CHAPTER 10

LIPIDS IN HERBAGE

Their fate in the rumen of dairy cows and implications for milk quality

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Abstract. This chapter describes the fatty-acid profile of lipids in milk and herbage normally included in dairy diets. The next section deals with the possible effects of forage management on lipid intake in dairy cows. Then a detailed account is given of the fate of fatty acids in the rumen, showing that important microbial transactions occur through which unsaturated fatty acids become partly hydrogenated and are converted into intermediates, some of which exert important metabolic functions in the cow. When such intermediates are incorporated in and excreted with the milk, some are now considered to have beneficial effects on human health. In the last section examples are presented on how fatty-acid metabolism in dairy cows can and should be represented in terms of their fate in the rumen, their absorption and metabolic role in the intermediary metabolism and finally how rumen fatty-acid metabolism can be simulated in a mechanistic dynamic model.
Keywords: fatty acids; herbage; microbes; rumen; modelling

PROFILE AND ORIGIN OF FATTY ACIDS IN MILK OF DAIRY COWS

Fatty acids (FA) in milk fat originate from a variety of different sources. A part of the fatty acids is ingested with the feed and reaches the mammary gland unmodified. Another part is also ingested with the feed but undergoes microbial modification in the rumen or is modified in the animal's metabolism. Yet another part of the milk fatty acids is synthesized *de novo* in the mammary gland. The *de novo* synthesis is restricted to fatty acids with carbon chain lengths of C16 and less. Finally, milk fat may contain fatty acids that were mobilized from body reserves. As a result of these different sources of origin, milk fatty acids comprise short-chain (C4-C10), medium-chain (C12-C14) and long-chain (>C16) fatty acids. An alternative way to present

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the FA profile is a division in saturated, mono-unsaturated (MUFA), and poly-unsaturated fatty acids (PUFA). In small amounts, milk fat contains odd-numbered and branched-chain fatty acids.

The bulk of nutrients utilized by ruminants like dairy cows are of vegetable origin, and plants and plant parts play an important role as suppliers of nutrients to dairy cows. For that reason the chemical and morphological characterization of plants and plant parts is very relevant. Morphologically plants are, above-ground, composed of stems, leaves and seeds or fruits.

Dairy diets are normally composed of a mix of fresh forages (mainly leaf blades), conserved forages (leaf blades, and also stems in spring) and concentrates (seeds), all of which contain lipids. These lipids can be characterized as structural or polar lipids (PL: glycolipids, phospholipids), free fatty acids (FFA), triacylglycerides (TG) and sterole esters (SE) (Yang and Fujita 1997). In forages and grains, structural lipids (glycolipids and phospholipids) predominate, whereas the main components in oil seeds and oils are storage lipids (triacylglycerides).

LIPIDS IN HERBAGE

FA in plants are hydrocarbon chains of various lengths and degrees of unsaturation that terminate with carboxylic-acid groups. FA in biological systems usually contain an even number of C atoms, typically between 14 and 24. The 16- and 18-carbon FA are most common.

Plants are composed of plant cells, the fundamental units of life. Plant cells are organized into tissues, which are further organized into organs (e.g., leaves and stems). Plant cells are not amorphous sacks of components, but complex structures filled with organelles (endoplasmic reticulum, mitochondria and nucleus), which are usually bound by membranes. Chemically, membranes are composed of lipids (cholesterol, phospholipid and sphingolipid), proteins and carbohydrates (glycoprotein). Oil bodies or lipid droplets give a granular appearance to the cytoplasm. They are widely distributed throughout the cells of the plant but are most abundant in fruits and seeds.

Membranes have a lipid bilayer structure with a hydrophobic interior. There are 2 major types of lipids in the membranes of plant cells: phospholipids (most abundant) and sterols, mainly stigmasterol. Most membranes are composed of 40-50 % lipid (by mass) and 50-60 % protein.

Membranes are currently pictured according to the fluid-mosaic model, according to which proteins and lipids diffuse in the membrane. This fluidity is believed to be maintained by the type of lipids, more specifically their melting point. Hence, plants in temperate regions or at a high altitude have a high content of unsaturated fatty acids.

Plant lipids have several functions, and four groups can be distinguished:

- fats and oils are triglycerides that store energy;
- phospholipids are modified triglycerides that are components of cellular membranes;

- waxes, cutin and suberin are lipid-like substances on the plant surface that form barriers to water loss;
- steroids have linked hydrocarbon rings and play a variety of roles in plants; they have acute biological activity.

Lipids in plants are not static entities, but are continuously subject to turnover, meaning that lipid degradation is a normal process in the living plant and that lipases are normally present. At the short term this will not have a big influence on the FA composition of the lipid fraction in plants. There are at least three occasions when the lipid fraction in plants or plant parts may significantly be modified, i.e., during senescence, immediately after detachment (grazing or cutting) and during storage after cutting.

In detached plants, immediately after cutting and perhaps during the early stages of ingestion and ensiling, the metabolism of plant cells can continue. Also, there is activity of the enzymes of dead tissue. The processes of respiration and proteolysis are best known, however, also lipolysis occurs.

In ruminants grazing fresh pastures, the first stages of lipolysis could be mediated by plant lipases (Lee et al. 2003). These enzymes are widely present in plants and their regulation might be altered due to the double stress of elevated temperature and anoxia imposed on the plant metabolism of intact plant cells after ensiling or ingestion by ruminants. A potential plant breeding goal to reduce the rate and extent of lipolytic activity in the rumen could be to select forage plants with reduced lipolytic activity.

