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The effects of feeding various forages on fatty acid composition of bovine milk fat: A review

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ABSTRACT: The nutritional image of bovine milk fat has suffered for years because of the association of saturated fatty acids and coronary heart disease. Thus the alteration of fatty acid composition has been a long-term strategy. Forages, even though containing a relatively low level of lipids, are the cheapest and often the major source of beneficial unsaturated fatty acids in ruminant diets. Recent progress in the research of factors affecting fatty acid content and composition in fresh and preserved forages and the associations between feeding such forages and milk fat profile are reviewed. Milk from cows grazed or fed fresh forage, especially from species-rich grasslands or forage legumes, has a considerably higher ratio of unsaturated to saturated fatty acids and a higher content of nutritionally beneficial *trans*-fatty acids (e.g. CLA, vaccenic acid) than milk from cows fed silage or hay. Grass and legume silages seem to affect the fatty acid profile more propitiously than maize silage.

Keywords: review; bovine milk fat; milk fat profile alteration; fatty acids; CLA; rumenic acid; vaccenic acid; pasture; fresh forage; silage; hay

List of abbreviations

ALA = α-linolenic acid
CLA = conjugated linoleic acids
DM = dry matter
FA(s) = fatty acid(s)
MUFA(s) = monounsaturated fatty acid(s)
PUFA(s) = polyunsaturated fatty acid(s)
SFA(s) = saturated fatty acid(s)
UFA(s) = unsaturated fatty acid(s)

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1. INTRODUCTION

There has been a considerable interest to alter the fatty acid (FA) composition of bovine milk with an overall aim to improve the long-term health of consumers.

The structural characteristics of the major saturated and *cis*-unsaturated FAs including their grouping are given in Table 1. Common names of the acids and the abbreviation ALA for α-linolenic acid will be used in the following text.

The predominant FAs in milk fat are saturated myristic, palmitic and stearic acids together with monounsaturated acids (MUFAs), among which oleic acid prevails. The usual proportion of polyunsaturated fatty acids (PUFAs), occurring preferably as linoleic, ALA and conjugated linoleic acids (CLA), is low. Important health targets include a reduction of medium-chain saturated FAs (SFAs; lauric, myristic and palmitic acids) connected with the cardiovascular risk, in favour of beneficial FAs, such as oleic acid reducing the risk. Some isomers of *trans*-unsaturated FAs with 18-carbon chain (CLA, vaccenic acid) have been shown to exert certain anticarcinogenic properties. Nevertheless, the altering of the saturated and unsaturated fatty acids (UFAs) ratio causes changes in oxidative stability of milk fat and of its sensorial properties such as butter texture (spreadability), whipping properties of cream etc. (Hillbrick and Augustin, 2002; Gonzalez et al., 2003; Henning et al., 2006). These aspects had to be also taken under consideration.

Fatty acid composition of bovine milk fat is affected by numerous factors which can be classified into three groups as animal, feed and environmental factors. Among animal (intrinsic) factors, the effects of breed, parity and stage of lactation have been widely studied. Further information has been available on environmental factors, such as season, farming system, herd management, milking frequency etc. (for reviews see Jensen, 2002; Samková et al., 2008). Within feed factors, forages and vari-

Table 1. Main saturated and *cis*-unsaturated fatty acids (FAs) occurring in forages and milk fat

Group and its abbreviation		Common name of acid	Designation[1]	Double bond position(s)[2]
Saturated	SFA	butyric	C4:0	–
		caproic	C6:0	–
		caprylic	C8:0	–
		capric	C10:0	–
		lauric	C12:0	–
		myristic	C14:0	–
		palmitic	C16:0	–
		stearic	C18:0	
Monounsaturated	MUFA	palmitoleic	C16:1n-7	9
		oleic	C18:1n-9	9
Polyunsaturated (with two and more double bonds)	PUFA			
n-6 family		linoleic	C18:2n-6	9,12
		γ-linolenic	C18:3n-6	6,9,12
		arachidonic	C20:4n-6	5,8,11,14
n-3 family		α-linolenic (ALA)	C18:3n-3	9,12,15
		stearidonic	C18:4n-3	6,9,12,15

[1] CX:Yn-Z X = number of carbons; Y = number of double bonds; Z = position of the first double bond numbered from the methyl end of the carbon chain (ω-Z has also been used frequently)

[2] positions of double bonds numbered from carboxylic group with number 1

ous types of rumen protected or unprotected fat supplements are the main sources of FAs (for reviews on supplemented fats see Dewhurst et al., 2003c; Chilliard and Ferlay, 2004; Dewhurst et al., 2006; Woods and Fearon, 2009).

Forages, either fresh or preserved, have been the staple component in different feeding systems. Thus, recent data on the effects of forages on bovine milk fat composition will be reviewed.

2. MAJOR FATTY ACIDS IN BOVINE MILK FAT

Among hundreds of FAs detected in bovine milk fat, only a limited number affects nutritional, sensorial and technological properties. The main FAs have straight chain and even number of carbons. Odd- and branched-chain FAs occur in bovine milk fat as minor components.

Comprehensive data on bovine milk lipids are available from a review of Jensen (2002). Among them, typical proportions of 69, 27 and 4% of SFAs, MUFAs and PUFAs in total FAs, respectively, were reported.

