Influence of Oilseed Supplementation on Ruminant Meat and Milk with Emphasis on Fatty Acids

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Cover: Limousin calves on pasture, courtesy of Stoneyview Limousin Farm, SK, Canada.
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Abstract

In the human diet, long-chain polyunsaturated fatty acids (LC-PUFA, ≥ C20) are supplied almost exclusively from animal sources. Red meat represents a significant source of LC-PUFA in many areas of the world. However, the health aspects of consuming ruminant tissues are often viewed critically due to the proportions of saturated fatty acids (SFA). The health benefits from the monounsaturated fatty acids (MUFA) and polyunsaturated fatty acids (PUFA) in ruminant muscle are often overlooked. Improvements to the lipid quality of ruminant tissues can be achieved through dietary manipulation.

This thesis focuses on oilseed supplementation as a means to alter the FA profile of ruminant tissues. The results obtained indicate that the FA profile of the oilseed supplement and the feeding duration affected the muscle FA profile. The diet and nutritional status of the lactating ewes affected the milk FA profile, in turn affecting the growth performance and *M. semispinalis capiti*s FA profile of the lambs after weaning. Modifying the FA profile of ruminant meat requires reducing the accessibility of dietary PUFA to ruminal biohydrogenation. Heat processing has been shown to alter the oilseed protein structure, increasing the post-ruminal supply of amino acids and PUFA. Roasting and extruding were equally effective processes for increasing the *in vitro* undegradable N fraction of linseed and soybean without affecting N digestibility. The *M. masseter* and *M. pars costalis diaphragmatis*, collected from animals fed roasted or extruded oilseeds had PUFA profiles which were influenced both by the dietary n-6/n-3 PUFA ratio and by the Δ5- (Δ5d) and Δ6- (Δ6d) desaturase enzymes responsible for forming LC-PUFA. Moreover, the Δ5d and Δ6d protein expressions were correlated within the muscles, whereas the dietary regulation of the Δ5d and Δ6d protein expression was muscle specific.

Based on these findings, dietary oilseed supplementation modified the FA profile of ruminant products, improving the health benefits from their consumption. Further studies into oilseed processing and influence of diet on the lipogenic pathways will assist in feeding strategies to further enhance the health benefits from ruminant meat consumption.

Keywords: Δ5-desaturase, Δ6-desaturase, MUFA, n-6/n-3 ratio, oilseeds, PUFA, processing, SFA

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Betydelse av oljefröväxter i foder till idisslare, påverkan på kött- och mjölkkvalitet med fokus på fettsyror

Sammanfattning

Långa fleromättade fettsyror (LC-PUFA, ≥ C20) i människans kost kommer från livsmedel av animaliskt ursprung. Rött kött är en betydande källa för dessa fettsyror i stora delar av världen där fisk inte konsumeras. Kött förknippas emellertid ofta med mättade fetter och de viktiga fleromättade och enkelomättade fettsyrorna glöms bort.

Förbättrad och anpassad fettkvalitet i djurfoder leder till nyttigare fettsammansättning i produkter från betande djur. I denna avhandling har fokus legat på en förbättrad fettsammansättning i foder till idisslare genom att utnyttja oljefröprodukter som fodertillsats. Studierna har visat att fettsyrasammansättningen hos oljefröerna och utfodringens längd påverkar musklernas fettsyrasammansättning. Tillsats av hampa förbättrade fettsyrasammansättningen i biff jämfört med sojatillsats, mot mer omega-3 fettsyror (FS). I en lammstudie var tackornas foder och utfodringsintensitet av största betydelse för lammens tillväxt och mjölksammansättning och avspeglades i lammköttets fettsyraprofil efter avvänjning. Anpassning av fettsyraprofilen i kött från idisslare förutsätter att mikrobiell nedbrytning av fleromättade FS minskar i vommen. Värmebehandling av oljefröer kan påverka proteinstrukturen och därmed också öka passagen av såväl aminosyror som FS genom vommen. Rostning och extrudering var lika effektiva i *in vitro* försök för att öka det icke nedbrytningsbara kvävet utan att påverka kvävets smältbarhet negativt. Vid uppfödning av nöt har extruderade och rostade oljefrön som tillsats i fodret resulterat i högre andel fleromättade fettsyror i musklerna. De muskler som undersöktes var *M. masseter* och *M. pars costalis diaphragmatis*. I studien visades att både fodrets omega-6/omega-3 fettsyrakvot och proteinuttryck av Δ5 och Δ6- desaturas i musklerna påverkade båda musklernas FS-profil. Dessutom kunde vi visa att musklernas desaturasuttryck var muskelspecifikt.

Tillsats av oljefröer kan påverka fettsyraprofilen i produkter från idisslare och därmed förbättra nyttan vid human konsumtion. Fortsatta studier för att optimera tillsatser i foder är önskvärda. Dessutom behövs undersökningar som kan visa hur foder påverkar de metabola mekanismer som bestämmer fettsyraprofilen i produkter från idisslare. Sådana undersökningar kommer att ha betydelse för nya och optimaliserade utfodringsstrategier som leder till hälsosammare produkter.

Nyckelord: Δ5- och Δ6-desaturas, omättade fettsyror, omega-6/omega-3 kvot, processning, mättade fettsyror

Dedication

To family and friends who have lent their support along the way, with my heartfelt thanks, this thesis is dedicated to you.

Contents

List of Publications

This thesis is based on the work contained in the following papers, referred to by Roman numerals in the text:

I Turner, T., Hessle, A., Lundström, K., Pickova, J. (2008). Influence of hempseed cake and soybean meal on lipid fractions in bovine *M. longissimus dorsi*. *ACTA Agriculturae Scandinavia, Section A* 58, 152-160.

II Bernes, G., Turner, T., Pickova, J. (2010). Sheep fed only silage or supplemented with concentrates. 2. Effect on lamb performance and fatty acid profile. (manuscript).

III Turner, T., McNiven, M.A. (2010). *In vitro* N degradability and N digestibility of raw, roasted and extruded canola, linseed and soybean. *(*manuscript).

IV T. Turner, Pickova, J., Duynisveld, J., Doran, O., McNiven, M.A. (2010). Δ5- and Δ6- desaturase protein expression in two bovine muscles as influenced by dietary n-6/n-3 ratio in feedlot steers fed processed oilseeds. (manuscript).

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The contribution of Tyler Turner to the papers included in this thesis was as follows:

I Performed the analytical work, evaluated the results and prepared the manuscript.

II Performed the analytical work, evaluated the results and prepared the manuscript for the lipid related sections.

III Participated in the analytical work, evaluated the results and prepared the manuscript.

IV Performed the analytical work, evaluated the results and prepared the manuscript.

Abbreviations

Δ5d	Δ5* desaturase
Δ6d	Δ6* desaturase
ADF	Acid detergent fibre
ADIN	Acid detergent insoluble nitrogen
CP	Crude protein
CLA	Conjugated linoleic acid
CVD	Cardiovascular disease
DM	Dry matter
FA	Fatty acid
HDL	High density lipoprotein
IMF	Intramuscular fat
LC-PUFA	Long-chain polyunsaturated fatty acid (≥ C20)
LDL	Low density lipoprotein
LBT	Leukotreines
LWG	Live weight gain
MUFA	Monounsaturated fatty acids
NDF	Neutral detergent fibre
NEFA	Non-esterified fatty acid
NL	Neutral lipid fraction
PG	Prostaglandins
PL	Polar lipid fraction
PUFA	Polyunsaturated fatty acids
RUP	Rumen undegradable protein
SCD	Sterol-CoA desaturase
SEM	Standard error of the mean
SFA	Saturated fatty acids
TXA	Thromboxanes
WB-SF	Warner-Bratzler shear force

1 Introduction

Red meat provides many essential nutrients, including fats, and can be an important part of a balanced diet (Williamson *et al.*, 2005). Globally, there has been a general trend towards increased meat consumption, rising by more than 10% since the 1960's (Valsta *et al.*, 2005). Today, health concerns have been raised about saturated fatty acids (SFA) as well as the amount and types of polyunsaturated fatty acids (PUFA) ingested. Consumers often have been critical of ruminant meat due to the high SFA and low PUFA/SFA ratio (Enser *et al.*, 1996). Moreover, the use of concentrates in modern livestock production has led to an increase in the n-6/n-3 PUFA ratio of ruminant meat. Cumulatively, this has led to a decline in beef sales during the last 40 years (Givens *et al.*, 2006).

To improve the public image of beef and increase consumption the nutritive qualities have been promoted, namely that it is a good source of protein, iron and B vitamins. Improvements to the nutritional quality of lipids in ruminant meat have focused on the animal's diet in efforts to reduce the SFA content while enhancing the monounsaturated fatty acid (MUFA) and PUFA content with an n-6/n-3 PUFA ratio favourable for human health. Howe *et al.* (2006) and Williamson *et al.* (2005) have emphasized the importance of meat as a source of long-chain polyunsaturated fatty acids; (LC-PUFA, $\geq$ C20), particularly for populations with traditionally low intake of food from marine sources. Additionally, conjugated linoleic acid (CLA), a lipid natural to ruminant products, has been shown to have many bio-functional properties (Park, 2009). Earlier studies have shown the CLA and LC-PUFA level in ruminant products can be increased via diet manipulation (Scollan *et al.*, 2005; Dannenberger *et al.*, 2004; Bauman *et al.*, 2000).

Precursors must be provided in the diet to increase the LC-PUFA content of ruminant tissue, namely the essential fatty acids (FA), C18:2n-6

and C18:3n-3. The main PUFA in most cereals and oilseeds is C18:2n-6, whereas C18:3n-3 is most abundant in forages and linseed. Oilseeds are an excellent protein source and contain as much as 45% lipids, whereas forages and cereals have lower lipid levels. The higher lipid content of oilseeds can potentially provide a higher post-ruminal flow of PUFA. One of the challenges faced when feeding PUFA to ruminants is the extensive biohydrogenation activity in the rumen. The proportion of PUFA lost due to biohydrogenation has been shown to be reduced by heat processing the oilseeds, thus increasing the post-ruminal PUFA available for absorption. Once integrated into the tissues, essential FA undergo a number of elongation and desaturation steps to produce LC-PUFA (Sprecher, 2000). The n-3 LC-PUFA level in the food supply could be increased if the animal's biosynthesis mechanisms could be influenced by diet to increase the formation of n-3 LC-PUFA within the tissue (Wood *et al.*, 2008a). Numerous trials have shown that supplementing finishing diets with oilseeds can be used to influence both the n-6/n-3 PUFA ratio and LC-PUFA content (Dawson *et al.*, 2010; Basarab *et al.*, 2006; Kronberg *et al.*, 2006; Maddock *et al.*, 2006; Choi *et al.*, 2000).

This work explored how oilseed supplementation could be used to modulate the FA profile of ruminant muscles and subsequently influence meat quality. The main focus has been on how the FA composition of oilseeds and heat processing of oilseeds influenced the muscle PUFA profile. During heat processing of oilseeds, the intermolecular protein bonds are altered resulting in the encapsulation of the oil droplets (Kennelly, 1996). Earlier studies have shown that reduced biohydrogenation of the protein-encapsulated droplets increased the post-ruminal PUFA available for absorption, in turn affecting the FA profile of the meat and milk (McNiven *et al.*, 2004; Reddy *et al.*, 1994). Feeding duration and nutritional quality have also been shown to influence the tissue FA profile. In agreement with other studies, the maternal diet of market lambs was shown to substantially influence the lamb's growth performance' and FA composition (Scerra *et al.*, 2007; Bas & Morand-Fehr, 2000). The capstone study investigated how the PUFA composition of the muscle can also influence the FA biosynthesis pathways, altering the LC-PUFA composition of the muscle.

Throughout the thesis, the potential to influence the FA profile of ruminant muscle by supplementing oilseeds has been shown. Cumulatively, small improvements to the human food chain could have substantial long-term health benefits. Increasing the intake of beneficial FA while reducing the proportion of FA considered detrimental to human health in raw products would represent a positive step forward. From an agricultural

perspective, this would mean investigating production methods and feeding strategies which address these health concerns.

1.1 Fatty acid nomenclature

A brief description of the lipids presented in this thesis, and the nomenclature used to describe them, is necessary to understand their functional properties. Lipid is a generalized term used to describe water insoluble compounds consisting of carbon, oxygen and hydrogen atoms. Christie (1982) provided a more comprehensive overview of nomenclature for the various lipid sub-groups found in the biological world. In the animal kingdom, FA and cholesterol play important roles in cellular structure and signalling. The focus throughout this thesis has been on how dietary FA affects the FA profile of ruminant meat and milk. Cholesterol is essential for lipid metabolism and transport as well as being a precursor for bile salts, facilitating the uptake of dietary lipids (Christie, 1982). In relation to human health, FA are known to affect the various cholesterol lipoprotein levels.

Fatty acids exist as carbon chains with a carboxyl group at one end and a methyl group at the other (Fig 1A). Fatty acids can be found in muscle tissues linked together via a glycerol backbone either as mono-, di- or triacylglycerols or as non-esterified fatty acids (NEFA) or in combination with a phosphate group. The two main groups of FA focused on during the course of this thesis were polar (PL) and neutral (NL) lipids. Polar lipids are defined as lipids with a higher affinity for polar solvents. Typical PL include NEFA that have a non-esterified carboxyl group (Fig. 1A) or phospholipids that consist of two hydrocarbon chains linked to a phosphate group (Fig. 1C). The polar nature of phospholipids allows them to form membrane bi-layers, an essential part of cells. Conversely, NL have no electron charge and are associated with storage lipids, predominantly in the form of triacylglycerols (Fig. 1B). The FA composition of NL and PL varies in carbon chain length, number of double bonds as well as geometric and positional isomers, all of which influence the physical properties of the molecule. The three main families of FA include SFA, MUFA and PUFA, which will be discussed in more detail below.

In order to identify the FA presented in this thesis, two nomenclature systems are required. Common to both systems is the number of carbons in the chain, followed by the number of double bonds in the chain, separated by a colon, presented as such: C18:1. The position of the double bond and geometrical shape taken by the FA is determined by the suffix cis- or trans- followed by the carbon position of the double bond counted from the

carboxyl group (Christie, 1982). Implications of geometric shape are discussed further in the MUFA section. An alternate system uses the omega- (n-) designation based on the position of the first double bond relative to the methyl end.

