Factors Affecting Fatty Acid Composition in Forage and Milk

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Abstract

The aims of the studies underlying this thesis were to evaluate variations in fatty acid (FA) contents of plants used as forage for dairy cows in northern Sweden, and their effects on the FA contents of the milk. Initially, samples of timothy (*Phleum pratense* L.) were subjected to different pre-treatments prior to analysis. Freezing with liquid N was not necessary to obtain apparently reliable FA profiles of the samples examined. The prevailing method for handling samples before analysis, *i.e.* freeze-drying and grinding, was satisfactory. However, heat drying samples at 60°C was just as good, or even better in some cases. Neither wilting (to 330-350 g dry matter/kg) nor application of additives (acid additive or bacterial inoculant) to timothy had any substantial effects on the proportions of FAs in silage.

In addition, seasonal variations in FA concentrations were evaluated in timothy and meadow fescue (*Festuca pratensis* Huds.) subjected to three different N fertilization regimes and harvested at different stages of maturity during their spring and summer growth periods. FA concentrations in both grasses declined over time in both growth periods. There were positive linear relationships between FAs and concentrations of crude protein (CP) and crude fat, which might be useful as tools to predict FA concentrations in the forage. Furthermore, the two grass species examined showed differences in FA profiles, notably timothy had higher concentrations of C18:2 n-6 and meadow fescue higher concentrations of C18:3 n-3, but the total FA concentrations were similar in both grasses.

In addition, three timothy silages subjected to different N-fertilization regimes and a red clover/timothy silage (60:40 on DM basis) were fed to 24 dairy cows in a change-over design. Higher levels of N-fertilization led to higher concentrations of CP accompanied by higher concentrations of FAs in silage. However, these differences did not affect the concentrations of either protein or 18:3n-3 in the milk of cows fed these grass silages, but including red clover in the cows' diet led to increased concentrations of C18:3 n-3 and cis-9, trans-11 18:2 in their milk.

Keywords: Fatty acids, *Festuca pratensis* Huds., Milk production, Pre-treatments, N-fertilization, *Phleum pratense* L., Seasonal variation, Silage, *Trifolium repens* L, Wilting

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Ju mer man tänker, ju mer inser man att det inte finns något enkelt svar.
A.A. Milne

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 K. Arvidsson, A-M. Gustavsson and K. Martinsson (2009). Fatty acids in forages: A comparison of different pre-treatments prior to analysis. *Animal Feed Science and Technology* 151, 143-152.

II K. Arvidsson, A-M. Gustavsson and K. Martinsson (2009). Effects of conservation method on fatty acid composition of silage. *Animal Feed Science and Technology* 148, 241-252.

III K. Arvidsson, A-M. Gustavsson and K. Martinsson. Fatty acid concentrations in timothy (*Phleum pratese* L.)and meadow fescue (*Festuca pratensis* Huds.): effects of seasonal variations at different nitrogen fertilization levels (manuscript).

IV K. Arvidsson, A-M. Gustavsson and K. Martinsson. The effect of crude protein level and fatty acid concentration in silage on the fatty acid composition of dairy cow milk (manuscript).

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The contribution of Katarina Arvidsson to the papers included in this thesis was as follows:

I Planning the research jointly with the co-authors, collecting samples and conducting the experimental treatments, processing data and responsible for compiling the manuscript.

II Planning the research jointly with the co-authors, collecting samples and conducting the experimental treatments, processing data and responsible for compiling the manuscript.

III Planning the research jointly with the co-authors, collecting samples, processing data and responsible for compiling the manuscript.

IV Planning the research jointly with the co-authors, collecting samples, processing data and responsible for compiling the manuscript.

Abbreviations

CLA	Conjugated linoleic acid
CP	Crude protein
DM	Dry matter
FA	Fatty acid
MUFA	Monounsaturated fatty acids
PPO	Polyphenol oxidase
PUFA	Polyunsaturated fatty acids
SFA	Saturated fatty acids
TFA	Total fatty acids

1 Introduction

Milk is a valuable source of nutrients, providing humans with energy, protein, essential minerals and vitamins. In many countries, milk is also an important fat source in the human diet. However, the healthiness of dairy products for humans has been questioned since they constitute a considerable fraction of the diet for many people (Mann, 2002) and the majority of fatty acids (FAs) in milk are saturated (Bauman *et al.*, 2006). Even if dairy products only provide 15-25 % of the total fat in the human diet, they provide about 25-35 % of the total intake of saturated fatty acids (SFA) (O'Donnell, 1993). From the consumer's point of view, there is also growing interest in the connection between diet and health; the public often associates SFA with obesity and coronary heart disease (Bauman *et al.*, 2006). According to Jensen (2002), SFA, monounsaturated fatty acids (MUFA) and polyunsaturated fatty acids (PUFA) account for, on average, 69 %, 27 %, and 4 % of the total contents of milk fat, respectively. MUFA and PUFA are regarded as beneficial constituents of the human diet (*e.g.* Banni & Martin, 1998; Leaf & Weber, 1988) and may have positive health effects on health (*e.g.* Simopoulos, 2001). There is also evidence of positive effects of *trans*-11 18:1, the major *trans* FA in milk, in animal models (Lock *et al.*, 2004). Milk also contains conjugated linoleic acids (CLA), the major isomer being *cis*-9, *trans*-11 18:2, which reportedly has a range of health-promoting properties (Parodi, 2002). Linoleic acid (C18:2n-6) and α-linolenic acid (C18:3 n-3) are referred to as the essential FAss, meaning they cannot be endogenously synthesised by humans, and hence need to be supplied by the diet. This is because mammals are not able to introduce double bonds between the terminal methyl group and the ninth carbon atom in FA chain (McDonald *et al.*, 1995), while plants have the ability to synthesise C18:3 n-3 acid *de novo* (Dewhurst *et al.*, 2003).

Dietary C18:3 n-3 is the best precursor of C18:3 n-3 in milk, while CLA derives from both C18:2 n-6 and C18:3 n-3. Hence, there has been growing interest in the concentration and composition of unsaturated FAs in forage. Intense research has been carried out to understand and improve the FA composition of forage plants, which contribute considerable proportions of the PUFA in ruminant diets. Since many metabolic factors affecting the conversion of PUFA in the rumen have not yet been identified, it is important to quantify the variations in FA concentration in grasses. This will help to identify breeding goals and management strategies to increase PUFA in forages and animal-derived products (Dewhurst *et al.*, 2001).

1.1 Nomenclature

FAss can be named according to the IUPAC (International Union of Pure and Applied Chemistry) nomenclature, by their trivial names or by shorthand abbreviations. (Figure 1). The carboxyl carbon is referred to as C-1 in IUPAC nomenclature, the following carbon atom is number 2 and so forth. Often Greek letters are used instead of numbers to identify the carbon atoms. The carbon next to the carboxyl carbon is called α, and the following carbon atoms are called β, γ, δ and so forth. However, the last carbon atom is always called omega (ω), regardless of the length of the hydrocarbon tail (IUPAC, 2009). In addition, the letter n is often used instead of ω (McDonald *et al.*, 1995), and this convention will be applied throughout this thesis.

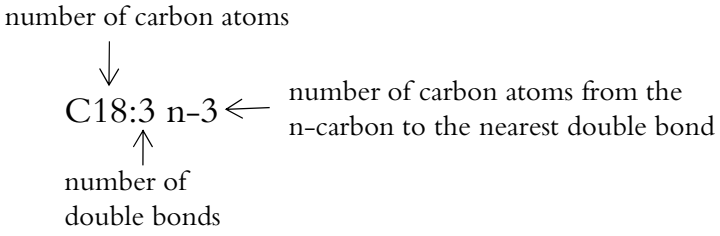

Figure 1. To identify a fatty acid, often two numbers separated by a colon are used. The first number refers to the number of carbon atoms in the fatty acid and the other indicates the number of carbon-carbon double bonds.