Melting temperature is considerably higher in SFAs (from –7.9 to 69.6°C for butyric acid and stearic acid, respectively) than in unsaturated acids (16.0, –5.0 and –10.6°C for oleic, linoleic and ALA acids, respectively) (Velíšek and Hajšlová, 2009). Thus, the texture of milk fat is largely affected by the FA composition.

Fatty acids in milk fat are mostly bound with glycerol preferably as various triglycerides. The content of free FAs is very limited in milk, which did not fall lipolytic or oxidative processes due to inappropriate storage conditions or due to a mechanical stress during milking and transport (Hanuš et al., 2008). The released free FAs, mostly C4–C12, participate in undesirable aroma of milk and milk products, mainly of butter.

The proportion of various *trans*-unsaturated FAs in bovine milk fat varies between 2% and 8% of total FAs. Among them, the isomeric acids C18:1 with different position of double bond are common. *Trans*-vaccenic acid (C18:1 acid with double bond between carbons 11 and 12) is present in the highest proportion. The great interest has been focused on conjugated linoleic acids, designated commonly as CLA (in total, 28 positional and geometric isomers). Rumenic acid (9-*cis*, 11-*trans*-C18:2 as being frequently reported), the most abundant isomer, and 10-*trans*, 12-*cis*-C18:2 acid were proved to have anticarcinogenic effects in a range of human cell lines and animal models. For more information on rumenic acid see Kramer et al. (1998).

In a study from France (Ledoux and Laloux, 2008), the mean annual content of rumenic acid was 0.59% in milk fat, ranging between 0.38% and 0.96% in winter and summer, respectively. Moreover, differences in rumenic acid content between regions were observed.

The nutritional image of milk fat has suffered for years because of the association of saturated fat, cholesterol and *trans*-fatty acids with coronary heart disease. Nevertheless, such association does not apply to *trans*-fatty acids of animal origin. These acids are mainly vaccenic and rumenic acids that may possess antiatherogenic properties. Moreover, rumenic acid has probably a role in the prevention of mammary tumorigenesis (for detailed information see comprehensive reviews of Parodi (2004) and Dhiman et al. (2005)).

3. FATTY ACIDS IN FRESH AND PRESERVED FORAGES

3.1 Fresh forages

The usual total FA content in different forages is in the range of 20–50 g/kg dry matter (DM). This is a relatively low level, however, forages have often been the major and also the cheapest and safest source of FAs in ruminant diets.

Fatty acid content and composition are affected by numerous factors such as plant species and variety, climate, day length, rainfall, fertilisation and stage of growth.

The highest level is in young plants at the first cut, then it decreases during summer, particularly around flowering. Such level and trends were reported by Dewhurst et al. (2001, 2003c), Elgersma et al. (2003, 2005), Clapham et al. (2005) and Van Ranst (2009a). Information on a recovery during autumn was presented in some papers (e.g. Witkowska et al., 2008). On the contrary, Boufaied et al. (2003a) observed a higher content of linoleic, ALA and total fatty acids in cocksfoot (*Dactylis glomerata*) and timothy (*Phleum pratense*) in summer regrowth than in spring cut. Similarly, Lee et al. (2006) reported a higher concentration of ALA in cocksfoot, timothy and red clover (*Trifolium pra-tense*) in summer regrowth, while there were

Table 2. Mean content of major fatty acids (g/kg dry matter) in fresh forages (mostly cut in July)

Forage	Fatty acid						Country	Reference
	total	palmitic	stearic	oleic	linoleic	α-linolenic		
Cocksfoot/*Dactylis glomerata*	19.67	3.91	0.92	0.45	2.85	10.56	Wales	Dewhurst et al. (2001)
	37.51	4.38	0.48	0.45	3.98	26.70	USA	Dierking et al. (2010)
Tall fescue/*Festuca arundinacea*	21.65	4.21	1.06	0.96	2.55	11.98	Wales	Dewhurst et al. (2001)
	39.28	4.46	0.53	0.61	3.56	28.40	USA	Dierking et al. (2010)
Meadow fescue/*Festuca pratensis*	20.61	4.09	0.99	1.04	2.74	10.95	Wales	Dewhurst et al. (2001)
Italian ryegrass/*Lolium multiflorum*	14.66	3.05	0.94	0.84	2.26	6.94	Wales	Dewhurst et al. (2001)
Perennial ryegrass/*Lolium perenne*	21.82	4.30	1.01	1.24	2.90	11.42	Wales	Dewhurst et al. (2001)
	21.70	3.43	0.29	0.39	2.61	14.0	Belgium	Van Ranst et al. (2009a)
	44.40	5.07	0.65	0.63	3.89	31.87	USA	Dierking et al. (2010)
Festulolium spp.	20.39	3.96	0.87	1.22	3.04	10.39	Wales	Dewhurst et al. (2001)
Timothy/*Phleum pratense*	20.77	4.05	1.05	1.05	3.18	10.43	Wales	Dewhurst et al. (2001)
Red clover/*Trifolium pratense*	30.24	5.89	1.15	1.07	6.47	11.73	Finland	Vanhatalo et al. (2007)
	24.10	3.53	0.43	0.44	5.07	13.30	UK	Lee et al. (2009)
	29.40	4.17	0.62	0.79	5.21	17.70	Belgium	Van Ranst et al. (2009a)
White clover/*Trifolium repens*	28.80	4.56	0.63	0.93	5.43	16.40	Belgium	Van Ranst et al. (2009a)
Lucerne/*Medicago sativa*	38.79	5.66	0.74	0.71	6.22	24.79	USA	Dierking et al. (2010)

comparable levels in lucerne (*Medicago sativa*) from both cuts. A progressive increase in total FAs and in the proportion of ALA was observed in the stay-green trait of perennial ryegrass (*Lolium perenne*) from early to late season (Dewhurst et al., 2002).