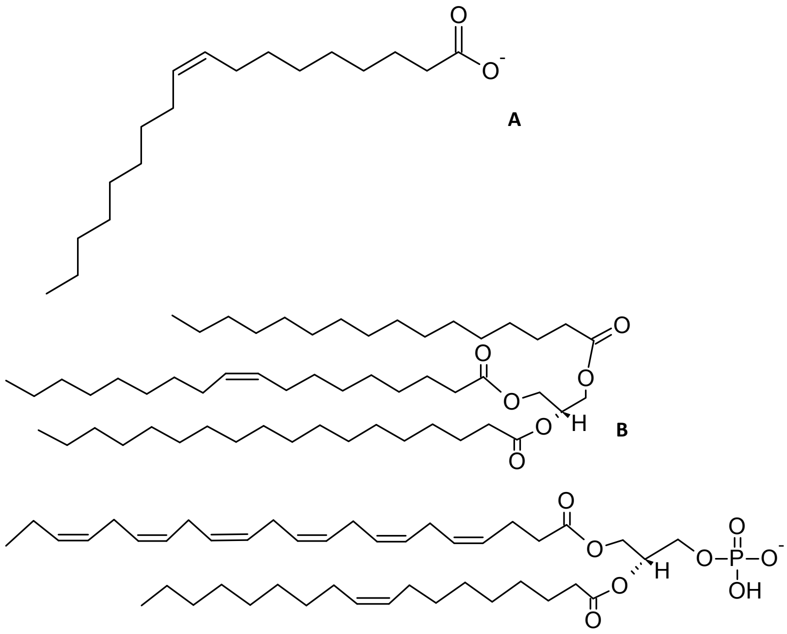

Figure 1. Chemical structure of: (A) non-esterified fatty acid C18:1*c*-9, indicating the structural 30° bend caused by the cis- double bond; (B) triacylglycerol with C16:0, C18:1*c*-9 and C18:0 side chains; and (C) phospholipid with C22:6n-3 and C18:1*c*-9 side chains. (Image created with ACD Labs freeware ChemSketch ver. 12.0).

1.2 Fatty acid groups in ruminant tissues

Saturated fatty acids contain no double bonds, resulting in a straight-chain FA that can be densely packed. As the molecular weight increases, in this case carbon chain length, the viscosity and melting point increases. The majority of ruminant SFA are associated with adipose tissue as storage of excess dietary energy.

Monounsaturated fatty acids contain a single double bond either in cis- (*c*-) or trans- (*t*-) form, existing predominantly in the biological world in the cis- form. The cis- conformation results in a 30° bend in the FA chain that decreases the packing density and melting point (Fig. 1A). The double bond in trans-MUFA maintains the straight chain structure as found with SFA.

For this reason, the physical properties of trans-FA are similar to SFA. Trans-MUFA result in reduced fluidity and a higher melting point than cis-MUFA at an equivalent bond position (Valenzuela & Morgado, 1999). As with SFA, both MUFA isoforms are more abundant in ruminant adipose tissue than membrane tissue. Both cis- and trans- isomers can be directly absorbed from the digesta or cis-isomers can be formed by desaturation of the SFA with tissues. Trans-MUFA in ruminant tissues are predominantly of microbial origin as a result of incomplete biohydrogenation (Jenkins, 1994).

Polyunsaturated fatty acids contain two or more double bonds, generally separated by two carbons. As the number of double bonds increases so does the extent of chain-bending, considerably reducing the packing density and melting point in comparison to MUFA. The majority of PUFA in mammalian tissues are associated with membrane phospholipids. The FA composition of membranes is static to maintain cell fluidity and function (Chapkin, 2008).

Conjugated linoleic acid (CLA) is the cumulative name for a series of PUFA predominantly associated with ruminant products. A conjugated bond implies that the double bond is separated by a single carbon bond. During the biohydrogenation of C18 PUFA, a wide range of C18 trans-MUFA are produced (Chilliard *et al.*, 2007). Within ruminants, C18 trans-MUFA provide the precursors for *de novo* production of the different CLA isomers in adipose and mammary tissue (Bauman *et al.*, 2000).

1.2.1 Desaturation of fatty acids

Enzyme desaturases create cis-double bonds by removing two hydrogen atoms at specific locations on an FA chain. The physical structure and properties of the FA changes based on the number of double bonds inserted. The Δ denotes the carbon position from the carboxyl end where the double bond is inserted. In mammals, the Δ9-desaturase (SCD) is responsible for converting SFA to MUFA, with the enzyme activity increasing as the animal accumulates adipose tissue (Smith *et al.*, 2009; Duckett *et al.*, 1993). Dietary PUFA tend to reduce the activity of desaturase enzymes in human and rat models (Nakamura & Nara, 2004).

Mammals lack the desaturase enzymes to insert a double bond at either the n-6 or n-3 position; hence C18:2n-6 and C18:3n-3 are essential dietary FA. The inability of mammals to synthesis these essential dietary FA gives rise to two distinct LC-PUFA subgroups, namely the n-3 and n-6 series. These n-3 and n-6 LC-PUFA can be synthesised from their respective precursors through a series of Δ5- (Δ5d) and Δ6- (Δ6d) desaturase steps common to both FA series (Sprecher *et al.*, 1995)(Fig. 2).

Preference by the desaturase enzymes for n-3 over n-6 FA precursors in mammalian tissue is widely accepted (Chapkin, 2008). The Δ6d enzyme has been reported to have a 2- to 3-fold higher affinity for C18:3n-3 over C18:2n-6 (Rodriguez *et al.*, 1998). However, mammalian membranes have a stronger affinity toward the incorporation of C18:2n-6 than C18:3n-3 (Wood *et al.*, 2008a; Enser *et al.*, 1998). Thus the proportion of n-6 LC-PUFA is generally higher than n-3 LC-PUFA in ruminant tissues with the most abundant LC-PUFA in decreasing order being: C20:4n-6, C22:5n-3 and C20:5n-3 (Wood *et al.*, 2008a).

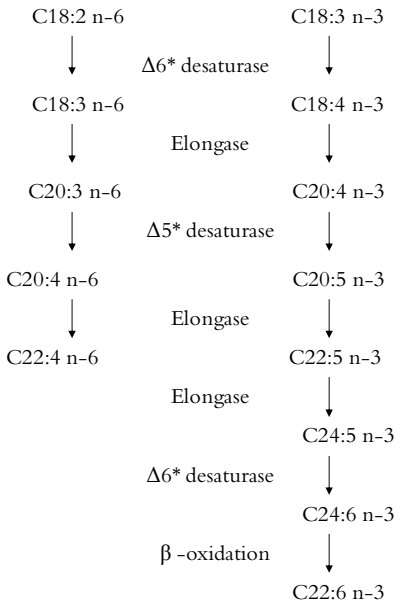

Figure 2. Pathways showing the formation of n-3 and n-6 LC-PUFA via a series of elongation and desaturation steps. Note that the Δ-5desaturase and Δ-6 desaturase are common to both FA series (adapted from Raes *et al.* (2004)).

1.3 Current health trends and ruminant products

1.3.1 Saturated fatty acids and cholesterol

Generally, SFA are the most abundant FA in the human diet owing in part to the SFA content of vegetable oils used in cooking and processed foods (Ailhaud *et al.*, 2006). Increased globalization has led to cheap foreign processed products now competing with local foods, which has altered

traditional diets. This has led to a concomitant increase in obesity, cardiovascular disease (CVD) and other metabolic disorders coined "diseases of the developed world" emerging in the developing world. The greatest health risk to humans posed by dietary SFA is by increasing the plasma total cholesterol and low-density lipoprotein (LDL) levels. High LDL and total cholesterol levels are known risk factors for the development of CVD (Lefevre *et al.*, 2004). The increased risk comes from oxidized LDL becoming adhered to the arterial epithelial layer leading to the accumulation of cholesterol esters and formation of plaque deposits (Christie, 1982). High density lipoproteins (HDL) play a crucial role in reducing these arterial deposits by absorbing the cholesterol esters and transporting them back to the liver. Studies generally have implicated C12:0, C14:0 and C16:0 in elevating total cholesterol and LDL levels (Williams, 2000), whereas, C18:0 has shown neutral or slightly beneficial effects on cholesterol levels and associated CVD development (German & Dillard, 2004).

1.3.2 Health effects of cis- and trans- monounsaturated fatty acid

Compared to plasma LDL raising SFA, cis-MUFA are considered to be more beneficial for human health by reducing inflammation and blood coagulation factors with no associated reduction to plasma HDL levels (Williamson *et al.*, 2005). Alternatively, dietary trans-FA act like SFA by raising plasma LDL levels; however trans-MUFA are considered to be more potent than SFA due to associated reductions to the plasma HDL level (Williamson *et al.*, 2005). Trans-MUFA found in commercial vegetable oils are formed during the refining process and have been shown to reduce plasma HDL level, whereas trans-MUFA from ruminant origin have not shown to alter plasma HDL levels (Williams, 2000).

1.3.3 Conjugated linoleic acid and associated health benefits

A number of beneficial physiological effects have been attributed to CLA *c*-9, *t*-11 and CLA *t*-10, *c*-12 including anti-carcinogenic and anti-obesity effects, as well as lowering the risk of developing atherosclerosis by lowering plasma LDL levels (Park, 2009; Huang *et al.*, 2008; Riserus *et al.*, 2004; Akahoshi *et al.*, 2003; MacDonald, 2000). Whereas supplementing CLA in animal trials has shown positive effects, results from human studies using supplemented CLA are limited and often contradictory. Differences in trial design, subject selection, administered dose and gender have all contributed to the contradictory results (Plourde *et al.*, 2008). The general consensus is that further investigations are warranted before recommending CLA as a therapeutic agent (Park, 2009).

Milk has been shown to have a higher CLA content than meat, though both are a dietary source for humans (Mir *et al.*, 2004). The proportion of each major isomer in ruminant products can vary with ruminant diet. In general, the CLA *c*-9, *t*-11 isomer has been shown to constitute between 75 to 90% of the CLA found in ruminant products, with CLA *t*-10, *c*-12 accounting for an additional 1 to 2% (Lock & Bauman, 2004). Levels of CLA *c*-9, *t*-11 in ruminant products have been enhanced by forage feeding or supplementation with oilseeds (Chilliard *et al.*, 2007; Bolte *et al.*, 2002). Increased levels of CLA *t*-10, *c*-12 in ruminant products are generally associated with feeding diets containing high levels of concentrate (Chilliard *et al.*, 2007).

1.3.4 Polyunsaturated fatty acids and inflammatory response

Dietary PUFA help to reduce the risk of developing CVD by raising plasma HDL levels while lowering both plasma total cholesterol and LDL levels (Wijendran & Hayes, 2004). Besides affecting cholesterol levels, dietary PUFA, particularly LC-PUFA, play an important role in producing eicosanoids. Eicosanoid is a term used to describe biologically active lipids that modulate the body's inflammatory response. The conversion of C18:2n-6 and C18:3n-3 to their LC-PUFA derivatives is considered to be low in mammals; hence humans rely considerably on dietary sources of LC-PUFA (Chapkin, 2008).

As mentioned previously, C18:2n-6 and C18:3n-3 undergo a series of elongation and desaturation steps in the endoplasmic reticulum membrane yielding LC-PUFA (Sprecher, 2000). Eicosanoids are produced from LC-PUFA released from tissue phospholipids via phospholipase (Zhou & Nilsson, 2001). The relative proportions of tissue n-3 and n-6 LC-PUFA act as a regulator for localized eicosanoid production and activity, ultimately influencing the physiological functions of the body. The LC-PUFA are divided into two families based on their initial substrate and can be generalized as either n-6 pro-inflammatory or n-3 anti-inflammatory LC-PUFA based on the eicosanoids derived from them (Fig. 3). Prostaglandins are generally considered to be pro-inflammatory (PGE_2, PGF, PGD) but can also reduce blood platelet aggregation (PGE_1, PGI), further blurring the line between pro-inflammatory and anti-inflammatory responses (Lee & Hwang, 2008). Thromboxanes (TXA) induce blood platelet aggregation, whereas leukotreines (LTB) elicit allergic reactions. The antagonistic actions of the n-6 series eicosanoids are generally many times higher than those derived from the n-3 series (Lands, 2008).

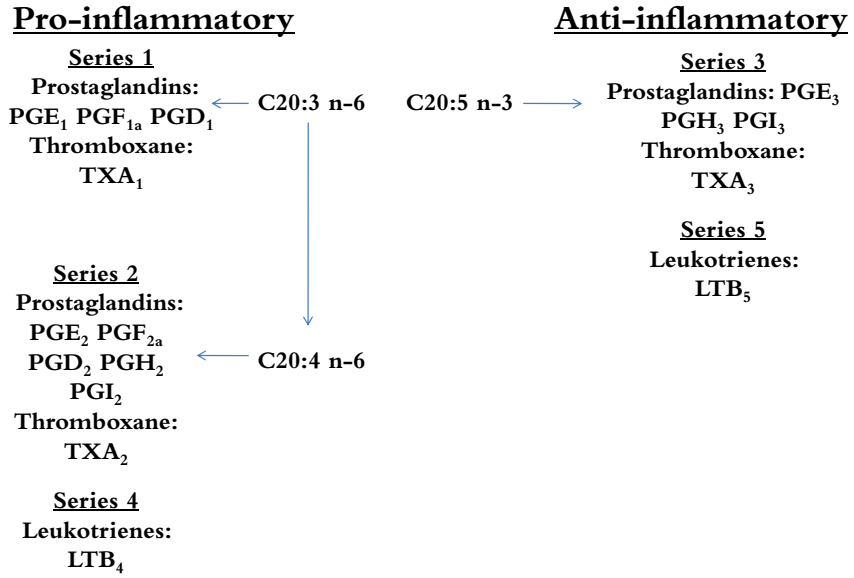

Figure 3. Eicosanoids are produced from LC-PUFA of the n-3 and n-6 series. The relative proportion of LC-PUFA precursors regulates the intensity of the body's inflammatory response. Pro-inflammatory eicosanoids are produced from the n-6 series; C20:3n-3 produces series 1 prostaglandins (PG_1) and thromboxane (TXA_1), C20:4n-6 produces series 2 prostaglandins (PG_2), thromboxane (TXA_2) and series 4 leukotrienes (LTB_4). Anti-inflammatory eicosanoids produced from C20:5n-3 include series 3 prostaglandins (PG_3) and series 5 leukotrienes (LTB_5) (adapted from Bonis *et al.* (2005)).

