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Digestion of fatty acids in ruminants: a meta-analysis of flows and variation factors: 2. C18 fatty acids

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In ruminants, dietary lipids are extensively hydrogenated by rumen micro-organisms, and the extent of this biohydrogenation is a major determinant of long-chain fatty acid profiles of animal products (milk, meat). This paper reports on the duodenal flows of C18 fatty acids and their absorption in the small intestine, using a meta-analysis of a database of 77 experiments (294 treatments). We established equations for the prediction of duodenal flows of various 18-carbon (C18) fatty acids as a function of the intakes of their precursors and other dietary factors (source and/or technological treatment of dietary lipids). We also quantified the influence of several factors modifying rumen metabolism (pH, forage : concentrate ratio, level of intake, fish oil supplementation). We established equations for the apparent absorption of these fatty acids in the small intestine as a function of their duodenal flows. For all C18 unsaturated fatty acids, apparent absorption was a linear function of duodenal flow. For 18:0, apparent absorption levelled off for high duodenal flows. From this database, with fatty acid flows expressed in g/kg dry matter intake, we could not find any significant differences between animal categories (lactating cows, other cattle or sheep) in terms of rumen metabolism or intestinal absorption of C18 fatty acids.

Keywords: digestion, fatty acids, meta-analysis, rumen, small intestine

Introduction

There is a growing interest in the lipid composition of ruminant products. They have often been highlighted as a source of saturated fat in human diet, which could be deleterious for cardio-vascular diseases (Mann, 2002). In Europe, dairy products represent around 50% of saturated fat intake. Hence, there are advantages in decreasing the saturated fatty acid content of milk and meat from ruminants. On the other hand, conjugated linoleic acid isomers (CLA), mainly found in ruminant products, could have beneficial effects on human health (Wahle *et al.*, 2004). Therefore, there are advantages in increasing the levels of CLA as well as other polyunsaturated fatty acids in milk and meat by natural means, i.e. by modifications to animal diets.

Ruminants are characterised by major ruminal transformations of ingested feedstuffs before they reach the duodenum and are absorbed and used to produce tissues or products. Fatty acids (FA), in particular, are extensively isomerised and hydrogenated by rumen microbes, and this phenomenon is responsible for the low levels of unsaturated fat in ruminant products. Lipids entering the rumen are first lipolysed by bacteria, then released unsaturated FA are isomerised and saturated (Harfoot and Hazlewood, 1997). To reach a high transfer efficiency of dietary unsaturated FA to animal tissues, various strategies of lipid protection have been used since the 1970s, with inconsistent results. Besides the lipid composition of the diet, other diet characteristics and various components of the rumen ecosystem are thought to influence the extent of biohydrogenation (BH), including fibre (or forage) content of the diet, rumen pH, etc.

Many studies over the last four decades have dealt with these aspects, but only a few synthetic quantitative evaluations are available (Doreau and Ferlay, 1994; Sauvant and Bas, 2001). The present article is based on a meta-analysis of a larger set of published data from *in vivo* experiments, including recent data on trans-FA and CLA.

The objectives of this study were (1) to estimate the duodenal flows of the stearic and major unsaturated 18-carbon (C18) FA based on their intakes and diet characteristics, (2) to assess the influence of potential dietary or ruminal factors on the ruminal metabolism of

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these FA and (3) to estimate the absorbed flows of C18 fatty acids in the small intestine from their duodenal flows.

Material and methods

We used the same procedures and the same set of publications as those described in the companion paper (Schmidely *et al*., 2008). The following describes only the specific methods used for the various C18 FA studied in the present paper.

Inclusion and expression of data

For duodenal flow studies, we included all the published experiments in the database, quantifying intakes and duodenal flows of C18 FA and dry matter intake (DMI). We also included experiments providing data allowing the computation of these flows, together with six experiments from our laboratory (INRA Theix) either partially published or awaiting publication. We did not produce any estimations of FA flows, and we only used calculations from the values given in the publications. DMI values were estimated from fresh matter intakes in only two publications (Hogan *et al*., 1972; Chikunya *et al*., 2004).

For C18 FA apparent absorption in the intestine, we kept only the publications reporting duodenal and terminal ileal flows (24 experiments), excluding publications with duodenal and faecal flows (31 experiments). Indeed, even if there is little or no absorption of long-chain FA in the large intestine, FA can be biohydrogenated, which could lead to bias in apparent absorption values for unsaturated FA. Published data reporting both ileal and faecal flows are too few and scattered to enable a reliable assessment of these biases. Apparent absorption was thus calculated as the difference between duodenal and terminal ileal flows.

All FA flows were expressed in g/kg DMI, in order to obtain similar levels for sheep, lactating cows and other cattle, which otherwise highly differ when flows are expressed in g/day. Experiments with only one experimental treatment or less than three animals per experimental group were excluded.

Finally, the database studied for duodenal C18 FA flows comprised 294 treatments from 77 experiments, of which 35 involved lactating cows, 25 involved other cattle and 17 involved sheep. The complete list of publications used is available from the corresponding author and on the journal's website.

Encoding of experiments, various C18 FA, rations and dietary lipid supplements

For publications reporting several experiments, each experiment was assigned a specific code. The study faced a major problem due to discrepancies in FA analysis methods and changes in these methods according to publication year. For example, it was almost impossible to determine whether the 'linoleic acid' reported in some publications was a chromatographic peak of pure linoleic acid (cis9,cis12-18:2, hereafter c9c12-18:2) or a coelution of different isomers of 18:2. Hence, for publications reporting only one isomer of 18:1, 18:2 or 18:3, we encoded the isomers into global 18:1, 18:2 or 18:3, respectively; we limited oleic (c9-18:1), linoleic (c9c12-18:2) and α-linolenic (c9c12c15-18:3) acids to publications reporting several isomers of each unsaturated C18.

Experimental diets were assigned two codes according to the main lipid source ($i = 1$–13) and the technological treatment (form) of the dietary lipids ($j = 1$–12). These two codes correspond, respectively, to the rows and columns of Table 1. Diets were first encoded according to the origin of the main lipid source (animal fat, hydrogenated fats, fish oil, linseed, soybean, sunflower, cottonseed, rapeseed, palm, other vegetal lipids, animal–vegetal blend). Control diets (no lipid supplement) and 100%-forage diets were also assigned specific codes in this coding system. The second coding system defined the technological treatment ('form') of the dietary lipids: amides, encapsulated oils, whole seeds, crushed, ground or cracked seeds, heated seeds, extruded seeds, alkaline treatment, formaldehyde treatment, triglycerides (TGs; oils), calcium soaps, free fatty acids. Control diets without specific treatment were also assigned a specific code within this second coding system. The distribution of experimental treatments according to lipid source and technological treatment is presented in Table 1.

Statistical analyses

Data were analysed using the Proc Mixed component of the SAS/STAT software package (Sas Institute, 2000), as described by St-Pierre (2001). We defined a random experiment effect on both the intercept and the coefficient (slope) of each independent variable studied in order to distinguish within-experiment effects ('biological effects' of the variable) and experiment effects (not linked to the variable studied). We used the Akaike Information Criterion (AIC) to assist in the selection of alternative models for the same dependent variable (Wang and Goonewardene, 2004).

Unless otherwise stated, model coefficients are mean $\pm$ s.e. of the coefficient. When there was a lipid source or treatment effect on the slope of the models, the adjusted coefficient given in the equations is the value obtained for control diets.

Selection of experiments

For each dependent variable, we compared various models including several combinations of independent variables or factors. To study the effect of an independent variable, it was necessary to select a subset of experiments exhibiting a within-experiment variation for this variable. The statistical model adjustments were obtained from these experimental subsets. We included experiments with a within-experiment s.d. of above 4 g/kg DMI for 18:1 intake,

Table 1 *Number of experimental treatments in the database according to lipid source and technological treatment ('form') of the dietary lipids*

	Technological treatment of the dietary lipids											
Lipid source	Amides	Calcium salts	Encapsulated oils	Extruded seeds	Free fatty acids	Whole seeds	Heated seeds	Crushed or ground seeds	Formaldehyde-treated	Alkaline-treated	Fats or oils	Others (control)	Total
Animal fat		1	2		1						24		28
Fish oil					1				2		16		19
Hydrogenated fats					3						8		11
Vegetal + animal fats											8		8
Cottonseed						9	1	2					12
Linseed				1		2	1	1	3		5		13
Palm oil		12			1					1	1		15
Rapeseed	2	4		1		1		6		2	5		21
Soybean	1	2		2	1	4	4	2			8		24
Sunflower						3					5		8
Other plants			1			3					6		10
Forage diets												39	39
Others (control)									4			82	86
Total	3	19	3	4	7	22	6	11	9	3	86	121	294

above 1.5 g/kg DMI for 18:2 intake and above 0.5 g/kg DMI for 18:3 intake. Fish oils are known to disrupt ruminal FA metabolism. For this reason, we excluded all the experimental treatments containing fish oil or fishmeal from the data used for C18 duodenal flow prediction, and studied the specific effects of fish oils in a separate section.

Clustering analysis of C18 isomers

In order to examine the links between dietary polyunsaturated FA and their various BH products, we performed a clustering analysis of 18:2 and 18:3 intakes and the proportions of the major 18:1 and 18:2 isomers at the duodenum. We selected six experiments from the same laboratory (INRA Theix) in order to ensure common methods and procedures in FA determination: three published experiments (Doreau *et al.*, 2003; Loor *et al.*, 2004 and 2005), from which we excluded the fish oil treatment reported in Loor *et al.* (2005), and three unpublished experiments (comparisons of cocksfoot or red clover given to sheep as fresh grass, hay or silage). All data were expressed as percentage of C18 FA (intake or duodenum). We selected the 18:1 and 18:2 isomers accounting for more than 0.5% of duodenal C18 FA on average, and t10c12-18:2 for its important role in mammary metabolism (Baumgard *et al.*, 2000). The variables were centred on the experiment's mean for each experiment in order to obtain within-experiment variations and thus avoid clustering due to experiment-to-experiment variation. These centred variables were then clustered in Minitab® 14 (Minitab Inc., State College, PA, USA) using the correlation distance and the average linkage method (the linkage method did not modify the resulting clusters).

