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Digestion of fatty acids in ruminants: a meta-analysis of flows and variation factors. 1. Total fatty acids

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A database built from 95 experiments with 303 treatments was used to quantify the ruminal biohydrogenation (BH) of fatty acids (FA), efficiency of microbial protein synthesis (EMPS), duodenal flow and intestinal absorption of total FA and of FA with 12 to 18 C units, in response to variations in dietary FA content, source or technological treatment of fat supplement. Flows of FA were expressed relative to dry matter intake (DMI) to compile data from bovine and ovine species. BH tended to increase curvilinearly with FA intake, whereas dietary FA did not affect EMPS. A linear relationship between FA intake and duodenal flow of total FA was obtained, with a coefficient of 0.75 ± 0.06 g duodenal FA/kg DMI for each g FA intake/kg DMI. Between experiments, positive balances of total FA (intake – duodenum) were related to low EMPS. Relationships between duodenal flows of FA with 12 to 18 C units and their respective intakes were linear, with a coefficient that increased with the number of C units. Duodenal flow of bacterial FA was linearly related to FA intake (coefficient 0.33 ± 0.13), whereas contribution of bacterial lipid to duodenal flow decreased as FA intake increased. For each FA with 12 to 16 C units, prediction of FA absorption from its respective duodenal flow was linear. For total FA and FA with 18 C units, apparent absorption levelled off at high duodenal flows. All these relationships were discussed according to current knowledge on microbial metabolism in the rumen and on the intestinal digestibility of FA in the intestine.

Keywords: biohydrogenation, fatty acids, meta-analysis, microbes, rumen

Introduction

Over the last decade, the positive effects that dietary fat can exert on animal performance and the quality of ruminant-derived products for human consumption have led to a resurgence of interest in feeding supplemental fat. Human dietary guidelines recommend decreasing the intake of saturated fats, while increasing the content of polyunsaturated fatty acids (PUFA) and the isomers of the conjugated linoleic acid (CLA) from animal products (Williams, 2000). However, in ruminants, the possibilities for manipulation of fatty acid (FA) content are limited, because dietary FA may affect the efficiency of microbial protein synthesis (EMPS) and fibre digestion (Elizalde *et al*., 1999; Ueda *et al*., 2003). Moreover, PUFA undergo extensive biohydrogenation (BH) in the rumen (Harfoot and Hazlewood, 1997), which increases the duodenal flow of saturated FA at the expense of PUFA. The BH of FA is also characterized by isomerization and hydrogenation, which produces trans-FA and CLA. It has been claimed that some CLA have a positive impact on human health, at least the *c*9,*t*11-C18:2 isomer (Martin and Valeille, 2002), while some trans-FA possibly have negative effects (EFSA, 2004). In addition, duodenal flows of FA also include dietary cis-FA that escaped BH, and FA synthesized *de novo* by ruminal bacteria.

Manipulations of diet composition such as lipid content, FA profile of added lipids, percentage and/or nature of the concentrate and addition of buffers are key factors that influence EMPS, BH and FA profiles in the duodenum. Consequently, different forms of protection of FA such as Ca salts, amides, encapsulation and formaldehyde treatment of protein-rich lipid supplements have been developed to protect PUFA from BH and/or prevent the negative effects of PUFA on microbial metabolism in the rumen.

There are numerous reviews dealing with the digestion of dietary fat in ruminants (Jenkins, 1993; Doreau and Ferlay, 1994; Sauvant and Bas, 2001), but there is also a growing body of data that could be compiled to establish a more quantitative and more precise prediction of the response of rumen FA metabolism to dietary factors. Consequently, the

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objectives of this meta-analysis are to predict duodenal flows and intestinal absorption of total FA as well as of individual FA in relation to their chain length and their response to changes in dietary FA content, source or technological treatment of the fat supplement. The consequences of changing dietary fat characteristics on BH and EMPS are also quantified. The companion paper (Glasser *et al*., 2008) focuses on the major saturated and unsaturated forms of C18.

Data selection and calculation

Data inclusion

A database was compiled based on studies published between 1970 and 2006 dealing with *in vivo* ruminal metabolism of FA and including the key words 'fatty acids', 'ruminal fermentation', 'biohydrogenation' and 'duodenum'. Criterion for selecting publications was the reporting of both daily FA intake and duodenal (or omasum) FA flows. Two publications were eliminated because reported duodenal flows of FA represented less than 20% of FA intake (Chang *et al*., 1991) or FA digestion in the intestine was very low compared with the other publications in the database (Aldrich *et al*., 1997). Publications reporting several experiments were dealt with by assigning a specific code for each experiment. This resulted in a database of 74 publications reporting 95 experiments (N_{exp}) with a total of 303 treatments (N_{trt}) reporting FA intake, partitioned between dairy cows ($N_{exp} = 43$; $N_{trt} = 147$), growing cattle ($N_{exp} = 31$; $N_{trt} = 96$) and sheep (mostly fed at a maintenance level; $N_{exp} = 21$ and $N_{trt} = 60$). The database consisted of 625, 596 and 339 observations for dairy cows, growing cattle and sheep, respectively. Each observation corresponded to the mean of each treatment group.

Most of the studies investigated the effects of dietary lipid supplementation on duodenal flows of FA, with lipids from different sources (palm oil, soybean, rapeseed, sunflower or linseed for oils and seeds; tallow, yellow grease or blends for animal fats) and/or the physical form of fat source (raw or processed seeds, extracted oil) and/or protection of the lipid supplements (Ca salts of FA (CaSalt), FA encapsulated in a formaldehyde-treated protein matrix or FA-amides). Although it has been established that direct treatment of oilseeds with formaldehyde is ineffective without a prior emulsification procedure in protecting PUFA from BH (Petit, 2003), this aspect was generally not detailed in the studied publications. We also included publications reporting the effects of forage and its conservation, forage-to-concentrate ratio, nature of the energy supplement (wheat *v.* barley *v.* corn), the physical process applied to the cereal (dry-rolling, steam-flaking, etc.) and the formaldehyde treatment of dietary protein on duodenal flows of FA.

