*-3 fatty acids.

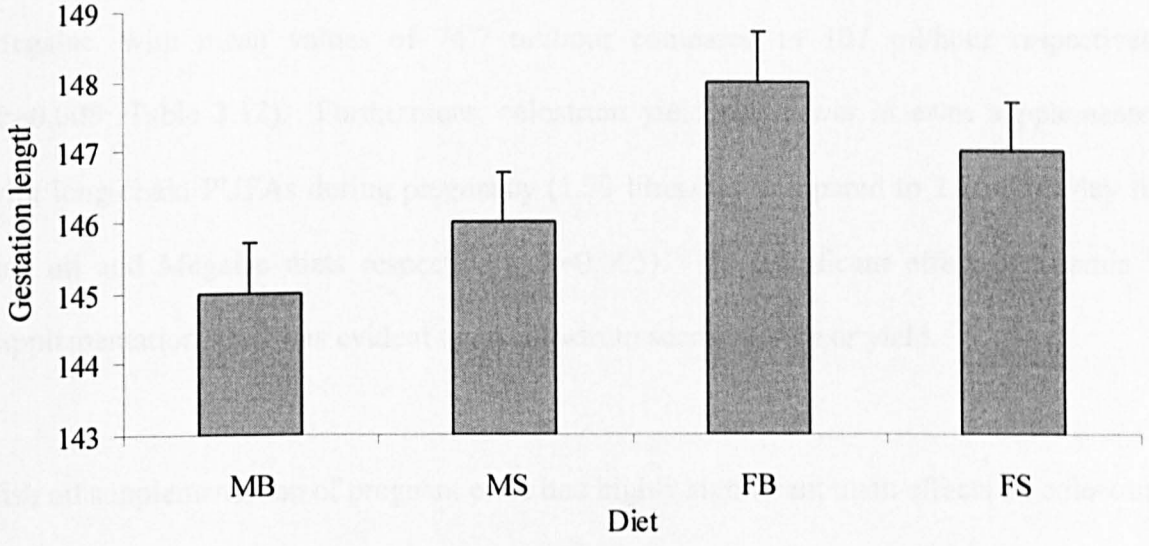


Figure 3.2. Effect of PUFA and vitamin E supplementation of ewes on ewe gestation length

3.4.2.7. Gestation length

Ewe gestation length was significantly increased by the addition of fish oil to the diet with mean values of 148 days compared with 146 days for ewes fed diets containing Megalac (P<0.001; Figure 3.2). There was no main effect of dietary vitamin E concentration upon gestation length, and no interactions between fat source and vitamin E concentration were observed upon this parameter.

Table 3.12. Effect of PUFA and vitamin E supplementation of ewes on colostrum parameters

	Diet					P		
	MB	MS	FB	FS	s.e.d.	Fat	Vit E	FxV
Secretion rate (ml/hour)	94.7	119	77.3	72.1	15.17	0.005	0.383	0.179
Yield (l/day)	2.27	2.85	1.86	1.73	0.364	0.005	0.383	0.179
Fat concentration (g/kg)	123	135	101	104	8.7	<0.001	0.225	0.475
Fat yield (g/hour)	12.7	16.2	7.63	7.66	2.245	<0.001	0.274	0.282
Protein concentration (g/kg)	85.1	85.7	74.2	71.6	9.59	0.076	0.888	0.813
Protein yield (g/hour)	8.69	10.7	5.61	5.00	1.635	<0.001	0.548	0.264
Vitamin E concentration (mg/kg)	8.23[a]	27.4[b]	6.93[a]	9.26[a]	1.814	<0.001	<0.001	<0.001
Vitamin E yield (mg/hour)	0.95[a]	2.85[b]	0.91[a]	1.06[a]	0.187	<0.001	<0.001	<0.001

MB = Megalac + 50 mg/kg vitamin E; MS = Megalac + 500 mg/kg vitamin E; FB = Fish oil + 50 mg/kg vitamin E; FS = Fish oil + 500 mg/kg vitamin E
Means without common superscripts are significantly different at the P<0.05 level

3.4.2.8. Colostrum production

Colostrum secretion rate was reduced in ewes offered diets containing fish oil compared to Megalac, with mean values of 74.7 ml/hour compared to 107 ml/hour respectively (P=0.005; Table 3.12). Furthermore, colostrum yield was lower in ewes supplemented with long-chain PUFAs during pregnancy (1.79 litres/day compared to 2.56 litres/day for fish oil and Megalac diets respectively, P=0.005). No significant effect of vitamin E supplementation level was evident upon colostrum secretion rate or yield.

Fish oil supplementation of pregnant ewes had highly significant main effects on colostrum fat concentration and yield with means of 103 g/kg compared to 129 g/kg for fat concentration and fat yields of 7.65 g/hour compared to 14.46 g/hour, for fish oil and Megalac diets respectively. Although slight numerical differences in fat concentration and yield occurred as a result of supranutritional vitamin E supplementation, these differences were not statistically significant. Long-chain PUFA supplementation tended to reduce colostrum protein concentrations (72.9 g/kg compared to 85.4 g/kg for fish oil and Megalac respectively, P=0.076) and significantly reduced protein yield (5.30 g/hour for fish oil diets, 9.71 g/hour for Megalac diets, P<0.001). Vitamin E supplementation level had no significant effect on colostrum protein parameters.

Supranutritional vitamin E supplementation during pregnancy had highly significant main and interaction effects upon the colostral vitamin E concentration and yield. A main effect of vitamin E supplementation was evident with ewes fed concentrates containing supranutritional concentrations of vitamin E having higher concentrations (18.3 mg/kg compared to 7.58 mg/kg, $P<0.001$) and yields (1.95 mg/hour compared to 0.93 mg/hour, $P<0.001$) of the vitamin in colostrum. In addition, fish oil supplementation reduced the concentration (8.10 mg/kg) and yield (0.99 mg/hour) of colostral vitamin E compared to supplementation with Megalac (17.8 mg/kg and 1.90 mg/hour). A highly significant interaction between fat source and dietary vitamin E concentration was observed for vitamin E concentration and yield; ewes fed diet MS had significantly higher values for both parameters than ewes fed the other three treatment diets.

3.4.2.9. Colostrum fatty acids

The total concentration of fatty acids within ewe colostrum was not significantly altered by dietary treatment (Table 3.13). The proportions of all short-chain saturated fatty acids apart from C4:0 within colostrum were significantly increased by long-chain PUFA supplementation of the ewe. A diminishing effect of supranutritional vitamin E supplementation was observed upon the proportions of C12:0 in colostrum (1.65 g/100 g fatty acids for ewes fed supranutritional concentrations vs. 2.13 g/100 g fatty acids for those fed basal concentrations, $P=0.005$). A similar effect was observed upon C14:0 within colostrum with means of 6.83 g/100 g fatty acids in contrast to 8.59 g/100 g fatty acids for supranutritional and basal diets respectively ($P<0.001$). Furthermore, a significant interaction between dietary vitamin E concentration and fat source was observed upon the proportion of C12:0 in colostral fat, the highest values being found in colostrum from ewes offered diet FB.

Table 3.13. Effect of PUFA and vitamin E supplementation of ewes on the proportions of fatty acids in colostrum

	Diet					P		
Fatty acid (g/100 g fatty acid)	MB	MS	FB	FS	s.e.d.	Fat	Vit E	FxV
C4:0	4.07	4.05	3.60	4.12	0.384	0.475	0.370	0.338
C6:0	1.24	1.31	1.87	1.81	0.212	0.001	0.977	0.665
C8:0	0.73	0.72	1.29	1.17	0.142	<0.001	0.562	0.602
C10:0	1.70	1.63	3.31	2.80	0.346	<0.001	0.252	0.381
C12:0	1.60[a]	1.47[a]	2.66[b]	1.83[a]	0.214	<0.001	0.005	0.034
C14:0	6.77	5.77	10.4	7.89	0.629	<0.001	<0.001	0.107
C16:0	27.5	23.9	25.2	23.6	1.32	0.181	0.012	0.288
C16:1*n*-7	0.92	0.93	1.30	1.26	0.077	<0.001	0.758	0.697
C18:0	8.26	8.38	5.23	6.54	0.781	<0.001	0.209	0.296
C18:1 *trans*	2.91	2.99	4.36	4.78	0.282	<0.001	0.219	0.411
C18:1*n*-9 *cis*	31.0	29.2	19.9	23.3	2.12	<0.001	0.599	0.098
C18:2*n*-6 *cis*	2.72	2.21	1.96	1.87	0.202	0.001	0.053	0.159
CLA (*cis*-9,*trans*-11)	1.92	1.92	2.84	2.86	0.174	<0.001	0.951	0.969
C18:3*n*-3 *cis*	0.48	0.45	0.56	0.65	0.053	0.001	0.397	0.096
C20:4*n*-6	0.14	0.13	0.09	0.18	0.045	0.899	0.221	0.147
C20:5*n*-3	0.14	0.11	0.54	0.45	0.083	<0.001	0.299	0.609
C22:6*n*-3	0.09	0.00	0.54	0.51	0.084	<0.001	0.255	0.611
RFA[¶]	7.90	14.9	14.4	14.4	4.200	0.322	0.251	0.250
Total fatty acids (mg/ml)	90.8	72.1	76.2	71.1	11.90	0.366	0.172	0.428

MB = Megalac + 50 mg/kg vitamin E; MS = Megalac + 500 mg/kg vitamin E; FB = Fish oil + 50 mg/kg vitamin E; FS = Fish oil + 500 mg/kg vitamin E

[¶] RFA = All remaining fatty acids

Means without common superscripts are significantly different at the P<0.05 level

The proportion of the medium-chain fatty acid C16:0 within colostral fat was reduced by the addition of supranutritional amounts of vitamin E to the diet of the ewe, with mean values of 23.7 g/100 g fatty acids compared to 26.3 g/100 g fatty acids for ewes fed basal concentrations of vitamin E (P=0.012). Although unaffected by dietary vitamin E concentration, the amount of C18:0 present in colostrum was reduced by the addition of long-chain PUFAs to ewe diets (5.88 g/100 g fatty acids vs. 8.32 g/100 g fatty acids for fish oil and Megalac diets respectively, P<0.001).

The proportion of C16:1*n*-7 within colostrum fat was significantly increased by fish oil supplementation at 1.28 g/100 g fatty acids in contrast to 0.93 g/100 g fatty acids resulting from Megalac supplementation. Supplementation of ewes with fish oil during pregnancy also reduced the proportions of C18:1*n*-9 *cis* within colostrum, with mean values of 21.6 g/100 g fatty acids in ewes fed fish oil compared to 30.1 g/100 g fatty acids in those fed

Megalac ($P<0.001$). The proportion of C18:1 *trans* isomers in colostrum fat were increased in ewes fed fish oil compared to Megalac (4.57 g/100 g fatty acids compared to 2.95 g/100 g fatty acids respectively, $P<0.001$). There were no significant main effects of vitamin E supplementation or interactions between dietary fat source and vitamin E concentration upon the proportions of monoenoic fatty acids in colostrum.

The concentration of CLA within colostrum was increased by the addition of fish oil to ewe diets with means of 2.85 g/100 g fatty acids for ewes fed fish oil compared to 1.92 g/100 g fatty acids for those supplemented with Megalac ($P<0.001$). No significant effect of vitamin E supplementation was observed upon this parameter.

The predominant *n*-6 fatty acid observed in ewe colostrum samples was C18:2*n*-6 *cis*. A reduction in the proportion of this fatty acid within colostral fat was exhibited by ewes fed diets containing fish oil (FB or FS) compared to Megalac (1.91 g/100 g fatty acids vs. 2.47 g/100 g fatty acids, $P=0.001$), however, there was no significant effect of dietary vitamin E upon this parameter. Neither dietary fat source or vitamin E supplementation had any significant effect upon the proportional contribution of C20:4*n*-6 to colostrum fat.

The proportion of C18:3*n*-3 in colostral fat was increased in ewes offered a long-chain PUFAs during pregnancy with mean values of 0.60 g/100 g fatty acids compared to 0.46 g/100 g fatty acids for fish oil and Megalac diets respectively ($P=0.001$). Long-chain PUFA supplementation of the pregnant ewe also conferred increases in the proportions of C20:5*n*-3 in colostrum with means of 0.49 g/100 g fatty acids in ewes fed fish oil compared to 0.13 g/100 g fatty acids in ewes offered Megalac ($P<0.001$). Similarly, proportions of C22:6*n*-3 within colostrum fat were higher in ewes supplemented with fish oil (0.52 g/100 g fatty acids) compared to Megalac (0.04 g/100 g fatty acids, $P<0.001$).

The amount of dietary vitamin E offered to pregnant ewes had no significant main or interaction effect upon the proportions of individual *n*-3 fatty acids within ewe colostrum.

3.4.2.10. Plasma fatty acids (two weeks *post partum*)

Dietary treatment had no significant effect upon the total concentration of fatty acids in ewe plasma at two weeks *post partum* (Table 3.14). Fish oil supplementation of ewes conferred a reduction in the proportion of C16:0 within plasma when compared to Megalac (17.4 g/100 g fatty acids compared to 21.1 g/100 g fatty acids respectively, P<0.001), however, there was no significant difference between treatments on the proportion of C18:0 in plasma.

Table 3.14. Effect of PUFA and vitamin E supplementation of ewes on the proportions of fatty acids in ewe plasma samples collected at two weeks[¶] post partum

	Diet					P		
Fatty acid (g/100 g fatty acid)	**MB**	**MS**	**FB**	**FS**	**s.e.d.**	**Fat**	**Vit E**	**FxV**
C16:0	21.1	21.2	17.1	17.7	0.56	<0.001	0.409	0.455
C16:1*n*-7	1.11	1.49	2.02	1.54	0.485	0.177	0.885	0.227
C18:0	19.9	19.8	19.1	18.4	0.85	0.067	0.497	0.610
C18:1 *trans*	3.06	2.80	4.64	5.01	0.289	<0.001	0.789	0.139
C18:1*n*-9 *cis*	19.4	19.6	15.5	14.0	1.15	<0.001	0.465	0.300
C18:2*n*-6 *cis*	19.2	20.0	11.6	12.6	0.95	<0.001	0.194	0.969
CLA (*cis*-9,*trans*-11)	0.12	0.10	1.84	0.78	0.402	<0.001	0.075	0.082
C18:3*n*-3 *cis*	1.78	1.20	2.68	3.40	0.691	0.005	0.890	0.199
C20:4*n*-6	2.84	2.82	2.34	2.21	0.380	0.051	0.781	0.837
C20:5*n*-3	1.99	2.11	6.96	7.64	0.445	<0.001	0.216	0.383
C22:6*n*-3	0.26	0.15	3.13	3.82	0.421	<0.001	0.341	0.194
RFA[§]	9.25	8.67	13.12	12.96	1.478	<0.001	0.725	0.845
Total fatty acids (mg/ml)	1.44	1.30	1.12	1.18	0.187	0.110	0.754	0.443

MB = Megalac + 50 mg/kg vitamin E; MS = Megalac + 500 mg/kg vitamin E; FB = Fish oil + 50 mg/kg vitamin E; FS = Fish oil + 500 mg/kg vitamin E

[¶] two weeks *post partum* = day 14 of lactation

[§] RFA = All remaining fatty acids

Small numerical increases in the amount of C16:1*n*-7 in plasma as a result of fish oil supplementation were not statistically significant. Ewes fed fish oil had lower proportions of C18:1*n*-9 *cis* in plasma than ewes offered Megalac (14.7 g/100 g fatty acids vs. 19.5 g/100 g fatty acids respectively, P<0.001). By contrast, the proportion of C18:1 *trans* isomers within ewe plasma lipid was increased by the provision of fish oil (4.84 g/100 g

fatty acids) when compared to Megalac (2.93 g/100 g fatty acids, P<0.001). Offering diets containing fish oil to pregnant and lactating ewes had an augmenting effect upon the proportion of CLA within ewe plasma (1.31 g/100 g fatty acids for ewes offered diets containing fish oil compared to 0.11 g/100 g fatty acids for those fed diets based on Megalac, P<0.001). There were no significant effects of dietary vitamin E supply upon individual polyenoic fatty acid proportions.

Ewe offered fish oil as the main fat source had lower proportions of C18:2*n*-6 *cis* in plasma than those fed diets containing Megalac (12.1 g/100 g fatty acids compared to 19.6 g/100 g fatty acids, P<0.001). By contrast, the proportion of C20:4*n*-6 within plasma was unaffected by dietary treatment, although ewes supplemented with fish oil tended to have higher concentrations of this fatty acid in plasma compared to ewes fed Megalac (P=0.051).

A two-fold increase in C18:3*n*-3 was observed in ewe plasma lipid as a result of fish oil supplementation (3.04 g/100 g fatty acids) when compared to Megalac supplementation (1.49 g/100 g fatty acids, P=0.005). The proportion of C20:5*n*-3 in ewe plasma was increased by a factor of 3.5 by fish oil supplementation of ewes (7.30 g/100 g fatty acids) when contrasted with Megalac (2.05 g/100 g fatty acids, P<0.001). A highly significant increase in the proportional contribution of C22:6*n*-3 to plasma fatty acids was conferred by fish oil supplementation with means of 3.47 g/100 g fatty acids in ewes offered diets containing fish oil compared to 0.21 g/100 g fatty acids for ewes fed Megalac.

3.4.2.11. Milk production

There was no significant effect of dietary fat source or vitamin E concentration observed upon milk secretion rate or yield (Table 3.15). Offering fish oil to pregnant and lactating ewes conferred a increase in the fat concentration of milk samples (96.8 g/kg for diets FB

+ FS compared to 74.6 g/kg for diets MB + MS, P=0.031). Moreover, a tendency for ewes fed diet FB to have a higher milk fat content than those offered diet MB (P=0.053) was observed. Milk fat yields were higher in ewes fed diets containing long-chain PUFAs, however, the differences were not significantly different. There was no significant main effect of dietary vitamin E concentration upon milk fat concentration or yield.

Table 3.15. Effect of PUFA and vitamin E supplementation of ewes on milk parameters

	Diet					P		
	MB	**MS**	**FB**	**FS**	**s.e.d.**	**Fat**	**Vit E**	**FxV**
Secretion rate (ml/hour)	96.7	82.9	91.8	102	11.13	0.382	0.817	0.143
Yield (l/day)	2.32	1.99	2.20	2.44	0.267	0.382	0.817	0.143
Fat concentration (g/kg)	62.2	87.0	104	89.5	13.76	0.031	0.604	0.053
Fat yield (g/hour)	5.35	7.31	8.51	8.00	1.464	0.075	0.492	0.244
Protein concentration (g/kg)	31.2^{a}	40.1^{b}	40.9^{b}	38.5^{ab}	3.59	0.122	0.211	0.034
Protein yield (g/hour)	2.41^{a}	3.52^{ab}	4.25^{b}	3.39^{ab}	0.585	0.048	0.764	0.02
Lactose concentration (g/kg)	39.9	47.7	46.6	48.6	4.28	0.222	0.118	0.348
Lactose yield (g/hour)	1.52	1.91	1.89	1.83	0.167	0.232	0.169	0.072
Vitamin E concentration (mg/kg)	0.95^{a}	3.44^{c}	0.65^{a}	1.96^{b}	0.329	0.001	<0.001	0.020
Vitamin E yield (mg/hour)	0.11	0.20	0.06	0.22	0.046	0.756	<0.001	0.334

MB = Megalac + 50 mg/kg vitamin E; MS = Megalac + 500 mg/kg vitamin E; FB = Fish oil + 50 mg/kg vitamin E; FS = Fish oil + 500 mg/kg vitamin E
Means without common superscripts are significantly different at the P<0.05 level

Milk protein concentrations and yields were lower in ewes fed diet MB compared to the other three treatment diets (P=0.034). No significant main effects of fat source or dietary vitamin E concentration were observed upon milk protein concentration, however, protein yield (g/hour) was increased by the addition of long-chain PUFAs to the ewes' diet (3.82 g/hour vs. 2.96 g/hour for fish oil and Megalac diets respectively, P=0.048). There was no significant effect of dietary treatment upon milk lactose concentration or yield.

The concentration of vitamin E within milk was significantly increased by supplementing the ewe with a supranutritional level of vitamin E (2.70 mg/kg) compared to a basal dietary concentration (0.80 mg/kg). However, long-chain PUFA supplementation had an antagonistic effect upon milk vitamin E concentrations, with means of 1.30 mg/kg in milk

from ewes fed fish oil compared to 2.20 mg/kg in milk from those fed Megalac (P=0.001). A significant interaction between dietary fat source and vitamin E concentration was observed with ewes fed diet MS having higher milk vitamin E concentration than ewes fed any of the other treatment diets. By contrast, fat source had no significant effect on vitamin E yield although this was increased by the addition of supranutritional concentrations of vitamin E to the diet (0.21 mg/hour for diets MS + FS compared to 0.09 mg/hour for diets MB + FB, P<0.001).

Table 3.16. Effect of PUFA and vitamin E supplementation of ewes on the proportions of fatty acids in milk

	Diet					P		
Fatty acid (g/100 g fatty acid)	**MB**	**MS**	**FB**	**FS**	**s.e.d.**	**Fat**	**Vit E**	**FxV**
C4:0	4.78	4.70	3.93	3.86	0.237	<0.001	0.638	0.970
C6:0	2.04	1.93	2.12	1.94	0.217	0.800	0.356	0.823
C8:0	1.37	1.24	1.63	1.45	0.194	0.111	0.273	0.869
C10:0	3.18	2.95	3.80	3.32	0.489	0.170	0.317	0.721
C12:0	1.79	1.61	2.40	2.03	0.231	0.002	0.066	0.493
C14:0	5.69	5.21	7.05	6.70	0.341	<0.001	0.103	0.792
C16:0	25.9	25.9	21.4	21.5	1.30	<0.001	0.910	0.947
C16:1*n*-7	1.10	1.18	1.45	1.71	0.208	0.007	0.270	0.536
C18:0	12.9	14.1	8.78	9.36	0.722	<0.001	0.089	0.526
C18:1 *trans*	3.43	3.28	5.04	5.54	0.680	0.002	0.892	0.411
C18:1*n*-9 *cis*	26.3	27.5	21.9	22.8	0.946	<0.001	0.130	0.797
C18:2*n*-6 *cis*	1.49	1.10	1.35	1.69	0.333	0.336	0.922	0.140
CLA (*cis*-9,*trans*-11)	1.09	0.90	1.48	1.16	0.264	0.098	0.175	0.734
C18:3*n*-3 *cis*	0.32	0.31	0.45	0.41	0.076	0.044	0.608	0.750
C20:4*n*-6	0.08	0.10	0.19	0.19	0.097	0.172	0.868	0.884
C20:5*n*-3	0.05	0.00	0.29	0.13	0.073	0.002	0.049	0.283
C22:6*n*-3	0.03	0.00	0.46	0.62	0.162	<0.001	0.572	0.435
RFA[¶]	8.29	7.89	16.3	15.7	1.95	<0.001	0.699	0.920
Total fatty acids (mg/ml)	86.9	96.6	68.0	66.5	9.56	0.002	0.550	0.414

MB = Megalac + 50 mg/kg vitamin E; MS = Megalac + 500 mg/kg vitamin E; FB = Fish oil + 50 mg/kg vitamin E; FS = Fish oil + 500 mg/kg vitamin E

[¶] RFA = All remaining fatty acids

3.4.2.12. Milk fatty acids

The total concentration of fatty acids within ewe milk at three weeks *post partum* was reduced by the provision of long-chain PUFAs to pregnant and lactating ewes (67.3 mg/ml in ewes fed fish oil compared to 91.7 mg/ml in those offered Megalac, P=0.002; Table 3.16). Vitamin E supplementation had no significant main or interaction effect upon this parameter.

Long-chain PUFA supplementation of ewes reduced the proportion of C4:0 in ewe milk fat (3.89 g/100 g fatty acids for diets FB + FS compared to 4.74 g/100 g fatty acids for diets MB + MS, P<0.001). Dietary fat source had no significant effect upon the proportion of C6:0, C8:0 or C10:0 within milk fat. However, the proportions of C12:0 (2.22 g/100 g fatty acids vs. 1.70 g/100 g fatty acids, P=0.002) and C14:0 (6.88 g/100 g fatty acids vs. 5.45 g/100 g fatty acids, P<0.001) in milk fat were increased by the addition of long-chain PUFAs to the ewe diet when compared to Megalac supplementation. There was no significant effect of the amount of vitamin E offered to ewes upon the proportions of the saturated fatty acids C4:0 – C14:0 within milk. The predominant saturated fatty acid in milk fat, C16:0, was lower in ewes fed diets containing long-chain PUFAs compared to those fed Megalac (21.4 g/100 g fatty acids compared to 25.9 g/100 g fatty acids respectively, P<0.001), however, there was no significant main or interaction effect of dietary vitamin E concentration. Similar effects of dietary treatment were observed upon the proportion of C18:0 in ewe milk with mean values of 9.07 g/100 g fatty acids in ewes offered diets containing fish oil compared to 13.5 g/100 g fatty acids in those fed Megalac (P<0.001).

The addition of long-chain PUFAs to the ewe diet increased the proportion of C16:1*n*-7 (1.58 g/100 g fatty acids for ewes fed fish oil compared to 1.13 g/100 g fatty acids in those fed Megalac, P=0.007) and C18:1 *trans* (5.28 g/100 g compared to 3.36 g/100 g fatty acids for fish oil and Megalac diets respectively, P=0.002) in milk fat. The proportion of C18:1*n*-9 *cis* within milk fat was lower (22.3 g/100 g fatty acids vs. 26.9 g/100 g fatty acids, P<0.001) in ewes offered diets containing fish oil compared to those fed Megalac. Dietary vitamin E concentration had no effect upon the amount of C18:1*n*-9 *cis* in milk. Neither dietary fat source nor vitamin E concentration conferred any significant change upon proportions of milk CLA, although ewes fed fish oil tended to have higher proportions of this fatty acid in milk fat (P=0.098).

No significant main or interaction treatment effects were observed upon the proportions of C18:2*n*-6 or C20:4*n*-6 in milk. By contrast, a significant difference in the proportion of C18:3*n*-3 within milk lipids was evident, with ewes offered fish oil (0.43 g/100 g fatty acids) having higher values for C18:3*n*-3 than those fed Megalac (0.32 g/100 g fatty acids). Furthermore, the proportion of C20:5*n*-3 within milk was enhanced by the addition of long-chain PUFAs to the ewe diet with means of 0.21 g/100 g fatty acids compared to 0.02 g/100 g fatty acids in ewes fed diets containing Megalac (P=0.002). The proportion of C22:6*n*-6 within milk was also increased by long-chain PUFA supplementation of the ewe when contrasted with supplementation with Megalac (0.54 g/100 g fatty acids compared to 0.01 g/100 g fatty acids, P<0.001). There was no significant effect of dietary vitamin E concentration upon the amounts of *n*-3 PUFAs in milk, save for a reduction in the concentration of C20:5*n*-3 effected by supranutritional supplementation (0.170 mg/ml for diets MS + FS compared to 0.130 mg/ml for diets MB + FB, P=0.049).

3.4.3. Lamb performance

3.4.3.1. Neonatal lamb behaviour

Ewe and lamb behavioural parameters are presented in Table 3.17. Maternal behaviour scores were similar for all treatments, regardless of dietary fat source or vitamin E concentration. An interaction effect was observed between fat source and dietary vitamin E concentration upon the latency of standing in neonatal lambs: supranutritional vitamin E supplementation reduced the time taken to stand in lambs from ewes fed fish oil (diet FS) but increased the latency of standing in lambs borne by ewes offered Megalac (diet MS; P=0.048). In addition, maternal fish oil supplementation tended to reduce the time taken to stand with mean values of 16.3 minutes compared to 20.3 minutes resulting from Megalac supplementation (P=0.082).

Table 3.17. Effect of PUFA and vitamin E supplementation of ewes on maternal behaviour scores and latencies of neonatal lamb behaviours

	Diet					P		
	MB	MS	FB	FS	s.e.d.	Fat	Vit E	FxV
Maternal measurements:								
Maternal behaviour score	134	132	135	129	4.9	0.882	0.302	0.552
Neonatal measurements:								
Latency of standing (min)	17.0^{ab}	23.6^{b}	17.6^{ab}	14.9^{a}	3.23	0.082	0.388	0.048
Latency of searching for the udder (min)	24.7	26.0	24.3	19.8	4.01	0.253	0.580	0.316
Latency of successful suckling (min)	38.2	48.7	33.6	34.5	4.92	0.009	0.105	0.171

MB = Megalac + 50 mg/kg vitamin E; MS = Megalac + 500 mg/kg vitamin E; FB = Fish oil + 50 mg/kg vitamin E; FS = Fish oil + 500 mg/kg vitamin E
Means without common superscripts are significantly different at the $P<0.05$ level

No significant main effects of fat source or vitamin E concentration were observed on the time taken by lambs to search for the udder. Nevertheless, lambs produced by ewes fed diets containing fish oil suckled significantly faster at 34.0 minutes when compared with lambs from ewes fed Megalac diets at 43.4 minutes. No significant main or interaction effect of dietary vitamin E concentration was observed upon this parameter.

3.4.3.2. Neonatal lamb plasma vitamin E

Vitamin E concentrations in neonatal plasma were only detectable in three out of twelve plasma samples, the measurable values being in lambs from treatment MS (2 samples) and FS (1 sample).

Table 3.18. Effect of PUFA and vitamin E supplementation of ewes on vitamin E concentrations in neonatal lamb tissues

	Diet					P		
	MB	MS	FB	FS	s.e.d.	Fat	Vit E	FxV
Brain vitamin E (mg/kg)	1.59	2.85	1.57	2.11	0.389	0.216	0.017	0.238
Muscle vitamin E (mg/kg)	0.69	1.18	0.57	0.95	0.128	0.099	0.003	0.573

MB = Megalac + 50 mg/kg vitamin E; Megalac + 500 mg/kg vitamin E; FB = Fish oil + 50 mg/kg vitamin E; FS = Fish oil + 500 mg/kg vitamin E

3.4.3.3. Neonatal lamb tissue vitamin E

Supranutritional vitamin E supplementation of the pregnant ewe significantly increased the concentration of vitamin E within neonatal brain tissue with mean values of 2.48 mg/kg for

supranutritional diets compared to 1.58 mg/kg for ewes offered basal diets (Table 3.18). Although a numerical reduction in brain vitamin E concentration existed in lambs from ewes fed fish oil compared to Megalac (1.84 mg/kg compared to 2.22 mg/kg respectively), this difference was not statistically significant.

Vitamin E concentrations in neonatal muscle tissue were also increased by the provision of supranutritional dietary concentrations of vitamin E to the ewe during pregnancy at 1.06 mg/kg in contrast to 0.63 mg/kg for ewes offered basal dietary concentrations of vitamin E (P=0.003). Furthermore, a tendency for maternal long-chain PUFA supplementation to reduce neonatal muscle vitamin E concentrations was in evidence; lambs from ewes fed fish oil had a mean vitamin E concentration of 0.76 mg/kg compared to 0.93 mg/kg for ewes fed Megalac (P=0.099). No significant interaction between fat source and maternal dietary vitamin E concentration was observed upon neonatal muscle vitamin E concentrations.

3.4.3.4. Neonatal lamb plasma fatty acids

The total concentration of fatty acids within neonatal lamb plasma was reduced by fish oil supplementation with means of 0.280 mg/ml compared to 0.413 mg/ml for Megalac supplementation (P=0.041; Table 3.19). Maternal dietary vitamin E concentration had no significant effect upon total fatty acid concentrations within neonatal lamb plasma. No significant effect of dietary treatment was observed upon the proportions of individual saturated fatty acids within plasma. The only monoenoic fatty acids of significance detected in neonatal plasma were C16:1*n*-7 and C18:1*n*-9 *cis*, however, neither fatty acid was subject to manipulation by maternal diet. The proportion of CLA within neonatal lamb plasma was not altered by dietary supplementation of ewes with long-chain PUFAs, vitamin E, or their combination.

Table 3.19. Effect of PUFA and vitamin E supplementation of ewes on the proportions of fatty acids in neonatal lamb plasma

	Diet					P		
Fatty acid (g/100 g fatty acid)	**MB**	**MS**	**FB**	**FS**	**s.e.d.**	**Fat**	**Vit E**	**FxV**
C16:0	21.3	21.1	22.6	21.2	1.93	0.624	0.559	0.671
C16:1*n*-7	6.02	5.83	8.06	5.50	1.763	0.518	0.313	0.377
C18:0	9.89	9.37	10.20	10.50	0.821	0.262	0.849	0.507
C18:1 *trans*	ND	0.44	ND	ND	0.157	-	-	-
C18:1*n*-9 *cis*	34.2	40.6	33.0	32.3	5.67	0.280	0.503	0.407
C18:2*n*-6 *cis*	3.23	3.25	5.60	3.57	0.768	0.046	0.118	0.104
CLA (*cis*-9,*trans*-11)	1.66	0.47	0.68	1.49	0.960	0.979	0.792	0.190
C18:3*n*-3 *cis*	1.63	2.20	1.03	4.54	2.180	0.592	0.234	0.378
C20:4*n*-6	2.81	2.40	1.57	1.99	0.828	0.209	0.993	0.506
C20:5*n*-3	1.85	0.42	1.80	2.64	0.692	0.069	0.573	0.059
C22:6*n*-3	0.00	1.03	2.62	3.64	0.981	0.009	0.192	0.992
RFA[¶]	17.4	12.9	12.8	12.7	1.80	0.104	0.116	0.133
Total fatty acids (mg/ml)	0.352	0.474	0.271	0.289	0.0726	0.041	0.221	0.347

MB = Megalac + 50 mg/kg vitamin E; MS = Megalac + 500 mg/kg vitamin E; FB = Fish oil + 50 mg/kg vitamin E; FS = Fish oil + 500 mg/kg vitamin E
[¶] RFA = All remaining fatty acids; ND = not detected

The proportional contribution of C18:2*n*-6 to total fatty acids within plasma was increased by the addition of fish oil to the maternal diet with mean values of 4.59 g/100 g fatty acids in contrast to 3.23 g/100 g fatty acids for Megalac diets (P=0.046). There was no effect of maternal vitamin E supplementation upon the proportion of C18:2*n*-6 within neonatal lamb plasma. Numerical differences existed between the proportion of C20:4*n*-6 in plasma for fish oil compared to Megalac, however, these differences did not reach statistical significance.

No significant main or interaction treatment effects were observed upon the proportions of the fatty acids C18:3*n*-3 within plasma lipid. By contrast, the proportional contribution of C20:5*n*-3 to plasma fatty acids tended to be higher in lambs borne by ewes fed fish oil (2.22 g/100 g fatty acids) when compared to those produced by ewes fed Megalac (1.14 g/100 g fatty acids; P=0.069). Furthermore, ewes fed diet FS tended to bear lambs with higher proportions of this fatty acid within plasma when compared to those fed diet MS. An increase in C22:6*n*-3 was exhibited by lambs borne by ewes offered fish oil diets when

compared to ewes fed Megalac (3.13 g/100 g fatty acids vs. 0.51 g/100 g fatty acids respectively, P=0.009).

Table 3.20. Effect of PUFA and vitamin E supplementation of ewes on the proportions of fatty acids in neonatal lamb brain

	Diet					P		
Fatty acid (g/100 g fatty acid)	**MB**	**MS**	**FB**	**FS**	**s.e.d.**	**Fat**	**Vit E**	**FxV**
C16:0	19.2	20.0	18.3	18.9	0.72	0.095	0.202	0.917
C16:1*n*-7	0.75	0.80	0.78	0.78	0.062	0.979	0.538	0.599
C18:0	15.3	15.2	14.6	15.2	0.48	0.366	0.453	0.375
C18:1 *trans*	0.10	0.13	0.14	0.08	0.046	0.940	0.692	0.209
C18:1*n*-9 *cis*	14.6	14.4	13.5	15.0	1.44	0.815	0.538	0.408
C18:2*n*-6 *cis*	0.24	0.30	0.27	0.23	0.105	0.748	0.896	0.526
CLA (*cis*-9,*trans*-11)	1.11	0.94	1.05	0.99	0.241	0.947	0.530	0.759
C18:3*n*-3 *cis*	0.19	0.41	0.65	0.43	0.181	0.111	0.955	0.141
C20:4*n*-6	4.33	2.98	2.20	3.13	1.045	0.228	0.788	0.174
C20:5*n*-3	0.27	0.00	0.94	0.67	0.167	0.011	0.104	0.011
C22:6*n*-3	10.4	10.5	11.8	11.3	0.62	0.055	0.631	0.533
RFA[¶]	33.5	34.4	36.8	33.6	1.77	0.347	0.375	0.148
Total fatty acids (mg/g)	26.1	25.5	26.6	25.0	1.29	0.986	0.282	0.654

MB = Megalac + 50 mg/kg vitamin E; MS = Megalac + 500 mg/kg vitamin E; FB = Fish oil + 50 mg/kg vitamin E; FS = Fish oil + 500 mg/kg vitamin E
[¶] RFA = All remaining fatty acids; ND = not detected

3.4.3.5. Neonatal lamb brain fatty acids

The total fatty acid concentration of neonatal lamb brain tissue was equivalent between treatments (Table 3.20). Fish oil supplementation of the ewe tended (P=0.095) to reduce the proportional contribution of C16:0 to total lamb brain fatty acids, however, there was no significant effect of dietary vitamin E supply to the ewe. The proportions of C18:0 within neonatal brain tissue were similar between treatments with no significant effect of dietary treatment. The proportions of all recorded monoenoic fatty acids (C16:1*n*-7, C18:1 *trans* and C18:1*n*-9 *cis*) were equivalent between treatments, with no significant effect of maternal dietary fat source or vitamin E concentration. The *cis*-9,*trans*-11 CLA isomer was found in concentrations approximately equal to 1% of brain fatty acids within each sample, with no statistically significant effects of either maternal dietary fat source or vitamin E concentration.

The proportion of C18:2*n*-6 within brain tissue lipids was equivalent between treatments with no significant effect of maternal dietary fat source or vitamin E concentration. Feeding fish oil to pregnant ewes appeared to confer a decrease in the proportion of C20:4*n*-6 within neonatal lamb brains when compared to those offered Megalac, however, this difference was not statistically significant.

The mean proportion of C18:3*n*-3 within neonatal brain fatty acids varied from 0.19 g/100 g fatty acids (diet MB) to 0.65 g/100 g fatty acids (diet FB), however, these differences did not reach statistical significance. Fish oil supplementation of the ewe conferred a significant increase in the proportion of C20:5*n*-3 within neonatal lamb brain tissue with mean values of 0.81 mg/100 g fatty acids compared to 0.14 mg/100 g fatty acids in samples from lambs produced by ewes fed Megalac. Lambs born to ewes offered diets containing fish oil tended to have increased proportions of C22:6*n*-3 within brain tissue with mean values of 11.50 g/100 g fatty acids compared to 10.5 g/100 g fatty acids in lambs borne by ewes fed Megalac, a difference that approached significance at P=0.055.

3.4.3.2. Lamb liveweight

No significant effect of long-chain PUFA supplementation was observed upon lamb birthweight or liveweight (Table 3.21). However, ewes fed fish oil during pregnancy and lactation tended to have lower individual lamb growth rates than those fed Megalac (0.253 kg/day compared to 0.270 kg/day respectively, P=0.054). Individual lamb birthweights were increased by supranutritional vitamin E supplementation of pregnant ewes (Table 3.21), with mean values of 4.17 kg for lambs compared to 3.86 kg for lambs from ewes offered diets containing a basal concentration of vitamin E (P=0.023). This difference persisted as a significant main effect of vitamin E supplementation until three weeks of age, after which there was no significant effect of dietary vitamin E concentration on lamb liveweight.

Table 3.21. Effect of PUFA and vitamin E supplementation of ewes on lamb birthweights (kg) and lamb and litter growth rates (kg/day)

	Diet					P		
	MB	MS	FB	FS	s.e.d.	Fat	Vit E	FxV
Lamb liveweight:								
At birth	3.87	4.01	3.85	4.33	0.190	0.268	0.023	0.215
At 1 week of age	5.70	5.95	5.50	6.17	0.262	0.960	0.015	0.264
At 2 weeks of age	7.58	8.09	7.64	8.23	0.309	0.646	0.014	0.832
At 3 weeks of age	9.41	9.90	9.44	9.93	0.391	0.909	0.082	0.991
At 4 weeks of age	11.0	11.6	11.1	11.6	0.48	0.824	0.117	0.869
Lamb growth rate	0.26	0.28	0.25	0.25	0.012	0.054	0.270	0.333
Litter growth rate	0.51	0.56	0.50	0.48	0.026	0.013	0.374	0.074

MB = Megalac + 50 mg/kg vitamin E; Megalac + 500 mg/kg vitamin E; FB = Fish oil + 50 mg/kg vitamin E; FS = Fish oil + 500 mg/kg vitamin E

Litter growth rates from birth until four weeks of age were reduced in lambs suckling ewes fed fish oil (mean of 0.487 kg/day) compared to those feeding from ewes offered Megalac (0.536 kg/day, P=0.013). Supranutritional vitamin E supplementation of the ewe also tended to reduce litter growth rates when fed in combination with long-chain PUFAs (diet FS) in contrast to Megalac (diet MS; P=0.074).

3.4.3.3. Suckling lamb antioxidant status

At two weeks of age, long-chain PUFA supplementation of the ewe had an antagonistic effect upon lamb plasma vitamin E concentrations with concentrations of 2.82 mmol/l (diets FB + FS) compared to 4.97 mmol/l (diets MB + MS, P=0.001; Table 3.22). Lambs borne by ewes offered supranutritional concentrations of vitamin E had higher plasma vitamin E concentrations (5.75 mmol/l) compared to those suckling ewes fed diets containing a basal vitamin E concentration (2.04 mmol/l, P<0.001). An interaction between fat source and maternal dietary vitamin E concentration was also in evidence (P=0.036) with supranutritional dietary concentrations of vitamin E resulting in a greater increase in plasma vitamin E in lambs suckling ewes fed Megalac (diet MS) compared to fish oil (diet FS).

Table 3.22. Effect of PUFA and vitamin E supplementation of ewes on indicators of vitamin E and selenium status and of cellular damage in lambs at two weeks of age

	Diet					P		
	MB	MS	FB	FS	s.e.d.	Fat	Vit E	FxV
Plasma vitamin E (mmol/l)	2.47[a]	7.48[c]	1.60[a]	4.02[b]	0.816	0.001	<0.001	0.036
Erythrocyte GPx (U/ml PCV)	289	308	273	277	16.3	0.049	0.336	0.514
Serum CK (U/l)	194	143	456	421	162.7	0.022	0.709	0.944

MB = Megalac + 50 mg/kg vitamin E; Megalac + 500 mg/kg vitamin E; FB = Fish oil + 50 mg/kg vitamin E; FS = Fish oil + 500 mg/kg vitamin E

Means without common superscripts are significantly different at the P<0.05 level

Lambs suckling ewes fed long-chain PUFAs had significantly lower activities of GPx in erythrocytes than feeding from ewes fed Megalac (275 U/ml PCV compared to 299 U/ml PCV respectively, P=0.049). By contrast, Vitamin E supplementation of the ewe had no effect upon the activity of GPx in lamb erythrocytes. Maternal fish oil supplementation conferred an increase in the serum CK concentrations in lambs at two weeks of age at 439 U/l compared to 168 U/l resulting from Megalac supplementation (P=0.022). Vitamin E supplementation of the ewe had no significant effect upon CK concentrations in lamb serum.

3.4.3.4. Suckling lamb plasma fatty acids

Offering fish oil to pregnant and lactating ewes reduced the total fatty acid concentration in lamb plasma at two weeks of age (1.85 mg/ml for diets FB + FS, 2.42 mg/ml for diets MB + MS, P=0.003; Table 3.23). However, there was no significant effect of maternal vitamin E supplementation upon this parameter. Maternal long-chain PUFA supplementation reduced the proportions of C16:0 (20.7 g/100 g fatty acids compared to 22.6 g/100 g fatty acids, P=0.002) and C18:0 (12.9 g/100 g fatty acids compared to 15.8 g/100 g fatty acids, P<0.001) within lamb plasma lipid when compared to supplementation with Megalac. No significant effect of vitamin E supplementation of the ewe was in evidence upon these parameters.

Table 3.23. Effect of PUFA and vitamin E supplementation of ewes on the proportions of fatty acids in lamb plasma samples collected at two weeks of age

	Diet					P		
Fatty acid (g/100 g fatty acid)	**MB**	**MS**	**FB**	**FS**	**s.e.d.**	**Fat**	**Vit E**	**FxV**
C16:0	23.3	21.9	20.3	21.0	0.79	0.002	0.499	0.068
C16:1*n*-7	0.96	1.32	1.50	1.59	0.311	0.081	0.322	0.549
C18:0	15.6	16.0	13.4	12.3	0.71	<0.001	0.473	0.135
C18:1 *trans*	2.80	2.76	4.33	4.44	0.212	<0.001	0.823	0.599
C18:1*n*-9 *cis*	25.1	25.8	20.4	21.0	1.11	<0.001	0.398	0.964
C18:2*n*-6 *cis*	12.6	12.6	10.1	9.07	0.856	<0.001	0.416	0.401
CLA (*cis*-9,*trans*-11)	0.19	0.14	0.78	1.43	0.283	<0.001	0.153	0.095
C18:3*n*-3 *cis*	1.16	1.08	2.05	1.96	0.273	<0.001	0.664	0.963
C20:4*n*-6	3.09	3.50	2.15	1.94	0.299	<0.001	0.639	0.165
C20:5*n*-3	1.06[a]	1.13[a]	5.20[c]	4.16[b]	0.304	<0.001	0.036	0.018
C22:6*n*-3	0.66[a]	1.64[b]	2.57[c]	2.32[c]	0.329	<0.001	0.129	0.015
RFA[¶]	13.4	12.1	17.2	18.8	1.20	<0.001	0.888	0.105
Total fatty acids (mg/ml)	2.60	2.23	1.84	1.86	0.240	0.003	0.296	0.270

MB = Megalac + 50 mg/kg vitamin E; MS = Megalac + 500 mg/kg vitamin E; FB = Fish oil + 50 mg/kg vitamin E; FS = Fish oil + 500 mg/kg vitamin E

[¶] RFA = All remaining fatty acids

Means without common superscripts are significantly different at the P<0.05 level

Dietary treatment had no significant main or interaction effect upon the proportion of C16:1*n*-7 in plasma at two weeks of age. The proportion of C18:1*n*-9 was lower in lambs borne by ewes offered diets containing fish oil (20.7 g/100 g fatty acids) when compared to those containing Megalac (25.4 g/100 g fatty acids, P<0.001). By contrast, adding fish oil to the diets of ewes during pregnancy and lactation significantly increased the proportional contribution of C18:1 *trans* to lamb plasma fatty acids (4.38 g/100 g fatty acids for fish oil treatments vs. 2.78 g/100 g fatty acids for Megalac treatments). A significant increase in the proportion of CLA was observed within lamb plasma as a consequence of supplementing maternal diets with long-chain PUFAs (1.10 g/100 g fatty acids compared to 0.17 g/100 g fatty acids for fish oil and Megalac diets respectively).

The proportion of C18:2*n*-6 in lamb plasma at two weeks of age was reduced by long-chain PUFA supplementation of the dam with means of 9.58 g/100 g fatty acids compared to 12.6 g/100 g fatty acids (P<0.001) for maternal diets containing fish oil and Megalac respectively. The proportion of C20:4*n*-6 within plasma lipids was lower in lambs produced by ewes supplemented with long-chain PUFAs when compared to those suckling

ewes offered Megalac (2.05 g/100 g fatty acids vs. 3.30 g/100 g fatty acids, P<0.001). However, no significant effect of vitamin E supplementation of the dam was observed upon the proportions of individual *n*-6 fatty acids within plasma.

Proportions of C18:3*n*-3 were increased from 1.12 g/100 g fatty acids in lambs suckling ewes fed Megalac to 2.00 g/100 g fatty acids in those suckling ewes offered fish oil (P<0.001). In addition the proportion of C20:5*n*-3 within suckling lamb plasma lipid was increased from 1.09 g/100 g fatty acids (diets MB + MS) to 4.68 g/100 g fatty acids (diets FB + FS; P<0.001). The proportion of C20:5*n*-3 was also lower in lambs produced by ewes fed supranutritional levels of vitamin E (2.64 g/100 g fatty acids) compared to those fed diets containing basal vitamin E concentrations (3.13 g/100 g fatty acids, P=0.036). Lambs suckling ewes fed diet FB had higher proportions of C20:5*n*-3 than those feeding from ewes fed diets MB or MS (P=0.018). The proportion of C22:6*n*-3 within suckling lamb plasma was significantly augmented from 1.15 g/100 g fatty acids in lambs suckling ewes fed Megalac to 2.45 g/100 g fatty acids in those suckling ewes offered diets containing fish oil (P<0.001). Furthermore, the highest proportions of C22:6*n*-3 were observed in lambs produced by ewes fed diets FB or FS compared to diet MB (P=0.015).

3.5. Discussion

3.5.1. Ewe parameters

3.5.1.1. Straw Intake

Within the current study, neither dietary fat source nor vitamin E concentration had any significant effect upon the daily straw intake of pregnant and lactating ewes. This is in direct contrast to the results of Donovan *et al.* (2000), Keady and Mayne (2000) and Annett *et al.* (2004) who demonstrated significant decreases in dry matter intake as a result of supplementing ruminants with fish oil. Szumacher-Strabel *et al.* (2001a; 2001b) concluded that this effect may be a consequence of the toxic effects of long-chain PUFAs upon rumen microflora with subsequent reductions in fibre digestibility. However, supplementing ruminant diets with fatty acids protected from ruminal biohydrogenation may reduce or eliminate the adverse effects of long-chain PUFAs upon DM intake. Kitessa *et al.* (2001a; 2001b) supplemented lactating ewes and goats with either protected or unprotected tuna oil and reported no significant effect of protected oil on DM intake although intake was reduced in animals offered unprotected oil. Moreover, Sanz Sampelayo *et al.* (2002) demonstrated that the DM intake of goats was unaffected by protected PUFA supplementation when compared to a control concentrate. It is postulated that the adsorbent nature of the vermiculite carrier included in the treatment concentrate may have rendered the long-chain PUFAs unavailable to rumen microflora, thereby negating the effects observed in studies employing unprotected fish oils.

3.5.1.2. Nutritional status

Supplementing pregnant sows with salmon oil or tuna oil had no significant effect upon liveweight or backfat thickness in the studies of Rooke *et al.* (2000) and Rooke *et al.* (2001b) respectively. Similarly, adding fish oil to the diet of pregnant and lactating ewes had no effect upon ewe body condition score in the current study. However, the extent of liveweight loss *post partum* was significantly lower in ewes supplemented with long-chain

PUFAs. Furthermore, plasma βHB and NEFA, metabolic indicators of adipose tissue mobilisation, were significantly lower in ewes supplemented with fish oil. These results are in agreement with those reported by Ahnadi *et al.* (2002) as a result of feeding protected fish oil to dairy cattle. Annett *et al.* (2004) also observed reduced liveweight change *pre-partum* in ewes supplemented with fish oil, however, both Keady *et al.* (2000) and Lacasse and Anhadi (1998) described increased liveweight loss in lactating cattle supplemented with fish oil. In the aforementioned studies (Annett *et al.*, 2004; Keady *et al.*, 2000; Lacasse and Anhadi, 1998), dry matter intake was significantly reduced by PUFA supplementation with consequent effects upon total energy intake, an effect which was not observed in the current experiment. By contrast, Chilliard and Doreau (1997) and Whitlock *et al.* (2002) described no significant effect of fish oil supplementation on the liveweight or body condition score of lactating cows, despite decreases in feed intake. The lower rate of liveweight loss observed in fish-oil supplemented ewes within the current experiment may be attributed to the reduced colostrum and milk production and consequent improvement in energy balance *post partum*.

Supplementing ewes with supranutritional dietary concentrations of vitamin E increased body condition score loss during pregnancy, a difference that became significant during lactation. As vitamin E acts as an antioxidant within cells (Gonzalez-Corbella *et al.*, 1998), the increase in oxidative challenge to the ruminant resulting from mobilisation of adipose tissue to meet requirements for milk production may increase the dietary vitamin E requirement (Allison and Laven, 2000). However, this result does not concur with the effects of vitamin E supplementation reported by both Kott *et al.* (1998), and Merrell (1998) who observed no significant influence of vitamin E on ewe body condition score.

3.5.1.3. Ewe antioxidant status

Within the current study, supranutritional supplementation of the pregnant and lactating ewe resulted in a significant increase in plasma vitamin E concentrations. Vitamin E concentrations in maternal plasma have been shown to increase with dietary supplementation of ewes (Hidiroglou *et al.*, 1969; Njeru *et al.*, 1994; Gabryszuk and Klewiec, 2002), pigs (Hidiroglou *et al.*, 1993a; Farnworth *et al.*, 1995; Lauridsen *et al.*, 2002), rats (Martin and Hurley, 1977) and humans (Mino and Nishino, 1973; Léger *et al.*, 1998). The magnitude of the differences in plasma vitamin E concentration between ewes offered supranutritional and basal supplementation were demonstrably higher during pregnancy than during lactation. Similar results were reported by Hidiroglou *et al.* (1993a) who supplemented pregnant and lactating sows with dietary vitamin E and attributed the lower plasma vitamin E concentrations found in lactation to the partitioning of vitamin E into colostrum and milk. By contrast, Mahan (1991) reported an increase in serum vitamin E as lactation progressed, in sows supplemented with either 22 or 44 mg dietary vitamin E/kg diet. The total daily supply of vitamin E during lactation was considerably higher than that fed during pregnancy and it is therefore probable that the lower concentrations observed in plasma are due to an increased requirement during lactation as a consequence of milk production. The index proposed by Hidiroglou *et al.* (1992b) to indicate satisfactory plasma concentrations in ruminants suggests that ewes supplemented with supranutritional concentrations of vitamin E had minimal or marginal concentrations of vitamin E in plasma throughout. By contrast, ewes fed concentrates formulated to satisfy the basal vitamin E requirement were classified as deficient.

The animals' requirement for vitamin E is positively correlated with the oxidative challenge posed to the animal as a result of the unsaturated fatty acid supply (Farnworth *et al.*, 1995). Wang *et al.* (1996), McGuire and Fritsche (1997) and Chikunya *et al.* (2004) have described the abrogating effect of dietary long-chain PUFAs upon the vitamin E

status of various animals. The reduced plasma vitamin E concentrations observed in ewes fed fish oil within the current study concur with these results. By contrast, Rochester and Caravaggi (1971) reported no significant effect of fish oil supplementation upon serum vitamin E concentrations in lambs.

The plasma vitamin E concentration also has a complex interrelationship with the selenium status of the animal, specifically with the enzyme glutathione peroxidase, which acts to prevent the oxidation of unsaturated fatty acids within the cell (Van Metre and Callan, 2001). Suarez *et al.* (1999) reported that supplementing rats with dietary vitamin E reduced the total amount of GPx present in liver and brain and attributed this reduction to a sparing effect of vitamin E upon the synthesis of GPx; supplemental vitamin E increasing the antioxidant status of the cell and thereby reducing the requirement for GPx. By contrast, within the current study, no effect of vitamin E supplementation was observed upon the activity of GPx in ewes although this activity was significantly reduced in ewes offered fish oil. Increasing the peroxidative challenge to the animal by the addition of long-chain PUFAs to the diet has a concurrent effect upon the cellular requirement for antioxidants. Consequently, it is logical to attribute the reduction in GPx activity in ewes supplemented with fish oil, to a higher rate of GPx oxidation within the cell and corresponding reduction in active enzyme availability. Indeed, Smith *et al.* (1994) reported reduced GPx activity in erythrocytes of sheep supplemented with protected PUFAs.