Results and discussion

Models of duodenal flows

For all the statistical analyses performed, we tested the fixed effect of animal category (lactating cows, other cattle and sheep) on the intercepts and on the slopes of the equations. This effect was not significant in any of the relations reported below. The companion paper (Schmidely *et al.*, 2008) reports the global equation for the duodenal flow of C18 FA according to their intake, with a mean $\pm$ s.d. slope of 0.84 ± 0.034. The mean duodenal flows of the different C18 FA for diets with and without fish oil are given in Table 2. All FA intakes (FA_{int}), duodenal flows (FA_{duo}) and root mean square of error (RMSE) values for the adjusted models are expressed in g/kg DMI. The significance of the model coefficients was tested against 0. The number of experiments (N_{exp}) and treatments (N_{trt}) used for each model adjustment is also indicated.

18:0. The model of 18:0 duodenal flow was adjusted with data from experiments with within-variation in intakes of 18:1, 18:2 and/or 18:3. We excluded three experiments with obviously aberrant data (Hogan *et al.*, 1972 and

Table 2 *Duodenal C18 flows for diets with or without fish oil or fish meal (diets without fish oil:* n = *132 for cis-18:1 flows,* n = *138 for trans-18:1 flows, and* n = *244 to 257 for the other flows; diets with fish oil:* n = *14 for all the flows)*

	g/kg dry matter intake mean ± s.d. (range)		g/100 g total C18 mean ± s.d. (range)	
Fatty acid	No fish oil	With fish oil	No fish oil	With fish oil
18:0	23.2 ± 14.1 (1.5 to 99.0)	9.7 ± 8.2 (2.2 to 34.3)	69.5 ± 10.4 (28.5 to 94.0)	34.1 ± 12.9 (16.5 to 61.5)
Cis-18:1	3.2 ± 2.5 (0.2 to 16.6)	2.5 ± 1.4 (1.2 to 5.7)	9.1 ± 4.7 (1.4 to 31.9)	10.3 ± 3.5 (6.1 to 19.9)
Trans-18:1	4.0 ± 3.9 (0.5 to 19.9)	10.6 ± 3.5 (5.1 to 17.1)	11.3 ± 7.0 (2.6 to 34.6)	44.7 ± 11.4 (22.3 to 64.0)
Global 18:2	2.3 ± 2.0 (0.0 to 12.7)	1.8 ± 1.1 (0.5 to 3.4)	7.4 ± 4.7 (0.0 to 23.1)	7.9 ± 5.1 (2.4 to 14.5)
18:3	0.5 ± 0.5 (0.0 to 3.5)	0.7 ± 0.5 (0.1 to 1.5)	1.9 ± 2.1 (0.0 to 13.8)	3.1 ± 2.3 (0.5 to 6.7)

White *et al*., 1987, with duodenal 18:2 flows higher than 18:0 flows, and Chang *et al*., 1991, with 80% of ingested FA disappearing in the rumen). There were significant correlations between intakes of 18:0 and 18:1 ($r = 0.15$, $P = 0.03$), 18:0 and 18:3 ($r = -0.16$, $P = 0.02$) and 18:1 and 18:2 ($r = 0.41$, $P < 0.001$). The intercept and coefficients of 18:0, 18:1 and 18:2 intakes were significantly different from 0 ($P < 0.0001$), similar to the coefficient of the 18:3 intake ($P = 0.002$). The adjusted model was

$$\begin{aligned}\text{18:0}_{\text{duo}} = {} & (5.34 \pm 0.64) \\ & + (0.747 \pm 0.082) \times \text{18:0}_{\text{int}} \\ & + (0.535 \pm 0.056) \times \text{18:1}_{\text{int}} \\ & + (0.624 \pm 0.085) \times \text{18:2}_{\text{int}} \\ & + (0.247 \pm 0.060) \times \text{18:3}_{\text{int}}\ (\text{in g/kg DMI})\end{aligned}$$

($N_{\text{exp}} = 57$, $N_{\text{trt}} = 205$, RMSE = 3.17).

cis-18:1. Reported values for global 18:1 are difficult to interpret because there is no way of knowing whether they included only cis-18:1 (mainly dietary 18:1 having escaped BH), or both cis- and trans-18:1 (that reflect totally different processes, trans-18:1 being a product of the partial BH of unsaturated FA). We thus studied cis- and trans-18:1 duodenal flows separately.

In the model adjustment of cis-18:1 duodenal flow, we found a significant intercept ($P = 0.002$) and a highly significant influence of 18:1 intake ($P < 0.0002$), but no significant effects of 18:2 ($P > 0.05$) or 18:3 ($P > 0.5$) intakes. The adjusted model was

$$\begin{aligned}\text{cis-18:1}_{\text{duo}} = {} & (0.84 \pm 0.16) \\ & + (0.240 \pm 0.025) \times \text{18:1}_{\text{int}}\ (\text{in g/kg DMI})\end{aligned}$$

($N_{\text{exp}} = 32$, $N_{\text{trt}} = 121$, RMSE = 0.775).

trans-18:1. We studied the effects of intakes of 18:1, 18:2 and 18:3 on duodenal flows of trans-18:1. For the adjustment of this model, we excluded seven treatments with high levels of dietary trans-18:1 (diets containing hydrogenated fats for which trans-18:1 represented more than 15% of total the 18:1 intake). The adjusted model gave a significant intercept ($P = 0.008$) and highly significant effects of intakes of 18:1 ($P = 0.001$), 18:2 ($P < 0.0001$) and 18:3 ($P < 0.0001$). There was a significant correlation between 18:1 and 18:2 intakes ($r = 0.35$, $P < 0.001$) in the dataset. The adjusted model was

$$\begin{aligned}\text{trans-18:1}_{\text{duo}} = {} & -(1.83 \pm 0.65) \\ & + (0.087 \pm 0.026) \times \text{18:1}_{\text{int}} \\ & + (0.262 \pm 0.029) \times \text{18:2}_{\text{int}} \\ & + (0.244 \pm 0.034) \times \text{18:3}_{\text{int}}\ (\text{in g/kg DMI})\end{aligned}$$

($N_{\text{exp}} = 31$, $N_{\text{trt}} = 107$, RMSE = 1.69).

This was the only model with a negative intercept. This may have occurred due to the influence of the 18:2 intake, which could be non-linear (curvilinear trend). This may also partly explain the higher RMSE (compared with the cis-18:1 model), as well as the higher variability of trans-18:1 duodenal flows. We identified a contribution of dietary cis-18:1 to duodenal trans-18:1 flow, which is consistent with data obtained *in vivo* (Bickerstaffe *et al*., 1972) and *in vitro* (Hazlewood *et al*., 1976; Mosley *et al*., 2002; Vossenberg and Joblin, 2003), or as suggested by the increase of trans-18:1 in milk from cows supplemented with oleic acid (Selner and Schultz, 1980). Based on our data, the coefficient from dietary cis-18:1 to duodenal trans-18:1 was 8.7%, although we cannot exclude some overestimation bias due to the correlation between 18:1 and 18:2 intakes in the dataset.

To study the fate of dietary trans-18:1, we selected experiments with within-variation in trans-18:1 intake (24 treatments in eight experiments with a within-experiment SD higher than 0.5 g/kg DMI). In the model of trans-18:1 duodenal flow from intakes of trans-18:1 and 18:2 (there was no significant variation of 18:3 intake in the selected experiments), we found a coefficient of 0.70 ± 0.17 for trans-18:1 intake, which means that only 30% of dietary trans-18:1 is expected to be metabolised in the rumen (hydrogenated to 18:0 and/or isomerised to cis-18:1 isomers).

Individual 18:1 isomers. We selected publications reporting more than two individual isomers of cis- and/or trans-18:1 in order to evaluate the contribution of these isomers to the total flow of duodenal 18:1. The results are presented in Table 3, using values from lipid-supplemented and unsupplemented diets. The main isomers were t11-, c9-, t13 + 14- and t10-18:1.

Table 3 *Proportions of 18:1 isomers at duodenum (in g/100 g duodenal 18:1), in a selection of papers reporting more than two cis- or trans-isomers, and isomers with more than 10 available data*

Isomer	Unsupplemented diets	Lipid-supplemented diets	Minimum	Maximum
c9	19.6 ± 10.8	21.2 ± 17.6	4.8	58.7
c11	4.8 ± 3.1	6.1 ± 3.7	1.9	16.2
c12	1.5 ± 0.6	1.8 ± 0.9	0.4	3.4
c13	0.5 ± 0.1	0.4 ± 0.1	0.3	0.7
c15	3.7 ± 3.3	3.2 ± 2.3	0.8	11.8
t4	0.8 ± 0.3	0.5 ± 0.1	0.3	1.4
t5	0.7 ± 0.5	0.7 ± 0.6	0.2	1.9
t6 + 7 + 8	2.5 ± 0.9	2.3 ± 1.7	0.3	4.4
t9	1.8 ± 0.5	2.1 ± 1.4	0.1	4.6
t10	5.0 ± 3.2	16.2 ± 18.4	0.9	53.3
t11	30.8 ± 12.3	20.4 ± 16.7	2.3	60.2
t12	3.9 ± 1.1	2.9 ± 1.7	0.5	6.1
t13 + 14	11.1 ± 3.4	10.5 ± 4.9	5.3	18.1
t15	4.9 ± 2.5	4.5 ± 2.3	0.3	9.4
t16	6.0 ± 2.6	3.1 ± 2.1	1	10.7
Sum of trans	63.9 ± 16.1	71.7 ± 17.4	25.2	90.5

Data are mean ± s.d., minimum and maximum values are across all diets.

The proportion of t10-18:1 increased three-fold under lipid-supplemented diets, but was nevertheless highly variable.

There were too few experiments reporting data on individual isomers to adjust the models of their duodenal flows without a risk of bias from correlations between independent variables or leverage of some experiments. Several significant effects were found, but need to be confirmed when more data are available: for t10-18:1 duodenal flow, an increase by 18:2 intake; for t11- and t13 + 14-18:1, a contribution from all the dietary unsaturated C18; for t9-18:1, the only significant positive effect was 18:1 intake, which is in agreement with *in vitro* results showing a major contribution of oleic acid to t9-18:1 (AbuGhazaleh *et al.*, 2005).