Experiments in the database were mainly designed as Latin squares (85% of the designs). Wide ranges in duration of experimental periods, in methods to estimate total duodenal or microbial FA flows and in methods of FA analysis were observed. For instance, durations of experimental periods (including the adaptation period to experimental diets) were between 14 and 44 days. Flows of FA to the duodenum were assessed using triple-, double- or single-marker methods, representing 4%, 28% and 70% of the observations, respectively. With the single-marker method, chromic oxide either in the diet or infused into the rumen was the most used molecule, whereas combination of polyethylene glycol, Cr-ethylenediamine tetraacetic acid (EDTA), rare earths and chromic oxide was used for the double-marker method. Duodenal samples were generally collected over 3 to 5 days of the experimental period, so that every 90 to 240 min in a 24-h period were represented; such a time schedule could have affected the representativeness of the pooled samples. For FA analysis, in older publications total FA were extracted with chloroform–methanol (Folch *et al*., 1957) or petroleum ether including saponification with ethanol–NaOH and acidification with HCl. In the most recent experiments, total FA content of feed and duodenal samples were determined according to Sukhija and Palmquist (1988) by a one-step extraction and trans-esterification procedure with BF_3–methanol. In some instance, direct trans-methylation procedure using methanol–HCl (Park and Goins, 1994) was also used. No attempt was made to take into account such experimental factors of variation. Duodenal flow of microbial FA was calculated from microbial FA content and duodenal flow of microbial organic matter (OM) (flow of duodenal microbial N divided by the ruminal bacterial N : OM ratio). Duodenal microbial N was derived from the ratio of purine to N in isolated bacteria and in duodenal samples (Zinn and Owens, 1986).

Methods used to isolate bacteria were highly variable, suggesting that representation of solid-associated bacteria (SAB) and liquid-associated bacteria (LAB) was not homogenous between publications. This may have led to bias the estimation of microbial FA flows, as FA composition and response to lipid supplementation are different between SAB and LAB bacteria (Vlaeminck *et al*., 2006).

Data processing and calculation

FA intake expressed relatively to metabolic body weight (BW) is a more reliable indicator of changes in intestinal FA digestion than percentage FA in the diet; however, BW in the database ($N_{trt} = 246$) was unreported in numerous studies conversely to dry matter intake (DMI) ($N_{trt} = 303$). Consequently, FA intake, duodenal FA flows and absorbed FA flows were expressed relatively to DMI to pool data obtained on both ovine and bovine species. Overall, BH of FA in the rumen was evaluated as the difference between the intake (DB_{int}) of double bonds (DB) and their flow to the duodenum (DB_{duo}). DB_{int} and DB_{duo} were obtained by multiplying the daily flow of each FA (intake or duodenum, g/day) by their respective number of DB. Overall, BH of FA in the rumen was expressed as a proportion of the DB_{int} entering the rumen (BH_{pc}, %).

To determine apparent FA absorption in the intestine, only publications reporting flows of FA at both the duodenal and the terminal ileum were considered. To be consistent

with the approach described in the companion paper (Glasser *et al.*, 2008), and although there is little or no absorption of long-chain FA in the large intestine, we excluded publications that provided duodenal and only faecal flows.

Encoding and statistical analysis

Data were encoded according to the lipid source with two codes. The first code indicated the origin of the lipid source (complete forage diet, control diets without lipid supplementation, cottonseed, linseed, palm, rapeseed, soybean, sunflower, other plant lipids, animal fat (tallow, yellow grease), fish, hydrogenated fats, animal–vegetal blend). The category 'animal–vegetal blend' included associations of tallow (partially hydrogenated or not) with whole seeds (soybean or cottonseed) or with vegetal oils. The second code indicated the 'physical form' of the lipid source, i.e. mode of protection of oils or seeds (amide or encapsulated), whole *v.* extruded *v.* cracked *v.* heated seeds, alkaline treatment, formaldehyde treatment, triglycerides (oils), calcium soaps and free FA. Table 1 gives the partition of experimental data according to dietary lipid sources and their physical form. Tables 2 and 3 give cross-table representations of the experimental treatments (N_{trt}) and the level of FA supplementation (FA_{int}) according to studied species and dietary lipid sources. As fish oils are supposed to affect ruminal fermentation patterns and FA metabolism (mainly C18), all data dealing with fish oils or combining fish oils and vegetable oils ($N_{trt} = 20$) were analysed separately from data relative to the other lipid sources. Experiments dealing with forage ($N_{trt} = 27$) were encoded according to forage species (mainly clover without distinction between red or white clover, orchardgrass and ryegrass) and conservation (as silage, hay, green forage), and they were analysed separately. Other experimental protocols studied the effects of forage-to-concentrate ratio ($N_{trt} = 15$), nature of energy supplement ($N_{trt} = 8$), physical process of cereals ($N_{trt} = 10$, only studied in dairy cows) and formaldehyde treatment of the protein fraction of the diet ($N_{trt} = 8$, only studied in sheep) on the duodenal flows of FA. Consequently, the effects of the two latter factors may have been confounded with animal species; however, as these experiments had iso-lipid diets, they were not included to predict duodenal flows of FA, but they were used to validate the obtained equations (see below).