FAss without any carbon-carbon double bonds are classified as SFA and FAss with at least one carbon-carbon double bond are classified as

unsaturated FAss. Unsaturated FAss with one carbon-carbon double bond are called MUFA and those with more than one carbon-carbon double bond are referred to as PUFA.

1.2 Unsaturated FAs

The unsaturated FAss are grouped into three families, named omega-9 (n-9), omega-6 (n-6) and omega-3 (n-3), according to the position of the double bond nearest the omega (n) carbon atom in them. Precursors of these families are oleic (18:1 n-9), 18:2 n-6, and 18:3 n-3 acids, respectively (Figure 3).

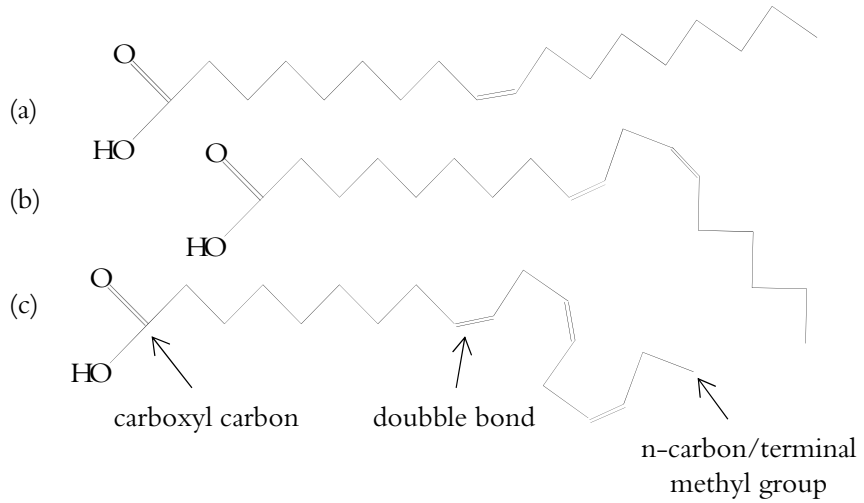

Figure 2. Schematic diagram of (a) oleic acid (C18:1 n-9), (b) linoleic acid (C18:2 n-6), and (c) α-linolenic acid (C18:3 n-3).

The double bonds found in FAs are nearly always in the *cis* configuration (Figure 4). As shown in Figure 3 this causes a bend in the FA chain. This bend has important consequences for the structure of biological membranes. SFA chains can pack closely together to form ordered, rigid arrays while unsaturated FAs prevent such close packing and produce flexible, fluid aggregates.

As mentioned above, n-3 FAss include C18:3 n-3, the predominant FA in grasses. The group also includes eicosapentaenoic acid (EPA; C20:5 n-3) and docosahexaenoic acid (DHA; C22:6 n-3), which are found in fish oils. Apart from C18:2 n-6, the long chain n-6 FAss are arachidonic acid (ARA; C20:4 n-6), and adrenic acid (C22:4 n-6) (Raes *et al.*, 2004). C18:2 n-6 and

C18:3 n-3 in feed are the precursors of these beneficial FAss in milk and meat.

1.2.1 *Trans*-FAs

Ruminant products are the only edible sources of natural *trans* FA, mainly *trans*-18:1, for humans. *Trans*-FAss have one or several double bonds in the *trans* configuration, which means that the hydrogen atoms are on opposite sides of the double bond (Figure 3) (McDonald *et al.*, 1995). This results in straight chains and gives the *trans*-FAss properties similar to SFAs . In addition to their production by ruminal biohydrogenation, *trans*-FAs are industrially manufactured by hydrogenation of vegetable oils and fats (Livsmedelsverket, 1997). However, industrially produced *trans*-FAs have been shown to have adverse effects on low-density lipoprotein cholesterol levels (Voigt & Hagemeister, 1993), and Willett *et al.* (1993) found consumption of *trans* isomers from vegetable fats, but not *trans* isomers from animal fats, to be associated with increased risks of coronary heart disease in humans. The amounts of *trans*-9 and *trans*-10 18:1 FAs, but not *trans*-11 18:1, in food have also been positively correlated with the risk of coronary heart disease (Hodgson *et al.*, 1996). Therefore, it is desirable to strive for high contents of PUFA and CLA in milk, together with low contents of *trans*-9 18:1 and *trans*-10 18:1 FAss (Voigt & Hagemeister, 1993).

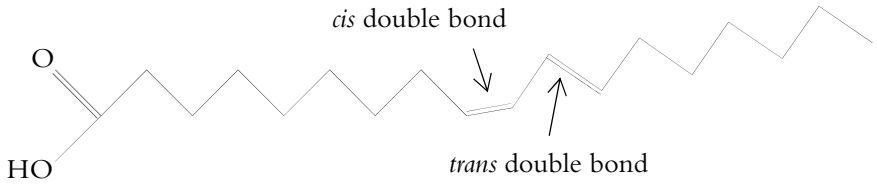

Figure 3. Schematic diagram of *cis*-9, *trans*-11 18:2 (CLA) showing the *cis* and *trans* configuration of an unsaturated fatty acid

1.2.2 CLA

Conjugated linoleic acid (CLA) is the collective term for isomers of C18:2 with conjugated double bonds (Parodi, 2002), which means that two double bonds are immediately adjacent to each other (compare Figure 2 with Figure 3). Many stereo and positional isomers of CLA are found in ruminant products, of which *cis*-9, *trans*-11 18:2 (Figure 3) and *trans*-10, *cis*-12 18:2 are the most abundant (Raes *et al.*, 2004). Of these two, the *cis*-9, *trans*-11 18:2 isomer is the major isomer in ruminant fat, and represents 80 to 90 % of the total CLA in milk fat. It is also the most common isomer in meat fat

from cattle, but represents a lower percentage (60-85 %) of the total CLA content in the meat (Shantha *et al.*, 1994; Chin *et al.*, 1992). *Cis*-9, *trans*-11 18:2 has been stated to be the most biologically active CLA isomer since it is the predominant isomer incorporated in the phospholipids of cell membranes (Ip *et al.*, 1991; Ha *et al.*, 1990). CLA has been shown to have multiple health benefits at amounts of 1 % or less of the diet. CLA inhibits the growth of cancer cells and it also possesses antiatherogenic, antidiabetic, antiallergenic, and immunomodulating, properties (Parodi, 2002).

Cis-9, *trans*-11 18:2 is produced by the biohydrogenation of dietary C18:2 n-6 in the rumen, and is also derived from dietary C18:3 n-3, via vaccenic acid (*trans*-11 18:1), by endogenous synthesis (see section 1.5). Research has shown that endogenous synthesis of *cis*-9, *trans*-11 18:2 from ruminally-derived *trans*-11 18:1 may provide 60-75 % of the *cis*-9, *trans*-11 18:2 in milk. Since this is the isomer that is found in highest concentrations, and is the most common CLA isomer in ruminant products, it has been given the trivial name 'rumenic acid' (Bauman & Griinari, 2001; Palmquist, 2001). Thus, CLA refers to *cis*-9, *trans*-11 18:2 hereafter, unless otherwise stated.

1.3 Lipids in plants

Dairy cow diets usually consist of fresh and/or conserved forages supplemented with concentrates (*i.e.* cereal grains). The lipid fraction in leaf tissue ranges from 3 to 10 % of dry matter (DM). Most of the lipids are located in the chloroplasts, where they comprise up to 30 % of DM (Hawke, 1973).