Global 18:2. Most publications reported amounts of '18:2', without any details to which isomer(s) were measured. Two experiments were excluded (Hogan *et al.*, 1972; White *et al.*, 1987) due to obviously aberrant duodenal 18:2 flows (>30 g/kg DMI and higher than 18:0 duodenal flows).

The best adjustment included a significant intercept ($P = 0.0001$), a significant effect of 18:2 intake and an effect of lipid form on the coefficient (interaction, $P < 0.0001$):

$$18{:}2_{duo} = (0.89 \pm 0.21) + (0.117 \pm 0.026) \times 18{:}2_{int} \text{ (in g/kg DMI)}$$

($N_{exp} = 41$, $N_{trt} = 160$, RMSE = 0.83).

Two classes of lipid supplements had a significant effect on the linear coefficient: alkaline treatment (+0.107) and encapsulated oils (+0.194, but based on two treatments only). The coefficients from all other lipid forms were not significantly different from control diets. We did not find any significant effect of 18:3 intake on the duodenal flow of 18:2.

c9c12-18:2. In this section, only publications that separated c9c12-18:2 from other 18:2 isomers were used. Mean c9c12-18:2 duodenal flow was 1.59 ± 1.36 g/kg DMI ($n = 75$, minimum 0.12, maximum 7.21). We calculated the apparent BH of c9c12-18:2 as (intake – duodenum flow)/intake in these publications. The apparent BH of c9c12-18:2 was 87 ± 7% ($n = 60$, range 65% to 95%, no protected lipid sources in these data). Among the experiments with sufficient within-experiment variation in 18:2 intake, only nine (27 treatments) reported duodenal flows of c9c12-18:2. No effect of lipid form or 18:3 intake could be detected on these flows.

The adjusted model had a significant intercept ($P = 0.002$) and a significant effect of 18:2 intake ($P = 0.004$), as follows:

$$\text{c9c12-18:2}_{duo} = (1.33 \pm 0.28) + (0.054 \pm 0.013) \times 18{:}2_{int} \text{ (in g/kg DMI)}$$

($N_{exp} = 9$, $N_{trt} = 27$, RMSE = 0.29).

Other 18:2 isomers. The database comprised 21 experiments (72 treatments) reporting duodenal flows of 18:2 isomers other than c9c12-18:2. The number of isomers reported varied between one (most frequently c9t11-18:2) and 18 (Doreau *et al.*, 2003; Shingfield *et al.*, 2003). The proportion of these isomers in total 18:2 varied between 0% (not detected) and 95% (Shingfield *et al.*, 2003), with a mean ± s.d. of 24 ± 21%. This proportion was not related to the number of isomers measured, which probably indicates that the chromatographic peaks identified in reports with few isomers were in fact the sum of several isomers. The mean proportions of the various 18:2 isomers in both unsupplemented and lipid-supplemented diets are given in Table 4. The major 18:2 isomers were c9c12 and t11c15.

Most authors have reported c9t11-18:2 as the major 18:2 isomer (apart from c9c12), but several recent studies report levels of less than 10% of the non-c9c12-18:2 isomers (Doreau *et al.*, 2003; Lee *et al.*, 2004; Loor *et al.*, 2004 and 2005). This probable overestimation of c9t11-18:2 flows in many publications could stem from a combination of factors: c9t11-18:2 was the only CLA isomer studied by many authors, the discrimination between chromatographic peaks was better in recent publications, and what appeared before as c9t11-18:2 was probably the coelution of several isomers and other FA like C21:0 (Kramer *et al.*, 2001): 'c9t11-18:2' represented a mean of 12% of total 18:2 in publications reporting only this isomer, *v.* 2% in publications reporting other 18:2 isomers.

Indeed, c9t11-18:2 is a primary product of c9c12-18:2 isomerisation, but is probably very rapidly hydrogenated to vaccenic acid (t11-18:1), resulting in very low levels at the

duodenum. With diets rich in 18:2 (i.e. more than 80% of polyunsaturated FA intake, $n = 16$), 18:2 duodenal flow was 90% c9c12-18:2, and c9t11-18:2 on average represented 41% of the other isomers. With diets rich in 18:3 (i.e. more than 66% of polyunsaturated FA intake, $n = 14$), 18:2 duodenal flow was 59% c9c12-18:2, and c9t11-18:2 on average represented 3% of the other isomers (58% were t11c15-18:2). The database comprised 68 data of c9t11-18:2 and t11-18:1 duodenal flows. In a large majority of these data (56/68), c9t11-18:2 flow represented less than 6% of t11-18:1 flow. The 12 data with ratios higher than 6% were obtained with rations containing high levels of grass and/or clover silages (Dewhurst *et al.*, 2003; Lee *et al.*, 2003; Shingfield *et al.*, 2003). Based on our database, we could find no significant effects of the various unsaturated FA intakes on the duodenal flow of c9t11-18:2, nor any relation between duodenal c9t11-18:2 and t11-18:1 flows.

It was impossible to study the dependence of the duodenal flows of other individual 18:2 isomers on the intake of their precursors (dietary 18:2 and 18:3), as there were too few published reports on these isomers, and most of the reports available contained confounding factors such as forage : concentrate ratio, forage type, and positive or negative correlations between 18:2 and 18:3 intakes. The adjustments drawn from these data led to aberrant coefficients (negative or higher than 1).

For the sum of non-c9c12 isomers, the adjusted model had a non-significant intercept and a significantly positive effect of 18:3 intake ($P = 0.002$):

$$\text{Non-c9c12 18:2}_{\text{duo}} = (0.12 \pm 0.40) + (0.086 \pm 0.023) \times 18{:}3_{\text{int}} \text{ (in g/kg DMI)}$$

($N_{\text{exp}} = 8$, $N_{\text{trt}} = 26$, RMSE = 0.272).

This suggested that non-c9c12 18:2 originates mainly from dietary 18:3. This is probably due to the fact that t11c15-18:2, a BH intermediate of 18:3, is the main non-c9c12 18:2 isomer (Table 4). However, we did not find a significant effect of dietary 18:3 on global 18:2 flow (see above), probably because non-c9c12 isomers are often minor compared with c9c12-18:2.

18:3. The apparent BH of 18:3 for diets with formaldehyde- or alkaline-treated lipids was 67 ± 19% ($n = 15$, range 41% to 93%), compared to 86 ± 12% ($n = 222$, range 40% to 100%) for the other diets. Contrary to a previous review (Doreau and Ferlay, 1994), mean 18:3 BH obtained from the whole database was not significantly different from mean c9c12-18:2 BH ($P = 0.85$).

For duodenal flow model adjustment, we used the experiments with either sufficient within-variation of 18:3 intake or various lipid forms, i.e. intakes of 18:3 between 0.4 and 32.7 g/kg DMI in this dataset (Figure 1).

The adjustment showed a significant intercept ($P < 0.0001$), a significant effect of 18:3 intake ($P = 0.0009$) and an interaction between lipid form and 18:3 intake ($P < 0.0001$) (i.e. a significant effect of dietary lipid form on the slope). The adjusted model for control diets was

$$18{:}3_{\text{duo}} = (0.19 \pm 0.03) + (0.052 \pm 0.015) \times 18{:}3_{\text{int}} \text{ (in g/kg DMI)}$$

($N_{\text{exp}} = 60$, $N_{\text{trt}} = 224$, RMSE = 0.20).

Three lipid forms led to a significant slope alteration compared with the control rations: calcium salts (+0.064), formaldehyde treatment (oils or seeds: +0.052) and whole seeds (+0.048). Alkaline treatment also had a significant effect (+0.258), but this effect was based on two experiments only (points with $18{:}3_{\text{duo}} > 2$ g/kg DMI in Figure 1), with a risk of confounding lipid-form effect and experiment effect. We did not find any significant effect of 18:2 intake on the duodenal flow of 18:3.

Clustering analysis of major 18:1 and 18:2 isomers. The subset of data used for the clustering analysis comprised 17 treatments from six experiments. We included the intakes of 18:2 and 18:3 (as percentage of total C18 intake), and duodenal proportions of c9-, c11-, c15-, t9-, t10-, t11-, t12-, t13 + 14-, t15-, t16-18:1 and c9c12-, and t11c15- and t10c12-18:2. The experiment effects were removed by centering the data in each experiment, otherwise global correlations were sometimes contrary to within-experiment relations, reflecting biological phenomena. However, this clustering was obtained from a limited set of experiments

Table 4 *Proportions of 18:2 isomers at duodenum (in g/100 g duodenal 18:2), in a selection of papers reporting more than two isomers, and isomers with more than 10 available data*

Isomer	Unsupplemented diets	Lipid-supplemented diets	Minimum	Maximum
c9c11	0.6 ± 0.4	3.3 ± 5.5	0.1	7.7
c9c12	65.2 ± 18.8	80.3 ± 24.0	5.2	98.4
c9c15	1.0 ± 0.3	No data	0.3	1.5
c9t11	1.7 ± 4.2	3.1 ± 3.2	0.2	22.7
c9t12	4.1 ± 2.2	2.2 ± 1.2	0.9	8.4
c9t13	1.4 ± 0.7	2.2 ± 0.9	0.0	3.9
c10c12	0.6 ± 0.4	3.3 ± 5.5	0.1	22.3
c10t12	0.1 ± 0.1	0.2 ± 0.1	0.0	0.6
c11c13	0.1 ± 0.1	0.1 ± 0.1	0.0	0.3
c11t13	0.1 ± 0.1	0.3 ± 0.2	0.0	0.6
t8c10	0.4 ± 0.3	0.3 ± 0.1	0.0	0.9
t8c13	5.6 ± 1.5	3.3 ± 1.5	1.3	8.8
t9c11	0.5 ± 0.5	0.3 ± 0.1	0.1	2.1
t9c12	1.6 ± 1.0	2.7 ± 1.1	0.1	4.6
t9t11	1.0 ± 0.9	0.4 ± 0.6	0.1	2.4
t9t12	5.9 ± 10.0	2.2 ± 1.1	0.2	31.3
t10c12	1.2 ± 2.0	1.7 ± 1.3	0.0	6.6
t11c15	20.6 ± 11.4	36.5 ± 16.8	3.5	53.4
t11t13	1.8 ± 2.1	0.9 ± 1.1	0.1	7.9

Data are mean ± s.d., minimum and maximum values are across all diets.