Studying changes in the content of three UFAs (oleic, linoleic and ALA acids), Mir et al. (2006) reported a higher availability in young plants of cocksfoot and perennial ryegrass as compared with tall fescue (*Festuca arundinacea*). However, dry matter yields of both former grasses were too low in that period.

As results from data in Table 2, ALA is the prevailing acid with the usual proportion of about 50–60% in total FAs, followed by palmitic and linoleic acids. Red clover and white clover (*Trifolium repens*) seem to have a higher total level of FAs than grasses. Low content of ALA of about 6 g/kg DM was determined in lucerne (Boufaied et al., 2003a; Lee et al., 2006). The proportion of ALA 64.7, 56.3 and 53.3% in total FAs decreased in the order red clover, white clover and lucerne, respectively (Wiking et al., 2010). In other forage legumes, common vetch (*Vicia sativa*), hairy vetch (*V. villosa*), crimson clover (*Trifolium incarnatum*) and Egyptian clover (*T. alexandrinum*), FA profiles were affected mainly by forage species and phenological stage, however, numerous interactions among these factors occurred. Linoleic acid content and proportion increased considerably from vegetative to reproductive stage, whereas ALA decreased to a lower extent. During this period, the ratio of SFAs: UFAs increased (Cabiddu et al., 2009).

Also in sunflower (*Helianthus annuus*) forage PUFAs accounted for 81–75% of total FAs during the growth cycle. Major FAs were ALA, linoleic, palmitic and stearidonic acids with 54.9–44.6, 16.4–21.8, 10.0–12.8 and 6.5–8.8% in total FAs, respectively (Peiretti and Meineri, 2010).

Nitrogen fertilisation (120 vs. 0 kg/ha) increased the content of ALA in timothy at all tested maturity stages, while phosphorus (45 vs. 0 kg/ha) had no significant effect (Boufaied et al., 2003a; Lee et al., 2006). In a report of Elgersma et al. (2005), nitrogen application (45 or 100 vs. 0 kg/ha) significantly increased the content of all five determined FAs (palmitic, palmitoleic, oleic, linoleic and ALA) in perennial ryegrass, however, the composition of the acids was not affected. Moreover, a strong positive linear correlation was found between the content of total FAs or ALA with the crude protein content of herbage. The highest FA contents were determined in perennial ryegrass which was applied high rates of nitrogen and the herbage of which was harvested after short regrowth intervals (Witkowska et al., 2008).

According to Dewhurst et al. (2003c), forage breeding to increase the supply of beneficial FA from plants into ruminant products is an important long-term strategy. However, the situation is complicated by the large genotype × management interactions, as was recently reported by Palladino et al. (2009) for perennial ryegrass.

3.2 Changes during wilting and drying

Forage wilting prior to ensiling and drying for hay production have been widely used technological operations. Mechanical damage to plant tissues combined with air access cause extensive oxidation of PUFAs. The processes are started by lipolysis catalysed by plant lipases. The PUFAs released from membranes are then oxidised by air oxygen under the catalysis with lipoxygenases. Many of the products participate in emissions of volatile organic compounds.

Higher oxidative losses of ALA in hay as compared with silage of Italian ryegrass (*Lolium multiflorum*) were reported by Aii et al. (1988). Similar considerable losses of both total FAs and a decrease in ALA were caused by an extended wilting of perennial ryegrass for 68 h prior to ensiling. Shading of the stand by a black plastic sheet for 24 h before the cut had a less dramatic effect (Dewhurst and King, 1998). The detrimental effects of prolonged wilting and field drying on the content of both total FAs and PUFAs were proved by further studies (Boufaied et al., 2003a; Shingfield et al., 2005b; Lee et al., 2006; Van Ranst et al., 2009a,b). Nevertheless, in a report of Arvidsson et al. (2009), wilting of timothy shorter than 24 h did not affect the FA composition.

Plant lipids are mainly associated with the thylakoid membranes of chloroplasts. An alternative strategy for reducing losses is to produce more resilient chloroplasts, e.g. “stay-green” varieties. Only a small reduction in losses of FAs during wilting for 48 h was found in stay-green perennial ryegrass as compared with normal herbage (Dewhurst et al., 2002).

Overall data on the FA composition in hay and silage are given in Table 3.

Table 3. Total content (g/kg dry matter) and composition (g/100 g total fatty acids) of major fatty acids in hay and silage

Forage	Fatty acid						Country	Reference
	total	palmitic	stearic	oleic	linoleic	α-linolenic		
Hay								
Cocksfoot	1.8	24.1	2.8	3.4	15.5	35.0	France	Ferlay et al. (2006)
	–	23.7	4.5	4.4	16.8	27.4	Australia	Kitessa et al. (2004)
Lucerne	–	37.1	6.6	3.1	10.1	0.4	USA	Whitlock et al. (2006)
	1.1	30.0	6.0	8.0	24.4	23.2	USA	AbuGhazaleh et al. (2007)
Mountain grassland	3.0	19.2	1.5	2.3	16.2	50.4	France	Ferlay et al. (2006)
Perennial ryegrass	3.2	15.8	1.8	2.0	14.0	55.9	France	Ferlay et al. (2006)
Silage								
	4.0	15.6	2.4	23.7	48.6	3.4	France	Ferlay et al. (2006)
Maize	–	29.5	3.5	4.0	18.7	4.9	USA	Whitlock et al. (2006)
	1.2	16.6	2.9	18.8	48.5	11.1	USA	AbuGhazaleh et al. (2007)
Perennial ryegrass	3.7	21.2	2.0	2.8	13.4	52.2	France	Ferlay et al. (2006)