Plant lipids can be grouped into structural and storage compounds. The structural lipids are present in various membranes and protective surface layers (McDonald *et al.*, 1995). The surface lipids are mainly waxes with small proportions of long hydrocarbons, FAs and cutin. The membrane lipids, which are present in the mitochondria, endoplasmic reticulum, plastids and plasma membranes, consist mainly of glycolipids and phospholipids (McDonald *et al.*, 1995). The leaf lipids are mainly galactolipids, a subgroup of the glycolipids that consist of glycerol, unsaturated FAs, one or more galactose units and in some cases either a hydroxyl group or a sulfonic acid group (Van Soest, 1994). The storage lipids occur in seeds and are mainly triglycerides (Van Soest, 1994). Since the triglycerides are confined to the seeds they are negligible constituents of forages and will not be further discussed here.

There are five major FAs in forage plants (C16:0, C18:0, C18:1 n-9, C18:2 n-6 and C18:3 n-3), which comprise up to 95 % of their total fatty acids (TFA). The FAs associated with galactolipids contain high amounts of C18:2 n-6 and C18:3 n-3 (Van Soest, 1994). The chloroplast membranes are the most abundant membranes in green leaves, comprising up to 70 % of the lipids in green tissue. Thus, the galactolipids dominate the lipids in photosynthetic tissue (Taiz & Zeiger, 2002), hence are the leaves rich in C18:3 n-3 (between 60 % and 75 % of TFA), C18:2 n-6 and C16:0 (6% - 20 %), while C18:1 n-9 is a minor component (Hawke, 1973).

The galactolipids are highly important for the metabolism of the plant, which might explain why their composition is quite constant. C18:3 n-3 is the major FA of the galactolipids in grass (95 %), while C18:2 accounts for just 2-3 %. The concentration of galactolipids declines with age and varies with the proportion of leaves to stems and other metabolically active plant tissues (Van Soest, 1994).

In roots, the phospholipids dominate, with C16:0 and C18:2 n-6 as major FAs (Harwood, 1980).

1.4 Factors affecting FA concentrations in forage plants

FA concentrations and composition of forages are influenced by a multitude of factors, some of which are highlighted below. FA concentration varies over the season, and the seasonal effects are associated with changes not only in climatic variables, such as daylength, light intensity, and temperature, but also with physical properties of the sward (Elgersma *et al.*, 2005). The level of FA varies considerably during the season as the plants develop, mature, and senesce (Harwood, 1980). There are also variations in FA concentrations amongst species.

1.4.1 Stage of maturity

Several studies have shown declined FA concentrations with increasing maturity (Boufaïed *et al.*, 2003; Barta, 1975). Barta (1975) noticed reductions in TFA of more than 30 % in six grass species as the plants increased in maturity. This was confirmed by Boufaïed *et al.* (2003), who found reductions in C16:0, C16:1, C18:2 n-6, C18:3 n-3 and TFA with advancing maturity of timothy from stem elongation to early flowering. In addition, in an experiment conducted by Dewhurst *et al.* (2001), the highest levels of TFA concentrations in *Lolium* L. *spp.* (*L. perenne, L. multiflorum and L. boucheanum*) were noted when the grass was in vegetative growth stages.

A similar pattern of seasonal change in perennial ryegrass (*L. perenne*) was found by Gilliland *et al.* (2002).

There is some evidence indicating that the flowering process can also cause a reduction in FA concentrations (Dewhurst *et al.*, 2002a). Thus, a management program that prevents flowering should increase the FA concentration of the forage (Dewhurst *et al.*, 2003).

Changes in leaf:stem ratios can partly explain the declining proportions of FA as plants mature (Boufaïed *et al.*, 2003). The proportion of leaves to stems decreases with maturity in timothy (*Phleum pratense* L.) (Bélanger & McQueen, 1996) and stems have 50-70 % lower FA concentrations than leaves (Jarrige *et al.*, 1995, according to Boufaïed *et al.*, 2003). Dewhurst *et al.* (2003) also concluded that leaf content is a very important determinant of FA concentrations.

With this in mind, the FA concentration of the feed could be manipulated through management of the sward, through either grazing or cutting. Ctting at an early stage of development may result in a feed with higher FA concentration than later cutting.

1.4.2 Temperature

Exposure to chilling temperatures induces substantial changes in plants' membrane lipids, notably their degree of FA unsaturation and phospholipid contents increase during cold acclimatization. These changes enhance membrane fluidity, maintain membrane integrity, permit essential cross-membrane trafficking to continue, and thus minimize disruption to cellular functions (Uemura *et al.*, 1995). However, there has been little investigation of the effect of variations in temperature during the growing season in forage plants, and most of the temperature-related investigations that have been done to date have focused on the cold hardiness of plants.

Falcone *et al.* (2004) showed that FA concentrations in *Arabidopsis* are affected by changes in growth temperature. Following a reduction in temperature, from 36°C to 17°C, increases in concentrations of C16:3 and C18:3 n-3 were found while the concentrations of C16:1 and C18:2 n-6 decreased. Somewhat contradictory results were obtained by Kuiper (1970), who found that lucerne (*Medicago sativa* L.) grown at 15°C contained higher proportions of both C18:2 n-6 and C18:3 n-3 than plants grown at 30°C.

1.4.3 Light intensity

Light intensity is another factor that may affect the FA composition of forage plants, *inter alia* by influencing their chloroplast contents. For instance, Dewhurst & King (1998) found that shading grass with a black plastic sheet

for 24 hours prior to cutting resulted in reductions of the TFA contents and proportion of C18:3 n-3 in silage obtained from it.

In addition, Witkowska *et al.* (2008) found a positive relationship between FA concentrations and photosynthetic activity. However, the highest concentrations of TFA and proportions of C18:3 n-3 coincided with the lowest daily total radiation (because, they hypothesised, more N needs to be invested in the pigment-protein complexes embedded in thylakoid membranes to optimise radiation capture at lower light intensities). The relationship between N and TFA (described below), the presence of FA in the thylakoid membranes that host the photosynthetic pigment-protein complexes and the positive relationship between TFA and chlorophyll concentrations (Hawke, 1973) all indicate that changes in FA concentrations occur in parallel to changes in N distribution in response to changes in solar radiation (Witkowska *et al.*, 2008). Gregorini *et al.* (2008) found that concentrations of C18:1 n-9 increased from sunrise to sunset in meadow fescue and orchard grass (*Dactylis glomerata* L.). However, this increase in C18:1 n-9 did not result in any time-of-day effect in TFAs.

Some studies have also assessed effects of light on the lipid contents of pea (*Pisum sativum* L.) (Trémolières & Lepage, 1971) and barley (*Hordeum vulgare* L.) (Newman *et al.*, 1973; Grey *et al.*, 1967). Trémolières & Lepage (1971) discovered that pea sprouts grown in the dark had a different FA composition from those grown in the light. The longer the plants were exposed to light, the higher their PUFA contents. Gray *et al.* (1967) cultivated barley under three different light regimes (natural light, low light and darkness) for seven days. Plants grown in natural light contained higher proportions of C18:3 n-3 than plants grown in low light or darkness. Newman (1971) also concluded that leaves of barley grown in the dark had lower concentrations of C18:3 n-3 than plants grown with access to light.

1.4.4 Genetic variation

Differences in FA concentrations have been found both between families, species and cultivars. When harvested at the same stage of development (early heading and 10 % bloom for grasses and legumes, respectively), Boufaied *et al.* (2003) found significant differences both within species of the same family (grass or legumes) and between the two families. Legumes had higher concentrations of C14:0, C16:0, C18:0, C18:1 n-9, C18:2 n-2 and TFA and lower concentrations of C18:3 n-3, but large variations among species within each family were also observed.