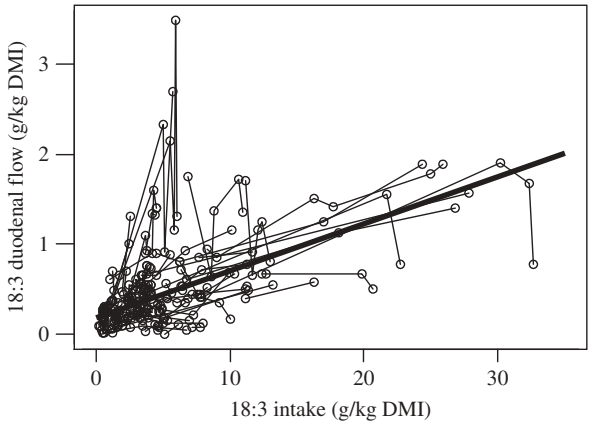

Figure 1 Intake and duodenal flows of 18:3, based on the experiments with a within-experiment variation in 18:3 intake or different lipid technological treatments. The thin lines join the experimental treatments of a same experiment, and the thick line represents the adjusted model for control diets.

and the correlation distance gives weight to highly variable experiments, which could distort the distances obtained. The resulting clustering is presented in Figure 2. Two main clusters appeared, one containing the 18:2 intake and the other containing the 18:3 intake. The duodenal isomers included in each cluster could thus be interpreted as products of the ruminal metabolism of the respective dietary FA (exclusion of 18:2 and 18:3 intakes from the analysis did not modify the clustering of the duodenal isomers). Duodenal t10-18:1 and t10c12-18:2 levels were strongly linked to 18:2 intake, like duodenal c9-, c11-, c12-18:1 and c9c12-18:2. Duodenal t13 + 14-, t15- and t16-18:1 were the most closely related to the 18:3 intake, followed by c15-18:1 and t11c15-18:2, and then a group formed by t6 + 7 + 8-, t9-, t11- and t12-18:1. This approach confirms on *in vivo* data the results observed *in vitro* (Jouany *et al.*, 2007). The fact that duodenal t11-18:1 is linked to 18:3 intake reflects the fact that the diets that induced higher proportion of t11-18:1 in the dataset were the diets richer in 18:3, and similarly for duodenal t10-18:2 and 18:2 intake. The results of the cluster analysis depend on the dataset used, and when other detailed data are available, the stability of the present clustering could be studied on a larger dataset.

Balance of the different C18 FA. For each dietary FA, we compiled the coefficients obtained from the different duodenal flow equations in order to estimate the mean fate of these dietary FA (Table 5). The sums of the coefficients (62% to 93%) were compatible with the global coefficient for C18 (0.84, see companion paper). Moreover, the proportions of the identified products of dietary 18:3, i.e. 40% 18:0 (calculated as 0.25/0.62, see Table 5), 39% trans-18:1, 15% 18:2 and 6% 18:3, were close to the results obtained *in vivo* with labelled 18:3, i.e. 43% 18:0, 4% cis-18:1, 34% trans-18:1 and 19% 18:2 + 18:3 (Scott and Cook, 1975). A majority of 18:2 and 18:1 intakes were hydrogenated to 18:0, and smaller amounts reached the duodenum as

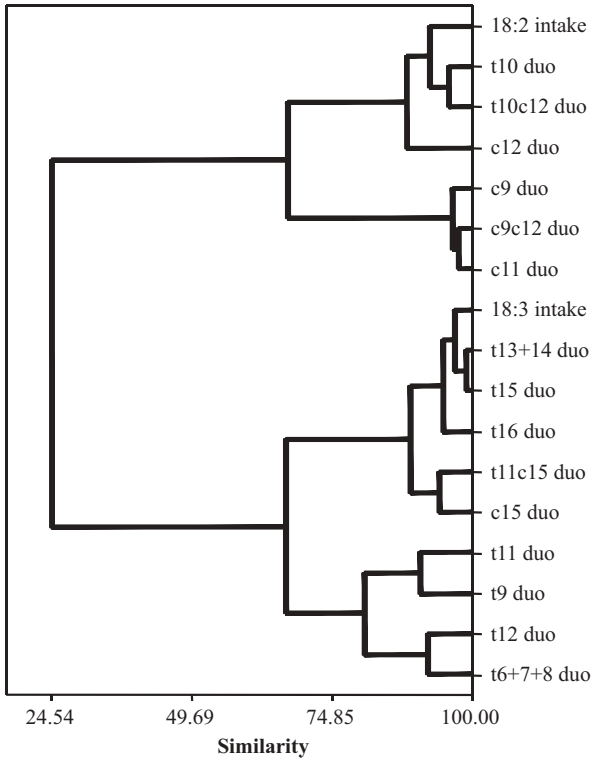

Figure 2 Clustering of 18:2 and 18:3 intakes and major 18:1 and 18:2 isomers at the duodenum, expressed in % C18 FA (17 data from six experiments, using correlation distance and average linkage method on centred data).

trans-18:1 (estimated at 26% of 18:2 intake and 9% of 18:1 intake). These estimates reveal a somewhat less-complete BH than those determined in goats by the rumen infusion of labelled 18:1, 18:2 and 18:3 (Bickerstaffe *et al.*, 1972). This approach is subject to numerous potential biases: the coefficients were obtained for control diets and did not take into account various effects (lipid form, etc.); they represented only marginal coefficients and did not take into account the intercepts; and some coefficients of small magnitude could have been masked by the high experiment effects on the data (for example, we found no significant contribution of dietary 18:2 to duodenal 18:2 isomers other than c9c12-18:2). However, this table could provide a rough estimate of the mean extent of total or partial BH of the different dietary FA. The validity or limits of these estimates may be challenged by dynamic modelling of the different FA pools (Harvatine and Allen, 2004; Moate *et al.*, 2004).

Some factors influencing FA ruminal metabolism

The meta-analysis of the effects of various dietary or ruminal factors on C18 ruminal FA metabolism was limited by the low number of experiments with within-experiment variation of the factors without concomitant variations of other variables. Since this condition was rarely met, the conclusions drawn are only indicative of these effects, since the currently available experimental data did not allow to formally exclude potential confounding factors.

Table 5 *Summarization of the coefficients of the different equations predicting duodenal C18 flows from dietary intake of precursors (diets without fish oil)*

Dietary intakes (g/kg DMI)	Duodenal flows (g/kg dry matter intake, DMI)						Sum of coefficients
	18:0	cis-18:1	trans-18:1	c9c12-18:2	Other 18:2	18:3	
18:0	0.75	–	–	–	–	–	0.75
18:1	0.54	0.24	0.09	–	–	–	0.87
Trans-18:1	–	–	0.70	–	–	–	0.70
18:2	0.62	ns	0.26	0.05	ns	–	0.93
18:3	0.25	ns	0.24	ns	0.09	0.04	0.62

DMI = dry matter intake; ns = not significant.

Ruminal pH effect. We selected the 14 experiments with within-experiment differences in ruminal pH higher than 0.2 units, from which we excluded treatments containing fish oil. There was a concomitant variation of forage : concentrate ratio in three of these 14 experiments (Kalscheur *et al*., 1997; Kucuk *et al*., 2001; Harvatine *et al*., 2002). In this subset, mean pH values varied between 5.5 and 6.6. In the models of duodenal flow of the C18 FA according to the intake of their precursors, we tested the significance of pH introduced both as a simple effect and as an interaction with the other variables of the model (i.e. as a modifier of the coefficients of precursor intake). We found no pH effect on the duodenal flows of cis-18:1, trans-18:1 and global 18:2, neither as a simple effect (on the intercepts of the models) nor as an interaction with intakes of 18:1, 18:2 or 18:3 (effect on the coefficients of the models). A significant interaction was found in the model of c9c12-18:2 duodenal flow ($P = 0.0005$, 20 treatments in five experiments): the coefficient of c9c12-18:2 intake was increased by 0.0109 ± 0.0014 for each 0.1 pH unit decrease (linear relationship, quadratic component of pH not significant). Concerning the duodenal flow of 18:3, there was also a significant interaction between 18:3 intake and pH ($P < 0.05$, 52 treatments in 12 experiments): the coefficient of 18:3 intake was increased by 0.0075 ± 0.0035 for each 0.1 pH unit decrease (linear relationship).

We could thus detect a significant protective effect of low pH on c9c12-18:2 and 18:3: one pH unit decrease more than doubled the mean coefficients. This effect of pH could be explained in diets supplemented with calcium salts of polyunsaturated FA by its influence on the dissociation of calcium salts. For other diets, pH could influence lipolysis (Demeyer and Van Nevel, 1995) and/or BH itself (Troegeler-Meynadier *et al*., 2003). Indeed, a pH sensitivity of lipases has been observed (Henderson, 1971; Faruque *et al*., 1974), and isomerase activity has also been shown to be dependent on pH (Kepler and Tove, 1967; Yokoyama and Davis, 1971; Troegeler-Meynadier, 2004), but modifications in bacterial populations or bacterial activity may also modify the BH process. Some authors have argued that the pH effect could be more important on lipolysis than on hydrogenation, without major effects on bacterial biomass (Van Nevel and Demeyer, 1996). In *in vitro* continuous cultures, the effects of pH on BH depend on other factors, including dilution rates or soluble carbohydrates (Martin and Jenkins, 2002).