In the harvested plant, biosynthetic reactions are limited and it is assumed that virtually all the energy resulting from respiration is converted into heat. In an isolated plant this heat energy would be dissipated into the atmosphere, but in the silo or in a heap of cut grass, most of the heat arising from the action of respiratory enzymes is retained in the mass of herbage, causing an increase in temperature.

Dewhurst and King (1998) studied the effect of ensiling on the fatty acids in the material. Wilting prior to ensiling reduced the content of total fatty acids by almost 30 % (from 24.6 to 17.5 g kg^{-1} DM), with a reduction of up to 40 % for linolenic acid. These authors suggested that the ensiling process itself has little influence, provided compaction and sealing of the silo is good. This may not always be the case in big bale silages. Adding silage additives (formic acid, formalin) resulted in much smaller losses, which was also found for formic acid by Doreau and Poncet (2000). Hay making reduced total FA by over 50 %, with a greater loss of linolenic acid (Doreau and Poncet 2000). Similar observations were made for haylage (70 % DM) by Elgersma et al. (2003b).

EFFECTS OF FORAGE MANAGEMENT AND DIET ON LIPID INTAKE

Increasing the concentration of desired FA in ruminant products nowadays receives much attention. In contrast to short- and medium-chain FA, ruminants cannot endogenously synthesize these long-chain C18 FA that are desired in meat and milk. Therefore, ruminants have to ingest long-chain FA with the feed. In herbage, C18:3 predominates. In oil seeds, linoleic acid predominates as the major FA, except for

linseed, which contains mainly α-linolenic acid. Animal diets could also be supplemented with fish oil, containing predominantly FA of 20 or 22 carbons as the major FA, but this might negatively affect milk taste and harm the image of dairy farming and dairy products while plant sources, especially forage, would represent the most natural and environmentally sustainable source.

In the rumen, linoleic acid (C18:2 *cis*-9, *cis*-12) and α-linolenic acid (C18:3 *cis*-9, *cis*-12, *cis*-15) are extensively subjected to microbial biohydrogenation. There are two possible ways to reach the objective of increasing the concentration of desired FA in ruminant products, either to increase the concentration of substrate in the feed or to reduce the extent of biohydrogenation in the rumen.

Some information is available in the literature about the lipid concentration and composition of forages and factors influencing them. For example, it has been established that the lipid fraction in leaves of herbs and grasses ranges from 30 to 100 g kg^{-1} DM, much of which is contributed by lipids in the chloroplasts (Bauchart et al. 1984). Sources of variation in lipid concentration are plant species, growth stage, temperature and light intensity (Hawke 1973). There are five major fatty acids in grasses, but approximately 95 % consist of C18:3, C18:2 and C16:0 (Hawke 1973). Fresh grass contains a high proportion (50-75 %) of its total fatty-acid content in the form of C18:3. Levels of α-linolenic acid vary with plant and environmental factors such as stage of maturity, genetic differences (Elgersma et al. 2003a; 2003c), season and light intensity (Dewhurst and King 1998).

Quantifying the concentrations and composition of fatty acids in grasses in response to environmental factors could help to design management strategies to increase precursors for beneficial FA in products from ruminants.

Cows on pasture (Dhiman et al. 1999; Elgersma et al. 2003b) grazing lush green grass (Dewhurst et al. 2003; Elgersma et al. 2003d; Khanal and Olson 2004) at a high herbage allowance (Elgersma et al. 2004a) produce milk with the highest concentrations of PUFA. Under these circumstances, cows would be able to select and to ingest upper layers of the canopy, where young leaf blades predominate. Elgersma et al. (2005) therefore hypothesized that the protein concentration in the herbage, the leaf-blade proportion of the canopy and regrowth period of the sward might affect the concentration of fat and the proportions of FA in the herbage. Regrowth period indeed affected the total FA concentration, and significantly lower concentrations of C18:3 and C16:1 were found after a longer period of regrowth. N application on the other hand resulted in higher concentrations of all FA. The FA composition was not affected by N application, but a longer regrowth period significantly decreased the proportion of C18:3 and increased those of C18:2 and C16:0. A strong positive overall linear relation was found between the concentrations of total FA and C18:3 with the N concentration in the herbage (Figure 1).

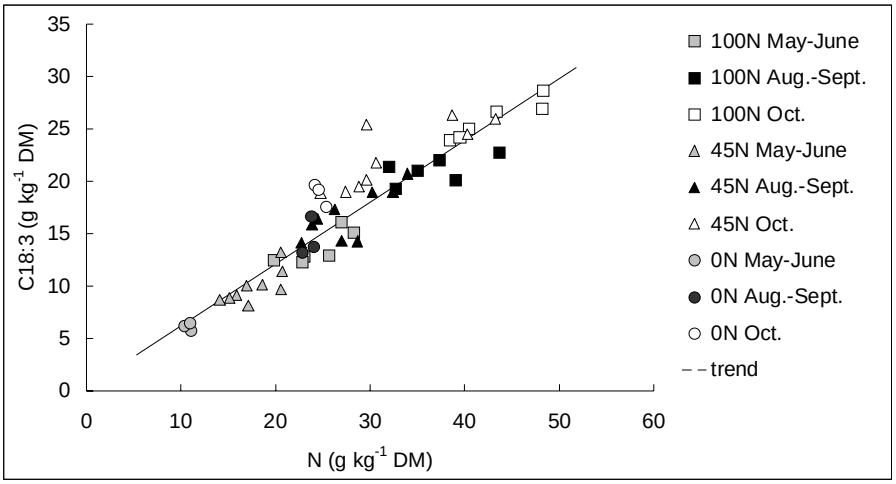

***Figure 1**. The relation between N and C18:3 concentration (g kg^{-1} DM) of Lolium perenne during three periods in 2004; C18:3 concentration = 0.49 + 0.59 * N; R^2 = 0.87 (Witkowska et al. in prep.)*

THE FATE OF FATTY ACIDS IN THE RUMEN

A biologically sound description of rumen function is possible by a mathematical presentation of the dynamics of its pools, their inputs, the digestive events in these pools, their interactions and their outputs (Dijkstra et al. 2002).