In a study by Dewhurst *et al.* (2001) eight different forage grasses were compared under the same management regime. They found distinct

between-species differences in FA profiles of plants cut at the same date, *e.g.* cook's foot (*Dactylis glomerata* L.) had relatively low levels of C18:1n-9 and timothy had relatively high levels of C18:2 n-6, but there was also a significant interaction effect between species and cutting date (Dewhurst *et al.*, 2001). Evidence for genetic effects on FA contents has also been found within species. Notably, Elgersma *et al.* (2003b) found differences between cultivars of perennial ryegrass in concentrations of both individual FA and TFAs (which they were unable to relate to heading date, yield, DM concentration or leaf proportion). Loyola *et al.* (2002) also found differences in the CLA concentration of milk from cows grazing different ryegrass cultivars, despite strong similarities in the C18:2 n-6 and C18:3 n-3 acid concentrations of the grasses.

Gilliland *et al.* (2002) compared different diploid and tetraploid varieties of perennial ryegrass. Although the varieties with the highest C18:2 n-6 contents were diploids and the two with the lowest contents were tetraploids, the overall differences were not significant. However, there were significant between-variety and between-ploidy differences in the C18:3 n-3 concentration, which were higher in tetraploids than diploids. On the other hand, no effect of ploidy on FA contents of perennial ryegrass were found in analyses reported by Dewhurst *et al.* (2001).

The above mentioned results show that there are significant differences in individual and total FAs among species and cultivars, thus there is scope to increase FA contents through breeding. However, genetic differences in FA concentrations will probably be more pronounced in young growing plants, while flowering and senescence become increasingly important in the more mature grasses used to make conserved products such as silage (Dewhurst *et al.*, 2006). Further, strong effects of environmental factors, such as light, cutting interval, seasonal variations and fertilization regimes have also been detected in studies in which evidence of genetic effects has been found.

1.4.5 Fertilization

In grasses, N-fertilization has been found to increase FA concentrations in several species. Boufaïed *et al.* (2003) found that concentrations of palmitic acid (C16:0), C18:2 n-6, C18:3 n-3 and TFA were higher in timothy fertilized with 120 kg N/ha than in controls that received no N fertilization. In addition, Barta (1975) and Mayland *et al.* (1976) found positive correlations between N-fertilization and concentrations of TFA in a range of species. However, in contrast to Boufaïed *et al.* (2003), Mayland *et al.* (1976) observed no significant fertilization-related differences in the distribution of individual FAs. Boufaïed *et al.* (2003) also found that the decrease of certain

FAs (C16:1 and C18:3 n-3) with maturity occurred at a later growth stage when timothy was fertilized with N. In addition, Elgersma *et al.* (2005) found that higher N fertilization resulted in higher FA concentrations in perennial ryegrass, but the proportions of FAs were not affected. Witkowska *et al.* (2008) also studied perennial ryegrass and found a positive linear relationship between N concentrations and TFA in the plants.

Several studies have reportedly found phosphorus fertilization to have little apparent effect on the FA concentrations of grasses shown (Lee *et al.*, 2006; Boufaïed *et al.*, 2003; Barta, 1975).

1.4.6 Preservation

Oxidation during field wilting and biohydrogenation in the rumen have been identified as two of the main factors responsible for losses of herbage PUFA. In both cases lipolysis, mediated by the action of either plant or microbial lipases, is the first step. During field wilting plant lipases are the predominate agents, but there is also some evidence that plant lipases participate in rumen lipolysis (Dewhurst *et al.*, 2003). Ueda *et al.* (2002) found no differences in total FA concentration and composition between direct cut silage of legumes and the fresh material, but for lucerne hay and red clover (*Trifolium pratense* L.) haylage there were large decreases compared with fresh legumes in both TFA and C18:3 n-3 contents (Ueda *et al.*, 2002). Combined effects of wilting and ensiling also reduced the concentration of TFA (especially concentrations of C18:1 n-9 and C18:3 n-3) in perennial ryegrass examined by Elgersma *et al.* (2003a). The same authors compared effects of two regrowth intervals, and found greater losses of FAs during ensiling in grass with 33 days of regrowth than in grass with 23 days of regrowth (Elgersma *et al.*, 2003a). In addition, French *et al.* (2000) found that although grass and grass silage had similar FA profiles, the grass contained a lower proportion of SFAs and a higher proportion of unsaturated FAs than the silage.

Use of additives may also affect FA concentrations, but reported findings in this respect conflict. For instance, Dewhurst & King (1998) found that although additives had major effects on overall fermentation parameters, their effects on levels and proportions of FAs in grass silage made from perennial ryegrass were significant but relatively minor. In contrast, (Warren *et al.*, 2002) found that use of formic acid or bacterial inoculants decreased total FA concentrations in perennial ryegrass and red clover silages. Also, Boufaïed *et al.* (2003) found that use of a formic acid and bacterial inoculant resulted in declines in the concentrations of C18:3 n-3 and total FAs in grass silage and haylage made from timothy.

1.5 Feeding and utilization

The CLA found in milk fat and meat fat originate from two sources (Griinari & Bauman, 1999): ruminal biohydrogenation of C18:2 n-6 (Figure 4), and endogenous synthesis in the animal's tissues from *trans*-11 18:1 (Bauman *et al.*, 2000). In the rumen, bacteria are largely responsible for the biohydrogenation of unsaturated FAss (Harfoot & Hazlewood, 1997). Rumen bacteria have extremely high levels of PUFA biohydrogenation activity so the recovery of PUFAs in dairy products is very low (Dewhurst *et al.*, 2002b). The proportions of C18:2 n-6 and C18:3 n-3 generally biohydrogenated in dairy cows have been estimated to be ca. 80 and 92 %, respectively (Doreau & Ferlay, 1994). The bacteria are divided into two groups (A and B), based on the reactions and end-products of their biohydrogenation. The bacteria in group A hydrogenate C18:2 n-6 and C18:3 n-3 with *trans*-11 18:1 as their major end-product. Group B bacteria utilize the *trans*-11 18:1 as one of their main substrates and transform it into stearic acid (C18:0), which is their end-product (Kemp & Lander, 1984). The group B bacteria can also carry out the first hydrogenation step to some extent (Harfoot & Hazlewood, 1997). Since both group A and group B bacteria can undertake the first isomerization and hydrogenation steps, the final step has been found to be rate-limiting because of the small number of bacteria known to carry it out (Harfoot & Hazlewood, 1997).

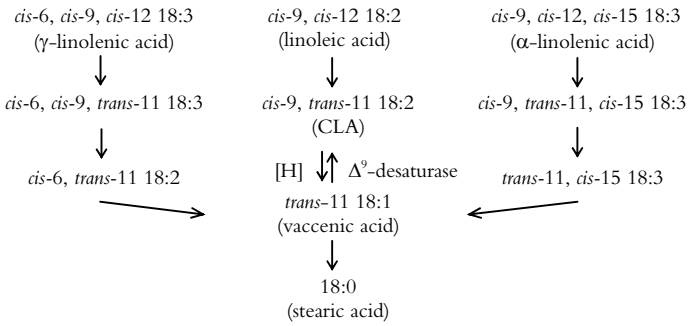

Figure 4. Pathways for ruminal biohydrogenation of PUFA (adapted from Griinari and Bauman, 1999).