Level of intake. Some authors have suggested that the level of intake could influence BH rates through a modification of ruminal outflow rates. There were three experiments (11 treatments) in our database with protocols comparing different levels of intake of the same diet (Sutton *et al*., 1970; Weisbjerg *et al*., 1992; Qiu *et al*., 2004a). Among these, Sutton *et al*. (1970) reported zero duodenal flows for 18:2 and 18:3 in several treatments, which were therefore excluded for these FA. We expressed levels of intake as percentage of the maximum observed intake for each diet, since animal body weights were not available in all these experiments. The duodenal flow of 18:3 was significantly decreased in high-intake treatments ($P = 0.03$). No effect was detected on 18:2 or 18:1 total flows, and there was only a tendency to decrease duodenal flows of cis-18:1 ($P = 0.1$). Similar results were obtained when rumen liquid outflow rate (in % per hour) was used as a proxy of level of intake (data not shown): a significant decrease of the coefficients for 18:3 ($P = 0.02$) and cis-18:1 ($P = 0.04$) was observed for increasing outflow rates.

Both approaches thus led to the same result: high levels of intake decreased duodenal flows of 18:3 (increased BH), and also decreased duodenal flows of cis-18:1. This result is surprising, since we could have expected a high ruminal turnover rate to decrease micro-organism activity on feedstuffs and thus decrease BH and increase duodenal flows of unsaturated FA; but it is consistent with *in vitro* continuous culture results (Martin and Jenkins, 2002; Qiu *et al*., 2004b). The various environmental conditions (pH, ruminal turnover rate, diet composition, etc.) may interact to cause modifications in bacterial populations and/or activity, which might be at the origin of these effects.

Forage : concentrate ratio. We selected the experiments (or treatment groups within experiments) implementing protocols aimed at comparing different forage proportions in the diet.

On 18:3 duodenal flow, the model including 18:3 intake and its interactions with lipid form and forage percentage identified significant effects of these three variables (26 treatments in seven experiments, $P = 0.03$ for forage

effect). The coefficient of 18:3 intake was decreased by 0.00128 ± 0.00031 for each forage percentage increase in the diet. The same model applied to the 18:2 duodenal flow model showed the same significant effects (29 treatments in eight experiments, $P = 0.03$ for the effect of forage percentage on the 18:2 intake coefficient). The coefficient of the 18:2 intake was decreased by 0.00149 ± 0.00038 for each forage percentage increase in the diet. In both cases, introducing rumen pH into the models had no significant effect. For cis-18:1 duodenal flow, the model including 18:1 intake and its interaction with forage percentage gave a significant effect for this interaction (18 treatments in five experiments, $P = 0.03$). The coefficient of 18:1 intake was decreased by 0.00340 ± 0.00089 for each forage percentage increase in the diet.

In all three cases, the replacement of the variable 'forage percentage' in the models by a binary factor (forage percentage higher or lower than 50% DMI, which corresponds to the bimodal distribution of the forage proportions in this set of data) also led to a significant effect on the coefficients of the respective FA intakes (high forage *v.* low forage: −0.040 ± 0.010 for 18:3, −0.055 ± 0.015 for 18:2, −0.094 ± 0.027 for cis-18:1).

High forage proportion in the diet thus had a negative effect on 18:1, 18:2 and 18:3 duodenal flows. This effect has been observed by numerous authors, and could stem from several phenomena known to influence BH, including ruminal pH, starch content of the diets and changes in bacterial populations. An increase in lipolysis, BH and bacterial biomass was observed with an increase in the forage proportion in the diet (Latham *et al*., 1972). Dietary fibre increases lipolysis and BH (Gerson *et al*., 1985), which could be explained by a higher population of cellulolytic bacteria that are thought to be the most active in the BH process (Harfoot and Hazlewood, 1997), or a higher food particles content, which are the preferred sites for BH (Harfoot and Hazlewood, 1997), or both. The relative effects on the coefficients were more pronounced on 18:2 and 18:3 (25% decrease in the coefficients for each 10% increase in forage proportion) than on 18:1 (15% decrease).

Fish oil. As seen in Table 2, fish oil inclusion significantly decreased the proportion of 18:0 and increased the proportions of trans-18:1 and 18:3 in total C18 duodenal flows. We selected experiments comparing treatments with and without fish oil or fishmeal, and calculated the intake of fish oil in % DMI. In the models of duodenal flows of c9-18:1, c9c12-18:2, global 18:2 and 18:3, there was no significant effect of fish oil intake, either as simple effect or as interaction with the coefficients of the respective FA intakes (with and without inclusion of the effect of technological treatment of the lipid in the models). The absence of significant effects in the models of cis-18:1, c9c12-18:2 and 18:3 duodenal flow excludes major inhibitory effects of fish oil on the BH of these FA. The only significant effects were obtained on trans-18:1 duodenal flow, i.e. a positive effect on the coefficients of 18:2 and 18:3 intakes (28 treatments in eight experiments). Each percentage increase of fish oil in DMI increased the coefficient of 18:2 intake to duodenal trans-18:1 by 0.166 ± 0.053 ($P = 0.03$) and the coefficient of 18:3 intake to duodenal trans-18:1 by 0.161 ± 0.051 ($P = 0.03$). The model for 18:0 duodenal flows included too many variables and potential interactions to be tested on this limited dataset. However, the decrease of 18:0 duodenal flow induced by inclusion of fish oil (Table 2) suggests that fish oil inhibits trans-18:1 BH. The same influence was observed in *in vitro* studies (Chow *et al*., 2004). The inhibition of the final step of BH could be triggered by changes in bacterial populations (Wachira *et al*., 2000), particularly group B bacteria (Harfoot and Hazlewood, 1997). Both 20:5 n-3 and 22:6 n-3 can inhibit trans-18:1 BH (AbuGhazaleh and Jenkins, 2004).

Table 6 *Apparent digestibilities in the small intestine (%)*

Fatty acid	Apparent digestibility
18:0	74.0 ± 16.9 ($n = 79$)
Global 18:1	79.1 ± 16.2 ($n = 74$)
Cis-18:1	77.9 ± 11.9 ($n = 40$)
Trans-18:1	82.4 ± 18.1 ($n = 45$)
Global 18:2	71.7 ± 20.1 ($n = 84$)
c9c12-18:2	77.2 ± 10.0 ($n = 17$)
c9t11-18:2	44.8 ± 49.9 ($n = 17$)
18:3	70.2 ± 20.5 ($n = 63$)

Data are mean ± s.d.

Absorption in the small intestine

We calculated apparent small intestine digestibilities for the different C18 FA as (duodenum flow − ileum flow)/duodenum flow. The mean ± SD, expressed in %, are given in Table 6. We studied the models of FA apparently absorbed in the small intestine (duodenum flow − ileum flow) as a function of their respective duodenal flows.

We excluded three experimental treatments from Aldrich *et al*. (1997) (whole canola seeds, crushed canola seeds and alkaline-treated canola seeds) that exhibited obviously aberrant intestinal absorption results for several FA compared with the other publications. Indeed, due to the small amount of data on intestinal absorption, a few atypical observations may have a major influence on parameter values in adjusted models.

There were 22 experiments (84 treatments) with measurements of duodenal and ileal flows. For all C18 FA, intercepts of mixed-model adjustments were not significantly different from 0. We thus set intercepts at zero (NOINT option of Proc Mixed) for the adjustments presented below. Preliminary examination of the data showed a significant difference in absorption for diets containing hydrogenated tallow (HT) (Moller and Borsting, 1987; Pantoja *et al*., 1996; Elliott *et al*., 1999), but no significant differences among other lipid supplements. We thus assigned a specific code to these diets for the absorption studies.

18:0. The dataset was characterised by an extreme experiment (Kucuk *et al*., 2004), which is represented by the four points in the upper right angle in Figure 3. Inclusion or exclusion of this experiment did not significantly modify the coefficients of the model.

The adjusted model retained for intestinal absorption had significant linear ($P < 0.0001$) and quadratic ($P < 0.0001$) effects of 18:0 duodenal flow, as well as a significant effect of HT on the linear coefficient ($P < 0.0001$). The adjusted model for diets without HT (see Figure 3) was

$$18{:}0_{abs} = (0.840 \pm 0.033) \times 18{:}0_{duo} - (0.0044 \pm 0.0005) \times 18{:}0^2{}_{duo} \text{ (in g/kg DMI)}$$

($N_{exp} = 21$, $N_{trt} = 76$, RMSE = 1.67).

The linear coefficient for diets with HT was 0.644 ± 0.045. Apparent digestibility calculated from this model varied between 81.8% (for a duodenal flow of 5 g/kg DMI) and 40.0% (for a duodenal flow of 100 g/kg DMI). The same model without the quadratic effect had a linear coefficient of 0.630 ± 0.046 for diets without HT, and a significantly lower coefficient for diets with HT (0.433 ± 0.057). The significant quadratic effect suggested a saturation of 18:0 intestinal absorption at high duodenal flows. This saturation was specific to 18:0 and was not the result of a saturation of total FA absorption capacities, since the absorption of other FA is not modified by high duodenal flows of 18:0 (Kucuk *et al*., 2004).

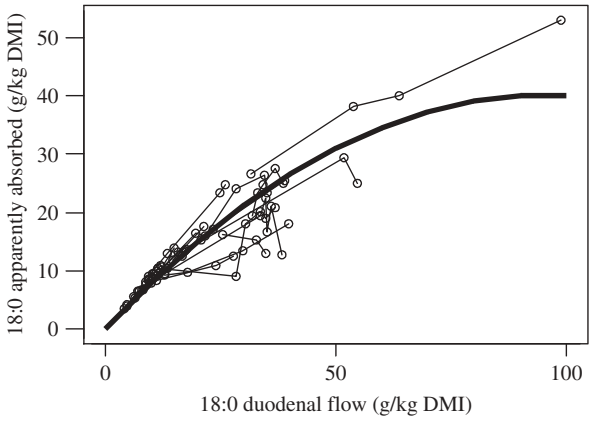

Figure 3 Apparent absorption of 18:0 in the small intestine as a function of duodenal flow: the points are experimental treatments, thin lines link treatments within the same experiment, and the thick line represents the adjusted model for diets without hydrogenated tallow.

Low absorption of saturated FA has been observed by many authors, and various hypotheses have been put forward, including lower micelle formation (Doreau, 1992) or slower TG formation from saturated FA in intestinal cells that could reduce absorption rates (Borsting *et al*., 1992). In publications reporting both terminal ileum and faecal flows, there was no significant difference in apparent digestibilities in the small intestine or total intestine (paired *t* test, $n = 36$, $P = 0.7$), meaning that BH in the colon is not responsible for previous reports of low apparent digestibility of 18:0.