Overstimulation of the inflammatory system has been linked to a number of human chronic diseases including atherosclerosis, asthma, and a number of cardiovascular related events (Calder, 2006). Moreover, C20:4n-6 has been shown to be a potent promoter of adipogenesis, leading towards obesity (Ailhaud *et al.*, 2006). Alternatively, studies have indicated C22:6n-3 is essential for neurological function and furthermore, exhibits strong positive cardiovascular effects (Connor, 2000). The function of C22:5n-3 has not been investigated as extensively as C20:5n-3 or C22:6n-3; however there are indications that C22:5n-3 is a more potent inhibitor of platelet aggregation than either C20:5n-3 or C22:6n-3 (Akiba *et al.*, 2000).

Many mechanisms are involved in the modulation of the relative proportions of LC-PUFA within the membranes. The formation of n-6 LC-PUFA is known to be reduced through competitive inhibition by n-3 LC-PUFA (Lee & Hwang, 2008). The higher affinity of desaturase enzymes

towards n-3 FA has also been shown to reduce the relative production of n-6 derived eicosanoid, thereby reducing the health risks associated with a pro-inflammatory state (Lee & Hwang, 2008). Moreover, n-3 LC-PUFA have been shown to be poor substrates for eicosanoid production, thus mitigating the intensity of the inflammatory response (Lands, 2008). A comprehensive review of the physiological functions exerted by n-3 or n-6 LC-PUFA through the production of eicosanoids has been compiled by Lee and Hwang (2008).

1.4 Health recommendations

Recommendations for optimal lipid intake to minimize metabolic related disease in humans are strongly related to lifestyle and constantly being re-evaluated through new research findings. General guidelines have recommended that total fat intake does not exceed 30% of the daily energy intake (Linseisen *et al.*, 2009). Recommendations for the different FA classes have suggested that SFA contribute less than 10% daily energy intake and that the PUFA/SFA ratio is higher than 0.4 (Smit *et al.*, 2009). Substituting MUFA for SFA is encouraged; however, the intake of trans-MUFA should not exceed 3 g/day (Weggemans *et al.*, 2004), or the equivalent of 1% of the daily energy intake (Willett & Mozaffarian, 2008). The total PUFA intake is recommended to be 18 g/day or about 6 to 7% of the daily energy intake (Chapkin, 2008).

The recommended intake of PUFA is more complicated as the composition of the PUFA has been shown to play an important role in determining the associated health benefits. Simopoulos (2002) advocates a return to a pre-industrial n-6/n-3 PUFA ratio of 4:1 for optimal health benefits, compared to the current estimated 15:1 n-6/n-3 PUFA ratio typical of a Western diet. The dramatic change in the human n-6/n-3 ratio over time has been largely attributed to lifestyle changes. The *ad hoc* medical advice of substituting animal fat with vegetable oils since the 1960's has coincided with a rapid increase in diet related diseases (Ailhaud, 2006; Williams, 2000). Wijendran and Hayes (2004) emphasized that the n-6/n-3 PUFA ratio was not as important as the absolute intake of PUFA to modulate metabolic risk factors. An intake of 450 mg/day of n-3 LC-PUFA has been recommended to meet the needs of an adult human (Givens & Gibbs, 2006). Suggested optimal PUFA intakes expressed as a percent of daily energy are as follows: 6% C18:2n-6, 0.75% C18:3n-3 with 0.25 to 0.5% of combined C20:5n-3 and C22:6n-3 (Wijendran & Hayes, 2004).

1.5 Meat eating quality

Dikeman *et al.* (2005) identified the most important factors that determine the satisfaction from a meat eating experience as tenderness, juiciness and flavour. Age and sex are two of the major factors influencing animal muscle fibre cross-linkages, in turn influencing post-mortem muscle tenderness. Diet influences meat tenderness in relation to animal growth and degree of finish achieved prior to slaughter. Fat content of the muscle has been shown to enhance the perception of tenderness, though marbling only accounts for 10% of the variation in muscle tenderness (Hocquette & Bauchart, 1999). Post-mortem ageing has the most substantial influence on meat tenderness. During the ageing process, proteolytic activity of μ-calpain has been shown to reduce the myofibrillar cross-linkages leading to tenderization of the meat (Koohmaraie, 1996). The cumulative activities of the calcium activated μ-calpain and calpastatin enzymes are known to play key roles both in animal growth and post-mortem tenderization (Goll *et al.*, 1998).

Meat tenderness depends on a number of intrinsic and extrinsic factors relating to the animal and can be interpreted differently by consumers (Destefanis *et al.*, 2008). Warner-Bratzler shear force (WB-SF) has become the most common instrument method used for assessing meat tenderness (Miller *et al.*, 1995). The breakdown of fibre cross-linkages as measured by the myofibrillar fractionation index has also been used to assess meat tenderness and it has been found to correlate well with WB-SF assessments (Olson *et al.*, 1976). The correlation between WB-SF and sensory panel assessments for tenderness is highly dependent on the variability of the material and has been reported to range between -0.26 and -0.60 (Caine *et al.*, 2003; Lorenzen *et al.*, 2003). The precision becomes weaker when using untrained consumer panels. Aalhus *et al.* (2004) reported that a minimum WB-SF tenderness value difference of 1.92 kg (19 N) could be detected by a consumer panel.

Boleman *et al.* (1997) assessed consumer acceptability of meat tenderness to vary between what would be perceived as "tender" (22 to 35 N) and "intermediate tenderness" (50 to 53 N) as assessed by WB-SF. Shackelford *et al.* (1991) stated that a WB-SF value less than 38.3 N would be necessary to assure a consumer perception of tenderness with a 68% confidence level. Smith *et al.* (1978) reported that maximal tenderization of prime cuts can be achieved with seven days of ageing. Further investigations into optimizing the eating quality of subprime cuts indicated that there was a benefit from ageing up to 28 days (Gruber *et al.*, 2006).

While a number of factors have been shown to contribute to meat quality, diet has been identified as the most influential external component

affecting meat flavour (Melton, 1990). Meat quality from forage finishing systems has been criticized in terms of consumer acceptance due to negative organoleptic properties (Nuernberg *et al.*, 2005). Common complaints have included less tender meat, darker meat, less marbling and more yellowness of the fat present as either intermuscular or intramuscular deposits (Valsta *et al.*, 2005). An average carcass fat content of less than 2.5% has been considered too low to provide an enjoyable beef eating experience (Jurie *et al.*, 2007).

Certain fatty acids have been associated with having positive and negative correlations with taste. Higher phospholipid levels (i.e. leaner meat) have been negatively associated with muscle flavour, whereas higher triacylglycerol content (i.e. marbling) positively influences flavour (Dryden & Maechello, 1970). Consumer preferences for forage vs. concentrate finished meat exist and they have been shown to be highly dependent on traditional farming practices inherent to the region. Within Europe, a taste preference was shown towards beef produced under low intensity production, i.e. leaner meat higher in C18:3n-3, with favourable correlations towards fishy, grassy, milky and sour tastes (Campo *et al.*, 2003; Priolo *et al.*, 2001). The function of the muscle, thus the proportion of "red" or "white" muscle fibre, also slightly influences the FA composition (Wood *et al.*, 2003). Red muscles are characterised as having more mitochondria, hence higher proportions of PUFA. The oxidation of MUFA and PUFA has also been shown to lead to the formation of a number of volatile products which could produce off-flavours in meat (Campo *et al.*, 2003). Aldehydes and ketones produced during lipid oxidation have been implicated in the emergence of off-flavours in meats (Dewhurst *et al.*, 2003). Many of these compounds are formed as a result of over-cooking, emphasising the importance of meat preparation in relation to consumer perception of a "good experience".

1.6 Ruminant nutrition and physiological functions influencing tissue fatty acid profile

1.6.1 Muscle lipid content and fatty acid composition

The total lipid content of ruminant meat can vary depending on muscle, feeding programme and breed, but usually is less than 5 g/100 g wet tissue (Wood *et al.*, 2008b). In general, the total lipid content of ruminant tissues is comprised of about 40% SFA, 40% MUFA and 2 to 25% PUFA (Wood *et al.*, 2008b). The main SFA found in ruminant meats include C14:0, C16:0 and C18:0; with smaller amounts of C10:0 and C12:0 (Wood *et al.*, 2008b).

The C18:1*c*-9 level accounts for more than 30% of the total lipid in meat, proportionally increasing as the animal fattens (Duckett *et al.*, 1993). The trans-MUFA content in meat is generally less than 4% of the total lipid, with proportions shifting depending on dietary energy level (Daley *et al.*, 2010). The C18:2n-6 and C18:3n-3 levels in meat can vary substantially depending on diet; however C18:2n-6 is always more abundant (Wood *et al.*, 2008a). The health quality of the ruminant meat FA profile could benefit from feeding programmes designed to reduce the SFA content while increasing the MUFA and PUFA content, particularly n-3 PUFA.

1.6.2 Lipolysis in the rumen

Once ingested, the initial step in lipolysis involves the hydrolysis of the ester linkages by microbial lipolytic enzymes, resulting in the release of NEFA into the rumen environment (Harfoot & Hazlewood, 1997). The cellulose components of plant cell walls are resistant to microbial degradation and lipolysis. Similarly, whole raw oilseeds rely extensively on the seed hull for protection against lipolysis. Lipolysis can also be hindered by the forage quality (lignification), exposed surface area of the feed particle and structural modifications that inhibit microbial contact and enzyme activity (Jenkins, 1993). The microbial population within the rumen is mediated by the fermentation substrates available in the rumen. *Butyrivibrio fibrisolvens* predominate with high cellulose diets, whereas *Anaerovibrio lipolytica* are more adapted to starch-rich diets. Likewise, *Butyrivibrio fibrisolvens* are more apt at hydrolysing phospholipids and galactolipids whereas *Anaerovibrio lipolytica* microbial species hydrolysed mainly triacylglycerols (Bauman *et al.*, 2003). The release of NEFA is a prerequisite for the subsequent enzymatic activities of the microbes. Rapid hydrogenation of the NEFA occurs once exposed to the microbial environment in a process called biohydrogenation.

Oilseeds used in ruminant diets provide a rich source of protein and the lipid content is generally more than 20% PUFA, whereas the lipid content of cereals and forages is generally less than 5% dry matter (DM). Cereal crops, oils and oilseeds, with the noted exception of linseed, are generally higher in C18:2n-6 than C18:3n-3. Forages contain a high proportion of C18:3n-3 localized in the chloroplasts, with the proportion of C18:3n-3 decreasing as the plant matures (Boufaied *et al.*, 2003). The C18:2n-6 proportion in total lipids of feed oilseeds can range from 20% in canola to 70% in sunflowers, whereas the proportion of C18:3n-3 in linseed is higher than 50% (White, 2008). Some oilseeds, i.e. canola, olive and palm, also contain substantial proportions of C18:1*c*-9.

1.6.3 Rumen biohydrogenation

The rumen fauna consists of dynamic populations of microbes, fungi and protozoa sensitive to substrate and shifts in the rumen environment. Microbial populations are primarily responsible for the biohydrogenation activity in the rumen, with protozoal populations playing a minimal role (Harfoot & Hazlewood, 1997). The biohydrogenation process involves the removal of double bonds through microbial enzyme activity. During biohydrogenation, PUFA are converted into MUFA and ultimately to SFA. Fermentation of carbohydrates releases hydrogen which can then be used for hydrogenation processes. The biohydrogenation process consumes only 1 to 2% of the hydrogen released from ruminal fermentation (Jenkins, 1993). Once in NEFA form, both C18:2n-6 and C18:3n-3 undergo extensive hydrogenation, estimated to be about 79% and 86% respectively (Jenkins & Bridges Jr., 2007). Acute changes to the rumen pH or diets containing too much lipid result in substantial disruptions in rumen function and fibre digestion. The lipid content of ruminant diets is generally recommended not to exceed 6 to 7% (Bauman *et al.*, 2003). The hydrogenation of PUFA can be reduced under conditions where the passage rate is increased, generally related to reduced particle size and decreased rumen pH. Diets containing concentrates at more than 70% DM have been reported to reduce the biohydrogenation of C18:2n-6 and C18:3n-3 up to 50% and 65% respectively (Schmidely *et al.*, 2008; Chilliard *et al.*, 2000; Doreau & Ferlay, 1994).

The reason for the extensive biohydrogenation is that PUFA disrupt the cell structure of rumen microbes, in a sense being toxic (Harfoot & Hazlewood, 1997). Microbes capable of performing biohydrogenation can be divided into two groups based on the substrates being hydrogenated. Group A consists of an extensive range of microbes capable of hydrogenating PUFA to C18:1*t*-11, whereas Group B has a more limited species range, but are capable of converting C18:1*t*-11 to C18:0 (Fig. 4). The conversion of C18:1*t*-11 to C18:0 is the rate limiting step, leading to a build-up of intermediates in the rumen. Incomplete biohydrogenation results in a number of intermediates, with C18:1*t*-11 being the predominant intermediate leaving the rumen (Chilliard *et al.*, 2007).

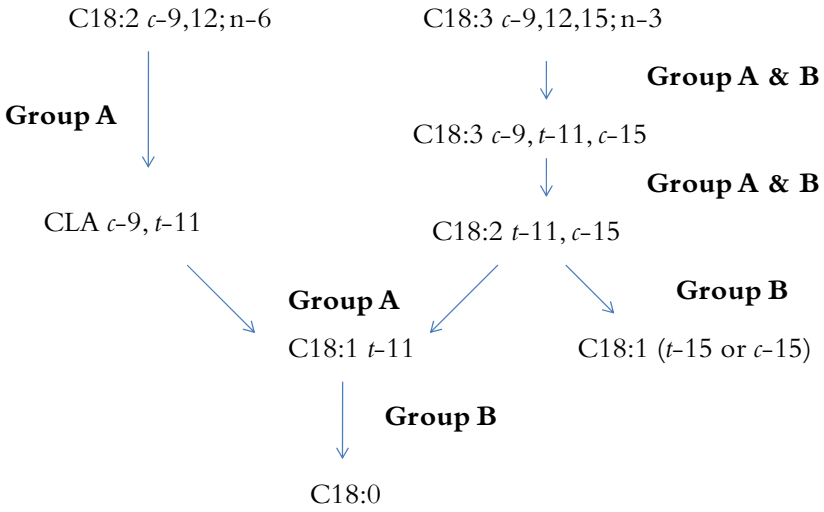

Figure 4. Biohydrogenation of essential dietary PUFA depicting the specific role of group A and group B microbial populations. (Adapted from Bauman *et al.* (2003)).