As a consequence, *trans*-11 18:1 accumulates in the rumen (Keeney, 1970) and is, therefore, more available for absorption (Bauman *et al.*, 2000). Under certain conditions, especially when high concentrations of C18:2 n-6 are present in the feed, hydrogenation of *trans*-11 18:1 to form C18:0 seems to

be inhibited (Noble *et al.*, 1974), which enhances accumulation of *trans*-11 18:1 (Palmquist, 2001).

However, several factors affect rates of biohydrogenation, for instance they are reportedly lower in cows fed high-concentrate diets than in cows fed high-forage diets, apparently because of the associated reductions in ruminal pH (Kalscheur *et al.*, 1997). Hence, altering the forage:concentrate ratio may affect biohydrogenation parameters and, thus, the production of CLA and *trans*-11 18:1 (Griinari & Bauman, 1999).

The PUFAs that escape from the rumen are absorbed from the intestine into the circulation system, where they are transported to the mammary gland and adipose tissue and then used in the synthesis of triacylglycerols and phospholipids (Griinari & Bauman, 1999). Ruminants mammary glands and adipose tissues contain the enzyme Δ^9-desaturase (Bauman *et al.*, 2000; Griinari & Bauman, 1999), which introduces a *cis*-double bond between carbons 9 and 10 in FAss. The adipose tissue seems to be the major site of endogenous synthesis of CLA in growing animals, but in lactating ruminants the mammary gland is the main apparent site of endogenous synthesis of CLA (Kinsella, 1972; Bickerstaffe & Annison, 1970). It has been confirmed that CLA can be produced from *trans*-11 C18:1 in the mammary gland by the Δ^9-desaturase enzyme, as well as in the rumen through incomplete hydrogenation, since high levels of CLA have been found in milk from cows fed diets containing both low and high C18:2 n-6/C18:3 n-3 ratios (Lock & Garnsworthy, 2002).

1.6 Summary

Summarizing the above mentioned factors, one can conclude that FA concentrations and proportions in forage plants are influenced by a multitude of factors, and that management is a crucial determinant of the FA composition of forage. There may be wide variations in the FA profiles of ruminant products, since the plant genotypes, harvest times, cutting intervals, fertilization regimes and ensiling techniques applied to forage fed to the animals will all influence the FA composition of their milk and meat. Thus, quantifying the FA concentrations and proportions in forage plants and the influence of environmental factors on them would be extremely helpful for identifying management strategies that are likely to promote increases in the amounts of PUFA in forage plants and subsequently in ruminant products. However, many metabolic factors that affect the conversion of PUFA in the rumen remain to be identified.

2 Objectives

Timothy is the most important forage grass species in northern Sweden, often cultivated in mixtures with meadow fescue and red clover, but little is known about the factors affecting their FA concentrations and proportions. The overall aims of the studies this thesis is based upon were to describe and evaluate the variations of FA contents in these forage plants and their effects on FA concentrations in the milk of dairy cows fed on them. More specific aims were:

- To study the effects of different methods of handling and storing samples, in an attempt to identify the optimal approach for preparing them prior to analysis of the FA composition.
- To investigate the effects of wilting and additives on the FA composition of grass silage made from timothy.
- To evaluate the seasonal variation and the effects of N-fertilization on the FA concentrations in timothy and meadow fescue.
- To study the effects of crude protein (CP) and FA concentrations in silages on milk FA concentrations.

3 Material and methods

The crops in all the studies described in Papers I to IV were grown at Röbäcksdalen Research Centre, Swedish University of Agricultural Sciences, Umeå (63°45′N; 20°17′E). The materials used in the studies were timothy (*Phleum pretense* L., cv. Grindstad) (all papers), red clover (*Trifoulium pretense* L., cv. Betty) (Papers I and IV) and meadow fescue (*Festuca pratensis* Huds., cv Kasper) (Paper III). In all studies the crops were grown on a silt loam.

3.1 Paper I

Samples were taken from the first and second cuts of a first year ley, consisting mainly of timothy. In both cuts the grass was harvested when it was mainly in the sheath elongation stage. Two replicates for each pre-treatment were taken. The material was then used in two handling experiments.

3.1.1 Sampling and storage

Ten pre-treatments were included in the study, as described in Table 1. In order to stop biological activity as quickly as possible, treatments 1–3 and 10 were applied in the field. In treatments 6 and 7 the samples were wilted on a laboratory bench at room temperature, in treatments 5-7 the samples were cut after wilting or storage at 4°C, respectively, while in treatments 8 and 9 they were cut into 5 cm pieces before drying. The samples subjected to treatments 1-4 and 10 were not cut at all.

Table 1. *Pre-treatments used in the study described in Paper I.*

Pre-treatments	
1.	Frozen in liquid N: the samples were lowered into liquid nitrogen in plastic cans in which holes had been drilled to allow the liquid N to enter.
2.	Frozen in liquid N followed by dry ice: the samples were lowered into liquid N, as above, then after 5 min they were placed in a cooling box containing dry ice.
3.	Frozen with dry ice: the samples were put in plastic bags and placed in a cooling box containing dry ice.
4.	Frozen at -20°C: the samples were put in plastic bags and placed in a -20°C freezer.
5.	Stored at 4°C for 24 h, then cut into pieces (5 cm) and placed in a -20°C freezer.
6.	Wilted in the lab for 24 h, then cut into pieces (5 cm) and placed in a -20°C freezer.
7.	Wilted in the lab for 6 h, then cut into pieces (5 cm) and placed in a -20°C freezer.
8.	Dried at 60°C in a forced-air oven for 24 h: samples were cut into pieces (5 cm) and dried.
9.	Dried at 30°C in a forced air oven for 6 d: samples were cut into pieces (5 cm) and dried.
10.	Conserved with acid: samples were put in net bags and lowered into concentrated acid.

3.1.2 Freeze-drying and grinding

In a complementary study, first-cut samples that had been subjected to treatments 4 and 8 and samples of a second cut of red clover were subjected to the following further treatments to investigate the effects of grinding and freeze-drying. Samples dried at 60°C in an air-forced oven for 24 h were either analysed without further treatment or ground to pass through a 1 mm screen prior to analysis. Frozen (−20°C) samples were (1) cut with a paper cutter just before analysis, (2) freeze-dried or (3) freeze-dried and ground to pass through a 1 mm screen.

3.2 Paper II

A pure grass sward, consisting mainly of timothy, was used for sampling. Both the first and the second cuts were studied and the grass was mainly in the sheath elongation developmental stage when harvested. After cutting, the fresh material was either ensiled within 2 h or immediately placed on a tarpaulin for wilting to a DM content of 300–350 g/kg. Just prior to ensiling the grass was treated with an acidic additive, bacterial inoculant or water (control). The silos used were 1700 ml glass jars with screw tops, provided with water seals to release gas. The silos were stored in a dark room at 20°C for 92 days. To obtain reference data on the fresh material, samples were collected from both unwilted and wilted material just before treatment with

additives and ensiling. Part of this material was immediately frozen with liquid N and the rest was dried in an air-forced oven at 60°C for 24 h.

3.3 Paper III

First-year leys with pure timothy and pure meadow fescue was harvested at four different stages of maturity in both spring growth and summer growth periods. The plots for summer growth were harvested with a ley harvester at the third harvest time in the spring growth period. Three different N-fertilization regimes were applied in the experiment: 30, 90 and 120 kg N/ha for the spring growth and 30, 90 and 90 kg N/ha, respectively, for the summer growth (N-30, N-90 and N-120). N-90 plots were harvested on all eight occasions, while N-30 and N-120 plots were harvested on three occasions during the spring growth and twice during the summer growth periods (Table 2).