Current views are that the microbial population in the rumen comprises three distinct groups or pools of micro-organisms (Nagorcka et al. 2000). These pools are bacteria attached to or associated with feed particles or solids (SAB), bacteria that are free-floating in and hence associated with the rumen liquid (LAB) and protozoa, either free-floating or attached to particles. The latter are assumed to predate on bacteria. The role of protozoa in dietary lipolysis is still subject of debate, whereas current consensus is that protozoa are of minor importance in biohydrogenation of unsaturated fatty acids (review by Harfoot and Hazlewood 1997). Therefore, protozoa will not be discussed further. Bacteria in the SAB pool have a long rumen residence time (20-40 h), are assumed to ferment predominantly cell walls and grow relatively slowly, but with a low maintenance requirement per unit of time. Bacteria in the LAB pool, on the other hand, have a much shorter rumen residence time (10-15 h), ferment predominantly cell contents, grow at a fast rate, but have a high maintenance requirement (Russell and Strobel 2005).

Based on *in vitro* continuous-culture experiments, Czerkawski (1986) observed lipid and fatty-acid concentrations in rumen bacteria to range between 50 and 150 g kg^{-1} DM, with in SAB usually around twice as many lipids as in LAB. This difference was confirmed *in vivo* in beef cattle (Merry and McAllan 1983; O'Kelly and Spiers 1991), in dairy cattle (Bauchart et al. 1990) and in sheep (Rodriguez et al.

2000). After feeding diets with a lipid (total of long-chain fatty acids) content of over 25 g kg^{-1} DM, fatty-acid contents in both microbial pools became enhanced (Bauchart et al. 1990), but it remained uncertain if this enhancement and the differences between SAB and LAB resulted from an increased adherence or from an increased incorporation of fatty acids.

Fatty acids in bacterial lipids are predominantly C16:0, C18:0 and C18:1 (Bauchart et al. 1990). These authors demonstrated that the addition to the diet of dairy cows of lipids from widely different origin (rapeseed oil at two levels, tallow, soybean oil and palmitostearin) had little effect on the polar-lipid content in SAB or LAB, but could increase their free fatty-acid (FFA) content by up to 150 %. Total lipid content was 1.7 to 2.2 times higher in SAB than in LAB. From the results of a similar experiment with steers, O'Kelly and Spiers (1991) concluded that, following the uptake of unsaturated fatty acids, some protection against biohydrogenation developed.

Different bacterial strains have different capacities to biohydrogenate poly-unsaturated fatty acids in the rumen. They are usually divided into a group A and a group B. Members of group A can hydrogenate linolenic and linoleic acid to (mostly) *trans*-11-octadecenoic acid but appear incapable to further hydrogenate octadecenoic acids, whereas members of group B can hydrogenate a wide range of octadecenoic acids, including *cis*-9-oleic and *trans*-11(*trans*-vaccenic) and linoleic acid, with stearic acid (C18:0) as the ultimate end product. Members belonging to group A are *Butyrivibrio fibrisolvens*, *Ruminococcus albus*, *Eubacterium* sp., *Treponema*, a rumen spirochaete and some gram-negative rods.

Only three isolates of group B are known, two species of *Fusocillus* and an unknown gram-negative rod (Harfoot and Hazlewood 1997). Figure 2 shows the major isomerization and hydrogenation pathways of unsaturated fatty acids. Three important intermediates are the C18:2 t11c15, the C18:2 c9t11 (CLA isomer rumenic acid) and the C18:1 t11 (vaccenic acid).

The fate of dietary lipids in the rumen depends on a number of events. These are: their release from the feed, their adherence to or incorporation in bacterial cells, their biohydrogenation and their passage to the lower gut. A model developed by Dijkstra et al. (2000) described lipid metabolism in the rumen with three state variables, representing a lipid pool (1) and pools of unsaturated (2) and saturated long-chain fatty acids (3).