Data distribution between dietary lipid sources, level of lipid supplementation and animal species was not homogenous; e.g., vegetable oils were mostly studied in dairy cows, whereas animal fats were mostly studied in growing cattle. Moreover, when no lipid was added, DMI expressed relatively to BW was $1.76 \pm 0.28\%$ ($N_{trt} = 17$), $1.95\% \pm 0.37$, ($N_{trt} = 37$) and $3.08\% \pm 0.55$, ($N_{trt} = 22$) for sheep, growing cattle and lactating cows, respectively ($P < 0.001$). Consequently, it cannot be ruled out that effects of lipid supplementation may be partly confounded with animal species and/or level of intake. It should also be emphasized that in order to compare dietary treatments at an

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

	Physical form of the dietary lipid source											
Lipid source	Amides	Calcium salts	Encapsulated oils	Extruded seeds	Free fatty acid	Whole seeds	Heated seeds	Crushed or ground seeds	Formaldehyde-treatment	Alkaline-treated seeds	Fats or oils	Others (control)	Total
Animal fat		2			3						35		40
Fish									2		18		20
Hydrogenated fats					3						8		11
Animal–vegetal blend											14		14
Cottonseed						9	1	2					12
Linseed				1		2	1	1	4		6		15
Palm oil		13			1						1		15
Rapeseed	2	4		1		1		6		3	5		22
Soybean	2	2		2	1	3	3	1			11		25
Sunflower						3					5		8
Other plant lipids			1			2					8		11
All forage diets												27	27
Others (control)									4			79	83
Total	4	21	1	4	8	20	5	10	10	3	111	106	303

Table 2 *Distribution of the experimental treatments according to animal category and source of lipid supplement, and mean levels of fatty acid intake (g/kg dry matter intake)*

Source of lipids studied*	Animal category		
	Growing cattle	Sheep	Lactating cows
Control [No lipid added]	24.3 ± 9.1 [20]	22.1 ± 12.4 [4]	23.6 ± 6.5 [29]
Vegetable oils			
Rapeseed	36.6 ± 2.5 [2]	–	64.3 ± 18.4 [9]
Palm	68.5 [1]	48.9 [2]	62.4 ± 15.6 [7]
Linseed	–	61.5 ± 11.1 [7]	54.7 ± 10.0 [3]
Soybean	24.3 [1]	74.4 ± 23.6 [8]	54.5 ± 18.5 [7]
Sunflower	52.6 ± 6.0 [5]	–	65.8 ± 0.2 [3]
Other oils or blends of oils	51.8 ± 0.2 [2]	32.0 [1]	62.5 ± 0.4 [4]
Animal fats		–	
Tallow	58.0 ± 14.8 [9]	–	56.6 ± 16.1 [13]
Yellow grease	68.3 ± 22.6 [12]	–	50.1 [1]
Blend of animal fats	67.2 ± 10.3 [10]	–	77.3 ± 18.6 [2]
Oil seeds		–	
Rapeseed	82.3 ± 13.1 [7]	–	67.9 ± 16.0 [4]
Linseed	58.6 [1]	48.0 [1]	72.3 ± 2.5 [3]
Soybean	64.5 ± 13.5 [2]	–	44.2 ± 16.3 [7]
Safflower	73.0 ± 1.3 [2]	–	–
Cotton	45.0 [1]	–	47.3 ± 6.8 [11]
Fish			
Fish oil/ fish meal	53.4 ± 4.7 [4]	47.0 ± 17.8 [10]	35.5 ± 2.9 [6]
Blend of animal and vegetable fats	59.9 ± 8.9 [3]	–	60.5 ± 7.5 [10]

*Data are expressed as mean ± s.d. (when appropriate). Data in brackets indicate the number of experimental treatments.

Table 3 *Level of lipid intake (g fatty acid /kg dry matter intake) in experiments without dietary lipid supplementation*

Experimental Factor*	Animal category		
	Growing cattle	Sheep	Lactating cows
Forage type and conservation	20.0 ± 2.5 [7]	13.5 ± 4.0 [14]	28.6 ± 2.2 [6]
Forage-to-concentrate ratio	52.6 ± 3.3 [3]	16.2 ± 1.8 [4]	26.8 ± 13.3 [8]
Nature of energy supplement	29.9 ± 11.5 [4]	–	14.5 ± 2.6 [4]
Physical process of cereals	–	–	51.3 ± 2.3 [10]
Formaldehyde treatment of the protein fraction of the diet	–	18.7 ± 1.4 [8]	–

*Data are expressed as mean ± s.d. (when appropriate). Data in brackets indicate the number of experimental treatments.

approximately similar theoretical value of energy intake, the increase in FA_{int} content occurred mainly at the expense of starch content in the diet (g/kg DMI)

$$FA_{int} = 58.2\,(\pm 6.6) - 0.031\,(\pm 0.019) \times \text{starch},$$
$$N_{trt} = 93,\ N_{exp} = 34,\ \text{RMSE} = 76.7.$$

Fitting a higher polynomial model provided a higher RMSE and a higher AIC (Akaike's Information Criterion, see below). Lipid supplementation had no significant influence on the other dietary components (NDF, ADF and CP) in the database.

Data for a dependent variable Y were processed using variance–covariance analysis with the MIXED procedure of the SAS statistical software package (release 8.01, 1999; SAS Institute Inc., Cary, NC, USA) including the source of added lipid, physical form and the overall slope of Y *v.* X (covariate) as fixed factors. Random part of the model was constituted by experiment effect and a slope clustered on the experiment effect. It has previously been shown that considering experiment effect as a random factor results in a better prediction of coefficients and a more accurate description of prediction error (St-Pierre, 2001). Consequently, the model of analysis was

$$Y_{ij} = B_0 + B_1 X_{ij} + B_2(\text{lipid source} \times \text{physical form}) X_{ij} + s_i + b_i X_{ij} + e_{ij},$$

where i = number of experiments, j = number of groups, $B_0 + B_1 X_{ij} + B_2$(lipid source × physical form), X_{ij} is the fixed effect part of the model and $s_i + b_i X_{ij} + e_{ij}$ is the random effect part of the model. In this model, B_1 is the overall slope of Y *v.* X, and B_2 is the difference from the overall slope for a given combination of lipid source × physical form. Statistical significance of B_2 (significant interaction between X_{ij} and lipid source × physical form) was reported only when there were at least three observations (N_{trt}).