Table 2. *Harvest times for material subjected to each of the N-fertilization regimes*

	Spring growth				Summer growth			
	7 June	16 June	22 June	30 June	13 July	20 July	03 Aug	18 Aug
N-30	x	x	x		x		x	
N-90	x	x	x	x	x	x	x	x
N-120	x	x	x		x		x	

3.4 Paper IV

A 3-year-old grass ley, consisting mainly of timothy, was fertilized with N at three different rates (30, 90 and 120 kg N/ha) to produce three different grass silages (G-30, G-90 and G-120, respectively). Also included was a clover-grass crop (RC-G), consisting of red clover and timothy (60:40 on a DM basis). The silages were fed to 24 dairy cows in a change-over design with three periods, each four weeks long, and four dietary treatments. The cows were offered 11 kg DM of silage and 7 kg in total of two commercial standard concentrates. The milk yield of each cow was automatically measured at every milking, and samples for determining fat, protein, lactose, and urea were taken each week on two consecutive days (morning and evening). At the same time, samples for determining FA concentrations were taken in the two last weeks of each experimental period.

4 Summary of results

4.1 Paper I

There were no clear between-treatment differences in the determined FA proportions and significant interactions between treatments and cuts were detected. Freezing with liquid N was not necessary to obtain apparently reliable FA profiles of the samples examined. Furthermore, drying samples did not result in any significant differences in the relative proportions of the measured FAs.

In addition, there were no major differences in the FA proportions of either the grass or the clover samples subjected to the comparative series of freezing at -20°C, freeze-drying ± grinding or drying in an air-forced oven ± grinding. No significant differences between ground and non-ground samples were detected, except slightly higher proportions of C18:2 n-6 in the clover. Freeze-drying had some minor, but significant, effects on the FA proportions in clover samples. Simply freezing at -20°C resulted in higher propositions of C18:0 and C18:1 in the grass samples than the other treatments.

4.2 Paper II

All FAs investigated in the study were affected by cut, but no differences in the proportions of FAs appeared to be associated with either the degree of wilting or use of the different additives. However, the ensiling process did affect the FA proportions. After ensiling there were lower proportions of C16:0, C18:1 and C18:3 n-3 and higher proportions of C16:1, C18:2 n-6 and other identified FAs than in the fresh material.

4.3 Paper III

The harvest date had a strong influence on FA concentrations. Both individual and total FAs declined over time in both timothy and meadow fescue and during both spring and summer growth periods, irrespective of the N fertilization level. The proportions of all FAs, except C18:0, were also affected by the harvest date. There were no direct effects of N fertilization level on FA concentrations, but positive linear relationships were found between CP and the concentrations of individual (except C18:1 n-9) and total FAs for both grasses. Positive linear relationships were also found between crude fat and concentrations of individual (except C18:1 n-9) and total FAs. Differences in FA concentrations between the two grasses were found. Overall, meadow fescue contained higher concentrations of C16:0, C18:1 n-9 and C18:3 n-3, while the opposite was true for C18:0 and C18:2 n-6.

4.4 Paper IV

In the silages, the CP concentration increased with increases in the N fertilization level, being significantly higher in G-120 than in G-30, and intermediate in G-90. RC-G had the highest CP concentration, but not significantly higher than G-120. The concentrations of all individual FAs, except C16:0, and TFAs were differed between the silages. Feeding the G-30 and RC-G silages resulted in higher concentrations of C18:2 n-6 in milk than the other two. The highest levels of C18:3 n-3 and CLA were found in milk from cows offered the RC-G silage. Even though the intake of C18:2 n-6 was comparable and the intake of C18:3 n-3 was lower from the RC-G diet than from the G-120 diet, feeding the RC-G silage resulted in higher concentrations of both FAs in milk. The G-30 diet resulted in higher concentrations of C18:2 n-6 and the same concentration of C18:3 n-3 as the other grass silages, despite lower intake levels of these FAs. The apparent recoveries of C18:2 n-6 and C18:3 n-3 from feed to milk were 0.10 and 0.05, respectively, for the G-90 and G-120 silages, 0.12 and 0.06 for G-30, and 0.11 and 0.06, respectively, for the RC-G silage.

5 Discussion

The public has high awareness of the healthiness of many food products. Since dairy products are often associated with obesity and coronary heart disease, due to their high concentrations of SFA, it would be desirable to increase their concentrations of PUFA. Hence, it is important to be able to give farmers advice about appropriate forage crops, fertilization regimes, harvest times and preservation techniques in order to produce good quality silage (in terms of yield/ha, energy and CP concentrations *etc.*) with high concentrations of PUFA. Such advice must, of course, be locally relevant. Hence, more research on the effects of local conditions (climatic, environmental and financial) is required. We also need to give the farmers tools to predict the FA concentrations in forage.

In order to evaluate the FA concentrations of forage crops in the field it is important to use appropriate sampling and handling methods. Rapid freezing with dry ice or liquid N followed by storage at -20°C in an inert (N_2) atmosphere have been considered to be the best methods to preserve plant tissues (Christie, 1993). However, comparison of various techniques showed limited effects on FA proportions and out of ten handling methods tested, there were no indications that any of them were superior to the others (Paper I). Drying in an air-forced oven at 60°C did not result in any significant differences in the relative proportions of the measured FAs, which is in accordance with results presented by Fievez *et al.* (2004), who found no significant differences in total FA contents or FA composition between samples dried for 24 h (at 50°C) and fresh material. Since this was shown to be a sufficient method for preparing samples before analysis, FA contents could be examined in large numbers of samples, collected in diverse experiments that were not designed for FA analysis. On the other hand, wilting for 24 h, or even storage at 4°C overnight, did not result in numerically large differences in FA proportions compared to drying in an

air-forced oven. Even though these methods are not recommendable in research contexts they would enable farmers to take samples of their forage for FA analysis. Drying in an air-forced oven is also less labour demanding and a cheaper method compared to many other methods. However, one must be aware that different species and even different cultivars of the same species may respond differently to different treatments. For instance, results from a study by Chow *et al.* (2004) indicate that susceptibility to oxidation during field wilting is cultivar-dependent. The cited authors compared three cultivars of perennial ryegrass and found that wilting had no significant effect on the proportions of C18:3 n-3 in one cultivar, but it reduced the proportions in the other two. In addition, Van Ranst *et al.* (2009) found variations in losses of C18:3 n-3, despite similarities in wilting conditions (temperature, duration) and final DM. These results indicate that other characteristics or constituents, *e.g.* lipoxygenase activity and/or anti-oxidant concentrations, of forage plants can also influence lipid oxidation during wilting (Van Ranst *et al.*, 2009). Furthermore, there are likely to be significant interactive effects, for instance, relationships between lipoxygenase activity and temperature have been reported, and its activity is reportedly lower during chilling responses in chilling tolerant species than in chilling sensitive species (Kaniuga, 2008). From a practical perspective, wilting is preferable, since it minimizes losses of nutrients through effluents (Steen *et al.*, 1998). Accordingly, the FA concentrations and proportions of the timothy cultivar Grindstad, which was examined in the studies this thesis is based upon, was not affected by wilting (Paper II), but they were affected by the ensiling process (Paper II and values from the study paper IV is based upon [Study IV], Table 3).