Specific cellular metabolites are released when tissues are damaged as a result of long-chain PUFA peroxidation (Lefebvre *et al.*, 1996). The enzyme CK is a reliable indicator of tissue damage (Vojtic, 2000) and it may be postulated that adding long-chain PUFAs to the ruminant diet would increase the concentration of CK within serum. Dietary supplementation with PUFAs increased the concentration of ewe serum CK in the current

study, however, this result did not reach statistical significance. Furthermore, no effect of vitamin E supplementation was observed, despite the potential differences in peroxidation challenge and dietary antioxidant supply provided by the four treatment diets. Reference values for CK in sheep serum proposed by Bostedt and Schramel (1990) suggested that the extent of tissue damage induced by long-chain PUFA supplementation was low, with values exceeding 2000 IU/l suggesting sub-clinical nutritional myopathy.

3.5.1.4. Plasma fatty acids

As ewes were supplemented with the same diet, albeit in differing quantities, during both pregnancy and lactation, the *pre-* and *post partum* fatty acid results are discussed together. Ewe plasma fatty acid concentrations may be manipulated by dietary fatty acid supply, although bound by the constraints imposed by ruminal biohydrogenation and differences in digestion and absorption of individual fatty acids. However, changes in ewe plasma fatty acids largely reflect the fatty acid composition of the dietary treatments fed within the current study.

The total fatty acid concentration within ewe plasma during pregnancy was significantly reduced by supplementation with long-chain PUFAs. As the total daily fatty acid supply was constant between experimental diets and straw intake was unaffected by dietary fat source, it can be suggested that the differences in total plasma fatty acid concentration may be as a result of differences in endogenous fatty acid synthesis. Indeed, long-chain PUFA supplementation has been shown to reduce the endogenous synthesis of short and medium-chain fatty acids for deposition into milk via direct inhibiting effects of metabolites produced by ruminal biohydrogenation of PUFAs (Romo *et al.*, 1996; Brzóska *et al.*, 1999; Bauman and Griinari, 2001). The results of Ahnadi *et al.* (1998) who reported that feeding fish oil to lactating cattle reduced the expression of enzymes responsible for fatty acid synthesis in the mammary gland concur with this hypothesis, however, it is not known

whether similar inhibition mechanisms occur in cells other than those of the mammary gland.

The replacement of Megalac by fish oil in the diets of pregnant and lactating ewes conferred a significant reduction in the amount of C16:0 within plasma. The predominant fatty acid contained within the Megalac diets was a protected form of C16:0 at concentrations approximately double those found in the fish oil diets, and proportional to those observed in ewe plasma. A depression in endogenous fatty acid synthesis may reduce concentrations of C16:0 in body fluids and tissues, however, this difference may be attributed to a combination of low dietary supply and endogenous synthesis. The increased amounts of C16:0 observed in plasma of ewes offered Megalac concur with research published by Petit (2002) who found higher amounts of C16:0 in plasma of dairy cows fed Megalac compared to linseed or soya beans. Moreover, according to Ashes *et al.* (1992), supplementing sheep with protected fish oil reduced the total amount of C16:0 in serum lipids.

Offer *et al.* (2001) reported lower concentrations of C18:0 in plasma lipid fractions of lactating cattle offered fish oil when compared to those fed a control diet, results which concur with those observed in pregnant ewes within the current study. Furthermore, values quoted by Ashes *et al.* (1992) for C18:0 are in agreement with the concentrations observed in the current study. The dietary supply of C18:0 was similar between treatment concentrates, consequently, the differences observed between plasma concentrations in pregnant ewes fed fish oil and Megalac may be a result of changes in the efficiency of ruminal biohydrogenation induced by feeding PUFAs. Long-chain PUFAs are reported to have cytotoxic effects upon ruminal microorganisms responsible for biohydrogenation (Donovan *et al.*, 2000), the addition of fish oil to ewe diets may have reduced the ability of the ruminal microorganism population to biohydrogenate fatty acids and thereby resulted

in lower amounts of C18:0 in plasma. However, the total daily supply of fatty acids which would be biohydrogenated to form C18:0 (C18:1 *cis*, C18:2*n*-6, C18:3*n*-3) also tended to be lower from diets containing fish oil, which may have depressed concentrations of C18:0 within plasma.

Addition of fish oil to the ruminant diet has been shown to increase the amount of both C18:1 *trans* isomers and CLA produced by the incomplete biohydrogenation of unsaturated fatty acids within the rumen (Baumgard *et al.*, 2000; Wachira *et al.*, 2000; Chikunya *et al.*, 2004). Furthermore, these fatty acids may be deposited into milk as a consequence of PUFA supplementation (Donovan *et al.*, 2000; Keady *et al.*, 2002 and Gulati *et al.*, 2003). The results of the aforementioned studies concur with the higher quantities of C18:1 *trans* and CLA isomers observed in plasma of both pregnant and lactating ewes during the current study.

The dietary supply of C18:1*n*-9 *cis* was lower in concentrates containing fish oil than those which included Megalac as the principal fatty acid source. Similarly, ewes supplemented with fish oil during pregnancy and lactation had lower proportions of C18:1*n*-9 *cis* within plasma than those offered diets containing Megalac. This is in direct contrast to the results of Ashes *et al.* (1992) who observed an increase in plasma C18:1*n*-9 *cis* concentrations in sheep supplemented with protected or unprotected fish oils. However, results similar to those observed in the current study were reported by Fritsche *et al.* (1993).

The diminishing effect of fish oil supplementation upon ewe plasma concentrations of C18:2*n*-6 within the current study concurs with the results published by Ashes *et al.* (1992) and Offer *et al.* (2001) and cannot simply be explained by differences in dietary supply. Although concentrations of this fatty acid were lower in experimental diets containing fish oil compared to Megalac, proportionally, the reduction in plasma concentrations was

significantly higher, suggesting significant disappearance of this fatty acid between ingestion and transport in plasma. Ruminal biohydrogenation of C18:2*n*-6 ranges from 70-95%, depending on the fat content of the diet (Chilliard *et al.*, 2000; Wachira *et al.*, 2000; Chikunya *et al.*, 2004). Thus, although the rate of biohydrogenation increases with increasing C18:2*n*-6 content of the diet, the proportion that resists hydrogenation tends to remain constant (Cieślak *et al.*, 2001). Consequently, it would be logical to predict a higher rate of biohydrogenation of C18:2*n*-6 in ewes offered Megalac compared to fish oil, a theory which does not entirely concur with the results of the current study. The lower proportions of C18:2*n*-6 within plasma could also be attributed to changes in the rate of endogenous synthesis of long-chain *n*-6 fatty acids between treatments. Despite the increased concentrations of C20:4*n*-6 in treatment diets containing fish oil, plasma concentrations of this fatty acid were similar between treatments. If appreciable quantities of C18:2*n*-6 were utilised by ewes fed Megalac to synthesise C20:4*n*-6, the relative concentration of C18:2*n*-6 would be expected to be increased within plasma of ewes fed fish oil.

Ewes supplemented with fish oil during pregnancy and lactation had higher C20:4*n*-6 intakes, although the amount of this fatty acid in plasma was similar between treatments. This is in contrast to the results of Kitessa *et al.* (2001a) who observed higher C20:4*n*-6 concentrations in plasma of cannulated sheep supplemented with fish oil. Kitessa *et al.* (2001c) reported that the biohydrogenation of long-chain PUFAs was increased by the provision of fish oil within the diet, therefore this difference may be due to an increased rate of biohydrogenation of C20:4*n*-6 in ewes offered diets FB and FS. Alternatively, Koletzko (1996) states that the Δ-desaturase enzymes responsible for the endogenous production of long-chain PUFAs have a higher specificity for C18:3*n*-3 than for C18:2*n*-6. Therefore, given equal amounts of precursor fatty acid, more C20:5*n*-3 will be produced than C20:4*n*-6. However, possibly as a consequence of the increased intake of C18:2*n*-6 in

Megalac-supplemented ewes, proportions of C20:4*n*-6 in plasma were higher than those of C20:5*n*-3.

The four experimental diets provided similar daily intakes of C18:3*n*-3; however, higher plasma concentrations were conferred by long-chain PUFA supplementation. This is in contrast to the results observed by Offer *et al.* (2001) as a result of adding fish oil to the diets of lactating cattle, Ashes *et al.* (1992) in sheep and Rooke *et al.* (2001b; 2000; 1998) in pregnant sows. The long-chain PUFAs C20:5*n*-3 and C22:6*n*-3 may be endogenously synthesised from C18:3*n*-3 (Koletzko, 1992); a mechanism which is significantly less effective in animals supplemented with a preformed dietary source of long-chain PUFAs (Sargent, 1997). Consequently, it can be postulated that the proportion of plasma fatty acids contributed by C18:3*n*-3 was lower in ewes supplemented with Megalac as a consequence of this fatty acid being utilised as an endogenous PUFA precursor. Voigt and Hagemeister (2001) suggest that the endogenous synthesis of long-chain PUFAs via elongation and desaturation in ruminants is negligible. However, this is not borne out by the results of the current study in which ewes supplemented with Megalac had proportions of C20:5*n*-3 at approximately half those found in fish oil-supplemented ewes, and C22:6*n*-3 proportions at about one-third of those found in ewes fed fish oil. The significant increase in amounts of C20:5*n*-3 and C22:6*n*-3 in plasma of ewes offered diets containing fish oil is a direct result of the increased dietary supply of preformed long-chain PUFAs. However, if, as suggested, endogenous synthesis of these fatty acids via elongation and desaturation is negligible in the ruminant, it would be predicted that they would be undetectable in plasma of ewes offered Megalac. These results also concur with those of both Wachira *et al.* (2002) and Chikunya *et al.* (2004), both studies reporting the presence of C20:5*n*-3 and C22:6*n*-3 within either muscle or plasma fatty acids of sheep supplemented with Megalac. Logically, a mechanism must exist to synthesise or mobilise long-chain PUFAs within the pregnant ewe to ensure the development of brain and nervous tissue in the foetal lamb. Consequently, the major source of C20:5*n*-3 and

C22:6*n*-3 within plasma of ewes fed Megalac and the reason for reduced C18:3*n*-3 concentrations in these ewes is likely to have been from endogenous desaturation and elongation of EFAs.

Dietary supplementation of ruminants and other animals with preformed dietary long-chain PUFAs has been demonstrated by several authors to increase the amounts of C20:5*n*-3 and C22:6*n*-3 within the plasma lipid fraction (Ashes *et al.*, 1992; Otto *et al.*, 2000; Chikunya *et al.*, 2004). However, despite significant increases in the amounts of both C20:5*n*-3 and C22:6*n*-3 in plasma as a response to fish oil supplementation, the increase was proportionally higher for C20:5*n*-3 than C22:6*n*-3 despite similar dietary intakes. Although several researchers have debated the extent to which C20:5*n*-3 and C22:6*n*-3 are biohydrogenated within the rumen, it appears that a greater proportion of C20:5*n*-3 may be saturated and isomerised when compared to a similar intake of C22:6*n*-3 (Gulati *et al.*, 1999; Wachira *et al.*, 2000; Cooper *et al.*, 2002). Furthermore, Doreau and Chilliard (1997a) suggested that increasing chain length may decrease fatty acid digestibility, this hypothesis concurs with the results observed in the current study, however, it is also postulated that absorption may increase with unsaturation, thereby an increased concentration of C22:6*n*-3 compared to C20:5*n*-3 would be predicted. The elongation and desaturation of C18:3*n*-3 produces C20:5*n*-3, a proportion of which is further elongated and desaturated to produce C22:6*n*-3 (Sprecher, 2000). Increased amounts of C20:5*n*-3 compared to C22:6*n*-3 in plasma could therefore be attributed to endogenous synthesis of long-chain PUFAs via elongation and desaturation, however, this synthesis has been demonstrated to be inhibited by supplementation with dietary long-chain PUFAs (Williard *et al.*, 2001). An alternative explanation is that C22:6*n*-3 may have been retro-converted to C20:5*n*-3 within the ewes, thereby increasing the relative proportion of this fatty acid within plasma (Cooper *et al.*, 2004). In addition, the quantities of C22:6*n*-3 needed for neural cell function and deposition into either the foetal lamb or milk may result in a higher

requirement for this fatty acid compared to C20:5*n*-3, therefore lower quantities of this fatty acid found in plasma may be the result of increased cellular uptake of C22:6*n*-3.

3.5.1.5. Gestation length

The estimates of gestation length within the current study include a large random component due to the uncertainty about the exact time of mating as, although oestrus was synchronized, the ewes and rams were kept together for 21 days. However, as ewes were allocated randomly to the treatment groups, there should be no bias in the comparison. A significant increase in the duration of gestation was demonstrated as a result of fish oil supplementation of ewes. Long-chain polyunsaturated fatty acids have long been implicated as a factor in the prolongation of gestation length (Olsen *et al.*, 1986). Observational studies found that women living in Sweden and the Faroe Islands who habitually consumed large amounts of fish, had increased gestation length and increased birthweights compared to women with low fish consumption (Olsen *et al.*, 1990; 1992). This has important implications for human health, as premature birth is a major factor in infant survival and viability (Wen *et al.*, 2004) and may also have implications for animal production, specifically neonatal vigour and mortality.

Olsen *et al.* (1992), supplemented pregnant women with fish oil, olive oil or no supplementary oil source and found that both gestation length and birthweight were significantly higher in the group supplemented with fish oil, with both effects strongest in women with a low habitual fish intake. Furthermore, the work of Rooke *et al.* (2001c) demonstrated increased gestation lengths in sows supplemented with salmon oil during pregnancy. The consumption of fish products leads to a concomitant increase in the daily intake of *n*-3 fatty acids, specifically C20:5*n*-3, and it is postulated that an augmented dietary supply of C20:5*n*-3 was the causative factor behind the increased gestation length in the current study. Both C20:4*n*-6 and C20:5*n*-3 act as prostaglandin precursors within

the ruminant, the bioactive dienoic prostaglandins PGE_2 and $PGF_{2\alpha}$ being synthesised from C20:4*n*-6 whereas prostaglandin PGE_3 is produced from C20:5*n*-3 (Calder, 2001). These two fatty acids compete within the cell for the activity of Δ-6 desaturase; for incorporation into phosphatidylglycerols and for the enzyme prostaglandin-H-synthetase (PGHS; Olsen *et al.*, 1986). Dietary *n*-3 PUFAs have been reported to attenuate the production of prostaglandins formed from *n*-6 fatty acids (Li *et al.*, 1994). Furthermore, an increase in the ratio of C20:5*n*-3:C20:4*n*-6 available to the animal, whether from dietary or endogenous synthesis, results in a shift in prostaglandin production from bioactive dienoic prostaglandins which have an established role in the induction of parturition (Hansen and Olsen, 1988), towards less active trienoic prostaglandins (Olsen *et al.*, 1986). Olsen *et al.* (1992) suggested that this effect may be dose-responsive until a saturation point is reached at which the initiation of parturition is delayed.

This hypothesis concurs with the work of Baguma-Nibasheka *et al.* (1998; 1999) and Hong-Ma *et al.* (2000) who demonstrated delays in the initiation of glucocorticoid-induced parturition in ewes supplemented with fish oil with concurrent reductions in maternal oestradiol and prostaglandin-H-synthetase-2, these chemicals are thought to initiate and maintain myometrial contraction during parturition (Liggins *et al.*, 1972). Galli *et al.* (1980) also reported decreased excretion of $PGF_{2\alpha}$ and PGE_2 in rats fed high concentrations of dietary PUFAs, these prostaglandins being associated with uterine contraction during parturition (Lye, 1996). Replacement of $PGF_{2\alpha}$ and PGE_2 with trienoic prostaglandins formed from the *n*-3 series fatty acids does not specifically inhibit parturition, rather, it is suggested that the trienoic prostaglandins are not biologically active enough to induce myometrial contractions (Abayasekara and Wathes, 1999).

The study of Smuts *et al.* (2003) reported increased gestation length in pregnant women supplemented with C22:6*n*-3, however this fatty acid is not a direct prostaglandin

precursor. The C20:5*n*-3 content of the supplements in the aforementioned study was not reported, however, the authors suggested that retroconversion of C22:6*n*-3 to C20:5*n*-3 could increase the proportion of *n*-3 prostaglandins produced or that *n*-6 prostaglandin formation may have been disrupted by C22:6*n*-3 deposition in membrane phosphatidylglycerols. The effects observed upon gestation length in the current study may therefore be due, in part, to the supplementation with both C20:5*n*-3 and C22:6*n*-3 in the form of fish oil.

3.5.1.6. Colostrum

Colostrum is produced in the mammary gland during the weeks immediately prior to parturition (Barrington *et al.*, 2001), the yield being governed by several factors including genetic influences and endocrine parameters (O'Doherty and Crosby, 1996). Colostrum composition differs from milk produced during an established lactation due to the high concentrations of nutritional and non-nutritional factors essential for the provision of energy and passive immunity to the neonate (Blum and Hammon, 2000). However, little research has been devoted to the effects of long-chain PUFA and vitamin E supplementation upon ruminant colostrum production, therefore, the results of the current study are discussed with reference to studies involving animals in established lactation.

Various effects of PUFA supplementation of lactating ruminants upon milk yield have been reported; many researchers described no effect of dietary fat type, regardless of whether fish oil (Cant *et al.*, 1997; Lacasse and Ahnadi, 1998; Kitessa *et al.*, 2003), vegetable oil (Benson *et al.*, 2001), whole oilseeds (Petit *et al.*, 2002a) or saturated fats (Grum *et al.*, 1996) were employed. By contrast, both Chilliard and Doreau (1997) and Keady *et al.* (2000) offered fish oil to lactating dairy cows and observed a consequent increase in milk yield. Furthermore, Chikunya *et al.* (2002) observed an increase in the milk yield of dairy ewes supplemented with either a prilled mixture of linseed and fish oil,

or with Incromega™. The results of Keady *et al.* (2000) also indicate that increasing the level of fish oil in the diet of dairy cows confers an increase in milk yield. Ewes within the current study exhibited significantly lower colostrum yields when supplemented with fish oil, results which concur with those of Donovan *et al.* (2000) and Whitlock *et al.* (2001). Furthermore, Annett *et al.* (2004) reported a significant decrease in colostrum yield as a result of fish oil supplementation of pregnant ewes. Colostrum production is associated with an increase in the basal energy and protein requirements of the ewe; reductions in colostrum yield may be attributed to lower forage intakes, as exhibited by animals in many studies involving fish oil supplementation (Chilliard and Doreau, 1997; Donovan *et al.*, 2000). However, as previously discussed, no significant effect of PUFA supplementation was observed upon straw intake in the current study. Donovan *et al.* (2000) suggested that the milk yield response to fish oil supplementation may be dependant on the amount fed, as yields were similar in cattle with zero or 2 % fish oil added to the diet, but lower at a 3 % inclusion rate. In the current study, the concentration of fish oil in diets of ewes at two weeks *pre-partum* was approximately 4.2 % which, if the hypothesis of Donovan *et al.* (2000) is correct, would induce a reduction in colostrum yield. The supplementation of dairy cattle with saturated fatty acids has been demonstrated by Dhiman *et al.* (2001) and Fahey *et al.* (2002) to increase milk yield. In consequence, the results observed in the current study may not be a direct result of fish oil lowering colostrum yield, but an increase conferred by Megalac supplementation in control diets.

Ruminant milk composition is plastic and inherently vulnerable to manipulation via the diet (Bauman and Griinari, 2001). Successful protection of PUFAs from ruminal biohydrogenation results in minimal disruption to the rumen environment, in consequence, milk fat concentrations should be unaffected (Kitessa *et al.*, 2003). Both the colostrum fat concentration and yield of ewes supplemented with fish oil were significantly reduced when compared to ewes fed Megalac. The significant impact of fish oil supplementation

upon milk fat concentrations in the current study implies that absorption into vermiculite was not a suitable mechanism to protect all PUFAs contained within fish oil from biohydrogenation. This concurs with the results of Cooper *et al.* (2004) who demonstrated that vermiculite provided partial protection against ruminal biohydrogenation of PUFAs contained within linseed oil.

The mechanism by which milk fat concentration is reduced by PUFA supplementation has been debated for some time. Current theories include a decrease in synthesis from acetate as a result of decreased fibre digestion (Palmquist, 1984), the inhibition of synthesis by *trans*-10,*cis*-12 CLA produced by ruminal biohydrogenation (Romo *et al.*, 1996) or specific long-chain PUFAs retarding the activity of enzymes involved in endogenous fat synthesis (Viswanadha *et al.*, 2003).

Straw intake was unaffected by dietary fat source in the current study and the concentration of individual short-chain fatty acids within colostrum was not significantly influenced by fish oil supplementation. Indeed, the proportion of short-chain fatty acids within milk fat was increased by the provision of fish oil within the ewe diet. Consequently, the hypothesis that milk fat concentration has been depressed as a result of reductions in acetate production or *de novo* acetate synthesis appears unfounded, although no direct measurements of ruminal acetate production were made. Interestingly, despite the decreased dietary and plasma concentrations of C16:0 in ewes offered fish oil, no difference in colostrum C16:0 concentrations was observed as a result of dietary fat source. As the principal endogenously synthesised fatty acid found within colostral fat, this adds weight to the argument that fatty acid synthesis was not significantly affected by fish oil supplementation.

Reduced concentrations of C18:0 are described in ewe milk by Kitessa *et al.* (2003) as a consequence of adding tuna oil to the diet. Furthermore, Keady *et al.* (2000) reported lower concentrations of C18:0 in milk from cattle supplemented with fish oil, a result in accordance with those reported by Cant *et al.* (1997) and Donovan *et al.* (2000). However, the concentrations of C18:1*n*-9 *cis* and C18:2*n*-6 within milk vary between the aforementioned studies, with no consistent effect of long-chain PUFA supplementation. The results observed in the current study reflect the plasma fatty acid concentrations at two weeks *pre-partum*, with lower concentrations of C18:0, C18:1*n*-9 *cis* and C18:2*n*-6 in colostrum of ewes fed diets containing fish oil. Within colostrum fat, these fatty acids are principally supplied by the diet or from mobilisation of adipose tissue, hence the similarity between concentrations in plasma and colostrum.

Examination of plasma samples taken at two weeks *pre-partum* reveals increased concentrations of *trans* C18:1 fatty acids. These fatty acids are considered by Keady *et al.* (2000) to inhibit the synthesis of fatty acids within the mammary gland, possibly as a result of reduced uptake by lipoprotein lipase. The increased concentration and proportion of *trans* C18:1 fatty acids within colostrum fat, in combination with the reduced total fat concentration concur with this hypothesis. Furthermore, the presence of higher concentrations and proportions of C20:5*n*-3, both in plasma and colostrum of ewes offered diets containing fish oil may lend weight to the suggestion by Chilliard *et al.* (2001b) that C20:5*n*-3 may inhibit fatty acid synthesis. However, this may be a result of increased *trans* fatty acid production from the biohydrogenation of C20:5*n*-3, rather than a direct effect of this long-chain fatty acid.

As with *trans* C18:1 fatty acids, fish oil supplementation of lactating ruminants has been reported to increase the concentration of CLA isomers within milk fat, a phenomenon described by Ramaswamy *et al.* (2001), Whitlock *et al.* (2002) and Gulati *et al.* (2003).

CLA have been associated with milk fat depression, specifically with inducing changes in endogenous milk fat synthesis, acting via a similar mechanism to *trans* C18:1 fatty acids (Chouinard *et al.*, 1999; Baumgard *et al.*, 2000). Only one CLA isomer was identifiable by the experimental method utilised in this study (*cis*-9,*trans*-11 CLA) which is produced within the mammary gland rather than as a product of ruminal biohydrogenation. However, as increased concentrations of CLA were also observed in plasma within the current study, the theory that CLA depresses milk fat concentrations should not be dismissed.

It is well established that supplementation of ruminants with long-chain PUFAs increases the concentrations of these fatty acids within milk fat (Palmquist, 1984). Ewes fed Megalac had very low concentrations of C20:5*n*-3 and C22:6*n*-3 within colostrum fat, therefore it can be assumed that the small increase in the concentrations of these fatty acids within colostrum of ewes fed fish oil was a result of dietary supplementation. Similar results have been reported by Kitessa *et al.* (2003) in sheep and Donovan *et al.* (2000), Ramaswamy *et al.* (2001), and Gulati *et al.* (2003) in cattle.

The majority of research involving the provision of long-chain PUFAs to lactating ruminants has concluded that dietary supplementation reduces milk protein concentrations. This is true of the results reported by Cant *et al.* (1997), Lacasse and Ahnadi (1998), and Petit *et al.* (2002a), although Kitessa *et al.* (2003) observed no significant effect of protected tuna oil upon ewe milk protein concentrations. The decrease in colostrum protein concentration observed in the current study concurs with the results of the aforementioned authors; furthermore, protein yield was significantly reduced as a consequence of reductions in both protein concentration and colostrum yield induced by fish oil supplementation. Dietary supplementation of lactating ruminants with long-chain PUFAs reduces acetate and increases propionate production within the rumen as a result of

changes in microbial populations (Fievez *et al.*, 2003). This increase in propionate production may lead to an increase in lactose synthesis with concurrent increase in colostrum yield, whereby the protein concentration is reduced. However, Wilkinson *et al.* (2000) demonstrated that, although milk yield was depressed by long-chain PUFA supplementation of milking ewes, protein concentration was increased. This is in contrast to the result observed within the current study, as both colostrum yield and protein concentration, and therefore protein yield, were reduced by long-chain PUFA supplementation of the ewe. No measure of the relative proportions of different colostrum proteins were made within the current study, therefore it is not possible to conclude whether the depression was a consequence of a decrease in casein synthesis due to PUFA supplementation, as purported by Chilliard and Doreau (1997). Furthermore, the hypothesis proposed by Demeyer and Doreau (1999), namely that *trans* fatty acids may cause changes in fatty acid uptake by the mammary gland, inducing insulin resistance and inhibiting milk protein synthesis may be the most suitable explanation for the lower colostrum protein concentrations observed in ewes fed fish oil, but cannot be verified.

The colostral vitamin E concentration mirrors the values observed in plasma at two weeks *pre-partum*, with significantly higher concentrations observed as a result of supranutritional supplementation. High concentrations of vitamin E in colostrum have been reported by Gentry *et al.* (1992), Hidiroglou *et al.* (1992) and Njeru *et al.* (1994) as a result of supplementation, although concentrations for ewes fed diet MS were considerably higher in the current study than values reported by other authors. This is in part due to the high concentrations fed via diets FS and MS in the current study, the maximum dietary supplemental amount used in previous studies being 150 mg/day. The supranutritional dietary vitamin E concentration combined with the low oxidative challenge induced by Megalac resulted in the highest colostrum vitamin E concentrations being found in ewes fed diet MS. This result was not unexpected, given the effects of an increased oxidative

challenge upon vitamin E concentrations as shown by the differences between diets MS and FS.

3.5.1.7. Milk

Adding fish oil to the diets of lactating ewes had no significant effect upon milk yield, although numerically yields were higher ($P>0.05$). Cant *et al.* (1997) suggested that an increase in lactose production may have explained the increased milk yields observed in cattle fed fish oil compared to an unsupplemented diet. The lack of an observed effect of dietary treatment upon milk lactose production within the current study may elucidate the reasons behind the similarity in milk yield between treatments.

It is generally agreed that feeding long-chain PUFAs in the form of fish oils to lactating ruminants reduces milk fat concentration, as reported by Cant *et al.* (1997), Chilliard and Doreau (1997) and Whitlock *et al.* (2002). However, the study of Kitessa *et al.* (2004) reported no significant effect of feeding protected fish oil to dairy cattle upon milk composition. By contrast, the fat concentration of milk produced by ewes supplemented with fish oil in the current study was increased when compared to ewes offered Megalac. This is in direct contrast to the results obtained by Chikunya *et al.* (2002) and was an unexpected result. Although it was not identified within milk or plasma samples and therefore cannot be substantiated, it is possible that a lack of the *trans*-10,*cis*-12 CLA isomer may have lessened the reducing effects of long-chain PUFAs upon milk fat concentration. However, the total milk fatty acid content, as measured by gas chromatography, was significantly lower in samples from ewes given diets containing fish oil, this is in direct contrast to the results obtained by near-infra-red spectrophotometry to estimate milk fat content. It is therefore possible that errors in sampling or analysis may have given rise to this anomalous result.

In contrast to the results previously discussed for colostrum fatty acid composition, changes in the concentration and proportion of C16:0 within milk fat as a result of long-chain PUFA supplementation can be attributed to the considerable decrease in the amount of this fatty acid fed via the treatment diets containing fish oil. A reduction in the efficacy of endogenous fatty acid synthesis would also confer a decrease in the secretion of this fatty acid into milk fat, however, there was little evidence of other medium- or short-chain fatty acids being reduced by fish oil supplementation.

Lower concentrations of C18:0 within milk fat as a result of fish oil supplementation of ewes concurs with the reduced amount of this fatty acid in plasma and with results reported by Donovan *et al.* (2000), Kitessa *et al.* (2001b) and Gulati *et al.* (2003). The augmentation of *trans* C18:1 fatty acids in milk fat as a consequence of long-chain PUFA supplementation of ruminants has been discussed by Donovan *et al.* (2000), Keady *et al.* (2002) and Gulati *et al.* (2003) and was exhibited by ewes fed fish oil in the current study. Concentrations of C18:1*n*-9 *cis* in treatment concentrates containing fish oil were approximately one-third of those based on Megalac, and were reflected by changes in ewe plasma and milk fatty acid composition; a result in agreement with those of Cant *et al.* (1997) and Kitessa *et al.* (2003).

As exhibited by the colostral fat results, the proportion of C18:1 *trans* fatty acids was higher both in plasma and milk of lactating ewes fed fish oil, this result would be expected to indicate a reduction in the total milk fat content as a result of inhibition of endogenous milk fat synthesis caused by these fatty acids. By contrast, although plasma CLA concentrations were increased by fish oil supplementation, the amount of this fatty acid in milk was similar between treatments.

A high proportion of plasma fatty acids was contributed by C18:2*n*-6, whereas this fatty acid was found in only small amounts in milk fat, furthermore, a six-fold decrease in C18:3*n*-3 in milk compared to plasma was observed. As both C18:2*n*-6 and C18:3*n*-3 are essential for the endogenous synthesis of C20:4*n*-6, C20:5*n*-3, C22:6*n*-3 and prostaglandins, the requirement for these fatty acids in metabolic processes may explain their low deposition into milk fat. Moreover, C18:2*n*-6 and C18:3*n*-3 tend to be present in the phosphatidylglycerol fraction rather than being incorporated into triacylglycerols. The low phosphatidylglycerol content of milk may therefore reduce the secretion of these fatty acids into milk fat.

Considerable research has been devoted to increasing the concentration of C20:5*n*-3 and C22:6*n*-3 within milk products. The study of Gulati *et al.* (2003) indicated that C20:5*n*-3 and C22:6*n*-3 may be transferred into milk at a rate between 6.8% and 8.1% of dietary intake. Given the duration of dietary supplementation in the current study and the probable incorporation of both fatty acids into body reserves that could later be mobilised, it is impossible to directly measure the transfer of these fatty acids from the diet into milk. However, the amounts of C20:5*n*-3 and C22:6*n*-3 were significantly higher in milk fat from ewes fed fish oil compared to Megalac-fed ewes where they were almost undetectable. These results are in agreement with those observed by Ramaswamy *et al.* (2001), Gulati *et al.* (2002), and Kitessa *et al.* (2003) as a result of fish oil supplementation of lactating ruminants.

Milk protein concentration and yield were significantly lower in ewes offered diet MB compared to the other three treatments. Decreases in milk protein concentrations are commonly observed when fish oil is added to the diet of lactating ruminants (Cant *et al.*, 1997; Lacasse and Ahnadi, 1998; Petit *et al.*, 2002a). Furthermore, the combination of low protein concentrations and a non-significant decrease in milk yield in ewes offered

Megalac led to a significant decrease in milk protein yields. The mechanism by which PUFA supplementation increased milk protein concentration is unclear: plasma urea concentrations were higher at two and four weeks *post partum* in ewes offered diets containing fish oil, although the difference was not statistically significant. This may indicate that these animals had a better protein status during lactation, leading to increased casein secretion into milk, however, neither plasma albumin or total protein concentrations were affected by treatment diet (data not shown).

Milk vitamin E concentrations were strongly positively correlated with plasma concentrations at two weeks *post partum* ($r = 0.894$) with lower values observed in ewes supplemented with fish oil. Results relating to the increase observed with dietary supranutritional vitamin E supplementation in the current study are in agreement with those of Charmley *et al.* (1993), Hidiroglou *et al.* (1993a) and Njeru *et al.* (1994). However, the abrogating effect of long-chain PUFA supplementation upon milk vitamin E concentrations observed within the current study is in contrast to the results published by Hidiroglou *et al.* (1993a) in which fat source (tallow, fish oil or no added fat) had no significant effect upon the vitamin E concentration of sows milk. The lower concentrations of vitamin E in milk compared to colostrum for all treatments are in agreement with the results of Hidiroglou *et al.* (1993a) and Njeru *et al.* (1994) who observed a three-fold increase in colostral vitamin E compared to that of milk. However, Csapó *et al.* (1995) reported that vitamin E concentrations in colostrum and milk were similar in unsupplemented mares. Mahan (1991) suggested that increased concentrations in colostrum compared to milk may be as a result of enhanced dietary supply to the labile tissue pool during pregnancy before exhaustion of this source for colostrum production with consequent reliance on dietary vitamin E for deposition in milk. Furthermore, the increased colostral fat concentration may have increased the requirement for antioxidant deposition into colostrum compared to milk.

3.5.2. Lamb parameters

3.5.2.1. Maternal and neonatal behaviour

Survival of the lamb past the immediate neonatal period is dependent upon the formation of an exclusive bond with the dam, facilitating the ingestion of nutrients in the form of colostrum (Dwyer and Lawrence, 1998). The formation of this bond relies on the performance of specific behaviours by the ewe and lamb. Failure to form a secure ewe-lamb attachment often leads to mis-mothering, rejection and increased lamb mortality rates (O'Connor and Lawrence, 1992; Nowak, 1996).

The maternal behaviour score used in the current study was a modified version of that described by O'Connor and Lawrence (1992), based upon behaviours normally exhibited by the ewe at parturition. Other researchers have assessed maternal behaviour using a score based on the readiness of the ewe to approach a human handler (O'Connor *et al.*, 1985), or upon the effect of a human approaching the ewe on lamb desertion (Wassmuth *et al.*, 2001). Within the current study, these tests were not practicable due to space restrictions and were only appropriate for use after the initial neonatal period. Furthermore, interruption of neonatal lamb behaviours by a human handler would significantly bias results.

Maternal behaviours directed towards the lamb before suckling are thought to be hard-wired and relatively inflexible, being similar between several species (Fraser and Broom, 1997). These behaviours are mainly influenced by the hormonal status of the ewe and may be induced in the non-pregnant ewe by the administration of hormones together with physical manipulation of the cervix (Caba *et al.*, 1995). Therefore, although studies have reported differences in maternal behaviour as a consequence of age (Lawrence and Dwyer, 1997) or breed (Dwyer and Lawrence, 1998), diet was not predicted to have a significant effect, a hypothesis borne out by the results observed in the current study.

The success of lamb suckling is directly influenced by both maternal and neonatal factors, ewes maintain grooming behaviours until signals from the lamb stimulate the facilitation of suckling. Indeed, inexperienced ewes often attempt to maintain grooming behaviour and circle when lambs attempt to suck (Dwyer and Lawrence, 1998). Although differences were observed in the time taken for lambs to suckle successfully, these were uninfluenced by maternal responses to suckling. Dwyer *et al.* (1999) suggested that neonatal lamb behaviours are unaffected by maternal behaviour, however, this may be debated, as extreme behaviours such as withdrawal and aggression have palpable effects upon the ability of the lamb to seek and obtain colostrum.

After birth, lambs first raise and shake the head, before making attempts to stand, standing successfully on all four legs, searching for the udder and finally, suckling from the ewe (Vince, 1993). The latencies of neonatal behaviours observed in the current study agree with those reported by Alexander and Williams (1966), Wassmuth *et al.* (2001) and Cloete *et al.* (2002). Addition of fish oil to the maternal diet significantly reduced the latencies of successful suckling in neonatal lambs. This result concurs with those reported by Rooke *et al.* (2001a) in which neonatal piglets from sows supplemented with tuna oil during late pregnancy tended to make contact with the udder and teats more quickly than control piglets. By contrast, piglet viability scores based on heart rate and latencies of breathing and standing were reduced in piglets borne by sows fed tuna oil (Rooke *et al.*, 1998).

O'Connor and Lawrence (1992) postulated that delays in suckling may, in part, be due to myopia reducing the ability of the lamb to successfully locate the udder. Brain and retinal tissues contain a high proportion of lipid including particularly high concentrations of C20:4*n*-6 and C22:6*n*-3 within membrane phosphatidylglycerols (Lauritzen *et al.*, 2001). Studies of long-chain PUFA supplementation in various mammals have demonstrated that retinal function is adversely affected in animals deprived of *n*-3 fatty acids. Furthermore,

research published by Birch *et al.* (2000; 2002) and Bouwstra *et al.* (2003) using human infants have related improved visual acuity, cognitive development and motor skills during infancy to the intake of *n*-3 PUFAs, specifically C22:6*n*-3, before weaning, suggesting that supplementation with these fatty acids improves neural development. However, neither Gibson *et al.* (1997), Jensen *et al.* (1999) nor Lucas *et al.* (1999) reported any significant effect of C22:6*n*-3 supplementation upon visual acuity in human infants.

Higher concentrations of C22:6*n*-3 were observed within brain tissue of lambs produced by ewes fed fish oil within the current study when compared to ewes fed Megalac. This concurs with the results published by Bouwstra *et al.* (2003) in a study involving human infants, and suggests that the selective accumulation of this fatty acid within brain tissue may have led to measurable differences in motor development. Learning behaviour is improved in rats (Yamamoto *et al.*, 1987; Bourre *et al.*, 1993; Ikemoto *et al.*, 2001) and pigs (Ng and Innis, 2003) by *n*-3 fatty acid supplementation. As successfully locating and returning to the teat involves an aspect of learning behaviour on the part of the neonatal lamb, the reduced time taken to successfully suckle in those lambs borne by ewes supplemented with fish oil may have been a consequence of differences in cognitive development between treatments.

The majority of C22:6*n*-3 accumulation in the brain occurs during the brain growth spurt (Green and Yavin, 1998; Lauritzen *et al.*, 2001), which, in the lamb, occurs *in utero*. Both the total amount and concentration of C22:6*n*-3 within brain tissue increases during the growth spurt, concurring with synaptogenesis – the formation of synapses essential for conducting nerve impulses (Lauritzen *et al.*, 2001). Indeed, Ahmad *et al.* (2002) suggested that C22:6*n*-3 deficiency reduces neuron size which may be linked to loss of optimal nerve function. If lambs produced by ewes offered diets containing Megalac were deficient in

C22:6*n*-3, this decrease in neuron size might have a significant impact on neonatal lamb behaviour.

Despite tissue vitamin E concentrations indicating that placental transfer was increased by supranutritional supplementation, any effect of vitamin E supplementation upon vigour may have been confounded by the addition of long-chain PUFAs to the diet. Indeed, a significant interaction between vitamin E and PUFAs was observed for the latency of lamb standing: supranutritional vitamin E supplementation had no effect when fed with a control fat, however, reduced this latency when fed in combination with long-chain PUFAs. Merrell (1998) reported that lambs borne by ewes supplemented with vitamin E during pregnancy tended to stand and suckle faster than those from control ewes. Williamson *et al.* (1995) also recorded increased vigour scores in neonatal lambs as a result of maternal vitamin E supplementation. The supranutritional vitamin E concentration used in the current study was higher than those employed in the aforementioned experiments although no significant main effect of vitamin E supplementation upon lamb vigour was observed. Dwyer *et al.* (2003) described reduced latencies of standing and suckling in lambs that were heavier at birth. The increase in birthweight conferred by vitamin E supplementation within the current study may therefore have been expected to enhance lamb behaviour.

3.5.2.2. Antioxidant status of the neonate

The majority of research conducted to date has suggested that the transfer of vitamin E across the placenta to the foetus or neonate is low and that adequate tissue and plasma concentrations are only obtained after the ingestion of colostrum (Njeru *et al.*, 1994). Lauridsen *et al.* (2002) concluded that low tissue concentrations of vitamin E in neonatal piglets provided evidence of inefficient placental transfer, and suggested that neonatal vitamin E status could not be manipulated by maternal supplementation. The high ratio between maternal and neonatal plasma vitamin E concentrations and the lack of a

treatment effect on foetal blood concentrations also lead to the conclusion that porcine placental transfer is inefficient in the study of Farnworth *et al.* (1995).

By contrast, Hidiroglou *et al.* (1993a) reported that although vitamin E concentrations in neonatal piglets were low, piglets born to supplemented sows tended to have increased plasma concentrations. Similar results in lambs have been published by Pehrson *et al.* (1990). Mahan (1991) demonstrated that increasing the amount of supplementary vitamin E available to the pregnant sow from 0 mg/kg to 44 mg/kg tended to increase the vitamin E concentration in piglet serum and liver before nursing. Equivalent concentrations of vitamin E in maternal and neonatal rat plasma were also described by Wei Cheng *et al.* (1961).

Significant transfer of radio-labelled α-tocopherol across the guinea pig placenta has been described by Hidiroglou *et al.* (2003) with plasma concentrations similar to or higher than those of the dams. However, it was suggested that these animals are unique in their ability to conserve dietary vitamin E for deposition into the foetus whilst Kelly *et al.* (1992) suggested that the foetal liver may act as a labile source of vitamin E and that plasma concentrations may not be a reliable indicator of neonatal vitamin E status.

Results from the current study support the hypothesis that placental transfer of vitamin E may be manipulated by maternal supplementation in ruminants. Although neonatal plasma vitamin E concentrations were low or undetectable in agreement with the findings of Hidiroglou *et al.* (1995), brain and *semimembranosis* concentrations were significantly increased by maternal supranutritional supplementation. It is debatable as to whether the low vitamin E concentrations observed in neonatal plasma are a reliable indicator of deficiency status or a measure of the increased rate of uptake from plasma in an attempt to maintain satisfactory tissue concentrations. Vatassery *et al.* (1988) hypothesised that the

rodent brain may be resistant to vitamin E depletion even when a vitamin E-deficient diet is supplied for a period of four months, therefore, it seems logical that organs with an increased requirement for antioxidant vitamins may have an enhanced capacity for uptake in times of diminished availability.

Supranutritional vitamin E supplementation of ewes within the current study resulted in neonatal lamb muscle concentrations similar to those reported for unsupplemented growing lambs by Ochoa *et al.* (1992). However, Hidiroglou and Batra (1996) observed a mean value of 2.70 mg/kg in hip muscle of lambs with no supplemental vitamin E added to the diet. This suggests that, although supplementation increased the concentration of vitamin E in neonatal tissues within the current study, these animals may still have been sub-clinically deficient in vitamin E. Furthermore, brain concentrations observed in neonatal lambs within the current study were significantly lower than values reported by Vatassery *et al.* (1988) for rats fed vitamin E-deficient diets. Although it may be suggested that the liver acts as a reservoir for vitamin E (Kelly *et al.*, 1992), this does not concur with the results of Lauridsen *et al.* (2002) who found higher vitamin E concentrations in brain tissue compared to liver in neonatal piglets. Differences between treatment diets as a result of fat source in the current study with fish oil supplementation abrogating the neonatal tissue vitamin E concentration concur with the results of Hidiroglou *et al.* (1970), Farnworth *et al.* (1995) and Wang *et al.* (1996) and with patterns observed in ewe plasma and colostrum.

3.5.2.3. Plasma fatty acids in the neonate

Elphick *et al.* (1979) described reduced plasma fatty acid concentrations in the foetal lamb compared to the dam, a result which concurs with that observed in the current study, in which neonatal plasma fatty acid concentrations were approximately one-third of those found in the ewe. These results also support the hypothesis proposed by Leat and Harrison (1980), namely that placental fatty acid transfer may be limited in the ruminant. However,

the total fatty acid concentration within neonatal lamb plasma was proportional to that within maternal plasma at two weeks *pre-partum* indicating that the extent of fatty acid transfer may be affected by maternal plasma fatty acid concentration and consequently, by treatment diet.

Feeding fish oil to pregnant ewes reduced the amount of C16:0 and C18:1*n*-9 *cis* in ewe plasma; however, proportions of these fatty acids in neonatal plasma samples were equivalent between treatments. Transfer of radio-labelled C16:0 across the ovine placenta has been demonstrated to be low (Elphick *et al.*, 1979; Leat and Harrison, 1980), leading the latter authors to conclude that the placenta is impermeable to medium-chain fatty acids. However, the high proportional contributions of these fatty acids to neonatal lamb plasma within the current study appear to disprove this hypothesis.

A considerable amount of research has been devoted to the examination of EFA concentrations within the neonate. Both C18:2*n*-6 and C18:3*n*-3 have been reported by Leat and Harrison (1980) to be present in very small quantities within neonatal lamb plasma. This result is in agreement with those of Elphick *et al.* (1979), who considered that the low concentrations of C18:2*n*-6 within plasma may be the result of elongation and desaturation of this fatty acid to produce C20:4*n*-6. Elphick *et al.* (1979) suggested that only free fatty acids present in maternal plasma may cross from maternal to foetal circulation, and that those incorporated into triacylglycerols and phosphatidylglycerols are unable to transverse the placenta. By contrast, Ramsay *et al.* (1991) suggested that, in the pig, fatty acids are absorbed from maternal plasma and released into foetal circulation as free fatty acids. The proportion of C18:2*n*-6 in plasma of lambs produced by ewes supplemented with fish oil during the current study was approximately half the equivalent measurement made in ewe plasma at two week *pre-partum*. Noble *et al.* (1982) described higher concentrations of C20:4*n*-6 within plasma of the foetus compared to the pregnant

ewe and concur that this increase is due to elongation and desaturation of C18:2*n*-6 to C20:4*n*-6 by the placenta. However, the results of the current study do not concur with this hypothesis, as proportions of C20:4*n*-6 were lower in the neonate than those observed in ewes.

A mechanism selecting against C18:2*n*-6 may exist within the placenta as although the EFA C18:3*n*-3 was also found in low quantities in neonatal lamb plasma, as a proportion of total fatty acids, it was similar to maternal concentrations. The high requirement for C20:5*n*-3 and C22:6*n*-3 within the neonate and the potential for production of these fatty acids via elongation and desaturation within the placenta may induce a selection pressure for the precursor *n*-3 fatty acid. This has been suggested by Payne (1978) as the reason behind the relatively high concentrations of C18:3*n*-3 and its metabolic derivatives in neonatal plasma.

Maternal plasma concentrations of C20:5*n*-3 and C22:6*n*-3 were significantly higher as a consequence of fish oil supplementation. However, the proportion of C20:5*n*-3 within plasma was similar for ewes fed Megalac and their offspring, whilst a four-fold decrease in C20:5*n*-3 proportion was seen in lambs borne by ewes offered fish oil when compared to their dams. The reason for this decrease is unclear, although it may be related to a down-regulation of C20:5*n*-3 production from C18:3*n*-3 within the placenta as a consequence of increased maternal dietary supply.

Notional placental supply of C22:6*n*-3 to the lamb was increased in ewes fed long-chain PUFAs, resulting in higher concentrations of this fatty acid in neonatal plasma. Although the research conducted by Elphick *et al.* (1979) and Leat and Harrison (1980) suggested that the placental is impermeable to long-chain PUFAs, Knipp *et al.* (1999) reported that fatty acid binding proteins facilitate the directional transport of fatty acids across the

placenta from maternal circulation to the foetus. Furthermore, Campbell *et al.* (1998) described a human placental protein which preferentially binds C22:6*n*-3 compared to C18:0 or C18:3*n*-3. If such a protein exists within the ruminant placenta, this could elucidate the mechanism behind the transfer of C22:6*n*-3 to foetal lambs, even when dietary supply or maternal endogenous synthesis is low. However, this does not explain why C22:6*n*-3 was not detected in samples from lambs borne to ewes fed treatment MB, despite the presence of this fatty acid in plasma of ewes fed this diet. Furthermore, this casts doubt on the hypothesis of Noble *et al.* (1985) that the placenta may elongate and desaturate C18:3*n*-3 to form C22:6*n*-3, as the concentration of C18:3*n*-3 in lambs from treatment MB was similar to those of the other treatments. Sinclair *et al.* (2002) suggested that plasma C22:6*n*-3 may not be an accurate indicator of tissue C22:6*n*-3 status due to the biosynthetic capacities of brain and retina. This concurs with results within the current study, as brain C22:6*n*-3 concentrations tended to be higher in lambs produced by ewes fed fish oil, although the magnitude of the difference was far less than the differences in plasma C22:6*n*-3 between treatments.

3.5.2.4. Neonatal brain fatty acids

Brain tissue contains a significant lipid component, of which more than one-third of fatty acids are polyunsaturated, functioning to modulate the structure, fluidity and function of neural cellular membranes (Bourre *et al.*, 1993). Total fatty acid concentrations within neonatal brain tissue were equivalent between treatments at approximately 26 mg/g fresh tissue. It may be suggested that the total brain fatty acid concentration is relatively inflexible given its pivotal role within the animal, hence the lack of a treatment effect upon this parameter.

The fatty acid composition of lamb brain tissue appears to mirror that of plasma, with no differences in concentrations of individual fatty acids save for C16:0, C20:5*n*-3 and

C22:6*n*-3. The amount of C16:0 within maternal body fluids (plasma, colostrum) was reduced by fish oil supplementation of the ewe within the current study. As previously discussed, this may be attributed to a lower rate of cellular fatty acid synthesis induced by either the lack of a 2-carbon precursor or by the inhibitory effect of long-chain PUFAs upon enzymes necessary for synthesis to occur. It is possible that the tendency for the amount of C16:0 within neonatal lamb brain to be reduced by fish oil supplementation of the ewe may be a result of either mechanism. Plasma C16:0 concentrations were similar between treatments in the neonatal lamb, however, as suggested by Sinclair *et al.* (2002), plasma fatty acid concentrations may not accurately reflect tissue concentrations, especially in an animal vulnerable to fatty acid deficiency.

Given the low dietary, and therefore placental, supply of long-chain PUFAs to the lamb in ewes fed conventional diets, it is logical to suggest that a mechanism for the synthesis of these fatty acids must be present within brain or other tissues. Indeed, Williard *et al.* (2001) demonstrated that astrocytes within rat brain tissue are capable of synthesising C22:6*n*-3 from C18:3*n*-3. Furthermore, studies in rats (Green and Yavin, 1993; Pawlosky *et al.*, 1996), humans (Salem *et al.*, 1996) and primates (Sheaff Greiner *et al.*, 1997; Su *et al.*, 1999; 2001) have reported that long-chain PUFAs are biosynthesised within the foetus and neonate. In consequence, a concentration gradient would be expected to exist between plasma and brain tissue with lower concentrations of C18:2*n*-6 and C18:3*n*-3 and higher concentrations of long-chain PUFAs within brain tissue compared to plasma. This concurs with the research of Berlin *et al.* (1998) who observed that whole brain fatty acid composition is not significantly correlated with erythrocyte fatty acid composition.

The proportion of C18:2*n*-6 was reduced approximately ten-fold in brain tissue across all treatments when compared to plasma with a corresponding, but lower (approximately two-fold) increase in C20:4*n*-6 concentration. Although theoretically one molecule of C18:2*n*-

6 would be converted into one molecule of C20:4*n*-6, the efficiency of the desaturation and elongation process and competition between individual fatty acids for desaturase and elongase enzymes reduces the amount of C20:4*n*-6 formed per molecule of C18:2*n*-6. The augmented concentration of C20:4*n*-6 within brain tissue compared to plasma may also be facilitated by preferential uptake and incorporation of this fatty acid at times when the placental or dietary supply is low.

Salem *et al.* (1996) suggested that the conversion of *n*-6 fatty acids to their long-chain derivatives may be more efficient than the conversion of *n*-3 fatty acids despite the accumulation of higher concentrations of *n*-3 fatty acids in plasma of human infants. However, within the current study, the magnitude of the difference between concentrations of C18:3*n*-3 in plasma and brain tissue was lower than that of C18:2*n*-6 despite the four- or five-fold increase in C22:6*n*-3 concentrations in brain compared to plasma. Although C18:3*n*-3 is the principal precursor of C22:6*n*-3, C20:5*n*-3 is produced as an intermediate during desaturation and elongation (Sprecher, 2000). Dietary C20:5*n*-3 (as compared to endogenously produced C20:5*n*-3) may therefore be utilised for the production of C22:6*n*-3, which would explain the low concentrations of this fatty acid within brain tissue compared to plasma.

The range of C20:5*n*-3 concentrations within brain tissue across treatments concur with those reported by Rooke *et al.* (1999; 2000; 2001a; 2001b; 2001c) in various studies. Furthermore, in Rooke's aforementioned studies, C20:5*n*-3 was found in very low concentrations in brain tissue compared to C20:4*n*-6 and C22:6*n*-3. Concentrations of C20:5*n*-3 within plasma tended to be higher in lambs borne to ewes fed fish oil and the amount of this fatty acid in brain tissue was significantly increased by fish oil supplementation of the ewe. The low concentrations of C20:5*n*-3 observed in brain tissue of lambs produced by ewes fed Megalac compared to fish oil may therefore have resulted

from a combination of low dietary supply and endogenous conversion to longer chain fatty acids. However, although fatty acids of the *n*-3 series are known to be important for the correct development and function of nervous tissue, C20:5*n*-3 is not generally found in high concentrations within brain tissue (Joshi *et al.*, 2004). Therefore, the low C20:5*n*-3 concentrations may simply be a result of the low cellular demand for this fatty acid within the brain.

Published values for C22:6*n*-3 concentrations within mammalian brain tissue vary from 11 g/100g fatty acids (Joshi *et al.*, 2004) to 22 g/100g fatty acids (Rooke *et al.*, 2001a). The proportional contribution of C22:6*n*-3 to brain fatty acids within the current study concur with values those reported by Lauritzen *et al.* (2001). However, Rooke *et al.* (2001c) observed significantly higher proportion of C22:6*n*-3 within brain tissue (approx 20 g/100g fatty acids) in piglets borne by ewes fed salmon oil than those seen within the current study. Similar results were observed by Rooke *et al.* (2001a) as a consequence of tuna oil supplementation of the pregnant sow. By contrast, previous studies conducted by Rooke *et al.* (1999) demonstrated that either salmon or tuna oil supplementation of the pregnant sow resulted in brain C22:6*n*-3 concentrations only marginally higher than those observed within the current study. Variation between these studies may be due to differences in fatty acid supply and the duration of supplementation. Goustard-Langelier *et al.* (1999) reported higher C22:6*n*-3 concentrations in brain tissue of suckling piglets than those observed within the current study, the concentration of C22:6*n*-3 varying between brain compartments (192 g/100g fatty acids in cortex, 153 g/100g fatty acids in cerebellum). Palowsky *et al.* (1996) concluded that separate areas of the brain differed both in growth rate and ability to synthesise or absorb C22:6*n*-3, which would further explain variation in C22:6*n*-3 concentrations between the current study, in which the entire brain was homogenised and analysed for fatty acids, and that of Goustard-Langelier *et al.* (1999).