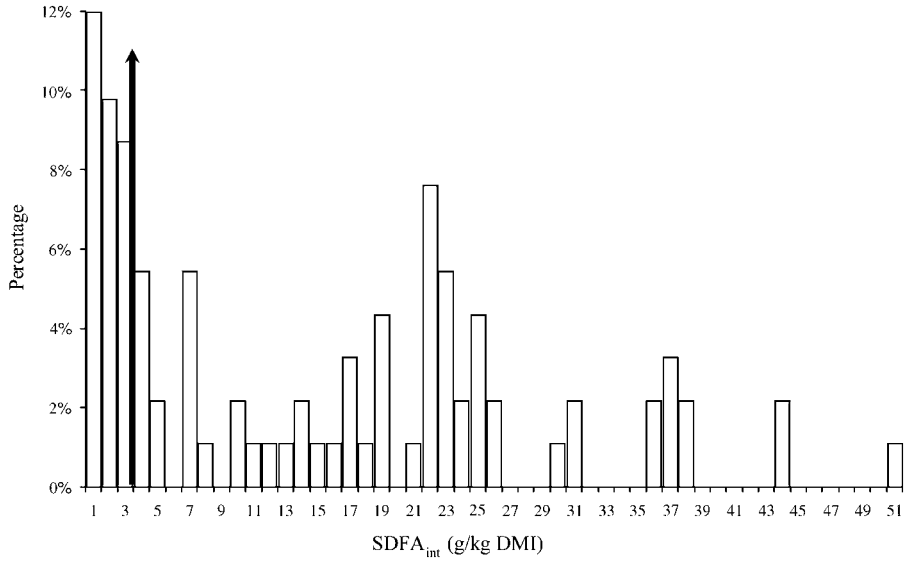

Figure 1 Distribution of the intra-experiment standard deviations of dietary fatty acid content ($SDFA_{int}$, g FA/kg dry matter intake (DMI)) in the database. The arrow indicates the threshold ($SDFA_{int} \geqslant 3$ g/kg DMI) used to select experiments retained for the analysis of the effect of dietary fatty acid content.

In some cases, quadratic models were also tested for the same dependent variable: the selected model was the one with the lowest Akaike's Information Criterion (Wang and Goonewardene, 2004). Statistical analyses were not weighed for several reasons. First, studies in the database had similar number of animals (between 3 and 8), and most of them had the same experimental design (see below). Moreover, optimal weighing of the observations would require identical expression of the variability of the studied parameters. Unfortunately, this was not the case: variability was expressed either as SEM (or SE), or SD of the difference between means, or SD for the mean of each treatment group; in some publications variation was even not reported.

Statistical analysis was performed in two successive steps. The first step included all experiments (N_{exp}) of the database, while the second step selected only those experiments that had an intra-experiment variation (standard deviation, s.d.) above a predefined threshold. Theoretically, the inclusion of all available data would provide a more precise relationship because of an improved prediction of the random intercept of each experiment. However, this was not the case in our database (see below); moreover, the aim of our analysis focused on the response of different flows (Y) to variation in X, rather than on an explanation of between-experiment variations. Consequently, the threshold of inclusion was arbitrarily set at a value above which intra-variation of the independent variable was considered relevant, with regard to its overall distribution in the database. For example, the distribution of SD for the independent variable FA_{int} ($SDFA_{int}$) is presented in Figure 1: the experiments with $SDFA_{int}$ lower than 3 g FA/kg DMI were excluded, i.e. approximately 30% of the observations (N_{trt}). Theoretically, these two approaches estimate a 'true' intra-experiment effect of independent variable on the selected dependent variables (except if there was a covariation of other variables). However, because the independent variable (FA_{int} mainly) was generally affected by an experiment effect on this data set, the calculated intra-experiment coefficients have to be interpreted with caution.

Unless otherwise stated, parameter estimates are mean $\pm$ s.e., whereas for all other data they are presented by mean $\pm$ s.d. Mean $\pm$ s.d. of the studied variables for the whole database, or sorted by species, are presented in Appendix 1. A preliminary analysis of the data indicated no significant effect of animal species on any of the variables studied (except on DMI). Consequently, this factor was not discussed in the text.

Results and discussion

Ruminal phenomena

Hydrogenation capacity of the rumen and ruminal pH. From the whole database, ruminal pH was not affected by FA_{int} or by the source of added lipid. When experiments with $SDFA_{int}$ lower than 3 g/kg DMI were excluded, BH_{pc} tended to increase linearly with FA_{int} according to Equation (1) in Table 4. Compared with the coefficient of control diets (0.38, expressed as the increase in BH_{pc} for an increase of 1 g FA/kg DMI), the coefficient of animal fat (0.10) and especially hydrogenated tallow (0.03), CaSalt of palm oil (0.08) was lower, whereas it was higher for soybean oil (0.58) and linseed oil (0.53) either protected or not. Coefficients for all other physical forms of added lipids were not significantly different from that of control diets. Obviously, low BH_{pc} occurred with fat that was already partly saturated or hydrogenated, whereas a high rate of BH occurred on lipids that contained PUFAs, mainly linolenic acid that has the highest BH_{pc} compared with other unsaturated FA (Glasser *et al.*, 2008).