3.3 Changes during forage ensiling and silage feed out period

Extensive lipolysis of membrane lipids occurs during ensiling. The mean lipolysis level of 90.3, 86.4 and 85.7% in perennial ryegrass, red clover and white clover, respectively, was reported by Van Ranst et al. (2009a). The extent of lipolysis was affected by cutting date, species and to some extent by cultivar. In red clover, lipolysis is partially reduced by the enzyme polyphenol oxidase through deactivation of lipolytic enzymes and/or through formation of protein-phenol-lipid complexes (Lee et al., 2004, 2008).

The application of formic acid at ensiling of red clover, white clover and perennial ryegrass resulted in lower lipolysis as compared with a commercial inoculant of lactic acid bacteria or carbonate buffer (Van Ranst et al., 2009b).

As compared with prolonged wilting, the application of additives seems to have only a limited effect on changes in the FA composition. It was reported for formic acid (Dewhurst and King, 1998; Boufaied et al., 2003b), its mixture with propionic acid (Arvidsson et al., 2009) and lactic acid bacteria inoculants (Boufaied et al., 2003b; Lee et al., 2008; Arvidsson et al., 2009) used for ensiling of perennial ryegrass, timothy, red clover or white clover.

The anaerobic reductive environment of silage is changed after silo opening. Free fatty acids are exposed to air and light and their oxidisation is induced. In a study of Khan et al. (2009), maize silages of various dry matter and grass silages of various quality were exposed to air for up to 24 h. In maize silages, a significant decrease in ALA, linoleic acid, oleic acid and in total FA contents was determined after 24-h exposure. The relative drop in total FAs decreased with increasing dry matter content. Similarly, a decrease in ALA and linoleic acid and in total FA contents was observed in grass silages.

Thus, both forage wilting and silage aeration during the feeding out period increase losses of UFAs and total FAs. The majority of the FAs occur as free fatty acids in silage as opposed to fresh herbage.

4. CHANGES OF DIETARY LIPIDS IN DAIRY COW'S ORGANISM

All milk FAs are either derived, almost equally, directly from preformed FA in the diet or are produced by *de novo* synthesis. Fatty acids with the carbon chain length C4–C14 and about a half of C16 are formed *de novo*, while the second half of C16 and FAs with longer chains originate from feed and body adipose tissues. It is hypothesised that the transcription of enzymes involved in milk fat synthesis is affected by grazing fresh forage rich in legumes, containing large concentrations of PUFAs (Wiking et al., 2010).

The digestive tract exerts a profound influence on the fate of dietary FAs. The short-chain free SFAs are absorbed through the walls of the rumen or abomasum into the bloodstream. The medium- and longer-chain SFA pass into the small intestine, diffuse across the membrane wall where they are incorporated into lipoproteins and enter the bloodstream via the lymphatic system. The majority of UFAs are extensively biohydrogenated in the rumen (Mansbridge and Blake, 1997; Bauman et al., 2006).

Dietary lipids entering the rumen are mainly triglycerides and in a lower proportion phospholipids and galactolipids. The initial transformation step is lipolysis releasing free FAs. Lipolysis is considered to be rate-limiting for biohydrogenation. Free linoleic acid and ALA are then saturated via CLA or conjugated linolenic acids to stearic acid. The saturation pathway in biohydrogenation is carried out almost exclusively by rumen bacteria, while the role of protozoa and anaerobic rumen fungi is limited (for more details see reviews of Chilliard et al., 2007; Jenkins et al., 2008 and Kim et al., 2009). Within CLA, geometric isomers 10,12-CLA are synthesised by a mechanism that differs from the synthesis of 9,11-isomers (Wallace et al., 2007).

A varying proportion of PUFAs, ranging usually between 2 and 20%, is recovered in milk as vaccenic acid, an important product of biohydrogenation of both linoleic acid and ALA. There is a strong relationship between the contents of rumenic and vaccenic acids in bovine milk fat, with the content of vaccenic acid being 2–2.5 times higher than that of rumenic acid (Elgersma et al., 2006).

The extent of structural plant lipid lipolysis is lower than that of supplemented unprotected oils due to the need to remove surrounding cellular matrices before lipolysis can occur. According to the meta-analysis of Glasser et al. (2008), ruminal pH, forage concentrate ratio, level of intake and fish oil supplementation are the main factors influencing the level of biohydrogenation. Increased biohydrogenation occurs at a high proportion of forage in the diet, causing a negative effect on the flow of linoleic acid and ALA to the duodenum.

A beneficial increase in CLA and n-3 PUFAs in milk fat is reported at grazing species-rich grasslands as compared with intensively managed swards, zero-grazed or silage-fed cows (e.g. Lock and Garnsworthy, 2003; Lourenço et al., 2005; Frelich et al., 2009; Mohammed et al., 2009). Species-rich forages probably affect the ruminal microbial population or the activity of lipases and hence reduce the extent of biohydrogenation. The inhibition activity is associated with polyphenol oxidase (in red clover; for an overview see Kim et al., 2009) or some secondary plant metabolites, namely essential oils, saponins, polyphenols and catecholamines (for a review see Lourenço et al., 2008).