Table 3. *Fatty acid proportions and concentrations in fresh and ensiled material reported in Paper II and obtained from Study IV*

	Paper II (g/100 g FA)		Study IV[1] (g/100 g FA)		Study IV[1] (g/kg DM)	
	Fresh	Silage	Fresh	Silage	Fresh	Silage
C16:0	16.94	15.67	17.11	13.48	2.50	2.22
C16:1	0.02	1.64	0.14	1.21	0.02	0.20
C18:0	1.32	1.26	1.40	1.10	0.20	0.18
C18:1	2.29	2.04	3.87	3.71	0.57	0.61
C18:2	16.35	16.98	16.85	16.74	2.46	2.75
C18:3	61.96	60.49	51.70	50.55	7.56	8.32

[1] Only G-90, i.e. grass fertilized with 90 kg N/ha, included

There were lower proportions of C16:0 and higher proportions of C16:1 after ensiling in the material examined in the study reported in Paper II and

Study IV and lower proportions of C18:0 after ensiling in the latter. Ensiling also resulted in changes in concentrations of individual FAs relative to the fresh material (Study IV), including reductions in concentrations of C16:0 and increases in concentrations of C16:1 and C18:2 n-6. These differences were, however, numerically small and would be unlikely to have any major effects on FA profiles of milk or meat of the ruminants consuming the forages as silages. In addition, there were no further significant changes in FA concentrations or proportions in the silages after ca. 30 weeks of ensilage (Study IV). Thus, since there were differences between fresh matter and silage, but no further changes during the 12-week study, the biggest changes appear to occur early in the ensiling process, after which FA contents seem to remain quite stable. Furthermore, only minor differences between fresh and ensiled material have been found by Dewhurst & King (1998), and Steele & Noble (1983), Chow *et al.* (2004) and Van Ranst *et al.* (2009) have all found no significant changes in the concentration of TFA during the ensiling process. In addition, Cone *et al.* (2008) found that TFA concentrations, and proportions, of individual FAs in grass silage did not change after opening and exposure to air up to 24 h. Thus, together with the finding that the choice of additive did not affect the FA proportions (Paper II), these results indicate that the prospects are good for farmers to produce good quality silage without losing essential FAs, despite local variations in practices. However, in some studies (Boufaïed *et al.*, 2003; Warren *et al.*, 2002) lower concentrations of TFA and C18:3 n-3 have been found in silages treated with formic acid and bacterial inoculant compared to no additive. A more limited fermentation observed with additives may reduce the loss of fermentable components and in-silo DM losses and hence cause reduced concentrations of TFA (Boufaïed *et al.*, 2003; Warren *et al.*, 2002). There were no significant differences between additive dosage of either the bacterial inoculant or the acid additive in the study by Boufaïed *et al.* (2003).

The highest concentrations of FAs were found early in the season, when the grass was still in the sheath elongation stage (Paper III), and the DM yield/ha was low. At normal harvest time for silage production, the TFA concentrations were about 50-60 % of those at the earliest harvest occasion during spring growth (Paper III). During summer growth reductions in FA concentrations were observed even though the grass remained in the sheath elongation stage for a longer period. Other studies have also observed a decline in FA concentrations in late summer despite an increase in leaf proportion (Elgersma *et al.*, 2004; Elgersma *et al.*, 2003b). Hence, there is a conflict between high concentrations of FAs and a high yield/ha.

Consequently, the leaf:stem ratio may play a more important role early in the season, while other factors such as light intensity and temperature are more important in late summer, even though the proportion of leaf biomass is high (Elgersma *et al.*, 2003b)

Differences in forage quality can affect rumen metabolism and there could be opportunities to optimize the composition of ruminant products by optimizing the choice of species and cultivar (Dewhurst *et al.*, 2006). There were overall differences in FA concentrations between timothy and meadow fescue. Meadow fescue contained higher concentrations of C16:0, C18:1 n-9 and C18:3 n-3, while timothy had higher C18:0 and C18:2 n-6 concentrations, but the concentration of TFA was the same in both grasses (Paper III). In Sweden, farmers usually cultivate these two grasses in mixtures, together with red clover. Silage made of red clover and timothy (60:40 on DM basis) contained higher concentrations of C18:0, similar or lower concentrations of C18:1 n-9 and C18:3 n-3, and similar or higher concentrations of C18:2 n-6, than the timothy silages (Paper IV). The total content of FAs was the same for all silages (Paper IV). However, despite the lower concentrations of C18:3 n-3 in the red clover/timothy silage it resulted in higher concentrations of both C18:3 n-3 and CLA in milk than the other silages. This was probably due to the action of polyphenol oxidase (PPO), an enzyme found in red clover that has been suggested to inhibit lipolysis in silage, leading to lower concentrations of free FAs in silages (Lee *et al.*, 2004). Hence, PPO activity can reduce the biohydrogenation of PUFA in the rumen through lipolysis in the silage and/or the rumen since lipolysis is a prerequisite for biohydrogenation. However, Van Ranst *et al.* (2009) examined three cultivars of red clover with significant differences in PPO activity, and found no link between differences in lipolysis and measured PPO activities. Higher recoveries of C18:2 n-6 and C18:3 n-3 from feed to milk were found for the G-30 silage than for the G-90 and G-120 silages, indicating that the G-30 diet in some way inhibited the biohydrogenation pathways, but not the other grass silages (Paper IV). The three grass silages were very similar in chemical composition and fermentation characteristics, so the cause of the higher recoveries needs to be investigated further.

Analysis of FAs is currently too expensive for routine practice. Therefore it is desirable to identify forage characteristics that enable its FA concentrations to be predicted. In this context, the strong linear relationships between FA concentrations and both crude fat and CP found in these studies may be of interest, especially since the variations amongst samples was greater for samples in the sheath elongation stage ("grazing stage") than

in more mature grass (Paper III). Hence, the relationships were not very strong for young grass, but when it was time to harvest for silage production the relationships between FAs and both crude fat and CP were strong. The relationships between FAs and crude fat contents were also strong when both grasses were included in the same regression analysis (as were the relationships between individual FAs and TFA). Since timothy and meadow fescue, as mentioned earlier, are often cultivated in mixtures in Sweden, these relationships could be used as possible tools to predict the FA concentrations in the forage.

However, it might not always be sufficient to analyze the forages to tell which would provide the best silages in terms of FA contents. In the study described in Paper IV, three very similar grass silages, in terms of energy concentration, neutral detergent fibre concentration and fermentation characteristics were examined. Even though, made from the same cultivar and grown on the same field, there were significant differences in FA contents of the milk of cows fed on them that cannot be related only to the differences in FA concentrations of the silages. Thus, other factors have to be identified in order to obtain robust predictions of milk FA concentrations from the FA concentrations in silage.

6 Conclusions

The timothy used in the studies this thesis is based upon seems to be a variety in which FAs were affected relatively little by drying and wilting. Hence, the prevailing method for handling samples before analysis, *i.e.* freeze-drying and grinding, was satisfactory, but drying samples in an air-forced oven was just as good, or even better in some cases. Drying in an air-forced oven is also less time consuming. In addition, the relatively short wilting had no effect on FA proportions in the resultant silage. The FAs were, however, affected by the ensiling process, even though the differences between the fresh material and the silages were numerically small.

FA concentrations declined in both timothy and meadow fescue over time in both spring and summer growth periods. There were positive linear relationships between both crude fat and CP and several individual and total FAs, which might be useful as tools to predict FA concentrations in the forage. Higher levels of N-fertilization led to higher concentrations of CP accompanied by higher concentrations of FAs in silage. However, these differences did not affect the concentrations of either protein or 18:3 n-3 in the milk of cows fed these grass silages, but including red clover in the cows' diet led to increased concentrations of C18:3 n-3 and CLA in their milk.

There are possibilities to improve the FA profile and concentration of forage through optimizing the fertilization regime, choice of species and harvest time but there are other factors that have to be identified in order to predict milk FA concentrations from FA concentrations in silage.