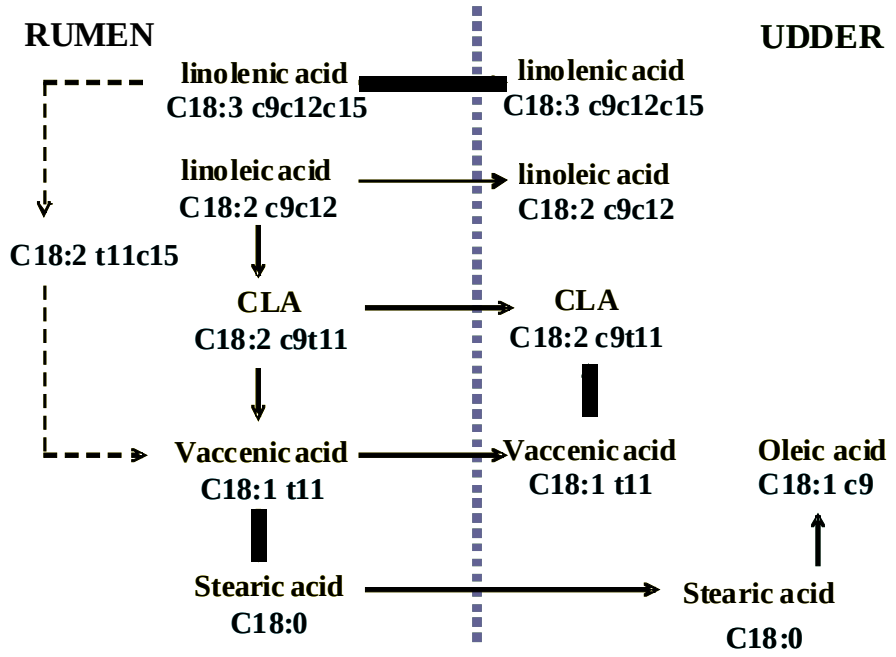

***Figure 2**. Simplified scheme of biohydrogenation and desaturation pathways of C18 fatty acids in rumen and udder of dairy cows*

Table 1 summarizes *in vivo* data on the biohydrogenation of polyunsaturated fatty acids (PUFA), derived from duodenal flow measurements. Feed intake and percentage forage differed between and within the experiments with dairy cows, but the data base is too small to draw conclusions on the effects of level of feed intake and proportion of forage in the diet. Regardless of type of animal, level of intake and type of diet, linoleic (C18:2) acid seems to be biohydrogenated to a smaller extent than linolenic (C18:3) acid. This appears also true for grass-fed animals, as demonstrated by Scollan et al. (2003) and Lee et al. (2003) in beef cattle. A varying proportion of PUFA, ranging from less than 2 to over 20 %, is recovered as vaccenic acid (C18:1 t11), an important product of the biohydrogenation of both linoleic and linolenic acid.

An important source of PUFA in herbages, including grass, are the chloroplasts, the release of which is probably mimicked by the release of chlorophyll. This release has been studied by Waghorn et al. (1989), Boudon and Peyraud (2001) and Boudon et al. (2002). Waghorn et al.(1989) observed a high (50-60 %) immediate release, followed by a slow gradual fractional release of 0.05 – 0.08 h^{-1}. Boudon and Peyraud (2001) and Boudon et al. (2002) on the other hand observed an immediate release of

Table 1. *Degree of in vivo biohydrogenation of polyunsaturated fatty acids (PUFA) in the rumen of beef and dairy cattle*

Species	Diet	Intake			% Biohydrogenation		Duodenal flow		Reference
		DM (kg/d)	C18:2 (g/d)	C18:3 (g/d)	C18:2	C18:3	C18:1 t11 (g/d)	% PUFA	
Beef	Mixed	10.4	162.4	21.0	77.8	89.3	5.0	2.7	Duckett et al. 2002
		10.7	254.1	34.5	79.2	91.3	9.0	3.1	
		10.6	287.1	37.1	83.7	92.8	5.2	1.6	
Beef	Grass	4.2	10.6	35.7	86,4	92.5	3.2	7.0	Lee et al. 2003
	Red clover	6.4	27.8	67.8	84.3	83.8	4.6	4.8	
	White clover	8.4	38.0	91.9	82.6	87.6	6.9	5.3	
	Grass	6.7	20.3	51.3	78.3	89.2	9.1	12.7	Scollan et al. 2003
	Grass	9.3	29.2	108.5	81.5	91.8	11.1	8.1	
Dairy	Mixed	5.9	77	73	85	95	24	16.0	Lock and Garnsworthy 2002
		5.9	79	117	85	97	24	12.2	
		5.9	125	75	90	95	27	13.5	
		5.9	130	122	91	97	29	11.5	
Dairy	Mixed	19.8	96.7	82.1	77.7	89.9	21.4	15.3	Loor et al. 2004b
		19.1	180.9	444.9	88.8	97.1	61.7	9.9	
		20.8	142.2	54.7	74.5	83.9	26.0	13.7	
		20.3	238.7	442.7	82.0	93.2	138.8	20.4	
Dairy	Mixed	20.6	379	83	79.0	87.8	61	13.2	Kalscheur et al. 1997
		21.9	381	85	79.3	88.1	57	12.2	
		23.7	501	58	73.3	81.9	120	21.5	
		24.1	510	58	79.3	85.9	66	11.6	

only between 20 and 35 %, followed by a faster fractional release of between 0.10 and 0.15 h^{-1}. In concentrates, fatty acids are either included as part of the original seed or added to it as oil. The fate of fats and fatty acids (FA) in the rumen has been studied with nylon-bag incubations for raw and treated soybeans (Perrier et al. 1992; Chouinard et al. 1997), canola seeds (Enjalbert et al. 2003) and sunflower seeds (Mustafa et al. 2003; Sarrazin et al. 2003). The results in Table 2 show that FA disappear from nylon bags at a 2 to 4 times faster rate than dry matter, and that polyunsaturated FA (PUFA) disappear faster than saturated FA, also because PUFA not only leave the bags with feed particles, but are also biohydrogenated into less unsaturated FA. Fractional rates of disappearance of total FA varied between 0.10 and 0.25 h^{-1} and processing (extrusion, roasting, moist heat treatment) slowed down the fractional rate of disappearance, notably because the size of the washout fraction became reduced.