1.6.4 Lipid transport in ruminants

Fatty acids in the small intestine predominantly originate from the diet or microbial *de novo* synthesis; however a significant proportion (roughly 10 to 20%) of the FA arise from microbial phospholipids (Bauchart, 1993). Once lipids reach the duodenum, FA blend with bile salts to form micelles which are then absorbed across the mucosal cells (Bauchart, 1993). The capacity to absorb FA diminishes with increasing lipid supplementation in the diet, assumably due to limitations in the production of pancreatic lipases and bile salts (Bauchart, 1993). Once absorbed, medium-chain FA (C6 to C12) are transported as NEFA via the portal vein and can be either be absorbed in the liver or peripheral tissues. In the liver, the NEFA can undergo oxidation to form acetate, or be formed into triacylglycerols and re-excreted as very low density lipoproteins (Hocquette & Bauchart, 1999). Fatty acids greater than C12 are transported as triacylglycerols by lipoproteins called chylomicrons through the lymphatic system to the peripheral tissues. Lipoprotein lipases hydrolyse the triacylglycerols, releasing NEFA, which are then absorbed and incorporated into membrane or adipose tissue (Hocquette & Bauchart,

1999). Further elongation and desaturation of the absorbed lipids occurs via lipogenic enzymes (Hocquette *et al.*, 2010).

1.6.5 Relationship between diet and the △5d and △6d enzymes in ruminant tissues

Currently, there is limited knowledge available about the protein expression and activity of Δ5d and Δ6d in ruminant tissues and factors that can modulate their gene expression. The role of Δ5d and Δ6d enzymes in the formation of LC-PUFA from C18:2n-6 and C18:3n-3 was described earlier (*see section 1.2.2.*). Initial research has indicated that both Δ5d and Δ6d are correlated with changes to the muscle intramuscular fat (IMF) content. Ward *et al.* (2010) reported that the Δ5d and Δ6d desaturase protein expression increased in relation to FA synthesis and IMF content. Findings from their trial showed that a higher n-6 PUFA level in *M. semimembranosus* increased Δ5d and Δ6d protein expression, whereas the n-3 PUFA level resulted in a lower expression level (Ward *et al.*, 2010). In a similar study, the dietary n-6/n-3 PUFA ratio was tested in relation to the IMF; again the diet higher in n-6 PUFA had a higher IMF content (Herdmann *et al.*, 2010). The diet higher in n-6 PUFA was positively correlated to increased SCD and Δ6d protein expression in *M. longissimus dorsi* (Herdmann *et al.*, 2010). In agreement, Waters *et al.* (2009) found that SCD gene expression was negatively correlated to n-3 PUFA and positively correlated to n-6 PUFA. The increase in IMF content has been linked to a subsequent increase in SCD activity in a number of ruminant studies (Smith *et al.*, 2009; Chung *et al.*, 2007; Smith *et al.*, 2006). Taken together, n-6 PUFA up-regulated the lipogenic desaturase enzymes and led to increased IMF. Possible mechanisms for this can be extrapolation from human studies, which indicates that the proportion of C18:3n-3 in the diet hinders the formation of C20:4n-6 through competitive inhibition of Δ6d. This reduces the C20:4n-6-catalyzed maturation of pre-adipocytes and adipose development (Ailhaud *et al.*, 2006). Caution must be taken when extrapolating between gene expression and protein expression as an up-regulation of gene expression does not necessarily correspond to increased protein expression (Ward *et al.*, 2010).

1.6.6 Factors influencing protein digestion in the rumen

Oilseeds are an excellent protein source for ruminants; however, the protein quality for the animal may be reduced if the protein is readily available for degradation in the rumen. The protein content of oilseeds can be divided into rumen degradable and rumen undegradable protein (RUP). Van Soets

(1981) further subdivided these classes into non-protein N (fraction A), true protein (fraction B_1, B_2, B_3) and bound or indigestible protein (fraction C). Rumen protein degradability is a function of the degradability rate and digesta passage rate (Sniffen *et al.*, 1992). Fractions A and B_1 are rapidly degraded (120 to 400% /h), contributing to microbial protein synthesis. The RUP fraction is comprised of slowly degradable fractions (B_2 and B_3) which degrade at rates of 3 to 16% /h and 0.06 to 0.55% /h respectively, and the indigestible C fraction. The slower degradation rates result in an increased supply of protein to the small intestine where it is digested by intestinal enzymes. Heat treatment of feedstuffs have been shown to decrease the protein degradability while increasing the RUP fraction (Satter, 1986; Mir *et al.*, 1984). The rate of digesta passage also contributes to the relative proportion of protein which escapes rumen degradation. Passage rate is affected by intrinsic feed factors such as particle size, density and hydration rates as well as extrinsic factors such as intake (Sniffen *et al.*, 1992). The indigestible C fraction of protein is estimated by measuring the acid detergent insoluble N (ADIN), which is used as an estimation of heat damaged protein in feedstuffs (Faldet *et al.*, 1992a). The ADIN fraction represents the N fraction which would not be digestible by rumen microbes or intestinal enzymes (Clipes *et al.*, 2006).

Estimating protein degradability and digestibility *in vivo* is costly and labour intensive. *In vitro* models are a cost effective means of estimating rumen protein degradability. *In vitro* models measure the amount of N remaining in a sample after incubation to represent the proportion of protein that would be degradable in the rumen. *In vitro* N digestibility estimates the proportion of protein which would be digestible by the animal, including the RUP fraction. Similar to N degradability estimates, N digestibility measures the difference in the N content of a feedstuff after an enzymatic incubation. Calsamiglia and Stern (1995) used a buffered pepsin-pancreatin solution to simulate *in vivo* conditions to estimate N digestibility; however their method required the feedstuffs to be incubated in the rumen prior to *in vitro* analysis. The *in vitro* method presented by McNiven *et al.* (2002) modifies this procedure by combining an N degradability step followed by an N digestion step. This method has been shown to accurately reflect *in sacco* and mobile bag results (McNiven *et al.*, 2002).

The RUP fraction of oilseeds can be increased by altering the protein structure thereby reducing the susceptibility of the proteins to microbial degradation. Heat treatments such as extrusion, infrared micronisation and roasting are effective methods for increasing the undegradable N fraction of oilseeds (Gonthier *et al.*, 2004; Wang *et al.*, 1999; Chapoutot & Sauvant,

1997; Chouinard *et al.*, 1997). The effectiveness of heat processing to increase the undegradable N fraction is a function of the heating method, moisture content of the feedstuff and time (Doiron *et al.*, 2009). During the heating process, the protein structure is altered creating cross-linkages within and between peptide chains (Deacon *et al.*, 1988). Changes to the protein structure are usually referred to in terms of proportional shifts in the globulin and albumin fractions as they are more susceptible to heat denaturing (Park *et al.*, 2010; Ganesh & Grieve, 1990). The albumin and glutelin protein fractions contain higher proportions of sulphur-containing amino acids, methionine and cystine. Higher concentrations of these amino acids increase the formation of di-sulfide bonds during the heating process (Lykos & Varga, 1995). Di-sulfide bonds are known to be highly resistant to microbial degradation (Mahadevan *et al.*, 1980). Recently, synchrotron microspectroscopy has been used to identify changes in protein secondary structures as a result of heating in an effort to determine optimal conditions under which oilseeds should be processed to maximize the undegradable N fraction without increasing the indigestible fraction (Yu, 2007). Heat treatment that increases the α-helix/β-sheet ratio has been shown to increase the undegradable N fraction. However, overheating results in the formation of indigestible Maillard reaction products by fusing proteins with sugars. Heat damage represented by these Maillard products can be measured by the ADIN content of a feedstuff (Goering *et al.*, 1972). In relation to modifying the FA profile of tissues, encapsulation of the oil micelles is a concomitant benefit of the protein reconfiguration induced by heat treatment.

2 Objectives

The objective of this work was to use dietary oilseeds supplementation to influence the FA profile of ruminant muscles. This thesis summarizes the findings from investigations where the dietary FA composition was manipulated by supplementing processed oilseeds and how the FA profiles of the oilseeds affected the muscle in terms of technological and nutritional quality. The influence of dietary FA composition on protein expression of the enzymes responsible for forming LC-PUFA was also investigated. The goal was to enhance the proportion of beneficial FA while decreasing the proportion of those FA considered detrimental to human health. The overall hypothesis was that the FA profile of ruminant products can be influenced by the FA profile of the oilseed.

Specific objectives were to use dietary manipulation to:

- Assess whether substituting cold-pressed hempseed cake for soybean meal as a protein source would have consequential effects on the FA profile or meat tenderness of Swedish Red breed steers (Paper I)

- Evaluate the influence of pre- and post-weaning diets on the FA profile of market lambs using ensiled forage and a concentrate including rapeseed cake (Paper II)

- Compare the effect of heat processing on the N degradability and N digestibility of oilseeds using an *in vitro* method (Paper III)

- Investigate how the dietary n-6/n-3 PUFA ratio can influence the protein expression of Δ5d and Δ6d desaturase enzymes in different beef muscles (Paper IV)

3 Materials and methods

The following section describes the materials and methods used to obtain the results compiled for the presentation of this thesis. A more detailed description of the procedure presented below can be found within the methods section of Paper I to IV.

3.1 Trial design and sample collection

Paper I

The trial objective in Paper I was to determine what influence feeding cold-pressed hempseed cake (*Cannabis sativa* L.) compared to soybean meal (*Glycine max* L.) had on the *M. longissimus dorsi* FA profile and meat tenderness. Swedish Red breed steers (n=16), initial weight of 396.4 kg (SD 29.7 kg) were bunk fed a 45:55 DM grass silage: rolled barley basal diet, with a protein supplement top-dressed and hand-mixed daily. Supplement for each animal for the first 50 days included 0.2 kg as-fed hempseed cake (HC) or 0.1 kg soybean meal (SM) + 0.1 kg rolled barley. Supplement for the subsequent 126 days (SD 18 days) of the trial included 1.4 kg as-fed HC or 0.7 kg SM + 0.7 kg rolled barley. Further description of diet and feeding conditions are reported by Hessle *et al.* (2008). At processing, 24 h post-slaughter, entire *M. longissimus dorsi* were collected and a 100 g sample from the anterior section was removed for lipid analysis. Remaining *M. longissimus dorsi* were vacuum-packed, stored at 5 °C and transferred to Uppsala, SE. At 48 h post-slaughter, pH was measured, then loins were vacuum packed, aged seven days at 5 °C and frozen at -20 °C until shear force analysis.

Differences between dietary supplements for the parameters measured in this study were tested by an analysis of variance model using the SAS Mixed Procedure (SAS, 2002) with animal as the experimental unit. Comparisons between fresh and heat treated values with dietary supplement included

animal as a random effect. Estimates of the measured variables were reported as least square means and standard error of the mean (SEM) with a significance level of $P<0.05$.

Paper II

In Paper II the objective was to determine the influence of a post-weaning diet cross-over on the FA profile of lamb *M. semispinalis capiti*. White Swedish Landrace / Texel crossbreds ewes 2007 (Y1) and 2008 (Y2) were fed silage (SIL) or silage + concentrates (CON). The silage (Y1, 11.2 MJ/kg, 15.6% CP, 45.2% NDF; Y2, 10.9 MJ/kg, 12.0% CP, 53.6% NDF) was fed *ad lib* whereas the concentrates were limited to less than 50% of the DM intake in adherence with organic production protocols (KRAV, 2010). The ewe concentrates fed during lactation (14 MJ/kg, 19.7% CP, 12% NDF) included about 40% barley, 40% peas and 20% rapeseed cake (*Brassica napus* L.) (Ekologisk rapskaka, Vegolia AB, Sweden). Milk samples were collected at 2 and 4 weeks post-lambing from 39 ewes in Y1 and 41 ewes in Y2 and frozen at -20 °C until FA analysis.

The cross-bred lambs were fed forage or forage + concentrates during Y1 and Y2. Before weaning the lambs had access to creep-feeders with the same diets as their dams. The CON lamb creep-feed consisted of 40 to 50% whole barley, crushed peas and 20% Y1 or 35% Y2 cold-pressed rapeseed cake (Ekologisk rapskaka, Vegolia AB, Sweden). The higher rapeseed level Y2 was to compensate for the lower nutrient content in the silage in Y2. At weaning, (67 d), half the animals were switched over to the alternate feeding regime. In Y1, 34 ram lambs were fed 5 weeks and in Y2, 36 ram lambs were fed 7 weeks prior to slaughter. The lamb treatments were as follows:

SS = Only silage both pre- and post-weaning.
SC = Only silage until weaning, silage and concentrates thereafter.
CC = Silage and concentrates both pre- and post-weaning.
CS = Silage and concentrates before weaning, only silage thereafter.

Post-weaning, the concentrate composition in Y1 was about 40% barley, 40% peas and 20% rapeseed cake (14.0 MJ/kg, 19.7% CP, 12.6% NDF) and in Y2 was 48% barley, and 26% of both peas and rapeseed cake (14 MJ/kg, 19.5% CP, 8.6% NDF). Lamb rations were adjusted regularly to adhere to the Swedish organic production regulations (KRAV, 2010). The KRAV regulations stipulate that concentrates should not exceed 50% of the daily DM intake the first month after weaning and thereafter should be maximum 30%. At slaughter samples of *M. semispinalis capiti*s were collected and frozen at -20 °C until FA analysis.

The milk FA of the ewes were tested by an analysis of variance model reporting the least square means with the significance set at ($P<0.05$) using the SAS Mixed Procedure (SAS, 2002). Comparisons were made between dietary treatments on each sampling occasion within each year. Comparison of main effect of dietary treatment and sampling occasion within years were also tested. The interaction between dietary treatment and sampling occasion within each year were found to be non-significant. The difference between years was also tested using averaged values within year. The FA profiles of lamb tissue were compared between the dietary groups within each year, as well as between the mean values for the two years, by analysis of variance at a significance level of $P<0.05$, using the SAS Mixed Procedure (SAS, 2002). Estimates of the FA in milk and muscle are presented as the least square means and SEM. A multivariate analysis of factors influencing the C18:2n-6 and C18:3n-3 level within lamb muscle was made using Unscrambler v9.5 software (CAMO Process AS, Oslo, Norway).