7 Future research

Since drying of samples has been shown to be an adequate method for preparing samples for FA analysis it would be possible to analyze FA contents of samples collected in diverse experiments, both field and feeding experiments, which were not specially designed to investigate FA concentrations. That would enable FA concentrations in a range of forage plant species, and varieties within species, to be assessed, and the effects of both climatic and management factors on their FA contents and profiles to be mapped. All of the samples that were ground in the studies this thesis is based upon, were ground just prior to analysis. The effect of storing ground samples was not examined, but it would be interesting to conduct such an investigation. It would also be valuable to find tools to predict the FA concentration of forages. The relationships between FAs and concentrations of CP and crude fat found in the studies reported in this thesis could be useful in this context, but this possibility requires further investigation.

There is also a need for more understanding of processes that occur in the rumen. The high recoveries of C18:2 n-6 and C18:3 n-3 from one of the grass silages investigated in these studies show that factors other than FAs influence the amounts of PUFA that escape from the rumen, and these factors warrant further study.

8 Populärvetenskaplig sammanfattning

Mjölk är en värdefull källa till energi, fett, protein och essentiella mineraler och vitaminer. I genomsnitt består mjölkfettet till 69 % av mättat fett, vilket gjort att den länge förknippats med bl.a. hjärt- och kärlsjukdomar. Dock innehåller mjölk även 27 % enkelomättat och 4 % fleromättat fett, som anses vara nyttiga beståndsdelar i vår kost och kan ha positiva effekter på vår hälsa. Omättade fettsyror av särskilt intresse är konjugerad linolsyra (CLA), vars positiva hälsoegenskaper har påvisats i en rad olika studier, samt α-linolensyra (C18:3, omega-3). Den sistnämnda räknas, tillsammans med linolsyra (C18:2, omega-6), som essentiell, vilket betyder att kroppen själv inte kan tillverka dem utan att de måste tillföras via kosten. Mjölk och kött från idisslare är bland de rikaste källorna till naturligt förekommande CLA och är även källa för omega-3 fettsyror. Ökade kunskaper om olika fettsyrors biologiska egenskaper, medför ett ökat intresse att påverka mjölkens fettsyrasammansättning för att på så vis förbättra dess hälsosamma effekter.

Vallfoder svarar för en stor del av de fettsyror en idisslare konsumerar. Koncentrationen av fettsyror i vallfoder varierar över säsongen och beror på faktorer som grödans utvecklingsstadium, genetiska faktorer och produktionsmetoder, t.ex. gödsling och konservering. Även temperatur och ljusintensitet under växtsäsongen har betydelse. Vallgräs innehåller de fleromättade linol- och α-linolensyrorna, den senare svarar för inte mindre än 55-75 % av växternas totala fettsyrainnehåll. Linolsyra och den mättade palmsyran (C16:0) bidrar med 6-20 % vardera och endast en mindre del består av stearin- och oljesyra (C18:0 respektive C18:1).

I norra Sverige är timotej och ängssvingel de viktigaste vallgräsen, och de samodlas ofta med rödklöver, men kunskapen om hur fettsyra-sammansättningen i dessa påverkas av olika faktorer är begränsad. Syftet med avhandlingen var därför att studera hur olika faktorer påverkar vallväxternas fettsyrasammansättning och hur den i sin tur påverkar fettsyras-

ammansättningen i mjölken. De fyra studierna genomfördes vid institutionen för norrländsk jordbruksvetenskap, Röbäcksdalen, SLU i Umeå.

I en första studie jämfördes tio olika metoder för hantering av växtprover innan analys av fettsyrasammansättning, bl.a. nedfrysning med flytande kväve direkt i fält, torkning i torkskåp i 60°C och frysning i -20°C. Även effekten av frystorkning och malning studerades. Ingen metod visade sig vara överlägsen de andra vilket betyder att torkning i torkskåp i 60°C, som är den enklaste metoden, var tillräckligt bra för att ge tillförlitliga analyssvar. Inte heller frystorkning eller malning påverkade fettsyrasammansättningen nämnvärt vilket tyder på att den gängse metoden att frystorka och mala proven innan analys är tillfredsställande.

För att undersöka effekten av förtorkning och ensilering studerades dels direktskördat och dels förtorkat (33-35 % torrsubstanshalt [ts]) material från både första och andra skörd av timotej. Två olika tillsatsmedel, ett syrapreparat (Proens™, Perstorp AB) och ett medel innehållande bakteriekultur (Siloferm® Plus, Medipharm AB) utvärderades. Vare sig förtorkning eller tillsatsmedel påverkade fettsyrasammansättningen i ensilaget. Däremot visade det sig att ensileringsprocessen i sig påverkade fettsyrorna, men skillnaderna mellan färskt och ensilerat material var numerärt små.

Hur koncentrationen av fettsyror varierar över växtsäsongen och hur de påverkas av kvävegödsling studerades i timotej och ängsvingel, som gödslats med tre olika kvävegivor (30+30, 90+90 eller 120-90 kg N/ha till första skörd respektive återväxt). Gräsen skördades vid olika mognadsstadier under både första skörd och återväxt. Koncentrationen av fettsyror sjönk med tiden i båda gräsen och under båda växtperioderna oavsett gödslingsnivå. De båda gräsen skilde sig åt på så vis att ängssvingel innehöll högre koncentrationer av palm-, olje- och α-linolensyra och lägre koncentration av stearin- och linolsyra. Den totala mängden fettsyror var emellertid densamma för de två gräsen.

Det fanns inget direkt samband mellan kvävegödsling och mängden fettsyror i gräsen, däremot fanns ett tydligt samband mellan råprotein-koncentrationen och innehållet av fettsyror. Det fanns även tydliga samband mellan mängden råfett och individuella fettsyror.

Slutligen genomfördes ett utfodringsförsök med mjölkkor där fyra olika ensilage jämfördes. Vi ville undersöka om det samband vi sett mellan råprotein och fettsyror kunde användas för att påverka fettsyra-sammansättningen i mjölken. Följaktligen gödslades en ren timotejvall även denna gång med 30, 90 och 120 kg N/ha till förstaskörd för att åstadkomma tre ensilage med olika råproteinkoncentration. Vi valde även att ta med ett

ensilage bestående av rödklöver och timotej (60 respektive 40 % på ts-basis), då klöver generellt innehåller mer råprotein än gräs. Högre kvävegiva resulterade i högre råproteinkoncentration och till viss del också högre koncentration av α-linolensyra i gräsensilagen. Dessa skillnader var dock utjämnade i mjölken, där inga sådana skillnader kunde ses. Däremot gav utfodring med klöver/gräsensilaget högre halter av både α-linolensyra och CLA i mjölken.

Från dessa resultat kan vi dra följande slutsatser:

- Förtorkning av grödan påverkade inte fettsyresammansättningen nämnvärt. Detta är positivt då förtorkning är att föredra ur ett praktiskt perspektiv eftersom man då minimerar problemet med att näringsämnen försvinner med pressvattnet.
- Fettsyrakoncentrationen i både timotej och ängssvingel sjönk med ökat mognadsstadium vilket gör att det uppstår en konflikt mellan hög avkastning/ha och högt innehåll av fettsyror. Högst andel fettsyror återfanns i gräs som var i ”betesstadium”.
- Det finns ett linjärt samband mellan koncentrationerna av råprotein och fettsyror, och även mellan råfett och fettsyror. Då det i dagsläget är för dyrt att rutinmässigt analysera fettsyrekoncentrationen i foder skulle halterna av råprotein och råfett kunna användas som indikatorer på hur mycket fettsyror det finns i grödan/ensilaget.
- Den ökade koncentrationen av α-linolensyra som återfanns i timotejensilaget till följd av högre råproteinkoncentration återfanns inte i mjölken. Däremot ökade halterna av både α-linolensyra och CLA i mjölken till följd av utfodring av rödklöver/timotejensilaget.

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