Unsaturated C18. There was a high variability in apparent digestibility data (Table 6), especially for trans-18:1, c9t11-18:2 and 18:3.

For all the unsaturated C18, we did not find any significant quadratic effect in the mixed models of absorption in relation to duodenal flow. Linear coefficients for 18:1 and 18:3 absorption were significantly lower for diets containing HT. The adjustments for unsaturated C18 absorption models are shown in Table 7.

These coefficients were ranked similarly to the apparent digestibility values, but their variability was lower (except for c9t11-18:2). The most easily absorbed unsaturated C18 was 18:1, with coefficients slightly higher for trans-18:1 than for cis-18:1, followed by global 18:2 and 18:3, which had similar coefficients, although c9c12-18:2 may be slightly more absorbed than other 18:2 isomers. The lowest and most variable coefficient was obtained for c9t11-18:2. This high variability can be explained by the low duodenal and ileal flows of this isomer and probable analytic inaccuracies or biases.

There are thus two different effects (that may be linked or independent) that may affect small intestine absorption: a decrease in intestinal digestibility of 18:0 for high duodenal flows, and an inhibiting effect of saturated fats on 18:0, 18:1 and 18:3 absorption.

A widely accepted hypothesis is lipolysis-limited intestinal absorption, where, due to the slow pH increase throughout the small intestine, the various lipases may only

Table 7 *Adjusted coefficients of apparent absorption in the small intestine (g/kg dry matter intake (DMI), dependent variable) of selected unsaturated fatty acids according to their duodenal flows (g/kg DMI, independent variable)*

Fatty acid	Diets containing hydrogenated tallow	Other diets	No. of treatments (experiments)	Residual s.d.
Global 18:1	0.564 ± 0.045	0.845 ± 0.018	71 (20)	0.44
Cis-18:1	0.659 ± 0.037	0.861 ± 0.012	40 (10)	0.20
Trans-18:1	0.416 ± 0.073	0.889 ± 0.040	45 (11)	0.27
Global 18:2[a]	0.734 ± 0.038		81 (22)	0.14
c9c12-18:2[a]	0.797 ± 0.068		17 (5)	0.07
c9t11-18:2[a]	0.710 ± 0.187		17 (5)	0.004
18:3	0.529 ± 0.050	0.741 ± 0.036	61 (18)	0.04

[a]For 18:2 isomers, all diets were pooled (the adjustment was better without hydrogenated tallow effect).

be active at the end of the small intestine, and so remain unable to hydrolyse high amounts of duodenal TG (Jenkins and Jenny, 1992). However, this hypothesis is not valid for plant oils, since there is no decrease in fat digestibility with high quantities (up to 1 kg/day) of post-ruminally infused oils (Chilliard *et al*., 1991; Bandara, 1997; Chelikani *et al*., 2004). Proportions of FA in the esterified form at the duodenum differ between studies, with the % of total FA values cited at 13–15% (Moller and Borsting, 1987), 20–40% (Legay-Carmier, 1989) or 30–98% (Elliott *et al*., 1999). HT increased the proportion of esterified FA at the duodenum in one study (Elliott *et al*., 1999) but not in another (Moller and Borsting, 1987); but in both cases HT increased the proportion of esterified FA at the ileum, thus supporting the hypothesis of lipolysis-limited absorption for HT. This could be due to the physical form of the supplement, such as the particle size of hydrogenated fats (Doreau, 1992; Pantoja *et al*., 1995), or due to the fact that lipolysis is inhibited when saturated FA are in positions sn-1 or sn-3 of the TG (Mattson *et al*., 1991; Fouw *et al*., 1994; Brink *et al*., 1995), which is most probably the case in HT. Saturated FA are less incorporated in micelles (due to a decreased solubility), their melting point is above body temperature, and they can complex with minerals to form insoluble salts in the intestine (Brink *et al*., 1995). All these characteristics are deleterious to their absorption in the intestine. Differences in absorption of the different FA could also arise from differences in enterocyte FA uptake from micelles (linked to FA solubility in water), differences in transport across the enterocyte membrane (Hamilton, 1998) or from different post-absorptive mechanisms (enterocyte FA metabolism). Monounsaturated FA are mainly integrated in TG, whereas polyunsaturated and trans-FA are mainly incorporated in phospholipids (Bernard *et al*., 1987, Carlier *et al*., 1991). The re-esterification of FA thus demands specific ratios of saturated and unsaturated FA, or its kinetics can differ between FA (Ockner *et al*., 1972). This could explain the decrease in uptake with an increasing degree of unsaturation as well as the phenomenon of 'coabsorption', in which the absorption of a given FA is influenced by absorption of the others (McDonald and Weidman, 1987). These phenomena may explain the limitation of 18:0 absorption at high 18:0 duodenal flows.

General discussion

The meta-analysis approach was highly useful in extracting information from the large set of quantitative data used in the present study. The distinction between experiment effects and within-experiment effects (linked to the controlled factors) increases the accuracy of the models (St-Pierre, 2001; Sauvant *et al*., 2005). The use of mixed models should broaden the range of model applications and also more efficiently detect potential correlations, e.g. between experiment intercepts and slopes (St-Pierre, 2001). In this study, the within-experiment and inter-experiment relations were coherent, so the within-experiment effects obtained from the models, despite integrating a small variability linked to the differences among experiments, must be very close to pure within-effects (biological effects). Moreover, the coefficients retained in the equations, which were obtained from control treatments when there were other modifying effects (such as lipid supplement form, etc.), were very close to the mean coefficients, thus validating the use of the equations for a wide range of lipid supplements. In some cases, there were significant correlations between independent variables in the same model. Even if this could introduce a bias in coefficient adjustment, it is probably minor for several reasons: the correlation coefficients were always low (<0.5); correlations did not exist between all independent variables; the correlations did not reflect a systematic linkage between two variables in all experiments, but rather arose from the combination of two groups of experiments in which each variable varied independently from the others; the sums of coefficients (Table 5) were consistent with the global C18 coefficient, excluding major over- or underestimation bias.

Experimental variability can arise from methods (e.g. FA analysis, total FA quantities, flow measurements, etc.) and from individual variability between the animals, particularly those with high-lipid diets. Hence, the conclusions and tendencies reported here represent the best projections of a highly variable set of experiments conducted in various countries, on various types of animals, with various diets, under various environmental conditions. The results obtained in any given experiment can thus diverge from these mean results (but it could prove particularly interesting to find out why), and it is important not to lose sight of the variability around the mean coefficients provided by our analyses.

Duodenal flows

For all the flows studied, there was a large experiment effect (intercept or slope) on the data, and an increasing variance of the flows with increasing intakes of the various FA. This somewhat limits the applicability of the results for technical purposes. These results are more accurate in predicting variations in duodenal flows in response to a modification of the diet from a known level, rather than in predicting absolute duodenal flows. Residue dispersion was in general larger for high FA intakes, which could arise from a greater biological variability for high-lipid diets. We did not find any significant effect of species or animal category (lactating cows, other cattle or sheep) on the coefficients of the different models. This represents a great advantage for future model applications.

The positive intercept in global 18:2, c9c12-18:2 and 18:3 models do not reflect a synthesis of these FA in the rumen for zero intake but likely reflect the structure of the data: most experiments had significant amounts of these FA in their control diets, meaning that the validity range of the models cannot be extended to zero intake. Two estimates of polyunsaturated FA disappearance in the rumen were produced, which differ in their meaning: apparent BH data

are a global estimate of the disappearance in the rumen (around 86% to 87% for both c9c12-18:2 and 18:3), whereas model coefficients are within-experiment slopes, reflecting the proportion of supplemental FA intake that escapes ruminal BH and reaches the duodenum. Here again, coefficients are similar between c9c12-18:2 (0.054) and 18:3 (0.052), but higher when duodenal flow of global 18:2 is considered (0.117). The contrast between apparent BH and model coefficients is related to the positive intercepts of the models, which could also reflect a difference in BH between the FA of the basal diet and the supplemental FA added to the diet. Previous studies reporting a lower BH (higher duodenal flow) for 18:2 compared with 18:3 (e.g. Doreau and Ferlay, 1994) were probably biased by the fact that their 18:2 data included isomers other than c9c12-18:2, leading to an overestimation of c9c12-18:2 duodenal flow. This is confirmed by the higher coefficient obtained in the present study for the model of global 18:2 duodenal flow (0.117) compared with c9c12-18:2 duodenal flow (0.054). When c9c12-18:2 is separated from other 18:2 isomers, its ruminal metabolism seems very similar to that of 18:3, both from apparent BH data and from within-experiment coefficients.

The study of dietary lipid treatment effects was limited by the low number of experiments for each treatment, and a possible confusion between experiment and treatment effects. We could detect significant effects of some technological treatments on the coefficients of 18:2 and 18:3 but no effect on 18:1 in the models tested. We found protective effects of alkaline-treated seeds and encapsulated oils on 18:2, and effects of alkaline-treated seeds, calcium salts, formaldehyde treatment (encapsulated oils or treated seeds) and whole seeds on 18:3. However, these results arose from a meta-design that was not totally suitable for the determination of such effects. Only those experiments that include several intake levels of several lipid supplements would be able to give definitive conclusions on this point. The other dietary factors faced the same problem of scarcity of experiments studying these factors, or groups of experiments with sufficient variation among treatments in the studied factors. We quantified the protective effect of low pH on c9c12-18:2 and 18:3 observed in numerous *in vivo* and *in vitro* studies, as well as the protective effect of a low forage : concentrate ratio on cis-18:1, 18:2 and 18:3. High levels of intake appeared to decrease duodenal flows of 18:3, and probably also 18:1.