Forage legumes were reported to show a higher transfer efficiency of PUFA to bovine milk fat in comparison with grasses. For instance, although the biohydrogenation of ALA was still high for red clover silage (86.1% compared with 94.3% for grass silage), there was a 240% increase in the proportion of ALA passing through the rumen (Dewhurst et al., 2003b). Limited lipolysis and biohydrogenation are attributed, among others, to the content of various polyphenols. As reported Van Ranst et al. (2010), red clover silages showed lower biohydrogenation in the rumen as compared with fresh or wilted forages. This suggests that lipids in ensiled red clover were more protected.

Cabiddu et al. (2010) compared in an *in vitro* study the effects of freeze-dried crimson clover and common vetch, both at vegetative and reproductive stages, on lipolysis and PUFAs biohydrogenation in the rumen. Vetch was characterised by a higher level of tannic polyphenols than clover in both stages, whereas bound phenol levels were low in both forages. Both types of phenols had a negative effect on lipolysis and biohydrogenation, but tannic phenols were more effective than bound phenols.

More future data on the fate of chloroplast lipids (released or intact) within the rumen could improve the understanding of the processes.

5. ASSOCIATIONS BETWEEN FEEDING OF VARIOUS FORAGES AND FATTY ACID COMPOSITION OF MILK FAT

The nature of forages consumed by dairy cows has a large effect on both nutritional and sensorial characteristics of milk and dairy products.

Dairy cows on herbage-based diets derive FAs for milk fat synthesis from the diet and rumen microorganisms (400–450 g/kg), from adipose tissues (< 100 g/kg), and from *de novo* biosynthesis in the mammary gland (about 500 g/kg). The relative contributions of these FA sources to milk fat production are highly dependent upon feed intake, diet composition, and stage of lactation. High intake of starch is associated with a higher level of *de novo* synthesis resulting in more saturated milk fat. In contrast, higher intakes of PUFAs from pasture result in higher concentrations of oleic acid, vaccenic acid and CLA in milk fat (Walker et al., 2004).

It is not easy to categorise literature data by the individual types of forages, as the effects of different diets were compared in virtually all experiments.

5.1 Effects of season, different nutrition and management systems

In temperate and cold regions, summer feeding has usually been based on pasture or green fodder for housed dairy cows, while preserved forages have been fed during the winter season. Thus, large seasonal variations exist in the content of the main bovine milk components and in FA composition. It was demonstrated in a comprehensive year-round study in the Netherlands (Heck et al., 2009) by weekly sampling of bulk milk. The proportion of ALA, vaccenic, rumenic and oleic acids was highest during the summer grazing period and lowest during the winter housing period. The changes were continuous, probably because of the variability of both summer and winter diets. In the grazing period, grass quality and supply vary and the intake of FAs need not be stable during this period. Surprisingly, comparing data from this study in 2005 with a previous investigation in 1992 carried out under the same methodology, a considerable increase in SFAs was observed.

The more beneficial FA composition during summer months in temperate regions was reported also in other studies (e.g. Lock and Garnsworthy, 2003; Ferlay et al., 2008; Frelich et al., 2009).

A low-input feeding system based on grazing increases the proportion of beneficial FAs, however, daily milk yields are lower (Frelich et al., 2009; Slots et al., 2009). Energy deficient diet decreased milk production and protein level whereas fat content increased as did the proportion of oleic acid and the sum of n-3 FAs (Morel et al., 2010).

Differences in the FA composition between organic and conventional milks result from different feeding management. Bulk milk samples from 19 conventional and 17 organic dairy farms located in north-west England and in Wales were collected during a 12-month period. Organic dairy farms produced milk with higher PUFA content, particularly n-3 PUFAs, and lower MUFAs content (Ellis et al., 2006). In a Dutch study of raw milk at the end of the winter housing period, organically managed cows were fed grass-clover silage and hay as compared with maize silage and concentrates on conventional farms. The proportions of CLA and n-3 FAs were higher in organic milk (Bloksma et al., 2008).

Minor odd- and branched FAs in milk fat are interesting for a better understanding of rumen processes and also due to their effects on cancer cells and on milk fat texture. Their contents, mainly of iso-C14:0 and iso-C15:0 acids, were elevated in milk from cows with an increasing proportion of dietary grass or grass silage. Maize silage feeding increased the level of C17:0 and 9-*cis*-C17:1 acids (for more information see a review of Vlaeminck et al., 2006).

5.2 Effects of grazing and green forage feeding

As reviewed by Martin et al. (2009), numerous papers reported considerable differences in the sensorial and nutritional characteristics of milk and dairy products from cows grazed, especially in spring, or fed hay or grass silage.

Data on the content of selected FAs in milk fat of cows either grazed or fed fresh forages are given in Table 4. However, such data should be assessed with caution, because of numerous further factors affecting the fatty acid profile of milk fat. As can be deduced from Tables 4 and 5, green fodder causes a decrease in the proportion of SFAs, mainly of palmitic acid, and an increase in vaccenic and rumenic acid content as compared with silage feeding. Nevertheless, the proportion of linoleic acid seems to be higher in the milk fat of cows fed maize silage than of grazed cows.

A negligible effect of grazing vs. barn feeding freshly cut grass sward was reported by Leiber et al. (2005).