The effect of pH on BH was studied in the companion paper (Glasser *et al.*, 2008). Even in trials where effect of

Table 4 *Prediction of rumen biohydrogenation of total fatty acid (FA), duodenal flows, apparent absorbed flows of total FA and FA with 12, 14, 16 or 18 C units (diets without fish oils or only forage-based diets)*

Dependent variable (Y)	Independent variable(s) (X, g/kg DMI*)	Eq.	Intercept	Coefficient (B_1)	N_{exp}	N_{trt}	RMSE (R^2)
Hydrogenation of FA, BH_{pc} (%)	FA intake, FA_{int}	1	62.0 ± 3.0	0.38 ± 0.13	48	158	0.074 (0.82)
Duodenal flows (g/kg DMI)							
Total FA, FA_{duo}	FA intake, FA_{int}	2	9.69 ± 1.37	0.75 ± 0.06	60	194	3.56 (0.99)
C12, $C12_{duo}$	C12 intake, $C12_{int}$	3	0.09 ± 0.02	0.24 ± 0.10	19	56	0.05 (0.97)
C14, $C14_{duo}$	C14 intake, $C14_{int}$	4	0.42 ± 0.21	0.55 ± 0.17	32	98	0.24 (0.98)
C16, $C16_{duo}$	C16 intake, $C16_{int}$	5	1.94 ± 0.39	0.73 ± 0.09	45	147	0.91 (0.99)
C18, $C18_{duo}$	C18 intake, $C18_{int}$	6	7.30 ± 1.34	0.75 ± 0.08	46	160	3.14 (0.98)
Duodenal flow of microbial FA, FA_{mic} (g/kg DMI)	FA intake, FA_{int}	7	10.8 ± 3.0	0.33 ± 0.13	14	49	4.40 (0.93)
Proportion of microbial FA in duodenal flow of total FA, FA_{mic}/FA_{duo} (%)	FA intake, FA_{int}	8	71.0 ± 6.4	-0.51 ± 0.15	14	49	6.5 (0.73)
Absorbed flows (g/kg DMI)							
Total FA, FA_{abs}	FA_{duo}, FA^2_{duo}	9	–	0.83 ± 0.03, -0.0011 ± 0.0003	16	61	2.80 (0.99)
C12, $C12_{abs}$	$C12_{duo}$	10	–	0.62 ± 0.12	6	15	0.02 (0.90)
C14, $C14_{abs}$	$C14_{duo}$	11	–	0.71 ± 0.05	11	34	0.07 (0.99)
C16, $C16_{abs}$	$C16_{duo}$	12	–	0.79 ± 0.07	11	39	0.84 (0.97)
C18, $C18_{abs}$	$C18_{duo}$, $C18^2_{duo}$	13	–	0.85 ± 0.03, -0.0017 ± 0.0003	14	57	1.70 (0.98)

*DMI = dry matter intake; N_{exp} = number of trials included; N_{trt} = number of treatments (observations); RMSE = root mean square error of the model; Eq. = equation.
Values are expressed as mean ± standard error. Presented equations correspond to the fixed part of the model of analysis according to: $Y = \text{Intercept} + B_1X$. Significant difference from B_1 for a given lipid form (B_2(Lipid source × technological treatment) X) is indicated in the text. For total FA and C18 absorbed flows, the model was quadratic and it included FA_{duo} and FA^2_{duo}, and $C18_{duo}$ and $C18^2_{duo}$, respectively.

dietary treatments on pH was not significant (Ueda *et al*., 2003), variations in BH_{pc} were observed, thus indicating that BH may be affected by changes in microbial populations or in EMPS without a concomitant change in pH. There is suggestive evidence that BH is more pronounced in experiments where rumen fermentations are less intense (higher pH and higher Acetate-to-propionate ratio) and associated with a lower EMPS due to a higher ruminal supply in apparently fermented OM. This is supported by the positive inter-experiment relationship between BH_{pc} and OM truly digested in the rumen (RDOMt, $+0.40 \pm 0.25$%) for an increase in 1% in OM ruminal digestibility, $N_{trt} = 67$, RMSE = 6.6) and the negative relationship with EMPS (-0.54 ± 0.30%) for an increase in 1 g N/kg RDOMt, $N_{trt} = 97$, RMSE = 6.9). High EMPS have been shown to reduce methane production (Preston and Leng, 1987) that may increase availability of H_2 in the rumen and BH, though methanogenesis is a more efficient process to dispose excess reducing power, at least *in vitro* (Joyner *et al*., 1977). Furthermore, it cannot be ruled out that in these conditions (high EMPS), bacteria may increase their incorporation of long-chain FA (Sasaki *et al*., 2001), which are consequently protected from BH; in our database, BH_{pc} was negatively related to FA_{mic} with a 1.1% (± 0.23, $N_{trt} = 30$, RMSE = 8.7) decrease in BH_{pc} for an increase in 1 g FA_{mic}/kg DMI. The relevance of this relationship remains unclear, since BH is generally considered as a means to decrease the toxicity of PUFA (Kemp *et al*., 1984). Changes in bacterial and protozoa populations may also account for changes in ruminal BH. For instance, the absence of rumen protozoa has been shown to affect bacterial population through a reduced diversity of bacterial groups (Yanez-Ruiz *et al*., 2007). Contribution of protozoa to BH and changes in bacterial populations in response to dietary lipids supplementation have not been extensively studied until now.

EMPS. Mean values of EMPS and RDOMt for the whole database are given in Appendix 1. When experiments with an $SDFA_{int}$ lower than 3 g FA_{int}/kg DMI were excluded, FA_{int} showed no effect on EMPS, except for soybean seeds with $+0.19$ ($N_{trt} = 6$) g microbial N/kg RDOMt for an increase of 1 g FA_{int}/kg DMI. It should be emphasized that the increase in EMPS was related to a reduction in RDOMt due to lipid supplementation (Tice *et al*., 1994; Howlett *et al*., 2003; Scholljegerdes *et al*., 2004), whereas the daily flows of microbial N to the duodenum were either not affected or slightly increased in these experiments. The lack of effect of lipid addition on EMPS, except when RDOMt was reduced, is in agreement with the review of Doreau and Ferlay (1995), who studied the effect of lipid addition on ruminal N metabolism. The reduction of RDOMt has been shown to reflect the decrease in ruminal NDF digestion observed in the whole database (data not shown), especially when unsaturated fat was fed (Doreau and Ferlay, 1994). A toxic effect of fat on the protozoa population has been reported (Bock *et al*., 1991; Onetti *et al*., 2001), which may reduce bacteria lysis and predation, and increase EMPS (Ushida *et al*., 1984).