Dietary fish oil largely increased trans-18:1 and decreased 18:0 duodenal flows. Even without fish oil, the last step of BH (from trans-18:1 to 18:0) seems limiting, since trans-18:1 levels were high in duodenal digesta. This step is thought to be achieved by group B rumen bacteria (Kemp and Lander, 1984; Harfoot and Hazlewood, 1997). Based on experiments with high levels of dietary trans-18:1, we found that only around 30% of this trans-18:1 was metabolised in the rumen. Cis-18:1, 18:2 and 18:3 contribute to the duodenal flow of trans-18:1. The accumulation of trans-18:1 with polyunsaturated fatty acid-rich diets probably arises from both an increased flow of precursors and an inhibition of trans-18:1 BH by dietary polyunsaturated fatty acids (particularly 18:3) and/or BH intermediates (Troegeler-Meynadier, 2004). The various ruminal micro-organisms have different sensitivities or affinities to the various polyunsaturated fatty acids, which could modify their biomasses or activities. The balance between the different populations can influence the outcomes of BH (Kemp and Lander, 1984). Passage of c9c12-18:2 and 18:3 at the duodenum seemed very low, with coefficients of around 5% of the supplemental intake. It did not seem to be modified by fish oil inclusion, but could be increased by rumen environment (pH, forage : concentrate ratio) and certain lipid technological treatments.

Concerning the different isomers of 18:1 and 18:2, there is currently a shortfall in *in vivo* data, and the experimental conditions reported to date are too diverse to ensure representative conclusions from model adjustments without confounding effects linked to diet or animal characteristics other than FA intakes. For the isomers that are transferred into animal tissues without further transformation, analysis of data on milk or adipose tissue FA profiles could provide deeper insight into their ruminal production. *In vitro* data could also give information on these processes, even if there are differences between *in vivo* and *in vitro* data for BH products.

Intestinal absorption

We present here the first study focussed on small intestine absorption alone, since the previous studies (Doreau and Ferlay, 1994; Sauvant and Bas, 2001) combined small intestine and whole intestine data. Experiment effects were less important for intestinal absorption than for ruminal digestion. All models had non-significant intercepts, which means that based on this set of data, we could not detect any endogenous flow (due to cell desquamation, for example, which would appear as a negative intercept for null duodenal flow). For all FA except 18:0, we obtained linear models, and absorption coefficients were thus close to the apparent digestibilities. Nonetheless, variability of absorption coefficients was lower than that of apparent digestibilities, since the latter included experiment effects. There was a major negative effect of HT on intestinal absorption, from −23% to −53% on 18:0, 18:1 and 18:3.

In conclusion, this study is the first meta-analysis on C18 FA digestion. We provided equations to predict duodenal FA flows from dietary FA intakes and synthesised *in vivo* data on the various trans-FA and 18:2 isomers (including CLA). We found that the apparent BH of 18:2 and 18:3 are probably closer than previously thought, and we provided a first synthesis on FA apparent absorption in the small intestine, leading to more accurate estimates of apparent absorption coefficients for the various C18 FA.

Finally, these data could constitute a first step towards being able to predict absorbed FA based on diet composition and characteristics in order to modulate animal product

FA profiles. At the rumen level, the complex regulation of the ruminal ecosystem by interaction of dietary and other environmental factors introduced a large biological variability that somewhat limits the utilisation of mean values. At the small intestine level, the experiment effects were lower, but the underlying mechanisms still require better understanding. The study of existing data revealed a lack of data in three main areas: experiments with several supplementation levels of several lipid supplements, which are essential to generating definitive conclusions on the differential BH of the supplements; experiments with detailed duodenal FA profiles (individual isomers of 18:1 and 18:2), which could also enhance our understanding of the potential metabolic effects of these FA on animal metabolism and product quality; and experiments comparing the effects of different forages on duodenal FA flows and on the BH of concentrate-supplied lipids. More experimental data in these fields could enable a better understanding of ruminal FA metabolism and thus establish the means to modulate ruminant product FA profiles.

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References

AbuGhazaleh AA and Jenkins TC 2004. Disappearance of docosahexaenoic and eicosapentaenoic acids from cultures of mixed ruminal microorganisms. Journal of Dairy Science 87, 645.

AbuGhazaleh AA, Riley MB, Thies EE and Jenkins TC 2005. Dilution rate and pH effects on the conversion of oleic acid to trans C18:1 positional isomers in continuous culture. Journal of Dairy Science 88, 4334–4341.

Aldrich CG, Merchen NR, Drackley JK, Gonzalez SS, Fahey JGC and Berger LL 1997. The effects of chemical treatment of whole canola seed on lipid and protein digestion by steers. Journal of Animal Science 75, 502–511.

Bandara ABPA 1997. Modifying fatty acid composition of bovine milk by abomasal infusion or dietary supplementation of seed oils or fish oil. Virginia Polytechnic Institute and State University.

Baumgard LH, Corl BA, Dwyer DA, Saebo A and Bauman DE 2000. Identification of the conjugated linoleic acid isomer that inhibits milk fat synthesis. American Journal of Physiology – Regulatory, Integrative and Comparative Physiology 278, R179–R184.

Bernard A, Echinard B and Carlier H 1987. Differential intestinal absorption of two fatty acid isomers: elaidic and oleic acids. American Journal of Physiology 253, G751–G759.

Bickerstaffe R, Noakes DE and Annison EF 1972. Quantitative aspects of fatty acid biohydrogenation, absorption and transfer into milk fat in the lactating goat, with special reference to the cis- and trans-isomers of octadecenoate and linoleate. Biochemical Journal 130, 607–617.

Borsting CF, Weisbjerg MR and Hvelplund T 1992. Fatty acid digestibility in lactating cows fed increasing amounts of protected vegetable oil, fish oil or saturated fat. Acta Agriculturae Scandinavica. Section A, Animal Science 42, 148–156.

Brink EJ, Haddeman E, de Fouw NJ and Weststrate JA 1995. Positional distribution of stearic acid and oleic acid in a triacylglycerol and dietary calcium concentration determines the apparent absorption of these fatty acids in rats. Journal of Nutrition 125, 2379–2387.

Carlier H, Bernard A and Caselli C 1991. Digestion and absorption of polyunsaturated fatty acids. Reproduction, Nutrition, Development 31, 475–500.

Chang JH-P, Sturdivant CA, Greene LW, Lunt DK and Smith SB 1991. Fatty acid absorption of cattle fed diets containing high-oleate sunflower seed. Journal of Animal Science 69, 547–548.

Chelikani PK, Bell JA and Kennelly JJ 2004. Effects of feeding or abomasal infusion of canola oil in Holstein cows 1. Nutrient digestion and milk composition. Journal of Dairy Research 71, 279–287.

Chikunya S, Demirel G, Enser M, Wood JD, Wilkinson RG and Sinclair LA 2004. Biohydrogenation of dietary n-3 PUFA and stability of ingested vitamin E in the rumen, and their effects on microbial activity in sheep. British Journal of Nutrition 91, 539–550.

Chilliard Y, Bauchart D, Gagliostro G, Ollier A and Vermorel M 1991. Duodenal rapeseed oil infusion in early and midlactation cows. 1. Intestinal apparent digestibility of fatty acids and lipids. Journal of Dairy Science 74, 490–498.

Chow TT, Fievez V, Moloney AP, Raes K, Demeyer D and De Smet S 2004. Effect of fish oil on in vitro rumen lipolysis, apparent biohydrogenation of linoleic and linolenic acid and accumulation of biohydrogenation intermediates. Animal Feed Science and Technology 117, 1–12.

Demeyer DI and Van Nevel CJ 1995. Transformations and effects of lipids in the rumen: three decades of research at Gent University. Archives of Animal Nutrition 48, 119–134.

Dewhurst RJ, Evans RT, Scollan ND, Moorby JM, Merry RJ and Wilkins RJ 2003. Comparison of grass and legume silages for milk production. 2. In vivo and in sacco evaluations of rumen function. Journal of Dairy Science 86, 2612–2621.

Doreau M 1992. Effects of supplementation with hydrogenated fish fat on digestion in dairy cows. Annales de Zootechnie 41, 137–143.

Doreau M and Ferlay A 1994. Digestion and utilisation of fatty acids by ruminants. Animal Feed Science and Technology 45, 379–396.

Doreau M, Ueda K and Poncet C 2003. Fatty acid ruminal metabolism and intestinal digestibility in sheep fed ryegrass silage and hay. Tropical and Subtropical Agroecosystems 3, 289–293.

Elliott JP, Drackley JK, Beaulieu AD, Aldrich CG and Merchen NR 1999. Effects of saturation and esterification of fat sources on site and extent of digestion in steers: digestion of fatty acids, triglycerides, and energy. Journal of Animal Science 77, 1919–1929.

Faruque AJMO, Jarvis BDW and Hawke JC 1974. Studies on rumen metabolism. VIII. Characteristics of lipases in rumen contents and rumen bacteria. Journal of the Science of Food and Agriculture 25, 439–449.

Fouw NJD, Kivits GAA, Quinlan PT and Nielen WGLv 1994. Absorption of isomeric, palmitic acid-containing triacylglycerols resembling human milk fat in the adult rat. Lipids 29, 765–770.

Gerson T, John A and King ASD 1985. The effects of dietary starch and fibre on the in vitro rates of lipolysis and hydrogenation by sheep rumen digesta. Journal of Agricultural Science 105, 27–30.

Hamilton JA 1998. Fatty acid transport: difficult or easy? Journal of Lipid Research 39, 467–481.

Harfoot CG and Hazlewood GP 1997. Lipid metabolism in the rumen. In The rumen microbial ecosystem (ed. PN Hobson and CS Stewart), pp. 382–426. Blackie Academic & Professional, London.

Harvatine KJ and Allen MS 2004. Kinetic model of rumen biohydrogenation: fractional rates of fatty acid biohydrogenation and passage. Journal of Animal and Feed Sciences 13, 87–90.

Harvatine DI, Firkins JL and Eastridge ML 2002. Whole linted cottonseed as a forage substitute fed with ground or steam-flaked corn: digestibility and performance. Journal of Dairy Science 85, 1976–1987.

Hazlewood GP, Kemp P, Lander DJ and Dawson RMC 1976. C18 unsaturated fatty acid hydrogenation patterns of some rumen bacteria and their ability to hydrolyze exogenous phospholipids. British Journal of Nutrition 35, 293–297.

Henderson C 1971. A study of the lipase produced by *Anaerovibrio lipolytica*, a rumen bacterium. Journal of General Microbiology 65, 81–89.