Couvreur et al. (2006) tested four isoenergetic diets with an increasing proportion of fresh perennial ryegrass to replace maize silage (0, 30, 60 and 100% dry matter of forage). The increasing proportion of fresh grass in the diet induced a linear decrease in milk fat content and a linear increase in UFA

Table 4. The mean proportion of selected fatty acids (g/100 g of total fatty acids) and the ratio of saturated to unsaturated fatty acids (S/U) in milk fat from cows grazed or fed fresh herbage (adapted from Samková et al., 2008)

Botanical composition[1]	Fatty acid						S/U	Reference
	palmitic	oleic	vaccenic	linoleic	α-linolenic	rumenic		
Perennial ryegrass (76), weeds (17)	24.0	21.3	2.89	0.58	0.90	1.21	2.09	Wijesundera et al. (2003)
Perennial ryegrass (51), white clover (19), weeds (19)	27.5	19.9	3.54	0.75	0.65	1.44	2.11	Wijesundera et al. (2003)
Perennial ryegrass (68), *Poaceae* spp. (28), white clover (2), weeds (3)	27.6	21.6	3.93	0.73	0.95	1.84	1.80	Kay et al. (2005)
Perennial ryegrass, white clover	24.1	21.1[2]	4.70	1.26	0.70	1.65	1.81	Couvreur et al. (2006)
Perennial ryegrass (75), white clover (4), *Poaceae* spp. (2)	26.5	18.4	4.16	1.28	1.05	1.87	1.89	Kay et al. (2007)
Perennial ryegrass (76), smooth meadow-grass/*Poa pratensis* (14), white clover (5), weeds (5)	26.0	23.9	3.06	1.05	0.63	1.43	1.69	Rego et al. (2008)
Perennial ryegrass (60), white clover (40)	26.8	22.2	2.72[3]	1.59	1.09	1.27	1.74	Van Dorland et al. (2008)
Perennial ryegrass (60), red clover (40)	28.0	20.0	3.57[3]	1.46	0.92	1.23	1.95	Van Dorland et al. (2008)
Poaceae spp. (77), clovers (6) (19 species)	25.3	18.3	4.01[3]	0.94	0.70	1.71[4]	2.19	Leiber et al. (2005)
Poaceae spp. (36), clovers (23) (71 species)	25.4	24.1	3.12[3]	1.57	1.15	1.34[4]	1.65	Leiber et al. (2005)
Poaceae spp. (50) (32 species)	26.2	22.9	2.72	1.16	0.84	1.26	2.01	Ferlay et al. (2006)
Smooth bromegrass/*Bromus inermis* (50), cocksfoot (33), smooth meadow-grass (7)	23.8	31.9	3.37	2.35	1.14	1.35	1.18	Bargo et al. (2006)
Lucerne (50), red clover (20), fescue (20), weeds (10)	23.3	25.4	3.39	1.93	0.59	1.12	1.34	Flowers et al. (2008)

[1] numbers in brackets mean the weight percentage of a species (in dry matter except Leiber et al., 2005); [2] sum of 9-*cis* and 13-*trans*-C18:1; [3] sum of 10-*trans* and 11-*trans*-C18:1; [4] sum of 9-*cis*, 11-*trans*, 8-*trans*, 10-*cis* and 7-*trans*, 9-*cis*-C18:2

percentages at the expense of SFAs. The respective relationships were +0.38, +0.12, +0.05 and –0.69 points per 10% of fresh grass in the diet for vaccenic, rumenic, ALA and palmitic acid.

Grazing or feeding of floristically diversified swards results in the production of milk fat richer in ALA and CLA (Martin et al., 2009), e.g. in Alpine milk (Leiber et al., 2005; Chion et al., 2010). The increased content and proportion of CLA and vaccenic acid were observed in milk fat from high altitudes (above 900 m) as compared to that from upland (600–650 m). This indicates increased biohydrogenation in the rumen probably due to the high content of PUFAs in some fodder species, such as *Leontodon hispidus*, birdsfoot trefoil (*Lotus corniculatus* and *alpina*) and red clover (Collomb et al., 2002). Only three CLA isomers (rumenic acid, *trans*-11, *cis*-13 and *trans*-8, c*is*-10), particularly the isomer *trans*-11, *cis*-13, in milk fat showed a linear increase with elevated pasture altitudes (between 600 and 2 120 m) (Collomb et al., 2004). Changes in stearic, linoleic, vaccenic acids and CLA were reported also during transhumance of cows among Alpine pastures (1 400–2 200 m) in the grazing period (De Noni and Battelli, 2008).

Proportions of myristic and palmitic acids were significantly lower and those of stearic, oleic, linoleic and ALA acids were higher in the milk fat of cows fed fresh lucerne compared with those fed lucerne silage (Whiting et al., 2004).

Transition from a fresh grass diet on pasture to a winter diet of mixed grass/maize silage altered the milk FA composition within two days. Most changes took place within four days after the transition. The nutritional FA profile worsened. The proportions of myristic and palmitic acids increased, while those of stearic, oleic, rumenic acids and CLA declined (Elgersma et al., 2004).

There exists a general agreement that grazing or feeding of fresh forages produces milk fat with the nutritionally beneficial FA profile as compared with hay or silage.

5.3 Effects of hay feeding

Data on hay effects on the milk fat profile have been scarce as compared with fresh forage or silage.