It is interesting to note that in humans, brain C22:6*n*-3 accretion tapers off at approximately 2 years of age at a concentration of 3 mg/g freshweight (Lauritzen *et al.*, 2001). The proportion of C22:6*n*-3 (expressed as g/100g fatty acids) within lamb brain tissue was similar to that of neonatal human brain tissue (Lauritzen *et al.*, 2001). However, lambs within the current study had a C22:6*n*-3 concentration of approximately 3 mg/g of brain tissue (freshweight) at birth, underlining the difference in neural development, precocity and motor coordination in neonatal lambs when compared to human infants.

Maternal dietary vitamin E concentration had no significant effect upon the deposition of fatty acids into brain tissue within the current study, although it could be hypothesised that increasing the antioxidant supply would retard oxidation and promote the incorporation of long-chain PUFAs into cellular membranes. Çelik *et al.* (1999) demonstrated increased concentrations of all fatty acids in brain tissue of growing lambs (eight to nine months of age) as a result of dietary supplementation with vitamin E, selenium or a combination of the two antioxidants. However, the elevated fatty acid requirement of the neonatal lamb in relation to the supply of fatty acids from placental transfer may have negated any effects of maternal vitamin E supply.

3.5.2.5. Lamb growth rate

Several studies have reported that long-chain PUFA supplementation of the dam increases birthweights in human and animal neonates (Olsen *et al.*, 1992; Crawford *et al.*, 1997; Smuts *et al.*, 2003). However, Annett *et al.* (2004) observed no significant effect of fish oil supplementation of ewes upon lamb birthweight, whilst Rooke *et al.* (2001c) described a decrease in piglet birthweight as a result of salmon oil supplementation of the pregnant sow. Mean lamb birthweights were numerically higher as a result of fish oil supplementation of the ewe in the current study although these results did not reach statistical significance. This increase may be ascribed to the increase in gestational age in

these lambs: as foetal growth follows an exponential curve during late pregnancy, an increase in the duration of gestation would be expected to increase neonatal birthweight (Agricultural Research Council, 1980).

An increase in lamb birthweight was conferred by maternal vitamin E supplementation in the current study. This concurs with research conducted by Merrell (1998) and Kott *et al.* (1996) who suggested that supranutritional vitamin E supplementation of pregnant ewes may improve lamb survival and growth rate. Gentry *et al.* (1992) reported that lambs from vitamin E-supplemented ewes tended to have higher birthweights and increased pre-weaning liveweight gains. Moreover Bass *et al.* (2001) described higher average weaning weights in beef cattle as a result of maternal vitamin E supplementation during gestation. Conversely, neither Williamson *et al.* (1995) or Kott *et al.* (1998) observed any effect of vitamin E supplementation of the ewe on lamb liveweight or growth rate.

The mechanism by which birthweight may be increased by vitamin E supplementation has not been investigated; however, it may, in part, be due to the effects of antioxidant vitamins upon the immune system, as reviewed by McDowell *et al.* (1996). Vitamin E has been reported to augment immune status and reduce the incidence and symptoms of disease in ruminant animals (Reddy *et al.*, 1986). Consequently, improving the maternal immune status during pregnancy may promote the partitioning of additional nutrients towards the growing foetus, thereby increasing growth *in utero*.

Milk composition and yield are important regulators of pre-weaning lamb growth rate, as milk is the only source of energy and protein in the diet of the suckling lamb. The effect of long-chain PUFA supplementation of the ewe upon lamb growth rate in the current study may be attributed to reduced energy intakes as a consequence of decreased milk fat concentrations. By contrast, Rooke *et al.* (2001c) fed salmon oil to sows during pregnancy

and lactation, but observed no effect of this supplementation upon piglet weaning weight. Similar results are described by Rooke *et al.* (2000) as a consequence of feeding tuna oil during pregnancy and lactation.

Koletzko (1992) reviewed the effect of long-chain PUFA supplementation upon human infants and described a retarded growth syndrome induced by C20:4*n*-6 deficiency. Differences in milk C20:4*n*-6 concentrations between treatments were non-significant, nonetheless, plasma concentrations of this fatty acid in lambs suckling ewes fed fish oil were half those of the Megalac group. However, these differences were biologically insignificant and this explanation is unlikely to account for changes in growth rate observed within the current study.

3.5.2.6. Lamb antioxidant status

Significant increases in the concentration of plasma vitamin E observed in lambs as a consequence of maternal supranutritional supplementation reflect the differences observed in maternal plasma and milk. Indeed, ewe and lamb plasma vitamin E concentrations were similar at two weeks *post partum*. Similar results were described by Njeru *et al.* (1994) as a a consequence of supplementing pregnant and lactating ewes with various concentrations of dietary vitamin E.

Adding 1000 I.U. of d-α-tocopherol to the daily diet of three-month old lambs increased plasma vitamin E concentrations from 0.65 μg/ml to 1.90 μg/ml in the study of Hidiroglou and Batra (1996), values consistent with the lowest concentrations observed in lambs within the current study. The concentrations within the current study were within the range of plasma values reported by Doncon and Steele (1988), Håkansson *et al.* (2001) and Hatfield *et al.* (2002). However, offering diets containing Megalac and supranutritional dietary vitamin E to ewes within the current study resulted in significantly higher values

being detected. This may be due to differences in vitamin E absorption between experiments; the high fat content of ewe milk promoting micellular absorption in lambs compared to absorption from an intramuscular injection. Nonetheless, lambs from treatments MB, FB and FS were clinically deficient in vitamin E according to the index of Hidiroglou *et al.* (1992b) with only those borne by ewes fed diet MS achieving minimal vitamin E status.

Erythrocyte GPx activity is a reliable indicator of the selenium status of the animal (Carlstrőm *et al.*, 1990). GPx activities were approximately doubled in lamb erythrocytes at two weeks of age when compared to ewe erythrocyte activities. Placental transfer of selenium appears to be limited in the ewe (Jacobsson and Oksanen, 1966; Bostedt and Schramel, 1990) although it can be increased by maternal supplementation (Van Metre and Callan, 2001). However, there appears to be no barrier to mammary transfer, which may explain the higher concentrations in suckling lambs compared to ewes. The activity of GPx in lamb erythrocytes was reduced by maternal long-chain PUFA supplementation, concurring with patterns observed in maternal plasma during lactation. Increasing the cellular oxidative challenge by augmentation of the diet with unsaturated fatty acids confers an increase in the antioxidant requirement of the cell and may reduce GPx activity (Smith and Isopenko, 1997). Milk total unsaturated fatty acid concentrations were similar between treatments, although proportional increases in long-chain PUFA supply to the lamb may have further increased the antioxidant requirement.

Serum CK concentrations observed in lambs at two weeks of age concur with the results of maternal plasma taken at two weeks into lactation, in that the concentrations of this enzyme were significantly augmented by long-chain PUFA supplementation of the ewe. El-Neweehy *et al.* (2000) reported low concentrations of CK in animals free of nutritional myopathy (37 IU/l); sub-clinically affected animals had concentrations ranging from 1186-

3740 IU/l with detectable symptoms being seen at a mean CK concentration of 4291 IU/l. By these criteria, although lambs suckling from ewes fed fish oil were potentially more susceptible to nutritional myopathy, clinical disease was unlikely to be present. As plasma vitamin E concentrations were within reported ranges, the differences in CK concentrations may be a result of reduced activities of other antioxidants including GPx or vitamin C.

3.5.2.7. Plasma fatty acids in the suckling lamb

The lamb diet at two weeks of age consisted solely of ewe milk, consequently the fatty acid composition of lamb plasma would be expected to mirror that of milk. The milk fatty acid yield, calculated from milk yield and fatty acid content suggest that dietary fatty acid supply was reduced in lambs suckling ewes fed fish oil. This concurs with the observed results, in which lambs suckling from ewes offered fish oil had lower concentrations of total fatty acids in plasma.

As previously discussed, the concentration of C16:0 within milk fat was decreased by long-chain PUFA supplementation, this result being attributed both to changes in dietary C16:0 intake between treatments and to suppression of endogenous fatty acid synthesis. Proportions of plasma C16:0 were reduced in lambs suckling ewes supplemented with fish oil, a result which may therefore be attributed to the significantly lower dietary intake. Rumen function in the lamb is stimulated by ingestion of dietary fibre with complete rumen function usually being achieved as a consequence of weaning (McDonald *et al.*, 1988). Suckling lambs are therefore effectively monogastrics and differences in C18:0 are unlikely to be a result of changes in ruminal biohydrogenation. The same theory applies to the concentrations of C18:1 *trans* and CLA within lamb plasma. In the adult ruminant, these fatty acids derive from ruminal modification of dietary unsaturated fatty acids and may have been transferred to lamb plasma via milk.

Modification of ewe plasma and milk C18:1*n*-9 *cis* concentrations in the current study was achieved by addition of long-chain PUFAs to treatment concentrates. These differences were further reflected in the concentration of this fatty acid within lamb plasma, as a consequence of reduced dietary intakes from milk fat. By contrast, although a positive relationship exists between ewe and lamb plasma concentrations of C18:2*n*-6 between treatments, this difference in fatty acid composition was not observed in milk fat. The proportions of individual fatty acids were similar between ewe milk and lamb plasma for C16:0, C18:0, C18:1 *trans* and C18:1*n*-9 *cis* although the proportion of C18:2*n*-6 was approximately eight-fold higher in lamb plasma than in milk fat. Payne (1978) described the transfer of EFAs to the foetal lamb, suggesting that the neonate is deficient in C18:2*n*-6 but that this deficiency is rectified after three days of milk consumption. By contrast, Rajion *et al.* (1985) postulated that the quantities of EFA metabolites within plasma signify that suckling lambs are not deficient in EFAs. The consensus view appears to be that EFAs are not transferred across the placenta in any appreciable quantity, therefore, the presence of this fatty acid in plasma is unlikely to result from mobilisation of stored reserves. When the EFA supply is low, Noble *et al.* (1971) suggested a preferential utilisation of C18:2*n*-6 rather than C18:3*n*-3 for plasma phosphatidylglycerol synthesis, which may explain the higher concentrations seen in the current study.

The reduction in plasma C20:4*n*-6 exhibited by lambs suckling ewes fed fish oil does not appear to be related to plasma and milk concentrations, but may result from reduced endogenous synthesis within the lamb. Competition between C18:2*n*-6 and C18:3*n*-3 for the enzymes involved in the Δ-6-desaturase enzyme occurs, which is further complicated by the inhibiting effect of preformed dietary C20:5*n*-3 and C22:6*n*-3. Therefore, the increased dietary supply of long-chain *n*-3 PUFAs in lambs suckling ewes offered fish oil diets may have reduced the synthesis of the *n*-6 series PUFAs with concurrent effects upon labile C20:4*n*-6 supply.

Variation in the proportion of C18:3*n*-3 within lamb plasma between treatments may be ascribed to differences in dietary fatty acid intakes, as the results concur with ewe plasma and milk concentrations of this fatty acid. Throughout the current study, addition of fish oil to the diet of pregnant and lactating ewes has increased the amount of C20:5*n*-3 and C22:6*n*-3 within plasma. Again, lamb plasma concentrations concur with these results, with approximately double the amount of C20:5*n*-3 within plasma compared to C22:6*n*-3. As observed with C18:2*n*-6, the proportions of C20:5*n*-3 and C22:6*n*-3 within lamb plasma were higher than those within milk fat. Given the conditional essentiality of these fatty acids for the development and function of neural tissues, a mechanism by which they may be conserved and preferentially utilised must exist, especially in animals with a low dietary supply. Payne (1978) suggested that placental transfer of long-chain PUFAs was minimal. Nonetheless, the increased concentration of C22:6*n*-3 within neonatal lamb plasma and brain tissue observed during the current study indicates that this transfer may be significant in the maintenance of a satisfactory long-chain PUFA status in the neonate. The increased concentrations of C20:5*n*-3 and C22:6*n*-3 observed in plasma compared to milk may therefore, in part, be conferred by transfer of these fatty acids during foetal development.

3.6. Conclusion

Long-chain PUFA supplementation of pregnant ewes increased gestation length and augmented the deposition of C22:6*n*-3 into lamb brain tissue with a concomitant improvement in neonatal lamb vigour. However, milk fat concentration and yield were significantly reduced by PUFA supplementation, with concurrent effects upon lamb growth rate. The next logical step would be to replace dietary PUFAs with a saturated fatty acid source during lactation, in an attempt to negate the effect observed on lamb growth rate, whilst maintaining effects upon gestation length and lamb behaviour.

Neonatal lamb behaviour was unaffected by maternal dietary vitamin E concentration, although supranutritional supplementation increased lamb birthweight. In contrast to previously published research, it appears that the vitamin E status of the neonatal lamb may be manipulated via the maternal diet. Furthermore, lamb plasma vitamin E concentrations are not reliable indicators of the deposition of this vitamin in tissue.

4. SUPPLEMENTATION OF PREGNANT AND LACTATING EWES WITH VARIOUS FAT SOURCES: EFFECTS UPON EWE AND LAMB PERFORMANCE

4.1. Introduction

Since the BSE crisis of 1996, considerable consumer concern has existed regarding feeding sources of animal protein to ruminants (Verbeke, 2001). Both meat and bone meal and fishmeal have been banned in ruminant diets in the EU (Matthews and Cooke, 2003) and it is not unreasonable to suggest that feeding fish oil to ruminants may also be prohibited in future. Marine algae is an alternative dietary source of preformed long-chain *n*-3 PUFAs that may be included in ruminant diets (Papadopoulos *et al.*, 2002). However, the exact proportions of C20:4*n*-6, C20:5*n*-3 and C22:6*n*-3 within algae vary according to species (Sargent and Henderson, 1995). Using an algal species that provides a high dietary supply of C22:6*n*-3 in combination with a low C20:5*n*-3 supply may elucidate the biochemical mechanisms behind the effects of long-chain PUFA supplementation upon gestation length and lamb behaviour observed in Experiment One. Both C20:5*n*-3 and C22:6*n*-3 may be endogenously synthesised within the ruminant via elongation and desaturation of dietary C18:3*n*-3 (Voigt and Hagemeister, 2001). Linseed (*Linum usitatissimum*) contains high concentrations of C18:3*n*-3 and is suitable for use in ruminant diets; moreover, its protein-based seed coat may act as a barrier against the ruminal biohydrogenation of long-chain PUFAs (Doreau and Ferlay, 1994; Szumacher-Strabel *et al.*, 2001a). Studies in primates and human infants have attempted to quantify the synthesis of C22:6*n*-3 from C18:3*n*-3 (Koletzo *et al.*, 1996; Crawford *et al.*, 1997; Su *et al.*, 1999). However, there is no conclusive evidence as to whether endogenous synthesis of these fatty acids by pregnant ewes provides sufficient C22:6*n*-3 for optimal deposition in neonatal brain and nervous tissues.

The first experiment demonstrated that long-chain PUFA supplementation of pregnant ewes had a beneficial effect on neonatal lamb vigour although milk composition and lamb growth rate were adversely affected by PUFA supplementation during lactation. Feeding a saturated fat source during lactation may negate the changes in milk composition resulting from long-chain PUFA supplementation during pregnancy, thereby improving lamb growth rate.

4.2. Objective

The objective of the current experiment was to investigate the targeted supplementation of pregnant and lactating ewes with various fat sources, upon ewe and lamb behaviour and performance.

4.3. Materials and methods

4.3.1. Experimental animals and housing

Forty two twin-bearing and eighteen triplet-bearing ewes from the Harper Adams early lambing flock (Edgmond, Newport, Shropshire, UK) with a mean age of 4.6 years (s.d. 1.80), mean liveweight of 76.6 kg (s.d. 8.60 kg) and mean condition score of 3.1 units (s.d. 0.50) were used in a six-treatment randomised block design. Ewes were either Friesland x Lleyn (n = 36) or Suffolk x North of England Mule (n = 24). Ewes were housed, individually penned and bedded on sawdust from week 15 (designated week -6) of pregnancy until week 4 (week +4) of lactation. An additional eight ewes were housed in a group-pen and bedded on straw to provide foster lambs for any ewes that did not bear two or three live lambs. The building was continually lit and all ewes had free access to fresh water.

4.3.2. Experimental diets

A basal diet was formulated containing barley, sugar beet pulp, soyabean meal, Sopralin™, rapeseed meal, urea and molasses (Table 4.1). To this diet was added 177 g/kg of a long-chain PUFA (algae; A), precursor (linseed; L) or control saturated fat (Megalac®; M) premix. The PUFA premix comprised marine algae (Chance and Hunt Nutrition, Runcorn, Cheshire, UK) as the principal source of C22:6*n*-3, combined with Megalac® (Volac UK Ltd, Royston House, Royston, UK), soyabean meal, rapeseed meal and straw pellets. This premix was formulated to provide 6.32 g C22:6*n*-3/kg concentrate (36 g marine algae/kg concentrate). Using the figure of 43% published for ruminal biohydrogenation of C22:6*n*-3 by Cooper *et al.* (2002), this would provide 3.60 g C22:6*n*-3/kg concentrate to the pregnant animal, as recommended by Rooke *et al.* (2001b). The linseed premix contained only whole linseed, the premix inclusion rate was calculated to provide a dietary concentration of C18:3*n*-3 between seven and eight-fold higher than that of C22:6*n*-3 within the algal premix. According to the results of Su *et al.* (1999; 2001) this would deposit equivalent concentrations of C22:6*n*-3 into neonatal tissues, via the elongation and desaturation of C18:3*n*-3, as the algae premix. The control fat premix was made up of Megalac® as the principal fat source, plus soyabean meal, rapeseed meal and straw pellets. The latter components (soyabean meal, rapeseed meal and straw pellets) within the algae and Megalac® premixes balanced the protein component supplied by whole linseed in the precursor premix. The three premixes were formulated to provide equal concentrations of fat (62.2 g/kg freshweight) and protein (34.6 g/kg freshweight), and similar ME concentrations (averaged as 3.28 MJ ME/kg freshweight) within the concentrates. All concentrates also contained a vitamin and mineral premix (30 g/kg) providing 500 mg/kg vitamin E. The resulting concentrates were formulated to be isoenergetic and isonitrogenous with a predicted nutrient content of 14.0 MJ/kg DM metabolisable energy, 213 g/kg DM crude protein and 83.0 g/kg DM fatty acids, formulated according to the guidelines laid out by AFRC (1993).

The treatment concentrates were fed within six dietary strategies in an attempt to reduce the detrimental effect of long-chain PUFA supplementation upon milk composition and lamb growth rate (Table 4.3). During pregnancy, 30 ewes were fed the algae concentrate, 20 fed the linseed concentrate and 10 fed the Megalac® concentrate. Twenty of the ewes fed algae during pregnancy were then changed to either the linseed (10 ewes) or Megalac® (10 ewes) diets at +12 hours *post partum* and 10 of the ewes fed linseed during pregnancy were changed to the Megalac® diet at +12 hours *post partum*.

Table 4.1. Raw material and chemical composition of the three treatment concentrates

	Algae	Linseed	Megalac®
Raw material composition (g/kg)			
Barley	500	500	500
Sugar beet pulp	100	100	100
Soyabean meal	121	77	126
Rapeseed meal	75	48	78
Sopralin	10	10	10
Straw pellets	19	-	29
Marine algae	36	-	-
Whole linseed	-	177	-
Megalac®	51	-	69
Molasses	50	50	50
Urea	8	8	8
Vitamins/Minerals	30	30	30
Predicted chemical composition (g/kg DM)¶			
DM (g/kg)	868	858	867
CP	213	215	213
ERDP*	131	136	132
DUP*	55	51	54
EE	82	84	83
NDF	171	173	172
Ash	69	70	69
Vitamin E (mg/kg in premix)	500	500	500
ME (MJ/kg DM)	14.0	13.7	14.0
FME (MJ/kg DM)	10.8	11.1	11.0
ERDP:FME Ratio	12.1	12.3	12.0

Vitamin/Mineral supplement (Hac Ewe 25, Roche Products Limited, Heanor, Derbyshire, UK) supplied per kg of diet: Calcium 7.06 g; Sodium 2.67 g; Phosphorus 1.65 g; Selenium 0.36 mg; Vitamin A 14,400 IU; Vitamin D 30,000 IU; Vitamin E 500 mg.

¶ (AFRC, 1993)

* calculated according to AFRC (1993) at a rumen outflow rate of 0.08 ml/hour

Table 4.2. The structure of the six dietary strategies fed to ewes

Strategy	Pregnancy diet	Lactation diet
AA	Algae	Algae
AL	Algae	Linseed
AM	Algae	Megalac®
LL	Linseed	Linseed
LM	Linseed	Megalac®
MM	Megalac®	Megalac®

Ewes were fed a stepped daily amount of concentrate feed (Table 4.3) in two equal meals per day (at 08:00 and 16:00) during pregnancy, and were fed at a flat-rate of 1.7 kg/day in three meals per day (at 08:00, 12:00 and 16:00) during lactation. Barley straw was initially offered at 0.80 kg/day and subsequently fed *ad libitum* at intake levels calculated according to the method described in Chapter Two.

Table 4.3. Daily concentrate allowance for twin- and triplet-bearing ewes

Day of gestation	110	117	124	131	138	145	Lactation
Daily concentrate allowance for twin-bearing ewes (kg freshweight)	0.65	0.75	0.85	0.95	1.05	1.15	1.7
Daily concentrate allowance for triplet-bearing ewes (kg freshweight)	0.7	0.8	0.9	1.05	1.15	1.25	1.7

4.3.3. Experimental Procedure

Concentrate and straw samples were taken weekly and stored in airtight bags at –20 °C until analysis. Ewe liveweight, body condition score, straw intake, maternal and neonatal behaviour, lamb birthweight and liveweight, colostrum production and milk production were measured as previously described in Chapter Two.

4.3.3.1. Blood sampling

Blood samples were obtained from ewes by jugular venepuncture at 11:00 at six weeks (103 days, before the experimental concentrates were fed) and one week (138 days of gestation) *pre-partum*; at 12 hours *post partum* and at one and three weeks *post partum*.

Blood and tissue samples were taken from neonatal triplet lambs immediately after cessation of the heartbeat as described in section 2.2.4. Blood samples were taken by jugular venepuncture from growing lambs at +12 hours of age and again at 11:00 at one and three weeks of age. Plasma and tissue samples were prepared as described in Chapter Two.

4.3.4. Sample analysis

Concentrate and straw samples were analysed for DM, ash, CP and NDF. In addition, concentrate samples were analysed for fatty acid composition and for vitamin E. Ewe blood samples were analysed for urea, βHB, CK and GPx at all time points and for fatty acids at 103 and 131 days of gestation. Neonatal lamb blood samples were analysed for fatty acids; suckling lamb blood samples were analysed for CK and GPx at all time points and for fatty acid composition at 21 days of age. All analyses are described in Chapter Two.

4.3.5. Statistical analysis

Data were analysed as a six-treatment randomised block design by the ANOVA function within Genstat 6 version 6.2 (Lawes Agricultural Trust, 2002). Sex was used as a co-variate when analysing lamb birthweight, liveweight and growth rate data. Plotting lamb liveweight against time revealed linear growth rates, therefore overall growth rates were calculated using linear regression. Orthogonal contrasts were employed upon selected data to determine differences between treatment diets fed *pre-* or *post partum* (Genstat 6 version 6.2, Lawes Agricultural Trust, 2002). Contrasts employed were "algae versus Megalac", "algae versus linseed" and "linseed versus Megalac", according to the diet fed either *pre-* or *post partum*. Data collected *pre-partum* included 30 samples from ewes fed algae, 20 samples from those fed linseed and 10 from those fed algae. The same totals were used to calculate the effects of *pre-partum* diet upon *post partum* performance. The

effect of *post partum* diet upon *post partum* performance was analysed using 10 samples from ewes fed algae, 20 from those fed linseed and 30 from those fed algae. Contrasts comparing algae with Megalac and algae with linseed were calculated together, the analysis was re-run to calculate the effect of linseed compared to Megalac.

4.4. Results

Data from ten ewes were excluded from the analysis: three suffered from mastitis (one each from treatments AA, LL and MM), six reared single lambs (one from each of treatments AL, LL, MM AM, and two from treatment LM) and one did not rear any lambs (treatment MM). Data from lambs reared by excluded ewes were not included in statistical analyses.

4.4.1. Diet composition

The DM content was lowest for the algae (A) concentrate followed by the linseed (L) and Megalac (M) concentrates, whilst the ash fraction was similar between concentrates A and M and was lowest in concentrate L (Table 4.4). Crude protein concentrations were similar for diets A and M but lower for concentrate L, whilst the NDF content was similar amongst all three concentrates. The total fatty acid content of concentrate L was higher than that of concentrates A or M. Vitamin E concentrations were similar between concentrates A and M and slightly higher in concentrate L.

Table 4.4. Chemical composition of the three treatment concentrates and the straw

	Concentrate			Straw
	A	L	M	
Dry matter (g/kg)	814	845	861	872
Crude protein (g/kg DM)	199	181	199	54
Organic matter (g/kg DM)	925	942	921	921
Ash (g/kg DM)	75	58	79	79
Neutral detergent fibre (g/kg DM)	169	164	163	756
Vitamin E (mg/kg DM)	563	585	560	-
Total fatty acids (g/kg DM)	105	113	94	-

A= Algae diet; L = Linseed diet; M = Megalac diet

The principal fatty acids within concentrate M were C16:0, C18:1*n*-9 and C18:2*n*-6 with small amounts of C18:0 and C18:3*n*-3 present (Table 4.5). The long-chain *n*-3 PUFAs C20:5*n*-3 and C22:6*n*-3 were not detectable within concentrate M.

Table 4.5. Fatty acid composition of the three treatment concentrates

	Concentrate		
Fatty acid (g/kg DM)	**A**	**L**	**M**
C16:0	32.8	10.8	39.8
C16:1*n*-7	0.46	ND	0.24
C18:0	2.76	2.81	3.07
C18:1 *trans*	ND	ND	ND
C18:1*n*-9 *cis*	21.3	14.7	25.6
C18:2*n*-6 *cis*	17.6	25.7	18.2
C18:3*n*-3 *cis*	2.53	54.9	1.86
C18:3*n*-6	0.07	0.35	ND
C20:4*n*-6	0.21	ND	ND
C20:5*n*-3	ND	ND	ND
C22:6*n*-3	7.71	ND	ND
RFA[1]	19.2	3.94	5.11

A= Algae diet; L = Linseed diet; M = Megalac diet
[1] RFA = All remaining fatty acids; ND = not detected

The predominant fatty acid contained within concentrate L was C18:3*n*-3, contributing 0.48 of the total fatty acids. Furthermore, significant amounts of C18:1*n*-9 and C18:2*n*-6 were found, although no C20:5*n*-3 or C22:6*n*-3 were detected. The major fatty acids contained within concentrate A were C16:0, C18:1*n*-9 and C18:2*n*-6 with small amounts of C18:0 and C18:3*n*-3. By contrast, a significant amount of C22:6*n*-3 was present, contributing 0.07 of the total fatty acid content.

4.4.2. Ewe performance parameters

4.4.2.1. Straw intake

The daily straw intake (Tables 4.6 and 4.7, Figure 4.1) of all ewes remained relatively constant between the start of the experiment and parturition. When data were analysed as a randomised block design with six dietary strategies, there was no significant effect of strategy upon *pre-partum* daily straw intake. Analysis of the contrasts among dietary strategies revealed that ewes supplemented with either algae or linseed *pre-partum* had significantly lower daily straw intakes than ewes fed Megalac-based diets at one week *pre-partum.*

Table 4.6. Effect of supplementing the diets of ewes with various fat sources on daily straw intakes

	Diet						s.e.d.	P
	AA	AL	AM	LL	LM	MM		
Mean daily intake (kg DM):								
6 weeks[¶] *pre-partum*	0.57	0.51	0.54	0.59	0.51	0.54	0.056	0.677
1 week[¶] *pre-partum*	0.43	0.48	0.44	0.57	0.48	0.69	0.099	0.090
Pre-partum intake (kg/day)	0.51	0.50	0.53	0.62	0.52	0.63	0.085	0.464
0 weeks[¶] *post partum*	0.50^{ab}	0.58^{b}	0.40^{a}	0.55^{ab}	0.48^{ab}	0.85^{c}	0.081	<0.001
3 weeks[¶] *post partum*	1.04	1.14	0.85	0.95	1.04	1.37	0.223	0.294
Post partum intake (kg/day)	0.77	0.84	0.65	0.72	0.73	1.04	0.137	0.104

AA = Algae diet fed throughout pregnancy and lactation; AL= Algae diet fed during pregnancy followed by Linseed diet; AM = Algae diet fed during pregnancy followed by Megalac diet in lactation; LL = Linseed diet fed throughout pregnancy and lactation; LM = Linseed diet fed during pregnancy followed by Megalac diet in lactation; MM = Megalac diet fed throughout pregnancy and lactation

Means without common superscripts are significantly different at the P<0.05 level

[¶] 6 weeks *pre-partum* = mean straw intake on days 103-110 of gestation; 1 week *pre-partum* = mean straw intake on days 138-145 of gestation; 0 weeks *post partum* = mean straw intake on days 0 – 7 of lactation, 3 weeks *post partum* = mean straw intake on days 21 – 28 of lactation

Table 4.7. Effect of supplementing the diets of ewes with various fat sources pre- and post partum on daily straw intakes

	Diet			s.e.d.	P		
	Algae	Linseed	Megalac		C_1	C_2	C_3
Mean daily intake (kg DM):							
Effect of pre-partum diet							
Number per treatment	30	20	10				
6 weeks[¶] *pre-partum*	0.54	0.55	0.54	0.043	0.902	0.602	0.809
1 week[¶] *pre-partum*	0.45	0.52	0.69	0.075	0.004	0.846	0.008
Pre-partum intake (kg DM/day)	0.51	0.57	0.63	0.065	0.094	0.645	0.147
0 weeks[¶] *post partum*	0.67	0.62	0.94	0.078	<0.001	0.207	<0.001
3 weeks[¶] *post –partum*	0.99	0.97	1.51	0.205	0.053	0.448	0.040
Post partum intake (kg/day)	0.74	0.71	1.14	0.127	0.014	0.249	0.008
Effect of post partum diet							
Number per treatment	10	20	30				
0 weeks[¶] *post partum*	0.50	0.53	0.57	0.084	0.228	0.866	0.460
3 weeks[¶] *post –partum*	1.07	0.97	1.11	0.198	0.775	0.812	0.712
Post partum intake (kg DM/day)	0.79	0.73	0.81	0.125	0.731	0.817	0.685

C_1 = Algae vs Megalac; C_2 = Algae vs Linseed; C_3 = Linseed vs Megalac

[¶] 6 weeks *pre-partum* = mean straw intake on days 103-110 of gestation; 1 week *pre-partum* = mean straw intake on days 138-145 of gestation; 0 weeks *post partum* = mean straw intake on days 0 – 7 of lactation; 3 weeks *post partum* = mean straw intake on days 21 – 28 of lactation

A significant difference was evident during the week in which parturition occurred (week 0), where ewes offered the dietary strategy AM ate significantly less, and ewes offered the strategy MM significantly more, straw per day than ewes from the other four dietary strategies. However, daily straw intakes were not significantly different among any of the dietary strategies at any other time point *post partum.*

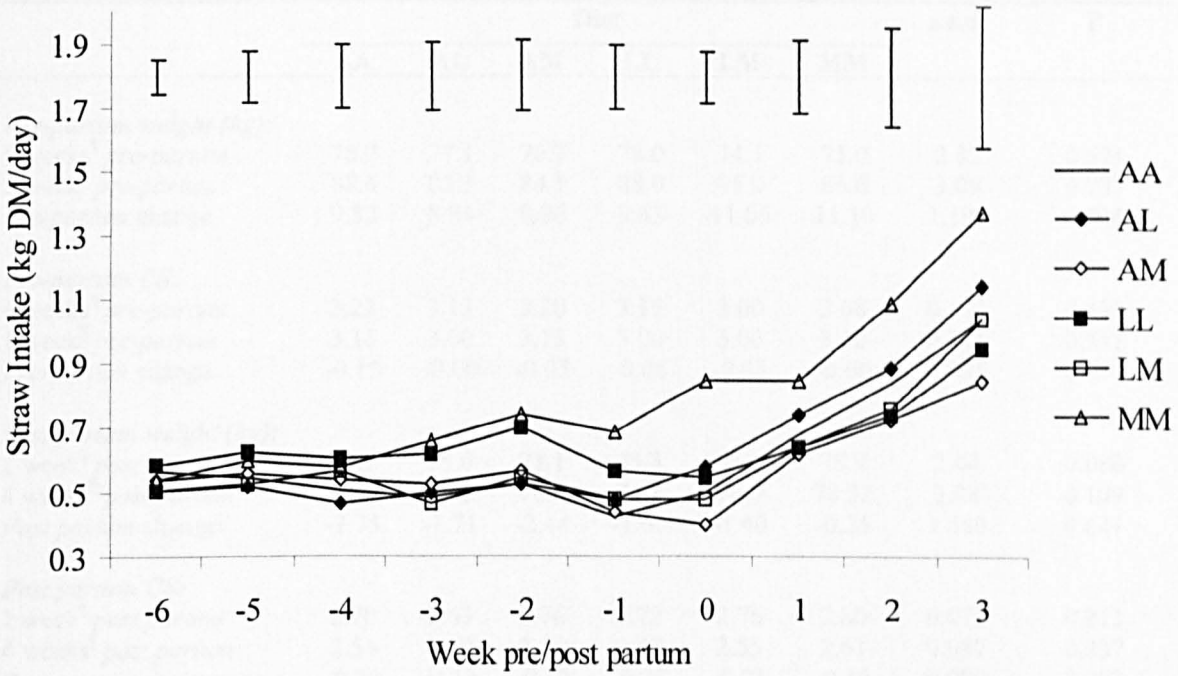


Figure 4.1. Effect of supplementing the diets of ewes with various fat sources on daily straw intakes

When the contrasts among the three fat sources were analysed as an effect of *pre-partum* dietary supplementation on *post-partum* straw intake, significant differences were elucidated between ewes fed algae (AA, AL, AM) and Megalac (MM) *pre-partum* at weeks 0 and +2 and between ewes fed linseed (LL, LM) and Megalac (MM) at all time points *post partum.* Furthermore, ewes fed algae or linseed *pre-partum* had significantly lower mean daily straw intakes *post partum* (averaged as 0.73 kg/day) than those fed Megalac (1.14 kg/day) during pregnancy. No significant effect of dietary fat source fed *post partum* was observed upon *post partum* straw intakes.

4.4.2.2. Liveweight and condition score

There were no significant differences in ewe liveweight among the six dietary strategies at the start of the experiment, at one week *pre-partum* or in total *pre-partum* liveweight change (Table 4.8). However, contrasts within the six strategies showed that ewes fed diets containing algae as the main fat source *pre-partum* gained significantly less weight than those fed Megalac-based diets (Table 4.9).

Table 4.8. Effect of supplementing the diets of ewes with various fat sources on liveweight and body condition score change

	Diet						s.e.d.	P
	AA	AL	AM	LL	LM	MM		
Pre-partum weight (kg):								
6 weeks[1] *pre-partum*	78.7	77.1	76.7	78.0	74.1	75.0	2.82	0.571
1 week[1] *pre-partum*	88.4	85.3	84.5	88.0	85.0	86.6	3.09	0.733
Pre-partum change	9.52	8.84	8.96	9.63	11.05	11.10	1.197	0.246
Pre-partum CS:								
6 weeks[1] *pre-partum*	3.23	3.13	3.20	3.15	3.00	3.08	0.114	0.392
1 week[1] *pre-partum*	3.15	3.00	3.13	3.00	3.00	3.10	0.111	0.557
Pre-partum change	-0.15	-0.00	-0.05	-0.08	-0.03	-0.00	0.095	0.616
Post partum weight (kg):								
1 week[1] *post partum*	74.7	75.0	73.1	75.3	71.5	78.9	2.44	0.088
4 weeks[1] *post partum*	72.4	73.5	70.6	74.8	70.0	78.32	3.08	0.109
Post partum change	-2.75	-1.71	-2.44	-1.60	-1.40	-0.35	1.460	0.647
Post partum CS:								
1 week[1] *post partum*	2.70	2.63	2.76	2.72	2.76	2.80	0.073	0.311
4 weeks[1] *post partum*	2.54	2.50	2.56	2.43	2.55	2.61	0.087	0.437
Post partum change	-0.20	-0.13	-0.19	-0.27	-0.21	-0.19	0.089	0.753

AA = Algae diet fed throughout pregnancy and lactation; AL= Algae diet fed during pregnancy followed by Linseed diet; AM = Algae diet fed during pregnancy followed by Megalac diet in lactation; LL = Linseed diet fed throughout pregnancy and lactation; LM = Linseed diet fed during pregnancy followed by Megalac diet in lactation; MM = Megalac diet fed throughout pregnancy and lactation

[1] 6 weeks *pre-partum* = day 103 of gestation; 1 week *pre-partum* = day 138 of gestation; 1 week *post partum* = day 7 of lactation; 4 weeks *post partum* = day 28 of lactation

Table 4.9. Effect of supplementing the diets of ewes with various fat sources pre- and post partum on liveweight change

	Diet			s.e.d.	P		
	Algae	Linseed	Megalac		C_1	C_2	C_3
Effect of pre-partum diet (kg)							
Number per treatment	30	20	10				
6 weeks[1] *pre-partum*	77.5	76.0	75.0	2.17	0.294	0.633	0.397
1 week[1] *pre-partum*	86.1	86.5	86.6	2.39	0.843	0.874	0.890
Pre-partum change	9.10	10.3	11.1	0.920	0.047	0.323	0.111
1 week[1] *post partum*	74.0	73.8	81.3	2.26	0.025	0.178	0.012
4 weeks[1] *post partum*	72.2	72.1	80.4	2.88	0.020	0.501	0.016
Post partum change	2.33	1.58	0.90	1.320	0.110	0.732	0.157
Effect of post partum diet (kg)							
Number per treatment	10	20	30				
1 week[1] *post partum*	74.9	75.0	74.7	2.13	0.932	0.685	0.729
4 weeks[1] *post partum*	73.5	73.3	72.9	2.79	0.813	0.432	0.740
Post partum change	2.93	1.64	1.73	1.240	0.264	0.929	0.465

C_1 = Algae vs Megalac; C_2 = Algae vs Linseed; C_3 = Linseed vs Megalac

[1] 6 weeks *pre-partum* = day 103 of gestation; 1 week *pre-partum* = day 138 of gestation; 1 week *post partum* = day 7 of lactation; 4 weeks *post partum* = day 28 of lactation

Ewes fed strategy MM tended to have higher liveweights at one week *post partum* than ewes offered strategic diet LM. No other differences among dietary strategies were

observed on *post partum* liveweight or liveweight change. By contrast, fat source offered *pre-partum* had a significant effect upon ewe liveweights *post-partum*: ewes fed algae or linseed during pregnancy had significantly lower liveweights at one and four weeks *post partum* than those fed Megalac. However, there was no effect of *pre-partum* fat source on *post partum* liveweight change. Ewe condition score and condition score change *pre-partum* and *post partum* were unaffected by dietary strategy or dietary fat source (data not shown).

4.4.2.3. Metabolic profiles

Ewes offered dietary strategy MM had the highest mean plasma βHB concentrations *pre-partum* when compared to strategies AM, LL and LM (Table 4.10). Furthermore, significant differences in mean *pre-partum* βHB concentrations were observed within all three contrasts, the lowest concentrations being found in ewes offered diets containing linseed and the highest in those fed diets based on Megalac (Table 4.11). Ewes supplemented with strategies AA or AM had significantly higher plasma βHB concentrations *post partum* compared to ewes offered strategies LL or LM. Furthermore, the mean plasma βHB concentration *post partum* was lower in ewes fed linseed-based diets when compared to those offered algae as the fat source when data were analysed according to the fat source fed *pre-* ($P<0.001$) or *post partum* ($P=0.017$).

Mean *pre-partum* plasma urea concentration was highest in ewes offered diets containing algae during pregnancy, the difference being statistically significant compared to ewes offered Megalac. Mean plasma urea concentrations recorded *post partum* were not significantly different. A significant carry-over effect of algal supplementation during pregnancy was observed upon mean *post partum* plasma urea concentrations when compared to Megalac supplementation *pre-partum*.

Table 4.10. Effect of supplementing the diets of ewes with various fat sources on plasma concentrations of β-hydroxybutyrate and urea

	Diet						s.e.d.	P
	AA	AL	AM	LL	LM	MM		
Pre-partum concentration (mmol/l):								
Mean* plasma βHB	0.79^{bc}	0.72^{abc}	0.67^{ab}	0.58^{a}	0.63^{ab}	0.87^{c}	0.085	0.016
Mean* plasma urea	6.61	6.94	7.36	6.56	6.95	6.12	0.509	0.249
Post partum concentration (mmol/l):								
Mean¶ plasma βHB	0.80^{c}	0.66^{bc}	0.78^{c}	0.41^{a}	0.54^{ab}	0.70^{bc}	0.110	0.009
Mean¶ plasma urea	8.27	8.14	8.22	7.82	7.16	7.00	0.625	0.186

AA = Algae diet fed throughout pregnancy and lactation; AL= Algae diet fed during pregnancy followed by Linseed diet; AM = Algae diet fed during pregnancy followed by Megalac diet in lactation; LL = Linseed diet fed throughout pregnancy and lactation; LM = Linseed diet fed during pregnancy followed by Megalac diet in lactation; MM = Megalac diet fed throughout pregnancy and lactation
Means without common superscripts are significantly different at the P<0.05 level
* Mean value = average of all measured values *pre-partum*
¶ Mean value = average of all measured values *post partum*

Table 4.11. Effect of supplementing the diets of ewes with various fat sources pre- and post partum on plasma concentrations of β-hydroxybutyrate and urea

	Diet			s.e.d.	P		
	Algae	Linseed	Megalac		C_1	C_2	C_3
Effect of pre-partum diet (mmol/l)							
Number per treatment	30	20	10				
Mean* plasma βHB *pre-partum*	0.72	0.61	0.87	0.066	0.040	0.004	0.005
Mean* plasma urea *pre-partum*	6.97	6.75	6.12	0.394	0.047	0.984	0.059
Mean¶ plasma βHB *post partum*	0.75	0.50	0.74	0.098	0.581	<0.001	0.500
Mean¶ plasma urea *post partum*	8.11	7.31	6.72	0.542	0.023	0.278	0.065
Effect of post partum diet (mmol/l)							
N	10	20	30				
Mean¶ plasma βHB *post partum*	0.80	0.55	0.70	0.096	0.154	0.017	0.459
Mean¶ plasma urea *post partum*	8.27	7.93	7.20	0.516	0.121	0.416	0.097

C_1 = Algae vs Megalac; C_2 = Algae vs Linseed; C_3 = Linseed vs Megalac
* Mean value = average of all measured values *pre-partum*
¶ Mean value = average of all measured values *post partum*

4.4.2.4. Antioxidant status

The mean activity of GPx in erythrocytes *pre-partum* was unaffected by dietary strategy or dietary fat source (Table 4.12). Furthermore, there was no effect of dietary strategy or dietary fat source upon the mean activity of erythrocyte GPx during lactation. Supplementing ewes with any of the six dietary strategies had no significant effect upon the concentration of CK in serum at any time point when data were analysed as a six

treatment randomised block design (Table 4.12). Moreover, there was no effect of *pre-partum* or *post partum* dietary fat source upon serum CK concentrations.

Table 4.12. Effect of supplementing the diets of ewes with various fat sources on indicators of selenium status and of cellular damage

	Diet						s.e.d.	P
	AA	AL	AM	LL	LM	MM		
Pre-partum:								
Mean* erythrocyte GPx activity (U/ml PCV)	115	113	102	96	88	105	14.4	0.421
Mean* serum CK activity (U/l)	148	123	122	119	138	157	33.9	0.829
Post partum:								
Mean¶ erythrocyte GPx activity (U/ml PCV)	349	326	273	259	290	280	38.4	0.187
Mean¶ serum CK activity (U/l)	212	197	163	196	172	202	32.9	0.658

AA = Algae diet fed throughout pregnancy and lactation; AL= Algae diet fed during pregnancy followed by Linseed diet; AM = Algae diet fed during pregnancy followed by Megalac diet in lactation; LL = Linseed diet fed throughout pregnancy and lactation; LM = Linseed diet fed during pregnancy followed by Megalac diet in lactation; MM = Megalac diet fed throughout pregnancy and lactation

* Mean value = average of all measured values *pre-partum*

¶ Mean value = average of all measured values *post partum*

Table 4.13. Effect of supplementing the diets of ewes with various fat sources on the proportions of individual fatty acids in plasma samples collected at six weeks¶ pre-partum (pre-treatment)

	Diet						s.e.d.	P
Fatty acid (g/100 g fatty acids)	AA	AL	AM	LL	LM	MM		
C16:0	18.6	18.3	18.6	18.4	18.5	17.8	0.51	0.676
C16:1*n*-7	1.48	0.84	0.92	0.86	0.88	1.07	0.272	0.174
C18:0	24.2	24.5	23.5	24.5	24.0	23.8	0.69	0.662
C18:1 *trans*	3.52	3.70	4.04	3.56	4.12	4.04	0.324	0.261
C18:1*n*-9 *cis*	21.1	20.3	20.7	21.3	20.8	21.1	0.84	0.890
C18:2*n*-6 *cis*	7.92	8.05	8.56	8.06	8.25	8.01	0.564	0.883
CLA (*cis*-9,*trans*-11)	0.07	0.03	0.14	0.01	0.12	0.10	0.056	0.178
C18:3*n*-3 *cis*	2.69	2.79	2.73	2.42	2.55	2.89	0.168	0.096
C20:4*n*-6	2.74	3.19	2.67	2.77	2.77	2.68	0.274	0.471
C20:5*n*-3	2.00	2.37	2.24	2.08	1.92	2.23	0.255	0.503
C22:6*n*-3	1.81	2.16	1.79	1.84	1.59	1.96	0.269	0.435
RFA§	13.9	13.8	14.2	14.4	14.5	14.3	0.60	0.804
Total fatty acids (mg/ml)	1.04	1.02	1.12	1.07	1.08	1.14	0.075	0.612

AA = Algae diet fed throughout pregnancy and lactation; AL= Algae diet fed during pregnancy followed by Linseed diet; AM = Algae diet fed during pregnancy followed by Megalac diet in lactation; LL = Linseed diet fed throughout pregnancy and lactation; LM = Linseed diet fed during pregnancy followed by Megalac diet in lactation; MM = Megalac diet fed throughout pregnancy and lactation

¶ six weeks *pre-partum* = day 103 of gestation

§ RFA = All remaining fatty acids

3.4.2.5. Plasma fatty acids (six weeks *pre-partum*)

No significant differences in the proportions of individual or total fatty acids within plasma were seen among any of the six dietary strategies at six weeks *pre-partum* (Table 4.13).

4.4.2.6. Plasma fatty acids (one week *pre-partum*)

The proportional contributions of each fatty acid to 100 g of plasma fatty acids are presented in Table 4.14, and the effects of *pre-* and *post partum* dietary fat sources upon proportions of notable plasma fatty acids within Table 4.15. The total concentration of fatty acids within ewe plasma at one week *pre-partum* was significantly higher in ewes fed strategies LL or MM compared to the other four dietary strategies. When data were analysed according to the fat source fed pre- partum, ewes fed algae had the lowest total fatty acid concentrations within plasma followed by linseed, then Megalac.

Table 4.14. Effect of supplementing the diets of ewes with various fat sources on the proportions of individual fatty acids in plasma samples collected at one week[¶] pre-partum

	Diet						s.e.d.	P
Fatty acid (g/100 g fatty acids)	**AA**	**AL**	**AM**	**LL**	**LM**	**MM**		
C16:0	22.8^{b}	23.3^{bc}	24.6^{c}	12.8^{a}	13.0^{a}	24.4^{bc}	0.85	<0.001
C16:1*n*-7	0.68^{b}	0.72^{b}	0.66^{b}	0.56^{a}	0.65^{a}	0.56^{a}	0.043	0.003
C18:0	13.5^{a}	13.6^{a}	12.5^{a}	30.6^{c}	27.5^{c}	22.4^{b}	2.12	<0.001
C18:1 *trans*	0.90^{bc}	1.05^{c}	0.96^{c}	0.53^{a}	0.68^{ab}	0.57^{a}	0.133	<0.001
C18:1*n*-9 *cis*	10.8^{ab}	10.5^{a}	9.95^{a}	13.5^{c}	12.3^{bc}	15.6^{d}	0.81	<0.001
C18:2*n*-6 *cis*	13.0^{a}	13.2^{a}	13.4^{a}	11.5^{a}	13.4^{a}	19.5^{b}	1.20	<0.001
CLA (*cis*-9,*trans*-11)	ND	ND	0.06	0.11	0.06	ND	0.050	0.190
C18:3*n*-3 *cis*	0.89^{a}	0.90^{a}	0.83^{a}	5.11^{b}	4.56^{b}	0.92^{a}	0.751	<0.001
C20:4*n*-6	6.28^{b}	6.36^{b}	5.93^{b}	2.14^{a}	2.12^{a}	2.84^{a}	0.421	<0.001
C20:5*n*-3	2.93^{d}	2.74^{cd}	2.75^{cd}	2.21^{b}	2.30^{bc}	1.03^{a}	0.237	<0.001
C22:6*n*-3	5.49^{c}	4.91^{b}	4.78^{b}	1.35^{a}	1.33^{a}	1.06^{a}	0.276	<0.001
RFA[§]	22.8^{b}	22.7^{b}	23.6^{b}	19.6^{b}	22.2^{b}	11.2^{a}	2.36	<0.001
Total fatty acids (mg/ml)	0.876^{a}	0.914^{a}	0.844^{a}	1.28^{b}	0.951^{a}	1.31^{b}	0.1027	<0.001

AA = Algae diet fed throughout pregnancy and lactation; AL= Algae diet fed during pregnancy followed by Linseed diet; AM = Algae diet fed during pregnancy followed by Megalac diet in lactation; LL = Linseed diet fed throughout pregnancy and lactation; LM = Linseed diet fed during pregnancy followed by Megalac diet in lactation; MM = Megalac diet fed throughout pregnancy and lactation

[¶] one week *pre-partum* = day 138 of gestation

[§] RFA = All remaining fatty acids

Means without common superscripts are significantly different at the P<0.05 level

Table 4.15. Effect of supplementing the diets of ewes with various fat sources pre-partum on plasma fatty acid proportions at one week[1] pre-partum

Fatty acid (g/100 g fatty acids)	Diet			s.e.d.	P		
	Algae	Linseed	Megalac		C_1	C_2	C_3
Number per treatment	30	20	10				
C18:0	13.2	29.0	22.4	1.64	<0.001	<0.001	0.098
C18:1*n*-9 *cis*	10.4	12.9	15.6	0.63	<0.001	0.002	<0.001
C18:2*n*-6 *cis*	13.2	12.4	19.5	0.93	<0.001	0.003	<0.001
C18:3*n*-3 *cis*	0.87	4.84	0.92	0.564	0.936	<0.001	0.012
C20:4*n*-6	6.19	2.13	2.84	0.319	<0.001	<0.001	<0.001
C20:5*n*-3	2.81	2.26	1.03	0.179	<0.001	0.472	<0.001
C22:6*n*-3	5.06	1.34	1.03	0.227	<0.001	<0.001	<0.001
Total fatty acids (mg/ml)	0.878	1.12	1.31	0.087	<0.001	0.048	<0.001

C_1 = Algae vs Megalac; C_2 = Algae vs Linseed; C_3 = Linseed vs Megalac
[1] one week *pre-partum* = day 138 of gestation

The proportion of C16:0 in plasma at one week *pre-partum* was significantly reduced in ewes fed LL or LM when compared to the other four strategies. One of the principal saturated fatty acids found in ewe plasma, C18:0, was lowest in ewes fed strategies AA, AL or AM and highest in ewes offered strategies LL or LM (P<0.001). Analysing the data according to the fat source fed during pregnancy revealed reduced proportions of C18:0 in ewes fed algae compared to linseed (P<0.001) or Megalac (P<0.001) and in ewes offered linseed compared to Megalac (P=0.098).

The amount of C16:1*n*-7 in plasma at one week *pre-partum* was significantly lower in ewes supplemented with strategies LL, LM or MM. The predominant monoenoic fatty acid found within plasma, C18:1*n*-9 *cis*, was significantly reduced by the provision of strategies AA, AL or AM to ewes, intermediate in ewes offered strategies LL or LM and highest in ewes consuming strategy MM at one week *pre-partum*. When data were analysed according to the fat source fed *pre-partum*, significant differences in the mean proportions of C18:1*n*-9 *cis* found in plasma were found among treatments in the order algae < linseed < Megalac. The proportional contribution of C18:1 *trans* to plasma fatty acids was increased in ewes offered strategies containing algae (AA, AL, AM; P<0.001) compared to the other three dietary strategies. No significant differences in the proportion

of CLA within plasma fatty acids were observed among dietary strategies at one week *pre-partum.*

The proportion of C18:2*n*-6 *cis* within plasma was similar in ewes fed diets containing algae or linseed *pre-partum.* By contrast, it was increased in ewes offered the Megalac diet, whether data were analysed as a randomised block design ($P<0.001$) or by orthogonal contrast ($P<0.001$; $P<0.001$). Ewes offered diets containing algae had higher proportions of C20:4*n*-6 within plasma lipids than ewes fed the other three strategies ($P<0.001$), linseed ($P<0.001$) or Megalac ($P<0.001$) at one week *pre-partum.* Moreover, ewes fed linseed during pregnancy had a lower proportional contribution of C20:4*n*-6 to total plasma fatty acids than those offered the Megalac diet ($P<0.001$).

Ewes fed diets containing linseed during pregnancy had significantly higher amounts of C18:3*n*-3 within plasma at one week *pre-partum* compared to those fed diets based on algae or Megalac. Regardless of the method of statistical analysis, the proportions of C20:5*n*-3 were similar in plasma from ewes fed diets containing algae or linseed but were significantly reduced in ewes fed Megalac. The amount of C22:6*n*-3 in plasma was increased as a consequence of algal supplementation: proportions of this fatty acid were significantly higher in ewes fed strategies AA, AL or AM whether data were analysed as a randomised block design or orthogonal contrasts employed. Furthermore, feeding linseed to the pregnant ewe significantly increased the proportion of DHA within plasma compared to feeding Megalac.

4.4.2.7. Gestation length

Dietary fat source had no significant effect upon ewe gestation length, regardless of the method by which data were analysed (Figure 4.2).