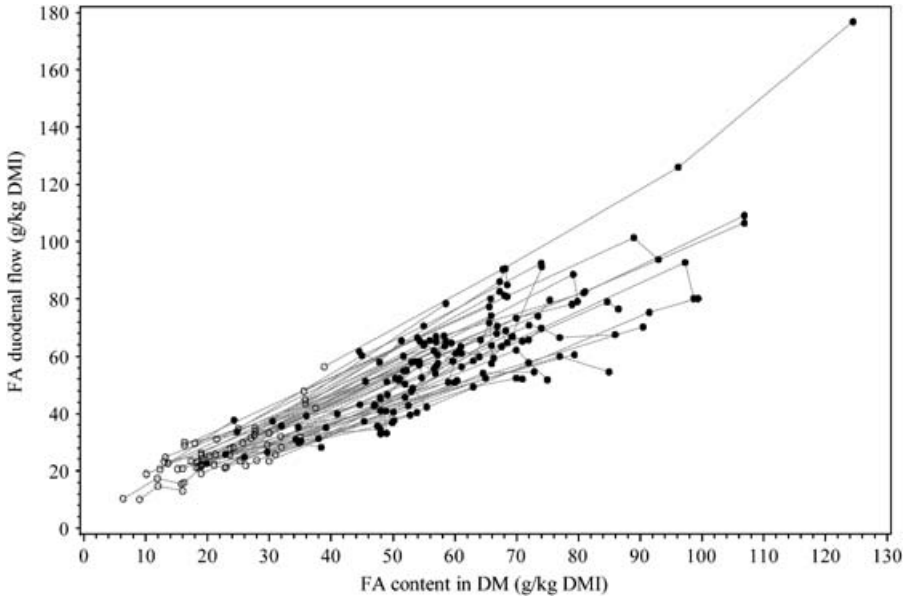

Figure 2 Influence of dietary fatty acid (FA) content in dry matter intake (DMI) on the duodenal flow of FA in ruminants fed control (○) or lipid-supplemented diets (●). Each point corresponds to an experimental treatment, and the lines link treatments from the same experiment.

Duodenal FA flow and ruminal FA balance

Duodenal flow of total FA. In the whole database, both FA_{duo} (mean value in Appendix 1) and its variability increased with FA_{int} (Figure 2). When experiments reporting a $SDFA_{int}$ lower than 3 g/kg DMI were excluded, the relationship between FA_{duo} and FA_{int} was linear (Equation (2), Table 4). A quadratic model using FA_{int}^2 did not improve the AIC of the model. Without selection on $SDFA_{int}$, the model was also linear and the intercept and the coefficient were 9.13 ± 1.28 and 0.77 ± 0.06, respectively, with RMSE = 3.61 ($N_{exp} = 79$; $N_{trt} = 252$; $R^2 = 0.98$), which shows that coefficients for the inter-experiment and intra-experiment equations were similar. The RMSE of the intra-experiment relation is close to that reported on smaller data sets without considering experiment effects ($N_{trt} = 113$, Doreau and Ferlay, 1994) or fixed effects for experiments ($N_{trt} = 116$, Sauvant and Bas, 2001). However, in these reviews, intra-experiment and inter-experiment effects might have been confounded since there was no selection of experiments based on their SD for FA_{int}. Interaction between FA_{int} and added lipid was significant: compared with control diets, soybean oil whatever its protection (+0.19), rapeseed raw (0.35) or ground (+0.10), unprotected blends of animal and vegetable fats (+0.10) and yellow grease (+0.12) all had higher coefficients. The coefficients from all other lipid presentations were not different from those of control diets.

Because of the selection of publications based on their variability for FA_{int}, a sensitivity analysis was performed on the data set of experiments that was excluded from the calculation of Equation (2). We predicted their duodenal flow of FA using Equation (2) and their FA_{int}, and we compared the prediction with the reported value of FA_{duo}: the regression between calculated and reported FA_{duo} had a slope of 0.95 ± 0.12, which was not different from 1 (the unit) and had no significant intercept. This indicated that Equation (2) might be used to predict FA_{duo} without any bias.

For diets containing fish oils, the relationship between FA_{duo} and FA_{int} ($N_{exp} = 11$; $N_{trt} = 24$; RMSE = 3.0) had a non-significant intercept (7.28 ± 6.18, $P < 0.30$) and a slope of 1.10 ± 0.31 ($P < 0.05$) that was higher than that presented in Table 4 (Equation (2)) for the other fat sources. However, this relationship should be considered with caution due to the low number of data. Protection of the fish oils did not affect the coefficient of the relationship.

For complete forage diets, FA_{int} was not affected by forage species. FA_{int} was lower for hays than for silages or green forages. Forage species did not affect FA_{duo} (17.8 ± 4.5 g/kg DMI, $N_{trt} = 25$). Compared with hay (FA_{duo}: 11.7 ± 2.6 g/kg DMI, $N_{trt} = 4$), green forages and silages had higher FA_{duo}, i.e. 17.6 ± 1.9 ($N_{trt} = 6$) and 20.0 ± 4.2 ($N_{trt} = 9$) g/kg DMI, respectively.

Based on the whole database, the mean calculated ruminal balance of FA (intake − duodenum, FA_{bal}, g/kg DMI) was -1.6 ± 9.8 g/kg DMI ($N_{trt} = 297$). The relationship between FA_{int} and FA_{duo} (Equation (2)) indicated that, independent of the source of lipid supplementation, a positive FA_{bal} occurred with a FA_{int} higher than 40 g/kg DMI, which is slightly lower than that previously reported (Doreau and Ferlay, 1994; Sauvant and Bas, 2001).

