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Animal Feed Science and Technology
131 (2006) 389–417
ANIMAL FEED SCIENCE AND TECHNOLOGY
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Review

Factors affecting odd- and branched-chain fatty acids in milk: A review

B. Vlaeminck [a], V. Fievez [a,*], A.R.J. Cabrita [b], A.J.M. Fonseca [c], R.J. Dewhurst [d]

[a] *Laboratory for Animal Nutrition and Animal Product Quality, Faculty of Bioscience Engineering, Ghent University, Proefhoevestraat 10, 9090 Melle, Belgium*
[b] *CECA/ICETA, Faculdade de Ciências, Universidade do Porto, Rua Padre Armando Quintas, 4485-661 Vairão VC, Portugal*
[c] *CECA/ICETA, ICBAS, Instituto de Ciências Biomédicas de Abel Salazar, Universidade do Porto, Rua Padre Armando Quintas, 4485-661 Vairão VC, Portugal*
[d] *Agriculture and Life Sciences Division, Lincoln University, Canterbury, New Zealand*

Accepted 6 June 2006

Abstract

Odd- and branched-chain fatty acids (OBCFA) in milk fat are largely derived from bacteria leaving the rumen. The main OBCFA in milk of dairy cows are isomers of tetradecanoic acid (*iso* C14:0), pentadecanoic acid (C15:0, *iso* C15:0 and *anteiso* C15:0), hexadecanoic acid (*iso* C16:0) and heptadecanoic acid (C17:0, *iso* C17:0 and *anteiso* C17:0). There is an increasing interest in OBCFA as potential diagnostic tools of rumen function (*e.g.*, rumen fermentation pattern, bacterial N). Other reasons for interest in OBCFA are their anticarcinogenic effects on cancer cells, their influence on milk fat melting point and their potential as indicators of dairy product intake by humans. In this paper, we review recent literature on the topic, particularly in relation to effects of dietary treatments on milk OBCFA. *De novo* synthesis of OBCFA in rumen bacteria and animal tissue is discussed briefly. Milk secretion of linear odd-chain fatty acids (C15:0, C17:0) was higher than their duodenal flow suggesting *de novo* synthesis from propionate in animal tissue, whereas regression analysis suggested *cis*-9 C17:1 to be a desaturation product of C17:0. Variation in milk OBCFA induced by

Abbreviations: C, concentrate; CoA, coenzyme A; CP, crude protein; CV, coefficient of variation; F, forage; NDF, neutral detergent fibre; OBCFA, odd- and branched-chain fatty acids

* Corresponding author. Tel.: +32 9 264 90 02; fax: +32 9 264 90 99.
E-mail address: Veerle.Fievez@UGent.be (V. Fievez).

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doi:10.1016/j.anifeedsci.2006.06.017

dietary treatments is further evaluated and related to OBCFA composition of pure strains of rumen bacteria. An increase in the proportion of dietary forage generally increased milk OBCFA with the strongest effect on *iso* C14:0 and *iso* C15:0. In addition, forage source substantially affected milk OBCFA pattern with a decrease in *iso* C14:0 and *iso* C16:0 and increase in C17:0 and *cis*-9 C17:1 upon replacement of grass silage by maize silage. Finally, we relate the variation in milk OBCFA to dietary composition and rumen hydrogenation intermediates of dietary polyunsaturated fatty acids. Milk content of medium-chain fatty acids (C12:0, C14:0 and C16:0) was positively related with the linear odd-chain fatty acids and milk content of major hydrogenation intermediates (*i.e.*, *trans*-11 C18:1; *cis*-9, *trans*-11 C18:2; *trans*-11, *cis*-15 C18:2) increased with increasing *iso* C17:0, whereas a negative relationship occurred with *iso* C14:0 and *iso* C16:0. This review illustrates the potential of OBCFA as a diagnostic tool for rumen function both in relation to nutrient supply and optimization of milk fatty acid composition.
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Keywords: Odd- and branched-chain fatty acids; Milk; Rumen; Bacteria

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1. Introduction

Odd- and branched-chain fatty acids (OBCFA) only occur at trace levels in most plants (Diedrich and Henschel, 1990), but are distinct components of milk and adipose tissue in cattle (Polidori et al., 1993), sheep (Jenkins, 1995) and goats (Rojas et al., 1994), as well as other animals with symbiotic fermentations, such as beavers (Kakela et al., 1996). Over 40 years ago, Keeney et al. (1962) suggested that rumen bacteria were the major source

of OBCFA in ruminant milk. Indeed, recent work showed that less than 100 g/kg of milk OBCFA could have been derived directly from feed, even if all dietary OBCFA had been transferred to milk (Dewhurst et al., submitted for publication). Bacterial OBCFA are mainly present in bacterial membrane lipids (Kaneda, 1991; Mackie et al., 1991) and include: *iso* tetradecanoic acid, *iso* C14:0; pentadecanoic acid, C15:0; 13-methyltetradecanoic acid, *iso* C15:0; 12-methyltetradecanoic acid, *anteiso* C15:0; *iso* hexadecanoic acid, *iso* C16:0; heptadecanoic acid, C17:0; 15-methylhexadecanoic acid, *iso* C17:0; 14-methylhexadecanoic acid, *anteiso* C17:0 and heptadecenoic acid, *cis*-9 C17:1, though only in trace amounts (Fievez et al., 2003b) for the latter.

Levels of C15:0 and C17:0 in subcutaneous adipose tissue (Wolk et al., 1998) and serum (Smedman et al., 1999; Warensjö et al., 2004) have been used as markers of intake of ruminant fat by humans. This has been useful in identifying relationships between intake of ruminant products and disease outcomes. OBCFA have also been used as markers of colonization of freshly ingested grass by rumen microorganisms (Kim et al., 2005). In addition, some recent research has studied their potential as markers to quantify bacterial matter leaving the rumen (Vlaeminck et al., 2005), to provide a qualitative description of the proportions of different classes of microbes leaving the rumen (Vlaeminck et al., 2004a, 2006a) and to predict rumen ratios of volatile fatty acids (Vlaeminck et al., in press-c).

The OBCFA in ruminant products have also been of further interest because of their low melting points relative to chain length (Enser, 1984). High concentrations of OBCFA have been associated with softer fat in lambs (Garton et al., 1972). *Anteiso* fatty acids have particularly low melting points and are used by some bacteria to maintain membrane fluidity when exposed to low temperatures (*e.g.*, Annous et al., 1997).

Recent studies have shown that branched-chain fatty acids have anti-cancer activity. Yang et al. (2000) showed that *iso* C15:0 extracted from a fermented soybean product effectively inhibited growth of various cancer cell lines both *in vitro* and *in vivo*. Further *in vitro* studies have shown activity for a series of *iso* fatty acids, as well as *anteiso* C15:0 against human breast cancer cells (Wongtangtintharn et al., 2004). Interestingly, the cytotoxicity of these fatty acids was comparable to that of conjugated linoleic acid—a generally lesser component of milk fat which has received much greater attention as a potential anti-cancer agent (Lock and Bauman, 2004). Wongtangtintharn et al. (2004) showed that branched-chain fatty acids inhibited fatty acid synthesis in tumour cells, which is recognised as a useful route for developing cancer treatments since cancer cells are more dependent on fatty acid biosynthesis than healthy cells (Kuhajda, 2000).

We first discuss the origin and synthesis of OBCFA in rumen bacteria and the mammary gland in addition to recent literature data reporting milk OBCFA concentrations, as well as variation relative to differences in dietary treatments and its linkage to microbial systematics. Finally, correlations between milk OBCFA and major intermediates in rumen hydrogenation of dietary polyunsaturated fatty acids are presented and related to differences in rumen conditions. Ultimately, data on multi-branched-chain milk fatty acids, besides the formerly discussed microbially derived OBCFA, are reviewed.

2. Odd- and branched-chain fatty acids in bacteria and milk

2.1. Bacteria

2.1.1. De novo synthesis

De novo synthesis of saturated fatty acids in bacteria is mediated out by two types of fatty acid synthetases, being straight-chain and branched-chain fatty acid synthetase (Kaneda, 1991). The difference between straight-chain and branched-chain fatty acid synthetase is mainly related to the substrate specificity of the acyl-CoA:ACP transacylase (Kaneda, 1991). In the former, *de novo* synthesis of fatty acids is achieved by repeated condensation of malonyl-coenzyme A (CoA) with acetyl-CoA as primer, yielding palmitic acid as the dominant end product (Fulco, 1983). Linear odd-chain fatty acids are formed when propionyl-CoA, instead of acetyl-CoA, is used as primer (Fulco, 1983; Kaneda, 1991). Hence, straight-chain fatty acid synthetase accepts both acetyl-CoA and propionyl-CoA and the balance to which extent acetyl-CoA and propionyl-CoA are used is probably a function of the relative availability of both primers, rather than a reflection of an altered specificity of the fatty acid synthetase (Fulco, 1983). Consequently, the only difference in synthesis of straight- and branched-chain fatty acids lies in their respective primers and products (Fig. 1; Kaneda, 1991). Further, Emmanuel (1978) reported that pure cultures of rumen bacteria synthesised linear odd-chain fatty acids through α-oxidation. However, the relative importance of α-oxidation reactions remains hypothetical *in vivo* (Bauchart et al., 1990).