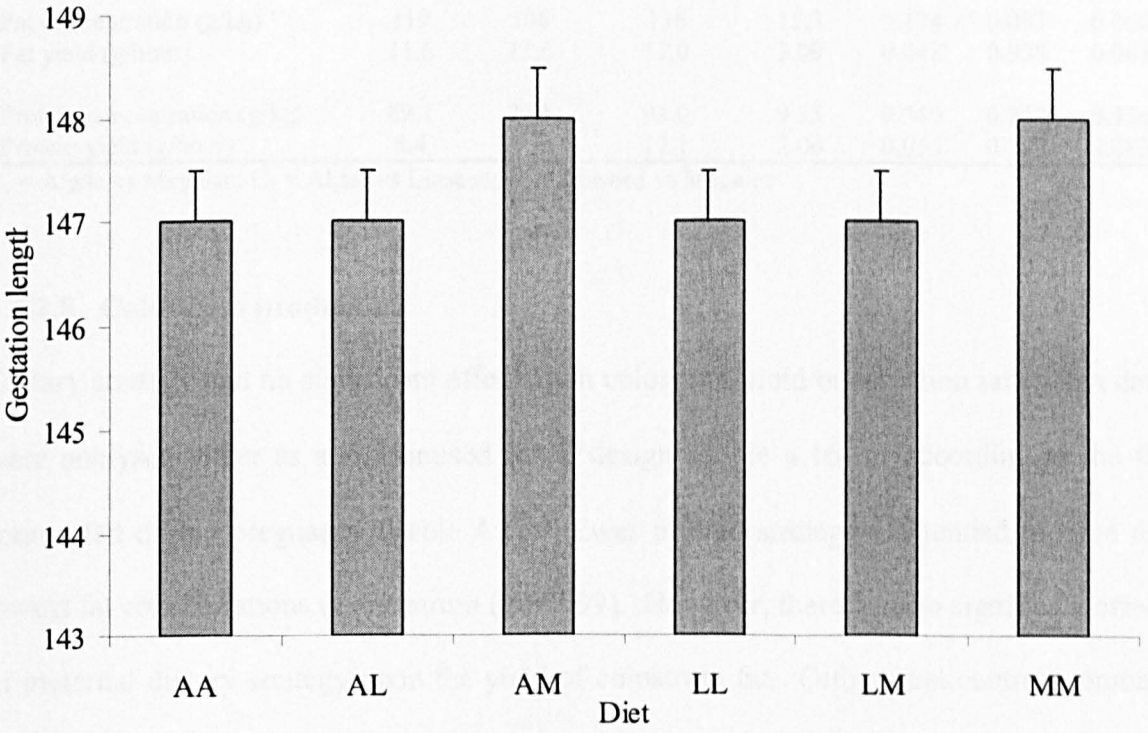


Figure 4.2. Effect of supplementing the diets of ewes with various fat sources on gestation length

Table 4.16. Effect of supplementing the diets of ewes with various fat sources on colostrum parameters

	Diet						s.e.d.	P
	AA	AL	AM	LL	LM	MM		
Secretion rate (ml/hour)	98.1	104	107	109	112	121	20.4	0.909
Yield (l/day)	2.36	2.48	2.56	2.61	2.68	2.91	0.490	0.909
Fat concentration (g/kg)	104	130	124	97.2	117	136	15.0	0.099
Fat yield (g/hour)	8.5	14.1	12.0	11.9	14.7	17.8	3.75	0.244
Protein concentration (g/kg)	83.9	88.5	88.5	77.1	83.5	93.0	11.98	0.831
Protein yield (g/hour)	6.25	9.14	8.79	8.70	10.4	12.1	2.523	0.329

AA = Algae diet fed throughout pregnancy and lactation; AL= Algae diet fed during pregnancy followed by Linseed diet; AM = Algae diet fed during pregnancy followed by Megalac diet in lactation; LL = Linseed diet fed throughout pregnancy and lactation; LM = Linseed diet fed during pregnancy followed by Megalac diet in lactation; MM = Megalac diet fed throughout pregnancy and lactation

Table 4.17. Effect of supplementing the diets of ewes with various fat sources pre-partum on colostrum parameters

	Diet			s.e.d.	P		
	Algae	Linseed	Megalac		C_1	C_2	C_3
Number per treatment	30	20	10				
Secretion rate (ml/hour)	102	110	120	15.9	0.267	0.814	0.327
Yield (l/day)	2.45	2.65	2.88	0.381	0.267	0.814	0.327
Fat concentration (g/kg)	119	106	136	12.3	0.174	0.087	0.069
Fat yield (g/hour)	11.6	13.4	17.0	3.09	0.048	0.938	0.063
Protein concentration (g/kg)	89.1	79.1	93.0	9.35	0.540	0.272	0.354
Protein yield (g/hour)	8.4	9.6	12.1	2.06	0.056	0.769	0.083

C_1 = Algae vs Megalac; C_2 = Algae vs Linseed; C_3 = Linseed vs Megalac

4.4.2.8. Colostrum production

Dietary strategy had no significant effect upon colostrum yield or secretion rate when data were analysed either as a randomised block design (Table 4.16) or according to the fat source fed during pregnancy (Table 4.17). Ewes offered strategy LL tended to have the lowest fat concentrations in colostrum (P=0.099). However, there was no significant effect of maternal dietary strategy upon the yield of colostrum fat. Orthogonal contrasts among the three fat sources revealed no significant differences between treatments in colostrum fat concentrations although fat yields were significantly lower for ewes fed algae compared to those fed Megalac (P=0.048). Ewes supplemented with linseed *pre-partum* also tended to have lower colostrum fat yields than ewes fed Megalac (P=0.063). There was no significant effect of dietary strategy or *pre-partum* maternal dietary fat source upon the concentration or yield of colostrum protein. Nonetheless, ewes offered Megalac *pre-partum* tended to have higher yields of colostrum protein compared to ewes fed either algae (P=0.056) or linseed (P=0.083).

4.4.2.9. Plasma fatty acids (three weeks *post partum*)

The total fatty acid concentration within ewe plasma at three weeks *post partum* was not significantly altered by dietary strategy or fat source fed either *pre-* or *post* partum (Tables 4.18 and 4.19). The addition of linseed to lactation diets (AL, LL) significantly reduced

the proportion of C16:0 within ewe plasma compared to the other four dietary strategies. By contrast, ewes supplemented with linseed during lactation had the highest proportions of C18:0 within plasma ($P<0.001$). Furthermore, ewes fed strategy AA had lower proportions of C18:0 within plasma than any of the other five dietary strategies ($P<0.001$). This pattern (algae < Megalac < linseed) was also observed when data were analysed according to the fat source fed during lactation.

Table 4.18. Effect of supplementing the diets of ewes with various fat sources on the proportions of individual fatty acids in plasma samples collected at three weeks[¶] post partum

	Diet						s.e.d.	P
Fatty acid (g/100 g fatty acids)	**AA**	**AL**	**AM**	**LL**	**LM**	**MM**		
C16:0	16.1^{b}	12.0^{a}	19.3^{c}	12.6^{a}	16.4^{b}	16.2^{b}	0.91	<0.001
C16:1*n*-7	0.08^{a}	0.38^{c}	0.32^{bc}	0.36^{c}	0.23^{b}	0.30^{bc}	0.062	<0.001
C18:0	12.4^{a}	22.6^{d}	19.8^{c}	22.8^{d}	17.3^{b}	17.2^{b}	1.142	<0.001
C18:1 *trans*	5.26^{b}	3.67^{a}	2.93^{a}	2.74^{a}	2.51^{a}	2.50^{a}	0.618	0.001
C18:1*n*-9 *cis*	9.10^{a}	12.1^{b}	13.9^{c}	13.4^{bc}	13.8^{bc}	12.9^{bc}	0.806	<0.001
C18:2*n*-6 *cis*	11.6^{a}	11.1^{a}	16.6^{b}	11.9^{a}	15.5^{b}	15.5^{b}	0.97	<0.001
CLA (*cis*-9,*trans*-11)	0.01	0.04	0.15	ND	ND	0.02	0.050	0.059
C18:3*n*-3 *cis*	0.82^{a}	4.04^{b}	1.12^{a}	3.49^{b}	1.19^{a}	1.19^{a}	0.347	<0.001
C20:4*n*-6	5.44^{c}	2.83^{b}	2.79^{b}	2.15^{a}	2.50^{ab}	2.11^{a}	0.292	<0.001
C20:5*n*-3	2.84^{d}	2.49^{cd}	1.59^{ab}	2.82^{d}	1.83^{bc}	1.04^{a}	0.371	<0.001
C22:6*n*-3	3.37^{c}	2.49^{b}	2.00^{b}	0.74^{a}	0.46^{a}	1.06^{a}	0.354	<0.001
RFA[§]	33.1^{c}	26.3^{ab}	19.6^{a}	27.1^{bc}	28.3^{bc}	30.0^{bc}	3.27	0.011
Total fatty acids (mg/g)	1.152	1.176	1.375	1.222	1.200	1.326	0.122	0.413

AA = Algae diet fed throughout pregnancy and lactation; AL= Algae diet fed during pregnancy followed by Linseed diet; AM = Algae diet fed during pregnancy followed by Megalac diet in lactation; LL = Linseed diet fed throughout pregnancy and lactation; LM = Linseed diet fed during pregnancy followed by Megalac diet in lactation; MM = Megalac diet fed throughout pregnancy and lactation

[¶] three weekz *post partum* = day 21 of lactation

[§] RFA = All remaining fatty acids; ND = not detected

Means without common superscripts are significantly different at the $P<0.05$ level

The fat source supplied to ewes *pre-partum* had no significant effect upon the proportion of plasma C18:0 at three weeks *post partum*, although there was a tendency ($P=0.055$) for ewes fed Megalac during pregnancy to have a lower proportion of C18:0 within plasma lipids than ewes fed linseed during pregnancy.

The proportion of C16:1*n*-7 within plasma lipids was significantly reduced in ewes fed strategy AA. By contrast, the proportion of C18:1 *trans* was significantly increased by the

supplementation of ewes with strategy AA when compared to the other five dietary strategies. The lowest proportional contribution of C18:1*n*-9 *cis* within ewe plasma was also conferred by feeding strategy AA (P<0.001). Supplementing ewes with algae during pregnancy had significant carry-over effects into lactation in that the proportion of C18:1*n*-9 *cis* within the plasma lipid fraction was reduced in these ewes when compared to those fed either linseed (P=0.005) or Megalac (P=0.078) *pre-partum*. A similar pattern was observed when data were analysed according to the fat source fed during lactation, with lowest values observed in ewes fed algae, intermediate for linseed and highest in those fed Megalac. Ewes fed either algae (P<0.001) or linseed (P=0.020) during lactation had lower proportions of C18:1*n*-9 *cis* in plasma compared to ewes offered Megalac.

Table 4.19. Effect of supplementing the diets of ewes with various fat sources pre- and post partum on plasma fatty acid proportions at three weeks[¶] post partum

Fatty acid (g/100 g fatty acids)	Diet			s.e.d.	P		
	Algae	Linseed	Megalac		C_1	C_2	C_3
Effect of pre-partum diet							
Number per treatment	30	20	10				
C18:0	18.3	19.8	17.5	2.32	0.268	0.456	0.055
C18:1*n*-9 *cis*	11.8	13.5	13.3	1.13	0.078	0.005	0.454
C18:2*n*-6 *cis*	12.9	13.7	15.1	1.51	0.006	0.971	0.009
C18:3*n*-3 *cis*	2.02	2.23	0.93	0.802	0.010	0.176	0.002
C20:4*n*-6	3.63	2.36	2.18	0.591	<0.001	<0.001	0.351
C20:5*n*-3	2.29	2.26	1.21	0.448	<0.001	0.150	<0.001
C22:6*n*-3	2.59	0.60	0.85	0.414	<0.001	<0.001	0.110
Total fatty acids (mg/g)	1.22	1.21	1.27	0.124	0.371	0.542	0.299
Effect of post partum diet							
Number per treatment	10	20	30				
C18:0	12.1	22.8	17.9	1.06	<0.001	<0.001	0.092
C18:1*n*-9 *cis*	9.15	12.8	13.7	0.730	<0.001	0.543	0.020
C18:2*n*-6 *cis*	11.3	11.4	15.7	0.86	<0.001	0.785	<0.001
C18:3*n*-3 *cis*	0.80	3.76	1.04	0.307	0.228	<0.001	<0.001
C20:4*n*-6	5.40	2.54	2.53	0.283	<0.001	<0.001	0.943
C20:5*n*-3	2.88	2.69	1.50	0.337	<0.001	0.656	<0.001
C22:6*n*-3	3.37	1.65	1.10	0.472	<0.001	0.002	0.243
Total fatty acids (mg/g)	1.12	1.19	1.28	0.108	0.152	0.402	0.112

C_1 = Algae vs Megalac; C_2 = Algae vs Linseed; C_3 = Linseed vs Megalac

[¶] three weekz *post partum* = day 21 of lactation

Negligible quantities of CLA (*cis-9,trans-11*) were present within ewe plasma at three weeks into lactation and was undetectable in samples from ewes fed strategies LL or LM. However, of the remaining results, ewes fed strategy AM had the highest proportion of CLA in plasma lipids, a difference that tended towards significance (P=0.059).

Feeding Megalac during lactation increased the proportion of C18:2*n*-6 within ewe plasma, the highest values being recorded in ewes fed strategies AM, LM or MM compared to either the other three dietary strategies. A similar pattern was seen when data were analysed according to the fat source fed *pre-* or *post partum* with Megalac supplementation significantly increasing the proportion of C18:2*n*-6 within plasma compared to feeding either algae or linseed. Ewes fed strategy AA had the highest proportion of C20:4*n*-6 within plasma at three weeks *post partum* when compared to the other five dietary strategies (P<0.001). Furthermore, ewes supplemented with algae during pregnancy or lactation had significantly higher proportions of C20:4*n*-6 within plasma at three weeks *post partum* than those fed either linseed or Megalac.

Adding linseed to the lactation diet resulted in a significant increase in the contribution of C18:3*n*-3 to total plasma fatty acids at three weeks *post partum* when data were analysed either as a randomised block design or by orthogonal contrasts. Carry-over effects of algae (P=0.010) and linseed (P=0.002) supplementation during pregnancy also increased the plasma C18:3*n*-3 at three weeks *post partum* when compared to supplementation with Megalac. The highest proportions of C20:5*n*-3 within plasma lipids were observed in ewes fed strategies AA, AL or LL at three weeks *post partum* (P<0.001). Feeding either algae or linseed *pre-partum* had a significant carry-over effect upon the proportional contribution of C20:5*n*-3 to plasma fatty acids at three weeks *post partum* when compared to feeding Megalac. Furthermore, the same pattern was observed when data were analysed according to the fat source supplied *post partum*, with highest values seen in ewes fed algae

(P<0.001) or linseed (P<0.001) compared to Megalac. The amount of C22:6*n*-3 within ewe plasma at three weeks into lactation was highest in ewes offered strategy AA, followed by strategies AL or AM, and the lowest values found in ewes fed strategies LL, LM or MM (P<0.001). Supplementing either the pregnant or lactating ewe with algae conferred significantly elevated proportions of C22:6*n*-3 within plasma lipids at three weeks *post partum*.

4.4.2.10. Milk production

Milk secretion rate and yield were increased by the provision of preformed long-chain PUFAs in the form of algae to the pregnant ewe, with those ewes offered strategies AL or AM having significantly higher yields than those fed strategies, LL, LM or MM (P=0.018; Table 4.20). Increases in milk secretion rate and yield were also observed when ewes offered diets containing algae during pregnancy were compared to those fed linseed (P=0.015) or Megalac (P=0.010; Table 4.21). By contrast, the fat source offered to ewes during lactation had no significant effect upon milk secretion rate or yield.

Table 4.20. Effect of supplementing the diets of ewes with various fat sources on milk parameters at four weeks[¶] post partum

	Diet						s.e.d.	P
	AA	AL	AM	LL	LM	MM		
Secretion rate (ml/hour)	109^{ab}	120^{b}	126^{b}	92.6^{a}	92.6^{a}	91.3^{a}	12.26	0.018
Yield (l/day)	2.62^{ab}	2.89^{b}	3.03^{b}	2.22^{a}	2.22^{a}	2.19^{a}	0.294	0.018
Fat concentration (g/kg)	65.2^{a}	99.2^{cd}	92.8^{bc}	78.9^{ab}	103^{cd}	113^{d}	9.24	<0.001
Fat yield (g/hour)	7.18^{a}	11.9^{b}	11.6^{b}	7.35^{a}	9.64^{ab}	10.4^{ab}	1.617	0.021
Protein concentration (g/kg)	35.4	38.6	38.5	37.5	38.8	41.0	2.00	0.173
Protein yield (g/hour)	3.95	4.61	4.80	3.47	3.61	3.76	0.526	0.083
Lactose concentration (g/kg)	47.1	48.4	47.7	48.6	47.4	45.5	1.19	0.162
Lactose yield (g/hour)	5.26^{ab}	5.84^{b}	6.06^{b}	4.49^{a}	4.50^{a}	4.16^{a}	0.631	0.021

AA = Algae diet fed throughout pregnancy and lactation; AL= Algae diet fed during pregnancy followed by Linseed diet; AM = Algae diet fed during pregnancy followed by Megalac diet in lactation; LL = Linseed diet fed throughout pregnancy and lactation; LM = Linseed diet fed during pregnancy followed by Megalac diet in lactation; MM = Megalac diet fed throughout pregnancy and lactation

Means without common superscripts are significantly different at the P<0.05 level

[¶] four weekz *post partum* = day 28 of lactation

The milk fat concentration was lower in ewes fed strategy AA when compared to the other five strategies (P<0.001). Analysing data according to the fat source offered to the ewe elucidated significant reductions in milk fat concentration for ewes fed diets containing algae or linseed compared to Megalac during either pregnancy or lactation. Furthermore, fat yields were lowest for ewes offered strategies AA or LL but highest in those fed strategies AL or AM (P=0.021) when data were analysed as a six-treatment randomised block design. No significant effect of fat source fed during pregnancy was observed upon milk fat yields, however, ewes offered diets containing algae *post partum* had lower fat yields than those fed diets containing Megalac *post partum* (P=0.017).

Table 4.21. Effect of supplementing the diets of ewes with various fat sources pre- and post partum on milk parameters at four weeks¶ post partum

	Diet			s.e.d.	P		
	Algae	Linseed	Megalac		C_1	C_2	C_3
Effect of pre-partum diet							
Number per treatment	30	20	10				
Secretion rate (ml/hour)	118	94.3	95.1	11.32	0.010	0.015	0.834
Yield (l/day)	2.84	2.26	2.28	0.272	0.010	0.015	0.834
Fat concentration (g/kg)	88.4	89.5	113	10.38	<0.001	0.794	0.001
Fat yield (g/hour)	10.5	8.5	10.2	1.67	0.907	0.087	0.507
Protein concentration (g/kg)	37.9	38.0	40.1	1.90	0.041	0.849	0.045
Protein yield (g/hour)	4.48	3.60	3.81	0.495	0.116	0.028	0.427
Lactose concentration (g/kg)	47.7	48.3	46.1	1.12	0.033	0.255	0.019
Lactose yield (g/hour)	5.71	4.59	4.40	0.586	0.005	0.038	0.365
Effect of post partum diet							
Number per treatment	10	20	30				
Secretion rate (ml/hour)	110	106	107	12.0	0.559	0.834	0.575
Yield (l/day)	2.65	2.53	2.57	0.289	0.559	0.834	0.575
Fat concentration (g/kg)	65.2	88.5	99.8	8.96	<0.001	0.005	<0.001
Fat yield (g/hour)	7.8	9.5	10.6	1.57	0.017	0.934	0.074
Protein concentration (g/kg)	35.9	38.2	39.0	1.78	0.019	0.766	0.060
Protein yield (g/hour)	4.03	4.02	4.16	0.518	0.811	0.984	0.877
Lactose concentration (g/kg)	47.2	48.4	47.4	1.08	0.824	0.039	0.102
Lactose yield (g/hour)	5.31	5.11	5.15	0.644	0.499	0.666	0.434

C_1 = Algae vs Megalac; C_2 = Algae vs Linseed; C_3 = Linseed vs Megalac

¶ four weekz *post partum* = day 28 of lactation

No significant effect of dietary strategy was observed upon protein concentrations in milk. Milk protein concentrations were lower in ewes offered diets containing algae or linseed *pre-partum* compared to those fed Megalac (P=0.041; P=0.045 respectively). Feeding diets containing algae *post partum* also reduced the protein concentration of milk (P=0.019). Milk protein yields tended to be higher in ewes fed strategies AL or AM. Ewes fed algae *pre-partum* had significantly higher protein yields than those fed linseed but there was no effect of fat source offered *post partum* upon this parameter. Analysing data as a six-treatment randomised block design revealed no significant effects of dietary strategy upon milk lactose concentrations. By contrast, lactose concentrations were higher in ewes offered diets containing algae (P=0.033) or linseed (P=0.019) *pre-partum* compared to Megalac. However, ewes fed linseed *post partum* had significantly higher lactose concentrations than those fed algae during lactation. Ewes fed dietary strategies AA, AL or AM had the highest mean lactose yields, this difference was significant as a difference among both dietary strategies and *pre-partum* fat sources. Nonetheless, there was no significant effect upon milk lactose yields of supplementing ewes with any of the three fat sources during lactation.

Table 4.22. Effect of supplementing the diets of ewes with various fat sources on maternal behaviour scores and latencies of maternal and neonatal lamb behaviours

	Diet						s.e.d.	P
	AA	AL	AM	LL	LM	MM		
Maternal measurements:								
Maternal behaviour score	121[a]	125[a]	134[b]	128[ab]	122[a]	121[a]	3.9	0.008
Latency of standing (sec)	119	16.2	99.6	153	33.0	82.8	61.38	0.217
Latency of vocalization (sec)	161	167	140	94.8	68.4	154	64.02	0.385
Latency of contact with the lamb (sec)	125	38	143	93.0	69.0	138	58.80	0.562
Neonatal measurements:								
Latency of standing (min)	19.0	15.5	14.6	17.8	16.5	12.0	2.63	0.123
Latency of searching for the udder (min)	29.9	21.6	18.0	23.6	23.5	24.7	4.48	0.195
Latency of successful suckling (min)	53.3	44.4	65.9	53.2	52.7	80.6	14.02	0.145

AA = Algae diet fed throughout pregnancy and lactation; AL= Algae diet fed during pregnancy followed by Linseed diet; AM = Algae diet fed during pregnancy followed by Megalac diet in lactation; LL = Linseed diet fed throughout pregnancy and lactation; LM = Linseed diet fed during pregnancy followed by Megalac diet in lactation; MM = Megalac diet fed throughout pregnancy and lactation
Means without common superscripts are significantly different at the P<0.05 level

4.4.3. Lamb performance parameters

4.4.3.1. Neonatal lamb behaviour

Ewes offered dietary strategy AM had significantly higher maternal behaviour scores immediately *post partum* than those ewes offered any of the other five dietary strategies; nevertheless, all other parameters relating to maternal behaviour were similar among dietary strategies (Table 4.22). Furthermore, no significant effects of maternal supplemental fat source were observed upon maternal behaviours (Table 4.23).

Table 4.23. Effect of supplementing the diets of ewes with various fat sources pre-partum on maternal and neonatal behaviours at and immediately after parturition

	Diet			s.e.d.	P		
	Algae	Linseed	Megalac		C_1	C_2	C_3
Number per treatment	30	20	10				
Maternal measurements:							
Maternal behaviour score	127	124	122	4.0	0.098	0.916	0.124
Latency of standing (sec)	1.26	1.46	1.26	0.829	0.934	0.717	0.971
Latency of vocalisation (sec)	2.45	1.52	2.58	0.874	0.382	0.386	0.270
Latency of contact with the lamb (sec)	1.66	1.35	2.40	0.768	0.953	0.062	0.590
Neonatal measurements:							
Latency of standing (min)	16.3	17.1	12.0	2.09	0.044	0.247	0.023
Latency of searching for the udder (min)	23.1	23.6	25.0	3.61	0.682	0.988	0.701
Latency of successful suckling (min)	54.9	53.0	80.9	11.36	0.025	0.350	0.016

C_1 = Algae vs Megalac; C_2 = Algae vs Linseed; C_3 = Linseed vs Megalac

Neonatal lamb behaviours were not significantly affected by maternal dietary strategy. By contrast, analysing data according to the fat source offered to the pregnant ewe revealed significantly lower latencies of lamb standing for Megalac compared to either algae or linseed. There was no significant effect of fat source offered to the dam upon the latency of lamb searching behaviour. By contrast, lambs produced by ewes offered diets containing algae (P=0.025) or linseed (P=0.016) during pregnancy had a lower latency of successful suckling compared to lambs from ewes fed Megalac.

4.4.3.2. Neonatal lamb plasma fatty acids

The total fatty acid concentration in neonatal lamb plasma was approximately two-fold higher in lambs from ewes fed strategy LL than any of the other five dietary strategies ($P<0.05$; Table 4.24). Analysing data according to the fat source fed to the ewe during pregnancy revealed a significantly higher concentration in lambs produced by ewes fed linseed compared to algae (Table 4.25). The contributions made by the individual saturated fatty acids C16:0 and C18:0 to total plasma fatty acids were unchanged by both dietary strategy and the maternal dietary fat source.

Table 4.24. Effect of supplementing the diets of ewes with various fat sources on the proportions of individual fatty acids in neonatal lamb plasma

	Diet						s.e.d.	P
Fatty acid (g/100 g fatty acids)	**AA**	**AL**	**AM**	**LL**	**LM**	**MM**		
C16:0	27.3	31.0	31.1	26.2	28.4	32.6	1.95	0.051
C16:1*n*-7	5.99	7.29	8.12	4.82	9.09	7.02	1.920	0.359
C18:0	15.6	13.0	12.5	12.5	11.2	12.5	1.70	0.286
C18:1 *trans*	3.14	3.16	5.09	3.30	5.27	4.15	1.201	0.332
C18:1*n*-9 *cis*	33.1	22.9	16.8	33.0	20.0	19.9	15.69	0.163
C18:2*n*-6 *cis*	0.45	0.62	0.71	2.47	3.35	1.72	1.229	0.194
CLA (*cis*-9,*trans*-11)	ND	ND	ND	ND	ND	ND	-	-
C18:3*n*-3 *cis*	ND	ND	ND	0.54	1.03	ND	-	-
C20:4*n*-6	3.57	5.14	4.09	1.65	4.76	3.47	1.760	0.497
C20:5*n*-3	2.70	6.03	2.58	1.30	2.43	2.60	1.265	0.055
C22:6*n*-3	1.83	2.36	1.81	1.42	1.58	0.62	1.239	0.816
RFA[¶]	6.40	8.60	16.8	12.8	12.9	15.4	4.430	0.238
Total fatty acids (mg/ml)	0.306[a]	0.215[a]	0.268[a]	0.578[b]	0.334[a]	0.207[a]	0.0877	0.016

AA = Algae diet fed throughout pregnancy and lactation; AL= Algae diet fed during pregnancy followed by Linseed diet; AM = Algae diet fed during pregnancy followed by Megalac diet in lactation; LL = Linseed diet fed throughout pregnancy and lactation; LM = Linseed diet fed during pregnancy followed by Megalac diet in lactation; MM = Megalac diet fed throughout pregnancy and lactation

[¶] RFA = All remaining fatty acids; ND = not detected

Means without common superscripts are significantly different at the $P<0.05$ level

There was no significant effect of maternal diet upon the proportions of C16:1*n*-7 or C18:1 *trans* within neonatal plasma samples, or upon the proportions of C18:1*n*-9 *cis* in neonatal plasma. Furthermore, the polyenoic fatty acids known collectively as CLA were undetectable within neonatal plasma samples.

Table 4.25. Effect of supplementing the diets of ewes with various fat sources pre-partum on neonatal lamb plasma fatty acid proportions

Fatty acid (g/100 g fatty acids)	Diet			s.e.d.	P		
	Algae	Linseed	Megalac		C_1	C_2	C_3
Number per treatment	30	20	10				
C18:0	13.7	11.9	12.5	1.39	0.411	0.179	0.729
C18:1*n*-9 *cis*	24.3	26.5	19.9	6.58	0.465	0.458	0.358
C18:2*n*-6 *cis*	0.59	2.91	1.72	0.860	0.288	0.022	0.840
C18:3*n*-3 *cis*	ND	0.78	ND	-	-	-	-
C20:4*n*-6	4.42	3.20	3.47	1.466	0.543	0.409	0.752
C20:5*n*-3	3.77	1.87	2.60	1.230	0.284	0.064	0.686
C22:6*n*-3	2.00	1.50	0.62	0.855	0.203	0.847	0.247
Total fatty acids (mg/ml)	0.263	0.456	0.207	0.0818	0.450	0.003	0.078

C_1 = Algae vs Megalac; C_2 = Algae vs Linseed; C_3 = Linseed vs Megalac
ND = not detected

The proportion of C18:2*n*-6 in plasma was approximately five-fold higher in lambs produced by ewes fed strategies LL or LM; although this difference did not reach statistical significance. However, orthogonal contrasts between linseed and algae revealed an increase in the proportion (P=0.022) of C18:2*n*-6 within plasma fatty acids in lambs produced by ewes fed diets containing linseed. Varying proportions of C20:4*n*-6 were observed amongst treatments although no significant influence of dietary strategy or maternal dietary fat source was observed.

Alpha-linolenic acid was only detectable in samples from ewes fed *pre-partum* diets containing linseed (LL, LM). There was no significant effect of dietary strategy upon the proportions of C20:5*n*-3 or C22:6*n*-3 within neonatal plasma although numerical differences were present, with the highest concentrations of C22:6*n*-3 being seen in lambs born to ewes fed algae or linseed.

4.4.3.3. Neonatal lamb brain fatty acids

The total amount of fatty acids within neonatal lamb brain varied from 20.6 mg/g to 25.5 mg/g, however, this parameter was unaffected by either maternal dietary strategy (Table 4.26) or fat source fed *pre-partum* (Table 4.27). The proportion of C16:0 within lamb

brain tissue was not significantly altered by maternal diet fed during pregnancy. Furthermore, there was no significant effect of either maternal dietary strategy or fat source fed *pre-partum* upon the proportional contribution of C18:0 to total neonatal lamb brain fatty acids.

Table 4.26. Effect of supplementing the diets of ewes with various fat sources on the proportions of individual fatty acids in neonatal lamb brain

	Diet						s.e.d.	P
Fatty acid (g/100 g fatty acids)	**AA**	**AL**	**AM**	**LL**	**LM**	**MM**		
C16:0	20.0	21.3	19.7	19.5	22.1	21.8	1.14	0.149
C16:1*n*-7	0.92^{a}	0.99^{ab}	0.94^{a}	1.02^{ab}	1.14^{c}	1.08^{bc}	0.061	0.035
C18:0	15.7	16.8	15.7	15.8	17.4	16.7	1.01	0.469
C18:1 *trans*	1.15	1.57	2.03	2.41	2.23	0.97	0.704	0.304
C18:1*n*-9 *cis*	13.5	15.4	14.5	14.2	10.7	15.6	2.63	0.496
C18:2*n*-6 *cis*	0.25	0.20	0.19	0.23	0.23	0.17	0.041	0.423
CLA (*cis*-9,*trans*-11)	0.79	1.01	1.12	0.96	1.07	0.95	0.170	0.504
C18:3*n*-3 *cis*	0.32	0.46	0.39	0.40	0.33	0.36	0.103	0.732
C20:4*n*-6	4.20^{abc}	4.55^{c}	3.66^{ab}	3.59^{a}	4.38^{bc}	4.87^{c}	0.338	0.022
C20:5*n*-3	0.75	0.46	0.36	0.66	0.68	0.34	0.259	0.503
C22:6*n*-3	12.1	11.8	12.0	12.5	12.5	12.7	1.03	0.938
RFA[1]	30.4	25.5	29.5	28.7	27.2	24.4	3.22	0.444
Total fatty acids (mg/g)	23.3	25.5	22.0	22.2	20.6	22.9	1.77	0.221

AA = Algae diet fed throughout pregnancy and lactation; AL= Algae diet fed during pregnancy followed by Linseed diet; AM = Algae diet fed during pregnancy followed by Megalac diet in lactation; LL = Linseed diet fed throughout pregnancy and lactation; LM = Linseed diet fed during pregnancy followed by Megalac diet in lactation; MM = Megalac diet fed throughout pregnancy and lactation

[1] RFA = All remaining fatty acids

Means without common superscripts are significantly different at the $P<0.05$ level

Table 4.27. Effect of supplementing the diets of ewes with various fat sources pre-partum on the proportions of individual fatty acids in neonatal lamb brain

	Diet			s.e.d.	P		
Fatty acid (g/100 g fatty acids)	**Algae**	**Linseed**	**Megalac**		C_1	C_2	C_3
Number per treatment							
C18:0	16.1	16.6	16.7	0.81	0.463	0.541	0.612
C18:1*n*-9 *cis*	14.5	12.4	15.6	1.99	0.607	0.181	0.360
C18:2*n*-6 *cis*	0.21	0.23	0.17	0.032	0.276	0.306	0.182
C18:3*n*-3 *cis*	0.39	0.37	0.36	0.078	0.710	0.774	0.793
C20:4*n*-6	4.14	3.99	4.87	0.345	0.025	0.137	0.013
C20:5*n*-3	0.52	0.67	0.34	0.197	0.416	0.253	0.262
C22:6*n*-3	12.0	12.5	12.7	0.71	0.412	0.572	0.545
Total fatty acids (mg/g)	23.6	22.1	22.9	1.51	0.646	0.093	0.890

C_1 = Algae vs Megalac; C_2 = Algae vs Linseed; C_3 = Linseed vs Megalac

The lowest proportional amount of C16:1*n*-7 within total neonatal lamb brain fatty acids was observed in lambs borne by ewes fed strategies AA or AM, and the highest proportion in lambs produced by ewes fed strategy LM (P=0.035). By contrast, maternal dietary strategy had no significant effect upon the proportion of C18:1 *trans* or C18:1*n*-9 nor was there a significant effect of maternal dietary fat source. Mean values for the proportion of CLA (*cis-9,trans-11*) within neonatal lamb brain varied from 0.79 g/100 g fatty acids to 1.12 g/100 g fatty acids but was not significantly different among dietary strategies.

The proportional contribution of C18:2*n*-6 to total neonatal lamb brain fatty acids was unaffected by either maternal dietary strategy or fat source supplied to the ewe during pregnancy. Lambs borne by ewes fed strategies AL or MM during pregnancy had a higher proportion of C20:4*n*-6 within brain tissue than those produced by ewes fed strategies AM or LL (P=0.022). There was also a significant effect of maternal dietary fat source, lambs born to ewes supplemented with Megalac during pregnancy having significantly higher proportions of C20:4*n*-6 within brain tissue than those fed algae or linseed.

The proportion of C18:3*n*-3 in neonatal lamb brain was similar among treatments. Furthermore, although mean values for C20:5*n*-3 and C22:6*n*-3 within lamb brain varied among treatments, there was no significant effect of dietary strategy or fat source upon either of these parameters.

4.4.3.2. Lamb liveweight

Ewes that were fed strategies AA or AM during pregnancy produced lambs that were numerically heavier at birth; however, this difference was not statistically significant (Table 4.28). No significant effects of maternal diet were observed upon lamb liveweights at any time point; although lamb growth rate was lower for ewes fed strategy AA, this

difference did not reach statistical significance. No significant effect of maternal dietary strategy was observed upon litter growth rates.

Table 4.28. Effect of supplementing the diets of ewes with various fat sources on lamb birthweights (kg) and on lamb and litter growth rates (kg/day)

	Diet						s.e.d.	P
	AA	AL	AM	LL	LM	MM		
Lamb liveweight:								
At birth	4.24	4.01	4.24	3.98	4.17	3.92	0.213	0.507
At 1 week of age	6.42	6.33	6.52	5.91	6.25	6.21	0.315	0.498
At 2 weeks of age	8.19	8.39	8.55	7.95	8.13	8.13	0.438	0.789
At 3 weeks of age	10.1	10.5	10.7	10.5	10.0	9.96	0.486	0.585
At 4 weeks of age	11.7	12.4	12.5	12.4	11.9	11.8	0.58	0.515
Lamb growth rate	0.26	0.29	0.29	0.28	0.28	0.29	0.017	0.504
Litter growth rate	0.57	0.58	0.57	0.58	0.54	0.54	0.024	0.310

AA = Algae diet fed throughout pregnancy and lactation; AL= Algae diet fed during pregnancy followed by Linseed diet; AM = Algae diet fed during pregnancy followed by Megalac diet in lactation; LL = Linseed diet fed throughout pregnancy and lactation; LM = Linseed diet fed during pregnancy followed by Megalac diet in lactation; MM = Megalac diet fed throughout pregnancy and lactation

Table 4.29. Effect of supplementing the diets of ewes with various fat sources on indicators of selenium status and of cellular damage in lambs

	Diet						s.e.d.	P
	AA	AL	AM	LL	LM	MM		
Erythrocyte GPx (U/ml PCV):								
12 hours of age	105	102	115	129	95	112	13.0	0.189
1 week of age	132	154	184	201	211	143	48.3	0.548
3 weeks of age	294	315	295	286	292	287	21.5	0.790
Serum CK (U/l):								
12 hours of age	714	696	689	618	708	910	136.7	0.414
1 week of age	219	163	211	170	210	163	46.9	0.667
3 weeks of age	151	176	152	177	145	185	28.1	0.617

AA = Algae diet fed throughout pregnancy and lactation; AL= Algae diet fed during pregnancy followed by Linseed diet; AM = Algae diet fed during pregnancy followed by Megalac diet in lactation; LL = Linseed diet fed throughout pregnancy and lactation; LM = Linseed diet fed during pregnancy followed by Megalac diet in lactation; MM = Megalac diet fed throughout pregnancy and lactation

4.4.3.3. Suckling lamb antioxidant status

The selenium status of suckling lambs, as indicated by GPx activity in erythrocytes increased between 12 hours and one week of age, and again at three weeks of age across all treatments (Table 4.29). However, there was no significant effect of maternal dietary strategy upon the activity of GPx in erythrocytes at any time point. Concentrations of CK

within plasma declined with increasing age but this parameter was unaffected by dietary strategy or fat source at all time points.

4.4.3.4. Suckling lamb plasma fatty acids (12 hours of age)

The total amount of fatty acids within lamb plasma at twelve hours of age was equivalent among treatments, with no significant effect of maternal dietary strategy (Table 4.30) or maternal dietary fat source fed *pre-partum* (Table 4.31). The proportional contribution of C16:0 to lamb plasma fatty acids at twelve hours of age was significantly lower in lambs borne by ewes supplemented with dietary strategies LL or LM compared to the other four dietary strategies. By contrast, lambs suckling ewes fed strategies AA, AL or AM had the lowest, and those fed strategies LL or LM the highest proportion of C18:0 within plasma ($P<0.001$). A similar pattern was observed when data were analysed according to the maternal dietary fat source fed *pre-partum*, with significant differences among all three contrasts analysed.

Table 4.30. Effect of supplementing the diets of ewes with various fat sources on the proportions of individual fatty acids in lamb plasma samples collected at 12 hours of age

	Diet						s.e.d.	P
Fatty acid (g/100 g fatty acids)	**AA**	**AL**	**AM**	**LL**	**LM**	**MM**		
C16:0	22.3^{b}	21.5^{b}	21.9^{b}	14.6^{a}	15.0^{a}	23.7^{b}	1.12	<0.001
C16:1*n*-7	0.87	0.76	0.74	0.81	0.85	0.90	0.083	0.854
C18:0	12.3^{ab}	12.0^{a}	12.7^{ab}	24.4^{c}	26.7^{c}	14.3^{b}	1.14	<0.001
C18:1 *trans*	4.97^{b}	5.03^{b}	4.76^{b}	3.01^{a}	2.85^{a}	2.64^{a}	0.622	0.007
C18:1*n*-9 *cis*	19.5^{a}	21.5^{ab}	20.7^{a}	25.6^{bc}	27.7^{c}	29.5^{c}	2.10	0.009
C18:2*n*-6 *cis*	11.2^{a}	10.9^{a}	10.5^{a}	12.3^{ab}	11.7^{a}	15.4^{b}	1.44	0.012
CLA (*cis*-9,*trans*-11)	0.46	0.33	0.57	0.58	0.41	0.39	0.143	0.674
C18:3*n*-3 *cis*	0.98^{a}	1.06^{a}	1.23^{a}	3.27^{b}	3.54^{b}	1.11^{a}	0.798	<0.001
C20:4*n*-6	6.02^{b}	5.43^{b}	5.89^{b}	2.78^{a}	2.56^{a}	2.99^{a}	1.034	<0.001
C20:5*n*-3	2.35^{b}	2.32^{b}	2.41^{b}	2.23^{b}	2.11^{b}	0.86^{a}	0.564	0.009
C22:6*n*-3	3.40^{b}	3.57^{b}	3.17^{b}	1.02^{a}	1.13^{a}	0.63^{a}	0.789	<0.001
RFA[1]	15.6^{b}	15.5^{b}	15.4^{b}	9.44^{ab}	5.47^{a}	7.60^{a}	3.67	0.007
Total fatty acids (mg/ml)	1.65	1.57	1.72	1.76	1.69	1.84	0.158	0.761

AA = Algae diet fed throughout pregnancy and lactation; AL= Algae diet fed during pregnancy followed by Linseed diet; AM = Algae diet fed during pregnancy followed by Megalac diet in lactation; LL = Linseed diet fed throughout pregnancy and lactation; LM = Linseed diet fed during pregnancy followed by Megalac diet in lactation; MM = Megalac diet fed throughout pregnancy and lactation

[1] RFA = All remaining fatty acids

Means without common superscripts are significantly different at the $P<0.05$ level

Table 4.31. Effect of supplementing the diets of ewes with various fat sources pre-partum on fatty acid proportions in lamb plasma samples collected at 12 hours of age

Fatty acid (g/100 g fatty acids)	Diet			s.e.d.	P		
	Algae	Linseed	Megalac		C_1	C_2	C_3
Number per treatment	30	20	10				
C18:0	12.3	25.6	14.3	0.98	0.032	<0.001	<0.001
C18:1*n*-9 *cis*	20.6	26.6	29.5	1.21	<0.001	<0.001	0.006
C18:2*n*-6 *cis*	10.9	12.0	15.4	1.50	<0.001	0.643	0.008
C18:3*n*-3 *cis*	1.09	3.41	1.11	1.022	0.976	0.003	0.003
C20:4*n*-6	5.78	2.67	2.99	1.139	<0.001	<0.001	0.810
C20:5*n*-3	2.36	2.17	0.86	0.548	0.003	0.772	0.004
C22:6*n*-3	3.38	1.08	0.63	0.876	<0.001	<0.001	0.089
Total fatty acids (mg/ml)	1.65	1.73	1.84	0.145	0.611	0.887	0.850

C_1 = Algae vs Megalac; C_2 = Algae vs Linseed; C_3 = Linseed vs Megalac

Maternal diet had no significant effect upon the proportion of C16:1*n*-7 within lamb plasma at twelve hours of age. By contrast, the amount of C18:1 *trans* within plasma was significantly higher in lambs suckling ewes fed strategies AA, AL or AM compared to the other three strategies. The mean proportion of C18:1*n*-9 *cis* within lamb plasma at twelve hours of age varied from 19.5 g/100 g fatty acids to 29.5 g/100 g fatty acids, the lowest values being observed in lambs borne by ewes fed strategies AA or AM, and the highest in lambs produced by ewes fed strategies LM or MM (P=0.006). When data were analysed according to the maternal dietary fat source fed during pregnancy, lowest proportions of C18:2*n*-6 were seen in lambs suckling ewes fed algae, intermediate for linseed and highest in lambs suckling ewes fed Megalac. There was no significant effect of maternal diet upon the proportion of CLA (*cis-9,trans-11*) within lamb plasma at twelve hours of age.

Lambs produced by ewes fed strategy MM had the highest proportion of C18:2*n*-6 within plasma lipids at twelve hours of age, a mean value that was significantly different from all other treatment means save for strategy LL. Analysing the data according to the maternal dietary fat source fed during pregnancy revealed that lambs suckling ewes fed Megalac had significantly higher proportions of C18:2*n*-6 within plasma than those suckling ewes fed either algae or linseed. Supplementing ewes with strategies AA, AL or AM conferred a

significant increase in the proportion of C20:4*n*-6 within lamb plasma at twelve hours of age compared to supplementation with strategies LL, LM or MM. Moreover, the same pattern was seen when data were analysed by orthogonal contrast, with significant differences within contrasts one (algae and Megalac) and two (algae and linseed).

Adding linseed to the diet of the pregnant ewe (strategies LL and LM) conferred an increase in the proportion of C18:3*n*-3 within plasma of lambs at twelve hours of age when compared to the other four dietary strategies ($P<0.001$). Moreover, analysing the data according to the fat source supplied to the ewe *pre-partum* revealed higher proportions of C18:3*n*-3 in plasma of lambs suckling ewes fed linseed when compared to those fed either algae ($P=0.003$) or Megalac ($P=0.003$). The amount of C20:5*n*-3 within the plasma lipid fraction of lambs at twelve hours of age was lowest in lambs produced by ewes fed strategy MM compared to the other five strategies. There was also a significant effect of maternal dietary fat source: plasma proportions of C20:5*n*-3 were lower in lambs suckling ewes fed Megalac during pregnancy when compared to those fed either algae ($P=0.003$) or linseed ($P=0.004$). The mean proportion of C22:6*n*-3 within lamb plasma at twelve hours of age ranged from 0.63 g/100 g fatty acids for strategy MM to 3.57 g/100 g fatty acid for strategy AL. Feeding dietary strategies AA, AL or AM to pregnant ewes conferred a highly significant increase in C22:6*n*-3 within lamb plasma compared to the other three dietary strategies. The proportion of C22:6*n*-3 within plasma was highest in lambs suckling ewes that had been supplemented with algae during pregnancy compared to linseed ($P<0.001$) or Megalac ($P<0.001$) supplementation. The contrast between linseed and Megalac also tended towards significance ($P=0.089$).

Table 4.32. Effect of supplementing the diets of ewes with various fat sources on the proportions of individual fatty acids in lamb plasma samples collected at three weeks of age

Fatty acid (g/100 g fatty acids)	Diet						s.e.d.	P
	AA	AL	AM	LL	LM	MM		
C16:0	24.8^{b}	17.9^{a}	24.8^{b}	17.1^{a}	24.9^{b}	24.7^{b}	0.90	<0.001
C16:1*n*-7	0.94	0.92	0.91	0.92	0.91	0.91	0.065	0.995
C18:0	11.7^{a}	18.5^{c}	15.0^{b}	19.6^{c}	14.9^{b}	15.4^{b}	0.94	<0.001
C18:1 *trans*	5.49^{c}	3.71^{b}	2.96^{ab}	2.56^{a}	2.65^{a}	2.51^{a}	0.470	<0.001
C18:1*n*-9 *cis*	18.3^{a}	25.4^{c}	22.5^{b}	26.9^{c}	24.4^{bc}	25.3^{c}	1.34	<0.001
C18:2*n*-6 *cis*	10.5	9.43	11.4	9.72	13.2	11.9	1.867	0.351
CLA (*cis*-9,*trans*-11)	0.13^{abc}	0.20^{bc}	0.03^{a}	0.28^{c}	0.05^{ab}	0.05^{ab}	0.076	0.018
C18:3*n*-3 *cis*	1.06	2.92	3.05	3.03	1.04	1.07	1.215	0.216
C20:4*n*-6	5.38^{c}	2.31^{a}	3.23^{b}	1.91^{a}	2.51^{ab}	2.57^{ab}	0.414	<0.001
C20:5*n*-3	2.27^{d}	1.08^{bc}	0.90^{abc}	1.29^{c}	0.81^{ab}	0.62^{a}	0.193	<0.001
C22:6*n*-3	2.19^{c}	1.12^{ab}	1.47^{b}	1.04^{a}	1.02^{a}	1.03^{a}	0.210	<0.001
RFA[¶]	17.3^{b}	16.5^{b}	13.8^{a}	15.3^{ab}	13.6^{a}	13.8^{a}	1.021	0.004
Total fatty acids (mg/ml)	2.56	3.22	2.61	2.85	2.23	2.84	0.528	0.563

AA = Algae diet fed throughout pregnancy and lactation; AL= Algae diet fed during pregnancy followed by Linseed diet; AM = Algae diet fed during pregnancy followed by Megalac diet in lactation; LL = Linseed diet fed throughout pregnancy and lactation; LM = Linseed diet fed during pregnancy followed by Megalac diet in lactation; MM = Megalac diet fed throughout pregnancy and lactation

[¶] RFA = All remaining fatty acids

Means without common superscripts are significantly different at the P<0.05 level

4.4.3.5. Suckling lamb plasma fatty acids (three weeks of age)

The total fatty acid concentration in lamb plasma was similar amongst all dietary strategies (Table 4.32) and there was no significant effect of either *pre-* or *post partum* maternal dietary fat source upon this parameter (Table 4.33). The proportion of C16:0 within plasma was lowest in lambs born to ewes fed strategies AL or LL (P<0.001) compared to those fed the other four dietary strategies. Dietary strategy had no significant effect upon the proportion of C16:1*n*-7 within lamb plasma at three weeks of age.

Offering dietary strategies AL or LL to ewes resulted in increased proportions of C18:0 in lamb plasma at three weeks of age compared to strategies AA and MM. Maternal dietary supplementation with linseed *pre-partum* increased the proportion of C18:0 in lamb plasma compared to algae supplementation (P=0.001). Furthermore, significant differences in the proportion of C18:0 within lamb plasma were observed among maternal

post partum dietary fat sources, with the lowest proportions found in those supplemented with algae and highest in those fed linseed.

Table 4.33. Effect of supplementing the diets of ewes with various fat sources pre- and post partum on fatty acid proportions in lamb plasma samples collected at three weeks of age

Fatty acid (g/100 g fatty acids)	Diet			s.e.d.	P		
	Algae	Linseed	Megalac		C_1	C_2	C_3
Effect of pre-partum diet							
Number per treatment	30	20	10				
C18:0	14.8	17.0	15.4	1.40	0.690	0.001	0.444
C18:1*n*-9 *cis*	21.8	25.5	25.3	1.49	0.007	0.002	0.102
C18:2*n*-6 *cis*	10.6	11.7	11.9	1.51	0.343	0.574	0.467
C18:3*n*-3 *cis*	2.34	1.98	1.07	1.009	0.211	0.992	0.234
C20:4*n*-6	3.76	2.30	2.57	0.568	0.004	<0.001	0.136
C20:5*n*-3	1.45	1.05	0.62	0.279	<0.001	0.171	<0.001
C22:6*n*-3	1.64	1.05	1.03	0.226	0.003	0.003	0.052
Total fatty acids (mg/ml)	2.71	2.42	2.84	0.416	0.916	0.425	0.724
Effect of post partum diet							
Number per treatment	10	20	30				
C18:0	11.7	19.1	15.1	0.73	<0.001	<0.001	0.010
C18:1*n*-9 *cis*	18.3	25.9	24.1	1.13	<0.001	<0.001	0.500
C18:2*n*-6 *cis*	10.5	9.74	12.2	1.438	0.281	0.069	0.044
C18:3*n*-3 *cis*	1.06	3.06	1.72	0.986	0.514	0.069	0.387
C20:4*n*-6	5.38	2.25	2.77	0.341	<0.001	<0.001	0.084
C20:5*n*-3	2.27	1.22	0.78	0.155	<0.001	0.780	<0.001
C22:6*n*-3	2.19	1.13	1.17	0.178	<0.001	0.012	0.034
Total fatty acids (mg/ml)	2.56	2.83	2.56	0.413	0.999	0.155	0.310

C_1 = Algae vs Megalac; C_2 = Algae vs Linseed; C_3 = Linseed vs Megalac

Higher proportions of C18:1 *trans* were found in lambs suckling ewes fed strategies AA or AL compared to LL, LM or MM (P<0.001). Maternal supplementation with algae significantly reduced the proportion of C18:1*n*-9 *cis* in lamb plasma, whether data were analysed according to dietary strategy, or to maternal fat source fed *pre-* or *post partum*. Lambs reared by ewes fed dietary strategy AM had the lowest and those offered strategy LL the highest, proportions of CLA within plasma fatty acids (P=0.018).

The proportions of C18:2*n*-6 within lamb plasma were unaffected by dietary treatment, regardless of whether data were analysed as a six-treatment randomised block design or according to the supplemental fat source offered to ewes during pregnancy. The only significant effect of *post partum* diet upon the proportion of C18:2*n*-6 within lamb plasma

at three weeks of age was an decrease conferred by supplementing ewes with linseed compared to Megalac. Feeding strategy AA to ewes resulted in a significant increase in the proportion of C20:4*n*-6 within lamb plasma at three weeks of age compared to the other five strategies. Furthermore, addition of algae to the diet either *pre-* or *post partum* significantly increased the proportional contribution of C20:4*n*-6 to lamb plasma fatty acids compared to feeding diets containing either linseed or Megalac.

The provision of various dietary strategies and fat sources to the pregnant and lactating ewe did not significantly affect the proportion of C18:3*n*-3 within lamb plasma at three weeks of age. The proportional contribution of C20:5*n*-3 to lamb plasma fatty acids was highest in lambs produced by ewes fed strategy AA and lowest in lambs born to ewes supplemented with strategy MM ($P<0.001$). Feeding algae to the ewe either *pre-* or *post partum* resulted in higher proportions of C20:5*n*-3 in lamb plasma, lambs suckling ewes fed linseed had intermediate values, with the lowest proportions found in lambs produced by ewes supplemented with Megalac. The proportion of C22:6*n*-3 within lamb plasma was increased by maternal supplementation with dietary strategy AA compared to any of the other five dietary strategies ($P<0.001$). Supplementing ewes with diets containing algae *pre* or *post partum* significantly increased the proportion of C22:6*n*-3 within plasma lipids of the suckling lamb compared to supplementation with either linseed or Megalac.

4.5. Discussion

4.5.1. Ewe parameters

4.5.1.1. Straw Intake

Papadopoulos *et al.* (2002) report that the protein capsule surrounding the lipid component of the algal cell protects the long-chain PUFAs within marine algae against ruminal biohydrogenation. Nonetheless, animals offered diets containing algae in the current study exhibited significantly lower forage intakes towards parturition and during lactation than those fed saturated fat sources. This is in contrast to the results of Cooper *et al.* (2004) who found no significant effect of supplementation with marine algae compared to linseed or fish oil upon the DM intake of lambs. Franklin *et al.* (1999) reported significant decreases in DM intake as a result of supplementing dairy cattle with marine algae; this was in part attributed to the reduced palatability of concentrates containing algae. However, as the straw fraction was fed separately from the concentrate component within the current study, any significant effect of concentrate palatability would not be expected to impact upon forage intake. This concurs with the results of Papadopoulos *et al.* (2002) who observed significant reductions in concentrate intake when the inclusion rate of marine algae was increased from 23.5 g to 47 g or 94 g, with no concurrent effect upon forage intake.