Paper III

The objective of the trial in Paper III was to investigate the influence of heat treatment on the N degradability and N digestibility of whole and ground oilseeds. Lots of canola (*Brassica napus* L.), linseed (*Linum usitatissimum* L.) and soybean (*Glycine max* L.) were divided into three batches for treatment: unprocessed, roasted or extruded. Canola and linseed were roasted at 121.1 °C for 45 s, whereas soybeans were roasted at 143.3 °C for 60 s, on a Cardinal fluidized bed roaster (Sweet Manufacturing, OH, USA). Oilseeds were extruded using a single screw InstaPro extruder (Triple F Inc., IA, USA). After treatment, each batch was divided into two, with one half whole and the other half ground through a 2-mm screen using a Retch Ultra Centrifuge Mill (ZM100; Fisher Scientific Co., ON, Canada). Six 1 g samples of ground and whole oilseeds from each oilseed type and heat treatment were analysed after the *in vitro* process described by McNiven *et al.* (2002). Three bags from each replicate were removed after 4 h protease incubation to be analysed for N degradability. The remaining samples were incubated 24 h in pepsin and pancreatin to be analysed for N digestibility. The bags from each oilseed type and heat treatment collected after the respective incubation points were used as the experimental unit.

The whole and ground sample results for N degradability and N digestibility were analysed by oilseed type and heat treatment in a two-way analysis of variance model using the SAS GLM Procedure (SAS, 2002). The Bonferroni adjustment was used for making protected comparisons of the means ($P<0.05$). The interaction between seed type and processing method

was investigated and found to be significant. Estimates of the interaction least square means are presented along with the SEM. The comparison between interaction subgroups were made within seed type and processing method respectively using the slice statement of SAS (2002).

Paper IV

The objective of the trial in Paper IV was to investigate the relationship between the protein expression of Δ5d and Δ6d desaturase enzymes within muscles and determine whether expressions were influenced by the dietary n-6/n-3 ratio using two bovine muscles. Two trials, each consisting of 40 crossbred steers with an initial live weight of 508 kg (SD 6.0 kg) were fed a basal diet consisting of 300 g/kg DM grass silage (13% CP, 34% ADF, 57% NDF) and 685 g/kg DM barley grain. Two animals were removed from the trial for reasons unrelated to the supplements. A roasted or extruded oilseed supplement was top-dressed at 2.0 kg per day and mixed by hand. Oilseed supplements were blended combinations of canola, linseed and soybean. Feeding treatments were designated by the oilseed supplement; canola-linseed, (C-L, n-6/n-3 ratio=0.6, n=19); soy-linseed, (S-L, n-6/n-3 ratio=1.0, n=20); soy-linseed-canola (S-L-C, n-6/n-3 ratio=1.2, n=20) and soy-canola, (S-C, n-6/n-3 ratio=3.6, n=19). Soybean meal and soybean oil were added to give the supplements equivalent lipid and protein contents. Within 30 min of exsanguination, duplicate *M. masseter* and *M. pars costalis diaphragmatis* samples were frozen in liquid nitrogen and transported on dry ice to the lab and stored at -80 °C. Lipid extractions and then PL fraction separation were made for FA analysis of the muscles. Microsomal extractions were made to determine Δ5d and Δ6d protein expression by Western blot. Protein expressions were normalized against β-actin (Santa Cruz Biotechnology, CA, USA) to compare between wells.

Differences in normalized protein expression and FA were tested by an analysis of variance model using the SAS Mixed Procedure (SAS, 2002). Variables in the model included trial, oilseed blend and processing method as well as interactions between oilseed blend and processing method. Based on studentized residuals, a log transformation of the normalized protein expression was warranted. Protein expression was presented as log values in the tables. The interaction effects between oilseed blend and processing method are reported for the protein expressions and individual FA using least square means and SEM at a significance level of $P<0.05$. Linear relationships between FA level and protein expressions were made using the SAS Regression Procedure (SAS, 2002) at a significance level of $P<0.05$.

3.2 Sample analysis

3.2.1 Lipid extraction and lipid fraction separation

Feed lipids in Paper I and II were extracted using chloroform:methanol (2:1 v/v) according to the Folch *et al.* (Folch *et al.*, 1957) method. Milk samples in Paper II and tissue lipids in Paper I and II were extracted using hexane:isopropanol according to the method of Hara & Radin (1978). The tissue lipid extractions in Paper IV followed the Bligh and Dyer (1959) method. The lipid classes in Paper I were separated by thin-layer chromatography using hexane:diethylether:acetic acid (85:15:2 v/v/v) as described in Dutta and Appelqvist (1989) modified by increasing the acetic acid content to aid in lipid separation. The NL and PL fractions in Paper I and II were separated using 500-mg silica SPE columns (Isolute SI, IST, UK) with NL eluted using 18 ml chloroform:methanol (50:1 v/v) while PL were eluted using 18 ml methanol. Lipid separation in Paper IV used NH_2 SPE columns (3 ml, 500-mg, 55 μm, 70a, Strata, Phenomenex Inc., USA), with PL being separated using 6 ml methanol. Lipids in Paper I and II were methylated according to Appleqvist (1968) whereas lipids in Paper IV were methylated as described in McNiven *et al.* (2004) prior to analysis by gas chromatography. Total lipids in Paper I and II were applied at a split ratio of 1:10, whereas PL were applied at a split ratio of 1:1 in Paper I and II and at 1:20 in Paper IV. Peak identification was based on retention times of reference standards (Nu-Chek Prep Inc., MN, USA).

3.2.2 Tissue α-tochopherol quantification

Tissue α-tocopherol content (Paper I) was determined according to the procedure of Högberg *et al.* (2002) using high performance liquid chromatography. Mobile phase consisted of 95% acetonitrile:methanol (1:1 v/v) and 5% chloroform with a flow rate of 1.2 ml/min. The HPLC column was a 4.0 x 250 mm RP-18 LiChroCART (Merck KGaA, Germany). Quantification of α-tocopherol was done by comparison to an external standard (Calbiochem, CA, USA). The α-tocopherol was identified at an excitation wavelength of 290 nm and an emission wavelength of 327 nm.

3.2.3 Warner-Bratzler shear force

M. longissimus dorsi samples (Paper I) were thawed for 24 h at 5 °C before heat treating in a 72 °C water bath to an internal temperature of 70 °C. Samples were cooled in running water to room temperature. Warner-Bratzler shear force analysis (Honikel, 1998) was performed on heat treated

M. longissimus dorsi by cutting the samples into 1 x 1 x 3 cm strips parallel to the muscle fibre. Maximum and total force measurements were based on the average of 12 samples from each animal measured perpendicular to the muscle fibre for using a Texture Analyser HD 100 (Stable Micro Systems, UK) equipped with a WB-SF blade. Blade dimensions include a cutting area of 11 x 15 mm with a blade thickness of 1-mm; blade speed was 0.83 mm/s.

3.2.4 Enzyme protein expression

Microsomal extracts of *M. masseter* and *M. pars costalis diaphragmatis* in Paper IV were done according to the procedure of Ward *et al.* (2010). Protein concentration of the extract was determined using a Bradford assay (Bio-Rad Laboratories Inc., Canada); average protein concentration was between 4 to 7 mg/ml in both muscles. The expressions of Δ5d and Δ6d microsomal protein were estimated after Western blot separation. Membrane was washed, then probed with a commercial goat anti-rabbit secondary (Santa Cruz Biotechnology Inc. CA, USA). Membranes were scanned using a Biospectrum AC Imaging System (UVP, CA, USA) and immunoreactive bands were quantified using Vision Works LS software v6.7.4 (UVP, CA, USA). Membrane was washed and re-probed using rabbit anti-β-actin as a housekeeping protein. Expression of β-actin was analysed, and the ratios of Δ5d/ β-actin and Δ 6d/ β-actin were calculated to normalize Δ5d and Δ6d signals on the blots.

4 Summary of results

4.1 Paper I

The C18:2n-6 level in hempseed was comparable to that of soybean meal, 53% and 55% respectively, whereas the C18:3n-3 level was 19% and 6% respectively (Paper I). The lipid content (g/kg DM) was 2.33 for the hempseed cake diet and 1.67% for the soybean meal diet. The different oilseed supplements did not affect animal growth performance (Hessle *et al.*, 2008) or *M. longissimus dorsi* tenderness. Heat treating the *M. longissimus dorsi* resulted in a reduction in triacylglycerols, while increasing the proportion of phospholipids, NEFA and cholesterol. Heat treating decreased the total lipid C18:0 level in *M. longissimus dorsi* of both supplement groups. The C18:2n-6, total n-6 and PUFA levels increasing, along with a concomitant, non-significant decrease in SFA after heat treatment. The hempseed cake supplement reduced the total lipid n-6/n-3 ratio. Within the PL fraction of *M. longissimus dorsi*, dietary hempseed cake supplement increased the PUFA level including both C18:2n-6 and C18:3n-3 while decreasing the SFA level (Table 1). Hempseed supplement also increased the C18:1*t*-11, leading to a higher proportion of CLA *c*-9, *t*-11 within the *M. longissimus dorsi* PL profile. The PL n-6/n-3 PUFA ratio did not differ between the two supplements.

Table 1. *Fatty acid polar and neutral lipid fractions (% identified FAME[1]) least square means comparison of fresh* M. longissimus dorsi *from hempseed cake (HC) or soybean meal (SM) supplemented steer*

	Polar Lipid				Neutral Lipid			
Fatty Acid	HC	SM	SEM[z]	*P*[y]	HC	SM	SEM	*P*
16:0	21.6	23.7	0.482	0.008	27.5	29.7	0.639	0.029
18:0	12.0	12.6	0.317	0.249	14.4	13.8	0.410	0.342
18:1 *t*-11	0.17	0.08	0.015	0.001	0.70	0.44	0.051	0.051
18:1 *c*-9	25.3	26.4	1.281	0.193	44.5	42.3	0.781	0.003
18:2 n-6	16.6	14.0	0.847	0.050	0.92	0.82	0.059	0.262
18:3 n-3	1.74	1.24	0.113	0.008	0.23	0.18	0.020	0.152
18:2 *c*-9, *t*-11	0.12	0.09	0.009	0.020	0.19	0.13	0.021	0.084
SFA	34.6	37.2	0.613	0.010	45.6	47.7	0.893	0.107
MUFA	30.1	31.4	1.284	0.499	51.8	49.8	0.802	0.103
PUFA	33.1	29.1	1.156	0.499	1.43	1.23	0.101	0.187
n-6	26.9	23.7	0.962	0.027	1.17	1.02	0.080	0.216
n-3	6.11	5.32	0.290	0.072	0.26	0.21	0.022	0.138
n-6/n-3	4.42	4.54	0.192	0.665	4.94	4.97	0.459	0.962

[1] abbreviations: FAME, fatty acid methyl-esters; MUFA, monounsaturated fatty acid; PUFA, polyunsaturated fatty acid; SFA, saturated fatty acid.
[z] Standard error of the mean (SEM).
[y] Level of significance ($P<0.05$).

4.2 Paper II

Silage feeding increased the C16:0, C18:1*t*-11, CLA *c*-9, *t*-11 and C18:3n-3 levels in the SIL ewe milk both years (Table 2). Concentrate feeding increased the C18:0 and C18:2n-6 levels in the CON ewe milk both years. The SFA level increased and the MUFA level decreased between the first and second sampling occasion both years. There was a higher level of MUFA and lower level of SFA and PUFA in the milk Y2 than in Y1. The plasma NEFA level was higher for the SIL ewes than the CON ewes at the first sampling occasion (Y1, 0.4 vs. 0.15 mmol/l and Y2, 0.7 vs 0.4 mmol/l), remaining significantly higher at the second sampling occasion in Y2 (0.36 vs. 0.25 mmol/l).

The average daily live weight gain (LWG) was highest for the CC and SC diets both years. The LWG was lowest for the SS diet both years, with a significantly lower weaning weight of the lambs receiving silage prior to

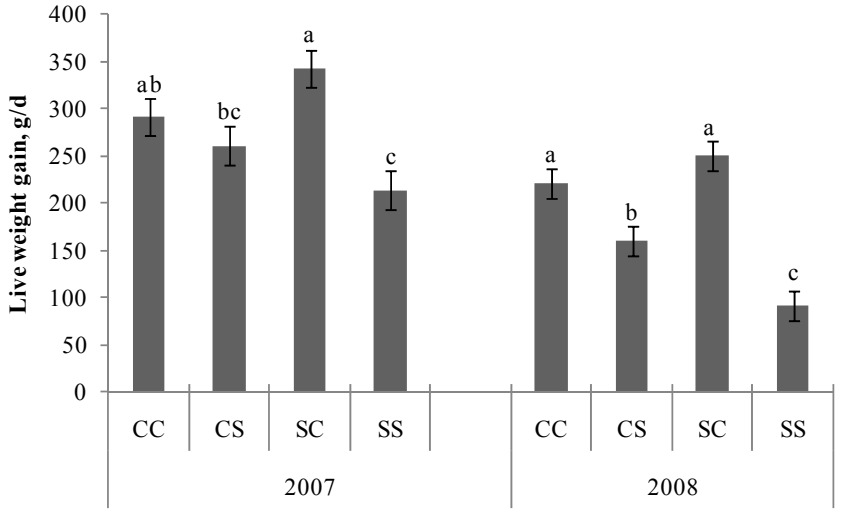

Figure 5. The average live weight gain of lambs showing comparisons between treatments within year for lambs fed either silage (S) or concentrates (C) pre-weaning followed by a post-weaning feeding period on the same diet or switching to the alternate diet. Difference between treatments within year indicated by different letters ($^{a-c}$), significance set at $P<0.05$, with error bars showing standard error of the mean.

weaning Y2 (Fig. 5). In the total lipid profile of *M. semispinalis capiti*s, the C18:3n-3 and n-3 FA levels were higher for the SS diet, with the levels decreasing in relation to the duration concentrates were fed (Table 3). The n-6/n-3 ratio was highest for the CC diet both years, with the ratio decreasing in relation to the duration silage was fed. The CLA *c*-9, *t*-11 level was higher for the SS diet than the CC diet both years. The SFA level was highest and the MUFA level lowest in Y1 compared to Y2.

In the PL fraction of the *M. semispinalis capiti*s, the SS diet had a higher C18:3n-3 level, whereas the CC diet had a higher C18:2n-6 level both years (Table 3). The total n-3 level increased with the duration of silage feeding both years. The total n-6 level and n-6/n-3 ratio increased with the duration of concentrate feeding both years.