Hogan JP, Connell PJ and Mills SC 1972. The digestion of safflower oil-casein particles protected against ruminal hydrogenation in sheep. Australian Journal of Agricultural Research 23, 87–95.

Jenkins TC and Jenny BF 1992. Nutrient digestion and lactation performance of dairy cows fed combinations of prilled fat and canola oil. Journal of Dairy Science 75, 796–803.

Jouany JP, Lassalas B, Doreau M and Glasser F 2007. Dynamic features of the rumen metabolism of linoleic acid, linolenic acid, and linseed oil measured in vitro. Lipids 42, 351–360.

Kalscheur KF, Teter BB, Piperova LS and Erdman RA 1997. Effect of dietary forage concentration and buffer addition on duodenal flow of trans-C18:1 fatty

acids and milk fat production in dairy cows. Journal of Dairy Science 80, 2104–2114.

Kemp P and Lander DJ 1984. Hydrogenation in vitro of alpha-linolenic acid to stearic acid by mixed cultures of pure strains of rumen bacteria. Journal of General Microbiology 130, 527–533.

Kepler C and Tove CB 1967. Biohydrogenation of unsaturated fatty acids. III. Purification and properties of a linoleate delta12-cis, delta11-trans-isomerase from *Butyvibrio fibrisolvens*. Journal of Biological Chemistry 242, 5686–5692.

Kramer JKG, Cruz-Hernandez C and Zhou J 2001. Conjugated linoleic acids and octadecenoic acids: analysis by GC. European Journal of Lipid Science and Technology 103, 600–609.

Kucuk O, Hess BW and Rule DC 2004. Soybean oil supplementation of a high-concentrate diet does not affect site and extent of organic matter, starch, neutral detergent fiber, or nitrogen digestion, but influences both ruminal metabolism and intestinal flow of fatty acids in limit-fed lambs. Journal of Animal Science 82, 2985–2994.

Kucuk O, Hess BW, Ludden PA and Rule DC 2001. Effect of forage : concentrate ratio on ruminal digestion and duodenal flow of fatty acids in ewes. Journal of Animal Science 79, 2233–2240.

Latham MJ, Storry JE and Sharpe ME 1972. Effect of low-roughage diets on the microflora and lipid metabolism in the rumen. Applied Microbiology 24, 871–877.

Lee MRF, Harris LJ, Dewhurst RJ, Merry RJ and Scollan ND 2003. The effect of clover silages on long chain fatty acid rumen transformations and digestion in beef steers. Animal Science 76, 491–501.

Lee MRF, Tweed JKS, Connelly PL, Merry RJ, Dewhurst R and Scollan ND 2004. Duodenal flow and biohydrogenation of C18 polyunsaturated fatty acids in beef steers fed high sugar grass, red clover or grass/red clover mix silages. In Annual Meeting of the BSAS, p. 66.

Legay-Carmier F 1989. Effet de rations riches en matières grasses sur le métabolisme lipidique des principaux compartiments microbiens du contenu de rumen chez la vache laitière; conséquences sur le flux duodénal des constituants microbiens. Université Clermont 2 Blaise Pascal.

Loor JJ, Ueda K, Ferlay A, Chilliard Y and Doreau M 2004. Biohydrogenation, duodenal flow, and intestinal digestibility of trans fatty acids and conjugated linoleic acids in response to dietary forage : concentrate ratio and linseed oil in dairy cows. Journal of Dairy Science 87, 2472–2485.

Loor JJ, Ueda K, Ferlay A, Chilliard Y and Doreau M 2005. Intestinal flow and digestibility of trans fatty acids and conjugated linoleic acids (CLA) in dairy cows fed a high-concentrate diet supplemented with fish oil, linseed oil, or sunflower oil. Animal Feed Science and Technology 119, 203–225.

Mann J 2002. Diet and risk of coronary heart disease and type 2 diabetes. Lancet 360, 783–789.

Martin SA and Jenkins TC 2002. Factors affecting conjugated linoleic acid and trans-C18:1 fatty acid production by mixed ruminal bacteria. Journal of Animal Science 80, 3347–3352.

Mattson FH, Nolen GA and Webb MR 1991. The absorbability by rats of various triglycerides of stearic and oleic acid and the effect of dietary calcium and magnesium. Journal of Nutrition 109, 1682–1689.

McDonald GB and Weidman M 1987. Partitioning of polar fatty acids into lymph and portal vein after intestinal absorption in the rat. Quarterly Journal of Experimental Physiology 72, 153–159.

Moate PJ, Chalupa W, Jenkins TC and Boston RC 2004. A model to describe ruminal metabolism and intestinal absorption of long chain fatty acids. Animal Feed Science and Technology 112, 79–105.

Moller PD and Borsting CF 1987. Fedtsyreomsaetningen hos malkekoer fodret med forsaebet, traditionelt eller maettet animalsk fedt. 3. Omsaetningen af flygtige fedtstyrer i formaverne. Statens Husdyrbrugsforsog Meddelelse 695, 1–4.

Mosley EE, Powell GL, Riley MB and Jenkins TC 2002. Microbial biohydrogenation of oleic acid to trans isomers in vitro. Journal of Lipid Research 43, 290.

Ockner RK, Pittman JP and Yager JL 1972. Differences in the intestinal absorption of saturated and unsaturated long chain fatty acids. Gastroenterology 62, 981–992.

Pantoja J, Firkins JL and Eastridge ML 1995. Site of digestion and milk production by cows fed fats differing in saturation, esterification, and chain length. Journal of Dairy Science 78, 2247–2258.

Pantoja J, Firkins JL, Eastridge ML and Hull BL 1996. Fatty acid digestion in lactating dairy cows fed fats varying in degree of saturation and different fiber sources. Journal of Dairy Science 79, 575–584.

Qiu X, Eastridge ML and Firkins JL 2004a. Effects of dry matter intake, addition of buffer, and source of fat on duodenal flow and concentration of conjugated linoleic acid and trans-11 c18:1 in milk. Journal of Dairy Science 87, 4278–4286.

Qiu X, Eastridge ML, Griswold KE and Firkins JL 2004b. Effects of substrate, passage rate, and pH in continuous culture on flows of conjugated linoleic acid and trans C18:1. Journal of Dairy Science 87, 3473–3479.

Sas Institute 2000. SAS User's Guide: Statistics. Version 8.1. Cary, NC.

Sauvant D and Bas P 2001. Lipid digestion in ruminants. Productions Animales 14, 303–310.

Sauvant D, Schmidely P and Daudin JJ 2005. Les méta-analyses des données expérimentales: applications en nutrition animale. Productions Animales 18, 63–73.

Schmidely P, Glasser F, Doreau M and Sauvant D 2008. Digestion of fatty acids in ruminants: a meta-analysis of flows and variation factors. 1. Rumen fermentation patterns, microbial efficiency and duodenal flows of total fatty acid. Animal 2, 677–690.

Scott TW and Cook LJ 1975. Effects of dietary fat on lipid metabolism in ruminants. In Digestion and metabolism in the ruminant (ed. GB McDonald and ACI Warner), pp. 481–495. University of New England, Armidale, Australia.

Selner DR and Schultz LH 1980. Effects of feeding oleic acid or hydrogenated vegetable oils to lactating cows. Journal of Dairy Science 63, 1235–1241.

Shingfield KJ, Ahvenjarvi S, Toivonen V, Arola A, Nurmela KVV, Huhtanen P and Griinari JM 2003. Effect of dietary fish oil on biohydrogenation of fatty acids and milk fatty acid content in cows. Animal Science 77, 165–179.

St-Pierre NR 2001. Invited review: Integrating quantitative findings from multiple studies using mixed model methodology. Journal of Dairy Science 84, 741–755.

Sutton JD, Storry JE and Nicholson JWG 1970. The digestion of fatty acids in the stomach and intestines of sheep given widely different rations. Journal of Dairy Research 37, 97–105.

Troegeler-Meynadier A 2004. Ruminal synthesis and mammary secretion of conjugated linoleic acid (CLA) in dairy cow: effect of polyunsaturated acids and of ruminal pH. Université Aix-Marseille III.

Troegeler-Meynadier A, Nicot MC, Bayourthe C, Moncoulon R and Enjalbert F 2003. Effects of pH and concentrations of linoleic and linolenic acids on extent and intermediates of ruminal biohydrogenation in vitro. Journal of Dairy Science 86, 4054–4063.

Van Nevel CJ and Demeyer DI 1996. Influence of pH on lipolysis and biohydrogenation of soybean oil by rumen contents in vitro. Reproduction, Nutrition, Development 36, 53–63.

Vossenberg JLCM and Joblin KN 2003. Biohydrogenation of C18 unsaturated fatty acids to stearic acid by a strain of *Butyrivibrio hungatei* from the bovine rumen. Letters in Applied Microbiology 37, 424–428.

Wachira AM, Sinclair LA, Wilkinson RG, Hallett K, Enser M and Wood JD 2000. Rumen biohydrogenation of n-3 polyunsaturated fatty acids and their effects on microbial efficiency and nutrient digestibility in sheep. Journal of Agricultural Science 135, 419–428.

Wahle KWJ, Heys SD and Rotondo D 2004. Conjugated linoleic acids: are they beneficial or detrimental to health? Progress in Lipid Research 43, 553–587.

Wang Z and Goonewardene LA 2004. The use of MIXED models in the analysis of animal experiments with repeated measures data. Canadian Journal of Animal Science 84, 1–11.

Weisbjerg MR, Borsting CF and Hvelplund T 1992. Fatty acid metabolism in the digestive tract of lactating cows fed tallow in increasing amounts at two feed levels. Acta Agriculturae Scandinavica. Section A, Animal Science 42, 106–114.

White BG, Ingalls JR, Sharma HR and McKirdy JA 1987. The effect of whole sunflower seeds on the flow of fat and fatty acids through the gastrointestinal tract of cannulated Holstein steers. Canadian Journal of Animal Science 67, 447–459.

Yokoyama MT and Davis CL 1971. Hydrogenation of unsaturated fatty acids by *Treponema* (*Borrelia*) Strain $B_2$5, a rumen spirochete. Journal of Bacteriology 107, 519–527.