Feeding whole oilseeds, in which a mucilaginous protein coat surrounds the lipid component, has been suggested as a strategy for enhancing the supply of unsaturated fatty acids to the duodenum (Doreau and Ferlay, 1994; Szumacher-Strabel *et al.*, 2001a). The majority of research involving the supplementation of lactating cattle with whole linseed has reported no significant effect of the fat source upon DM intake (Petit, 2002; AbuGhazaleh *et al.*, 2003; Soita *et al.*, 2003). Within the current study, ewes offered diets containing whole linseed exhibited lower daily straw intakes during pregnancy and lactation than those fed saturated fat sources. This is in contrast to the results of Wachira

et al. (2002) who observed no significant difference in DM intakes of growing lambs fed either whole linseed or Megalac. It may be suggested that supplementation of ewes with untreated whole linseed is ineffective as a method of protecting unsaturated fatty acids against ruminal biohydrogenation. This theory concurs with the results of Kennelley (1996) who demonstrated extensive biohydrogenation of C18:3*n*-3 when lactating cows were supplemented with whole linseed. Furthermore, Petit (2003) reported significant increases in dry matter intake as a result of feeding formaldehyde-protected whole linseed compared to unprotected linseed. Whether this is due to degradation of the protein coat within the rumen, or physical breakdown of the seed during mastication is not clear.

The rumen ecosystem is subjective to adaptive change as a result of environmental, metabolic and dietary influences, however, it is generally assumed that the toxic effects of unsaturated fatty acids are negated by dietary change and that the potential for carry-over is limited. Nonetheless, the reduction in daily straw intake conferred by supplementation with either marine algae or linseed during pregnancy persisted during lactation, regardless of whether the dietary regime was maintained or the principal dietary fat changed to an alternative or saturated source. It is possible that the observed effect of fat source upon straw intake may have resulted from the lysis of cellulolytic bacteria leading to a long-term reduction in fibre digestion (Szumacher-Strabel *et al.*, 2001a). Alternatively, it may have further reaching metabolic effects via the incorporation of long-chain unsaturated fatty acids into animal tissues during pregnancy.

Similar depressions in DM intake have been observed with intestinal infusion of unsaturated fatty acids, which are unlikely to have occurred as a result of effects on rumen microorganisms (Doreau and Chilliard, 1997b). A significant reduction in ruminant dry matter intake has been reported by Benson *et al.* (2001) as a consequence of abomasal infusion of unsaturated fatty acids. This was suggested to result from effects upon

metabolic controllers of feed intake or patterns of eating behaviour. The potential effects upon behaviour patterns may have been due to continual infusion of oils negating the "normal" peaks and troughs in nutrient supply, and is therefore unlikely to have occurred in the current experiment. An alternative explanation proposed by Benson *et al.* (2001) is that infused fatty acids interact with mobilised adipose tissue and depress intake via effects on metabolism. Incorporation of dietary long-chain PUFAs into body tissue during pregnancy, which are then released during lactation may therefore explain the continuing reductions in feed intake exhibited in ruminants changed to diets containing Megalac *post partum.*

4.5.1.2. Nutritional status

Treatment diets were formulated to be iso-energetic and iso-nitrogenous, therefore, as predicted, diet had little effect upon ewe liveweight, concurring with results reported by Goodridge *et al.* (2001), Johnson *et al.* (2002) and Petit (2003). However, ewes supplemented with algae during pregnancy gained less weight *pre-partum* than those fed diets containing Megalac. The reduction in straw intake exhibited by ewes fed algae was calculated to reduce ME supply by 1.5 MJ/day, a change that may explain the lower weight gain (AFRC, 1993). Although the difference was not statistically significant , the increase in lamb birthweight conferred by algal supplementation suggests that the lower rate of liveweight gain during pregnancy may have resulted from increased mobilisation of body reserves to meet foetal requirements, in combination with reduced rumen fill. Indeed, more condition was lost by ewes fed algae than those supplemented with Megalac, although, again, this difference was not statistically significant.

The mean plasma concentrations of βHB were lowest in ewes fed linseed and highest in ewes supplemented with Megalac *pre-partum*, with all values falling within the range proposed by Hamadeh *et al.* (1996). This may be interpreted as a result of differences in

the mobilisation of adipose tissue, however, this pattern does not concur with the body condition score results. Differences in βHB concentrations observed between ewes fed algae and linseed *pre-* and *post partum* may therefore be a result of changes in ruminal fermentation and volatile fatty acid production. The addition of linseed oil to the ruminant diet was shown by Cottyn *et al.* (1971), Ikwuegbu and Sutton (1982) and Ueda *et al.* (2003) to reduce the production of butyrate and increase the proportion of propionate produced within the rumen, which would have concurrent effects upon the concentration of βHB in plasma. However, neither Broudiscou *et al.* (1994) nor Chikunya *et al.* (2004) reported a significant effect of linseed oil or formaldehyde-treated linseed upon ruminal butyrate production. Fish oil supplementation of the ruminant diet has also been shown to reduce the concentration of acetate and increase propionate within the rumen (Fievez *et al.*, 2003), although no effects upon the concentration of butyrate were reported. This may explain the similarity in plasma βHB concentrations between ewes fed diets containing algae or Megalac.

Supplementing the pregnant ewe with algae during pregnancy increased the concentration of urea in plasma during both pregnancy and lactation, although values for all ewes were higher than those described by Dawson *et al.* (1999). Plasma urea originates mainly from ruminal protein degradation and deamination of amino acids; as all experimental diets contained similar protein concentrations, the protein component of the algal diet may have been more degradable than that within the linseed or Megalac diets. This concurs with the hypothesis propounded by Petit *et al.* (2002), who suggested that the mucilaginous protein coat surrounding oilseed fatty acids is a viable mechanism for protection of the fatty acids from biohydrogenation, and must thereby be resistant to ruminal degradation. Alternatively, it can be suggested that marine algae may have inhibited the growth of ruminal microorganisms, thereby increasing the amount of ammonia absorbed from the rumen. Doreau and Ferlay (1995) concluded that ruminant lipid supplementation either

maintains or reduces ruminal ammonia concentrations and increases microbial synthesis, thereby questioning the validity of this suggestion.

4.5.1.3. Ewe antioxidant status

Addition of unsaturated fatty acids in the form of linseed or algae to ruminant diets increases the peroxidation challenge to the animal. Erythrocyte GPx activities were similar among all treatments *pre-partum* although increased activity was observed in erythrocytes of ewes supplemented with algae *post partum*. The *pre-partum* results are in agreement with those of Smith and Isopenko (1997) who reported no significant differences in erythrocyte GPx as a result of protected PUFA supplementation of sheep. Observed GPx activities also concur with the range reported by Smith *et al.* (1994). Given the increased daily concentrate supply during lactation, the total dietary peroxide challenge to ewes fed algae would have been increased. The increased GPx activity may therefore have been in response to this challenge: Smith and Isopenko (1997) observed similar increases in GPx activity over time as a result of protected PUFA supplementation of sheep.

No difference in serum CK concentrations were observed amongst treatments, either *pre-* or *post partum*. The activity of this enzyme was higher than values observed by Vojtic (2000) in pregnant ewes and by Braun *et al.* (1993) in non-pregnant ewes. Nevertheless, it was lower than the range of values reported by Clemens *et al.* (1989) in pigs exposed to stressful conditions. This suggests that the observed values may have resulted from tissue damage induced by sampling techniques.

4.5.1.4. Plasma fatty acids *pre-partum*

A reduction in ewe plasma total fatty acid concentration resulting from PUFA supplementation of the diet was observed in Experiment One and repeated in the current study, the extent of the decrease appearing to be augmented by the degree of unsaturation of the supplementary fat source. This result concurs with the work of Offer *et al.* (2001) who described reductions in the total concentrations of fatty acids within serum lipoproteins conferred by long-chain PUFA supplementation of dairy cattle. Differences in this parameter between treatments may be attributed to variation in the digestibility of fat sources. Conflicting results upon the effect of fatty acid unsaturation on digestibility are reported by Doreau and Chilliard (1997a) and Wu *et al.* (1991), however, the results of the current study suggest that increasing the proportion of unsaturated fatty acids in the diet reduces digestibility. This is in contrast to the work of Powles *et al.* (1995) who reported that, in pigs, digestibility increased with augmentation of the unsaturated fatty acid concentration of the diet.

The saturated fatty acid profile of ruminant plasma is significantly affected by dietary fatty acid composition and by the degree of biohydrogenation of unsaturated fatty acids. The proportions of C16:0 within the plasma lipid fraction conferred by dietary strategies concurred with the fatty acid composition of experimental diets, in that the highest concentrations were found in ewes fed Megalac, and the lowest in ewes offered the linseed diet. Although Ashes *et al.* (1992) reported a reduction in C16:0 within plasma as a result of fish oil supplementation of sheep, the results of the current study may simply be attributed to differences in dietary supply.

Similarly, plasma C18:0 may originate directly from dietary supply or via the biohydrogenation of PUFAs within the rumen, specifically C18:2*n*-6 and C18:3*n*-3. Addition of long-chain PUFAs to the ruminant diet in the form of fish oil has been shown

by Offer *et al.* (2001), Cooper *et al.* (2002) and Chikunya *et al.* (2004) to reduce the concentration of C18:0 within plasma, results which concur with the effects of adding algae to the diet of pregnant ewes within the current study. Dietary C18:0 supply was equivalent across treatments, therefore the differences between plasma C18:0 concentrations of ewes fed algae and Megalac may be attributed to variation in the efficacy of ruminal biohydrogenation. As discussed with reference to the results observed in Experiment One, long-chain PUFAs have cytotoxic effects upon rumen microflora (Donovan *et al.*, 2000) and tend to disrupt ruminal biohydrogenation resulting in the production of *trans* isomers of C18:1 (Baumgard *et al.*, 2000). The proportions of these fatty acids in plasma were increased by algal supplementation of the ewe, suggesting that biohydrogenation may have been disrupted by PUFA supplementation.

Adding linseed to the ruminant diet has a significant augmenting effect upon the supply of C18:2*n*-6 and C18:3*n*-6 to rumen bacteria, thereby potentially increasing the products of unsaturated fatty acid biohydrogenation within plasma. The study of Petit (2002) reported an increase in the concentration of C18:0 within milk as a consequence of feeding whole linseed compared to Megalac, a result that is in accordance with those described by Goodridge *et al.* (2001). The rise in plasma C18:0 exhibited by ewes offered diets containing linseed concurs with the high concentrations of C18:2*n*-6 and C18:3*n*-6 within the diet, suggesting that the biohydrogenation of these fatty acids was not entirely prevented by the provision of whole oilseeds within the feed. Although the fatty acids within linseed may be protected from ruminal biohydrogenation by the mucilaginous protein seed coat, mastication during feeding may rupture the seeds and render PUFAs vulnerable to ruminal biohydrogenation. Supplementation of steers with whole linseed was observed by Scollan *et al.* (2001) to increase the flow of C18:3*n*-3 at the duodenum, however, a significant quantity of ingested C18:3*n*-3 was biohydrogenated with little protection offered by the seed coat. Wachira *et al.* (2000) and Chikunya *et al.* (2004)

reported similar results as a consequence of feeding whole linseed to sheep. The magnitude of ruminal biohydrogenation of fatty acids within whole linseed was not determined within the current study, Cooper *et al.* (2002) described biohydrogenation values of 83.8% for C18:2*n*-6 and 85.3% for C18:3*n*-3 in the rumen of sheep supplemented with whole linseed treated with formic acid.

Ewes fed the linseed diet had a lower dietary intake of C18:1*n*-9 *cis* compared to ewes supplemented with either the algae or Megalac diets. Differences in the concentration of this fatty acid within plasma at one week *pre-partum* between the linseed and Megalac treatments may therefore be attributed to variation in dietary supply. However, the proportion of C18:1*n*-9 *cis* within plasma fatty acids was significantly reduced by algal supplementation of the ewe, concurring with the effects observed in Experiment One as a result of fish oil supplementation. By contrast, the work of Ashes *et al.* (1992) and Offer *et al.* (2001) described increases in C18:1*n*-9 *cis* within plasma as a result of fish oil supplementation. Little data is available upon the effects of algal supplementation of ruminants upon plasma fatty acid composition, however, results by Franklin *et al.* (1999) and Papadopoulos *et al.* (2002) indicate that feeding algae to dairy cattle or lactating ewes significantly reduced the concentration of C18:1*n*-9 *cis* within milk fat. Nevertheless, Cooper *et al.* (2004) reported lower concentrations of C18:1*n*-9 *cis* within phosphatidylglycerols, but no differences in the concentration of this fatty acid in triacylglycerols in lambs fed a combination of algae and fish oil compared to fish oil alone.

Dietary C18:2*n*-6 intakes were similar between treatment diets containing algae or Megalac, but the proportion of this fatty acid within plasma lipid was reduced by the inclusion of algae in the diet. Again, this result concurs with that published by Franklin *et al.* (1999) and Papdopoulos *et al.* (2002), and with those observed in Experiment One as a consequence of fish oil supplementation. It may be suggested that the rate of

biohydrogenation of C18:2*n*-6 was affected by the provision of the algal supplement, however, long-chain PUFAs tend to have a toxic effect upon rumen microorganisms. Therefore they would be expected to either reduce the extent of ruminal biohydrogenation, or to increase the products of incomplete biohydrogenation, namely C18:1 *trans* and CLA. Franklin *et al.* (1999) observed a decrease in C18:2*n*-6 and a significant increase in the transfer of *trans* C18:1 fatty acids and CLA into milk fat as a result of algal supplementation of dairy cattle. Furthermore, Offer *et al.* (2001) described a significant decrease in plasma C18:2*n*-6 concentrations as a result of fish oil supplementation of cattle.

As discussed with reference to concentrations of C18:0 within plasma, the biohydrogenation of unsaturated fatty acids within the linseed diet appeared to be relatively high. This hypothesis is borne out by comparing the dietary intake of C18:2*n*-6 within the linseed diet with the amount found in plasma. Although the highest daily intakes of C18:2*n*-6 were conferred by linseed supplementation, plasma concentrations of C18:2*n*-6 were similar to those found in ewes supplemented with algae. This concurs with the work of Chikunya *et al.* (2004) who observed that although dietary supply of C18:2*n*-6 was similar among treatments, sheep fed a diet containing linseed had lower concentrations of C18:2*n*-6 than sheep fed Megalac. Petit (2002) published similar results with reference to the effect of whole linseed supplementation of dairy cows upon milk C18:2*n*-6 concentrations.

Endogenous synthesis of C20:4*n*-6 within the ruminant is achieved by the elongation and desaturation of C18:2*n*-6 (Wainwright, 2002). Consequently, an increased dietary supply of C18:2*n*-6 would be expected to increase the concentration of C20:4*n*-6 in plasma and tissues. As no detectable quantities of C20:4*n*-6 were observed in experimental diets containing linseed or Megalac it may be concluded that the presence of this fatty acid in

plasma of pregnant ewes was a result of endogenous synthesis. Indeed, Wiesenfeld *et al.* (2003) observed that adding linseed to the diet of pregnant rats reduced the ratio of C18:2*n*-6:C18:3*n*-3 within serum with a concomitant reduction in serum C20:4*n*-6. By contrast, addition of algae to the basal diet increased the dietary supply of C20:4*n*-6, significantly enhancing the proportion of this fatty acid within plasma.

The proportions of C18:3*n*-3 within plasma largely reflect the dietary intakes, however, the supply of C18:3*n*-3 was 25-fold higher in the linseed compared to the algae or Megalac diets, but only five-fold higher in plasma. This is in contrast to the result of Chikunya *et al.* (2004) who only achieved a two-fold increase in duodenal flow of C18:3*n*-3 at the small intestine as a consequence of supplementing sheep with whole linseed. However, the ten-fold increase in plasma C18:3*n*-3 concentrations in ewes fed linseed compared to algae or Megalac within the current study indicates that a substantial transfer of C18:3*n*-3 across the small intestine was achieved. Although a substantial amount of C18:3*n*-3 bypassed biohydrogenation and was absorbed at the small intestine, it is clear that C18:3*n*-3 within whole linseed was not wholly protected from ruminal biohydrogenation.

Hagemeister *et al.* (1991) concluded that the activity of Δ-desaturase and elongase enzymes within the dairy cow is low, as only modest increases in C20:5*n*-3 and C22:5*n*-3 were observed within milk fat as a consequence of linseed supplementation. No dietary C20:5*n*-3 was supplied by any of the treatment concentrates and the presence of this fatty acid within plasma can only be explained by the mobilisation of body reserves, endogenous desaturation and elongation from C18:3*n*-3 or retro-conversion from longer-chain fatty acids. As the pre-experimental diet did not contain a source of C20:5*n*-3, it seems unlikely that sufficient quantities could have been mobilised from stored reserves to account for the amounts found in plasma at one week *pre-partum*. Plasma C20:5*n*-3 concentrations were similar in ewes fed diets containing algae or linseed despite the

significant variation in dietary supply of C18:3*n*-3. It can therefore be suggested that the C20:5*n*-3 within plasma originated from two different sources: from the retroconversion of C22:6*n*-3 (algae diet) and the elongation/desaturation of C18:3*n*-3 (linseed diet).

The algae diet provided a high dietary supply of C22:6*n*-3, thus it is conceivable that the C20:5*n*-3 present in plasma of ewes fed this diet originated from the retro-conversion of C22:6*n*-3 to C20:5*n*-3. This hypothesis was proposed by Smuts *et al.* (2002) to explain the increases in gestation length observed in women supplemented with C22:6*n*-3. A similar explanation was offered by Papadopoulos *et al.* (2002) to explain the presence of C20:5*n*-3 within milk fat of ewes offered marine algae, and by Cooper *et al.* (2004) to explain the presence of this fatty acid in phosphatidylglycerols of sheep supplemented with algae. Although C20:5*n*-3 may have been synthesised via elongation and desaturation of C18:3*n*-3 within ewes fed algae, there was a substantial difference in plasma C18:3*n*-3 concentrations between these ewes and those offered linseed. Furthermore, dietary provision of long-chain PUFAs has been shown to inhibit the endogenous synthesis of C20:5*n*-3 and C22:6*n*-3 via the Δ-desaturase pathways (Sargent, 1997).

There is conflicting evidence regarding the efficiency of conversion of C18:3*n*-3 to C20:5*n*-3 and C22:6*n*-3 within mammals. Wiesenfeld *et al.* (2003) reported that the concentration of C20:5*n*-3 within serum of pregnant rats was significantly increased by the addition of linseed to the diet. Furthermore, both De Groot *et al.* (2004) and Francois *et al.* (2003) demonstrated increases in plasma C20:5*n*-3 as a consequence of dietary C18:3*n*-3 supplementation of women. Bazinet *et al.* (2003) also reported that the concentration of C20:5*n*-3 within sow milk was increased by linseed oil supplementation. It appears clear that the C20:5*n*-3 present in plasma of ewes fed the linseed or Megalac diets was a consequence of the elongation and desaturation of C18:3*n*-3. Therefore, the increased dietary supply of C18:3*n*-3 conferred by the linseed diet led to a concurrent increase in the

plasma C20:5*n*-3 concentration compared to the relatively low dietary supply and plasma concentrations in ewes fed Megalac. The published results of Francois *et al.* (2003) and De Groot *et al.* (2004) in humans, and those of Wachira *et al.* (2002), Cooper *et al.* (2004) and Demirel *et al.* (2004) in sheep indicate that although C18:3*n*-3 is elongated and desaturated to produce C20:5*n*-3, the production of C22:6*n*-3 is negligible. The observed concentrations of C22:6*n*-3 within plasma of ewes supplemented with either the linseed or Megalac diets concur with this hypothesis.

The concentration of C22:6*n*-3 within plasma of ewes fed algae was approximately three-fold higher than that of ewes offered linseed or Megalac, concurring with the results observed by Franklin *et al.* (1999) and Papadopoulos *et al.* (2002) in milk as a consequence of algal supplementation. However, the proportion of C22:6*n*-3 within plasma of ewes fed the algae diets was lower than that which would have been predicted from the dietary supply, indicating either a significant biohydrogenation of C22:6*n*-3 within the rumen or increased uptake by placental and foetal tissues during the last stage of pregnancy. As the latter suggestion does not appear to concur with the neonatal plasma C22:6*n*-3 concentrations, it appears that the C22:6*n*-3 within marine algae may not be invulnerable to ruminal biohydrogenation, as suggested by Papadopoulos *et al.* (2002).

4.5.1.5. Gestation length

The observation that human females from populations with a high habitual fish intake, such as the Eskimos or Danes, have longer gestation lengths than their counterparts in other areas of the world, led to the investigation of the effects of long-chain PUFA supplementation on this parameter. Subsequently, authors including Olsen *et al.* (1986; 1992) suggested that the *n*-3 long-chain fatty acids might be responsible for these effects. Investigations by Baguma-Nibasheka *et al.* (1998; 1999) and Hong Ma *et al.* (2000) using

pregnant sheep further reported that the initiation of parturition in the pregnant ewe may be inhibited by infusion of *n*-3 PUFAs into the bloodstream.

Within the current study, diet had no significant effect upon ewe gestation length. This is in contrast to the results of Olsen *et al.* (1990) in rats, Rooke *et al.* (2001c) in pregnant sows and to the results of Experiment One (section 3.4.2.7). Moreover, it does not agree with the results of Pickard *et al.* (2005) who demonstrated increased gestation lengths in ewes supplemented with algae during pregnancy. Trienoic prostaglandins produced from C20:5*n*-3 are thought to inhibit or delay the action of dienoic prostaglandins derived from C20:4*n*-6 (Hansen and Olsen, 1988). Smuts *et al.* (2003) observed a significant effect of C22:6*n*-3 supplementation upon the gestation length of pregnant women and attributed this effect to retroconversion of C22:6*n*-3 to C20:5*n*-3 with consequent effects upon prostaglandin production. No C20:5*n*-3 was detected within within feed samples and the presence of this fatty acid within ewe plasma at one week *pre-partum* derived either from the elongation and desaturation of C18:3*n*-3 (linseed diet) or the retroconversion of C22:6*n*-3 (algae diet). However, the amount of C20:5*n*-3 produced by this mechanism appears to be less than that required to generate a difference in gestation length. Indeed, plasma proportions of C20:5*n*-3 were 50% lower in ewes fed algae or linseed compared to those observed in Experiment One as a result of fish oil supplementation although concentrations of C20:4*n*-6 were similar to those reported for Experiment One. This adds weight to the hypothesis that an interaction between C20:5*n*-3 and C20:4*n*-6 is responsible for changes in gestation length as a result of altered prostaglandin production, indeed, the plasma C20:5*n*-3:C20:4*n*-6 ratio of ewes fed algae or linseed averaged 0.71, in comparison to 2.74 for ewe supplemented with fish oil in Experiment One. By contrast, Waltman *et al.* (1978) reported that C20:4*n*-6 extends gestation length in pregnant rats when compared to C18:0 or C18:1*n*-9 *cis* supplementation, but there is little other published evidence to support this result.

4.5.1.6. Colostrum

There is a paucity of information available upon the effects of PUFA supplementation upon colostrum yield and composition in ruminants, therefore results are discussed with reference to animals in established lactation. Colostrum composition figures were higher than the reference values for ewes proposed by Hadjipanayiotou (1995), however this difference is proposed to be a result of the high plane of nutrition supplied to ewes within the current study.

Colostrum yield is principally governed by energy and protein supply to the ewe, with significant but lesser effects of ewe breed and litter size (O'Doherty and Crosby, 1996; Knight, 2001; Pattinson and Thomas, 2004). Previous studies involving the supplementation of ruminants with long-chain PUFAs in the form of marine algae, fish oil or linseed have reported differing effects, ranging from an increase (Keady and Mayne, 1999a; Keady *et al.*, 2000; Petit, 2002a), to a significant decrease in milk yield (Jones *et al.*, 1998; Lacasse *et al.*, 2002; Whitlock *et al.*, 2002). However, concurring with the results published by authors supplementing lactating ruminants with marine algae (Franklin *et al.*, 1999; Papadopoulos *et al.*, 2002) or linseed (Brzóska *et al.*, 1999; Petit *et al.*, 2002; Petit, 2003), adding long-chain PUFAs to the diet had no significant effect upon colostrum yield of ewes within the current study.

The response in milk fat concentration and yield conferred by augmenting the ruminant diet with long-chain PUFAs is well documented. Although the addition of protected long-chain PUFAs has been shown by Kitessa *et al.* (2003) to have no significant effect on milk fat production, unprotected long-chain PUFA supplementation decreases milk fat concentration and yield. This effect of PUFA supplementation has been reported by Franklin *et al.* (1999) as a consequence of algal supplementation of dairy cattle, and by

both Petit (2002) and Petit *et al.* (2003) when linseed was added to the diet of the lactating ruminant.

One explanation for the reduced fat concentrations of ruminants supplemented with PUFAs is that reductions in forage intake lower the amount of acetate produced in the rumen, thus decreasing milk fat synthesis (Voigt and Hagemeister, 2001). This hypothesis concurs with the relationship between DM intake and milk fat concentration observed within the current study, however, the consensus view, as suggested by Chouinard *et al.* (1999), Baumgard *et al.* (2000) and Perfield *et al.* (2002), is that this effect is due to the action of *trans*-10,*cis*-12 CLA upon *de novo* fatty acid synthesis within the mammary gland. Identification of the *trans*-10,*cis*-12 isomer of CLA was not possible within the current experiment, therefore it is not possible to substantiate this hypothesis.

The protein component of colostrum contains an immunoglobulin component in addition to the principal milk protein casein, therefore values for this parameter are higher than the protein concentrations observed in studies measuring milk composition. The addition of marine algae or linseed to the diet may increase the undegradable digestible protein supply to the ruminant by virtue of the protein capsule or seed coat, therefore despite the iso-nitrogenous nature of the experimental diets, colostrum protein could conceivably have been increased by long-chain PUFA supplementation as observed by Papadopoulos *et al.* (2002) and Petit (2002; 2003). However, in agreement with the milk production results reported by Franklin *et al.* (1999) using marine algae, and Goodridge *et al.* (2001), Petit *et al.* (2002) and Soita *et al.* (2003) when feeding linseed, dietary treatment had no significant effect upon colostrum protein concentration within the current study. This is in direct contrast to the majority of research involving fish oil or linseed supplementation of lactating ruminants in which Lacasse and Ahnadi (1998), Keady *et al.* (2000) and Petit *et al.* (2002) observed decreases in milk protein concentrations.

4.5.1.7. Plasma fatty acids *post partum*

The total fatty acid concentration within the plasma lipid component of the lactating ewe was unaffected by either dietary strategy or fat source, a difference which is at odds with the effects of PUFA supplementation upon plasma fatty acid concentrations *pre-partum* and in Experiment One.

Linseed supplementation during pregnancy appeared to decrease the concentration of C16:0 within plasma of lactating ewes, however, this result may simply be attributed to the increased dietary supply of this fatty acid within the algae and Megalac diets. Nevertheless, changing from the linseed diet *pre-partum* to one containing Megalac (strategy LM) *post partum* did not raise proportions of this fatty acid in plasma to the levels found in ewes fed Megalac throughout the experiment. The carry-over effect of linseed supplementation during pregnancy upon proportions of C16:0 within plasma may therefore result from a combination of reduced dietary supply during pregnancy and lactation, and reduced labile supply from adipose tissue mobilisation.

Ewes fed diets containing either algae or Megalac *post-partum* had significantly lower plasma proportions of C18:0 when compared to ewes fed linseed. This has already been discussed with reference to *pre-partum* plasma results in section 4.5.1.4. Long-chain PUFA supplementation of ruminant diets in the form of fish oil demonstrably reduced the concentration of C18:0 within plasma of dairy cattle (Offer *et al.*, 2001), sheep (Ashes *et al.*, 1992) and steers (Ashes *et al.*, 1992), results which concur with the effects of algal supplementation of ewes within the current study. Ruminal biohydrogenation of long-chain fatty acids accounts for a significant proportion of the C18:0 absorbed at the small intestine and given the similar concentrations of this fatty acid among diets, reductions in the efficiency of biohydrogenation may have accounted for the differences in plasma concentrations between ewes fed algae or Megalac. Incomplete biohydrogenation of

unsaturated fatty acids also leads to an increase in the intestinal absorption of *trans* isomers of C18:1. The significantly higher concentrations of these fatty acids in plasma of lactating ewes fed diets containing algae during lactation adds weight to the suggestion that ruminal biohydrogenation was disrupted by algal supplementation.

Augmentation of the long-chain PUFA content of the diet with marine algae during pregnancy (regardless of the diet fed during lactation) significantly reduced the concentration of C18:1*n*-9 *cis* within ewe plasma at three weeks *post partum*. This effect of PUFA supplementation was also observed within Experiment One but is in contrast to the results published by both Ashes *et al.* (1992) and Offer *et al.* (2001) who reported an increase in plasma concentrations of C18:1*n*-9 *cis* as a result of long-chain PUFA supplementation of sheep and cattle. Demirel *et al.* (2004) reported lower concentrations of C18:1*n*-9 *cis* within neutral and polar lipid fractions of adipose tissue of lambs supplemented with a mixture of linseed and fish oil, furthermore, both Franklin *et al.* (1999) and Papdopoulos *et al.* (2002) observed low concentrations of this fatty acid in milk fat from ewes fed diets containing marine algae. The reasons behind this decrease are unclear, but may simply be the cumulative effect of increased amounts of long-chain PUFAs displacing C18:1*n*-9 *cis* within the plasma lipid fraction.

Reductions in the efficiency of ruminal biohydrogenation of PUFAs induced by algal supplementation of the ewe would be expected to increase the concentration of C18:2*n*-6 within plasma, a result in contrast to the lower proportions observed within the current study. Similar results were observed by Franklin *et al.* (1999) and Papadopoulos *et al.* (2002) within milk fat of dairy cows and sheep supplemented with algae and by Offer *et al.* (2001) in lactating cattle. Linseed supplementation of the ewe, during pregnancy and/or lactation also reduced the proportion of C18:2*n*-6 within plasma at three weeks *post partum*. This result concurs with those observed by Chikunya *et al.* (2004) in plasma of

dairy sheep and Petit (2002) in milk fat of lactating dairy cattle. It is interesting to note that both algae and linseed supplementation during pregnancy conferred significant carry-over effects on plasma C18:2*n*-6 into lactation. This may be attributed to reduced deposition of this fatty acid into lipid reserves during pregnancy with a consequent decrease in the labile supply from mobilisation of tissue during lactation. However, this hypothesis is not entirely in agreement with the results published by Demirel *et al.* (2004) who noted that the deposition of C18:2*n*-6 into lamb muscle phosphatidylglycerols was reduced by linseed supplementation, but deposition into muscular triacylglycerols and liver was unaffected by treatment diet.

Endogenous synthesis of C20:4*n*-6 may be inhibited by the supply of other long-chain PUFAs, specifically *n*-3 PUFAs to the ewe (Sargent, 1997). The presence of C20:4*n*-6 within ewes fed linseed and Megalac was due to endogenous synthesis via desaturation and elongation of C18:2*n*-6, it would be expected that it would be found in higher quantities in ewes fed Megalac compared to linseed given the increased supply of the precursor fatty acid and a lower rate of competition from C18:3*n*-3. By contrast, the proportions of C20:4*n*-6 within plasma of ewes at three weeks *post partum* were similar in ewes fed linseed or Megalac. Algal supplementation of the pregnant and lactating ewe increased the concentration of C20:4*n*-6 within plasma at three weeks *post partum*. In ewes supplemented with algae during pregnancy this may be attributed to an increased dietary supply. However, the biochemical reasons behind the carry-over effects of *pre-partum* supplementation upon this fatty acid in plasma warrant further investigation.

Supplementing the diet of the pregnant and lactating ewe with linseed increased both the dietary supply of C18:3*n*-3 and the concentration of this fatty acid within plasma at three weeks *post partum* compared to Megalac. This may be attributed to increased duodenal flow of this fatty acid as described by Wachira *et al.* (2000) and Chikunya *et al.* (2004).

Some investigators (Hagemeister *et al.*, 2001) have concluded that the synthesis of long-chain *n*-3 PUFAs from C18:3*n*-3 is negligible within the ruminant. Indeed, Wachira *et al.* (2002) observed no differences in muscular C20:5*n*-3 and C22:6*n*-3 concentrations between sheep fed a diet augmented with linseed and one containing Megalac. Furthermore, although Chikunya *et al.* (2004) observed that the concentration of C20:5*n*-3 within plasma of sheep supplemented with formaldehyde-treated linseed was higher than that of sheep fed Megalac, it was considerably reduced compared to sheep fed linseed mixed with fish oil. By contrast, ewes supplemented with linseed either *pre-* or *post partum* within the current study had similar proportions of C20:5*n*-3 within plasma to ewes fed algae, and higher than those fed Megalac. Long-chain *n*-3 PUFAs are stored within muscular phosphatidylglycerols rather than the triacylglycerols contained within adipose tissue (Ashes *et al.*, 1992). Although body tissues are mobilised in an attempt to maintain energy and protein supplies during pregnancy and lactation, the majority of the tissue mobilised is in the form of stored triacylglycerols rather than phosphatidylglycerols and would provide little, if any, C20:5*n*-3 or C22:6*n*-3. Therefore, the presence of C20:5*n*-3 within plasma suggests that endogenous synthesis of *n*-3 fatty acids is not, as previously suggested by Voigt and Hagemeister (2001), negligible.

Algal supplementation of the ewe during pregnancy and lactation increased the concentration of C20:5*n*-3 within plasma of ewes compared to those fed diets containing Megalac despite the low dietary supply of C18:3*n*-3 from the algal diet. The endogenous desaturation and elongation mechanism required to produce this fatty acid from C18:3*n*-3 should, according to Sargent (1997), Bougle *et al.* (1999) and Poumes-Ballihaut *et al.* (2001), have been inhibited by the considerable dietary supply of preformed C22:6*n*-3. Therefore it may be suggested that the presence of C20:5*n*-3 within plasma of ewes fed algae at three weeks *post partum* was the result of retroconversion of dietary C22:6*n*-3.

The increased dietary supply of C22:6*n*-3 conferred by algal supplementation of the ewe is likely to be the principal reason behind the increased proportion of this fatty acid within plasma *pre-partum*. This result concurs with those of Franklin *et al.* (1999) and Papadopoulos *et al.* (2002) resulting from the supplementation of lactating ruminants with marine algae. However, the reasons behind the continuing high concentrations of C22:6*n*-3 within plasma at three weeks *post partum*, despite the change to a diet based on Megalac or linseed at twelve hours *post partum* are more difficult to elucidate. As previously discussed, long-chain PUFAs are not deposited in significant quantities in triacylglycerols, but instead into phosphatidylglycerols which have a slower turnover rate (Wonsil *et al.*, 1994). Wonsil *et al.* (1994) also observed a significant carry-over effect of fish oil supplementation of lactating cattle upon subsequent plasma concentrations of C22:6*n*-3.

Linseed supplementation of the ewe provided sufficient C18:3*n*-3 to raise plasma concentrations of C20:5*n*-3 in the neonatal lamb to those observed in ewes fed marine algae. However, as evidenced by the current results, endogenous desaturation and elongation of C18:3*n*-3 was ineffective at increasing the concentrations of C22:6*n*-3 within plasma of the lactating ewe. The studies published by Hagemeister *et al.* (1991), Petit (2002) and Francois *et al.* (2003) also concluded that endogenous production of C22:6*n*-3 from C18:3*n*-3 is low whether observed within the ruminant or human. As C20:5*n*-3 concentrations were similar between algal and linseed treatments, the rate of endogenous synthesis of C22:6*n*-3 may be limited by the conversion of C20:5*n*-3 to C22:6*n*-3.

4.5.1.8. Milk

Given the plastic nature of the ruminal ecosystem, "cross-over" designs are generally considered to be valid for use within experiments employing ruminants. However, the effects observed upon milk production and composition as a result of PUFA supplementation during pregnancy throw doubt upon the validity of these experimental

designs. Milk yield was unaffected by fat source supplied to ewes during lactation, but there were significant carry-over effects of *pre-partum* algal supplementation upon milk yield. Neither the study of Franklin *et al.* (1999) nor work by Papadopoulos *et al.* (2002) observed any significant effect of algal supplementation of dairy cattle or ewes upon milk yield. However, both studies were carried out in animals during established lactation. No published studies have yet evaluated the effect of PUFA supplementation during pregnancy upon milk production and composition during lactation.

Chilliard and Doreau (1997) and Keady and Mayne (1999a; 1999b) reported increased milk yields in dairy cows offered varying concentrations of dietary fish oil. This was attributed by Chilliard and Doreau (1997) to an increase in energy supply by fish oil supplementation, but given the iso-energetic nature of the treatment diets and the similar body condition score loss among treatments, this hypothesis does not explain the results observed within the current study.

The production of lactose is a primary factor in the determination of milk yield, increased lactose production is associated with an increase in milk osmotic potential and concurrent increase in the secretion of water into milk (Ploumi *et al.*, 1998). Lactose yield was significantly increased by the provision of algae to pregnant ewes within the current study. Lactose in ruminant milk is produced from glucose and galactose, the former principally being derived from ruminal propionate production (Nielsen and Ingvartsen, 2004). Previous studies have noted decreases in ruminal acetate production with concurrent increases in propionate production as a result of long-chain PUFA supplementation (Chilliard, 1993; Doreau and Chilliard, 1997a; Doreau and Chilliard, 1997b). The hypothesis that milk yield is increased as a result of changes in rumen fermentation can therefore be considered valid, however, algal supplementation ceased four weeks before milk yield was recorded.

Infusing either CLA or *trans* C18:1 fatty acids into the abomasum of dairy cattle was shown by Mackle *et al.* (2003) to increase milk yield. Furthermore, Bernal-Santos *et al.* (2003) reported an increase in milk yield as a result of dietary supplementation of Holstein cattle with a mixture of CLA isomers. These authors concluded that a shift in nutrient partitioning from milk fat production towards milk synthesis was induced by CLA. If the effects observed within the current study are conferred by *trans* 18:1 or CLA, this indicates that either rumen function remains disrupted four weeks after the PUFA source is withdrawn from the diet, or that sufficient stores of these fatty acids are laid down during the period of PUFA supplementation to induce significant changes in milk production and composition during mobilisation of fat reserves in lactation. Shingfield *et al.* (2003) observed significant carry-over effects of fish oil supplementation of dairy cattle upon milk parameters two weeks after supplementation had ceased. By contrast, the studies of Kitessa *et al.* (2001a) and Kitessa *et al.* (2001b) employed nine and twelve day adaptation periods respectively and observed no carry-over effects of fish oil supplementation on ruminal metabolism or milk composition. However, there is little published data available to indicate whether such effects would be observed at four weeks post supplementation at the relatively high levels of PUFA supplementation employed within the current study.

Despite changes in dietary fat source at 12 hours *post partum*, milk fat synthesis during lactation was significantly reduced by provision of linseed or algae during pregnancy. Administration of either a mixture of CLA isomers (Perfield *et al.*, 2002; Bernal-Santos *et al.*, 2003) or *trans*-10, *cis*-12 CLA (Viswanadha *et al.*, 2003) has been conclusively demonstrated to reduce milk fat synthesis. The mechanism by which CLA acts is thought to be via inhibiting effects upon the enzymes necessary for milk fat synthesis, namely acetyl-CoA carboxylase, fatty acid synthase and stearoyl-CoA desaturase (Ahnadi *et al.*, 2002). *Trans* 18:1 fatty acids have also been implicated in the depression of milk fat synthesis by Wonsil *et al.* (1994), moreover, this fatty acid may be further desaturated

within the mammary gland by Δ-9 desaturase to form CLA (Griinari *et al.*, 2000). Indeed, Griinari *et al.* (2000) suggested that a significant proportion of the *trans*-10, *cis*-12 CLA found within milk is derived from desaturated *trans*-10 C18:1 fatty acids. If significant amounts of the *trans*-10,*cis*-12 isomer of CLA were stored during pregnancy and released during mobilisation of body condition during lactation, this may explain the reduction observed in milk fat synthesis.

Supplementing ewes with either algae or linseed during lactation also reduced milk fat concentration and yield within the current study. This concurs with the results observed by Franklin *et al.* (1999) as a consequence of algal supplementation and those of Petit (2002) who added linseed to the diet of lactating cattle. By contrast, the majority of studies involving linseed supplementation of ruminants report no significant effect upon milk fat concentration (Mansbridge *et al.*, 1999; Petit *et al.*, 2002; Petit, 2003; Soita *et al.*, 2003). Within the current study, the quantity of linseed added to the treatment diets was relatively high (177g/kg), however, variation in linseed inclusion rate among the aforementioned experiments suggests that the milk fat depression observed within the current study may not simply be a consequence of the level of supplementation, and may be attributed to changes induced by the products of ruminal PUFA biohydrogenation.

The synthesis of milk protein is dependent on the supply of amino acids and glucose from the diet (McDonald *et al.*, 1988). As previously discussed, the increase in milk yield conferred by algal supplementation of the pregnant ewe can be attributed to shifts in ruminal fermentation towards propionate at the expense of acetate. An increase in ruminal propionate production would theoretically increase the potential for milk protein synthesis. However, within the current study, feeding algae or linseed during pregnancy significantly reduced milk protein concentrations at four weeks *post-partum*. This is in contrast to the data published by Papadopoulos *et al.* (2002) who noted an increase in milk protein

concentration with algal supplementation. However, a non-significant decrease in milk protein concentration was observed by Franklin *et al.* (1999), concurring with the effects of fish oil supplementation described by Kitessa *et al.* (2001b), Ahnadi *et al.* (2002) and Lacasse *et al.* (2002).

Milk protein concentration may be reduced by an increase in milk yield, without a concurrent increase in protein synthesis, or by a reduction in casein synthesis as postulated by Chilliard and Doreau (1997). Algal supplementation of the pregnant ewe increased milk yield and reduced milk protein concentration within the current study. By contrast, milk yield was unaffected by algal supplementation of the lactating ewe although a reduction in milk protein concentration was observed. This lends weight to the casein hypothesis, however it is not possible to verify this theory as parameters of casein synthesis were not measured within the current study. The majority of studies involving linseed supplementation of the ruminant have reported either an increase in milk protein concentration (Petit, 2002; Petit, 2003) or no difference between linseed and control treatments (Brzóska *et al.*, 1999; Petit *et al.*, 2002; Soita *et al.*, 2003). Linseed appears to have acted by a similar mechanism to long-chain PUFA sources such as fish oil with regards to its observed effects upon milk fat and protein concentration, although no such effect was observed upon milk yield.

4.5.2. Lamb parameters

4.5.2.1. Maternal and neonatal behaviour

The principal factors affecting the quality of maternal care towards the lambs appear to be ewe breed and prior maternal experience (Dwyer *et al.*, 1999). Ewes traditionally used in extensive hill sheep systems tend to perform behaviours that enhance lamb survival, including vigorous grooming behaviour and facilitation of lamb suckling attempts (Dwyer and Lawrence, 1998). Furthermore, multiparous ewes exhibit less inappropriate

behaviours towards lambs during the first few hours after parturition than primiparous ewes (O'Connor *et al.*, 1992). However, there is little evidence to suggest that supplementation of maternal diet may affect maternal behaviour. As observed in Experiment One, maternal diet had no significant effect upon maternal behaviour scores or the latencies of various maternal behaviours, indicating that the quality of maternal care provided to the lamb was similar amongst treatments.

Observed latencies of neonatal behaviours concur with those published by Garcia-Gonzalez and Goddard (1998), Wassmuth *et al.* (2001) and Cloete *et al.* (2002). One of the first behaviours exhibited by the neonatal lamb is straightening of the hind limbs with concurrent attempts to stand (Vince, 1993), this behaviour being essential for exploratory behaviour, successful location of the udder and subsequent suckling. Within the current study, supplementation of the ewe with long-chain PUFAs in the form of algae or linseed delayed standing by the lamb when compared to ewes supplemented with Megalac. The observed results are in contrast to those of Pickard *et al.* (2005), who observed that supplementing ewes with algal biomass significantly reduced the latency of lamb standing. However, the latencies observed by Pickard *et al.* (2005) were considerably higher (mean value across all treatments of 25.8 minutes) than those recorded within the current study (mean value across all treatments of 15.9 minutes).

In contrast to suckling behaviours, which may be significantly affected by a combination of maternal behaviour, maternal posture and lamb sensory perception (O'Connor and Lawrence, 1992), the latency of lamb standing is primarily affected by ewe grooming behaviour. Dwyer and Lawrence (1999) noted that an increase in ewe grooming behaviour was related to delays in standing by neonatal lambs, furthermore, O'Connor and Lawrence (1992) concluded that ewe grooming behaviour within the first ten minutes *post partum* was positively correlated with lamb standing attempts. Although the maternal behaviour

scores utilised within the current study made use of qualitative measurements of ewe grooming behaviours, no quantitative measurements were made, therefore it is not possible to conclude whether the duration of grooming behaviours were increased in the ewes supplemented with algae and linseed compared to Megalac.

Searching behaviours in the neonatal lamb are usually performed after standing is achieved, thus following a similar pattern to the observed latencies of standing. However, the interval between standing and searching for the udder was increased in lambs from ewes supplemented with Megalac compared to the other treatments. Searching behaviour in the lamb usually begins at the neck of the ewe, the lamb making upward searching movements with the nose in search of a smooth, warm and hairless area, i.e. the udder (Vince, 1993). The success of locating the udder may be affected by maternal behaviour, in particular the amount of movement by the ewe, and a delay in searching behaviour indicates an increased degree of separation from the dam or a decrease in the sensory perception of the lamb. Visual and olfactory cues are essential in contributing to the ability of the lamb to successfully locate the udder (Vince, 1993), enhanced visual development or olfactory detection may have resulted in the lower latency of searching observed in lambs produced by ewes supplemented with algae or linseed.

Retinal cells contain a high proportion of long-chain PUFAs, specifically C22:6*n*-3, and some studies of long-chain PUFA supplementation of human infants have observed increases in visual acuity (Hoffman *et al.*, 1993; Gibson *et al.*, 1996; Birch *et al.*, 2002). This may explain not only the decreased interval from standing to searching in lambs from ewes fed diets containing algae or linseed, but also the lower latency of successful suckling observed in these lambs. This is in agreement with the results of Rooke *et al.* (2001a) who described a tendency for piglets to locate and grasp the teats as a consequence of tuna oil supplementation during the period immediately prior to parturition. However, there was

no significant effect of maternal dietary supplementation upon brain fatty acid composition in the neonatal lamb, which suggests that retinal fatty acid composition may have been similar across all treatments. The latency of successful suckling in lambs from ewes fed with Megalac was increased when compared to the normal range of values reported by Fahmy *et al.* (1997) and to results obtained within Experiment One. Therefore the reduced latencies exhibited by lambs borne by ewes fed either algae or linseed may have only been significant as a consequence of an increased latency to suckle exhibited by lambs produced by ewes fed Megalac.

4.5.2.2. Plasma fatty acids in the neonate

Prior to colostrum ingestion, the neonatal lamb is wholly dependent upon the placenta to supply fatty acids for energy and tissue development. There is considerable debate as to whether fatty acids are transferred across the placenta with equal efficiency or whether selection mechanisms exist.

Total fatty acid concentrations within neonatal plasma were similar to those observed in Experiment One, although considerably lower than values observed in ewes at one week *pre-partum*, a result in accordance concurring with the assertion by Leat and Harrison (1980) that fatty acid transfer from the dam to the foetus is limited in the ruminant. Lambs produced by ewes fed diet LL had a twofold increase in plasma fatty acid concentrations when compared to the other five treatments. This may be due to experimental error as there is no logical reason for this difference, especially given that the same diet was fed to ewes on treatments LL and LM during pregnancy.

Studies by Elphick *et al.* (1979) and Leat and Harrison (1980) concluded that the transfer of C16:0 across the ovine placenta was relatively low and that the placenta is impermeable to medium-chain fatty acids. As C16:0 was the principal fatty acid detected within ewe

plasma at one week *pre-partum* and was found in equivalent or higher concentrations in the neonatal lamb, it can be suggested that the concentration of this fatty acid may be influenced by maternal plasma concentrations, although it appeared to be unaffected by maternal dietary supply.

The proportional contribution of C18:0 to neonatal lamb plasma fatty acids was unaffected by maternal diet. Furthermore, no significant difference in the proportions of C18:1 *trans* existed among treatments, concurring with the observed results in ewe plasma at one week *pre-partum*. In the absence of an adequate EFA supply, C18:0 may be desaturated and elongated to form C20:3*n*-9 (Noble *et al.*, 1982). However, this fatty acid was not detectable in any of the plasma samples analysed, indicating that the supply of C18:2*n*-6 and C18:3*n*-3 via placental transport may have been adequate to satisfy the requirements of the foetal lamb.

The proportional contribution of C18:1*n*-9 *cis* to the lipid component of neonatal lamb plasma was higher than the proportions of this fatty acid within maternal plasma, suggesting that a concentration gradient selecting for this fatty acid may exist within the placenta. However, despite the wide range of values observed, maternal diet had no significant effect upon this parameter in the neonate. The concentrations observed were in agreement with the range of values reported by Noble *et al.* (1971) and Raijon *et al.* (1985) in lambs and Fritsche *et al.* (1993) and Rooke *et al.* (2001b) in neonatal pigs.

Supplementation of the pregnant ewe with linseed tended to increase the concentration of C18:2*n*-6 within neonatal lamb plasma. Noble *et al.* (1978) asserted that the supply of C18:2*n*-6 via the placenta to the foetal lamb was extremely low. Indeed, the proportional contribution of this fatty acid to total plasma fatty acids (average of 1.55 g/100g fatty acids across all treatments) was considerably less than that observed in maternal plasma at one

week *pre-partum* (average of 14.0 g/100g fatty acids across all treatments). The lamb plasma concentration of C18:2*n*-6 appeared to be affected by maternal diet, being highest in lambs born to ewes fed linseed, although this result did not directly concur with the maternal plasma concentrations in which the highest concentrations of C18:2*n*-6 were found in ewes fed Megalac. Leat and Harrison (1980) concluded that although transfer of long-chain fatty acids across the placenta is low, there was no evidence for a selection gradient against C18:2*n*-6. The results of the current experiment contradict this hypothesis. Although neonatal fatty acid concentrations are lower than those observed in the dam, if the selection pressure was equal for all fatty acids, similar proportions would be observed within maternal and neonatal plasma.

Elphick *et al.* (1979) suggested that low concentrations of C18:2*n*-6 within neonatal lamb plasma may result from the endogenous desaturation and elongation of C18:2*n*-6 to form C20:4*n*-6. Indeed, within the current study, the concentrations of C20:4*n*-6 were higher in neonatal plasma than might have been expected from amounts measured in maternal plasma. Huang and Craig-Schmidt (1996) stated that C20:4*n*-6 is conditionally essential within the foetal and neonatal animal for the development of neural tissues. Therefore, given the negligible concentrations of this fatty acid in commercial sheep diets, a biochemical mechanism may have evolved either to selectively transfer C20:4*n*-6 via the placenta or to produce it endogenously within the neonate.

Campbell *et al.* (1998) have identified a human placental membrane fatty acid binding protein that has higher binding capacities for C20:4*n*-6 and C22:6*n*-3 compared to C18:2*n*-6 and these authors concluded that this protein may serve to preferentially transport C20:4*n*-6 from the dam to the foetus. The presence of a similar protein within the ovine placenta would provide an explanation for the increased concentrations of C20:4*n*-6 observed within neonatal plasma.

Shand *et al.* (1978) reported that increasing the supply of C18:2*n*-6 to the foetal lamb conferred an increase in the concentration of C20:4*n*-6 within liver phospholipids. Furthermore, Noble *et al.* (1982) noted significant amounts of C20:4*n*-6 within neonatal lamb plasma and hypothesised that this was due to desaturation and elongation of C18:2*n*-6 within the placenta, as the maternal concentrations of this fatty acid were extremely low. Raijon *et al.* (1985) propose a similar theory, whilst Noble *et al.* (1985), concluded that desaturation and elongation of fatty acids may occur within the placenta. Noble *et al.* (1971) also suggested that foetal tissues were able to convert C18:2*n*-6 to C20:4*n*-6, and further postulated that the endogenous desaturation and elongation of C18:2*n*-6 to C20:4*n*-6 is more active within the foetal and neonatal lamb than in the ewe due to the increased cellular demand for this fatty acid (Noble *et al.*, 1978). Uauy *et al.* (2000) suggested that neonatal human infants are able to desaturate and elongate EFAs to their long-chain PUFA derivatives, furthermore, Salem *et al.* (1996) demonstrated that the synthesis of C20:4*n*-6 from C18:2*n*-6 within the human neonate was not only achieved within the liver and brain, but that it was more efficient than the synthesis of C22:6*n*-3 from C18:3*n*-3. It appears clear that the increased concentrations of C20:4*n*-6 in neonatal lambs compared to ewes within the current study were a consequence of elongation and desaturation of C18:2*n*-6 in response to an increased cellular requirement.

Previous studies have reported shown that the concentration of C18:3*n*-3 within neonatal lamb plasma is usually lower than that of C18:2*n*-6 (Noble *et al.*, 1982; Noble *et al.*, 1985; Raijon *et al.*, 1985), a difference which may be attributed to lower maternal plasma concentrations of C18:3*n*-3. Concurring with the aforementioned results, C18:3*n*-3 was not detectable in plasma of any neonatal lambs save for those borne by ewes supplemented with linseed during pregnancy. This result is not wholly unexpected given that C18:3*n*-3 is the principal fatty acid within linseed, although concentrations of this fatty acid were

extremely low when compared to maternal plasma results at one week *pre-partum*. As previously discussed, there is an increasing body of evidence to suggest that long-chain PUFAs may be endogenously synthesised from their EFA precursors in human infants (Salem *et al.*, 1996; Green and Yavin, 1998; Uauy *et al.*, 2000), primates (Su *et al.*, 1999; Su *et al.*, 2001), rodents (Kanazawa and Fujimoto, 1993; Woods *et al.*, 1996) and ruminants (Noble *et al.*, 1985; Raijon *et al.*, 1985). The magnitude of the difference between maternal and neonatal plasma C18:3*n*-3 concentrations may therefore, in part, be due to conversion of this fatty acid to C20:5*n*-3 and C22:6*n*-3 within the neonate.

Wiesenfeld *et al.* (2003) fed linseed to pregnant rats and observed an increase in the amount of C20:5*n*-3 within serum of neonatal rats. As C20:5*n*-3 is produced as an intermediate during the conversion of C18:3*n*-3 to C22:6*n*-3, it would be expected that the proportion of this fatty acid would be increased in lambs produced by ewes fed linseed. However, concentrations of C20:5*n*-3 in neonatal lamb plasma were similar among treatments. It may be suggested that the demand for C22:6*n*-3 during foetal and neonatal development is such that any excess C20:5*n*-3 may have been desaturated and elongated to C22:6*n*-3. Alternatively, the elongation and desaturation process that produces C20:5*n*-3 from C18:3*n*-3 may have been inhibited either by competition for Δ-desaturase enzymes or by other metabolic processes within the neonate.

Treatment diets containing algae or linseed were formulated to provide a similar potential supply of C22:6*n*-3 to the pregnant ewe (and subsequently to the foetus), either preformed (algae) or by endogenous synthesis (linseed). Estimates of the efficiency of conversion of C18:3*n*-3 to C22:6*n*-3 vary, but the consensus view appears to be that preformed C22:6*n*-3 is between seven and eight-fold more effective at providing C22:6*n*-3 accretion in tissues compared to C18:3*n*-3 (Su *et al*,. 1999; 2001). The similar concentrations of C22:6*n*-3 in plasma of neonatal lambs between the algae and linseed treatments suggests that this

formulation was successful and that the level of linseed inclusion within the diet provided equivalent concentrations of C22:6*n*-3 as the algal diet. Nonetheless, Sinclair *et al.* (2002) suggested that plasma C22:6*n*-3 may not be an accurate indicator of tissue C22:6*n*-3 status due to the biosynthetic capacities of brain and retina.