Lipolysis and biohydrogenation have been extensively studied *in vitro*, notably with soybean oil and to a lesser extent with linseed oil. The FA composition of linseed oil more or less resembles that of fresh grass, in that lipids in fresh grass also contain a high proportion of linolenic acid (C18:3 c9 c12 c15). Beam et al. (2000) estimated fractional lipolysis rate of soybean oil to vary between 0.30 and 0.44 h^{-1} and fractional rate of biohydrogenation of linoleic acid (C18:2) at 0.14 h^{-1}, and declining with the addition of C18:2. Lipolysis and biohydrogenation of linseed oil were studied by Chow et al. (2004) and fractional lipolysis rates of 0.073 and 0.087 h^{-1} and biohydrogenation rates of 0.224 and 0.309 h^{-1} could be calculated for linoleic and linolenic acid, respectively. Lipolysis and biohydrogenation of lipids in grass was also studied *in vitro* by Chow et al. (2003). After 6 h of incubation, 58 and 74 % of linoleic and linolenic acid had undergone lipolysis, of which 52.4 and 91.2 % had become biohydrogenated. Recalculating these figures to fractional rates results in 0.145 and 0.225 h^{-1} for lipolysis, and 0.124 and 0.405 h^{-1} for biohydrogenation, respectively.

In the experiments with linseed oil of Chow et al. (2004), an accumulation of the intermediates C18:2 t11c15 and C18:1 t 11 (vaccenic acid) was observed. After feeding linseed oil, Loor et al. (2004b) observed elevated levels of up to 100 g kg^{-1} of fat of the C18:2 t11c15 intermediate in rumen fluid. Levels increased from 30 to 90 g kg^{-1} of fat within 2 hours after the administration of about 300 g of linseed oil with the feed in the morning. During the same period vaccenic acid (C 18:1 t 11) increased only from 90 to 114 g kg^{-1} of fat. Because of its similarity with linseed oil, the C18:2 t11 c15 is most likely also an important intermediate in the rumen of grass-fed cows. It is likely that this is reflected in the FA profile of milk produced by grass-fed dairy cows. Comparing winter diets with diets on fresh grass revealed that in grass-fed cows the C18:2 t11 c15, when expressed as proportion of the sum of C18:2 + C18:3 in milk, ranged between 0.30 and 0.46, whereas on winter diets this was only between 0.029 and 0.042 (Vlaeminck, pers. communication). Changing from a grass and maize silage-based winter diet to fresh grass elevated the level of this intermediate from 0.046 to 0.155 after 5 days .

***Table 2**. In situ disappearance of fats and fatty acids from soybeans (1,2), sunflower seeds(3,4) and canola seeds (5)*

		Soybeans				Soybeans	Sunflower seed 1		Sunflower seed 2				Canola seed	
		Raw	E120	E130	E140	Raw	Raw	Roasted	Raw	Heated	Heated	Heated	Raw	E140
% Crude fat		6.0	6.2	5.8	6.1		44.3	43.3	45.4	48.2	48.5	48.6	42.7	47,7
C16:0 (% CFat)		11.6	10.8	10.9	10.6		5.8	5.8	5.9	5.9	5.8	5.8		
C18:0 (% CFat)		3.7	3.5	3.6	3.3		4.3	4.2	4.7	4.7	4.7	4.7	2.0	1,9
C18:1 (% CFat)		25.0	22.9	24.5	21.5		17.1	17.3	15.3	15.4	15.4	15.4	61.6	61,2
C18:2 (% CFat)		51.3	53.0	52.0	53.8		72.7	72.6	73.9	73.9	73.9	73.9	25.2	25,2
C18:3 (% CFat)		8.0	9.4	8.7	10.4		0.1	0.1	0.1	0.1	0.1	0.1	11.2	11,7
DM	a	17.9	18.3	18.3	20.2		48.2	36.8	59.5	43.6	43.3	40.7		
	b	84.0	70.5	77.8	76.9									
	c	0.06	0.05	0.04	0.05		0.13	0.07	0.09	0.05	0.05	0.05		
Fatty acids	a	15.3	6.0	3.0	0.2								27.4	46,5
	b	71.9	54.4	60.6	65.1									
	c	0.25	0.21	0.17	0.17	0.12	0.25	0.13	0.12	0.10	0.10	0.09	0.11	0,09
C16:0	a	5.16	12.22	4.14	-0.72									
	b	87.3	73.4	77.5	73.5									
	c	0.10	0.04	0.04	0.05		0.15	0.10	0.12	0.09	0.09	0.09		
C18:0	a	3.3	10.9	4.8	0.0									
	b	52.2	-92.0	-94.2	-86.4									
	c	0.28	0.11	0.06	0.12		0.13	0.07	0.11	0.08	0.08	0.08		

Table 2 (cont.)

E120, E130 and E140 extruded at 120, 130 and 140 °C respectively; a = fraction lost instantaneously *in situ*; b = potentially degraded *in situ*; c = rate constant for degradation of 'b' fraction *in situ*.

References: 1. Chouinard et al. 1997; 2. Perrier et al. 1992; 3. Sarrazin et al. 2003; 4. Mustafa et al. 2003; 5. Enjalbert et al. 2003

Table 2 (cont.)

		Soybeans				Soybeans	Sunflower seed 1		Sunflower seed 2				Canola seed	
		Raw	E120	E130	E140	Raw	Raw	Roasted	Raw	Heated	Heated	Heated	Raw	E140
C18:1	a	3.33	10.55	5.82	4.62									
	b	89.8	70.7	75.6	78.8									
	c	0.16	0.08	0.08	0.10		0.21	0.14	0.13	0.09	0.09	0.09		
C18:2	a	25.9	3.6	5.3	3.6									
	b	70.9	86.1	85.9	83.9									
	c	0.42	0.20	0.20	0.30	0.17	0.30	0.14	0.14	0.10	0.10	0.10		
C18:3	a	25.8	4.4	1.3	2.9									
	b	70.4	83.5	87.4	84.0									
	c	0.39	0.21	0.22	0.32									

E120, E130 and E140 extruded at 120, 130 and 140 °C respectively; a = fraction lost instantaneously *in situ*; b = potentially degraded *in situ*; c = rate constant for degradation of 'b' fraction *in situ*.