The relationship between FA_{int} and FA_{duo} indicated that 6% to 16% of FA_{int} disappeared between the mouth and the duodenum when FA_{int} varied between 50 and 120 g/kg DMI. Doreau and Ferlay (1994) suggested that a positive FA balance might stem from absorption through the rumen wall or metabolism (oxidation) in the rumen wall or by microbes. Rumen absorption of medium-chain FA has

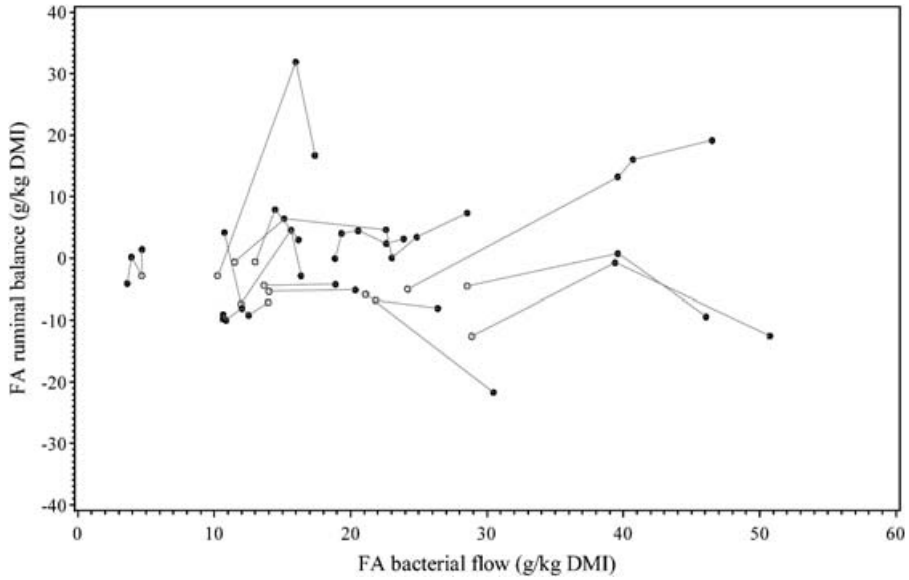

Figure 3 Influence of the bacterial flow of fatty acids (FA) to the duodenum on the ruminal balance of FA in ruminants fed control (○) or lipid-supplemented diets (●). Ruminal balance of FA was calculated as: FA intake − duodenal flow of total FA. Each point corresponds to an experimental treatment, and the lines link treatments from the same experiment.

previously been demonstrated (Hagemeister *et al*., 1979), and absorption of long-chain FA through diffusion in the rumen wall remains theoretically possible, although this process is probably of minor importance since FA are essentially associated with feed particles (Harfoot and Hazlewood, 1997). However, it has been suggested that absorption is possible when there is a high lipid content in the rumen (Doreau and Ferlay, 1994). Theoretically, the lack of oxygen in the rumen would indicate that oxidation of FA by ruminal microbes is probably low, but Doreau and Chilliard (1997) suggested that microbes adherent to the rumen may derive oxygen from the rumen wall to oxidize FA, and *in vitro* data indicated that partial oxidation of palmitate in isolated rumen cells or in ruminal epithelium may occur with the production of ketone bodies (Cook *et al*., 1967; Jesse *et al*., 1992). However, the extent of this process is not known, and it remains to be established whether this phenomenon is related to the quantity of FA in the rumen. Moreover, this phenomenon is probably not sufficient to explain a positive balance with low-lipid or forage diets. Indeed, even forages, which are low in lipids, gave positive ruminal balances particularly with fresh forage or silages (Dewhurst *et al*., 2003; Doreau *et al*., 2003; Lee *et al*., 2003 and 2006). Reasons for such positive balance when no fat is added are not known.

Finally, part of the variation of FA_{bal} could be due to a variable contribution of bacterial lipids to duodenal FA flow. However, this does not explain the positive balances of FA in our database, since negative or positive FA_{bal} values were obtained independent of duodenal flows of bacterial FA (Figure 3). The nature of bacterial lipids flowing to the duodenum is discussed below.

As for FA_{duo}, FA_{bal} increased in response to FA_{int}, partly due to inter-experiment variations. A curvilinear inter-experiment relationship was established between FA_{bal} and EMPS (Figure 4), which was adjusted with the NLIN procedure of SAS (release 8.01, 1999):

$$FA_{bal} = 36 - 45.9(\pm 3.1) \times (1 - \exp(-0.065(\pm 0.012) \times \text{EMPS})),\ N_{trt} = 185,\ \text{RMSE} = 70.$$

This model indicates that positive ruminal FA balances occurred at a calculated EMPS below 25 g N/kg RDOMt. It suggests that when ruminal conditions increased EMPS, dietary FA were less catabolized, leading to a negative ruminal FA balance. However, it could be argued that positive FA_{bal} were obtained because of erroneous (underestimated) duodenal FA flows. In this case, OM digested in the rumen and duodenal N flows would also have been equally biased, which would have led to an underestimation or overestimation of EMPS, preventing to obtain any relationship between EMPS and FA_{bal}. Moreover, in most experiments with positive FA_{bal}, duodenal flows of N and OM were in the range of usually reported values, which suggests that the relation between FA_{bal} and EMPS was not due to methodological errors.