Three series of branched-chain fatty acid products can be distinguished, even *iso* acids (*iso* C14:0, *iso* C16:0), odd *iso* acids (*iso* C15:0, *iso* C17:0) and odd *anteiso* acids (*anteiso*

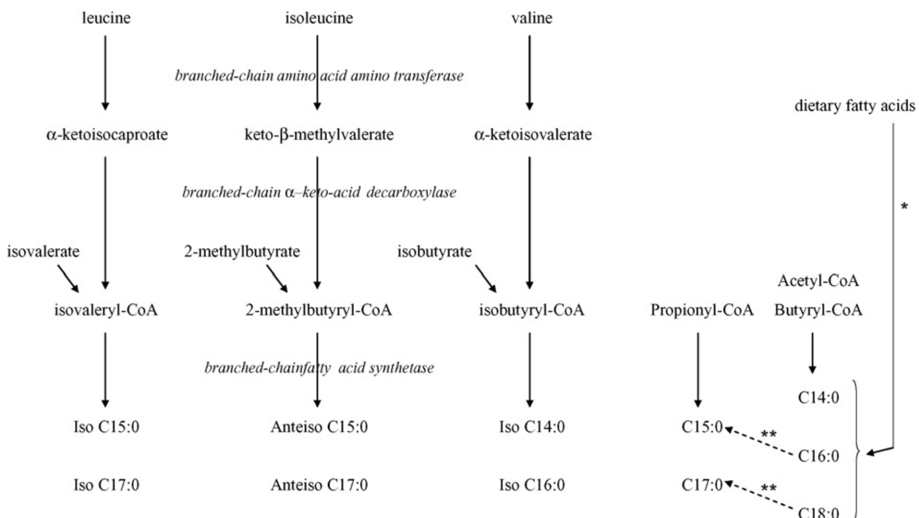

Fig. 1. Synthesis of odd- and branched-chain fatty acid in bacteria (based on Kaneda, 1977, 1991; Emmanuel, 1978; Annous et al., 1997). Asterisk (*) represents uptake of dietary fatty acids by rumen bacteria (Bauchart et al., 1990; Vlaeminck et al., 2006a); Double asterisk (**) represents α-oxidation of even-chain fatty acids (Emmanuel, 1978).

C15:0, *anteiso* C17:0) (Kaneda, 1977). According to Kaneda (1977), factors affecting the relative proportions of branched-chain fatty acids may be grouped into three categories, being: (i) the relative activity of primers towards the fatty acid synthetase; (ii) the relative availability of primers and (iii) the amount of chain extender. Factor (i) is characteristic of fatty acid synthetase and is fixed for a given micro-organism. Factors (ii) and (iii) are variable, depending upon physiological and culture conditions. Ifkovitz and Ragheb (1968) showed that the fatty acid composition was not altered appreciably by changes in the growth media used. In addition, there appeared to be no effect when bacteria were harvested either in the early or late logarithmic phases of growth (Ifkovitz and Ragheb, 1968). In contrast, Saluzzi et al. (1993) reported that addition of vitamins and volatile fatty acids to the culture medium resulted in changes in the relative proportions of some fatty acids. Nevertheless, they concluded that these changes were generally small relative to differences among species (Saluzzi et al., 1993). Hence, the OBCFA profile of rumen bacteria seems largely determined by the fatty acid synthetase of the micro-organism, and to a lesser extent by physiological and culture conditions. This suggests that variations in the profile of OBCFA leaving the rumen are mainly a reflection of changes in the relative abundance of specific bacterial populations in the rumen rather than altered bacterial fatty acid synthesis related to the availability of primers, as the latter differences are assumed to be small compared to the former.

2.1.2. Bacterial systematics

The analysis of microbial lipids has been used to estimate relative proportions of microbial species and groups in natural and complex bacterial communities (see Busse et al., 1996; Zelles, 1999). Although the OBCFA profile of many cultivable rumen bacteria has been reported (*e.g.*, Ifkovitz and Ragheb, 1968; Harfoot, 1978; Miyagawa et al., 1979; Miyagawa and Suto, 1980; Miyagawa, 1982; Minato et al., 1988; Saluzzi et al., 1993; Whitford et al., 2001; Kopecny et al., 2003), few attempts have so far been made to use these fatty acids for the analysis of mixed cultures of rumen bacteria. However, only a limited number of selected species have been studied and attempts to describe the composition of rumen samples in detail to a species level based on the OBCFA profile is not yet feasible. Nevertheless, such non-culture techniques, based on analysis of molecules of microbial origin, offers an elegant means to study shifts in rumen microbial ecology in response to, for example, dietary changes (Dehority and Orpin, 1988).

Cellulolytic bacteria contain high amounts of *iso*-fatty acids with *Ruminococcus flavefaciens* enriched in odd-chain *iso*-fatty acids and *Ruminococcus albus* in even-chain *iso*-fatty acids (Table 1), with *Prevotella* strains enriched in *anteiso* C15:0. The OBCFA profile for *Butyrivibrio* strains seems much more heterogeneous compared to the profile of other rumen bacteria (Table 1) suggesting that the genus *Butyrivibrio* is a heterogeneous bacterial taxon (Miyagawa, 1982). Marinsek Logar et al. (2001) reported that the grouping of *Butyrivibrio* strains based on fatty acids matched closely analysis based on 16S rDNA and RFLP analysis. The amylolytic bacteria *Succinivibrio dextrinosolvens*, *Succinimonas amylolytica*, *Ruminobacter amylophilus*, *Selenomonas ruminantium* and *Streptococcus bovis* showed low levels of branched-chain fatty acids and were relatively enriched in linear odd-chain fatty acids (Table 1).

Table 1
Odd- and branched-chain fatty acid profile of some predominant rumen bacteria (g/100 g fatty acids)

	Anteiso			Odd-*iso*			Even-*iso*		Odd linear				References
	C13:0	C15:0	C17:0	C13:0	C15:0	C17:0	C14:0	C16:0	C13:0	C15:0	C17:0	C17:1	
Ruminococcus flavefaciens													
Mean	–	2.3	2.9	–	35.7	5.2	2.5	7.3	0.1	3.2	0.5	–	Ifkovitz and Ragheb (1968), Harfoot (1976), Minato et al. (1988), Saluzzi et al. (1993)
FD1	–	3.5	2.3	–	42.2	2.3	1.8	1.1	0.1	6.9	0.2	–	Ifkovitz and Ragheb (1968), Saluzzi et al. (1993)
C94	–	4.6	1.4	–	32.6	0.7	2.9	13.2	0.7	2.3	2.4	–	Harfoot (1976), Minato et al. (1988)
Ruminococcus albus													
Mean	–	9.4	1.3	–	–	0.7	20.6	11.0	–	10.3	1.4	–	Ifkovitz and Ragheb (1968), Harfoot (1976), (4) Minato et al. (1988)
7	–	9.3	2.0	–	–	1.1	11.1	12.2	–	15.4	2.4	–	Ifkovitz and Ragheb (1968), Minato et al. (1988)
Fibrobacter succinogenes													
Mean	3.9	7.7	1.2	–	0.1	0.2	3.6	3.4	9.0	30.2	2.1	–	Ifkovitz and Ragheb (1968), Minato et al. (1988), Miyagawa et al. (1979), Saluzzi et al. (1993)
19169	0.7	10.1	2.0	0	0.1	0.3	1.6	2.3	2.0	37.8	2.9	–	Minato et al. (1988), Miyagawa et al. (1979)
S85	8.9	6.7	1.6	–	–	0.2	3.5	2.5	11.0	21.5	3.1	–	Ifkovitz and Ragheb (1968), Saluzzi et al. (1993)