Table 2. *Milk fatty acid profile (% identified FAME[l]) from ewes fed silage (SIL) or silage + concentrates (CON) during lactation in 2007 and 2008. Milk samples collected 2 and 4 weeks post-lambing during 2007 and 2008*

	2007								2008								2007				2008			
	Week 2				Week 4				Week 2				Week 4				Dietary		Sampling		Dietary		Sampling	
	CON	SIL			CON	SIL			CON	SIL			CON	SIL			Effect		Occasion		Effect		Occasion	
	n-20	n-16	SEM[z]	*P*[y]	n-21	n-18	SEM	*P*	n-22	n-19	SEM	*P*	n-18	n-19	SEM	*P*	SEM	*P*	SEM	*P*	SEM	*P*	SEM	*P*
% Lipid	6.08	6.37	0.35	0.56	5.88	5.73	0.25	0.68	7.57	7.26	0.50	0.66	6.34	7.49	0.55	0.15	0.25	0.86	0.21	0.09	0.38	0.53	0.37	0.34
C16:0	24.1	26.1	0.46	0.00	25.4	29.3	0.48	0.00	23.8	26.0	0.47	0.00	24.2	27.9	0.61	0.00	0.39	0.00	0.34	0.00	0.43	0.00	0.38	0.03
C18:0	20.3	15.3	0.50	0.00	18.8	13.5	0.47	0.00	19.8	16.1	0.49	0.00	19.8	15.7	0.59	0.00	0.37	0.00	0.34	0.00	0.38	0.00	0.38	0.68
C18:1 *c*-9	27.6	28.5	0.88	0.49	25.3	23.7	0.54	0.05	32.1	34.0	1.40	0.15	30.0	30.1	1.00	0.94	0.62	0.59	0.51	0.00	0.94	0.22	0.88	0.04
C18:2 n-6	1.63	1.29	0.03	0.00	1.59	1.27	0.04	0.00	1.50	1.05	0.04	0.00	1.47	0.95	0.04	0.00	0.03	0.00	0.02	0.12	0.03	0.00	0.03	0.04
C18:3 n-3	1.19	1.70	0.05	0.00	1.26	1.95	0.06	0.00	0.86	1.00	0.04	0.01	0.77	0.95	0.03	0.00	0.05	0.00	0.04	0.00	0.03	0.00	0.03	0.04
SFA	62.9	60.5	0.91	0.06	65.4	64.7	0.54	0.34	58.3	56.2	0.94	0.11	60.5	60.6	1.01	0.96	0.62	0.09	0.52	0.00	0.78	0.34	0.69	0.00
MUFA	31.5	33.4	0.94	0.17	29.0	28.5	0.53	0.55	36.5	39.0	0.97	0.08	34.4	34.5	1.02	0.97	0.64	0.52	0.53	0.00	0.81	0.27	0.70	0.00
PUFA	3.86	4.35	0.08	0.00	3.82	4.66	0.10	0.00	3.61	3.40	0.09	0.12	3.38	3.23	0.07	0.16	0.09	0.00	0.07	0.05	0.07	0.08	0.06	0.00
n-6[x]	1.84	1.55	0.04	0.00	1.79	1.58	0.04	0.00	1.67	1.22	0.04	0.00	1.65	1.17	0.03	0.00	0.03	0.00	0.03	0.55	0.03	0.00	0.03	0.33
n-3[w]	1.56	2.14	0.05	0.00	1.60	2.40	0.06	0.00	1.23	1.38	0.06	0.06	1.11	1.35	0.04	0.00	0.05	0.00	0.04	0.00	0.04	0.00	0.04	0.13
n-6/n-3	1.19	0.73	0.03	0.00	1.15	0.66	0.03	0.00	1.39	0.91	0.04	0.00	1.52	0.89	0.05	0.00	0.02	0.00	0.02	0.01	0.04	0.00	0.03	0.23

[l] abbreviations: FAME, fatty acid methyl-esters; MUFA, monounsaturated fatty acid; PUFA, polyunsaturated fatty acid; SFA, saturated fatty acid.

[z] Standard error of the mean (SEM).

[y] Significance level set at $P<0.05$.

[x] n-6- C18:2n-6 + C18:3n-6 + C20:4n-6.

[w] n-3-C18:3n-3 + C20:5n-3 + C22:5n-3 + C22:6n-3.

Table 3. *Total lipid profile (% identified FAME[1]) of lamb* M. semispinalis capitis*, showing comparisons between treatments within year for lambs fed either silage (S) or concentrates (C) pre-weaning followed by a post-weaning feeding period on the same diet or switching to the alternate diet. Comparisons between Y1 and Y2 illustrate the differences in the plane of nutrition between Y1 and Y2*

	Y1						Y2						Year			
	CC n=9	CS n=8	SC n=9	SS n=8	SEM[z]	*P*[y]	CC n=6	CS n=6	SC n=6	SS n=5	SEM	*P*	Y1	Y2	SEM	*P*
Carcass wt., kg	15.5	15.1	14.6	13.5	0.693	0.225	14.2[a]	11.9[a]	12.2[a]	9.1[b]	0.889	0.009	14.6	11.9	0.717	0.000
% Lipid	5.49[a]	3.92[b]	3.94	3.59	0.415	0.012	3.73	3.17	2.57	2.30	0.400	0.101	4.25	2.96	0.400	0.000
Total lipid																
C16:0	23.7[b]	23.0[b]	25.0[a]	25.7[a]	0.509	0.004	21.7	21.2	22.4	23.9	0.658	0.055	24.4	22.2	0.619	0.000
C18:0	16.4	15.7	15.2	14.6	0.636	0.240	17.4[a]	17.4[a]	14.9[b]	14.3[b]	0.703	0.007	15.5	16.0	0.601	0.323
C18:1 *c*-9	36.0[a]	35.5[ab]	34.0[b]	31.3[c]	0.663	0.000	40.0	38.7	39.6	37.2	1.145	0.360	34.3	38.8	0.908	0.000
C18:2 n-6	3.17	3.55	3.08	3.27	0.253	0.603	3.98	3.89	4.18	2.87	0.397	0.147	3.31	3.75	0.163	0.064
C18:3 n-3	1.34[d]	1.84[c]	2.17[b]	2.87[a]	0.118	0.000	1.03[c]	1.35[bc]	1.51[ab]	1.87[a]	0.130	0.002	2.11	1.43	0.269	0.000
SFA	48.0	47.1	49.0	49.5	0.737	0.148	44.4	44.3	42.9	44.8	1.085	0.657	49.0	44.0	0.462	0.000
MUFA	40.9[a]	40.4[ab]	38.9[b]	36.3[c]	0.707	0.000	46.3	45.3	45.7	44.0	1.137	0.587	39.6	44.4	0.856	0.000
PUFA	6.75[c]	8.78[b]	8.44[bc]	10.1[b]	0.616	0.005	8.54	9.48	10.6	10.2	0.974	0.484	8.99	9.57	0.643	0.295
PUFA/SFA	0.14[b]	0.19[a]	0.17[ab]	0.21[a]	0.015	0.017	0.19	0.21	0.25	0.23	0.025	0.443	0.18	0.22	0.014	0.013
n-6[x]	4.10	5.03	4.10	4.54	0.393	0.304	7.78	8.00	8.49	7.59	0.676	0.806	4.63	5.75	0.257	0.003
n-3[w]	2.20[c]	3.30[b]	3.74[b]	4.95[a]	0.261	0.000	2.37[c]	3.06[bc]	3.67[ab]	4.40[a]	0.326	0.003	3.76	3.19	0.541	0.010
n-6/n-3	1.85[a]	1.53[a]	1.09[ab]	0.91[b]	0.064	0.000	3.3[a]	2.65[b]	2.36[c]	1.73[d]	0.086	0.000	1.32	1.93	0.240	0.000

(Table 3. continued)

Polar lipid																
C18:2n-6	16.0[a]	13.6[b]	13.1[b]	12.2[b]	0.536	0.001	16.4[a]	14.1[b]	14.0[b]	9.48[c]	0.720	0.000	13.7	13.6	1.095	0.709
C18:3n-3	3.44[c]	4.98[b]	4.63[b]	6.87[a]	0.248	0.000	2.71[b]	3.50[ab]	3.29[b]	4.22[a]	0.281	0.013	4.98	3.45	0.532	0.000
C20:4n-6	7.71[a]	7.77[a]	5.51[b]	5.35[b]	0.268	0.000	8.11[a]	8.69[a]	6.34[b]	6.25[b]	0.298	0.000	6.58	7.35	0.649	0.000
C20:5n-3	3.39[c]	3.82[c]	4.47[b]	5.09[a]	0.181	0.000	2.37[c]	2.79[bc]	3.19[b]	4.15[a]	0.156	0.000	4.19	3.12	0.377	0.000
C22:5n-3	3.08[c]	3.63	4.10[a]	4.08[a]	0.123	0.000	3.32[c]	3.77[b]	3.87[b]	4.65[a]	0.153	0.000	3.72	3.89	0.247	0.101
C22:6n-3	1.06	1.10	1.22	1.26	0.066	0.134	0.83[b]	0.96[ab]	1.06[a]	1.11[a]	0.060	0.017	1.16	0.99	0.058	0.000
n-6	26.4[a]	23.7[b]	21.2[c]	20.0[c]	0.685	0.000	27.8[a]	25.9[b]	24.0[b]	19.6[c]	0.764	0.000	22.8	24.4	1.573	0.006
n-3	11.0[c]	13.5[b]	14.4[b]	17.3[a]	0.468	0.000	9.21[c]	11.0[b]	11.4[b]	14.1[a]	0.476	0.000	14.1	11.5	1.173	0.000
n-6/n-3	2.42[a]	1.75[b]	1.47[c]	1.17[d]	0.072	0.000	3.04[a]	2.36[b]	2.11[b]	1.39[c]	0.088	0.000	1.71	2.23	0.300	0.000

[l] abbreviations: FAME, fatty acid methyl-esters; MUFA, monounsaturated fatty acid; PUFA, polyunsaturated fatty acid; SFA, saturated fatty acid.

[z] Standard error of the mean (SEM).

[y] Different letters within a row within a year indicate a significant difference, ($P<0.05$).

[u] n-6- C18:2n-6 + C18:3n-6 + C20:4n-6.

[t] n-3-C18:3n-3 + C20:5n-3 + C22:5n-3 + C22:6n-3.

4.3 Paper III

In vitro results indicated that soybean and linseed N degradability was reduced by extrusion and roasting (Table 4). The canola N degradability was increased by extrusion. The N degradability of raw soybean was higher than canola or linseed. Among the roasted oilseeds, linseed had the lowest N degradability. The N digestibility of the roasted and extruded was similar to the raw treatment within each oilseed. Comparing ground oilseeds, soybean had the highest N digestibility in raw, roasted or extruded form. The ADIN content of the oilseeds was reduced after heat treatment (Paper III).

Table 4. *In vitro N degradability and N digestibility (g/kg) of ground raw, roasted or extruded oilseeds*

	Raw	Roasted	Extruded	SEM[z]
N degradability				
Canola	548^{bx}	469^{bw}	808^{aw}	20.1
Linseed	469^{ax}	280^{bx}	352^{bx}	18.6
Soybean	863^{aw}	490^{bw}	456^{bx}	25.2
N digestibility				
Canola	880^{x}	883^{x}	897^{x}	9.34
Linseed	844^{x}	843^{x}	830^{y}	9.34
Soybean	966^{w}	966^{w}	955^{w}	9.34

[z] standard error of the mean (SEM).

4.4 Paper IV

In *M. masseter*, the Δ6d protein expression generally increased in relation to increasing dietary n-6/n-3 ratio (Table 5). The Δ6d protein expression was generally higher for roasted oilseeds, except in the S-C supplement where the extruded oilseed was higher. The Δ5d and Δ6d expressions in *M. masseter* were positively correlated to their n-6 PUFA products, C20:4n-6 and C18:3n-6 respectively.

In *M. pars costalis diaphragmatis*, the Δ5d protein expression generally decreased in relation to increasing dietary n-6/n-3 ratio (Table 6). The Δ5d protein expression was generally higher for roasted oilseeds, except in the S-C supplement where the extruded oilseed was higher. The Δ5d and Δ6d protein expressions in *M. pars costalis diaphragmatis* were negatively correlated with the LC n-6/n-3 PUFA ratio in the PL fraction (Table 7). In *M. masseter* and *M. pars costalis diaphragmatis* the Δ5d and Δ6d protein

expressions were correlated to each other, r=0.57 and r=0.28 respectively. Although the Δ5d and Δ6d expressions were related within muscle, the desaturases appeared to respond to the dietary n-6/n-3 ratio in an opposite fashion. The fresh tissue lipid content of *M. masseter* was roughly 5% and roughly 9.5% for *M. pars costalis diaphragmatis*.