Hay or straw supplements as fibre sources to grazing cows in early lactation had a low effect on the milk FA composition (Wijesundera et al., 2003).

In spite of lower intakes of linoleic acid and ALA, their contents were higher in the milk fat of cows fed hay than in that of cows fed silages prepared from the primary growth of mixed timothy and meadow fescue (*Festuca pratensis*). The forage conservation method had no clear effect on milk *trans*-18:1 acids or CLA contents (Shingfield et al., 2005b).

Comparing two diets with about one half of dry matter offered from meadow hay or from maize silage, Staszak (2007) observed a higher level of linoleic acid, CLA, ALA and total unsaturated acids in milk fat from cows fed hay. These results were proved by Bernardini et al. (2010) in a similar experiment.

5.4 Effects of silage feeding

Overall data are given in Table 5. Comparing the values with data in Table 4 on fresh forages, some differences are apparent. Milk fat of cows fed silages has a higher ratio of SFAs:UFAs and a higher palmitic acid proportion, whereas the proportion of beneficial vaccenic and rumenic acids is lower. Extensive lipolysis during forage ensiling can be among the causes of these differences.

Similarly like in fresh grass, feeding grass silage from semi-natural grasslands increases CLA content in milk due to reduced biohydrogenation in the rumen as compared with silage from intensively managed grasslands (Lourenço et al., 2005).

The evaluation of the reported data is complicated by numerous interactions between the type of silage and other factors (mainly the proportion and type of a concentrate) affecting the milk fat composition. In most articles, the effects of two or more silages prepared from different forages on the milk fatty acid composition were compared. Grass silages were usually compared with silages of other forages.

5.4.1 Effects of maize silage feeding

Maize silage is the main component of diet for winter feeding, and also for year-round feeding in some management systems. The following reports compared the effects of maize silage with grass silage feeding.

The proportions of saturated FA in total FAs in milk fat were 67.6% and 62.9% and those of PUFA 3.6% and 4.7% following feeding maize and grass silages, respectively (Samková et al., 2009). A similar

Table 5. The mean proportion of selected fatty acids (g/100 g of total fatty acids) and the ratio of saturated to unsaturated fatty acids (S/U) in milk fat from cows fed various silages and hay (adapted from Samková et al., 2008)

Silage composition[1] and proportion[2]		Fatty acid						S/U	Reference
		palmitic	oleic	vaccenic	linoleic	α-linolenic	rumenic		
One-species									
Lucerne	50	29.2	20.9	1.48	2.48	0.63	0.69	1.95	Benchaar et al. (2007)
	83	31.0	19.4[3]	0.85	1.55	0.22	0.48	2.50	Couvreur et al. (2006)
Maize	86	31.0	16.7	1.04	1.46	0.24	0.66	3.30	Ferlay et al. (2006)
	50	32.6	18.5	0.50	2.73	0.23	0.37	2.07	Benchaar et al. (2007)
	50	32.9	16.3[4]	0.94	2.30	0.24	0.54	2.98	Kliem et al. (2008)
	87	32.1	16.0	0.87	1.09	0.94	0.46	3.48	Ferlay et al. (2006)
Perennial ryegrass	*ad libitum*	29.7	19.2	1.92[6]	1.52	0.90	0.82	2.25	Van Dorland et al. (2008)
	ad libitum	38.5	21.5	–	1.00	0.56	0.45	–	Moorby et al. (2009)
	83	31.8	20.0[5]	1.31	1.47	1.51	0.42	2.72	Dewhurst et al. (2003b)
Red clover	*ad libitum*	26.3	18.7	0.91	1.73	1.11	0.39	2.14	Vanhatalo et al. (2007)
	ad libitum	36.5	24.7	–	1.63	1.49	0.39	–	Moorby et al. (2009)
White clover	70	32.9	17.9[5]	1.06	1.54	0.96	0.34	3.14	Dewhurst et al. (2003b)
Mixed forages									
Maize silage/lucerne hay (50/50)	50	28.6	17.8	1.12	2.85	0.67	0.55	2.30	Whitlock et al. (2006)
Maize silage/lucerne hay (60/40)	51	27.1	22.6	1.41	2.73	0.29	0.53	–	Liu et al. (2008)
Maize silage/perennial ryegrass/hay (48/41/11)	65	31.9	23.0	1.61	1.68	0.32	0.71	1.86	Kay et al. (2005)
Ryegrass (3 species)	80	34.3	19.0[5]	1.16	0.90	0.48	0.37	3.08	Dewhurst et al. (2003b)
Ryegrass (3 species)/red clover (50/50)	81	34.4	19.6[5]	1.38	1.08	0.77	0.45	2.86	Dewhurst et al. (2003b)
Perennial ryegrass/red clover (60/40)	*ad libitum*	31.3	17.1	1.78[6]	1.43	1.04	0.71	2.57	Van Dorland et al. (2008)
Perennial ryegrass/white clover (60/40)	*ad libitum*	23.1	16.7	1.55[6]	1.43	1.14	0.66	2.62	Van Dorland et al. (2008)
Timothy/meadow fescue	62	34.2	15.0	–	0.95	0.36	0.44	2.97	Shingfield et al. (2005b)
Timothy/meadow fescue	*ad libitum*	28.8	16.7	1.00	1.28	0.39	0.40	2.56	Vanhatalo et al. (2007)
Hay									
Ryegrass hay	90	30.2	15.4	1.83	1.00	1.02	0.87	3.26	Ferlay et al. (2006)
Mountain grassland hay	87	28.6	16.0	1.36	1.08	1.25	0.71	3.26	Ferlay et al. (2006)