Butyrivibrio fibrisolvens													
Mean	6.4	16.2	8.6	6.8	10.4	5.7	10.8	11.1	2.9	7.8	4.2	3.5	Ifkovitz and Ragheb (1968), Kopecny et al. (2003), Minato et al. (1988), Miyagawa (1982)
Group 1	6.4	22.3	11.5	–	1.4	1.6	13.9	17.3	2.1	3.0	1.0	–	Miyagawa (1982)
Group 2	–	2.4	5.1	–	1.8	3.2	1.5	0.2	1.9	5.6	3.6	3.6	Ifkovitz and Ragheb (1968), Kopecny et al. (2003), Minato et al. (1988), Miyagawa (1982)
Group 3	–	–	3.7	6.8	15.4	8.0	–	2.2	4.6	16.8	7.0	3.2	Miyagawa (1982)
Prevotella													
Mean	1.2	36.7	4.2	3.0	14.7	2.3	3.3	3.0	1.2	12.1	1.2	–	Minato et al. (1988), Miyagawa (1982), Miyagawa and Suto (1980), Miyagawa et al. (1979)
19188	0.9	28.4	5.9	1.7	16.5	4.9	2.2	4.5	0.8	16.9	0.6	–	Minato et al. (1988), Miyagawa and Suto (1980), Miyagawa et al. (1979)
19189	0.6	36.3	2.1	4.0	10.8	1.2	2.1	1.2	0.8	9.1	1.5	–	Minato et al. (1988), Miyagawa (1982), Miyagawa and Suto (1980), Miyagawa et al. (1979)
Succinivibrio dextrinosolvens													
Mean	0.8	3.6	1.0	–	0.1	–	0.6	1.5	0.5	4.0	0.7	–	Ifkovitz and Ragheb (1968), Harfoot (1976), Minato et al. (1988), Miyagawa (1982)
Group 1	4.6	19.6	5.2	–	–	–	3.0	8.0	0.1	8.6	1.3	–	Ifkovitz and Ragheb (1968), Harfoot (1976)
Group 2	0	0.1	0.1	0.1	0.1	–	0.1	0.1	0.5	3.0	0.6	–	Minato et al. (1988), Miyagawa (1982)
Succinimonas amylolytica													
N6	–	–	–	–	52.6	10.8	1.6	5.3	1.6	5	–	–	Ifkovitz and Ragheb (1968)
B24	–	–	–	–	0.1	0.3	–	0.6	1.4	3.3	1.2	0.6	Minato et al. (1988)

Table 1 (*Continued*)

	Anteiso			Odd-*iso*			Even-*iso*		Odd linear				References
	C13:0	C15:0	C17:0	C13:0	C15:0	C17:0	C14:0	C16:0	C13:0	C15:0	C17:0	C17:1	
Ruminobacter amylophilus													
Mean	–	1.1	–	–	–	–	–	–	0.5	1.1	0.3	0.1	Ifkovitz and Ragheb (1968), Minato et al. (1988), Miyagawa et al. (1979)
Selenomonas ruminantium													
Mean	–	0.1	–	–	0.2	–	0.3	0.1	1.3	6.0	2.9	2.6	Ifkovitz and Ragheb (1968), Harfoot (1976), Minato et al. (1988), Miyagawa (1982)
Streptococcus bovis													
Mean	–	0.9	–	–	–	–	0.4	0.2	0.6	1.7	1.2	0.2	Ifkovitz and Ragheb (1968), Harfoot (1976), Minato et al. (1988)
Megasphaera elsdenii													
Mean	–	2.8	–	0.1	0.2	0.2	1.5	0.5	1.5	6.0	4.5	3.0	Ifkovitz and Ragheb (1968), Harfoot (1976), Minato et al. (1988)
Lachnospira multiparus													
Mean	–	4.0	2.6	–	1.1	1.1	1.2	1.8	0.3	2.9	0.8	0.1	Ifkovitz and Ragheb (1968), Minato et al. (1988), Whitford et al. (2001)
Eubacterium ruminantium													
B1C23	–	–	–	–	17.7	1.4	–	–	5.4	49.0	1.5	–	Ifkovitz and Ragheb (1968)
GA195	–	30.1	1.7	–	0.4	0.2	6.1	3.7	0.4	6.5	0.4	–	Minato et al. (1988)

In conclusion, large differences in the OBCFA profile observed among rumen bacteria suggest they could be useful in assessment of the composition of, or shifts in, the rumen microbial population (Saluzzi et al., 1995; Marinsek Logar et al., 2001; Vlaeminck et al., 2004a).

2.2. Synthesis in the mammary gland

Keeney et al. (1962) suggested that substantial quantities of OBCFA in milk fat are derived from incorporation of OBCFA of rumen bacterial lipids whereas endogenous synthesis was limited. Although linear odd-chain fatty acids, or their *anteiso* isomers, can be *de novo* synthesised in the mammary gland through incorporation of propionyl-CoA instead of acetyl-CoA, or methylmalonyl-CoA instead of malonyl-CoA, respectively (Horning et al., 1961; Smith, 1994), the contribution of the latter processes to OBCFA in milk from dairy cows was negligible (Croom et al., 1981). Nevertheless, several studies have shown that linear odd-chain fatty acids (C15:0 and C17:0) can be synthesised *de novo* from propionate in adipose tissue and the mammary gland of ruminants (Scaife et al., 1978; Dodds et al., 1981; Massart-Leën et al., 1983). Indeed, by compiling literature data, it was found that milk secretion of linear odd-chain fatty acids was higher than duodenal flow of these fatty acids (Fig. 2). Moreover, the higher ratio of linear odd-chain fatty acids to C18:0 + *cis*-9 C18:1 in milk, compared to blood plasma (0.079 *versus* 0.065; $n = 17$; P=0.084; Loor et al., 2005b,c; Kay et al., 2005, Fievez et al., unpublished) is an additional indication for partial synthesis of linear odd-chain fatty acids in the mammary gland.

Propionate may also be indirectly involved in synthesis of some branched-chain fatty acids through incorporation of methylmalonyl-CoA, its carboxylation product. Indeed, incorporation of methylmalonyl-CoA at the first step of chain-elongation would result in *anteiso*-fatty acids. When methylmalonyl-CoA is incorporated during the subsequent steps

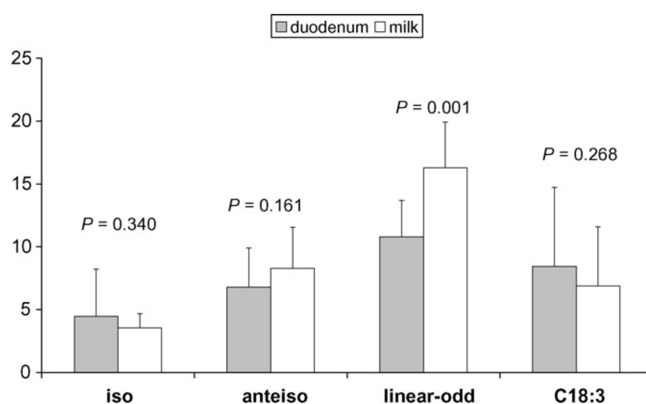

Fig. 2. Comparison of the duodenal flow and milk secretion of odd- and branched-chain fatty acids (g/d) ($n = 23$) (data from Dewhurst et al., 2003; Shingfield et al., 2003; Loor et al., 2004, 2005b,c,d; Vlaeminck et al., 2004d; Dewhurst et al., submitted for publication). Differences between duodenal flow and milk secretion of odd- and branched-chain fatty acids were evaluated with a paired *t*-test.

of chain-elongation, branched-chain fatty acids are formed with a methyl substituent on one of the carbon atoms between the antepenultimate carbon and the carboxyl end. The latter branched-chain fatty acids were found in adipose tissue triacylglycerols of young sheep and goats (Garton et al., 1972; Duncan and Garton, 1978; Berthelot et al., 2001) and in milk fat of goats (Massart-Leën et al., 1981; Alonso et al., 1999). However, analysis of cows milk demonstrated that branched-chain fatty acids other than *iso*- and *anteiso*-fatty acids are essentially absent (Duncan and Garton, 1978; Massart-Leën et al., 1981), consistent with low incorporation rates of methylmalonyl-CoA in fatty acids by incubation of bovine mammary tissue slices (Croom et al., 1981). Hence, there appear to be species differences between cattle and sheep or goats in utilization of methylmalonyl-CoA for fatty acid synthesis.

Less information is available on the ability of the mammary gland to use isovaleryl-CoA, 2-methylbutyryl-CoA and isobutyryl-CoA as primers for fatty acid synthesis. Verbeke et al. (1959), who evaluated incorporation of ^{14}C-isovaleric acid into milk fatty acids, found no selective incorporation of isovalerate into branched-chain fatty acids. Duodenal flow and milk secretion did not differ for the branched-chain fatty acids (Fig. 2). Assuming a duodenal fatty acid digestibility of 0.826 (Romo et al., 2000), milk secretion of *iso*-fatty acids was similar to the amount absorbed from the duodenum (3.69 ± 3.09 *versus* 3.56 ± 1.14, $n = 23$, P=0.868), suggesting limited *de novo* synthesis. The higher secretion into milk of *anteiso*-fatty acids, compared with their duodenal absorption (5.61 ± 2.56 *versus* 8.31 ± 3.25, $n = 23$, P=0.010), might be indicative of endogenous *de novo* synthesis. However, this observation was mainly due to an increase in *anteiso* C17:0 milk secretion (1.36 ± 0.45 *versus* 5.03 ± 0.99), whereas milk secretion and absorbed *anteiso* C15:0 were similar (4.14 ± 0.85 *versus* 4.58 ± 1.42, $n = 14$, P=0.261). In addition, the ratio of *anteiso* C15:0 to C18:0 + *cis*-9 C18:1 was comparable in milk and blood plasma (0.022 *versus* 0.029; $n = 14$; P=0.101; Loor et al., 2005b,c; Kay et al., 2005, Fievez et al., unpublished), suggesting *de novo* synthesis of *anteiso* C15:0 in the mammary gland is limited. Accordingly, high milk secretions of *anteiso* C17:0, reported in some studies, might have been due to erroneous analytical results rather than by *de novo* synthesis of this fatty acid in the mammary gland. Indeed, contamination of the peak with *cis*-9 C16:1, which can co-elute with *anteiso* C17:0, has been observed before (Precht and Molkentin, 2000).