Table 5. *Polar lipid fatty acid profile (mg/g lipid FAME[1]) and the Δ5d and Δ6d protein expression of* M. masseter *tissue from steers supplemented with either roasted (R) or extruded (E) combinations of canola-linseed (C-L), soy-linseed (S-L), soy-linseed-canola (S-L-C), or soy-canola (S-C) oilseeds in* 1:1 *ratio*

	C-L		S-L		S-L-C		S-C			*P*[y]		
	R	E	R	E	R	E	R	E	SEM[z]	Oilseed	Processing	Interaction
Dietary n-6/n-3	*0.55*	*0.61*	*0.96*	*1.12*	*1.08*	*1.25*	*3.55*	*3.61*				
PUFA	368	385	377	384	395	386	397	392	15.39	0.630	0.819	0.822
C18:2n-6	187	177	192	202	197	201	197	207	9.04	0.126	0.604	0.658
C18:3n-6	3.41	3.23	3.40	3.62	3.61	3.02	3.30	3.50	0.19	0.709	0.509	0.119
C20:3n-6	25.6	27.8	27.9	25.8	28.3	24.8	28.3	28.0	2.22	0.880	0.559	0.611
C20:4n-6	58.0	75.5	61.8	62.1	73.8	64.6	79.2	70.1	6.52	0.269	0.980	0.148
C22:4n-6	5.28	5.78	5.24	5.20	5.79	5.10	6.54	7.49	0.64	0.025	0.692	0.616
C22:5n-6	1.01	1.22	1.08	0.99	1.26	1.03	1.41	1.24	0.09	0.023	0.280	0.098
n-6	283	293	294	302	313	302	319	319	12.79	0.100	0.819	0.852
LC n-6	96	116	102	100	115	101	121	112	8.61	0.323	0.837	0.218
C18:3n-3	31.0	28.6	29.7	29.0	26.8	28.8	24.0	21.5	2.12	0.005	0.556	0.684
C20:5n-3	16.3	20.5	17.1	17.2	17.3	17.0	15.4	14.3	1.79	0.271	0.577	0.474
C22:5n-3	31.7	35.6	30.3	29.8	31.9	31.4	32.1	29.7	1.84	0.248	0.922	0.373
C22:6n-3	4.44	6.12	4.75	4.25	5.21	4.90	5.08	4.84	0.57	0.572	0.698	0.199
n-3	83.5	90.8	81.9	80.3	81.2	82.2	76.6	70.3	4.98	0.065	0.975	0.585
LC n-3	52.5	62.2	52.2	51.3	54.4	53.3	52.6	48.8	3.91	0.351	0.720	0.332

(Table 3 continued)

n-6/n-3	3.5	3.4	3.7	4.0	4.0	3.7	4.3	4.6	0.29	0.011	0.724	0.706
LC n-6/n-3	1.9	1.9	2.0	2.0	2.2	1.9	2.3	2.3	0.13	0.012	0.735	0.568
Δ5d expression[x]	2.34	2.32	2.28	2.26	2.50	2.42	2.31	2.54	0.11	0.327	0.754	0.541
Δ6d expression	1.23[abc]	1.11[bc]	1.14[bc]	1.09[c]	1.37[ab]	1.10[bc]	1.08[c]	1.39[a]	0.09	0.458	0.635	0.019

[1] abbreviations: FAME, fatty acid methyl-ester; PUFA, polyunsaturated fatty acid; n-6, sum of all n-6 FA; LC n-6, sum of n-6 FA > C18; n-3, sum of all n-3 FA; LC n-3, sum of n-3 FA >C18.

[z] standard error of the means (SEM)

[y] level of significance ($P<0.05$), different letters ([a-c]) within row indicate statistical difference.

[x] desaturase protein expressed as a log value.

Table 6. *Polar lipid fatty acid profile (mg/g lipid FAME[1]) and the Δ5d and Δ6d expression of* M. pars costalis diaphragmatis *tissue from steers supplemented with either roasted (R) or extruded (E) combinations of canola-linseed (C-L), soy-linseed (S-L), soy-linseed-canola (S-L-C), or soy-canola (S-C) oilseeds in* 1:1 *ratios*

	C-L		S-L		S-L-C		S-C			*P*[y]		
	R	E	R	E	R	E	R	E	SEM[z]	Oilseed	Processing	Interaction
Dietary n-6/n-3	*0.55*	*0.61*	*0.96*	*1.12*	*1.08*	*1.25*	*3.55*	*3.61*				
PUFA	304	268	315	320	319	303	296	285	10.44	0.006	0.052	0.273
C18:2n-6	167	146	190	184	186	179	177	166	8.57	0.003	0.073	0.787
C18:3n-6	3.01	3.03	3.17	3.13	3.00	2.54	3.30	3.19	0.32	0.485	0.507	0.883
C20:3n-6	16.7	15.6	16.5	17.3	18.7	15.8	17.8	18.1	1.19	0.493	0.383	0.439
C20:4n-6	39.2	36.4	35.9	40.9	41.1	37.8	38.2	37.0	2.41	0.867	0.739	0.287
C22:4n-6	4.12	4.42	3.90	4.26	4.37	3.86	4.91	4.74	0.28	0.037	0.971	0.374
C22:5n-6	0.84	0.79	0.77	0.73	0.93	0.65	0.78	1.01	0.14	0.732	0.716	0.365
n-6	232	207	251	252	255	241	244	231	9.82	0.009	0.075	0.614
LC n-6	65.3	61.3	61.2	67.6	69.0	61.9	66.6	65.2	3.37	0.872	0.525	0.224
C18:3n-3	28.0	22.3	28.1	26.0	25.8	24.4	19.1	18.6	1.38	0.000	0.014	0.264
C20:5n-3	12.3[a]	9.37[bcd]	9.23[cd]	11.3[ab]	10.1[bc]	9.75[bcd]	7.30[e]	8.22[de]	0.65	0.000	0.844	0.003
C22:5n-3	25.9	23.2	22.5	26.1	23.6	22.6	21.6	21.6	1.24	0.073	0.980	0.080
C22:6n-3	3.30	3.72	2.65	2.99	2.94	2.78	2.58	2.45	0.32	0.020	0.600	0.705
n-3	69.5[a]	58.6[c]	62.4[bc]	66.0[ab]	62.4[bc]	59.5[bc]	50.6[d]	50.8[d]	2.23	0.000	0.113	0.012
LC n-3	41.6[a]	36.3[bc]	34.3[cd]	40.0[ab]	36.6[bc]	35.1[bc]	31.6[d]	32.2[cd]	1.77	0.001	0.965	0.024

(Table 6 continued)

n-6/n-3	3.3	3.8	4.1	3.9	4.1	4.1	4.9	4.6	0.24	0.000	0.950	0.511
LC n-6/n-3	1.6	1.7	1.8	1.7	1.9	1.8	2.2	2.1	0.10	0.000	0.592	0.432
Δ5d expression[x]	2.20[ab]	2.10[abc]	2.20[ab]	2.12[abc]	2.21[ab]	2.02[bc]	1.95[c]	2.23[a]	0.07	0.689	0.639	0.004
Δ6d expression	1.24	1.24	1.29	0.98	1.30	0.97	1.07	1.05	0.09	0.252	0.009	0.090

[1] abbreviations: FAME, fatty acid methyl-ester; PUFA, polyunsaturated fatty acid; n-6, sum of all n-6 FA; LC n-6, sum of n-6 FA > C18; n-3, sum of all n-3 FA; LC n-3, sum of n-3 FA >C18.

[z] standard error of the means (SEM)

[y] level of significance ($P<0.05$), different letters ([a-c]) within row indicate statistical difference.

[x] desaturase protein expressed as a log value.

Table 7. *Linear relationships between Δ5d and Δ6d expression and FA proportions and PUFA n-6/n-3 ratio within* M. masseter *(cheek),* M. pars costalis diaphragmatis *(diaphragm) and polar lipid fraction from steers fed a grass silage-based diet supplemented with roasted or extruded oilseed combinations of canola-linseed, soy-linseed, soy-linseed-canola or soy-canola oilseeds in* 1:1 *ratios*

Y	Tissue	X	Intercept	b	P^{z}	r^2	MSE^{-2y}
Δ5d[1]	Cheek	C20:3n-6	2.05	0.10	0.066	0.05	0.30
		C20:4n-6	1.89	0.07	0.002	0.06	0.31
	Diaphragm	PUFA	1.80	0.01	0.038	0.06	0.21
		LC n-6/n-3	2.39	-0.14	0.045	0.05	0.21
Δ6d	Cheek	C18:2n-6	1.12	0.00	0.732	0.00	0.28
		C18:3n-6	0.65	1.59	0.000	0.17	0.26
	Diaphragm	LC n-6/n-3	1.63	-0.25	0.012	0.08	0.28
Δ5d	Cheek	Δ6d	1.59	0.65	0.000	0.32	0.29
Δ6d	Cheek	Δ5d	0.03	0.49	0.000	0.32	0.25
Δ5d	Diaphragm	Δ6d	1.87	0.21	0.015	0.08	0.22
Δ6d	Diaphragm	Δ5d	0.37	0.37	0.015	0.08	0.29

[1] abbreviations: Δ5d, Δ5-desaturase; Δ6d, Δ6-desaturase; PUFA, polyunsaturated fatty acid; LC, long chain (≥ C20).

[z] level of significance set at $P<0.05$.

[y] root mean standard error

5 Discussion

The extent to which PUFA are biohydrogenated in the rumen increases the challenge when trying to influence the PUFA profile of the products. The undegradable N fraction of oilseeds was shown to be altered after heat treatment (Paper III). An increase in the undegradable N fraction decreases the ruminal biohydrogenation of the PUFA in oilseeds, increasing the post-ruminal PUFA level. Inclusion of oilseeds in ruminant diets was shown to be an effective means to alter the FA profile of ruminant muscle and milk (Papers I, II, IV). Moreover, the Δ5d and Δ6d protein expression was shown to be influenced by the dietary n-6/n-3 ratio (Paper IV).

5.1 Hempseed cake as a protein and polyunsaturated fatty acid source

In Paper I, changes to the FA profile and meat tenderness were investigated in relation to the suitability of substituting hempseed cake for soybean meal as a protein supplement in finishing diets. Generally, oilseeds are included in ruminant diets as a protein source; however the contribution from the lipid content and FA profile should also be considered in ration formulation. Previously, feeding full-fat hempseeds at 9 to 14% dietary DM was shown to increase the C18:3n-3 level in total lipids (Gibb *et al.*, 2005). Similar growth performance between the supplemented groups in the present trial affirmed that hempseed cake was a suitable protein substitute with no deleterious nutritional characteristics at the present inclusion level. The higher lipid content of the hempseed cake, compared to the soybean meal, increased the proportion of PUFA in the diet. The degree in which PUFA undergo lipolysis/biohydrogenation has been shown to be inversely related to the dietary inclusion level (Beam *et al.*, 2000). While this may have not been the only influencing factor, the hempseed cake positively affected the total lipid

n-6/n-3 ratio and increased the PL C18:3n-3 level. The decrease in the muscle triacylglycerol level, after heat treatment, can be attributed to fat drippings. Overall, hempseed cake lowered the muscle n-6/n-3 ratio and had a positive influence on the FA profile in terms of FA suggested to have beneficial physiological effects on humans.

5.2 Feeding duration and influence on fatty acid profile

The findings from the lamb diet cross-over study (Paper II) illustrated the influence adequate nutrition has on lamb growth performance and FA profile. The milk FA profiles of the ewes were influenced by diet as well as changes related to lactation stage and nutritional status. Ensiled forage increased the C18:3 and C16:0 level in milk compared to rapeseed cake, which was higher in C18:2n-6. Milk FA profiles have been shown to be correlated to the duodenal concentrations of PUFA, which is related to the dietary FA profile and inclusion level (Glasser *et al.*, 2008). In both years of our study (Paper II), the C18:2n-6 and C18:3n-3 levels in the diet were reflected in the FA profile of the milk from CON and SIL ewes respectively. The higher C16:0 level from forage feeding was possibly related to the higher proportion of dietary neutral detergent fibre leading to longer rumen retention times and fermentation characteristics, increasing *de novo* FA synthesis. Higher rumen passage of C18:3n-3 with forage feeding was most likely due to the association of C18:3n-3 with chloroplasts and protection by the plant cell wall. Alternatively, rapeseed cake has been reported to depress the C16:0 level in milk with concurrent increases in C18:0 and C18:1*c*-9 levels (Kennelly, 1996). The expected increase in C18:1*c*-9 level associated with rapeseed supplementation (Petit *et al.*, 1997; Rule *et al.*, 1994; Khorasani *et al.*, 1991) was not apparent in this trial. This may be related to the dietary inclusion level or point in the lactation stage when the milk samples were collected.

The dietary induced differences in ewe milk FA profile was further compounded by the lamb diet, which further pronounced the differences in the total lipid FA profile of the muscle between treatments. In both years, the total lipid C18:3n-3 level in the *M. semispinalis capiti*s was positively related to the duration of silage feeding. Even after the diet cross-over point at weaning, the dietary influence during the suckling period was prevalent for the C16:0 and C18:1*c*-9 in the total lipid tissue FA profile in Y1 (Table 3). In Y2, the C18:0 level in muscle showed a strong connection to the pre-weaning diet, possibly also relating to the nutritional status of the ewes in Y2. Bas and Morand-Fehr (2000) reported that the FA profile of lambs

established during the suckling period tended to predominate several months post-weaning. The multi-component analysis presented in Paper II illustrates how the C18:2n-6 and C18:3n-3 levels within the tissue are highly related to ewe diet and milk profile both years.

Differences between the lamb diets for the PUFA levels were highlighted in the PL fraction of the *M. semispinalis capitis* (Table 3). As with total lipids, the C18:3n-3 level was higher in the PL fraction of lambs fed silage for longer durations, which led to higher n-3 LC-PUFA levels. Slight shifts in the PL FA profiles after the diet cross-over indicated that the post-weaning diet had an influence. The similarity of the two cross-over diets to their pre-weaning diets indicated that the feeding duration was not sufficient to substantially change the muscle PL PUFA profile.

Complications arising with the nutritional quality of the feed in Y2 exemplify the importance of feed testing and ration balancing. There were also shifts in the milk FA profile, which could be linked to lactation stage and a negative energy balance during early lactation. The higher proportions of C18:0 and C18:1*c*-9 observed at the first in Y1 could be related to the mobilization of body reserves during peak lactation. Ruminant body reserves are predominantly C18:1*c*-9 with lesser proportions of C16:0 and C18:0. The higher milk MUFA level in Y2 compared to Y1 was suggestive of a higher mobilization of body reserves, probably relating to the feed quality. In Y2 the negative energy balance experienced by the ewes appeared to be prolonged, with the milk C18:1*c*-9 level between diets being similar at both sampling periods (Table 2). Blood analysis results from the ewes showed a stronger spike in plasma NEFA in Y2 indicative of a higher draw on body reserves for milk production. Generally, during periods of negative energy balance, mobilization of body reserves can account for up to 10% of FA in milk; however this proportion can increase, within physiological limits, depending on the severity of the energy deficit (Bauman & Griinari, 2003; Palmquist *et al.*, 1993).

The nutritional status of the ewes between years was reflected in the growth performance of the lambs which had lasting effects on the FA profile of the lambs. Moreover, the nutritional quality in Y2 subsequently affected the lamb performance with lower weaning weights and average daily gain, resulting in lower slaughter weights compared to Y1. Both years, the lambs that switched from forage to concentrates exhibited substantial compensatory gain. Lambs that switched from concentrates to forage appeared to have impeded growth. In Y1, the lamb tissue total lipid FA profile responded in kind to the FA present in the diet. In Y2, the differences between the diets were less pronounced in the total lipids, possibly relating to less pronounced

differences in the milk FA profiles between diets. The lamb *M. semispinalis capitis* total lipid profile Y2 had a higher MUFA level with a lower SFA level compared to Y1 (Table 3). The proportions of LC-PUFA in the PL profile both years reflect the proportions of C18:2n-6 and C18:3n-3 in the diet. Although shifts in the FA profile were evident after weaning, the feeding duration necessary to induce a reversal in the FA profile was not achieved under the experimental conditions of this study. To affect the lamb FA profile more, a longer feeding period would be necessary or a more intensive feeding programme.