4.5.2.3. Neonatal lamb brain fatty acids

The range of lipid concentrations within brain tissue in the current study (from 20.6 mg/g freshweight to 25.5 mg/g freshweight) are in agreement with the values proposed by Lauritzen *et al.* (2001). Given the essential role of fatty acids within nervous tissue, it it logical to suggest that, unless dietary supply is severely compromised, the brain has evolved to maintaining a constant lipid concentration.

The proportions of the principal saturated (C16:0 and C18:0) and monoenoic (C18:1 *trans*, C18:1*n*-9 *cis*) fatty acids within lamb brain tissue were similar among treatments. Although saturated and short or medium-chain fatty acids are present within brain tissue and have minor functions, arguably the most important fatty acids supplied to the foetus for brain development are the EFA precursors of long-chain PUFAs (C18:2*n*-6 and C18:3*n*-3) and preformed long-chain *n*-6 and *n*-3 fatty acids (C20:4*n*-6, C20:5*n*-3, C22:6*n*-3).

Small amounts of C18:2*n*-6 were present within brain tissue with proportions similar across all treatment groups. Neonatal plasma results showed far greater concentrations of this fatty acid in lambs produced by ewes fed either linseed or Megalac diets. The lower brain concentrations of this fatty acid in the lambs borne by ewes fed linseed or Megalac may be attributed to its use as a precursor for the long-chain PUFA, C20:4*n*-6. Previous studies (Yamamoto *et al.*, 1987; Green and Yavin, 1993; Salem *et al.*, 1996) have demonstrated that cells within the brain are capable of synthesising long-chain PUFAs in

the absence of a preformed dietary supply. Therefore, neonatal lambs from ewes fed either linseed or Megalac diets should synthesise C20:4*n*-6 from C18:2*n*-6. If, by contrast, endogenous synthesis did not occur within the brain and fatty acid composition was only affected by placental supply, highest proportions of C20:4*n*-6 would be expected in neonatal brain tissue from lambs produced by ewes fed algae, with lower concentrations in those borne by ewes fed linseed or Megalac, as the amount of C20:4*n*-6 within ewe plasma at one week *pre-partum* was significantly higher in ewes supplemented with algae compared to linseed or Megalac.

Elongation and desaturation of C18:2*n*-6 may also be inhibited by other fatty acids, specifically C18:3*n*-3. Lambs produced by ewes fed linseed had proportions of C20:4*n*-6 within brain tissue similar to those of lambs borne by ewes given a preformed dietary supply of C20:4*n*-6. It can therefore suggested that the conversion of C18:2*n*-6 to C20:4*n*-6 was most efficient in lambs produced by ewes fed Megalac, was intermediate in those from ewes fed linseed due to competition from C18:3*n*-3 for Δ-desaturase enzymes and was lowest in those borne by ewes fed algae which was predicted to have the highest placental supply of C20:4*n*-6.

Su *et al.* (2001) evaluated the efficiency of supplementing foetal baboons with C18:3*n*-3 as an alternative to a preformed supply of C22:6*n*-3, and concluded that although C22:6*n*-3 is synthesised from C18:3*n*-3 and utilised by the brain, the process is eightfold less effective than supplementing the animal with a preformed supply. Within the current study, concentrations of C22:6*n*-3 were similar between neonatal lambs produced by ewes fed algae or linseed, indicating that endogenous synthesis was as effective as a preformed supply for deposition into brain. The experimental concentrate supplemented with algae contained 7.71 g/kg DM of C22:6*n*-3 whereas the linseed concentrate contained 54.9 g/kg DM C18:3*n*-3. Therefore, it can be seen that C18:3*n*-3 within the current study was just

over sevenfold less effective than preformed C22:6*n*-3 for deposition into brain, concurring with the results of Su *et al.* (2001).

Despite the lack of a dietary supply of C22:6*n*-3 in ewes fed Megalac *pre-partum*, lambs produced by these ewes had proportions of C22:6*n*-3 within brain tissue equivalent to lambs from ewes fed either algae or linseed. Given the low concentrations of this fatty acid within neonatal plasma, it may be suggested that, in the absence of a placental supply of either C18:3*n*-3 or preformed C22:6*n*-3, these lambs may have exhibited a preferential rate of uptake and deposition of C22:6*n*-3 into brain in an attempt to maintain long-chain PUFA concentrations. Both feral and domesticated sheep have a relatively low dietary supply of C18:3*n*-3 and no dietary preformed C22:6*n*-3 source, thereby a biochemical mechanism must exist to preferentially conserve and deposit C22:6*n*-3 into nervous tissue.

4.5.2.4. Lamb growth rate

Neonatal survival is dependent upon a combination of factors, including birthweight, perinatal vigour and the ability to ingest colostrum during the immediate postnatal period. Studies in neonatal lambs (Christley *et al.*, 2003) and piglets (Tuchscherer *et al.*, 2000) concluded that birthweight is an important factor; hypothermia may be increased by a high surface area: volume ratio (as exhibited by smaller animals) with a concurrent increase in mortality. Supplementation of the pregnant female with long-chain PUFAs has been shown to increase birthweight of both human (Olsen *et al.*, 1992; Crawford *et al.*, 1997; Smuts *et al.*, 2003) and animal (Rooke *et al.*, 2001a) offspring, however, no significant effect of adding algae or linseed to the diet of the pregnant ewe was observed upon lamb birthweight in the current study.

Post partum, one of the most important contributors to lamb growth rate is the energy and protein supplied by maternal milk. As long-chain PUFA supplementation of the lactating

animal has been shown to reduce milk yield, and particularly milk fat yield (Franklin *et al.*, 1999; Petit, 2002; Petit *et al.*, 2003), a decrease in lamb growth rate would have been expected to be exhibited by lambs suckling ewes offered diets AA and LL. However, no significant effects of dietary strategy were observed upon lamb growth rate, a result which concurs with those of Pickard *et al.* (2005). This result may be explained by the observation that although milk component concentrations were altered by dietary treatment, milk component yields were similar among dietary strategies.

One consequence of supplementing infant formulae with long-chain *n*-3 PUFAs is a concomitant reduction in C20:4*n*-6 supply (Hornstra *et al.* 1995). Koletzko (1992) concluded that C20:4*n*-6 deficiency is a significant factor in the reduced growth rate of human infants fed formulae. Therefore, it may be suggested that lambs with increased concentrations of not only *n*-3 fatty acids, but also C20:4*n*-6, may have an improved growth rate. However, despite the increased concentrations of C20:4*n*-6, C20:5*n*-3 and C22:6*n*-3 in plasma of lambs from treatment AA, this appears to have had little effect on lamb growth.

4.5.2.5. Lamb antioxidant status

Lamb erythrocyte GPx activities at birth were similar to values observed for pregnant ewes and gradually increased over time to reach a three-fold increase at three weeks of age. Studies indicate that the placental transfer of selenium may be limited in the ruminant (Jacobsson and Oksanen, 1966; Bostedt and Schramel, 1990). However, samples were taken from lambs at twelve hours *post partum* after considerable colostrum consumption. Selenium transfer from the ewe to the lamb is not constrained by any mammary barrier, hence the concurrence between concentrations in the lactating ewe and suckling lamb. Increasing the supply of long-chain PUFAs to the lamb, either by placental or mammary transfer, would be expected to increase the oxidative challenge with consequent reductions

in GPx activity. By contrast, Smith and Isopenko (1997) suggested that GPx activity increases in response to a peroxidative challenge. Results observed within the current study agree with those of the aforementioned authors in that PUFA supplementation of the ewe did not affect the activity of lamb erythrocyte GPx. The range of activities observed within the current study (95-315 U/ml PCV) concur with those reported by Smith *et al.* (1994) in weaner sheep.

Lamb serum CK concentrations were below the thresholds considered by El-Neweehy *et al.* (2000) to be indicative of sub-clinical (1186-3740 IU/l) or clinical (4291 IU/l) nutritional myopathy. High CK values observed in the neonatal lamb (12 hours of age) are likely to have been a consequence of the tissue damage incurred during parturition, with an increased margin for experimental error incurred by the difficulty of obtaining blood samples from these animals. Serum CK concentrations declined with age, reaching levels considerably lower than those reported by Clemens (1989) and Batra and Hidiroglou (1993) in pigs. Although the addition of linseed or algae to the maternal diet increased the dietary long-chain PUFA supply with a concurrent increase in peroxidative challenge to the animal, this challenge was not sufficient to induce tissue damage in the lamb, possibly as a consequence of the high maternal dietary vitamin E supply.

4.5.2.6. Suckling lamb plasma fatty acids at twelve hours of age

The rumen of the neonatal lamb is undeveloped and initially, free of the fanua needed for ruminal digestion (Skillman *et al.*, 2004). Consequently, the neonatal lamb, solely fed on colostrum, may be considered as a monogastic animal in terms of digestive process, without the potential to saturate PUFAs via the rumen. The fatty acid composition of the plasma lipid component is therefore directly linked to the fatty acids provided by colostrum from the ewe with a minor contribution made by fatty acids transferred across the placenta.

Total plasma fatty acids were increased approximately six-fold after the lamb had suckled, compared with the neonate. This result concurs with those of both Leat and Harrison (1980) and Raijon *et al.* (1985), both sets of authors concluding that lambs were deficient in EFAs at birth but achieved a satisfactory fatty acid status after ingestion of colostrum. Although the amount of colostrum ingested by the lamb over the first twelve hours after birth is difficult to assess, the high colostrum fat content, ranging from 97 g/kg to 136 g/kg will have been a significant factor in the enhancement of lamb fatty acid status.

The similarity in total plasma fatty acid concentrations among treatments suggests that lambs may be able to regulate their colostrum consumption according to their dietary requirements. Several studies have demonstrated that voluntary feed intake is depressed by the provision of long-chain PUFAs in adult animals, however, this tends to be a consequence of changes in ruminal function (Chilliard, 1993; Velasco *et al.*, 2001; Donovan *et al.*, 2000), which do not apply to the sucking lamb in the current situation. Studies in humans have reported that high-fat diets do not invoke the sensations of satiety after a meal produced by diets high in water, protein or carbohydrate (Blundell and MacDiarmid, 1997). If lambs are able to select their level of colostrum intake according to its fat content, it appears that this is not related to their level of satiation, but may be related to a biochemical or metabolic process. The enzyme cholecystokinin (CCK) has been related to the control of food intake, and its release into the monogastic stomach is stimulated by the presence of partly digested lipid and protein compounds (Crawley and Corwin, 1994). Therefore, it is possible that the lower fat content of the colostrum produced by ewes supplemented with diets containing linseed did not stimulate sufficient CCK production to limit colostrum intake compared to the other four diets.

By contrast, although the proportion of C16:0 within lamb plasma was similar between algae and Megalac treatments, lambs suckling ewes fed diets containing linseed had a

lower proportion of C16:0 within plasma. Long-chain PUFA supplementation of ruminants has been consistently demonstrated to reduce synthesis of C16:0 within the mammary gland and to lower the secretion of this fatty acid into colostrum and milk, therefore this result may have arisen as a consequence of effects of linseed supplementation on milk fatty acid composition. This result is in agreement with the work of Petit (2002), who reported reduced concentrations of C16:0 in milk of dairy cows fed whole linseed compared to Megalac. Ewes fed diets containing algae also had a significant dietary supply of long-chain PUFAs within the diet, but they were also supplemented with a preformed supply of C16:0 in the form of Megalac, and therefore may have been less reliant upon the endogenous synthesis of this fatty acid for secretion into colostrum and transfer to the lamb.

The proportions of C18:0 within plasma lipids of the suckling lamb at twelve hours of age reflect the fatty acid composition of ewe plasma at one week *pre-partum*, i.e. the lowest concentrations of C18:0 were found in lambs suckling ewes fed diets containing algae, intermediate in those suckling ewes consuming diets based on Megalac and highest in those suckling ewes supplemented with linseed. This may be attributed to changes in the deposition of C18:0 into milk and subsequent transfer to the lamb as a consequence of differences in ruminal biohydrogenation of unsaturated fatty acids among treatments. Augmenting the ruminant diet with long-chain PUFAs has been reported by Kitessa *et al.* (2001c) to disrupt ruminal biohydrogenation with consequent increases in the production of *trans* C18:1 fatty acids. This concurs with the results of the current study: the proportional contribution of C18:1 *trans* fatty acids to plasma fatty acids were significantly higher in lambs suckling ewes offered diets containing algae, and given the lack of a functioning rumen in these animals, may be attributed to the supply from colostrum. As demonstrated by Goodridge *et al.* (2001), feeding linseed to ruminants tends to increase the products of biohydrogenation within plasma and milk. The main product of ruminal

biohydrogenation of C18:3*n*-3 is C18:0, therefore, the increased dietary supply of C18:3*n*-3 to ewes fed linseed may be suggested to have significantly increased the proportion of C18:0 within colostrum and hence the concentration of this fatty acid in lamb plasma.

As observed for C18:0, the concentrations of C18:1*n*-9 *cis* within suckling lamb plasma mirrored those observed within ewe plasma at one week *pre-partum*, suggesting that similar patterns would have been observed in colostrum. Noble *et al.* (1978) observed lower concentrations of C18:1*n*-9 *cis* within the colostrum of ewes fed a protected PUFA supplement containing high concentrations of C18:2*n*-6, furthermore, Petit (2002) reported decreases in the concentrations of this fatty acid in milk of dairy cows supplemented with linseed compared to a Megalac control. In terms of algal supplementation, both Franklin *et al.* (1999) and Papadopoulos *et al.* (2002) observed decreases in C18:1*n*-9 concentration in milk fat as a consequence of adding algae to the ruminant diet. These effects may be extrapolated into colostrum and further into the plasma of suckling lambs.

Both Noble *et al.* (1978) and Raijon *et al.* (1985) described low concentrations of the EFAs C18:2*n*-6 and C18:3*n*-3 within neonatal lamb plasma, concluding that these fatty acids can only be transferred into plasma at satisfactory concentrations after ingestion and absorption of fatty acids from colostrum. These conclusions are borne out by the results of the current study, with considerably higher amounts of both C18:2*n*-6 and C18:3*n*-3 observed in suckling lambs at twelve hours of age compared to neonatal lambs immediately after birth. Concentrations of C18:2*n*-6 within lamb plasma reflect ewe plasma results at one week *pre-partum*, in that both sources of long-chain PUFAs (algae or linseed) depressed the concentration of this fatty acid within the plasma lipid fraction. This suggests a concurrent reduction in colostral C18:2*n*-6 concentration and subsequent drop in the efficiency of transfer to the suckling lamb. As previously discussed (section 4.5.1.4.), both Franklin *et al.* (1999) and Papdopoulos *et al.* (2002) demonstrated a reduction in the amount of

C18:2*n*-6 within milk of dairy cattle supplemented with marine algae. Petit (2002) also reported lower concentrations of C18:2*n*-6 in the milk fat of cows fed diets augmented with whole linseed. Therefore, it is likely that the positive relationship between lamb and ewe plasma C18:2*n*-6 is due to transfer of this fatty acid into milk at a concentration proportional to the amount found in ewe plasma.

As the precursor of C20:4*n*-6 within the ruminant, the proportion of C18:2*n*-6 within plasma of suckling lambs might have been expected to vary according to the demands for endogenous synthesis of long-chain *n*-6 PUFAs. Indeed, the study published by Shand *et al.* (1978) concluded that both neonatal and suckling lambs have the ability to elongate and desaturate C18:2*n*-6 in order to produce C20:4*n*-6 within the liver. However, potential differences in mammary transfer of C18:2*n*-6 into colostrum, with the added complication of the maternal dietary supply of preformed C20:4*n*-6 in ewes fed algae, make it difficult to draw meaningful conclusions regarding the efficiency of the endogenous production of C20:4*n*-6 in the suckling lamb.

Studies suggest that the suckling animal has the capacity to elongate and desaturate EFAs to their long-chain PUFA derivatives (Uauy *et al.*, 2000; Bazinet *et al.*, 2003) but the transfer of preformed long-chain n-3 PUFAs from colostrum may have depressed this synthesis. Dietary supply may have therefore been the major factor affecting the concentration of long-chain *n*-3 PUFAs in lamb plasma. The magnitude of the differences between dietary supply and plasma concentrations of C18:3*n*-3 in the ewe were considerable with a large proportion of the dietary C18:3*n*-3 appearing to be biohydrogenated or saturated. Further to this result, the five-fold increase in plasma C18:3*n*-3 concentrations of ewes fed linseed compared to those fed algae or Megalac was reduced to three-fold in plasma of lambs suckling ewes fed linseed compared to algae or Megalac. Suitable data were not available to calculate the transfer efficiency of ingested

C18:3*n*-3 into colostrum or milk within the current study, but the results suggest that C18:3*n*-3 may be transferred from the ewe to the lamb at an efficiency of approximately 70 %. This figure is only slightly higher than that suggested by Goodridge *et al.* (2002) who observed a transfer efficiency from a maternal diet high in C18:3*n*-3 into milk of 64 %.

A positive relationship appeared to exist between maternal plasma concentrations of C20:5*n*-3 and C22:6*n*-3 at one-week *pre-partum* with those in the suckling lamb at twelve hours *post partum*; the highest concentrations of C20:5*n*-3 observed in lambs suckling ewes fed algae or linseed compared to Megalac and the highest amounts of C22:6*n*-3 reported in lambs suckling ewes supplemented with algae compared to linseed or Megalac. Although the maternal supply of C18:3*n*-3 provided by the diets containing linseed was sufficient to provide equivalent concentrations of C22:6*n*-3 within brain tissue and plasma of neonatal lambs, lambs suckling ewes fed linseed had significantly less C22:6*n*-3 within plasma at twelve hours *post partum* than those suckling ewes fed algae. It can therefore be hypothesised that either the placenta has the ability to desaturate and elongate C18:3*n*-3 to C22:6*n*-3 as suggested by Noble *et al.* (1985), Koletzko (1992) and Williard *et al.* (2001) and that this characteristic is lacking in the mammary gland, or that the mechanisms by which C22:6*n*-3 is produced from C18:3*n*-3 are more effective in the ewe during pregnancy compared to lactation. Certainly, an endogenous mechanism for C22:6*n*-3 production must exist given the similarity between brain C22:6*n*-3 concentrations in lambs from ewes fed algae, linseed or Megalac, despite differing maternal dietary supply of C22:6*n*-3. As with the partitioning and synthesis of other metabolic products and chemicals during pregnancy, it is possible that there may be endocrine effects upon the rate of endogenous synthesis of C22:6*n*-3 which have not yet been investigated. As the greater part of nervous system and brain development occur during lamb foetal development, a biochemical mechanism by which C22:6*n*-3 synthesis is maximised during the last few weeks of pregnancy and minimised during lactation would maximise ewe and lamb

nutritional resources. However, the existence of such a mechanism has yet to be demonstrated in the ruminant.

4.5.2.7. Suckling lamb plasma fatty acids at three weeks of age

At three weeks of age, digestion in suckling lambs with no access to forage occurs by a similar process to that of the monogastric, with a high positive correlation being expected between the fatty acid composition of ewe milk and lamb plasma. Despite differences in milk fat yield among treatments, the concentration of total fatty acids in lamb plasma was similar among treatments, suggesting that all lambs had a similar dietary fat supply. Although a proportion of ingested fatty acids are utilised for specific metabolic purposes, the majority of ingested fat in the suckling lamb is oxidised and used as an energy source (Palmquist, 1984). Therefore, despite the variation in milk fat yield, the concurrence among lamb plasma fatty acid concentrations may explain the lack of a significant treatment effect upon lamb growth rate.

The relatively high concentration of C16:0 within lamb plasma was not unexpected as this fatty acid predominates within milk fat. However, various studies have demonstrated a reduction in fatty acid synthesis within the mammary gland as a result of long-chain PUFA supplementation (Brzóska *et al.*, 1999; Donovan *et al.*, 2000), therefore the lack of any treatment effect upon the plasma concentration of this fatty acid was surprising. It is notable that a lower proportion of C16:0 was present within the plasma of lambs sucking ewes supplemented with dietary strategies AL and LL, i.e. those fed linseed during lactation. Diets based on either algae or Megalac both contained a significant proportion of C16:0 as a result of Megalac being added to balance fatty acid concentrations. Therefore, the lower dietary supply of C16:0 conferred by the linseed diet may have reduced proportions of this fatty acids in milk with a concomitant reduction in C16:0 within lamb plasma.

Augmentation of maternal diets with linseed during lactation increased the proportion of C18:0 within lamb plasma fatty acids at three weeks *post partum*. This may be attributed to ruminal biohydrogenation of C18:2*n*-6 and C18:3*n*-3 within the linseed diet increasing the duodenal flow of C18:0 concurrent deposition into plasma and milk. *Trans* C18:1 fatty acids have been implicated as one of the major causes of milk fat depression in lactating ruminants fed long-chain PUFAs (Romo *et al.*, 1996; Brzóska *et al.*, 1999; Bauman and Griinari, 2001), their effect attributed to a decrease in the efficiency of enzymes responsible for fatty acid synthesis within the mammary gland (Ahnadi *et al.*, 1998). Increased concentrations of C18:1 *trans* were observed in colostrum and milk fat of ewes as a result of PUFA supplementation within Experiment One, with a concurrent reduction in milk fat concentration. The increase in C18:1 *trans* concentration within plasma of lambs suckling ewes offered dietary strategy AA positively correlates with the reduced milk fat yields of ewes fed this diet. Furthermore, as C18:1 *trans* is principally produced by ruminal biohydrogenation of PUFAs, the monogastric status of the suckling lamb indicates that this fatty acid which must have been supplied preformed from the diet. A significant carry-over effect of algal supplementation upon C18:1 *trans* concentrations within lamb plasma was observed when data were analysed according to maternal diet *pre-partum* (Algae vs. Megalac, $P<0.05$; Algae vs. Linseed, $P<0.05$, data not shown) which may explain the carry-over effects of algal supplementation during pregnancy upon milk fat concentration during lactation.

As observed within ewe plasma at one week *pre-partum*, supplementing the maternal diet with algae during lactation reduced the proportion of C18:1*n*-9 *cis* within plasma fatty acids of the suckling lamb. This implies that the amount of this fatty acid within milk was reduced by algal supplementation, a result which concurs with those observed within Experiment One as a result of fish oil supplementation. The reason behind the drop in C18:1*n*-9 *cis* concentrations in lambs supplemented with long-chain PUFAs is not clear,

and is in direct contrast to the results reported by Offer *et al.* (2001), in which dairy cows fed fish oil had higher concentrations of C18:1*n*-9 *cis* within the lipid fractions of plasma.

Concentrations of C18:2*n*-6 are relatively low within milk fat (Shingfield *et al.*, 2003), although, as observed within Experiment One, this was one of the predominant fatty acids within lamb plasma lipids. Neonatal lambs are generally considered to be deficient in EFAs (Leat, 1996; Payne, 1978), but Noble *et al.* (1971) suggested that C18:2*n*-6 may be preferentially accumulated in phosphatidylglycerols to overcome the deficiency effects observed before milk consumption. Therefore, a compensatory mechanism may exist to maintain the EFA status of the suckling lamb, despite the low dietary supply.

Although it is possible to suggest differences in the efficiency of C20:5*n*-3 and C22:6*n*-3 synthesis among treatments, only maternal dietary supply appeared to have any significant effect upon concentrations of C20:4*n*-6 within the suckling lamb. Furthermore, supplementation of the ewe within algae during pregnancy continued to have an augmenting effect upon the amount of C20:4*n*-6 within lamb plasma at three weeks of age. It is hypothesised that deposition of C20:4*n*-6 into maternal phosphatidylglycerols during the period of supplementation, followed by mobilisation of body reserves during lactation may have produced a labile source of C20:4*n*-6 for secretion into milk, thereby increasing the dietary supply to the lamb.

Bazinet *et al.* (2003) observed a significant increase in the amount of C18:3*n*-3 within piglet plasma and tissues as a consequence of supplementing the diet of the lactating sow with linseed oil. By contrast, C18:3*n*-3 was present as a minor constituent of the fatty acid fraction of suckling lamb plasma, at proportions comparable to those reported by Palmquist *et al.* (1977) in suckling lambs. Various studies have demonstrated increases in the concentration of C18:3*n*-3 within milk conferred by linseed supplementation

(Kennelly, 1996; Brzóska *et al.*, 1999; Petit *et al.*, 2002), however, if this phenomenon occurred, it was not carried further into an increase in the proportion of C18:3*n*-3 within lamb plasma.

One aim of the current study was to evaluate linseed as a replacement for fish oil within the diet of the pregnant and lactating ewe as a precursor for endogenous synthesis of C20:5*n*-3 and C22:6*n*-3. Bazinet *et al.* (2003) noted a significant increase in the proportion of C20:5*n*-3 and C22:6*n*-3 within plasma of piglets suckling sows supplemented with linseed oil. Furthermore, feeding formulas high in C18:3*n*-3 increased the deposition of C20:5*n*-3 and C22:6*n*-3 into neural tissues of piglets in the study published by Arbuckle and Innis (1992). However, studies in neonatal humans (Salem *et al.*, 1996), primates (Su *et al.*, 1999; Su *et al.*, 2001) and rats (Woods *et al.*, 1996) have reported that C18:3*n*-3 is significantly less effective as a source of C22:6*n*-3 for deposition into tissues compared to preformed C22:6*n*-3. No C20:5*n*-3 was present in any of the treatment diets supplied to the ewes, and the amounts present in plasma are suggested to be derived from C18:3*n*-3 via elongation and desaturation, or by peroxisomal retroconversion of C22:6*n*-3. The proportional contribution of C20:5*n*-3 to plasma fatty acids was increased by linseed supplementation of the ewe, suggesting that this fat source was effective in increasing C20:5*n*-3 deposition in the lamb, but concentrations were not as high as those observed in lambs suckling ewes fed algae. However, C22:6*n*-3 deposition in suckling lamb plasma was unaffected by linseed supplementation compared to Megalac, suggesting that the rate-limiting step in endogenous C22:6*n*-3 synthesis may be the conversion of C20:5*n*-3 to C22:6*n*-3 within microsomes. Despite differences in ewe plasma fatty acid composition, C22:6*n*-3 and C20:5*n*-3 concentrations were similar in suckling lamb plasma as a result of algal supplementation, suggesting either that a substantial amount of retroconversion occurred within the lamb to fulfil C20:5*n*-3 requirements, or that a selective transfer

against C22:6*n*-3 occurred within the mammary gland, thereby reducing the dietary C22:6*n*-3 supply to the lamb.

4.6. Conclusion

Both marine algae and linseed were effective in improving neonatal lamb vigour in terms of reducing the latency of suckling, however, the deposition of C22:6*n*-3 into neonatal lamb brain tissue was unaffected by treatment diet. Furthermore, neither preformed C22:6*n*-3 nor its precursor fatty acid C18:3*n*-3 had a significant effect upon ewe gestation length. Changing from a diet containing long-chain PUFAs to a saturated fatty acid source during lactation negated the decrease in lamb growth rate observed with long-chain PUFA supplementation during lactation as observed previously. Significant carry-over effects of *pre-partum* diet upon milk composition and yield warrant further investigation.

5. FISH OIL AND VITAMIN E SUPPLEMENTATION OF EWES DURING PREGNANCY AND LACTATION: EFFECTS UPON EWE AND LAMB PERFORMANCE

5.1. Introduction

The first experiment demonstrated that supplementing pregnant ewes with long-chain PUFAs in the form of fish oil significantly increased gestation length and reduced the latencies of neonatal lamb behaviours. Furthermore, increasing the vitamin E supply to the pregnant ewe augmented the concentrations of this vitamin within brain and muscle tissue, and improved lamb birthweight. However, continuing the long-chain PUFA supplementation into lactation significantly reduced milk fat concentration and yield and depressed lamb growth rate.

In an attempt to define the specific fatty acids responsible for changes in gestation length and neonatal behaviour, the second experiment employed a species of marine algae high in DHA within the diet. Linseed was used as an alternative fatty acid source to assess whether endogenous synthesis of C22:6*n*-3 from C18:3*n*-3 was adequate for the deposition of C22:6*n*-3 into lamb brain tissue. Although supplementation with either linseed or algae improved latencies of lamb behaviour, there was no effect upon gestation length, suggesting that C22:6*n*-3 supply was not wholly responsible for the effects observed in Experiment One. Changing to a diet containing Megalac during lactation negated the effects of PUFA supplementation upon lamb growth rate, however, significant carry-over effects of algal supplementation during pregnancy were observed upon milk production.

To determine the ideal dietary strategy for improving lamb vigour and birthweight, without reducing growth rate, the current study returned to the use of fish oil as the principal fatty acid source during pregnancy, followed by Megalac in lactation. This was considered to

provide a source of both C20:5*n*-3, thought to increase gestation length; and C22:6*n*-3 for deposition into lamb brain with concurrent effects upon vigour, whilst abrogating negative effects of PUFAs during lactation. Although promising results were observed within Experiment One, the extent of vitamin E transfer from the ewe to the lamb remains unclear. The effects of supplementing ewes with either basal or supranutritional dietary concentrations of vitamin E were therefore re-explored.

5.2. Objectives

1) To investigate the targeted supplementation of ewes with fish oil during pregnancy followed by Megalac® during lactation upon ewe and lamb behaviour and performance.

2) To investigate the supplementation of pregnant and lactating ewes with supranutritional concentrations of vitamin E upon ewe and lamb behaviour and performance.

5.3. Materials and Methods

5.3.1. Experimental animals and housing

Thirty twin-bearing ewes with a mean age of 3.2 years (s.d. 1.25), liveweight of 73.1 kg (s.d. 5.82) and body condition score of 3.1 units (s.d. 0.21) were selected from the Harper Adams University College early lambing flock (Edgmond, Newport, Shropshire, UK). All ewes were Suffolk x North of England mules, blocked according to age, condition score and liveweight and randomly allocated to one of three strategies within a randomised block design. Ewes were housed, individually penned and bedded on sawdust from week 15 of pregnancy (designated week -6) until week 4 (week +4) of lactation. An additional nine twin-bearing ewes were housed in three group pens until parturition, bedded on straw and fed one of three concentrates (three ewes per treatment, diets ML, FL or FH). These ewes provided nine neonatal lambs (one per ewe) for sacrifice. The building was continually lit and all ewes had free access to fresh water supplies.

5.3.2. Experimental diets

A basal ration was formulated containing barley, sugar beet pulp, soyabean meal, urea and molasses (Table 5.1). To this diet was added 59 g of long-chain PUFAs in the form of pilchard/mackerel oil with an *n*-3 fatty acid content of approximately 30 g/kg (United Fish Industries, Gilbey Road, Grimsby, South Humberside, UK) or a control saturated fat (C16:0; Megalac®; Volac UK Ltd, Royston House, Royston, UK). Butylated hydroxytoluene was added as an antioxidant to the fish oil at a rate of at 500 mg/kg. Exactly 30 g/kg of vitamin/mineral supplement containing either 50 mg/kg or 500 mg/kg vitamin E was also added to each diet. Numerous studies have demonstrated that protecting fatty acids from ruminal biohydrogenation increases their absorption and deposition in ruminant tissues and milk (Jenkins, 1993; Kitessa *et al.* 2001a; Gulati *et al.*, 2002), however, it is not clear whether adsorbing fish oil onto vermiculite is an efficient protection method. Therefore, unprotected fish oil was employed within the treatment concentrates. The resulting concentrates were isoenergetic and isonitrogenous with a predicted nutrient composition of 14.1 MJ/kg DM metabolisable energy, 255 g/kg DM crude protein and 80.3 g/kg DM fatty acids.

The treatment concentrates were fed in three dietary strategies in an attempt to reduce the detrimental effect of long-chain PUFA supplementation upon milk composition and lamb growth rate. Therefore, during pregnancy, 20 ewes were fed fish oil in combination with either a basal or supranutritional concentration of vitamin E, and 10 ewes were fed Megalac® with a basal concentration of vitamin E. The ewes fed fish oil were then changed to a Megalac®-based concentrate at 24 hours *post partum*, each concentrate again containing either a basal or supranutritional concentration of vitamin E.

The treatment diets were therefore:

MML: Megalac® + basal vitamin E during pregnancy and lactation (ML)

FML: Fish oil + basal vitamin E during pregnancy (FL)

Megalac® + basal vitamin E during lactation (ML)

FMH: Fish oil + supranutritional vitamin E during pregnancy (FH)

Megalac® + supranutritional vitamin E during lactation (MH)

Table 5.1. Raw material and chemical composition of the four treatment concentrates

	ML	MH	FL	FH
Raw material composition (g/kg)				
Barley	536	536	545	545
Sugar beet pulp	100	100	100	100
Soyabean meal	200	200	200	200
Fish oil	-	-	59	59
Megalac®	68	68	-	-
Molasses	50	50	50	50
Urea	16	16	16	16
Vitamins/Minerals	30	30	30	30
Predicted¶ chemical composition (g/kg DM)				
DM (g/kg)	864	864	862	862
CP	254	254	255	255
ERDP*	152	152	153	153
DUP*	83	83	81	81
EE	80	80	80	80
NDF	198	198	200	200
Ash	83	83	74	74
Vitamin E (mg/kg in premix)	500	500	50	500
ME (MJ/kg DM)	14.2	14.2	14.1	14.1
FME (MJ/kg DM)	11.1	11.1	11.2	11.2
ERDP:FME Ratio	13.7	13.7	13.7	13.7

Vitamin/Mineral supplement (Hac Ewe 25, Roche Products Limited, Heanor, Derbyshire) supplied per kg of diet: Calcium 7.06 g; Sodium 2.67 g; Phosphorus 1.65 g; Selenium 0.36 mg; Vitamin A 14,400 IU; Vitamin D 30,000 IU; Vitamin E 50 mg or 500 mg.

¶ (AFRC, 1993)

* calculated according to AFRC (1993) at a rumen outflow rate of 0.08 ml/hour

Ewes were fed a stepped concentrate ration (Table 5.2) in two equal meals per day (at 08:00 and 16:00) during pregnancy and at a flat-rate of 1.7 kg/day in three meals per day (at 08:00, 12:00 and 16:00) during lactation. Straw was fed at a flat rate of 0.8 kg/day (freshweight) during pregnancy and was increased from week +1 onwards (Table 5.3). Straw refusals were weighed back weekly.

Table 5.2. Daily concentrate allowance for ewes

Day of gestation	110	117	124	131	138	145	Lactation
Daily concentrate allowance (kg freshweight)	0.6	0.7	0.8	0.9	1.0	1.1	1.7

Table 5.3. Daily forage allowance for ewes

Day of lactation	*Pregnancy*	Day of lactation		
		7	14	21
Daily straw allowance (kg freshweight)	0.8	1.0	1.2	1.4

5.3.3. Experimental Procedure

Concentrate and straw sub-samples were taken weekly and stored in airtight bags at –20 °C until analysis. Ewe liveweight, body condition score, maternal and neonatal behaviour, lamb birthweight, lamb liveweight and milk production were measured as previously described in Chapter Two.

5.3.3.1. Blood sampling

Blood samples were obtained from ewes by jugular venepuncture at 11:00 at six weeks (day 103, before the experimental concentrates were fed) and one week (138 of gestation) *pre-partum*; at 12 hours *post partum* and at two weeks into lactation. Lamb blood and tissue samples were taken from nine neonatal lambs immediately after cessation of the heartbeat as described in section 2.2.4. Blood samples were taken from growing lambs by jugular venepuncture at +24 hours of age and at 11:00 at two weeks of age. The rectal temperature of each lamb was recorded at three hours *post partum* using a LifeSource flex-tip digital thermometer (A&D Medical, 1555 McCandless Drive, Milpitas, CA 95035, USA).

5.3.4. Sample analysis

Concentrate and straw samples were analysed for DM, ash, CP and NDF, and concentrate samples analysed for vitamin E and fatty acid composition. Ewe blood samples were

analysed for urea, βHB, CK and GPx at all time points and for fatty acids and vitamin E at days 103 and 131 of gestation and at 14 days into lactation. Neonatal lamb blood samples were analysed for fatty acids and vitamin E; suckling lamb blood samples were analysed for CK and GPx at all time points and for vitamin E and fatty acids at +24 hours and 14 days of age. All analyses are described in Chapter Two.

5.3.5. Statistical analysis

Data were analysed as a randomised block design. Lamb sex was used as a co-variate when analysing lamb birthweight, liveweight and growth rate data. Plotting lamb liveweight against time revealed linear growth rates, therefore overall growth rates were calculated using linear regression. All analyses utilised the ANOVA function within Genstat 6 version 6.2 (Lawes Agricultural Trust, 2002).

5.4. Results

Data from one ewe (treatment FMH) was excluded from the results before analysis as the subject lambed at 139 days of gestation. Data collected from the lambs produced by this ewe were also excluded from statistical analysis.

5.4.1. Diet composition

All parameters relating to feed chemical composition (DM, OM, CP, ash, NDF, total fatty acids) were similar between treatment concentrates (Table 5.). Vitamin E concentrations were similar to those predicted when formulating the diets.

Table 5.4. Chemical composition of the four treatment concentrates plus the straw

	Concentrate				Straw
	MML	MH	FML	FMH	
Dry matter (g/kg)	887	887	883	882	857
Crude protein (g/kg DM)	247	247	254	249	59
Organic matter (g/kg DM)	905	901	905	912	909
Ash (g/kg DM)	95	99	95	88	91
Neutral detergent fibre (g/kg DM)	189	192	188	192	790
Vitamin E (g/kg DM)	65	568	67	521	-
Total fatty acids (g/kg DM)	88	95	95	103	-

MML = Megalac + 50 mg/kg vitamin E; MH = Megalac + 500 mg/kg vitamin E; FML = Fish oil + 50 mg/kg vitamin E; FMH = Fish oil + 500 mg/kg vitamin E

Table 5.5. Fatty acid composition of the four treatment concentrates

	Concentrate			
Fatty acid (g/kg DM)	MML	MH	FML	FMH
C16:0	38.1	41.4	14.7	15.2
C16:1*n*-7	0.18	0.23	3.37	3.67
C18:0	3.69	3.82	2.79	2.68
C18:1 *trans*	ND	ND	0.60	0.85
C18:1*n*-9 *cis*	23.1	25.4	1.92	2.01
C18:2*n*-6 *cis*	18.0	18.4	13.7	14.7
C18:3*n*-3 *cis*	1.65	1.55	1.92	2.08
C20:4*n*-6	0.10	0.13	0.14	0.13
C20:5*n*-3	ND	ND	4.50	4.94
C22:6*n*-3	ND	ND	6.12	6.76
RFA[1]	3.18	4.07	45.2	50.0

MML = Megalac + 50 mg/kg vitamin E; MH = Megalac + 500 mg/kg vitamin E; FML = Fish oil + 50 mg/kg vitamin E; FMH = Fish oil + 500 mg/kg vitamin E

[1] RFA = Remaining fatty acids; ND = not detected

The predominant fatty acids contained within the Megalac concentrates (MML and MMH) were C16:0, C18:1*n*-9 *cis* and C18:2*n*-6, whilst the *n*-3 fatty acids C20:5*n*-3 and C22:6*n*-3

were not detected (Table 5.5). By contrast, the concentrates containing fish oil (FML and FMH) contained approximately 4.72 g/kg DM of C20:5*n*-3 and 6.44 g/kg DM of C22:3*n*-3. All experimental concentrates had a similar total fatty acid concentration at 95 g/kg DM.

Table 5.6. Effect of PUFA and vitamin E supplementation of ewes on daily straw intakes

	Dietary strategy			s.e.d.	P
	MML	FML	FMH		
Pre-partum intake (kg DM):					
6 weeks[¶] *pre-partum*	0.58	0.56	0.54	0.027	0.270
1 week[¶] *pre-partum*	0.55[b]	0.49[a]	0.51[a]	0.018	0.012
Pre-partum intake (kg DM/day)	0.58[b]	0.54[a]	0.52[a]	0.020	0.027
Post partum intake (kg DM):					
0 weeks[¶] *post partum*	0.61	0.55	0.59	0.035	0.218
3 weeks[¶] *post partum*	1.06	0.98	1.03	0.031	0.067
Post partum intake (kg DM/day)	0.80	0.76	0.78	0.037	0.546

MML = Megalac + 50 mg/kg vitamin E fed throughout pregnancy and lactation; FML = Fish oil + 50 mg/kg vitamin E in pregnancy followed by Megalac + 50 mg/kg vitamin E in lactation; FMH = Fish oil + 500 mg/kg vitamin E in pregnancy followed by Megalac + 500 mg/kg vitamin E in lactation

[¶] 6 weeks *pre-partum* = mean straw intake on days 103-110 of gestation; 1 week *pre-partum* = mean straw intake on days 138-145 of gestation; 0 weeks *post partum* = mean straw intake on days 0 – 7 of lactation, 3 weeks *post partum* = mean straw intake on days 21 – 28 of lactation

Means without common superscripts are significantly different at the $P<0.05$ level

5.4.2. Ewe performance parameters

5.4.2.1. Straw intake

Dietary treatment had no significant effect upon daily straw intake between weeks –6 and –4 (Figure 5.1); however, ewes fed diets FML or FMH had significantly lower daily intakes than ewes fed diets MML from week –3 until parturition. The mean *pre-partum* straw intake was significantly lower for ewes fed fish oil as the main fat source (diets FML and FMH) compared to those fed Megalac (diet MML; Table 5.6), but, there was no effect of vitamin E concentration. Daily straw intakes increased between parturition and week +3 of lactation for ewes on all treatments. Small numerical differences in daily straw intake were again observed *post partum*, however, no significant effect of dietary treatment was in evidence upon daily or mean *post partum* intakes.

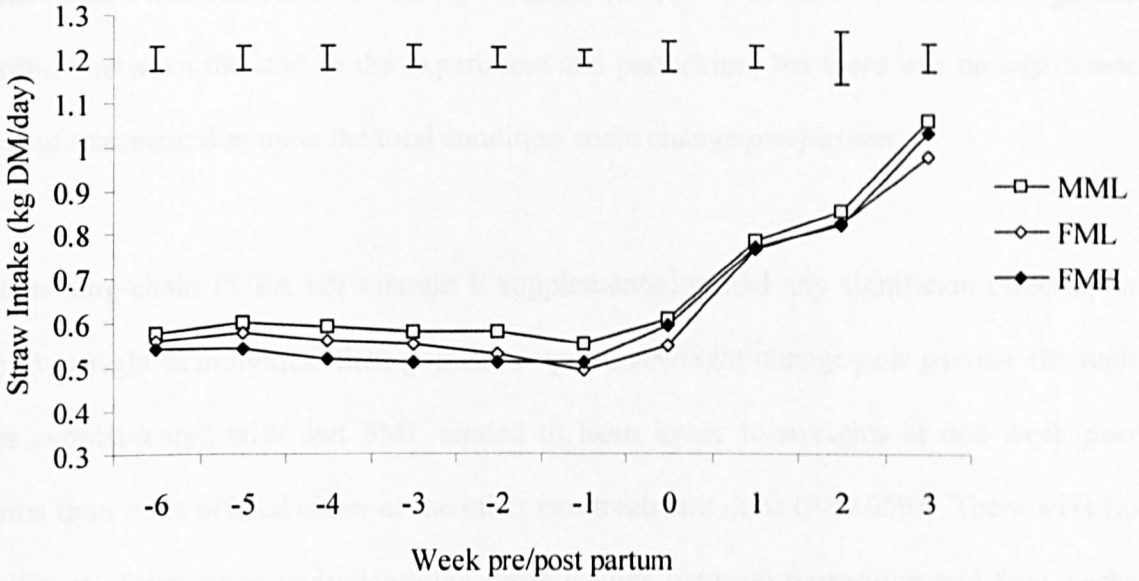


Figure 5.1. Effect of PUFA and vitamin E supplementation of ewes on daily straw intakes

Table 5.7. Effect of PUFA and vitamin E supplementation of ewes on liveweight and body condition score (CS) change

	Dietary strategy			s.e.d.	P
	MML	FML	FMH		
Pre-partum weight (kg):					
6 weeks¶ *pre-partum*	83.2	81.1	85.0	2.02	0.189
1 week¶ *pre-partum*	91.2	87.2	91.1	1.96	0.093
Pre-partum change	8.00	6.22	6.07	0.868	0.073
Pre-partum CS:					
6 weeks¶ *pre-partum*	3.12	3.12	3.12	-	-
1 week¶ *pre-partum*	3.38	3.25	3.38	0.095	0.338
Pre-partum change	0.25	0.17	0.25	0.091	0.584
Post partum weight (kg):					
1 week¶ *post partum*	77.1	73.9	78.0	1.68	0.059
4 weeks¶ *post partum*	72.4	71.0	73.7	2.06	0.436
Post partum change	-4.64	-2.89	-4.34	0.937	0.166
Post partum CS:					
1 week¶ *post partum*	2.93	2.83	2.85	0.113	0.662
4 weeks¶ *post partum*	2.38	2.28	2.43	0.097	0.311
Post partum change	-0.55	-0.56	-0.43	0.095	0.320

MML = Megalac + 50 mg/kg vitamin E fed throughout pregnancy and lactation; FML = Fish oil + 50 mg/kg vitamin E in pregnancy followed by Megalac + 50 mg/kg vitamin E in lactation; FMH = Fish oil + 500 mg/kg vitamin E in pregnancy followed by Megalac + 500 mg/kg vitamin E in lactation

¶ 6 weeks *pre-partum* = day 103 of gestation; 1 week *pre-partum* = day 138 of gestation; 1 week *post partum* = day 7 of lactation; 4 weeks *post partum* = day 28 of lactation

5.4.2.2. Liveweight and condition score

Dietary treatment had no significant effect upon ewe weights during pregnancy although ewes offered diets containing fish oil (FML and FMH) tended to gain less weight *pre-*

partum than ewes fed diets containing Megalac (P=0.073; Table 5.7). All ewes gained condition between the start of the experiment and parturition, but there was no significant effect of treatment diet upon the total condition score change *pre-partum*.

Neither long-chain PUFA nor vitamin E supplementation had any significant effect upon ewe liveweight at individual time points, or upon liveweight change *post partum* although ewes supplemented with diet FML tended to have lower liveweights at one week *post partum* than ewes offered either of the other two treatment diets (P=0.059). There were no significant effects upon body condition score change between parturition and four weeks into lactation.

5.4.2.3. Metabolic profiles

There was no significant effect of treatment strategy upon plasma βHB during pregnancy (Table 5.8). However, ewes supplemented with fish oil plus a low concentration of vitamin E (FML) tended to have increased βHB concentrations at two weeks *post partum* compared to ewes fed strategies FMH or MML (P=0.087).

Table 5.8. Effect of PUFA and vitamin E supplementation of ewes on concentrations of plasma β-hydroxybutyrate and urea

	Dietary strategy			s.e.d.	P
	MML	FML	FMH		
Plasma βHB concentration (mmol/l):					
6 weeks[1] *pre-partum*	1.12	0.98	0.95	0.137	0.437
1 week[1] *pre-partum*	1.53	1.00	0.72	0.373	0.117
2 weeks[1] *post partum*	1.01	1.39	0.91	0.208	0.087
Plasma urea concentration (mmol/l):					
6 weeks[1] *pre-partum*	6.41	5.70	5.38	0.531	0.171
1 week[1] *pre-partum*	7.05[a]	7.69[a]	8.98[b]	0.422	<0.001
2 weeks[1] *post partum*	9.39	9.54	10.41	0.695	0.318

MML = Megalac + 50 mg/kg vitamin E fed throughout pregnancy and lactation; FML = Fish oil + 50 mg/kg vitamin E in pregnancy followed by Megalac + 50 mg/kg vitamin E in lactation; FMH = Fish oil + 500 mg/kg vitamin E in pregnancy followed by Megalac + 500 mg/kg vitamin E in lactation

[1] 6 weeks *pre-partum* = day 103 of gestation; 1 week *pre-partum* = day 138 of gestation; 2 weeks *post partum* = day 14 of lactation

Means without common superscripts are significantly different at the P<0.05 level

The concentration of urea in ewe plasma increased during pregnancy (between week –6 and week –1) and again during lactation, regardless of dietary treatment (Table 5.8). Ewes fed fish oil plus a supranutritional concentration of vitamin E (strategy FMH) during pregnancy had higher levels of plasma urea at one week *pre-partum* compared to ewes offered strategies MML and FML ($P<0.001$). Although a similar pattern in plasma urea concentrations was evident between treatments during lactation, the differences were not significantly different.

5.4.2.4. Antioxidant status

Maternal plasma vitamin E concentrations at one week *pre-partum* were significantly increased by adding supranutritional concentrations of vitamin E to the fish oil based diet (Table 5.9), but no significant difference was observed between ewes fed fish oil or Megalac in combination with a basal vitamin E concentration. During lactation, highest plasma vitamin E concentrations were again observed in ewes offered strategy FMH compared to strategies FML or MML.

Table 5.9. Effect of PUFA and vitamin E supplementation of ewes on plasma vitamin E concentrations

	Dietary strategy			s.e.d.	P
	MML	FML	FMH		
Plasma vitamin E concentration (μmol/l):					
1 week[¶] *pre-partum*	2.94[ab]	1.75[a]	3.45[b]	0.425	0.004
2 weeks[¶] *post partum*	2.51[a]	1.99[a]	5.09[b]	0.349	<0.001

MML = Megalac + 50 mg/kg vitamin E fed throughout pregnancy and lactation; FML = Fish oil + 50 mg/kg vitamin E in pregnancy followed by Megalac + 50 mg/kg vitamin E in lactation; FMH = Fish oil + 500 mg/kg vitamin E in pregnancy followed by Megalac + 500 mg/kg vitamin E in lactation

[¶] 1 week *pre-partum* = day 138 of gestation; 2 weeks *post partum* = day 14 of lactation

Means without common superscripts are significantly different at the $P<0.05$ level

The selenium status of all ewes as indicated by the activity of GPx in erythrocytes, increased between the start of the experiment and parturition, and further increased in lactation (Table 5.10). No significant treatment effects were observed upon erythrocyte GPx activities at any time point. Serum CK concentrations declined between the start of

the experiment and week –1 in all ewes (Table 5.10). Neither long-chain PUFA nor vitamin E supplementation had any significant effect on this parameter during pregnancy or lactation.

Table 5.10. Effect of PUFA and vitamin E supplementation of ewes on indicators of selenium status and of cellular damage

	Dietary strategy			s.e.d.	P
	MML	FML	FMH		
Erythrocyte GPx activity (U/ml PCV):					
6 weeks[¶] *pre-partum*	129	138	140	23.1	0.881
1 week[¶] *pre-partum*	168	173	194	21.2	0.453
2 weeks[¶] *post partum*	208	225	229	19.3	0.503
Serum CK activity (U/l):					
6 weeks[¶] *pre-partum*	188	253	180	33.5	0.087
1 week[¶] *pre-partum*	155	191	125	38.5	0.254
2 weeks[¶] *post partum*	163	119	129	27.6	0.275

MML = Megalac + 50 mg/kg vitamin E fed throughout pregnancy and lactation; FML = Fish oil + 50 mg/kg vitamin E in pregnancy followed by Megalac + 50 mg/kg vitamin E in lactation; FMH = Fish oil + 500 mg/kg vitamin E in pregnancy followed by Megalac + 500 mg/kg vitamin E in lactation

[¶] 6 weeks *pre-partum* = day 103 of gestation; 1 week *pre-partum* = day 138 of gestation; 2 weeks *post partum* = day 14 of lactation

Table 5.11. Effect of PUFA and vitamin E supplementation of ewes on the proportions of individual fatty acids in ewe plasma samples collected at six weeks[¶] pre-partum (pre-treatment)

	Dietary strategy			s.e.d.	P
Fatty acid (g/100 g fatty acids)	**MML**	**FML**	**FMH**		
C16:0	17.6	17.6	17.5	0.47	0.987
C16:1*n*-7	0.92	0.86	0.86	0.030	0.083
C18:0	23.9	24.7	24.7	0.62	0.314
C18:1 *trans*	4.86	4.42	4.24	0.355	0.244
C18:1*n*-9 *cis*	22.2[b]	20.2[a]	20.9[a]	0.733	0.045
C18:2*n*-6 *cis*	7.05	8.27	8.23	0.532	0.063
CLA (*cis*-9,*trans*-11)	0.31	0.50	0.31	0.492	0.279
C18:3*n*-3 *cis*	2.45	2.56	2.34	0.197	0.558
C20:4*n*-6	3.21	3.43	3.80	0.268	0.119
C20:5*n*-3	2.05	1.92	1.90	0.201	0.733
C22:6*n*-3	1.38	1.63	1.55	0.261	0.633
RFA[¶]	14.2	14.0	13.6	0.49	0.562
Total fatty acids (mg/ml)	1.06	0.978	0.917	0.0798	0.199

MML = Megalac + 50 mg/kg vitamin E fed throughout pregnancy and lactation; FML = Fish oil + 50 mg/kg vitamin E in pregnancy followed by Megalac + 50 mg/kg vitamin E in lactation; FMH = Fish oil + 500 mg/kg vitamin E in pregnancy followed by Megalac + 500 mg/kg vitamin E in lactation

[¶] six weeks *pre-partum* = day 103 of pregnancy

[§]RFA = All remaining fatty acids

Means without common superscripts are significantly different at the P<0.05 level

5.4.2.5. Plasma fatty acids (six weeks *pre-partum*)

The proportions of all individual fatty acids within ewe plasma were similar between treatments at six weeks *pre-partum* (Table 5.11) save for the proportion of C18:1*n*-9 *cis* which was higher in ewes allocated to strategy MML compared to the other two strategies (P=0.045).

5.4.2.6. Plasma fatty acids (one week *pre-partum*)

Total fatty acid concentrations within ewe plasma samples at one week *pre-partum* were significantly lower in ewes fed diets containing long-chain PUFAs (FML and FMH) during pregnancy compared to those offered diets containing Megalac (MML), regardless of the dietary vitamin E concentration (Table 5.12). Proportions of C16:0 and C18:0 within plasma lipid were significantly lower in ewes offered diets containing fish oil (FML and FMH) compared to those fed diets containing Megalac as the principal fat source.

Table 5.12. Effect of PUFA and vitamin E supplementation of ewes on the proportions of individual fatty acids in ewe plasma samples collected at one week[¶] pre-partum

	Dietary strategy			s.e.d.	P
Fatty acid (g/100 g fatty acids)	**MML**	**FML**	**FMH**		
C16:0	24.0[b]	16.0[a]	16.0[a]	0.45	<0.001
C16:1*n*-7	0.55[a]	1.17[b]	1.13[b]	0.136	<0.001
C18:0	22.2[b]	18.3[a]	18.1[a]	0.77	<0.001
C18:1 *trans*	2.42[a]	7.77[b]	7.86[b]	0.915	<0.001
C18:1*n*-9 *cis*	16.4[b]	9.49[a]	9.27[a]	0.762	<0.001
C18:2*n*-6 *cis*	18.1[b]	9.38[a]	9.45[a]	0.415	<0.001
CLA (*cis*-9,*trans*-11)	0.19[a]	2.18[b]	1.98[b]	0.178	<0.001
C18:3*n*-3 *cis*	1.04[a]	2.52[b]	1.90[b]	0.321	0.001
C20:4*n*-6	3.40	2.70	2.67	0.340	0.086
C20:5*n*-3	1.20[a]	5.23[b]	5.55[b]	0.232	<0.001
C22:6*n*-3	1.14[a]	3.44[b]	3.44[b]	0.213	<0.001
RFA[§]	9.38[a]	21.8[b]	22.7[b]	1.62	<0.001
Total fatty acids (mg/ml)	1.20[b]	0.850[a]	0.838[a]	0.064	<0.001

MML = Megalac + 50 mg/kg vitamin E fed throughout pregnancy and lactation; FML = Fish oil + 50 mg/kg vitamin E in pregnancy followed by Megalac + 50 mg/kg vitamin E in lactation; FMH = Fish oil + 500 mg/kg vitamin E in pregnancy followed by Megalac + 500 mg/kg vitamin E in lactation

[¶] one week *pre-partum* = day 138 of pregnancy

[§]RFA = All remaining fatty acids

Means without common superscripts are significantly different at the P<0.05 level

Ewes offered dietary strategies containing fish oil (FML or FMH) during pregnancy had significantly higher proportions of C16:1*n*-7 and C18:1 *trans* within plasma than those fed diet MML. By contrast, the proportion of C18:1*n*-9 *cis*, the principal monoenoic fatty acid within plasma, was reduced in ewes fed diets FML and FMH compared to ewes offered diet MML ($P<0.001$). Ewes offered diets FML and FMH had approximately ten-fold higher proportions of CLA within plasma lipid than those fed diet MML ($P<0.001$).