Differences in the proportion of *cis*-9 C17:1 in duodenum, blood plasma and milk indicates this fatty acid is partially formed in the udder (Fievez et al., 2003b; Kay et al., 2005). Recently, Fievez et al. (2003b) suggested that this fatty acid was endogenously produced from C17:0 by Δ^9-desaturase activity, consistent with a product/precursor relationship, with the milk fat concentration of *cis*-9 C17:1 and C17:0 closely related (Fig. 3). Based on the model proposed by Palmquist et al. (2004), we calculated that 280 g/kg of C17:0 was desaturated in the mammary gland. Using the same data, we calculated that 286 g/kg of *trans*-11 C18:1 was desaturated to *cis*-9, *trans*-11 C18:2. A similar value of 278 g/kg was recently reported (Shingfield et al., 2005a), supporting the Palmquist et al. (2004) model. Similar values for proportions of C17:0 and *trans*-11 C18:1 desaturated in the mammary gland suggests the Δ^9-desaturase enzyme has a similar affinity for these fatty acids.

In conclusion, milk linear odd-chain fatty acids are partially synthesized in the mammary gland, as shown by the higher milk secretion of these fatty acids compared to duodenal flow,

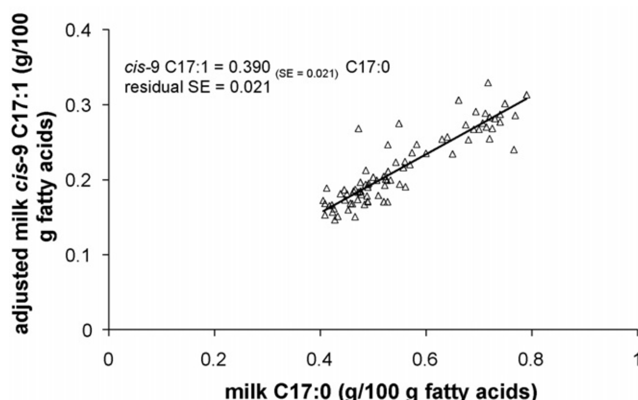

Fig. 3. Relation between milk C17:0 and *cis*-9 C17:1 ($n = 83$) (data from Khorasani et al., 2001; Dewhurst et al., 2003; Kraft et al., 2003; Shingfield et al., 2003; Adesogan et al., 2004; Al-Mabruk et al., 2004; Jurjanz et al., 2004; Nielsen et al., 2004; Vlaeminck et al., 2004c,d; Fievez et al., 2005; Jones et al., 2005; Loor et al., 2005a,b,c; Shingfield et al., 2005b; Van Nespen et al., 2005; Dewhurst et al., submitted for publication; Vlaeminck et al., unpublished).

whereas Δ^9-desaturase activity in the mammary gland is responsible for conversion of C17:0 to *cis*-9 C17:1.

3. Odd- and branched-chain fatty acids in milk

There are increasing numbers of publications reporting milk OBCFA. A total of 26 studies were found which presented data of milk OBCFA representing 138 treatment means (Khorasani et al., 2001; Salawu et al., 2002; Cabrita et al., 2003; Dewhurst et al., 2003; Kraft et al., 2003; Shingfield et al., 2003; Adesogan et al., 2004; Al-Mabruk et al., 2004; Collomb et al., 2004; Jurjanz et al., 2004; Nielsen et al., 2004; Singh et al., 2004; Vlaeminck et al., 2004c,d; Fievez et al., 2005; Jones et al., 2005; Loor et al., 2005a,b,c; Rego et al., 2005; Shingfield et al., 2005b,c; Van Nespen et al., 2005; Dewhurst et al., submitted for publication; Vlaeminck et al., unpublished). Only studies which reported all saturated fatty acids (*i.e.*, C4:0 to C18:0) were retained for statistical analysis. Milk fatty acid profiles as reported in these publications were used to study correlations between milk OBCFA and diet composition (Section 4.4) or hydrogenation intermediates in milk (Section 5).

Summary statistics (Table 2) suggest that linear odd-chain fatty acids accounted for the majority of milk OBCFA (494 g/kg of total OBCFA) followed by *anteiso*-fatty acids (272 g/kg). Branched-chain fatty acids with 15 carbon atoms were more abundant than shorter and longer branched-chain fatty acids. Table 2 also illustrates the large variation in milk OBCFA concentrations, although linear odd-chain fatty acids and *anteiso* C15:0 were the least variable (*i.e.*, coefficient of variation (CV) below 20%). Other branched-chain fatty acid CV's exceeded 20%, with a CV of more than 50% for *iso* C13:0 and *iso* C17:0.

Table 2
Overview of milk odd- and branched-chain fatty acid concentrations (g/100 g fatty acids)

	n	Mean	Median	S.D.	Minimum	Maximum
iso C13:0	64	0.040	0.027	0.036	0.011	0.144
iso C14:0	82	0.089	0.083	0.031	0.038	0.179
iso C15:0	103	0.224	0.210	0.059	0.124	0.510
iso C16:0	80	0.209	0.201	0.050	0.115	0.370
iso C17:0	93	0.272	0.214	0.164	0.124	0.910
anteiso C13:0	64	0.083	0.080	0.024	0.025	0.135
anteiso C15:0	101	0.462	0.451	0.090	0.290	0.760
anteiso C17:0	73	0.501	0.489	0.125	0.150	0.790
C15:0	103	1.104	1.097	0.164	0.780	1.640
C17:0	103	0.557	0.530	0.103	0.405	0.790
cis-9 C17:1	83	0.207	0.203	0.072	0.005	0.482

4. Dietary effects on milk odd- and branched-chain fatty acids

4.1. Forage:concentrate ratio

Increasing the forage:concentrate (F:C) ratio in the diet resulted in a higher proportion of milk OBCFA (Table 3). In addition, the increase of *iso* C14:0 and *iso* C15:0 in total OBCFA was higher than with *anteiso* C15:0, which decreased in some studies (*e.g.*, Shingfield et al., 2005b,c). These changes in milk concentrations of *iso*-fatty acids and *anteiso* C15:0 might reflect differences in the rumen bacterial populations induced by variation in the dietary F:C ratio. The effect of the F:C ratio on rumen bacterial populations was studied by Latham et al. (1971, 1972), Oshio et al. (1987) and Tajima et al. (2001). In general, decreasing the F:C ratio increased the relative importance of amylolytic bacteria (*e.g.*, *Prevotella ruminicola*, *Ruminobacter amylophilus*, *Succinivibrio dextrinosolvens*) and decreased cellulolytic bacteria (*e.g.*, *Ruminococcus flavefaciens*, *Butyrivibrio fibrisolvens*). Based on the OBCFA profile of rumen bacteria (Table 1), it might be expected that an increased proportion of cellulolytic bacteria would result in higher *iso*-fatty acids, whereas an increased proportion of amylolytic bacteria possibly increases *anteiso* and linear odd-chain fatty acids. Indeed, using the proportions of rumen bacteria reported by Latham et al. (1971, 1972), Oshio et al. (1987), Tajima et al. (2001) and the fatty acid profiles of these bacteria reported in Table 1, we calculate a relative increase in *anteiso*-fatty acids, and a relative decrease of *iso*-fatty acids upon replacement of a high forage diet with a high concentrate diet. These findings are consistent with results reported for mixed rumen bacteria (Bas et al., 2003; Vlaeminck et al., 2006a). Results of Bas et al. (2003) illustrate that the bacterial content of *iso* C14:0 increased the most when the F:C ratio increased. Vlaeminck et al. (2006a) reported a similar increase in *iso* C15:0 in bacteria isolated from the liquid and solid phases of the rumen due to an increased dietary F:C ratio. In addition, both studies indicated a strong negative correlation between dietary forage proportion and *anteiso* C15:0 in total OBCFA of the bacteria ($r_{\text{pearson}} = -0.771$, P=0.009, $n = 10$).