5.3 Effects of heat processing on oilseed undegradable N

Previously, this *in vitro* method was used to show differences in N degradability and N digestibility of soybean roasted for different times, and was found to be comparable to *in sacco* and mobile bag methods (McNiven *et al.*, 2002). *In vitro* results presented in Paper III indicated that roasting effectively increased the undegradable N fraction of linseed and soybean (Table 4). Moreover, while not statistically significant, there was an increase in the undegradable N fraction of roasted canola, suggesting a benefit from roasting. Similarly, the undegradable N fraction of raw linseed increased from 53% to 65% N after extrusion suggesting some benefit from extrusion processing. Previously, Mustafa *et al.* (2003) reported that the *in situ* undegradable N fraction of linseed was not increased by extrusion. Possible reasons for this may be due to differences in extrusion conditions, differences between *in situ* and *in vitro* methods or factors relating to the increased surface exposure of the extruded linseed. The undegradable N fraction of canola was decreased by extrusion, presumably due to increased surface area exposure facilitating enzymatic activity. Ferlay *et al.* (1992) reported that extrusion was not effective for altering the undegradable N fraction of canola due to the oil content which have prevented adequate heating during extrusion. Insufficient heating during extrusion would prevent protein denaturing, which would prevent changes to the peptide structure. In this trial, the oil content of linseed and canola were similar; however the undegradable N fraction of linseed was not increased after extrusion. Such a contradiction may have been related to the proportion or composition of protein secondary structures. As mentioned previously, the proportion of di-sulfide bonds and the α-helix/β-sheet ratio are known to influence N degradability. As no oilseed showed a decrease in N digestibility after heat treatment, we concluded that the heat treatments were not severe enough to cause the formation of Maillard reaction products. Furthermore,

the reduction in the ADIN fraction after heat treatment suggests an increase in the N available for digestion. Results from this study indicate that heat treatment will increase the post-ruminal supply of oilseed protein available for digestion without increasing the indigestible fraction. Further studies using higher processing temperatures could be made in an attempt to decrease the N degradability further; however decreases in N digestibility should be minimized.

5.4 Dietary influences on the formation of long-chain polyunsaturated fatty acids

The Δ5d and Δ6d desaturase enzymes are essential for forming the n-3 and n-6 series LC-PUFA derivatives from their respective C18:2n-6 and C18:3n-3 precursors. Previously, the protein expressions of Δ5d and Δ6d have been shown to not always coincide within tissues. In piglets, the Δ5d expression was reported to be higher in *M. longissimus dorsi* and lower in liver, whereas the Δ6d expression was higher in liver than *M. longissimus dorsi* (Missotten *et al.*, 2009) Information regarding the expression of Δ5d and Δ6d desaturase enzymes in bovine tissue is sparse. Contrary to porcine tissues, Herdmann *et al.* (2010) reported that bovine *M. longissimus dorsi* had higher Δ6d protein expression than subcutaneous adipose tissue.

The results presented in Paper IV are the first to relate Δ5d and Δ6d expression in two muscles over a range of dietary n-6/n-3 PUFA ratios using heat processed oilseeds. In general, roasted oilseeds elicited a higher Δ5d and Δ6d expression in both muscles compared to extruded oilseeds. This expression pattern may be related to a higher proportion of PUFA available in the duodenum, and higher incorporation into the muscle membrane. The inconsistency of Δ5d and Δ6d protein expression in relation to the roasted and extruded S-C supplement warrants further investigation into PUFA induced regulation of the lipogenic processes. In the muscles investigated; the Δ5d and Δ6d expressions showed a relationship to the PL FA profile, which was related to the dietary n-6/n-3 ratio.

In *M. masseter* tissue, the relationship of the Δ5d and Δ6d protein expressions to the desaturase products, rather than the respective precursors suggests that the proportion of LC-PUFA in the PL fraction was influenced more than what could be explained by the diet. The positive relation between desaturase products and Δ5d and Δ6d protein expression suggested that a higher dietary n-6/n-3 ratio increased desaturase expression in the *M. masseter*. This finding concurs with Herdmann *et al.* (2010) who found a diet

higher in n-6 PUFA increased the Δ6d protein expression in *M. longissimus dorsi*.

In *M. pars costalis diaphragmatis*, the protein expressions were most strongly related to the LC n-6/n-3 ratio rather than individual FA, though the reason for this is unclear. The negative relationship between Δ5d and Δ6d expressions and the muscle LC n-6/n-3 ratio suggests an increasing dietary n-6/n-3 ratio would decrease desaturase protein expressions within the *M. pars costalis diaphragmatis*. The apparent contradictory responses of the Δ5d and Δ6d desaturase expressions between the muscles is puzzling, but may be related to muscle function and metabolic activity in the muscles as suggested by Hocquette and Bauchart (1999).

Previously, Ward *et al.* (2010) found that the Δ5d and Δ6d protein expression was positively correlated to the IMF content in *M. semimembranosus*. Human studies have indicated that n-6 FA increases adipogenesis (Ailhaud *et al.*, 2006). This would explain the relationship between the increased Δ5d and Δ6d expression level to the n-6 LC-PUFA product levels in *M. masseter* and the positive correlation between Δ5d and Δ6d expression and increased IMF observed by Ward *et al.* (2010). In our study the lipid content of *M. masseter* was similar to that of retail muscles, whereas the *M. pars costalis diaphragmatis* content was about two-fold higher. Fatty acid binding protein expression and activity, oxidative enzyme activities and proportional presence of adipocyte cells can vary between muscles based on fibre types and proportions, cumulatively contributing to the muscles ability to synthesis and deposit of IMF (Jurie *et al.*, 2007). Such differences between muscles may help to explain the decrease in Δ5d and Δ6d expression in *M. pars costalis diaphragmatis* in relation to the lipid content of the muscle. Further investigations into factors that regulate the lipogenic processes and adipose formation are warranted to help understand the findings of this study.

5.5 General discussion

Dietary lipid composition can substantially influence the FA profile of ruminant products. The inclusion of oilseeds was shown to be an effective means by which the FA profile of ruminant products can be altered (Paper I, II, IV). Ruminal biohydrogenation of dietary PUFA poses a considerable hurdle when attempting to modify the PUFA profile of ruminant muscles. The dietary PUFA composition and inclusion level have been shown to correlate with the degree of influence exerted by the oilseeds (Griswold *et al.*, 2003). Moreover, the proportion of PUFA available post-ruminally

increases with dietary supplementation level (Beam *et al.*, 2000). The higher lipid content of the hempseed cake supplement and the higher C18:3 level of hempseed cake both probably contributed to the increased PUFA level and decreased n-6/n-3 ratio in *M. longissimus dorsi* (Paper I).

A number of studies have shown that forage enhances the muscle C18:3n-3 level and reduces the n-6/n-3 PUFA ratio in comparison to predominantly cereal diets (French *et al.*, 2000; Manner *et al.*, 1984). However, the use of fresh forage to alter the ruminant muscle FA profile typical of a winter concentrate-conserved forage diet in the study by Noci *et al.* (2005) was reported to be in the range of 158 days. After prolonged grazing, there was an increase in the n-3 level with no alteration to the n-6 level (Noci *et al.*, 2005). The changes to the FA profile using fresh forages illustrate the difficulty in reversing the influence of a diet containing concentrates.

While comparisons between fresh forage diets and oilseed supplementation are beyond the scope of this thesis, the challenges faced in meeting animal nutritional requirements with strictly conserved forages are similar. Compared to concentrate feeding, the ensiled forage diets of ewes and lambs increased the n-3 level of the milk and meat, but affected the lamb growth performance (Paper II). Comparisons between the years from the lamb diet cross-over study (Paper II) also illustrated the influence adequate nutrition has on lamb growth performance and FA profile, stressing the importance of feed testing. The small shifts in the lamb muscle FA profile after the diet cross-over indicated the post-weaning feeding duration was not sufficient to supplant the pre-weaning diet influence on the FA profile under these experimental conditions. In general, inclusion of oilseeds in conserved forage-based diets helps to meet animal nutritional needs for growth in addition to influencing the FA profile of the muscle in a predictable manner.

Heat processing of oilseeds has been shown to be an effective method to improve the utilization of high quality protein by increasing the duodenal flow of limiting amino acids (Kennelly, 1996; Aldrich *et al.*, 1995). Heat processing treatments have been shown to increase the rumen undegradable N fraction of canola, soybean and linseed including extruding, micronizing and roasting (Petit *et al.*, 2002; Dakowski *et al.*, 1996; Faldet *et al.*, 1992b). Similarly, in Paper III we found roasting and extruding increased the undegradable N fraction of soybean and linseed using an *in vitro* model. While changes in the protein structures were not investigated in Paper III, assumed restructuring of the peptide bonds reduced the susceptibility of the proteins to enzyme degradation. In general under-heating has no effect on

the undegradable N fraction, whereas overheating produces poorly digestible Maillard reaction products. Similar N digestibility between raw and roasted oilseeds in Paper III suggests the oilseed protein was undamaged by the heat treatments and would be available post-ruminally. Restructuring the peptide bonds after heat treatment has been shown to encapsulate the lipid droplets thereby reducing the extent of biohydrogenation (Kennelly, 1996). Heat treatments that reduce rumen protein degradability have been shown to increase the post-ruminal PUFA flow and alter the FA profile of milk (Chouinard *et al.*, 1997). The post-ruminal PUFA flow has been shown to be decreased after extrusion due to rupturing of the seed coat and increased exposure of the lipid to the rumen environment (Scott *et al.*, 1991).

Few studies have investigated the regulation of the Δ-5 and Δ-6 desaturases within ruminant tissues. Findings from Paper IV showed that the dietary n-6/n-3 ratio influenced the protein expression of the desaturases responsible for forming LC-PUFA within bovine *M. masseter* and *M. pars costalis diaphragmatis*. Roasted oilseeds were shown to generally increase the protein expression and increased the PUFA level in the *M. masseter* and *M. pars costalis diaphragmatis*. In relation to the dietary n-6/n-3 ratio, the protein expressions were found to be muscle specific. Protein expression in combination with FA profiles gave a plausible indication to the enzyme activity within the muscles. Further investigations relating to lipid metabolism, such as gene expression and enzyme activity, would contribute to the understanding of the factors determining the FA profile of ruminant tissue.

6 Conclusions

The benefits from increasing the n-3 LC-PUFA content in the human diet are well documented (Givens & Gibbs, 2006; Givens, 2005; Connor, 2000). There are strong indications that moderate intake of lean, trimmed ruminant meats can be beneficial in reducing the risk of developing chronic diseases associated with Western living (McAfee *et al.*, 2010; Williamson *et al.*, 2005). Moreover, ruminant meats contribute substantially to the human n-3 LC-PUFA intake, particularly in regions where marine sources are not typically consumed (Howe *et al.*, 2006; Scollan *et al.*, 2006; Wood *et al.*, 2003).

Within the scope of this work, factors influencing the FA profile of ruminant tissues in relation to oilseed supplementation, such as lipid source and processing, have been discussed. The FA profile of hempseed cake and proportion of PUFA in the diet was shown to increase the PUFA and CLA *c*-9, *t*-11 levels within the *M. longissimus dorsi* as well as reduce the n-6/n-3 ratio. Oilseeds have been shown to be an effective means in which to alter the FA composition of ruminant tissues, both within the context of this thesis as well as in other studies (Dawson *et al.*, 2010; Mahecha *et al.*, 2009; Maddock *et al.*, 2006; Scollan *et al.*, 2001). Heat treatment was shown to be an effective means to increase the undegradable N fraction of oilseeds, leading to increased post-ruminal concentrations of PUFA. Changes to the muscle FA profile were shown to be highly related to the FA profile of the diet as well as the feeding duration. The nutritive value of the feed significantly influences animal performance which also influences the FA profile of muscles as shown in the lamb study.

The subsequent influence of tissue FA profile on the protein expression of desaturases responsible for the formation of LC-PUFA in ruminants is a relatively new and innovative field of research. Previous studies have indicated that Δ5d and Δ6d are positively related to IMF accumulation in

bovine tissue (Herdmann *et al.*, 2010; Ward *et al.*, 2010). Our study showed that the Δ5d and Δ6d protein expressions pattern are tissue specific and differ in relation to the dietary n-6/n-3 ratio. Findings from this thesis provide clear evidence that the n-6/n-3 PUFA ratio of ruminant tissue can be altered via oilseed supplementation.

7 Main findings

- Oilseeds are an effective means by which the fatty acid profile ruminant products can be altered.
- The fatty acid profile of the oilseed exerts a relative influence on the fatty acid profile of the product.
- Feeding duration and nutritional status of the animal can influence the fatty acid profile.
- Processing of the oilseed affects the availability of the lipids to rumen biohydrogenation.
- Roasting decreased the ruminal hydrogenation of polyunsaturated fatty acids and increased the proportion of polyunsaturated fatty acids available for absorption into the tissues.
- The Δ5- and Δ6- desaturase protein expressions seem to be tissue specific and influenced by the dietary n-6/n-3 ratio.

8 Future considerations

This thesis explored the possibility of altering the FA profile of ruminant meat and milk through dietary manipulation. The degree to which dietary oilseed inclusion can exert an influence is a function of the FA profile and the extent to which the dietary lipids are protected from biohydrogenation. The tissue PUFA profile can incite tissue specific enzyme desaturase responses. In light of these findings, future research considerations include:

- Studying the interactions between oilseed moisture and processing temperature and time to decrease the degree of rumen biohydrogenation.

- Investigating alternate oilseeds, processing methods and oilseed substitutes to increase the polyunsaturated fatty acids level in ruminant muscles.

- Optimizing ruminant dietary oilseed inclusion levels to increase the level of fatty acids known to be beneficial for human health.

- Further investigating the influence of desaturase activity and lipid synthesis on tissue lipid composition.

- Attempting to induce desaturase activity within muscle tissue through dietary means and subsequent effects at the enzyme and gene regulation level.

- Investigating how desaturase activity changes in response to both physiological maturity and intramuscular fat content.

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