References: 1. Chouinard et al. 1997; 2. Perrier et al. 1992; 3. Sarrazin et al. 2003; 4. Mustafa et al. 2003; 5. Enjalbert et al. 2003

Members of the previously mentioned group-A bacteria are predominantly cell-wall degraders, although a number of group-A bacterial species and strains can utilize starch as well. Hence one would expect more biohydrogenation intermediates in the rumen with forage-rich diets than with concentrate-rich diets. Indeed, results of *in vitro* experiments indicate that, in contrast to readily fermentable carbohydrates, the amount of fibre positively affects rate of biohydrogenation (review Harfoot and Hazlewood 1997). However, dietary forage level did not affect concentrations or degree of saturation of FA in mixed rumen bacteria (MRB) harvested from the rumen of steers (Hussein et al. 1995). In experiments with goats, Bas et al. (2003) observed that the FA concentration in MRB harvested from duodenal contents varied with the proportion of forage in the diet. Notably the proportions of unsaturated even-chain FA decreased, whereas those of odd-and branch-chained FA increased with increasing forage. Kalscheur et al. (1997) observed in dairy cows a higher degree of biohydrogenation with high-forage diets than with low-forage diets. This discrepancy between mixed bacteria harvested from the rumen (Hussein et al. 1995) and those harvested from the duodenum (Kalscheur et al. 1997; Bas et al. 2003) can be explained by the different rate of passage between FAB and SAB.

IMPLICATIONS

In a mature dairy cow, consuming some 20 kg of organic matter per day, the size of the microbial organic-matter pool equals about 2 kg (Robinson et al. 1987; Beauchemin et al. 1999). The distribution between LAB and SAB in the rumen of such a dairy cow, when fed with a mixed diet, is 20-40 % LAB and 60-80 % SAB (Yang et al. 2001). No information is available yet on such distribution in grazing animals, but there are no indications that a fresh-grass diet will significantly deviate from this distribution.

Assuming microbial-pool sizes of 600 and 1400 g OM for LAB and SAB, lipid fractions of 80 and 120 g kg^{-1} OM and fractional turnover rates of 3.6 and 1.0 d^{-1} for LAB and SAB, respectively, the contribution to the intestinal lipid supply of LAB and SAB is 173 and 168 g d^{-1}, respectively. Recent results (Brito and Broderick 2004; Olmos Colmenero and Broderick 2006) indeed showed an almost equal duodenal flow of LAB and SAB in dairy cows.

QUANTITATIVE REPRESENTATION OF FA METABOLISM

Despite the impact of dietary FA on rumen metabolism and milk quality, its mathematical representation has received only limited attention in extant feed evaluation systems and mechanistic models of rumen fermentation and ruminant metabolism (Dijkstra et al. 2000). Current feed evaluation systems generally consider dietary lipid as a single entity, provide no or only a crude representation of lipolysis and biohydrogenation, largely ignore the changes in rumen microbial metabolism related to amount and type of FA, and do not represent udder metabolism of FA, including desaturase activities. Recently though, a sub-model

within the Cornell Net Carbohydrate and Protein System (CNCPS) has been developed that provides a mathematical representation of ruminal lipolysis and biohydrogenation and intestinal absorption of five individual FA (Moate et al. 2004). A mathematical model provides a way of bringing together knowledge about the parts of a system with several components, to give a coherent view of the behaviour of the whole system (Thornley and France 2005). Such an integrated quantitative approach is of high significance in attempts to manipulate FA composition of milk. For example, increasing the linoleic or linolenic acid content of the diet to increase CLA content of milk may also give rise to reduced fibre degradation and microbial protein synthesis in the rumen, because of the generally adverse effect of unsaturated LCFA upon fibrolytic bacteria (Jenkins 1993).

RUMEN FA METABOLISM

The presence of a free carboxyl group is required for biohydrogenation of unsaturated FA to take place. Thus, a mathematical representation of biohydrogenation requires representation of lipolysis as well.

Lipolysis is affected by a number of factors, including type of fat source, pH and feed intake level (see reviews by Jenkins 1993; Harfoot and Hazlewood 1997). In the model of Dijkstra et al. (2000), the default value for lipolysis is 90 % by setting the fractional rate of lipolysis at nine times the fractional passage rate of lipids. Dijkstra et al. (2000) indicated that the user can overrule this default value in case of specific circumstances, including feeding protected fat sources. The structure of this model, with pools of substrates and of microbes that change in time, potentially allows changes in lipolysis to occur based on the specific rumen conditions. Moate et al. (2004) derived substrate-specific fractional lipolysis rates. The fractional amount of lipolysis is calculated as fractional rate of lipolysis, divided by the sum of fractional lipolysis and passage rate. Therefore, a reduced degree of lipolysis is predicted when the retention time of lipid in the rumen decreases.