Besides changes in the rumen microbial population, the decreasing ratio of odd-chain *iso* to *anteiso*-fatty acids with decreasing F:C ratio also might be the result of stress stimuli, as shown in other biological systems (Kieft et al., 1994; Annous et al., 1997). Hence, changes

Table 3
Effect of forage:concentrate ratio (F:C) on milk odd- and branched-chain fatty acids (g/100 g fatty acids)

References	F:C	*iso* C14:0	*iso* C15:0	*iso* C16:0	*iso* C17:0	*anteiso* C15:0	*anteiso* C17:0	C15:0	C17:0	*cis*-9 C17:1
Salawu et al. (2002)	0.63:0.37	–	0.29	–	0.40	0.51	0.38	1.23	0.53	0.21
	0.53:0.47	–	0.28	–	0.36	0.50	0.37	1.13	0.44	0.16
Loor et al. (2005b)	0.64:0.36	0.133	0.356	0.298	–	0.750	–	1.29	0.720	0.205
	0.34:0.66	0.085	0.223	0.252	–	0.609	–	1.46	0.768	0.236
Shingfield et al. (2005b)[a]	0.74:0.26	0.08	0.21	0.21	0.74	0.39	–	1.22	0.63	–
	0.38:0.62	0.06	0.19	0.21	0.80	0.47	–	1.12	0.60	–
Shingfield et al. (2005c)	0.68:0.32	0.146	0.263	–	0.148	0.486	0.383	1.22	0.73	0.244
	0.57:0.43	0.137	0.243	–	0.147	0.493	0.380	1.17	0.71	0.238
Dewhurst et al. (submitted for publication)	0.80:0.20	0.101	0.304	0.184	0.230	0.477	0.666	1.43	0.661	0.365
	0.35:0.65	0.081	0.234	0.195	0.189	0.428	0.579	1.04	0.490	0.230

–, Not reported.
[a] Diets supplemented (30 g/kg DM) with a mixture (2:3, w/w) of fish and sunflower oils.

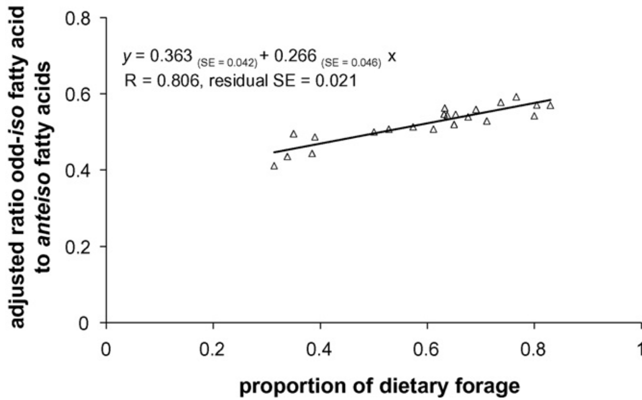

Fig. 4. Relation between proportion of dietary forage and the ratio odd-chain *iso*-fatty acids (*iso* C15:0 and *iso* C17:0) to *anteiso*-fatty acids (*anteiso* C15:0 and *anteiso* C17:0) ($n = 22$) (data from Salawu et al., 2002; Dewhurst et al., 2003; Adesogan et al., 2004; Loor et al., 2005b; Shingfield et al., 2005b,c; Dewhurst et al., submitted for publication).

in the rumen environment brought about by a changed F:C ratio (*e.g.*, decrease in rumen pH) possibly initiated mechanisms by rumen bacteria which resulted in a decreased ratio of odd-chain *iso*-fatty acids to *anteiso*-fatty acids (Fig. 4). Another response mechanism of some bacteria is isomerisation of *cis* to *trans* unsaturated fatty acids (Keweloh and Heipieper, 1996; Heipieper et al., 2003) which could partially explain the increased amount of *trans* fatty acids when F:C decreases (*e.g.*, Kalscheur et al., 1997; Loor et al., 2005b; Shingfield et al., 2005b) as previously suggested by Bessa et al. (2000). However, it remains unclear to what extent changes in the ratio of *iso* to *anteiso*-fatty acids and ruminal formation of specific hydrogenation intermediates are due to changes in the rumen bacterial population or a bacterial response to stress stimuli.

Effects of variation in the dietary F:C ratio on linear odd-chain fatty acids was not uniform over all studies (Table 3). Differences among studies might be related to the occurrence, or not, of changes in rumen fermentation. Indeed, the increasing F:C ratio affected molar proportions of ruminal propionate in the experiment of Loor et al. (2005b), whereas no changes in ruminal fermentation parameters occurred in the experiments of Shingfield et al. (2005c) and Dewhurst et al. (submitted for publication). Moreover, the nature of the forage supplied (*e.g.*, maize silage; Shingfield et al., 2005b) also might mask some expected changes in the milk OBCFA profile (see also Section 4.2).

4.2. *Influence of the nature of forages: maize silage versus grass silage*

Effects of forage type (*i.e.*, maize silage *versus* grass silage) on milk OBCFA (Table 4) could reflect changes in both substrates and the rumen environment. Replacing grass silage with maize silage increased dietary starch and decreased dietary neutral detergent fibre (NDF; Nielsen et al., 2004; Shingfield et al., 2005b), which could substantially affect rumen pH, microbial populations and proportions of volatile fatty acids produced.

Table 4
Effect of replacing grass silage (GS) with maize silage (MS) on milk odd- and branched-chain fatty acids (g/100 g fatty acids)

	Shingfield et al. (2005b)		Vlaeminck et al. (unpublished)			Nielsen et al. (2004)	
	GS	MS	GS	GS/MS	MS	GS	MS
iso C14:0	0.08	0.06	0.086	0.073	0.052	0.098	0.060
iso C15:0	0.21	0.18	0.241	0.218	0.166	0.202	0.152
iso C16:0	0.21	0.23	0.175	0.176	0.162	0.162	0.154
iso C17:0	0.74	0.91	0.188	0.184	0.230	0.215	0.291
anteiso C15:0	0.39	0.37	0.458	0.466	0.457	0.424	0.428
anteiso C17:0	–	–	0.456	0.479	0.553	0.430	0.471
C15:0	1.22	0.78	0.947	0.980	1.218	1.357	0.922
C17:0	0.63	0.54	0.478	0.490	0.549	0.521	0.390
cis-9 C17:1	–	–	0.196	0.186	0.291	0.223	0.207

–, Not reported.

When grass silage was replaced with maize silage, *iso* C14:0 and *iso* C15:0 decreased (Table 4). In addition, maize silage decreased the ratio of odd-chain *iso*-fatty acids to *anteiso*-fatty acids (0.490 *versus* 0.446, P=0.064, $n = 5$) and a negative relationship of this ratio with the dietary starch:NDF ratio has occurred ($r_{\text{pearson}} = -0.765$; P=0.004; $n = 12$). These effects are similar to those observed for a decrease in F:C ratio, as described above (Section 4.1), suggesting the relative contribution of amylolytic bacteria increased upon replacement of grass silage by maize silage and/or a reaction of rumen bacteria to stress stimuli (*e.g.*, a decrease in rumen pH) as described above.

Inclusion of maize silage increased milk levels of *iso* C17:0 and *anteiso* C17:0 (Table 4). Vlaeminck et al. (2006a) suggested bacterial *iso* C17:0 was related with bacterial growth rate as indicated by its strong relation with the adenine:N ratio in rumen bacteria. Microbial protein synthesis is energetically more efficient for maize silage *versus* grass silage based diets (Givens and Rulquin, 2004), which might explain the increase in milk *iso* C17:0. Cabrita et al. (2003) reported negative correlations between dietary crude protein (CP) content and milk proportions of *iso* C17:0 and *anteiso* C17:0. Indeed, in the studies in Table 4, dietary CP was similar (Shingfield et al., 2005b) and lower (Nielsen et al., 2004; Vlaeminck et al., unpublished) for the maize silage based diets, which promoted higher milk levels of *iso* C17:0 and *anteiso* C17:0, which will be further discussed in Section 4.4.

Inclusion of maize silage in the diet affected milk proportions of linear odd-chain fatty acids differently (Table 4). Differences among reported experiments in rumen propionate proportions and the dual origin of these fatty acids in milk (*i.e.*, rumen microbial and mammary gland; Section 2.2) could explain the inconsistency of results. The increase of *cis*-9 C17:1 (Vlaeminck et al., unpublished) suggests an increase in mammary Δ^9-desaturase activity, which could be induced by higher blood glucose levels (Ntambi and Miyazaki, 2004) due to higher ruminal propionate proportions (Fitzgerald and Murphy, 1999; Vlaeminck et al., 2004b) or higher duodenal glucose absorption (Nocek and Tamminga, 1991).