[1] numbers in brackets mean the weight percentage of a forage; [2] (%) of dry matter in a diet; [3] sum of 9-*cis* and 13-*trans*-C18:1; [4] sum of 9-*cis* and 15-*trans*-C18:1; [5] sum of 9-*cis* and 11-*cis*-C18:1; [6] sum of 10-*trans* and 11-*trans*-C18:1

trend of SFAs (mainly of lauric and myristic acids) and PUFAs was reported by Shingfield et al. (2005a). While the former authors found a significant difference in CLA content (0.48% and 0.92% after feeding maize and grass silage, respectively), the latter ones did not find any significant effect of silage type on total CLA and rumenic acid proportions.

However, in a report of Nielsen et al. (2006), maize silage diets resulted in a higher content of CLA isomers as compared with diets based on grass silage, but there was a significant interaction between the silage type and concentrate level for the content of rumenic acid, *trans*-10, *cis*-12-CLA, vaccenic acid and *trans*-10-C18:1 acid. The authors thus suggested that the high levels of grain did not significantly alter the pattern of PUFA biohydrogenation in the rumen, content of CLA and *trans*-C18:1 isomers in milk fat unless combined with forage naturally high in starch and linoleic acid such as maize silage.

Similar results, i.e. an increase in rumenic acid and *trans*-10, *cis*-12-CLA in milk fat following maize silage feeding compared to grass silage feeding, were reported by Norgaard et al. (2008).

5.4.2 Effects of feeding legume and grass silages

Two papers comparing the effects of red clover silage and silage from a mixture of timothy and meadow fescue (Vanhatalo et al., 2007) or of perennial ryegrass silage (Moorby et al., 2009) reported the increasing proportion of MUFA and PUFA at the expense of capric, lauric, myristic and palmitic acids in milk fat following red clover silage feeding. The effect on an increase in beneficial PUFAs in milk fat was higher in red clover silage prepared from forage cut at an early stage than at a late stage of growth (Vanhatalo et al., 2007). These changes could be partially explained by polyphenol oxidase activity in red clover silage (Lee et al., 2009).

Feeding perennial ryegrass silage or mixtures of this silage with red clover or white clover silages (both at 60:40 ratio based on DM basis), Van Dorland et al. (2008) observed an increased proportion of n-3 FAs but reduced proportions of CLA including rumenic acid and of *cis*-C18:1 isomers in milk fat of dairy cows fed the variants with clovers. The lowest n-6:n-3 ratio was in milk fat of cows fed perennial ryegrass silage.

Silages prepared from a sward of timothy and meadow fescue in three variants (no additive; formic acid + phosphoric acid; inoculant of lactic acid bacteria + cellulase and hemicellulase) did not cause any clear differences in CLA and *trans*-C18:1 acids (Shingfield et al., 2005b).

Thus the feeding of ensiled forage legumes seems to have similar effects on UFA proportion in milk fat as the feeding of fresh legumes.

5.5 Oxidative stability of milk fat

The evaluation of an increase in unsaturated nutritionally beneficial FAs should take into consideration also the oxidative stability of altered milk fat.

Milk fats of cows fed diets based on red clover and lucerne silages were more prone to oxidative deterioration in comparison with diets based on grass silage. The increased susceptibility was avoided by vitamin E supplementation in concentrates (Al-Mabruk et al., 2004; Kay et al., 2005).

Havemose et al. (2004) observed higher lipid oxidation in milk from cows fed grass silage compared to milk from cows fed maize silage, despite a higher antioxidative capacity of the former milk. Thus, the quenching of singlet oxygen by natural antioxidants did not prevent lipid oxidation. Different proportions of ALA (7% and 2% of total FA in milk fat from cows fed grass silage and maize silage, respectively) were proposed to be important for the formation of lipid hydroperoxides. On the contrary, milk from cows fed maize silage was more vulnerable to protein oxidation.

In a further study of Havemose et al. (2006), differences in the oxidative stability of milk from cows fed grass-clover silage or hay were examined. A higher degree of lipid oxidation was found in milk of cows fed the silage. Similarly like in the previous paper, the higher content of natural antioxidants did not prevent oxidation and different contents of ALA (8% and 4% of total FAs in silage and hay, respectively) were thought to be the cause.

6. CONCLUSION

The alteration of the FA composition of cow's milk fat has been a long-term strategy. Even though forages contain relatively low levels of FAs bound in lipids, they are the cheapest and often the major source of unsaturated FAs in ruminant diets.

Losses of PUFAs due to their oxidation occur during prolonged wilting prior to ensiling, field drying to hay and also during the silage feed out period. Extensive lipolysis during ensiling enhances the rate of PUFA biohydrogenation in the rumen. Milk from cows fed fresh forage, especially from species-rich grasslands or forage legumes, has thus a considerably higher ratio of UFAs to SFAs and a higher content of nutritionally beneficial *trans*-fatty acids (such as CLA and vaccenic acid) than milk from cows fed silage or hay. Milk fat from cows fed grass or legume silages seems to have the nutritionally more propitious composition than fat from cows fed maize silage. However, the former fats are more prone to oxidation.

Nevertheless, the milk fat composition is a result of complex effects of numerous feed, animal and environmental factors, among which the type of forage is the only one element.

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