The earlier rumen models of Baldwin include a crude representation of biohydrogenation of unsaturated FA during computation of ruminal methane production (see Baldwin 1995). Of all hydrogen sinks, though, biohydrogenation is only a minor contributor (Mills et al. 2001). Dijkstra et al. (2000) represented biohydrogenation of an aggregated pool of unsaturated FA in the rumen. The rate of biohydrogenation is assumed to follow saturation kinetics, in which increased ruminal concentrations of unsaturated FA reduce the rate of biohydrogenation whilst increased ruminal concentrations of fibre increase the rate of biohydrogenation. However, since this model does not represent the various intermediates in the biohydrogenation process, including *trans*-fatty acids, the model can merely give an indication of efficiency of biohydrogenation and formation of intermediates. Moate et al. (2004) represented ruminal biohydrogenation of five different unsaturated FA. In their model, fractional rate of biohydrogenation of some unsaturated FA is constant, whereas the rate of biohydrogenation of other unsaturated FA follows an exponential decline related to the ruminal production of that FA. This representation allows build-up of *trans*-fatty acids in the rumen when the ruminal production rate

of the *trans*-fatty acid exceeds a certain value, so that its fractional rate of biohydrogenation is smaller than the fractional rate of biohydrogenation of its precursor. However, unlike in the model of Dijkstra et al. (2000), the level of fibre does not affect the predicted rate and extent of biohydrogenation.

The model of Baldwin (1995) is the first one that represents the inhibition of fibre degradation and the enhanced microbial growth efficiency by added fat, though independent of the degree of LCFA saturation. Dijkstra et al. (2000) extended this representation, and in their model the metabolism of protozoa and fibrolytic bacteria is assumed to be negatively affected by the ruminal concentration of all FA or unsaturated FA, respectively. Protozoa are a major determinant of overall microbial efficiency in the rumen because of predation on bacteria (Dijkstra et al. 1998). Hence a rise in concentration of free FA will reduce simulated protozoal activity and increase microbial efficiency. Similarly, the reduced metabolism of fibrolytic bacteria upon increased levels of rumen unsaturated FA will result in predicted depression of fibre degradation. Amylolytic bacteria are assumed not to be affected by FA and consequently the ruminal degradation of starch remains largely unchanged by dietary lipid alterations, as the amylolytic bacteria will occupy the niche left by protozoa. In contrast, the model of Moate et al. (2004) does not provide a representation at all of the effects of FA on ruminal degradation of organic matter or on microbial metabolism.

ABSORPTION AND INTERMEDIARY METABOLISM OF FA

Absorption and metabolism of FA has received even less attention in ruminant models. Rate of absorption or digestion is usually a fixed percentage of the FA flow into the duodenum. For example, the model of Mills et al. (2001), a further development of the rumen model of Dijkstra et al. (2000), assumes a fixed absorption coefficient of 90 %. In the model of Moate et al. (2004), the digestion coefficients for lipids and FA are assumed to depend on type of diet and type of FA. Digestion coefficients of FA vary from 0.59 to 0.95, whereas digestion coefficients of lipids vary from 0 to 1. Remarkably, the digestion coefficient of stearic acid in lipids was set at zero for fat sources like tallow, vegetable oils and whole intact oil seeds, but set at one for Megalac.

No representations are available for the metabolism of different FA by the mammary gland. Specific *trans*-fatty acids, viz. *trans*-10 C18:1 FA, may well constitute the group of actual FA inhibitors of *de novo* milk fat synthesis and be responsible for milk fat depression (Bauman and Griinari 2001). The mathematical representation of this inhibition requires a description of the formation of these *trans*-10 fatty acids in the rumen and their absorption from the gut. In such a representation, individual animal variation should be included as well. Animals on the same diet showed a threefold difference in milk conjugated linoleic acid (CLA) content (Kelsey et al. 2003). Similar observations were made by Elgersma (2004b and unpublished results).

Also, consistency was shown in the ranking of individual cows for *cis*-9, *trans*-11 C18:2 content over time on the same diet or when cows were switched between

diets (Lock and Garnsworthy 2002). Since the majority of CLA in milk is produced by endogenous synthesis via delta-9 desaturases of the C18:1 precursors, individual cow variation in delta-9 desaturase activity should be represented as well. No polymorphisms in the mammary stearoyl-CoA desaturase promoter segment were observed, though (Keating et al. 2005). Thus, individual cow variation is most likely related to individual variation in ruminal synthesis of relevant CLA precursors or to differences in regulatory proteins themselves. Such aspects have not been described mathematically.

Scientific progress is partly hampered by the labour-intensive, slow and costly standard analysis of FA in milk and even more so in rumen digesta, using gas chromatography. A recently developed alternative method for estimating CLA in milk fat (Elgersma and Wever 2005) is more rapid and could be used for screening purposes.

SIMULATED RUMEN FA METABOLISM

To illustrate the effects of various grass-based diets on rumen fermentation and on FA metabolism, predictions using the model of Dijkstra et al. (2000)are presented in Table 3.

Table 3*. Simulated degradation of neutral detergent fibre (NDF), duodenal flow of fatty acids (FA) and of microbial non-ammonia nitrogen (NAN), apparent degree of saturation of FA flowing to the duodenum, and biohydrogenation of unsaturated FA in cows fed grass- or grass silage-based diets*

	Diet[1]				
	G	GS	GM	G70	GO
Ruminal NDF degradation (%)	73.1	75.0	67.0	70.9	63.7
Duodenal flow of: (g/d)					
Total FA	815	694	812	833	1399
Saturated FA (g/d)	580	525	602	620	775
Unsaturated FA (g/d)	235	169	210	212	624
Microbial NAN (g/d)	331	337	325	315	306
Apparent degree of duodenal FA saturation (%)	71.2	75.7	74.1	74.5	55.4
Apparent biohydrogenation of unsaturated FA (%)	61.7	62.6	65.6	61.0	49.6

[1]Default diet G consists of 85 % grass and 15 % concentrate; diet GS, grass replaced with grass silage; diet GM, grass replaced by mature grass; diet G70, 70 % grass, 30 % concentrate; diet GO, default grass supplemented with 30 g kg^{-1} DM pure oil. See text for further explanation of dietary characteristics.