Plasma C18:2*n*-6 was approximately two-fold higher in ewes offered strategy MML compared with FML or FMH ($P<0.001$). Furthermore, the amount of C20:4*n*-6 within plasma tended to be higher in ewes supplemented with Megalac than in those fed diets containing fish oil ($P=0.086$). Proportionally, the contribution of C18:3*n*-3 to total plasma fatty acids was similar between ewes fed diets FML or FMH, being more than two-fold higher than in ewes offered diet MML ($P=0.001$). The addition of fish oil to the diets of pregnant ewes significantly increased the proportion of C20:5*n*-3 within plasma at one week *pre-partum* compared to ewes supplemented with Megalac. Similar results were observed for C22:6*n*-3 as a result of fish oil supplementation with a three-fold difference in plasma proportions between ewes supplemented with fish oil and those fed Megalac.

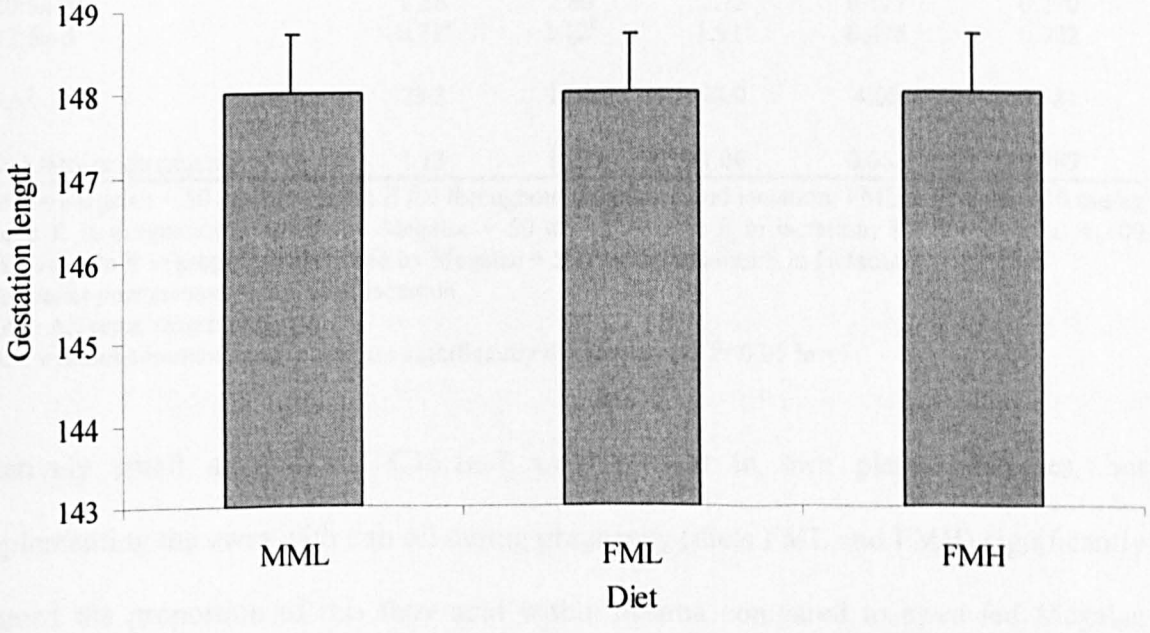


Figure 5.2. Effect of PUFA and vitamin E supplementation of ewes on ewe gestation length

5.4.2.7. Gestation length

No significant effect of dietary treatment was observed upon ewe gestation length (Figure 5.2).

5.4.2.8. Plasma fatty acids (two weeks *post partum*)

The total fatty acid concentration within ewe plasma at two weeks *post partum* was similar between treatments with no significant effect of dietary fat source or vitamin E concentration (Table 5.13). Individual proportions of the saturated fatty acids C16:0 and C18:0 within the plasma lipid fraction were similar between treatment diets with no significant effect of fat source or vitamin E concentration being observed.

Table 5.13. Effect of PUFA and vitamin E supplementation of ewes on the proportions of individual fatty acids in plasma samples collected at two weeks[¶] post partum

	Dietary strategy			s.e.d.	P
Fatty acid (g/100 g fatty acids)	**MML**	**FML**	**FMH**		
C16:0	18.1	19.1	18.6	1.12	0.683
C16:1*n*-7	0.34[b]	0.16[a]	0.19[a]	0.064	0.036
C18:0	17.7	19.7	19.0	0.91	0.109
C18:1 *trans*	2.56	2.86	2.73	0.177	0.270
C18:1*n*-9 *cis*	15.5	14.0	13.7	1.09	0.258
C18:2*n*-6 *cis*	14.0	15.5	14.3	0.68	0.826
CLA (*cis*-9,*trans*-11)	1.02	1.03	0.80	0.114	0.118
C18:3*n*-3 *cis*	0.88	0.92	0.83	0.150	0.826
C20:4*n*-6	3.42[b]	2.39[a]	2.32[a]	0.292	0.003
C20:5*n*-3	2.56	2.86	2.73	0.177	0.270
C22:6*n*-3	0.71[a]	2.12[b]	1.91[b]	0.478	0.022
RFA[§]	23.3	19.4	23.0	4.00	0.581
Total fatty acids (mg/ml)	1.13	1.02	1.04	0.083	0.387

MML = Megalac + 50 mg/kg vitamin E fed throughout pregnancy and lactation; FML = Fish oil + 50 mg/kg vitamin E in pregnancy followed by Megalac + 50 mg/kg vitamin E in lactation; FMH = Fish oil + 500 mg/kg vitamin E in pregnancy followed by Megalac + 500 mg/kg vitamin E in lactation

[¶] two weeks *post partum* = day 14 of lactation

[§]RFA = All remaining fatty acids

Means without common superscripts are significantly different at the P<0.05 level

Relatively small amounts of C16:1*n*-7 were present in ewe plasma samples, but supplementing the ewes with fish oil during pregnancy (diets FML and FMH) significantly reduced the proportion of this fatty acid within plasma compared to ewes fed Megalac

during pregnancy (diet MML). By contrast, the proportions of the minor plasma lipid constituent C18:1 *trans* and the principal plasma fatty acid C18:1*n*-9 *cis* were unaffected by either the dietary fat source or vitamin E concentration fed during pregnancy and lactation. Although ewes offered diet FMH appeared to have lower proportions of CLA (*cis*-9,*trans*-11) within plasma at two weeks *post partum* compared to ewes offered diets FML or MML, this difference did not approach statistical significance.

The proportion of C18:2*n*-6 within plasma lipid was unaffected by treatment diet. By contrast, supplementing ewes with fish oil during pregnancy had a significant carry-over effect upon the proportion of C20:4*n*-6 within plasma at two weeks *post partum*, with a mean value of approximately 2.36 g/100 g fatty acids (diet FML + diet FMH) compared to 3.42 g/100 g fatty acids (diet MML).

Neither the amount of C18:3*n*-3 nor C20:5*n*-3 within ewe plasma at two weeks *post partum* was significantly affected by dietary fat source or vitamin E concentration. Nonetheless, a significant carry-over effect of fish oil supplementation during pregnancy was observed upon the proportion of C22:6*n*-3 within ewe plasma at two weeks *post partum* with mean values of approximately 2.02 g/100 g fatty acids compared to 0.71 g/100 g fatty acids for ewes fed diets containing fish oil or Megalac respectively.

5.4.2.9. Milk production parameters

Supplementation of ewes with long-chain PUFAs and vitamin E during pregnancy had no effect on the secretion rate or calculated daily milk yield at four weeks into lactation (Table 5.14). The fat concentration in milk tended (P=0.091) to be lower in ewes supplemented with fish oil (diets FML and FMH) during pregnancy when compared to those fed the control diet (MML). A similar pattern was observed in fat yield (g/hour), however, the difference did not reach statistical significance. Milk protein concentrations and yields

were not significantly different between treatments. Furthermore, there was no significant effect of dietary fat source or vitamin E concentration upon milk lactose concentration or yield.

Table 5.14. Effect of PUFA and vitamin E supplementation of ewes on milk parameters at four weeks[¶] post partum

	Dietary strategy			s.e.d.	P
	MML	FML	FMH		
Secretion rate (ml/hour)	106	101	106	11.8	0.880
Yield (l/day)	2.55	2.42	2.55	0.284	0.880
Fat concentration (g/kg)	80.1	65.2	56.4	10.18	0.091
Fat yield (g/hour)	8.82	6.55	5.75	1.412	0.109
Protein concentration (g/kg)	38.6	37.8	36.5	1.10	0.189
Protein yield (g/hour)	4.10	3.80	3.86	0.451	0.793
Lactose concentration (g/kg)	47.5	48.9	48.7	0.86	0.230
Lactose yield (g/hour)	5.02	4.93	5.18	0.564	0.904

MML = Megalac + 50 mg/kg vitamin E fed throughout pregnancy and lactation; FML = Fish oil + 50 mg/kg vitamin E in pregnancy followed by Megalac + 50 mg/kg vitamin E in lactation; FMH = Fish oil + 500 mg/kg vitamin E in pregnancy followed by Megalac + 500 mg/kg vitamin E in lactation

[¶] four weeks *post partum* = day 28 of lactation

5.4.3. Lamb performance parameters

5.4.3.1. Neonatal lamb behaviour

Ewe maternal behaviour scores were similar for all treatments, regardless of dietary fat source or vitamin E concentration (Table 5.15). The latency of ewe standing was reduced by in ewes supplemented with long-chain PUFAs (diets FML and FMH) compared to the control fat source (MML; P=0.030). The latency of vocalisation was similar for treatments containing a basal concentration of vitamin E (MML and FML) but increased in treatment FMH (P=0.009). The time interval between expulsion of the lamb and the first contact between ewe and lamb was unaffected by dietary treatment.

Numerical differences in the latency of lamb standing existed between diets containing fish oil (FML and FMH) compared to the control fat diet (MML), but these differences were not statistically significant. These differences persisted in the latency of lamb searching

for the udder, which was lower in lambs produced by ewes fed diets FML and FMH compared to diet MML although again, this did not approach statistical significance. Similarly, although not statistically significant, a difference of approximately four minutes existed between the time taken for lambs on treatments FML and FMH to successfully suckle compared to those on treatment MML. Neonatal lamb body temperatures were not significantly affected by long-chain PUFA or vitamin E supplementation of the ewe.

Table 5.15. Effect of PUFA and vitamin E supplementation of ewes on maternal behaviour scores, latencies of maternal and neonatal lamb behaviours and neonatal lamb temperatures

	Dietary strategy			s.e.d.	P
	MML	FML	FMH		
Maternal measurements:					
Maternal behaviour score	118	117	118	5.2	0.959
Latency of standing (sec)	317^{b}	84.0^{a}	90.0^{a}	96.00	0.030
Latency of vocalisation (sec)	49.0^{a}	36.0^{a}	161^{b}	42.00	0.009
Latency of contact with the lamb (sec)	55.0	61.0	71.0	32.60	0.891
Neonatal measurements:					
Latency of standing (min)	15.6	12.9	11.8	2.46	0.296
Latency of searching for the udder (min)	17.5	13.8	14.9	3.21	0.508
Latency of successful suckling (min)	44.3	40.0	40.7	6.11	0.747
Lamb body temperature (°C)	38.98	39.04	39.03	0.224	0.959

MML = Megalac + 50 mg/kg vitamin E fed throughout pregnancy and lactation; FML = Fish oil + 50 mg/kg vitamin E in pregnancy followed by Megalac + 50 mg/kg vitamin E in lactation; FMH = Fish oil + 500 mg/kg vitamin E in pregnancy followed by Megalac + 500 mg/kg vitamin E in lactation
Means without common superscripts are significantly different at the $P<0.05$ level

5.4.3.2. Neonatal lamb plasma vitamin E

Plasma vitamin E concentrations were below the detectable limit (<0.1μmol/l) in neonatal lambs from any of the three treatments.

Table 5.16. Effect of PUFA and vitamin E supplementation of ewes on vitamin E concentrations of neonatal lamb brain tissue

	Dietary strategy			s.e.d.	P
	MML	FML	FMH		
Brain vitamin E concentration (mg/kg)	2.27	2.65	2.98	0.237	0.051

MML = Megalac + 50 mg/kg vitamin E fed throughout pregnancy and lactation; FML = Fish oil + 50 mg/kg vitamin E in pregnancy followed by Megalac + 50 mg/kg vitamin E in lactation; FMH = Fish oil + 500 mg/kg vitamin E in pregnancy followed by Megalac + 500 mg/kg vitamin E in lactation

5.4.3.3. Neonatal lamb tissue vitamin E

Lambs produced by ewes fed supranutritional concentrations of vitamin E tended to have higher brain vitamin E concentrations than those supplemented with basal vitamin E in combination with Megalac (P=0.051; Table 5.16). There was no significant difference between brain vitamin E concentrations in lambs borne by ewes fed strategy FML and either strategy MML or FMH.

Table 5.17. Effect of PUFA and vitamin E supplementation of ewes on the proportions of individual fatty acids in neonatal lamb plasma

	Dietary strategy			s.e.d.	P
Fatty acid (g/100 g fatty acids)	**MML**	**FML**	**FMH**		
C16:0	2.64	3.54	1.89	0.601	0.119
C16:1*n*-7	5.50	7.00	4.19	2.093	0.475
C18:0	9.38	9.29	9.30	0.890	0.993
C18:1 *trans*	3.68	3.87	2.98	0.529	0.317
C18:1*n*-9 *cis*	35.9^{b}	22.8^{a}	35.9^{b}	2.87	0.016
C18:2*n*-6 *cis*	1.70	1.43	3.00	0.945	0.312
CLA (*cis*-9,*trans*-11)	2.12	ND	ND	-	-
C18:3*n*-3 *cis*	ND	ND	1.64	-	-
C20:4*n*-6	1.50	2.88	0.77	0.979	0.206
C20:5*n*-3	0.72^{a}	5.21^{b}	2.36^{a}	1.004	0.027
C22:6*n*-3	ND	4.31^{b}	1.71^{a}	0.314	<0.001
RFA[¶]	36.9	39.7	36.2	2.12	0.329
Total fatty acids (mg/ml)	0.365^{a}	0.355^{a}	0.716^{b}	0.0725	0.012

MML = Megalac + 50 mg/kg vitamin E fed throughout pregnancy and lactation; FML = Fish oil + 50 mg/kg vitamin E in pregnancy followed by Megalac + 50 mg/kg vitamin E in lactation; FMH = Fish oil + 500 mg/kg vitamin E in pregnancy followed by Megalac + 500 mg/kg vitamin E in lactation

[¶] RFA = All remaining fatty acids; ND = not detected

Means without common superscripts are significantly different at the P<0.05 level

5.4.3.4. Neonatal lamb plasma fatty acids

Total plasma fatty acid concentrations were similar in neonatal lambs produced by ewes offered low dietary concentrations of vitamin E (MML and FML) but were approximately two-fold higher in lambs from ewes fed a high vitamin E concentration (diet FMH; P=0.012; Table 5.17). No significant differences in the proportions of individual saturated fatty acids were conferred by dietary supplementation of the ewe.

No significant effect of dietary treatment was evident upon the proportions of C16:1*n*-7 or C18:1 *trans* within neonatal lamb plasma. By contrast, the proportion of C18:1*n*-9 within plasma lipids was reduced in lambs produced by ewes fed diet FML during pregnancy compared to those fed diets MML or FMH (P=0.016). Neither dietary fat source or vitamin E concentration had any significant effect upon the proportion of CLA within neonatal lamb plasma.

Ewes offered diet FMH during pregnancy appeared to produce lambs with higher amounts of C18:2*n*-6 within plasma, however, this difference did not reach statistical significance. There was no significant effect of dietary treatment upon the proportion of C20:4*n*-6 within neonatal lamb plasma.

Plasma C18:3*n*-3 was only detected in samples from lambs suckling ewes fed strategy FMH. With reference to C20:5*n*-3, significant differences were observed between all three dietary treatments with samples from lamb on treatment FML having the highest proportion of C20:5*n*-3 compared to treatments FMH and MML. Proportions of plasma C22:6*n*-3 were below detectable levels in samples from treatment MML. Lambs born to ewes fed diet FML had plasma proportions of C22:6*n*-3 two-fold higher than those in lambs produced by ewes offered diet FMH (P<0.001). Neonatal lambs produced by ewes fed strategy MML did not have detectable quantities of C22:6*n*-3 within plasma.

5.4.3.5. Neonatal lamb brain fatty acids

Supplementing ewes with diets containing fish oil during pregnancy reduced the concentration of total fatty acids within neonatal brain tissue with mean values of approximately 30.1 mg/g (diets FML + FMH) compared to 34.8 mg/g (diet MML, P=0.016; Table 5.18). The proportions of the principal saturated fatty acids of note within

neonatal lamb brain tissue, C16:0 and C18:0, were unaffected by maternal dietary treatment.

Table 5.18. Effect of PUFA and vitamin E supplementation of ewes on the proportions of individual fatty acids in neonatal lamb brain

	Dietary strategy			s.e.d.	P
Fatty acid (g/100 g fatty acid)	**MML**	**FML**	**FMH**		
C16:0	19.3	20.0	19.1	0.41	0.199
C16:1*n*-7	0.99	1.01	1.07	0.050	0.361
C18:0	15.1	15.6	15.3	0.37	0.461
C18:1 *trans*	3.45[b]	3.02[a]	3.36[b]	0.085	0.016
C18:1*n*-9 *cis*	13.3	13.5	13.2	0.46	0.797
C18:2*n*-6 *cis*	0.15	0.28	0.22	0.038	0.060
CLA (*cis*-9,*trans*-11)	0.96	0.81	0.92	0.103	0.411
C18:3*n*-3 *cis*	0.38[b]	0.30[a]	0.37[b]	0.013	0.009
C20:4*n*-6	4.22	3.45	3.44	0.309	0.104
C20:5*n*-3	0.69	0.70	0.68	0.059	0.930
C22:6*n*-3	9.77	10.5	10.2	0.409	0.322
RFA[¶]	31.71	30.81	32.10	0.81	0.358
Total fatty acids (mg/g)	34.8[b]	30.9[a]	29.3[a]	1.45	0.043

MML = Megalac + 50 mg/kg vitamin E fed throughout pregnancy and lactation; FML = Fish oil + 50 mg/kg vitamin E in pregnancy followed by Megalac + 50 mg/kg vitamin E in lactation; FMH = Fish oil + 500 mg/kg vitamin E in pregnancy followed by Megalac + 500 mg/kg vitamin E in lactation

[¶] RFA = All remaining fatty acids

Means without common superscripts are significantly different at the P<0.05 level

There was no significant difference in the proportion of C16:1*n*-7 within neonatal lamb brain tissue between treatments. By contrast, supplementing pregnant ewes with fish oil in combination with a low concentration of vitamin E significantly reduced the proportion of C18:1 *trans* within neonatal lamb brain tissue when compared to the other two treatment strategies. The proportions of C18:1*n*-9 *cis* within neonatal lamb brain were similar between strategies. Although numerical differences were present, no significant effect of maternal dietary strategy was observed upon the proportion of CLA within lamb brain tissue.

Feeding diets containing fish oil to pregnant ewes marginally increased the proportion of C18:2*n*-6 within brain tissue, a result that tended towards significance (P=0.060) with mean values of 0.25 g/100 g fatty acids and 0.15 g/100 g fatty acids for fish oil and

Megalac treatments respectively. By contrast, the amount of C20:4*n*-6 within neonatal lamb brain tissue was reduced by fish oil supplementation of the pregnant ewe when compared to Megalac supplementation, however, this result only just approached statistical significance.

Feeding fish oil in combination with a low concentration of vitamin E to pregnant ewes reduced the proportion of C18:3*n*-3 within neonatal brain tissue compared to feeding strategies FMH or MML (P=0.009). The amount of C20:5*n*-3 found within neonatal lamb brain tissue was similar among different dietary strategies, with no significant effect of maternal dietary fat source or vitamin E concentration. Although numerical differences in the proportion of C22:6*n*-3 within brain tissue were observed, with higher values seen in lambs borne by ewes fed fish oil during pregnancy, these differences did not reach statistical significance.

Table 5.19. Effect of PUFA and vitamin E supplementation of ewes on lamb birthweights (kg) and on lamb and litter growth rates (kg/day)

	Dietary strategy			s.e.d.	P
	MML	FML	FMH		
Lamb liveweight:					
At birth	4.74	4.57	4.70	0.268	0.807
At 1 week of age	6.87	6.62	6.87	0.287	0.593
At 2 weeks of age	9.16	9.21	9.33	0.330	0.863
At 3 weeks of age	11.1	11.2	11.3	0.41	0.957
At 4 weeks of age	13.1	12.9	13.5	0.48	0.493
Lamb growth rate	0.30	0.30	0.31	0.013	0.651
Litter growth rate	0.60	0.59	0.62	0.027	0.667

MML = Megalac + 50 mg/kg vitamin E fed throughout pregnancy and lactation; FML = Fish oil + 50 mg/kg vitamin E in pregnancy followed by Megalac + 50 mg/kg vitamin E in lactation; FMH = Fish oil + 500 mg/kg vitamin E in pregnancy followed by Megalac + 500 mg/kg vitamin E in lactation

5.4.3.2. Lamb liveweight

Individual lamb birthweights were similar between treatments with no effect of maternal long-chain PUFA or vitamin E supplementation (Table 5.19). Moreover, no significant effect of treatment strategy was observed at any time point, or upon the individual or litter growth rate.

5.4.3.3. Suckling lamb antioxidant status

Lamb plasma vitamin E concentrations were unaffected by fat source, but maternal dietary vitamin E concentration had a significant effect upon plasma concentrations at both 24 hours and two weeks *post partum* with highest values exhibited by lamb suckling ewes fed diet FMH (Table 5.20).

Table 5.20. Effect of PUFA and vitamin E supplementation of ewes on indicators of vitamin E and selenium status and of cellular damage in lambs

	Dietary strategy			s.e.d.	P
	MML	FML	FMH		
Plasma vitamin E concentration (μmol/l):					
24 hours *post partum*	2.05[a]	1.94[a]	3.74[b]	0.625	0.032
2 weeks *post partum*	2.12[a]	2.05[a]	3.74[b]	0.430	0.004
Erythrocyte GPx activity (U/ml PCV):					
24 hours *post partum*	130[a]	143[a]	160[b]	8.0	0.003
2 weeks *post partum*	183	180	202	11.3	0.121
Serum CK concentration (U/l):					
24 hours *post partum*	401	398	470	62.3	0.425
2 weeks *post partum*	229	158	168	49.9	0.319

MML = Megalac + 50 mg/kg vitamin E fed throughout pregnancy and lactation; FML = Fish oil + 50 mg/kg vitamin E in pregnancy followed by Megalac + 50 mg/kg vitamin E in lactation; FMH = Fish oil + 500 mg/kg vitamin E in pregnancy followed by Megalac + 500 mg/kg vitamin E in lactation
Means without common superscripts are significantly different at the $P<0.05$ level

Lamb erythrocyte GPx activities were increased at two weeks of age compared to values at 24 hours of age. A significant increase in erythrocyte GPx was observed in lambs produced by ewes fed diet FMH compared to those fed either diet FML or MML at 24 hours of age. A similar pattern was seen at two weeks of age, but this difference was not statistically significant.

Serum CK concentrations were reduced in all lambs, regardless of maternal dietary treatment, at two weeks of age compared to values recorded at 24 hours old. Although numerical differences between treatments were observed, no significant effect of maternal long-chain PUFA or vitamin E supplementation was evident at any time point.

5.4.3.4. Suckling lamb plasma fatty acids at 24 hours of age

No significant effect of maternal treatment diet was evident upon the total fatty acid concentration in lamb plasma at 24 hours of age (Table 5.21). Expressing individual fatty acid data as a proportion of total plasma fatty acids revealed significant reductions in the contribution of C16:0 in lambs produced by ewes offered FML or FMH compared to those borne by ewes fed diet MML. Maternal dietary supplementation with fish oil or Megalac had no significant influence upon the proportion of C18:0 within lamb plasma at 24 hours of age.

Table 5.21. Effect of PUFA and vitamin E supplementation of ewes on the proportions of individual fatty acids in lamb plasma samples collected at 24 hours of age

	Dietary strategy			s.e.d.	P
Fatty acid (g/100 g fatty acids)	**MML**	**FML**	**FMH**		
C16:0	22.4[b]	20.7[a]	21.0[a]	0.65	0.045
C16:1*n*-7	1.40	1.64	1.58	0.170	0.374
C18:0	10.6	10.1	8.85	0.906	0.178
C18:1 *trans*	2.52[a]	4.77[b]	5.12[b]	0.380	<0.001
C18:1*n*-9 *cis*	32.8[b]	23.1[a]	23.0[a]	1.62	<0.001
C18:2*n*-6 *cis*	7.55[b]	5.35[a]	5.28[a]	0.754	0.014
CLA (*cis*-9,*trans*-11)	0.74[a]	1.70[b]	1.51[b]	0.198	<0.001
C18:3*n*-3 *cis*	0.95	1.06	1.24	0.160	0.237
C20:4*n*-6	1.82	2.30	1.96	0.259	0.194
C20:5*n*-3	0.81[a]	3.99[b]	3.45[b]	0.390	<0.001
C22:6*n*-3	0.32[a]	1.81[b]	1.38[b]	0.275	<0.001
RFA[¶]	18.1[a]	23.5[b]	25.6[b]	1.45	<0.001
Total fatty acids (mg/ml)	1.57	1.32	1.52	0.793	0.784

MML = Megalac + 50 mg/kg vitamin E fed throughout pregnancy and lactation; FML = Fish oil + 50 mg/kg vitamin E in pregnancy followed by Megalac + 50 mg/kg vitamin E in lactation; FMH = Fish oil + 500 mg/kg vitamin E in pregnancy followed by Megalac + 500 mg/kg vitamin E in lactation

[¶] RFA = All remaining fatty acids

Means without common superscripts are significantly different at the P<0.05 level

No significant effect of maternal diet was observed upon the proportion of C16:1*n*-7 in lamb plasma at 24 hours of age; by contrast, the proportion of C18:1 *trans* within total plasma fatty acids was significantly higher in lambs produced by ewes fed diets FML and FMH compared to diet MML. Furthermore, the proportion of C18:1*n*-9 *cis* as a component of total lamb plasma fatty acids was lower in lambs born to ewes supplemented with fish oil during pregnancy, than in those fed Megalac (P<0.001). The proportion of

CLA within plasma was significantly higher in lambs suckling ewes offered diets FML and FMH compared to diet MML.

Fish oil supplementation of the ewe conferred a significant reduction in the proportion of C18:2*n*-6 within plasma of lambs produced by ewes fed strategies FML and FMH compared to MML. Proportions of C20:4*n*-6 within lamb plasma at 24 hours of age were similar between treatments and unaffected by maternal dietary fat source or vitamin E concentration. Supplementing the diet of the pregnant ewe with fish oil (regardless of the dietary vitamin E concentration) resulted in a four-fold increase in the proportion of C20:5*n*-3 within lamb plasma at 24 hours of age when compared to supplementation with Megalac ($P<0.001$). Furthermore, the mean proportion of C22:6*n*-3 in lambs suckling ewes fed fish oil during pregnancy was significantly higher at 1.6 g/100 g fatty acids compared to 0.32 g/100 g fatty acids in lambs borne by ewes fed Megalac.

Table 5.22. Effect of PUFA and vitamin E supplementation of ewes on the proportions of individual fatty acids in lamb plasma samples collected at two weeks of age

	Dietary strategy			s.e.d.	P
Fatty acid (g/100 g fatty acids)	**MML**	**FML**	**FMH**		
C16:0	21.8	22.0	22.1	0.76	0.932
C16:1*n*-7	0.83	0.83	0.91	0.045	0.192
C18:0	16.4	16.2	15.6	1.35	0.410
C18:1 *trans*	2.96	3.25	2.45	0.203	0.095
C18:1*n*-9 *cis*	24.7	24.2	24.9	0.95	0.761
C18:2*n*-6 *cis*	14.1	14.2	13.9	1.28	0.965
CLA (*cis*-9,*trans*-11)	0.22	0.26	0.27	0.096	0.835
C18:3*n*-3 *cis*	1.06^{a}	1.40^{b}	1.35^{b}	0.106	0.016
C20:4*n*-6	3.64^{b}	2.07^{a}	2.08^{a}	0.396	0.002
C20:5*n*-3	0.71^{a}	1.54^{b}	1.47^{b}	0.098	<0.001
C22:6*n*-3	0.82^{a}	1.30^{b}	1.36^{b}	0.150	0.007
RFA[1]	12.8	12.9	12.6	0.49	0.880
Total fatty acids (mg/ml)	2.22	2.18	2.11	0.355	0.951

MML = Megalac + 50 mg/kg vitamin E fed throughout pregnancy and lactation; FML = Fish oil + 50 mg/kg vitamin E in pregnancy followed by Megalac + 50 mg/kg vitamin E in lactation; FMH = Fish oil + 500 mg/kg vitamin E in pregnancy followed by Megalac + 500 mg/kg vitamin E in lactation

[1] RFA = All remaining fatty acids

Means without common superscripts are significantly different at the $P<0.05$ level

5.4.3.5. Suckling lamb plasma fatty acids at two weeks of age

Total fatty acid concentrations within plasma of suckling lambs were similar between treatments, with no significant effects of maternal dietary fat source or vitamin E concentration (Table 5.22). No significant effect of maternal dietary treatment was evident upon the proportion of C16:0 or C18:0 within lamb plasma samples at two weeks of age. Minor numerical differences in the proportions of C16:1*n*-7, C18:1 *trans* and C18:1*n*-9 *cis* within lamb plasma were present as a result of maternal dietary supplementation, but these differences did not reach statistical significance. The proportion of CLA within lamb plasma at two weeks of age was similar between all maternal dietary treatments.

The proportions of C18:2*n*-6 within lamb plasma samples at two weeks of age were similar between maternal dietary treatments. By contrast, long-chain PUFA supplementation of the pregnant ewe significantly reduced the proportional contribution of C20:4*n*-6 to plasma fatty acids by a factor of 1.75 compared to supplementation with Megalac. Adding fish oil to the diet of pregnant ewes significantly increased the proportion of C18:3*n*-3 within lamb plasma with mean values of approximately 1.38 g/100 g fatty acids (strategies FML + FMH) compared to 1.06 g/100 g (strategy MML). The proportion of C20:5*n*-3 was approximately two-fold higher in lambs produced by ewes fed fish oil during pregnancy when contrasted with those offered diets containing Megalac ($P<0.001$). Amounts of C22:6*n*-3 within plasma were also two-fold higher in lambs suckling ewes fed fish oil compared to those fed Megalac during pregnancy ($P=0.007$).

5.5. Discussion

5.5.1. Ewe parameters

5.5.1.1. Straw Intake

In direct contrast to the results observed in Experiment One, fish oil supplementation significantly reduced the daily straw intake of pregnant ewes. This concurs with previous research in ruminants where feeding fish oil was associated with a decrease in DM intake (Donovan *et al.*, 2000; Keady *et al.*, 2000; Whitlock *et al.*, 2002). Moreover, similar results were observed by Annett *et al.* (2004), with a significant reduction in silage intake occurring as a result of fish oil supplementation of pregnant ewes. The differences between results observed here and those described in Experiment One indicate that vermiculite may have had a protective effect upon the unsaturated fatty acids contained within fish oil.

Changing from a dietary long-chain PUFA source during pregnancy to a saturated source during lactation appeared to negate the significant decrease in DM intake observed during pregnancy. Although considerable quantities of unsaturated fatty acids may be stored within lipid reserves during pregnancy and mobilised during lactation, the effects upon dry matter intake observed in previous studies appear to be via the cytotoxic effects of dietary long-chain PUFAs upon ruminal microorganisms. Although bacterial growth is significantly retarded by long-chain PUFAs, this effect has also been observed with medium-chain and saturated fatty acids (Doreau *et al.*, 1997). However, the addition of Megalac to the diet of ruminants has been demonstrated to have significantly less effect upon DM intakes than fish oil (Wachira *et al.*, 2002).

5.5.1.2. Nutritional status

Liveweight change *pre-partum* was significantly reduced as a consequence of fish oil supplementation of the pregnant ewe in the study of Annett *et al.* (2004), but little other

information upon the effects of fish oil supplementation upon ewe liveweight and condition score is available. The studies of Fritsche *et al.* (1993) and Rooke *et al.* (2000; 2001b) using domestic swine indicated that the addition of fish oil to the diet has no effect upon liveweight or backfat thickness. Although the addition of fish oil to the diet of pregnant ewes reduced straw intake during pregnancy, quantitatively, the difference was small. This would have reduced daily energy supply by 0.27 MJ (ARFC, 1993), which would not have significantly impacted upon ewe liveweight or condition score. The lack of a significant effect of vitamin E supplementation of the ewe upon these parameters is in agreement with the results published by both Kott *et al.* (1998) and Merrell (1998).

Metabolic indicators of ewe nutritional status were similar between treatments, although high co-efficients of variation indicated a wide range of values between individual animals. The mobilisation of body tissues during a period of negative energy balance leads to an increase in plasma βHB and NEFA (Nozière *et al.*, 2000). The lack of any effect of dietary supplementation upon these parameters concurs with the results observed for liveweight and condition score change during pregnancy and lactation. Ewes used in the current study were well-fed, with a ERDP:FME ratio slightly in excess of that recommended by AFRC (1993). Consequently, plasma concentrations of βHB and urea observed in the current study were higher than the reference values for ruminants proposed by Topps and Thompson (1984) and those reported by both Antunović *et al.* (2002) and Hamadeh *et al.* (1996). Furthermore, blood sampling occurred at approximately 2.5 hours after feeding, which may have increased the concentrations of these metabolites within plasma compared to sampling after a fasting period (Eicher *et al.*, 1999).

5.5.1.3. Ewe antioxidant status

Augmentation of the ewe dietary vitamin E supply conferred a significant increase in plasma concentrations, a response which has been documented in sheep (Hidiroglou *et al.*,

1969; Njeru *et al.*, 1994), pigs (Farnworth *et al.*, 1995; Lauridsen *et al.*, 2002), rats (Martin and Hurley, 1977) and humans (Mino and Nishino, 1973; Léger *et al.*, 1998). The observed concentrations concurred with those reported by Gentry *et al.* (1992), Hidiroglou *et al.* (1993a) and Gabryszuk and Klewiec (2002). By contrast to the results observed in Experiment One, ewes fed diet FMH had higher plasma vitamin E concentrations during lactation compared to pregnancy, which may, in part, be attributed to an increased dietary supply *post partum*. Chawla and Kaur (2004) observed a similar pattern in plasma vitamin E concentrations during pregnancy and lactation when dairy cattle were supplemented with 1000 IU dl-α-tocopherol acetate. This is in agreement with the results published by Mahan (1991) who described an improvement in vitamin E status of lactating sows as lactation progressed.

Hidiroglou *et al.* (1992) suggested that a ruminant plasma vitamin E concentration of >4 mg/kg indicates an adequate vitamin E status. However, by this definition, all ewes were clinically deficient in vitamin E during pregnancy, with only those supplemented with supranutritional vitamin E concentrations reaching marginal status during lactation. This underlines the suggestion that the basal vitamin E requirements of the ewe should be re-evaluated. A major factor in the determination of the dietary vitamin E requirement of any animal is the potential oxidative challenge posed as a result of PUFA supply from the diet. Several authors have reported that animals supplemented with long-chain PUFAs have a concurrent reduction in vitamin E status (Hidiroglou *et al.*, 1993a; Farnworth *et al.*, 1995; Wang *et al.*, 1996; McGuire and Fritsche, 1997), although no such effect was observed within the current study. This concurs with the results of Rochester and Caravaggi (1971) in lambs supplemented with fish oil.

In its role as a cellular antioxidant, vitamin E works in conjunction with vitamins A and C, and the selenoenzyme GPx, this enzyme being responsible for the reduction of lipid

hydroxyperoxide compounds within the cell (Putnam and Comben, 1987). The complex relationship between GPx and vitamin E was demonstrated in the study of Suárez *et al.* (1999) who described a reduction in liver and brain GPx concentration as a result of PUFA supplementation of rats, concluding that this effect is due to a sparing effect of vitamin E upon GPx synthesis. However, as observed in Experiment One, no significant effect of vitamin E supplementation was observed upon the ewe erythrocyte GPx activity, moreover, no effect of fat source was seen. It appears that the peroxidative challenge imposed by the experimental diets in the current study was insufficient to increase GPx activity, however, it is notable that GPx activity was increased by the additional daily selenium and vitamin E supply during lactation across all treatments.

It may therefore be suggested that dietary treatment would have no concurrent effect upon the cellular metabolites produced by peroxidation, i.e. creatine kinase. Indeed, provision of the treatment diets reduced the concentration of CK during pregnancy compared to baseline results, with little change between concentrations in pregnancy and lactation. When compared to reference values for CK in ovine serum (Bostedt and Schramel, 1990), observed values were tenfold lower than those indicative of clinical disease. The study published by Walsh *et al.* (1993) suggested that the threshold for sub-clinical myopathy may be as low as 250 IU/l, nonetheless, all ewes had serum CK concentrations below this threshold.

5.5.1.4. Plasma fatty acids *pre-partum*

The plasma fatty acid composition of the ruminant has a complex relationship with the dietary fatty acid supply (Jenkins and Thies, 1997). Daily fatty acid intakes were similar between treatments at one week *pre-partum*, but the total plasma fatty acid concentration was significantly lower in ewes offered diets containing fish oil. This may be attributed

either to differences in fatty acid absorption, or to changes in the endogenous synthesis of fatty acids within the ruminant.

Studies measuring the absorption of fatty acids within the ruminant intestine have produced conflicting results. Doreau and Chilliard (1997a) reported that fatty acid digestibility is reduced as acyl chain length increases, whilst Wu *et al.* (1991) suggested that digestibility may be increased with fatty acid unsaturation. Although the fish oil used in the current study was unprotected, the results observed were similar to those noted in Experiment One, using fish oil protected with vermiculite.

Several studies have reported the effect of long-chain PUFA supplementation upon endogenous fatty acid synthesis, specifically the depression of microbial (Loor *et al.*, 2002) and cellular fatty acid synthesis (Chilliard and Doreau, 1997). Microbial fatty acid synthesis may be reduced in ruminants due to the toxic effects of unsaturated fatty acids upon cellulolytic bacteria reducing ruminal production of the two-carbon fatty acid precursor acetate (Szumacher-Strabel *et al.*, 2001b). The reduction in straw intake observed in ewes fed fish oil concurs with this hypothesis. In addition, the *trans* C18:1 fatty acids (Ahnadi *et al.*, 1998) and CLA (Chouinard *et al.*, 1999) have been implicated in the suppression of cellular fatty acid synthesis and the concentration of these fatty acids were significantly increased in plasma of ewes offered fish oil within the current study.

The principal saturated fatty acid contained within plasma of ewes fed Megalac was C16:0, with concentrations of this fatty acid being more than two-fold lower in ewes offered diets containing fish oil. However, the magnitude of this difference was not as great as the differences in dietary supply conferred by the treatment diets. This may suggest that the endogenous production of C16:0 was not inhibited by fish oil supplementation within the current study. The amount of C18:0 within plasma was significantly reduced by the

addition of fish oil to the diet of the pregnant ewe. Dietary supply of this fatty acid was equivalent between treatment concentrates, therefore this reduction may have resulted as a consequence of alterations in the efficiency of ruminal biohydrogenation. Ruminal biohydrogenation of unsaturated fatty acids principally produces C18:0, but the addition of long-chain PUFAs to the diet demonstrably shifts the pattern of biohydrogenation towards the production of *trans* C18:1 fatty acids and CLA (Gulati *et al.*, 1999). This concurs with the proportions of C18:1 *trans* and CLA observed as a result of fish oil supplementation. Both Ashes *et al.* (1992) and Chikunya *et al.* (2004) reported similar results as a consequence of feeding protected fish oil to sheep, as did Offer *et al.* (2001) who supplemented lactating cattle with unprotected fish oil.

Differences in ewe plasma concentrations of C18:1*n*-9 *cis* between treatments are in agreement with those recorded as a result of fish oil supplementation of ewes in Experiment One. The amount of this fatty acid within plasma was increased by protected fish oil supplementation in the study of Ashes *et al.* (1992), when compared to a basal diet. However, the concentration of C18:1*n*-9 *cis* in experimental concentrates containing Megalac was high, therefore, the decrease in C18:1*n*-9 *cis* concentrations observed with fish oil supplementation may be attributed to variation in the concentration of this fatty acid between experimental diets.

The magnitude of the differences in plasma C18:2*n*-6 proportions between treatments was higher that would have been predicted from the daily fatty acid intake. Lower C18:2*n*-6 concentrations in plasma as a result of fish oil supplementation have been reported by Ashes *et al.* (1992) in sheep and Offer *et al.* (2001) in cattle. The extent to which C18:2*n*-6 is biohydrogenated is variable, with published values ranging from 70 to 95% (Chilliard *et al.*, 2000; Wachira *et al.*, 2000; Chikunya *et al.*, 2004), and appears to be dependent upon the fatty acid composition of the diet. Dohme *et al.* (2003) suggested that microbial

lipases may specifically target ester linkages associated with C18:2*n*-6 in preference to C20:5*n*-3 and C22:6*n*-3, thereby increasing the biohydrogenation of this fatty acid in preference to other long-chain PUFAs. This may explain the apparent increase in biohydrogenation of C18:2*n*-6 from fish oil compared to Megalac diets.

All treatment concentrates contained small amounts of C20:4*n*-6, but the proportion of C20:4*n*-6 within plasma fatty acids tended to be lower in ewes supplemented with fish oil compared to Megalac. This may be a consequence of inhibition of endogenous C20:4*n*-6 synthesis by the addition of preformed *n*-3 fatty acids to the diet, as described by Makrides *et al.* (1995) in humans. The decreases in C20:4*n*-6 within plasma observed throughout the Experiments One and Three as a consequence of fish oil supplementation may also have occurred due to competition between C20:4*n*-6, C20:5*n*-3 and C22:6*n*-3 for incorporation into phosphatidylglycerols.

As an EFA, C18:3*n*-3 must be supplied preformed from the ruminant diet, thus the presence of this fatty acid within plasma indicates that it has originated either from dietary supply or mobilisation of existing body fatty acid reserves. The small, but significant differences in plasma proportions of this fatty acid observed between treatments were related to the dietary intake of this fatty acid, i.e. lower intakes in ewes consuming diets containing Megalac. Biohydrogenation and absorption rates for C18:3*n*-3 are therefore assumed to have been similar between experimental diets. Voigt and Hagemeister (2001) suggested that the endogenous synthesis of C20:5*n*-3 and C22:6*n*-3 is negligible in the ruminant, however, this hypothesis does not concur with the results of any the three experiments within this study. Given the lack of a preformed source of C20:5*n*-3 or C22:6*n*-3 within the Megalac diet, it may be further suggested that the reduced proportion of C18:3*n*-3 within plasma of these ewes may result from C20:5*n*-3 and C22:6*n*-3

synthesis. Indeed, the exponential growth rate exhibited by the foetus in late pregnancy would increase the requirement for these fatty acids *pre-partum*.

Ashes *et al.* (1992) and Offer *et al.* (2001) demonstrated increases in the amount of C20:5*n*-3 and C22:6*n*-3 within plasma as a consequence of supplementing the ruminant diet with fish oil, and similar results have been observed in the current study. Protecting fish oil from ruminal hydrogenation is suggested to increase the amount of C20:5*n*-3 and C22:6*n*-3 absorbed at the small intestine. Despite increased daily intakes of C20:5*n*-3 and C22:6*n*-3 in the current study, the plasma proportions of these fatty acids were reduced in plasma compared to results within Experiment One. This suggests that the method of protection used in Experiment One (adsorption onto vermiculite) may have provided a degree of protection against the biohydrogenation of C20:5*n*-3 and C22:6*n*-3. Furthermore, Dohme *et al.* (2003) concluded that the extent of biohydrogenation of C20:5*n*-3 and C22:6*n*-3 from fish oils is dependent upon the inclusion rate and type of oil. Although inclusion rates were similar between Experiments One and Three, Experiment One employed unrefined Scandinavian fish oil, and Experiment Three used a mixture of mackerel and herring oil. Biohydrogenation rates of long-chain *n*-3 PUFAs may therefore have been higher in Experiment Three, independent of protection method.

Gulati *et al.* (2003) reported an increased transfer rate of C20:5*n*-3 compared to C22:6*n*-3 from dietary fish oil into milk of lactating cattle, suggested to be due to retroconversion of C22:6*n*-3 to C20:5*n*-3. The apparent transfer of C20:5*n*-3 from the diet to plasma of pregnant ewes in the current study appeared to be higher than that of C22:6*n*-3, concurring with plasma results from Experiment One. Whether this effect was due to retroconversion, changes in biohydrogenation and absorption or to a preferential transfer of C20:5*n*-3 from the small intestine into plasma lipid fractions, warrants further investigation.

5.5.1.5. Gestation length

In humans, both high dietary fish intakes and supplementation with fish oils have been observed to increase gestation length (Olsen *et al.*, 1986; 1992). The principal long-chain fatty acids within fish oil are C20:5*n*-3 and C22:6*n*-3, and it is assumed that one, or both of these are responsible for the reported effects. Several theories have been advanced to explain the mechanism by which these results occur, including effects upon the immune system (Waltman *et al.*, 1978) and on eicosanoid production (Olsen *et al.*, 1992). Consensus opinion appears to favour the hypothesis described by Olsen *et al.* (1992) and Abayasekara and Wathes (1999) proposing an effect of specific *n*-3 fatty acids upon the production of prostaglandins required for parturition. Trienoic prostaglandins, which have an inhibitory effect on the normal process of parturition, are endogenously synthesised from C20:5*n*-3, abrogating the effects of stimulatory dienoic prostaglandins produced from *n*-6 fatty acids (Abayasekara and Wathes, 1999). If the increase in gestation length can be attributed to the increased production of trienoic prostaglandins, it is suggested that C20:5*n*-3 alone is responsible. Supplementation of pregnant women with C22:6*n*-3 was reported to increase gestation length in the study of Smuts *et al.* (2003), but this effect was attributed to retro-conversion of C22:6*n*-3 to C20:5*n*-3 as C22:6*n*-3 is not a significant prostaglandin precursor. Nonetheless, C22:6*n*-3 may inhibit the formation of C20:4*n*-6-derived prostaglandins by binding to cellular sites or competing for enzymes required for prostaglandin production (Hansen and Olsen, 1988).

In contrast to the results of Rooke *et al.* (2001c) using pigs, Pickard *et al.* (2005) in sheep and the effects observed in Experiment One, fish oil supplementation of the pregnant ewe had no significant effect upon gestation length in the current study. This may simply be because the unprotected fish oil employed within the current study reduced the amount of C20:5*n*-3 absorbed by and available to the ewe for prostaglandin synthesis compared to that fed in Experiment One. Plasma concentrations of C20:5*n*-3 at one week *pre-partum*

were slightly reduced when expressed on a mg/ml basis and the proportional contributions were approximately 2.5 g/100g fatty acids lower than the equivalent results obtained in Experiment One. However, the absolute amount of C20:5*n*-3 within plasma and tissues may not be the instigator of changes in prostaglandin production, but rather the ratio of C20:5*n*-3 to C20:4*n*-6. The C20:5*n*-3:C20:4*n*-6 ratio in the current study for ewes supplemented with fish oil was 2.07 compared to 2.74 for Experiment One. Olsen *et al.* (1992) suggested that gestation length is increased only when C20:5*n*-3 saturation reaches a threshold level. It is therefore postulated that a threshold for the C20:5*n*-3:C20:4*n*-6 ratio exists, above which dienoic prostaglandin synthesis is reduced and gestation length increased.

Gestation length appeared to be increased across all treatments when compared to results observed in Experiment One, as the control (Megalac) group had a gestation length of 147.5 days in Experiment Three compared to 145.6 days in Experiment One. Although this appeared to have no effect upon gestation length in Experiment One, Cheng *et al.* (2003) propose that this fatty acid competes with C20:4*n*-6 for uptake and enzymes systems associated with prostaglandin production. Indeed, plasma concentrations of C18:2*n*-6 were significantly higher in ewes supplemented with Megalac in the current study. Nonetheless, concentrations of C20:4*n*-6 in plasma at one week *pre-partum* were significantly higher in the Megalac-supplemented group than in those fed fish oil, which was suggested by Olsen *et al.* (1990) to reduce gestation length. As ewe gestation length naturally ranges from 145-147 days, it is possible that the results observed were a consequence of prostaglandin inhibition across treatments by both C20:5*n*-3 (treatments FML and FMH) and C18:2*n*-6 (treatment MML), resulting in no significant difference between treatments.

5.5.1.6. Plasma fatty acids *post partum*

In an attempt to negate the effects of long-chain PUFA supplementation of pregnant and lactating ewes upon milk composition and lamb growth rate, ewes fed diets containing fish oil during pregnancy were changed onto a diet containing Megalac. However, it is interesting to note that some aspects of milk composition, namely fat concentration, were still affected by long-chain PUFA supplementation during pregnancy. Plasma fatty acids provide an indication of the fatty acid status of the ewe and may provide an insight into the mechanisms behind inhibition of milk fat synthesis. Nonetheless, within the current study, few significant differences were observed in the concentrations of plasma fatty acids as a consequence of dietary treatment during lactation.

In contrast to changes in plasma fatty acid composition observed during lactation within Experiment Two, fish oil supplementation of the ewe during pregnancy had no significant effect upon the concentrations of the major saturated fatty acids C16:0 and C18:0 within plasma at two weeks *post partum*. Furthermore, the concentrations of both C18:1*n*-9 *cis* and C18:1 *trans* were not significantly affected by dietary treatment. A considerable amount of research has been devoted to investigating the mechanisms behind milk fat depression in ruminants given long-chain PUFAs, and it appears that the incomplete biohydrogenation of fatty acids that leads to the production of trans octadecanoic fatty acids may be a major contributing factor (Wonsil *et al.*, 1994). However, given the tendency for ewes supplemented with fish oil during pregnancy to have lower milk fat concentrations at three weeks into lactation within the current study, a concurrent increase in the concentration of C18:1 *trans* fatty acids within plasma would have been expected.

In the absence of a preformed dietary source of long-chain PUFAs, the ewes requirement for C20:4*n*-6, C20:5*n*-3 and C22:6*n*-3 must be met by elongation and desaturation of the essential precursor fatty acids, C18:2*n*-6 and C18:3*n*-3. Concentrations of both C18:*2n*-6

and C18:3*n*-3 within plasma were similar between treatments, a result that may be attributed to similar dietary supply during lactation. However, provision of dietary fish oil during pregnancy had significant carry-over effects upon the amounts of long-chain PUFAs present within plasma at two weeks *post partum*. Little research has been directed towards the carry-over effects of PUFA supplementation upon ruminant plasma fatty acids. However Wonsil *et al.* (1994) suggested that the slow turn-over of phosphatidylglycerols and cholesterol esters within which long-chain PUFAs are preferentially incorporated may impart residual effects of supplementation upon plasma fatty acid concentrations once the PUFA source has been removed from the diet. This hypothesis would explain both the increase in C22:6*n*-3 concentration and decrease in C20:4*n*-6 concentration in plasma of ewes fed fish oil during pregnancy. Supplementation of the ewe with preformed dietary C22:6*n*-3 would facilitate the increased deposition of this fatty acid into plasma lipid components as reported by Ashes *et al.* (1992) and Chikunya *et al.* (2004). By contrast, the inhibition of C20:4*n*-6 synthesis induced by long-chain PUFA supplementation (Bougle *et al.*, 1999) would reduce the amount of this fatty acid deposited in plasma phosphatidylglycerols and cholesterol esters.

5.5.1.7. Milk

Milk yield and composition is affected by factors intrinsic to the ewe such as breed, age and stage of lactation, and by the dietary energy and protein supply (Hullar and Brand, 1993). Previous studies in which lactating ruminants were supplemented with fish oil have reported differing results: Keady *et al.* (2000) observed an increase in milk yield as a result of long-chain PUFA supplementation as did Chilliard and Doreau (1997), whereas the results of Jones *et al.* (1998), Kitessa *et al.* (2001b) and Shingfield *et al.* (2003) suggested that milk yield is depressed by the addition of fish oil to the ruminant diet. By contrast, no significant effect of dietary fish oil or vitamin E supplementation was observed upon milk yield or secretion rate within the current study, concurring with the results noted by Kitessa

et al. (2003) and Kitessa *et al.* (2004) using protected tuna oil and those of Cant *et al.* (1997), Offer *et al.* (2001) and AbuGhazaleh *et al.* (2003) using unprotected fish oils.

The consensus view is that isomers of CLA or *trans* C18:1 produced from incomplete ruminal fermentation of PUFAs are responsible for milk fat depression in animals fed long-chain PUFA sources via effects upon the enzymes required for milk fat synthesis within the mammary gland (Ahnadi *et al.*, 1998). However, the source of CLA and *trans* C18:1 in animals fed a diet devoid of long-chain PUFAs for a three-week period is a matter of contention. The industry standard for ruminal adaptation from a diet containing nutrients that have significant effects upon ruminal fermentation, is three weeks. However, milk composition was measured at four weeks after the diet change-over within the current study, at which point, carry-over effects were still present. Similar results were reported by Shingfield *et al.* (2003) after a 14-day diet adaptation period, but there is no comparable information available for longer time periods. No significant carry-over effect of fish oil supplementation during pregnancy was observed upon plasma concentrations of C18:1 *trans* or CLA within the current study. It is not possible to draw accurate conclusions regarding the effect of *trans*-10, *cis*-12 CLA upon milk fat concentrations due to difficulties in the identification of this fatty acid by gas chromatography.

A plausible explanation for the carry-over effect of fish oil supplementation upon milk fat concentration is that mobilisation of tissue reserves during lactation releases stored CLA and *trans* C18:1 fatty acids. However, long-chain PUFAs are normally stored in phosphatidylglycerols with only small amounts found in adipose tissue (Poumes-Ballihaut *et al.*, 2001) and it may be suggested that the low mobilisation of fatty acids from phosphatidylglycerols would not produce sufficient quantities of these fatty acids to induce changes in milk composition. By contrast, Scollan *et al.* (2003) suggested that triacylglycerols are the major storage depot for CLA, and Schrock and Connor (1975)

reported significant incorporation of *trans* C18:1 fatty acids into triacylglycerols compared to phosphatidylglycerols. Mobilisation of these tissues during lactation may provide a labile source of these fatty acids.