4.3. Influence of lipid supplements

Inhibitory effects of long-chain fatty acids on rumen bacteria is well documented. The severity of this inhibitory effect of fatty acids on rumen bacteria is higher with increasing unsaturation of long-chain fatty acids and with the *cis versus trans* configuration (Demeyer and Henderickx, 1967; Galbraith et al., 1971; Maczulac et al., 1981). However, not all types of bacteria are affected in the same way with growth of cellulolytic strains being more reduced than that of amylolytic ones, Gram positive being more sensitive than Gram negative, and large differences occurring between species or even strains (Galbraith et al., 1971; Maczulac et al., 1981). Hence, differences in the severity of these effects on the bacterial population could be expected when supplemented fat contains high amounts of either C18:2 *n*-6 (*e.g.*, sunflower oil), C18:3 *n*-3 (*e.g.*, linseed oil) or C20:5 *n*-3 and C22:6 *n*-3 (*e.g.*, fish oil) which is illustrated by changes in rumen fermentation patterns (*e.g.*, Fievez et al., 2003a, submitted for publication). Analysis of milk OBCFA seems to agree with the reported effects (Fig. 5). Dairy cows fed supplemental fat rich in C18:2 *n*-6 (Collomb et al., 2004; Fievez et al., 2005; Rego et al., 2005) and C18:3 *n*-3 (Collomb et al., 2004; Loor et al., 2005b) had lower proportions of milk OBCFA (Fig. 5). In contrast, supplementing fish oil or marine algae increased milk OBCFA (Fig. 5; Shingfield et al., 2003; Singh et al., 2004; Loor et al., 2005a). This increase in OBCFA might indicate increased duodenal flow of rumen bacteria (Vlaeminck et al., 2005). Indeed, feeding fish oil resulted in a numeric trend to an increase in fibre digestion (Doreau and Chilliard, 1997) and higher urinary excretion of purine derivatives (Fievez et al., 2003a). In contrast, Wachira et al. (2000) reported that dietary inclusion of fish oil decreased microbial protein synthesis.

Supplementation of C18:2 *n*-6 and C18:3 *n*-3 had only minor effects on even-chain *iso*-fatty acids whereas addition of fish oil or marine algae reduced these fatty acids to 60% *versus* the control diet (Fig. 5). The odd-chain *iso*-fatty acids increased with an increasing

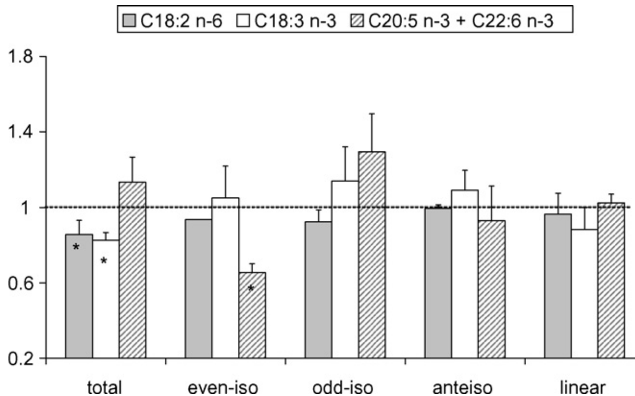

Fig. 5. Effect of supplementing fat on total milk odd- and branched-chain, odd-chain *iso* (*iso* C15:0, *iso* C17:0), even-chain *iso* (*iso* C14:0, *iso* C16:0), odd-chain *anteiso* (*anteiso* C15:0, *anteiso* C17:0) and linear odd-chain (C15:0, C17:0 and *cis*-9 C17:1) fatty acids. Values are presented compared to the control diet. Asterisk (*) significantly different from 1 (data from Shingfield et al., 2003; Collomb et al., 2004; Singh et al., 2004; Fievez et al., 2005; Jones et al., 2005; Loor et al., 2005a,b; Rego et al., 2005).

Table 5
Correlation coefficients between dietary proximate composition[a] (g/kg DM) and milk odd- and branched-chain fatty acids (g/100 g fatty acids) (values in parentheses are the number of observations)[b]

	NDF	Starch	CP	FA
iso C14:0	0.737 (50)	−0.886 (46)		−0.667 (49)
iso C15:0	0.830 (62)	−0.667 (55)	−0.537 (75)	−0.582 (56)
iso C16:0	0.297 (53)	−0.373 (44)		−0.587 (41)
iso C17:0			−0.328 (65)	0.855 (49)
anteiso C15:0	0.599 (62)	−0.538 (53)	−0.266 (73)	−0.698 (56)
anteiso C17:0	0.569 (37)		−0.806 (50)	−0.579 (43)
C15:0		0.279 (55)		−0.485 (56)
C17:0		0.296 (55)	−0.381 (75)	−0.811 (56)
cis-9 C17:1		0.658 (42)	−0.677 (59)	−0.462 (45)

[a] CP: crude protein; FA: fatty acids.
[b] Only significant (*i.e.*, $P<0.05$) correlations are shown.

degree of unsaturation of the supplemented fatty acids. When fish oil or marine algae were supplemented to the diet, the increase in odd-chain *iso*-fatty acids was mainly due to *iso* C17:0. Compared with the control diet, *iso* C17:0 increased by 95% (Shingfield et al., 2003; Jones et al., 2005) or 360% (Singh et al., 2004) when fish oil (Shingfield et al., 2003), fish and sunflower oil (Jones et al., 2005) or marine algae (Singh et al., 2004) were supplemented.

As fish oil or marine algae were reported to shift rumen fermentation to increased rumen propionate production (Fievez et al., 2003a, submitted for publication; Shingfield et al., 2003), an increased concentration of linear odd-chain fatty acids could have been expected in the milk, but this has not occurred (Fig. 5). It is possible that an increase in endogenous synthesis of linear odd-chain fatty acids, through increased precursor supply, was counteracted by reduced *de novo* synthesis in the mammary gland upon fish oil or micro-algae supplementation (Chilliard et al., 2001; Loor et al., 2005a).

4.4. Relation with dietary proximate composition

Variation in OBCFA profiles of milk fat were related to dietary nutrient composition. To assess potential effects of diet nutrients on milk OBCFA, a meta-analysis (St-Pierre, 2001) was conducted using a mixed model regression analysis in which milk OBCFA were the dependent variable. Adjusted observations were calculated by adding the residual from each individual observation to the predicted value of the study regression. These adjusted observations for study effects were used to calculate Pearson correlation coefficients. Results (Table 5) show relationships between dietary substrate supply and these milk fatty acids, suggesting different resultant classes of microbes in the rumen.

Dietary starch content was negatively correlated with *iso* C14:0, *iso* C15:0 and *iso* C16:0. As described in Sections 4.1 and 4.2 for varying concentrate or maize silage proportions, strong correlations of dietary NDF and starch with *iso* C14:0 and *iso* C15:0 probably indicates the relative importance of cellulolytic and amylolytic bacteria in the rumen. Indeed, Weimer et al. (1999) indicated that the ruminal cellulolytic bacterial population tended to increase with a higher NDF content of the diet. This might explain the higher levels of these fatty acids, as reported by Shingfield et al. (2005c), for a hay based diet (0.179 and

0.304 for *iso* C14:0 and *iso* C15:0, respectively) and by Kraft et al. (2003) where cows were grazing alpine pastures (0.168 and 0.340 for *iso* C14:0 and *iso* C15:0, respectively). Low milk levels of *iso* C14:0 (0.04) were reported by Jurjanz et al. (2004), and milk in the study reported by Van Nespen et al. (2005) showed low levels of both *iso* C14:0 and *iso* C15:0 (0.038 and 0.137, respectively). In the experiment of Jurjanz et al. (2004), cows were fed maize silage with a rapidly degraded starch that led to a strong postprandial drop in pH, probably inhibiting some cellulolytic bacteria. A similar effect could be expected with cows in early lactation receiving a highly digestible diet rich in starch (Van Nespen et al., 2005). Increasing starch content of the diet resulted in an increase in linear odd-chain fatty acids similar to shifts observed upon replacement of grass by maize silage (Section 4.2).

Although dietary NDF and starch contents were, respectively, positively and negatively correlated with *anteiso* C15:0, only NDF content correlated with *anteiso* C17:0. Additionally, decreasing dietary CP was associated with a lower milk OBCFA content, with the strongest negative correlation observed with *anteiso* C17:0. The current data set did not allow more detailed evaluation of effects of CP type, or its rate of rumen degradation, on milk OBCFA. Nevertheless, the negative correlation between ruminal NH_3 concentrations and milk *anteiso* C17:0 concentrations ($r_{pearson} = -0.644$, $P<0.05$, $n = 30$) confirms earlier suggestions that this fatty acid has potential as a marker of rumen CP availability and/or deficiency (Cabrita et al., 2003). Compared to *iso* and linear fatty acids, *anteiso* fatty acids have a greater effect on bacterial membrane properties (Kaneda, 1977). Hence, increased *anteiso* C17:0 concentrations might indicate the occurrence of some defence mechanisms of bacteria to maintain proper fluidity of membrane lipids in stress situations provoked by a deficit of rumen degradable CP.

It is well known that dietary fatty acids inhibit *de novo* synthesis of fatty acids by microbes (Demeyer et al., 1978; Emmanuel, 1978), and OBCFA decreased with increasing dietary fatty acid content, except for *iso* C17:0 (Table 5). Of note, this fatty acid is strongly correlated with biohydrogenation intermediates, which will be discussed further in Section 5.