In these simulations, the intake of a cow of 600 kg live-weight is 20 kg DM d^{-1}. The default diet (diet G) is largely based on Taweel et al. (2005) and Tas et al. (2005), and comprises (DM basis) 85 % fresh grass and 15 % concentrate. The chemical composition of the grass is (g kg^{-1} DM): NDF, 450; soluble sugars, 140; crude fat, 40; crude protein, 170. The FA content of the crude fat is 70 %, of which 95 % is esterified and 5 % in free FA form, and the degree of saturation of grass FA is 15 % (Tamminga et al. 2001). The chemical composition of the concentrate is assumed to be (g kg^{-1} DM): NDF, 270; soluble sugars, 120; crude fat, 40; crude protein, 145. In the concentrate, the FA content of the crude fat is 85 %, of which 30 % is in the free FA form. The degree of saturation of concentrate FA is 70 %.

Several modifications in this basal diet were made to illustrate dietary effects upon rumen fermentation and hydrogenation processes. In diet GS, the grass is replaced by grass silage to compare a grazing (summer) diet with a barn (winter) diet. The grass silage is assumed to differ from the grass in that the sugar content was lowered to 80 g kg^{-1} DM and the solubility and degradability characteristics of protein are set to that of an average grass silage quality. Furthermore, the fat content of grass silage is assumed to be 25 % lower than grass, and the esterified FA fraction lowered from 95 % to 60 %, because of lipolysis and oxidation processes during ensilage processes. The degree of saturation is reduced by 10% (Tamminga et al. 2001). In diet GM, the default grass is replaced by more mature grass of lower digestive value to evaluate the effect of high-fibre diets. The NDF, sugar and protein content of mature grass is set at 550, 80 and 150 g kg^{-1} DM, respectively. The solubility and degradability characteristics of NDF and protein are also adjusted. The fat profile of the mature grass is the same as in the default grass. In diet G70, the concentrate proportion of the diet is increased and the ratio of fresh grass:concentrates set to 70:30. Finally, in diet GO, pure oil (100 % free, unsaturated FA) at 30 g kg^{-1} DM is added to evaluate the effects of additional supply of unsaturated FA.

In the default situation (diet G), the predicted ruminal NDF degradation is 73.1 %. Assuming that total tract NDF degradation is some 15 % higher than ruminal NDF degradation, total tract NDF degradation is 85 %, in line with average values observed by Tas et al. (2005) for several ryegrass cultivars. Of the FA flowing to the duodenum, 71.2 % are saturated. Note that the unsaturated-FA flow originating from the diet comprises a mixture of FA not hydrogenated at all and partially hydrogenated FA (including *trans*-fatty acids). The apparent biohydrogenation of unsaturated FA (61.7 %) seems low. This is the result from the assumption in the model that 90 % of dietary lipids are hydrolysed and at maximum 90 % of unsaturated free FA are hydrogenated.

Replacing grass by grass silage (diet GS) results in a predicted reduction of the flow of unsaturated FA to the duodenum and higher degree of duodenal FA saturation. This reduction is due to the smaller dietary lipid content and higher ruminal biohydrogenation level of the grass silage diet. The reduced supply of FA and increased level of saturation is expected to impact milk FA composition in line with general trends observed between milk from cows on pasture or cows fed silage (Chilliard et al. 2000).

Experiments showed a quick response of the CLA content in milk to changing

cows from indoor feeding to pasturing (Kelly et al. 1998) and the reverse (Elgersma et al. 2004b). The maximum effect is reached within about 5 days (Chilliard et al. 2001).

In comparison to the default diet, feeding mature grass (diet GM) reduces predicted NDF degradation and, because of increased ruminal concentrations of fibre, increases predicted biohydrogenation. Hence the duodenal flow of unsaturated FA is lower, and the degree of FA saturation higher, than diet G, in accordance with the general observation that increased dietary fibre contents enhance ruminal biohydrogenation of unsaturated FA (Jenkins 1993). Similarly, increasing the concentrate to grass ratio (diet G70), and therefore increasing the starch content and reducing the fibre content of the diet, reduces predicted biohydrogenation. Finally, adding unsaturated FA to the default diet (diet GO) reduces predicted biohydrogenation, in line with general observations that complete biohydrogenation of unsaturated FA is inhibited irreversibly by high concentrations of unsaturated FA (Jenkins 1993; Chilliard et al. 2000). The high level of unsaturated FA addition in diet GO has a large negative impact on predicted fibre degradation (from 73.1 % to 63.7 %). The flow of microbial NAN however is much less affected (from 331 to 306 g/d) and indicates a higher microbial N efficiency on diet GO. Such an increase in microbial efficiency is related to the specific toxic effects of FA on protozoa (Harfoot and Hazlewood 1997), thereby reducing wasteful recycling of microbial N (Dijkstra et al. 1998).

The simulations indicate that the duodenal FA composition of forage-based diets may be altered by changes in diet composition. However, further improvements are required if the model is to predict accurately individual FA flows to the duodenum with the ultimate aim to predict FA composition of body and milk fat. This concerns in particular improved description of rates of lipolysis and biohydrogenation and further separation of the aggregated pool of unsaturated FA into individual FA.

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