Milk protein synthesis within the mammary gland is directly dependent upon sufficient supplies of amino acids and glucose. The iso-nitrogenous and iso-energetic nature of the experimental diets would suggest that milk protein concentration or yield should be unaffected by treatment diet. However, several authors have published data suggesting that milk protein concentration is depressed by fish oil supplementation of the lactating ruminant (Kitessa *et al.*, 2001b; Ahnadi *et al.*, 2002; Lacasse *et al.*, 2002) suggesting that these effects may be due to a decrease in casein synthesis or caused by a dilution effect of an increase in milk yield. Although the carry-over effects of fish oil supplementation during pregnancy were potent enough to affect milk fat concentrations, these effects may have been less significant upon milk protein.

5.5.2. Lamb parameters

5.5.2.1. Maternal and neonatal behaviour

Measurements of maternal behaviours and care have been shown to differ between breeds (Fahmy *et al.*, 1997; Lawrence and Dwyer, 1997; Lambe *et al.*, 2001) and to be affected by prior maternal experience (O'Connor *et al.*, 1985; Lambe *et al.*, 2001). However, diet is not generally considered to be a significant factor in maternal behaviours directed towards the neonatal lamb. The similar maternal behaviour scores exhibited by ewes on all treatments suggests that the quality of maternal care was unaffected by diet and would have little or no effect upon differences observed in lamb behaviours.

Differences in the latency of ewe vocalisation were unexpected, being significantly higher in ewes supplemented with fish oil plus a supranutritional level of vitamin E. Dwyer *et al.*

(1998) suggested that ewe vocalisation is intrinsically linked to maternal experience and breed, and unrelated to lamb characteristics. The aforementioned study discriminated between low-pitched bleating, emitted in the presence of the lamb and thought to reinforce the ewe-lamb bond, and high-pitched bleating, a distress signal related to lamb absence. Although these data were not recorded in the current study, lambs borne to ewes fed diet FMH may have spent more time apart from the lamb in the immediate *post partum* period thereby exhibiting increased vocalisation. A lack of vocalisation was correlated with reduced lamb standing attempts in the study of Vince (1986), but this effect was not observed in the current study.

Neonatal lamb behaviours follow an established pattern (Fraser and Broome, 1997), regardless of breed, although the latencies of these behaviours are directly related to lamb vigour, and may be affected by lamb breed (O'Connor and Lawrence, 1992; Wassmuth *et al.*, 2001) and birthweight (Tuchscherer *et al.*, 2000). The latencies observed for neonatal lambs within the current study concur with the time periods reported by Alexander and Williams (1966), Fahmy *et al.* (1997) and Wassmuth *et al.* (2001).

Supplementation of the pregnant ewe with long-chain PUFAs appeared to reduce the latencies of standing, searching for the udder and suckling in neonatal lambs, however, these differences were not significantly different. The underlying assumption behind the theory that long-chain PUFA supplementation will improve neonatal vigour, hinges upon the supposition that increasing the concentration of long-chain PUFAs within brain and nervous tissue will improve cognitive development and impact on behaviours. Studies involving the supplementation of animals with long-chain PUFAs upon neonatal behaviours have reported conflicting results. Rooke *et al.* (2001a) observed reduced latencies of contact with the udder and teats in piglets borne by sows fed tuna oil during late pregnancy. Pickard *et al.* (2005) also described lower latencies of standing in lambs

born to ewes supplemented with algal biomass. By contrast, the study of Rooke *et al.* (1998) reported reduced viability scores in piglets from tuna-oil supplemented sows. Brain tissue samples taken from neonatal lambs borne to ewes fed fish oil had higher proportions of C22:6*n*-3 compared to those from lambs produced by ewes fed Megalac, although these differences were not significant. When taken in combination with the reduced latencies of neonatal behaviour in these animals and the significant results observed in Experiment One, it could be suggested that a larger sample size might have led to these differences being significant.

Studies in humans, in which infants were fed formulae supplemented with long-chain PUFAs have reported significant improvements in infant visual acuity, intellectual development and motor skills (Hoffman *et al.*, 1993; Willatts *et al.*, 1998; Bouwstra *et al.*, 2003). However, human neonates are altricial by nature, dependent on maternal care during the first few months of life in contrast to sheep and pigs which are precocial, standing and following the dam within minutes of birth (Zelditch *et al.*, 2003). Therefore, it is possible that latencies of behaviours in lambs are not as precise indices of cognitive and motor development as they are in human infants.

Vitamin E supplementation of the pregnant ewe has been reported by Kott et al. (1998) to reduce lamb mortality and by Merrell (1998) to improve neonatal lamb vigour. No significant effect of maternal dietary vitamin E supplementation upon latencies of lamb behaviours was observed in the current study, furthermore, lamb mortality was unaffected by vitamin E supplementation.

5.5.2.2. Body temperature

The neonatal lamb is susceptible to substantial heat loss during the first hours of life as the skin is saturated with amniotic fluid (Fraser and Broom, 1997). Furthermore, lamb

mortality rates have been reported to be increased by inclement weather conditions when ewes lamb outside (Nowak, 1996; Kott *et al.*, 1998). The ability of the neonate to maintain homeothermy is therefore a significant factor in its survival (Herpin *et al.*, 2002). Homeothermy may be achieved in the neonate via three mechanisms: reducing evaporation, ingesting colostrum or thermogenesis (Herpin *et al.*, 2002). Grooming behaviour of the dam considerably reduces heat loss caused by the evaporation of amniotic fluids from the skin (Fraser and Broom, 1997). The ingestion of colostrum by the lamb then provides energy for heat production (Gonyou and Stookey, 1987). However, non-shivering thermogenesis (NST) is a major factor in the regulation of the lambs temperature with concurrent effects upon survival rate (Robinson, 1981). Thermogenesis occurs in brown adipose tissue (BAT), the extent of heat production dependent on the activation of the enzymes required for the uncoupling of oxidative phosphorylation (Palou *et al.*, 1998). Oudart *et al.* (1997) reported that the thermogenic activity of BAT is enhanced by the addition of long-chain PUFAs to the diet of rats. Therefore, it is logical to assume that supplementation of the ewe diet with fish oil may augment heat production via NST in the lamb. By contrast, Wrutniak and Cabello (1989) suggested that the increase in T_3 concentrations induced by the administration of soya oil to neonatal lambs may exert a negative feedback response upon the hypothalamus-pituitary-thyroid axis, thereby reducing NST.

Lamb rectal temperatures were similar between treatments in the current study, suggesting that maternal diet had no significant effect upon this parameter. However, in order to avoid biasing behavioural data, temperatures were measured at three hours *post partum* after the consumption of colostrum and considerable grooming from the dam, therefore may have been confounded by these factors. The rectal temperatures recorded in the current study are in agreement with those reported by Wassmuth *et al.* (2001) at three hours *post partum*, but it is not known whether differences would have been observed if

temperatures were recorded earlier. Furthermore, all lambs were housed and bedded on sawdust with little effect of climatic conditions, which may have masked potential differences in NST between treatments.

5.5.2.3. Antioxidant status of the neonate

Results observed during Experiment One suggested that transfer of vitamin E across the ovine placenta and subsequent deposition into neonatal tissues may be manipulated by the maternal dietary vitamin E supply. This is in contrast to the body of research which, to date, has concluded that placental transfer of vitamin E to the foetus is negligible and that a satisfactory vitamin E status is only achieved by the ingestion of colostrum (Njeru *et al.*, 1994). Plasma vitamin E concentrations are commonly regarded to be a reliable indicator of whole body vitamin E status, both in humans (Mino and Nishino, 1973; Léger *et al.*, 1998) and various animals (Njeru *et al.*, 1994; Hidiroglou *et al.*, 1995; Lauridsen *et al.*, 2002). Low neonatal plasma concentrations are therefore suggested to be indicative of a low vitamin E status. In agreement with results observed within Experiment One, Pehrson *et al.* (1990) reported increased plasma vitamin E concentrations in lambs borne by ewes supplemented with oral or intramuscular vitamin E, moreover, both Hidiroglou *et al.* (1993a) and Mahan (1991) demonstrated similar results in piglets.

Kelly *et al.* (1992) hypothesised that plasma concentrations may not fully represent foetal vitamin E status and that the liver may act as a labile source of vitamin E during periods of low placental supply. At first glance, the results of the current study appear to bear out the conclusions of previous researchers, in that neither fat source or maternal vitamin E supply had any significant effect on neonatal plasma vitamin E concentration. Indeed, all concentrations were below the level detectable by high performance liquid chromatography. However, supplementing pregnant ewes with supranutritional dietary concentrations of vitamin E significantly increased the deposition of vitamin E within the

brain of neonatal lambs, thereby rejecting the widely held hypothesis that placental transfer of vitamin E is impervious to manipulation by the maternal diet. Hidiroglou *et al.* (1993b) reported a similar pattern in piglet brain and liver as a consequence of increased maternal vitamin E supplementation.

Tissue vitamin E concentrations observed by Hidiroglou *et al.* (1993b) were substantially lower than those detected within the current study, possibly due to the relatively high dietary vitamin E concentrations used within the experiment. Moreover, brain vitamin E concentrations within the current study were approximately threefold higher than those reported by Lauridsen *et al.* (2002) in neonatal piglets. However, the current values were lower than those reported by Vatassery *et al.* (1988) in vitamin E-deficient rats.

High concentrations of long-chain PUFAs tend to abrogate the concentration of vitamin E within tissues and body fluids (Hidiroglou *et al.*, 1970; Farnworth *et al.*, 1995; Wang *et al.*, 1996). Although not significantly different, brain tissue concentrations of vitamin E were higher in lambs borne by ewes fed diet FML compared to MML. This is in contrast to the values observed within the Experiment One in which diets FB and MB conferred almost equivalent brain concentrations of vitamin E. Although maternal dietary vitamin E concentrations were similar between treatments MML and FML, this result may have been due to differences in absorption, placental transfer and incorporation into foetal tissue. Furthermore, it is possible to suggest that tissues with an enhanced PUFA concentration as a consequence of fish oil supplementation of the ewe may also have a concurrent increase in vitamin E deposition in order to prevent against oxidative stress.

5.5.2.4. Plasma fatty acids in the neonate

The effectiveness of the placental fatty acid supply to the neonatal lamb has been debated for some time. In essence, most reviewers have concluded that the only mechanism by

which the lamb attains a satisfactory fatty acid status is via the ingestion of colostrum (Raijon *et al.*, 1985). Analysis of plasma lipid fractions in the human female and neonatal infant by Matorras *et al.* (1999) showed that a high positive correlation exists between maternal and foetal plasma fatty acid concentrations, with particular reference to *n*-3 fatty acids. However, although fish oil supplementation reduced total fatty acid concentrations in ewes at one week *pre-partum*, treatment FMH (fish oil plus a high concentration of vitamin E) increased the concentration of fatty acids within neonatal plasma in the current study. Fatty acids are transported across the ovine placenta by specific fatty acid-binding proteins (Knipp *et al.*, 1999). Placental transfer has not been demonstrated to increase with vitamin E supplementation, therefore it appears that some mechanism exists by which PUFA transfer may be augmented. The total concentration of fatty acids within neonatal lamb plasma was approximately one quarter of that found in ewe plasma (treatments MML and FML) and almost equal for treatment FMH. The reduced concentrations concur with the results of Elphick *et al.* (1978) and Leat and Harrsion (1980) but indicate that although transfer is low, it may not be described as negligible.

The total concentration of saturated fatty acids within neonatal lamb plasma was increased by the provision of fish oil and supranutritional dietary concentrations of vitamin E to the ewe. However, the concentrations of the individual fatty acids C16:0 and C18:0 within neonatal lamb plasma were unaffected by ewe diet. The quantity of these fatty acids within ewe plasma was reduced by supplementation with fish oil, therefore the current results concur with those of Leat and Harrison (1980) who suggested that the placenta may differentiate between specific fatty acids. The preferential transfer of specific fatty acids across the placenta would explain the variation in fatty acid composition of neonatal lamb plasma compared to ewe plasma. For example, the concentration of C16:0 was high in experimental diet MML and concurrently high in ewe plasma, however, this fatty acid was

present in lower amounts in lambs produced by ewes offered diet MML compared to diet FML.

Neonatal lamb plasma concentrations of C18:1 *trans* were unaffected by maternal diet within the current study, but significant effects were observed upon C18:1*n*-9 *cis*, the lowest concentrations being found in lambs borne by ewes fed diet FML, compared to those fed diets MML and FMH. This appears to have resulted from the lower proportions of C18:1*n*-9 *cis* within ewe plasma, affecting the transfer of this fatty acid across the placenta. Supplementing the ewes' diet with fish oil during pregnancy (treatments FML and FMH) also significantly reduced the concentration of this fatty acid within plasma, concurring with the effect of diet MML compared to FML upon neonatal lamb plasma.

Several authors have described the EFA status of the neonatal lamb: Elphick *et al.* (1979) reported extremely low concentrations of C18:2*n*-6 in plasma as did Leat and Harrison (1980), Raijon *et al.* (1985), and Noble *et al.* (1978), the latter authors attributing this to the inability of complex lipids to transverse the placenta and only the unesterified fraction being available to the foetus via the placenta. By contrast, Payne (1978) suggested that placental transfer from dam to foetus is substantial and that the low concentrations of EFAs found in plasma are not representative of overall EFA status. The results of the current study appear to concur with the research of Elphick *et al.* (1979), Leat and Harrison (1980) and Raijon *et al.* (1985) in that the concentrations of C18:2*n*-6 within neonatal lamb plasma were extremely low and C18:3*n*-3 was virtually undetectable. However, this reduction in EFAs is often accompanied by an increase in C20:3*n*-9 (mead acid), the principal metabolite of the elongation and desaturation of C18:0 in the absence of C18:2*n*-6 or C18:3*n*-3 and a prime indicator of EFA deficiency (Noble *et al.*, 1982). Within the current study, C20:3*n*-9 was undetectable in neonatal lamb plasma samples (data not shown) and it is not possible to conclude whether lambs were deficient in EFAs.

Raijon *et al* (1985) suggested that plasma metabolites of EFAs are a pertinent indicator of EFA status, but within the current study, these were confounded by the supply of fish oil rich in C20:5*n*-3 and C22:6*n*-3 to pregnant ewes. The amounts of C20:4*n*-6, C20:5*n*-3 and C22:6*n*-3 within plasma were higher than those of the precursor fatty acids. Furthermore, the proportional contributions of C20:4*n*-6, C20:5*n*-3 and C22:6*n*-3 to plasma fatty acids were appreciably higher in the neonate than in maternal plasma at one week *pre-partum* indicating either a substantial fatty acid synthesis within the placenta or neonate, or a preferential transfer of these fatty acids across the placenta. Noble (1981) and Noble *et al.* (1985) postulated that increases in C20:4*n*-6 between the maternal and foetal circulation may be due to elongation and desaturation of maternally derived C18:2*n*-6. This is further confirmed by the presence of the Δ-6 and Δ-9 desaturase enzyme systems within the ovine placenta (Crawford, 2000). However, although small amounts of C20:4*n*-6 and C20:5*n*-3 were present in the plasma of lambs borne by ewes fed Megalac, C22:6*n*-3 was not detectable, which throws doubt upon the elongation-desaturation hypothesis.

Concurring with the hypothesis of Noble (1981), Campbell *et al.* (1998) described a fatty acid-binding protein present within the human placenta which preferentially binds and transfers C20:4*n*-6 and C22:6*n*-3 to the foetus. The presence of a similar protein within the ovine placenta would explain the increased concentrations of the *n*-3 and *n*-6 fatty acids within lamb plasma within the current study and warrants further investigation. The developing organs of the foetal and neonatal lamb may have an increased requirement for C20:5*n*-3 and C22:6*n*-3 compared to the adult animal, therefore, it may be hypothesised that, as a consequence of endogenous biosynthesis within brain and retina, plasma concentrations of these fatty acids may not be reliable indicators of their concentrations within tissue (Sinclair *et al.*, 2002).

5.5.2.5. Neonatal brain fatty acids

In Experiments One and Two it was suggested that the total fatty acid content of brain tissue was relatively inflexible due to the essential role played by the lipid component, hence the lack of significant effects of maternal treatment diet. However, within the current experiment, not only were the total fatty acid concentrations of neonatal brain tissue higher for all treatments than in the two previous studies but they were further augmented by the addition of Megalac to the maternal diet. Çelik *et al.* (1999) demonstrated that the addition of either vitamin E, selenium or their combination to the diet of growing lambs (at eight to nine months of age) significantly increased the lipid component of brain tissue. The higher vitamin E concentrations within brain tissue within the current study compared to that described in Experiment One concur with this result and suggest that the increased lipid component may have been facilitated by increased vitamin E deposition into brain tissue. However, two further questions are posed by the acceptance of this theory: dietary vitamin E concentrations (whether basal or supranutritional) were similar between Experiments One and Three and it is unclear whether the observed differences in plasma levels were due to variation in brain deposition, or to maternal transfer. Furthermore, the vitamin E concentration of brain tissue was lower in lambs produced by ewes fed diet MML, yet the brain fatty acid concentration was highest in these lambs, a fact which throws doubt on the validity of the theory that vitamin E deposition in the brain affects lipid concentration. It is not clear whether differences in total brain fatty acid concentration were observed in the studies in piglets published by Rooke *et al.* (1999; 2000; 2001a; 2001b) as all results were stated as proportions of total fatty acids.

Saturated and monoenoic fatty acids have a structural role within brain tissue, but, as discussed within section 4.5.2.3. play a minor functional role compared to the EFAs (C18:2*n*-6 and C18:3*n*-3) and the conditionally essential long-chain PUFAs (C20:5*n*-3, C20:4*n*-6 and C22:6*n*-3). The proportion of C18:2*n*-6 within neonatal lamb brain tissue

was considerably lower than that within plasma for all treatments, suggesting either a reduced uptake by brain tissue, or the conversion of this fatty acid to its long-chain derivative (C20:4*n*-6). Indeed, Innis (2003) suggested that the low concentrations of C18:2*n*-6 within human brain tissue are due to the high requirement of neural tissue for C20:4*n*-6 and C22:6*n*-3. However, the proportion of C18:2*n*-6 within the brain lipid component tended to be higher in lambs borne to ewes offered fish oil during pregnancy despite the lack of a treatment effect on neonatal plasma concentrations. From this result it could be hypothesised that C20:4*n*-6 was endogenously synthesised to a lesser extent in these lambs compared to those produced by ewes fed Megalac, a result which would be expected given the inhibitory nature of preformed long-chain *n*-3 PUFAs (C20:5*n*-3, C22:6*n*-3) upon synthesis of *n*-6 PUFAs. Indeed, the proportion of C20:4*n*-6 within lamb brain tissue tended to be lower in lambs borne to ewes fed fish oil during pregnancy compared to those from ewes offered Megalac. Depressions in C20:4*n*-6 status in neonatal piglets were related to reductions in birthweight by Cordoba *et al.* (2000). Furthermore, both Arbuckle and Innis (1992) and Su *et al.* (1999) reported reduced growth rate in animals with low concentrations of C20:4*n*-6 compared to *n*-3 fatty acids.

The proportion of C18:3*n*-3 within neonatal lamb brain tissue was reduced in lambs borne to ewes supplemented with diet FML compared to MML or FMH. The reason for this decrease is not immediately obvious. However, it may be hypothesised that it is a consequence of differing lamb antioxidant status between treatments. Lambs produced by ewes fed diet FML would be hypothesised to have the lowest vitamin E concentrations within brain tissue due to the combination of low maternal dietary supply and increased antioxidant challenge. Therefore, as demonstrated by Çelik *et al.* (1999), the deposition of specific fatty acids into brain tissue may have been reduced by the low vitamin E supply. However, Çelik *et al.* (1999) found that this reduced deposition was equivalent between fatty acids, with no significant effect of chain length or level of unsaturation. By contrast,

the effect of maternal vitamin E supplementation observed within the current study only appears to have affected the deposition of C18:3*n*-3 into brain tissue.

Various studies by Rooke *et al.* (1998; 1999; 2000) have demonstrated that the fatty acid composition of neonatal brain tissue may be manipulated by dietary supplementation of the dam with fish oils, with specific interest in increasing the brain C22:6*n*-3 concentration. In contrast to the results of Rooke *et al.* (1998; 1999; 2000), there was no concurrent increase in proportions of C20:5*n*-3 within lamb brain tissue despite maternal C20:5*n*-3 supply being increased by the addition of fish oil to the treatment diets. As previously discussed (sections 3.4.3.5 and 4.4.3.3), the brain does not appear to have a significant requirement for C20:5*n*-3, hence the concentration gradient that exists between plasma and brain tissue. Endogenous synthesis of C20:5*n*-3 from C18:3*n*-3 may occur in brain tissue, although the principal function of this synthesised fatty acid within the brain is as an intermediate in the synthesis of C22:6*n*-3 (Williard *et al.*, 2001). In contrast to lambs produced by ewes fed fish oil, ewes supplemented with the Megalac diet (MML) did not have a preformed dietary supply of C22:6*n*-3. Therefore it may be concluded that any C22:6*n*-3 present within the brain tissue of their offspring resulted from the endogenous elongation and desaturation of C18:3*n*-3. The pathway by which C20:5*n*-3 is converted to C22:6*n*-3 is not 100% efficient, therefore, it would be expected that higher C20:5*n*-3 concentrations would be present in the brains of lambs produced by ewes fed Megalac in order to accumulate sufficient C22:6*n*-3 for optimal function. This result was not observed within the current study, with similar proportions of C20:5*n*-3 in brain tissue between treatments. However, within the ruminant brain, it is not clear as to which step within the desaturation and elongation process is the rate-limiting step, therefore, there may have been a build-up of other intermediate fatty acids which have not been identified. Williard *et al.* (2001) stated that Δ-6-desaturase is the enzyme that limits the synthesis of C22:6*n*-3 as the binding-site of this enzyme is competed for by C18:1*n*-9, C18:2*n*-6 and C18:3*n*-3, however, both

C24:4*n*-6 and C24:5*n*-3 are also in competition with the aforementioned fatty acids for the synthesis of either C20:4*n*-6 or C22:6*n*-3. Therefore it is possible that the detection of differences in C24:5*n*-3 concentrations within brain tissue would have provided more evidence for the synthesis of C22:6*n*-3 within the brain of these lambs.

Payne (1978) suggested that there was a significant transfer of fatty acids across the ovine placenta, this assumption being based upon the presence of similar concentrations of *n*-3 fatty acids within brain tissue of neonatal and adult ruminants. Lauritzen *et al.* (2001) suggested that the human infant attains maximum *n*-3 deposition into brain (3 mg/g fresh tissue) at approximately two years of age. There is a paucity of data available upon the fatty acid composition of adult ovine brain. However, if the suggestion of Lauritzen *et al.* (2001) can be extrapolated to ruminants, it is likely that the proportions of C22:6*n*-3 observed in neonatal lamb brains within the current study were similar to that of the adult tissue.

The majority of research on infants concerning fatty acid supplementation to date has concentrated on the theory that increasing brain C22:6*n*-3 content has positive effects upon cognitive and motor development. Concurring with this research, the proportion of C22:6*n*-3 within lamb brain tissue, although not significantly different between treatments, was higher in lambs borne by ewes fed fish oil in the current study. These lambs also exhibited lower latencies of standing and suckling, although, again, the difference was non-significant. Therefore, although a link between the two parameters was demonstrated by the results within Experiment One, this link is more tenuous within the current study. This may be due to a number of factors, including variation in the fatty acid composition of fish oil, the absence of any protection against ruminal biohydrogenation and the non-factorial nature of the current experiment reducing the sample size.

5.5.2.6. Lamb growth rate

Lamb birthweight may have been a significant factor in the improvements in lamb vigour is response to maternal vitamin E supplementation as documented by authors including Gentry *et al.* (1992), Kott *et al.* (1996) and Merrell (1998). As observed within Experiment One, vitamin E supplementation of the ewe increases lamb birthweight, and this may have a significant positive effect upon lamb survival. Indeed, reduced levels of pre-weaning mortality in lambs from ewes supplemented with vitamin E were reported by Kott *et al.* (1983) and Gabryszuk and Klewiec (2002). By contrast, vitamin E supplementation of the pregnant ewe appeared to have no significant effect upon lamb birthweight within the current study, although numerically, lambs produced by ewes fed dietary strategy FML (who would be expected to have the lowest vitamin E status) had the lowest birthweights. These results are in agreement with those noted by Williamson *et al.* (1995) and Kott *et al.* (1998) who described no effect of vitamin E supplementation upon lamb liveweight or growth rate.

Long-chain PUFA supplementation of pregnant humans and animals may increase the birthweight of the offspring (Olsen *et al.*, 1992; Rooke *et al.*, 2001a; Smuts *et al.*, 2003), either as a result of improved nutritional status, or increased gestational age at birth. However, concurring with the lack of effects observed as a result of PUFA supplementation upon gestation length in the current study, lamb birthweights were unaffected by maternal dietary strategy. This is also in agreement with the results published by Annett *et al.* (2004) with regards to fish oil supplementation of pregnant ewes. Lamb growth rate was also unaffected by maternal diet, despite carryover effects of maternal *pre-partum* diet upon milk component yield. This may imply that PUFA supplementation of the dam may increase the efficiency of energetic conversion from milk to lamb growth. Low growth rates in human infants deficient in C20:4*n*-6 are reported by Koletzko (1992). Concentrations of this fatty acid were indeed lower in lambs suckling

ewes supplemented with fish oil during pregnancy, but this did not impact on growth rate. Similar results were observed by Rooke *et al.* (2001) and Rooke *et al* (2001c) as a result of augmenting the diet of pregnant and lactating sows with salmon or tuna oil. Therefore, despite differences in nutrient supply, it appears that replacing fish oil within the diet of pregnant ewes with saturated fats during lactation negates the negative effects of long-chain PUFAs upon lamb growth rate as observed during Experiment One. This concurs with the results published by Pickard *et al.* (2005). Alternatively, the methods by which milk yield and lamb growth rate were recorded may have led to overestimation of the nutrient supply to the lamb.

5.5.2.7. Suckling lamb antioxidant status

As demonstrated by the results of Experiment One, supranutritional vitamin E supplementation of the ewe significantly increases the dietary vitamin E supply to the lamb via colostrum and milk. Differences in lamb plasma vitamin E concentrations may therefore be attributed to variation in the colostrum and milk vitamin E yields between treatments, the highest values being seen in lambs suckling ewes fed diets containing a supranutritional vitamin E concentration. This concurs with the results of Njeru *et al.* (1994) who supplemented pregnant and lactating ewes with various levels of vitamin E.

Observed plasma vitamin E concentrations were in agreement with the range of values published by various authors as a result of vitamin E supplementation of sheep (Doncon and Steele, 1988; Gentry *et al.*, 1992; Hatfield *et al.*, 2002). When compared to the index suggested by Hidiroglou *et al.* (1992) for threshold values for vitamin E deficiency in cattle, lambs from all treatments appear to be deficient in vitamin E at both 24 hours and two weeks of age, regardless of the extent of maternal vitamin E supplementation. This concurs with the results of Wachira (1999) who observed poor intestinal absorption and consequent deposition of vitamin E into tissues of growing lambs.

Lambs solely dependent upon colostrum for nutrients had relatively high erythrocyte GPx activities, approximately two-fold higher than those observed in the ewe at one week *pre-partum*. This concurs with the theory that, although placental transfer of selenium may be limited, there is no barrier to mammary transfer (Jacobsson and Oksanen, 1966; Bostedt and Schramel, 1990). Concurring with ewe GPx results, maternal diet had no significant effect upon the activity of this enzyme in the lamb, at either 24 hours or two weeks of age. Although not reaching statistical significance, fish oil supplementation of the pregnant ewe depressed lamb GPx activity during lactation, which may be a consequence of the observed carry-over effect of fat source upon milk composition. Milk fat depression in lactating ewes resulting from fish oil supplementation may be associated with an increase in the proportion of long-chain PUFAs within milk. Lambs suckling from ewes fed diets FML or FMH may therefore have been subject to an increased oxidative challenge with consequent reduction in GPx activity. Lamb serum CK concentrations appeared to be increased by supranutritional vitamin E supplementation of the dam, although remaining below the threshold limits suggested by Bostedt and Schramel (1990) and Walsh *et al.* (1993) for sub-clinical myopathy. This is in direct contrast to the expected result, the biochemical mechanisms behind it being unclear.

5.5.2.8. Suckling lamb plasma fatty acids at 24 hours of age

The neonatal lamb effectively functions as a monogastric; ingested colostrum traveling down the oesophageal groove and forming an insoluble clot in the abomasum before digestion via lipase enzymes in the small intestine (Hocquette and Bauchart, 1999). Consequently, the plasma fatty acid composition at 24 hours of age is directly linked to the fatty acid composition of colostrum, although placental transfer of fatty acids also makes a small contribution. Neonatal animals are born with low concentrations of fatty acids within plasma, specifically EFAs, and researchers suggest that an adequate fatty acid status

is only attained after the consumption of colostrum (Leat and Harrison, 1980; Raijon *et al.*, 1985). Total plasma fatty acid concentrations at 24 hours of age were similar between treatments, but the fatty acids status of the lamb at this time point was considerably improved when compared to the neonate.

Lamb colostrum consumption was assumed to be similar between treatments and it can be suggested that differences in the amount of C16:0 within lamb plasma were the result of changes in colostrum composition. As previously discussed, long-chain PUFA supplementation of ruminants reduces the synthesis of C16:0 within the mammary gland with consequent effects upon the secretion of this fatty acid into colostrum. Furthermore, the maternal dietary supply of C16:0 was higher in ewes fed diet MML, corresponding with the increased concentrations of this fatty acid in lamb plasma. The proportion of C16:0 within suckling lamb plasma was increased when compared to that of the neonatal lamb, indicating a substantial transfer of this fatty acid from the maternal diet to colostrum. The proportions of both C16:0 and C18:0 within plasma were similar to those reported by Fritsche *et al.* (1993) in suckling piglets as a result of fish oil supplementation. Concentrations of C18:0 within lamb plasma were unaffected by maternal diet, a finding in contrast to the results observed for colostrum fatty acid composition within Experiment One.

Supplementation of the pregnant ewe with fish oil has been demonstrated to increase the concentration of C18:1 *trans* fatty acids within plasma and milk as a result of incomplete biohydrogenation of long-chain fatty acids within the rumen (Chilliard *et al.*, 2001a). Concurring with these results, augmentation of the maternal diet with fish oil increased the concentration of C18:1 *trans* within lamb plasma in the current study, at concentrations and proportions higher than those observed in the neonate. The principal fatty acid contained within lamb plasma was C18:1 *cis*, with lower concentrations observed as a

result of fish oil supplementation of the ewe. Ewe plasma concentrations of C18:1 *cis* were similarly reduced by fish oil supplementation at one week *pre-partum*. Although the concentration of this fatty acid may be increased in colostrum compared to plasma by virtue of Δ-desaturase enzymes within the mammary gland (Chilliard *et al.* 2001a), the proportions of this fatty acid within suckling lamb plasma were similar to those observed in the neonate.

A major research issue in neonatal ruminants has been the discovery that lambs have very low plasma concentrations of the EFAs C18:2*n*-6 and C18:3*n*-3, that are rectified by the consumption of colostrum and milk over the first few weeks of life (Raijon *et al.*, 1985). Indeed, the consumption of colostrum over the first 24 hours of life considerably increased the concentration of C18:2*n*-6 within suckling lamb plasma across all treatments. High plasma concentrations of C20:3*n*-9 indicate EFA deficiency in the lamb (Noble *et al.*, 1978) but this fatty acid was found in very low concentrations within lamb plasma (data not shown), therefore it can be concluded that lambs achieved an adequate fatty acid status by 24 hours *post partum*.

Despite differences observed in ewe plasma C20:4*n*-6 concentrations, the amount of this fatty acid within lamb plasma was similar between treatments. Dietary C20:4*n*-6 that escapes ruminal biohydrogenation is incorporated into phosphatidylglycerols and may be secreted into milk and colostrum, but the amount transferred to the lamb appears to be independent of the maternal dietary supply. Shand *et al.* (1978) reported that neonatal and suckling lambs were able to produce C20:4*n*-6 from C18:2*n*-6 within the liver via Δ-6 desaturase. Differences observed between maternal and lamb plasma concentrations may therefore be a result of C20:4*n*-6 synthesis within the lamb.

Both *n*-3 and *n*-6 PUFAs are essential for the correct formation and function of neural tissues both in the foetal and neonatal lamb (Goustard-Langelier *et al.*, 1999). Supplementation of the lactating ruminant with fish oil demonstrably increases the concentrations of C20:5*n*-3 and C22:6*n*-3 within milk as described by Lacasse *et al.* (1998), Keady *et al.* (2000) and Chilliard *et al.* (2001b) and it is logical to assume that the same mechanism occurs within colostrum, as observed within Experiment One. This concurs with the results observed for these fatty acids in lamb plasma, which, without being subject to modification by ruminal biohydrogenation, represent the concentrations of individual fatty acids in colostrum. This concurs with results observed in monogastric studies, including the study of Goustard-Langelier *et al.* (1999) where piglets fed formulae supplemented with *n*-3 fatty acids resulted in increased concentrations of C20:5*n*-3 and C22:6*n*-3 within plasma.

5.5.2.9. Suckling lamb plasma fatty acids at two weeks of age

Within the current study, lambs were solely dependent on maternal milk to fulfil their nutritional requirement at two weeks of age. Given the lack of long fibre, ruminal digestion and function would have been negligible in these suckling lambs. It may therefore be suggested that the fatty acid composition of lamb plasma at this time point provides an indication of milk fatty acid composition, especially given the positive relationship between maternal and lamb plasma fatty acid composition at two weeks *post partum*. Results from both Experiment One and studies published by Keady and Mayne (1999b), Chilliard *et al.* (2000) and Voigt and Hagemeister (2001) have demonstrated that long-chain PUFA supplementation of lactating ruminants reduces the fat concentration of milk. However, milk fat yield was unaffected by treatment diet and the similar total lamb plasma fatty acid concentrations between treatments suggest that all lambs had a similar fatty acid intake at two weeks *post partum*.

Lamb plasma fatty acid composition at two weeks *post partum* concurs with results observed in maternal plasma in that the concentrations of C16:0 and C18:0 were unaffected by treatment diet. There is some evidence to suggest that both long-chain PUFAs and products of the incomplete ruminal biohydrogenation of PUFAs (*trans* octadecanoic fatty acids, *trans*-10, *cis*-12 CLA) may act directly upon enzymes within the mammary gland and reduce milk fat synthesis (Peterson *et al.*, 2003), specifically reducing concentrations of C16:0. However, it appears that supplying a preformed dietary source of C16:0 in the form of Megalac to the lactating ewe may have negated any carry-over effect of PUFA supplementation upon the transfer of this fatty acid to the suckling lamb. Furthermore, the amounts of *trans* C18:1 and CLA, were present in similar proportions in all lamb plasma samples, suggesting that their concentrations within milk fat were equally unaffected by treatment diet.

Concentrations of C18:2*n*-6 within lamb plasma at two weeks *post partum* were similar between treatments, a result which implies that the dietary supply of this fatty acid was consistent between treatments. This would be expected given that all ewes were supplemented with diets of a similar fatty acid composition during lactation. As previously discussed (section 5.5.1.6.), a decrease in C20:4*n*-6 synthesis within ewes fed fish oil and subsequent low transfer to the lamb may have resulted from the inhibition of Δ^9-desaturase enzymes involved in the elongation and desaturation process (Demirel *et al.*, 2004). However, a relative increase in the proportion of the precursor fatty acid (C18:2*n*-6) within plasma would also have been expected to be observed. The proportion of this fatty acid within plasma may therefore be an inadequate indicator of the efficiency of the endogenous synthesis of *n*-6 fatty acids within the ewe or lamb.

The lower proportions of C18:3*n*-3 within plasma of lambs suckling ewes fed Megalac may be attributed to the utilisation of this fatty acid for C20:5*n*-3 and C22:6*n*-3 synthesis.

Despite the change to a dietary saturated fatty acid source during lactation, lambs suckling ewes which had been fed diets containing fish oil during pregnancy had significantly higher concentrations of C20:5*n*-3 and C22:6*n*-3 within plasma at two weeks *post partum*. This is in contrast to the results of Kitessa *et al.* (2003) who observed no significant carry-over effects of protected tuna oil upon milk composition at six days after fish oil was removed from the diet of lactating sheep. If, as suggested by Wonsil *et al.* (1994), long-chain *n*-3 PUFAs are incorporated into phosphatidylglycerols and cholesterol and slowly released as a result of the mobilisation of these lipid components, this increase in plasma long-chain *n*-3 PUFAs is not unexpected. Ewe plasma C20:5*n*-3 concentrations were similar between treatments and C22:6*n*-3 concentrations were approximately three-fold higher in ewes that were supplemented with fish oil during pregnancy. As the proportions of C20:5*n*-3 and C22:6*n*-3 were similar in suckling lambs, this appears to indicate a preferential transfer of C20:5*n*-3 into ewe milk lipids, with concurrent effects upon lamb dietary supply. Alternatively, a proportion of either maternal C22:6*n*-3 or that supplied to the lamb via mammary transfer may have been retroconverted to C20:5*n*-3. However, the reason behind the retroconversion of preformed C22:6*n*-3 to C20:5*n*-3 within the sucking lamb is as yet unknown as it would be expected that the lamb would have a relatively higher requirement for C22:6*n*-3 than for C20:5*n*-3 during this period of growth and neural tissue development.

5.6. Conclusion

Long-chain PUFA supplementation of the ewe in the form of fish oil had no significant effect upon ewe gestation length. The latencies of neonatal lamb behaviours appeared to be reduced by long-chain PUFA supplementation of the ewe, however, these differences did not reach statistical significance. Furthermore, deposition of C22:6*n*-3 within neonatal lamb brain tissue was unaffected by treatment diet. Changing the diet from one based on long-chain PUFAs during pregnancy, to one containing predominantly saturated fatty acids during lactation successfully negated effect of PUFA supplementation upon lamb growth rate. Nevertheless, milk composition tended to be affected by *pre-partum* diet even after a four-week interval. Supplementing pregnant ewes with supranutritional concentrations of vitamin E tended to increase the deposition of this vitamin in neonatal lamb brain tissue although no significant effect of maternal vitamin E supply was observed upon lamb behaviour.

6. GENERAL DISCUSSION

The objective of this series of experiments was to investigate the effects of long-chain PUFA and vitamin E supplementation of ewes upon lamb vigour and performance. This section concentrates on the discussion of parameters that have a direct impact upon this objective, and the possible implications for both animal and human nutrition.

One of the most notable findings of the current study was the significant effect of long-chain PUFA supplementation of pregnant ewes on gestation length in Experiment One. The supplementation of pregnant ewes with fish oil significantly increased gestation length by approximately two days within this experiment. By contrast, there was no effect of fat source upon this parameter in Experiments Two and Three. As described in section 1.2.3.3, endogenous synthesis of the prostaglandins that control parturition is governed by the supply of *n*-3 and *n*-6 long-chain PUFA precursors (Hansen and Olsen, 1988). Olsen *et al.* (1990) suggested that a threshold level might exist for the ratio of stimulatory to inhibitory prostaglandins, with ratios exceeding this level leading to the inhibition of parturition. The synthesis of stimulatory 2-series prostaglandins from C20:4*n*-6, which initiate parturition, may have been depressed or abrogated by C20:5*n*-3 supplementation and the production of inhibitory (3-series) prostaglandins in Experiment One. Certainly, the ratio of C20:5*n*-3:C20:4*n*-6 within ewe plasma at two weeks *pre-partum* was higher (2.71) within Experiment One than in the second (one week *pre-partum*; C20:5*n*-3:C20:4*n*-6 = 0.71) and third experiments (one week *pre-partum*; C20:5*n*-3:C20:4*n*-6 = 2.07).

Research in human neonates has demonstrated that parameters of neonatal vigour, specifically birthweight, increase with gestational age (Ayoubi *et al.*, 2002). It may therefore be suggested that the reductions in latencies of neonatal behaviours observed with long-chain PUFA supplementation of the ewe in Experiment One were conferred by

the concurrent increase in gestation length. As gestational age increases, foetal concentrations of EFAs and *n*-3 PUFAs are increased (Friedman, 1986). Consequently, if PUFA status is a significant contributing factor to lamb vigour during the perinatal period, an increase in ewe gestation length would be expected to improve latencies of neonatal behaviours. Nonetheless, gestation length was similar between treatments in Experiments Two and Three, therefore overall, the correlation between ewe gestation length and latency of lamb suckling was close to zero ($r^2 = 0.0014$; Figure 6.1).

The effects of long-chain PUFA supplementation of pregnant humans upon gestation length and neonatal vigour have been investigated in several studies including those of Olsen *et al.* (1986; 1992). However, one obvious drawback of human studies is the inability to relate behavioural characteristics such as improved cognitive or motor development to brain fatty acid composition (Bondia-Martinez *et al.*, 1998). Within the current study, providing preformed dietary sources of C20:5*n*-3 and C22:6*n*-3 to the pregnant ewe significantly improved lamb vigour in Experiment One and tended to improve it in Experiments Two and Three. Bondia-Martinez *et al.* (1998) suggested that augmentation of the C22:6*n*-3 concentration within neural tissue via dietary supplementation might improve human neonatal development. From the current study, it appears that neonatal lambs may be used as a sensitive model for the neonatal human, although differences in the repertoire of neonatal behaviours of the two species should be taken into account. Previous studies have assumed that the absolute amount of C22:6*n*-3 deposited in brain tissue is responsible for behavioural changes (Uauy *et al.*, 2003; Levant *et al.*, 2004). This hypothesis concurs with the results observed in Experiment One, where an increase in brain C22:6*n*-3 concentration with concurrent reductions in neonatal behavioural latencies was observed. However, this does not elucidate the mechanisms by which behavioural parameters appeared to be improved by PUFA supplementation in

Experiments Two and Three, despite the lack of a significant increase in brain C22:6*n*-3 concentrations.

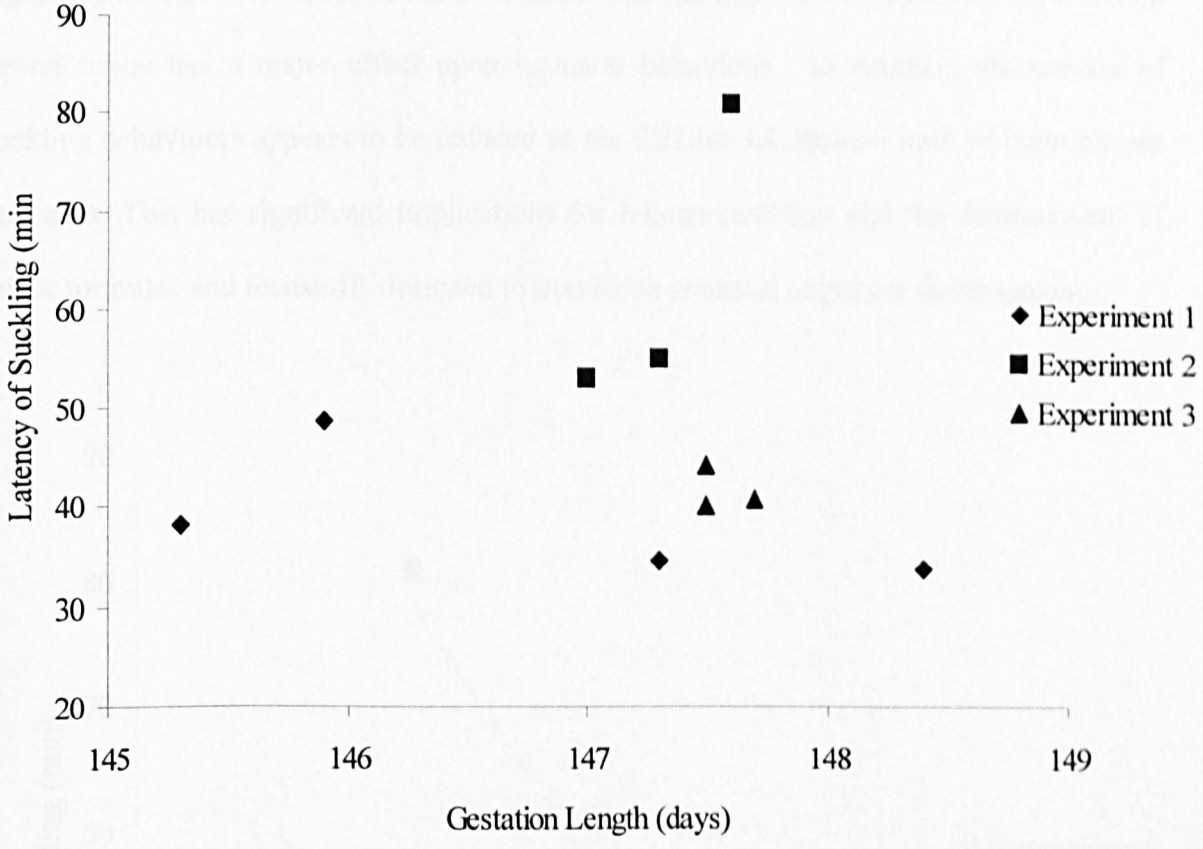


Figure 6.1 Effect of supplementing pregnant ewes with various fat sources and vitamin E concentrations upon the correlation of ewe gestation length to the latency of lamb suckling

Significant deposition of C20:4*n*-6 into foetal neural tissue also occurs during the brain growth spurt prior to birth (Arbuckle *et al.*, 1994). Therefore the lack of a significant effect of PUFA supplementation on the deposition of C22:6*n*-3 into lamb brain tissue in Experiments Two and Three may have resulted from competition with C20:4*n*-6. As previously discussed (section 4.5.2.3), both C20:4*n*-6 and C22:6*n*-3 have essential functions within the brain, therefore it may be difficult to manipulate the absolute amounts of these fatty acids deposited into brain tissue. Nonetheless, it may be appropriate to examine the ratio of C22:6*n*-3 to C20:4*n*-6 within neonatal lamb brain tissue across the three experiments when trying to elucidate the mechanisms behind behavioural changes. From the data presented in Figure 6.2, it can be observed that long-chain PUFA

supplementation of pregnant ewes increases the ratio of C22:6*n*-3:C20:4*n*-6 within the brain from approximately 2.44 (diets based on Megalac) to 3.24 (diets based on fish oil, algae or linseed). This leads to the conclusion that the ratio of C22:6*n*-3:C20:4*n*-6 within neural tissue has a major effect upon neonatal behaviour. In essence, the latency of suckling behaviours appears to be reduced as the C22:6*n*-3:C20:4*n*-6 ratio of brain tissues increases. This has significant implications for human nutrition and the development of infant formulae and feedstuffs designed to maximise neonatal cognitive development.

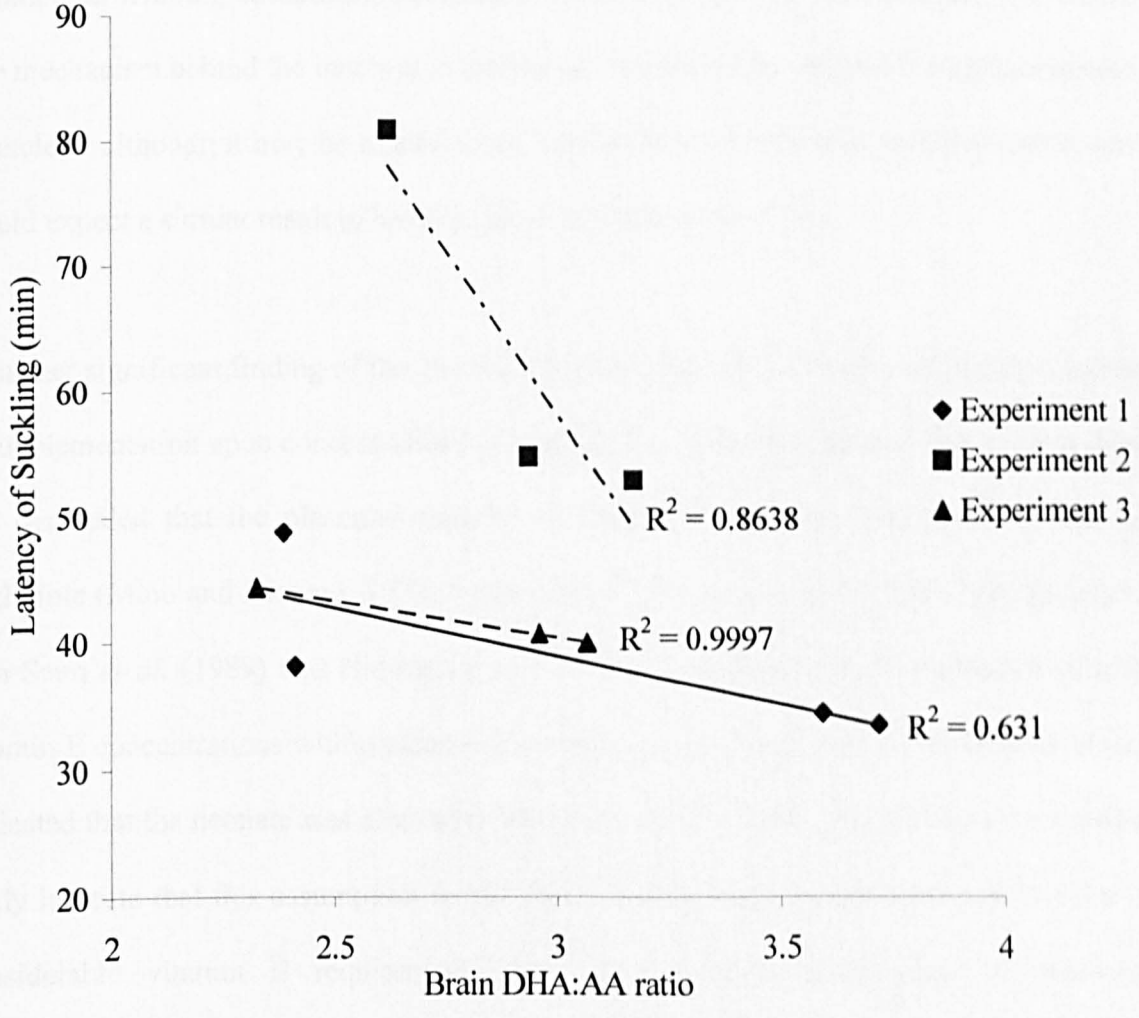


Figure 6.2 Effect of supplementing pregnant ewes with various fat sources and vitamin E concentrations upon the correlation of brain DHA:AA ratio to the latency of lamb suckling

Merrell (1998) and Kott *et al.* (1983; 1998) reported significant improvements in neonatal lamb vigour and reductions in lamb mortality rate as a consequence of vitamin E supplementation. By contrast, maternal dietary vitamin E concentration did not affect neonatal lamb behaviour in the current study. Within Experiment One, lamb birthweight was increased by vitamin E supplementation, which, according to the study of Tuchscherer *et al.* (2000), may improve survival. No differences in lamb mortality rates were observed across any of the experiments within this study. However, the level of animal husbandry and environmental conditions may be considered more conducive to survival than commercial lambing conditions, particularly those found in extensive husbandry systems. The mechanism behind the increase in birthweight conferred by vitamin E supplementation is unclear: although it may be related to an improvement in ewe immunocompetence, one would expect a similar result to have occurred in Experiment Three.

A further significant finding of the current study was the effect of maternal dietary vitamin E supplementation upon concentrations of vitamin E within lamb tissues. Research to date has concluded that the placental transfer of vitamin E from the dam to the foetus is negligible (Mino and Nishino, 1973; Njeru *et al.*, 1994; Léger *et al.*, 1998). Furthermore, Van Saun *et al.* (1989) and Hidiroglou *et al.* (1995) concluded that the presence of low vitamin E concentrations within plasma of neonatal animals compared to maternal plasma, indicated that the neonate was clinically deficient in this vitamin. Results from the current study indicate that this assumption is not valid. Tissues such as the brain, which have a considerable vitamin E requirement, may have evolved mechanisms to maintain satisfactory concentrations under conditions of sub-optimal supply. There is a paucity of data available relating to the concentration of vitamin E within neural tissue of suckling or adult ruminants and it is unclear whether the tissue concentrations achieved within Experiments One and Three were indicative of a satisfactory vitamin E status. Therefore, although placental transfer of vitamin E many be manipulated by maternal dietary regime,

it is not known whether this transfer alone may confer an adequate vitamin E status upon the lamb. However, it is clear, that the assumption made by previous researchers, namely that placental transfer of vitamin E from the ewe to the lamb is negligible, is invalid.

Concurring with the results of Cant *et al.* (1997), Keady and Mayne (1999b) and Petit *et al.* (2002a), Experiment One demonstrated that dietary supplementation of lactating ewes with long-chain PUFAs had significant effects upon milk composition and yield. A concurrent decrease in lamb growth rate was observed with long-chain PUFA supplementation, and the use of strategies, in which PUFAs were replaced with a saturated fat alternative during lactation were explored in Experiments Two and Three. Results from the latter two experiments indicated that it was possible to negate the effects of PUFA supplementation of lactating ewes upon lamb growth rate by changing to a saturated dietary fat source post partum. Nevertheless, significant effects of *pre-partum* diet upon milk composition were observed in both Experiments Two and Three, despite a period of four weeks elapsing between diet change-over and milk sampling. Similar results were observed by Shingfield *et al.* (2003) after a 14-day change-over period, but the study of Gulati *et al.* (2003) indicated that a shorter change-over period was sufficient to negate carry-over effects of PUFA supplementation. The results of the current series of experiments suggest that the observed carry-over effects may have major implications for previous and future nutritional experiments involving "cross-over" designs.

7. AREAS FOR FURTHER RESEARCH

The following questions have emerged from the current study and warrant further investigation:

- Is the increase in gestation length observed with long-chain PUFA supplementation the result of changes in prostaglandin synthesis? If so, are these changes dependent on the absolute amount of C20:5*n*-3 supplied to the ewe, or upon the ratio between C20:5*n*-3 and C20:4*n*-6 available to tissues?

- To what extent can changes in neonatal lamb behaviour be attributed to differences in gestation length as opposed to variation in brain C22:6*n*-3 concentration? Moreover, do behavioural differences simply result from differences in brain C22:6*n*-3 concentrations, or from differences in the ratio of C22:6*n*-3 to C20:4*n*-6 within the brain?

- Does a ceiling exist for the placental transfer and subsequent deposition of vitamin E into foetal tissues? To what extent may placental transport be manipulated by maternal dietary supplementation?

- What is the biochemical mechanism by which long-chain PUFAs continue to affect milk yield and composition after a significant dietary change-over period?

8. CONCLUSION

This study showed that long-chain PUFA supplementation of pregnant ewes can improve neonatal lamb vigour, probably mediated by effects upon ewe gestation length and lamb brain fatty acid composition. The negative effects of dietary supplementation with long-chain PUFAs upon milk composition and lamb growth rate may be negated by the provision of a saturated fat source during lactation. Placental transfer of vitamin E from the ewe to the foetal lamb may be enhanced by maternal dietary supplementation.

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