4.5. Potential of OBCFA as diagnostic tool for rumen function

Volatile fatty acids and microbial protein from ruminal degradation of feed constituents account for the majority of nutrients utilized by the host animal. The relative concentrations of the individual VFA, mainly acetate, propionate and butyrate, are important determinants of energy utilization by ruminants. In addition, duodenal flow of microbial protein supplies about 60% of the protein available for absorption in lactating dairy cattle. Hence, in practical dairy cattle feeding, as well as for research purposes, development of non-invasive methods that provide insight on rumen fermentation and duodenal supply of microbial protein is important.

4.5.1. Duodenal flow of bacterial CP

Keeney et al. (1962) suggested that bacterial OBCFA might be used as markers to quantify bacterial matter leaving the rumen. Since then, little attention has been paid to these fatty acids in relation with duodenal flow of microbial CP. Nevertheless, recent research showed duodenal flow of bacterial CP was closely related to duodenal flow (Vlaeminck et al., 2006b)

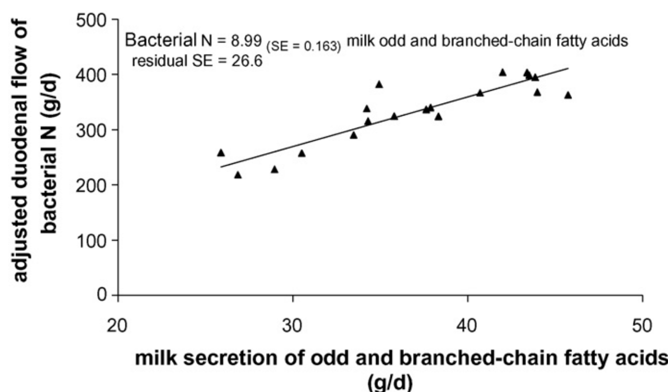

Fig. 6. Relation between milk secretion of odd- and branched-chain fatty acids and duodenal flow of microbial N (data from Cabrita et al., 2003; Dewhurst et al., 2003; Vlaeminck et al., 2005).

and milk secretion of OBCFA (Vlaeminck et al., 2005). In the latter study, relationships were examined using data from only one feeding experiment. However in the current review, data from Cabrita et al. (2003), Dewhurst et al. (2003) and Vlaeminck et al. (2005) were used to examine the relationship between duodenal flow of microbial CP and milk secretion of OBCFA (Fig. 6). The slope of the regression (Fig. 6) suggests the N:OBCFA ratio in mixed rumen bacteria is 9.0:1. Vlaeminck et al. (2006b) found this ratio was 11.1:1 and 10.4:1 for bacteria isolated from the solid and liquid phases of the rumen, whereas Bas et al. (2003) found a value of 6.2:1 for bacteria isolated from duodenal contents. This relationship between duodenal flow of bacterial N and milk secretion of OBCFA confirms that measurement of milk OBCFA could provide insight on microbial CP flow to the duodenum in non-fistulated dairy cows, although additional studies are needed to identify possible confounding factors, such as dietary lipid supplementation, lactation stage, contribution of OBCFA from body fat to milk OBCFA.

4.5.2. Rumen fermentation patterns

It is well accepted that the relative amounts of volatile fatty acids produced in the rumen, both *in vivo* and *in vitro*, are mainly determined by the diet. High concentrate rations and/or additions of large amounts of starch or soluble carbohydrates shift fermentation to high propionate proportions, whereas high forage diets favour acetate production (*e.g.*, Demeyer, 1981). As such shifts mainly reflect relative contributions of amylolytic and cellulolytic bacteria, it can be assumed that the rumen fermentation pattern is related to the pattern of OBCFA. Indeed, Vlaeminck et al. (2004b) reported a strong relationship between the OBCFA pattern and rumen proportions of volatile fatty acids with *in vitro* incubations. Later research showed that measurements of milk OBCFA were relatively accurate in predicting rumen proportions of volatile fatty acids (Vlaeminck et al., in press-c). Nevertheless, validation of the regression equations with an independent data set suggests dietary fatty acid content biased prediction accuracy (Vlaeminck et al., in press-c). Additional studies are needed to quantify effects of, for example, dietary lipid supplementation, lactation stage and contribution of OBCFA from body fat to milk OBCFA, on accuracy of the predictions.

Table 6
Overview of milk C18-fatty acids (g/100 g fatty acids)

	n	Mean	Median	S.D.	Minimum	Maximum
C18:0	103	9.36	9.67	2.43	2.71	19.54
trans-9 C18:1	84	0.32	0.23	0.23	0.096	1.16
trans-10 C18:1	78	1.18	0.36	2.15	0.099	12.73
trans-11 C18:1	95	1.92	1.23	2.16	0.35	14.36
cis-9 C18:1	90	17.38	17.75	3.90	4.84	27.72
cis-11 C18:1	90	0.76	0.65	0.52	0.28	2.74
cis-9, *trans*-11 C18:2	100	0.81	0.55	0.74	0.18	4.46
trans-10, *cis*-12 C18:2	74	0.018	0.014	0.014	0.001	0.076
trans-11, *cis*-15 C18:2	62	0.29	0.11	0.56	0.014	2.87
C18:2 *n*-6	103	1.53	1.51	0.47	0.80	3.57
C18:3 *n*-3	103	0.54	0.46	0.29	0.12	1.59

5. Relation of odd- and branched-chain fatty acids with other milk fatty acids

There is a great deal of interest in manipulation of the fatty acid profile of milk fat as concerns have been raised about the role of fatty acids in human health. In this respect, ruminant products have been criticized for their relatively high levels of medium chain saturated fatty acids (mainly C12:0, C14:0 and C16:0) and low levels of polyunsaturated fatty acids. These fatty acids result from *de novo* synthesis of short and medium chain fatty acids in the mammary gland from acetate and β-hydroxy-butyrate, and of extensive rumen biohydrogenation of dietary poly-unsaturated fatty acids, respectively. The latter results in ruminal formation of isomers of conjugated dienes and C18:3 and *trans* monoene intermediates (*e.g.*, Piperova et al., 2002; Shingfield et al., 2003; Lock and Bauman, 2004). The extent of accumulation of these hydrogenation intermediates seems largely dependent on the amount and type of lipid in the diet, and the rumen environment and bacterial population (*e.g.*, Harfoot and Hazlewood, 1997; Bauman and Griinari, 2003). As changes in the rumen microbial population are reflected in milk OBCFA, we investigated the relationship between hydrogenation intermediates and OBCFA. Summary statistics of milk C18-fatty acids in the studies are presented in Table 6. In addition, the relationship with *de novo* synthesised fatty acids were examined since Wongtangtintharn et al. (2004) suggested that branched-chain fatty acids lower fatty acid synthesis. Milk fatty acids were related to OBCFA using a mixed model regression analysis (St-Pierre, 2001) in which milk OBCFA were the independent variable. Adjusted observations were calculated by adding the residual from each individual observation to the predicted value of the study regression. These adjusted observations for study effects were used to calculate Pearson correlation coefficients.

Results show that an increase in milk *iso* C17:0 was accompanied by a decrease in C18:0, and an increase in *trans* monoene intermediates (Table 7). Based on observations with pure strains of rumen bacteria, the bacteria involved in hydrogenation have been classified into two groups (Harfoot and Hazlewood, 1997) with group ‘A’ bacteria hydrogenating polyunsaturated fatty acids to *trans* monoenes and group ‘B’ bacteria hydrogenating *trans* monoenes to C18:0. This suggests rumen conditions that promote synthesis of bacterial *iso* C17:0 are detrimental to group ‘B’ bacteria. The highest levels of *iso* C17:0 were observed in experiments of Shingfield et al. (2003), Jones et al. (2005) and Shingfield et al. (2005b).

Table 7

Correlation coefficients between milk odd- and branched-chain fatty acids and both *de novo* synthesized fatty acids[a] and C18-fatty acids in milk (g/100 g fatty acids) (values between brackets are the number of observations)[b]

	iso C14:0	*iso* C15:0	*iso* C16:0	*iso* C17:0	*anteiso* C15:0	*anteiso* C17:0	C15:0	C17:0	*cis*-9 C17:1
de novo		0.193 (99)		−0.672 (89)			0.491 (99)	0.535 (99)	
C18:0	0.280 (78)			−0.807 (89)	−0.207 (97)	−0.446 (69)	−0.465 (99)	−0.286 (99)	−0.276 (79)
trans-9 C18:1	−0.681 (70)	−0.553 (80)	−0.509 (76)	0.951 (70)	−0.435 (78)			−0.315 (80)	
trans-10 C18:1	−0.527 (67)	−0.288 (74)	−0.239 (70)	0.522 (64)					0.244 (68)
trans-11 C18:1				0.983 (81)		−0.475 (61)			0.325 (71)
cis-9 C18:1	0.322 (76)			−0.926 (76)	−0.396 (86)	0.504 (56)	−0.518 (86)	−0.361 (86)	
cis-11 C18:1	−0.760 (76)	−0.273 (86)	−0.493 (76)	0.858 (76)		0.868 (56)	0.371 (86)	0.431 (86)	
cis-9, *trans*-11 C18:2				0.895 (86)	0.264 (94)	−0.305 (69)			0.586 (79)
trans-10, *cis*-12 C18:2							0.365 (78)		0.390 (61)
trans-11, *cis*-15 C18:2	−0.503 (60)		−0.498 (60)	0.940 (52)		−0.693 (40)			
C18:2 *n*-6	−0.520 (78)	−0.755 (99)			−0.509 (97)	0.569 (69)			
C18:3 *n*-3	0.435 (78)				0.285 (97)	−0.416 (69)	0.363 (99)	0.396 (99)	0.392 (79)

[a] De novo = sum of C4:0 to C16:0.

[b] Only significant (*i.e.*, P<0.05) correlations are presented.

In these experiments, cows were fed a basal diet consisting of grass silage (Shingfield et al., 2003, 2005b) or maize silage (Jones et al., 2005; Shingfield et al., 2005b) and fish oil (Shingfield et al., 2003) or fish and sunflower oil (Jones et al., 2005; Shingfield et al., 2005b) as the lipid supplement. Hence, results suggest that long-chain polyunsaturated fatty acids in fish oil inhibit group 'B' bacteria thereby resulting in an elevation in *trans*-11 C18:1 (Wonsil et al., 1994; Scollan et al., 2001; Shingfield et al., 2003).

Iso C14:0 was positively related with C18:0 and had a negative relationship to *trans* monoene intermediates (Table 7). The ratio *trans*-11 C18:1 to C18:0 was negatively and positively related with *iso* C14:0 ($r_{pearson} = -0.400$, $n = 76$, $P<0.05$) and *iso* C17:0 ($r_{pearson} = 0.939$, $n = 76$, $P<0.05$), respectively, suggesting that a rumen bacterial population characterized by high levels of *iso* C14:0 is capable of completely hydrogenating polyunsaturated fatty acids to C18:0. Hence, high levels of *iso* C14:0 and low levels of *iso* C17:0 might indicate both group 'A' and 'B' bacteria are present in the rumen. As described above (see Section 4.4), *iso* C14:0 was positively related with dietary NDF content and increased with dietary F:C ratio. It is well known that diets high in NDF and/or dietary forage stimulate complete hydrogenation to C18:0 (Kalscheur et al., 1997; Kucuk et al., 2001). In contrast, *iso* C14:0 decreased with increasing starch content of the diet and/or upon replacement of grass by maize silage. Shingfield et al. (2005b) suggested that the amount of starch in the diet is an important factor determining milk *trans* C18:1 content, resulting in higher milk *trans* C18:1 content when maize silage replaced grass silage (Nielsen et al., 2004; Shingfield et al., 2005b). Hence, increased dietary starch seems to inhibit group 'B' bacteria, as suggested by a decrease in *iso* C14:0.

De novo synthesized fatty acids were negatively related to branched C17-fatty acids and showed a weak positive correlation with linear odd-chain fatty acids (Table 7). The latter being an additional indication that a part of the linear odd-chain fatty acids in milk derive from *de novo* synthesis in the mammary gland (see Section 2.2). The negative correlation between branched C17- and *de novo* synthesised fatty acids might indicate a direct inhibitory effect of these fatty acids on fatty acid synthesis in the mammary gland, as reported in breast cancer cells (Wongtangtintharn et al., 2004). However, the concomitant positive relationship between *de novo* synthesized fatty acids and *iso* C15:0 and *iso* C16:0 (Table 7), makes the latter hypothesis unlikely. Indeed, Wongtangtintharn et al. (2004) showed that all branched-chain fatty acids are equally effective against fatty acid synthesis. Hence, it is more likely that increased amounts of branched C17-fatty acids were associated with rumen conditions favouring formation and accumulation of specific hydrogenation intermediates (Bauman and Griinari, 2003).

6. Multi-branched-chain fatty acids in milk

Besides microbially derived OBCFA, other branched-chain fatty acids occur in milk. The occurrence of a multi-branched-chain fatty acid, 3,7,11,15-tetramethylhexadecanoic acid (phytanic acid), in milk fat was described in 1952 by Hansen and Shorland. Some years later, another multi-branched-chain fatty acid, 2,6,10,14-tetramethylpentadecanoic acid (pristanic acid), was found in milk fat (Hansen and Morrison, 1964). Interest in phytanic acid recently arose as a natural product with potentially positive effects on human health,

comparable to those of conjugated linoleic acid (*e.g.*, McCarthy, 2001; Schütler et al., 2002).

Phytanic acid is derived from phytol, the side chain of chlorophyll. After intracellular release, rumen micro-organisms effectively release phytol from chlorophyll, after which phytol is hydrogenated to yield dihydrophytol, which rumen micro-organisms can oxidize to phytanic acid (Hansen, 1966; Patton and Benson, 1966; Lough, 1973). Phytanic acid is further converted to pristanic acid in peroxisomes, a pathway extensively studied in humans in relation to genetic defects (Mukherji et al., 2003).

Summarizing literature data, Lough (1973) found an average value of 0.06 and 0.28 g/100 g fat for pristanic and phytanic acid, respectively. Recently, similar values were reported by Vlaeminck et al. (2004c,d), being 0.06 and 0.36 g/100 g fat for pristanic and phytanic acid, respectively, and Leiber et al. (2005), being 0.30 g/100 g fat for phytanic acid. Nevertheless, there was large variation in pristanic and phytanic acids in the experiments reported by Vlaeminck et al. (2004c,d) and Leiber et al. (2005), with phytanic acid ranging from 0.16 to 0.45 g/100 g fat in Leiber et al. (2005) and from 0.19 to 0.59 g/100 g fat in Vlaeminck et al. (2004c,d). Similar variation occurred for pristanic acid, ranging from 0.03 to 0.09 g/100 g fat (Vlaeminck et al., 2004c,d). Patton and Benson (1966) suggested that the large variation in phytanic acid content reported in published studies is probably due to differences in feed and rumen microflora. Indeed, lower values occurred in milk from cows fed low amounts of grass silage whereas an increase in dietary proportion of grass silage from intensively managed grassland or in cows grazing fresh grass increased pristanic and phytanic acid (Vlaeminck et al., 2004c,d; Leiber et al., 2005), which probably reflects the chlorophyll content of the diets. The strong relation of milk phytanic and pristanic acid (pristanic acid = $0.007_{(\text{S.E.}=0.003)} + 0.142_{(\text{S.E.}=0.007)} \times$ phytanic acid; $R^2 = 0.935$; $n = 32$; Vlaeminck et al., 2004c,d) is consistent with the formation of pristanic acid through α-oxidation of phytanic acid in peroxisomes (Mukherji et al., 2003).

Milk content of pristanic and phytanic acid seems to be largely determined by the dietary chlorophyll level. Nevertheless, Lee et al. (2004) found a lower duodenal flow of phytanic acid in steers fed red clover silage despite equal dietary chlorophyll content (2.51 and 2.64 mg/g DM for grass and red clover silage, respectively) and dry matter intake compared with grass silage. They suggested that this might be related to the enzyme polyphenol oxidase in red clover inhibiting hydrolysis of chlorophyll, or ruminal hydrogenation of phytol in a similar way to the postulated mechanism for the reduced biohydrogenation of C18:3 *n*-3 on red clover diets (Dewhurst et al., 2003; Lee et al., 2004; Lourenço et al., 2005). Similarly, the lower content of milk phytanic acid in a potato starch diet (Vlaeminck et al., 2004d) might be related to a lower rumen hydrogenation of phytol to dihydrophytol, which is consistent with the lower rumen biohydrogenation of dietary C18:2 *n*-6 and C18:3 *n*-3 when cows were fed a potato starch diet (Vlaeminck et al., 2004d).

7. Conclusions

OBCFA in milk fat largely derive from rumen bacteria, although *de novo* synthesis in the mammary gland of linear odd-chain fatty acids cannot be dismissed. Nevertheless, variation in milk OBCFA induced by diet changes could largely reflect the OBCFA composition of

rumen bacteria, which supports interest in these fatty acids as potential diagnostic tools of rumen function.

A reduction in the ratio of odd-chain *iso*-fatty acids to *anteiso*-fatty acids might be indicative for rumen stress stimuli associated with increased levels of dietary concentrate, such as reduced rumen pH. Changes in the ratio of *iso* to *anteiso*-fatty acids might be due to changes in the rumen bacterial population, or a bacterial response to stress stimuli. Increased milk concentrations of *anteiso* C17:0 might indicate a deficit of rumen degradable CP.

Milk content of *iso* C17:0 and hydrogenation intermediates (*trans*-11 C18:1; *cis-9*, *trans*-11 C18:2; *trans*-11, *cis*-15 C18:2) were positively related, whereas a negative relationship occurred with *iso* C14:0 and *iso* C16:0.

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