Duodenal flows of FA with 12, 14, 16 and 18 C units. Duodenal flows of FA with 12, 14, 16 or 18 C units (mean values in Appendix 1) were studied individually in relation to their corresponding intake (Table 4, Equations (3) to (6)), whereas results on individual duodenal flows of the various FA with 18 C units were reported in the companion paper (Glasser *et al*., 2008). It should be emphasized that only C18 exhibited an increased variability in duodenal flow in response to increased intake, as observed for total FA (data not shown). All intercepts and coefficients were statistically significant. The analysis of the relationships did not highlight a significant effect of the

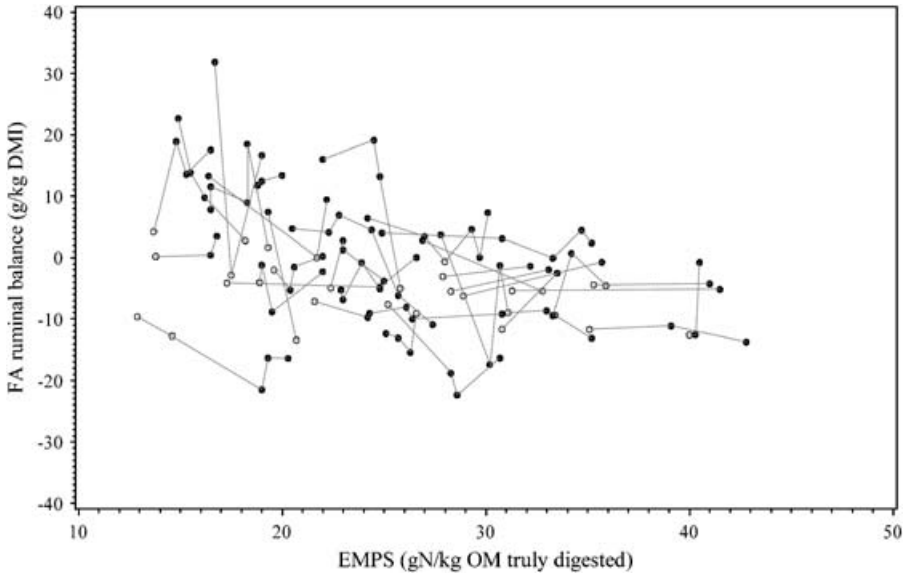

Figure 4 Influence of the efficiency of microbial protein synthesis (EMPS, g N/kg organic matter (OM) truly digested) in the rumen on the ruminal balance of fatty acids (FA) in ruminants fed control (○) or lipid-supplemented diets (●). Ruminal balance of FA was calculated as: FA intake − duodenal flow of total FA. Each point corresponds to an experimental treatment, and the lines link treatments from the same experiment.

source of added lipid or the lipid treatment, except for a higher coefficient of soybean oil (free or protected, +0.28) and raw rapeseed (+0.24) for $C18_{int}$. We observed that the coefficients increased with chain length, which could be due to the synthesis of C16:0 and C18:0 by rumen bacteria, which represented approximately two-thirds of bacterial FA (Sauvant and Bas, 2001). It may be suggested that catabolism or absorption of medium-chain FA (see above) may reduce their duodenal flows in comparison to C16 or C18. The significant intercepts in Equations (3) to (6) partially reflect the synthesis of C12 to C18 in the rumen, but they also represent the content of these FA in the control diets, indicating that extrapolation of these relationships to null intake provides gross estimates of true synthesis.

Duodenal flow of bacterial FA and contribution to total FA flow
Bacterial flow of FA to the duodenum. With complete forage diets, data reporting duodenal flow of bacterial FA are scarce; Hogan (1973) reported a value of 11.7 g FA/kg DMI in sheep fed clover. Data reporting FA_{mic} with fish-oil supplements are equally scarce ($N_{trt} = 3$; Qiu *et al*., 2004), and the $SDFA_{int}$ reported was low. Consequently, these data were not included in the relationship between FA_{mic} and FA_{int} (Figure 5, and Equation (7) in Table 4). The interaction between FA_{int} and lipid added on FA_{mic} was significant: compared with control diets, coefficients of animal fats and diets containing rice bran or wheat bran (Ieki *et al*., 1997) were −0.15, −0.35 and −0.42, respectively. For animal fats, the low coefficient was related to a decrease in FA_{mic} in response to increased FA_{int}, because the added fat was hydrogenated (Legay-Carmier, 1989; Elliott *et al*., 1999). The prediction model of FA_{mic} suggested that 10.8 g/kg DMI passing through the duodenum were of bacterial origin, but it must be emphasized that this estimation is a gross value, because coefficient of variation of the slope is high (Equation (7)). However, similar estimations were obtained from the equation predicting FA_{duo} (9.69 g/kg DMI) and from previous reviews, i.e. 9.3 g/kg DMI (Doreau and Ferlay, 1994) and 8.43 g/kg DMI (Sauvant and Bas, 2001), which did not provide the s.d. of the slope.

Distribution of duodenal FA flow between bacterial FA and dietary FA. In the experiments ($N_{trt} = 4$) that had values slightly higher than 100 for the FA_{mic}-to-FA_{duo} ratio (Legay-Carmier, 1989), this ratio was arbitrarily set to 100: mean value of the FA_{mic}-to-FA_{duo} ratio is presented in Appendix 1. When experiments with $SDFA_{int}$ lower than 3 g/kg DMI were excluded, lipid addition linearly decreased the contribution of bacterial flow to total duodenal FA flow (Equation (8) in Table 4). This equation indicates that for diets poor in FA (less than 20 g/kg DMI), the proportion of bacterial lipids in duodenal FA flow was above 60%, whereas with diets rich in FA (>40 g/kg DMI) the proportion fell below 50%. It is likely that these values were overestimated for the highest values of FA_{int}, because part of the dietary FA was probably directly incorporated into bacteria, as shown by the increase in the ratio of FA_{mic} to duodenal flow of microbial N (N_{mic}) with increasing FA_{int}:

$$FA_{mic}/N_{mic} = 0.99\,(\pm 0.18) + 0.011\,(\pm 0.002) \times FA_{int},$$
$$N_{exp} = 11,\ N_{trt} = 38,\ \text{RMSE} = 0.16,\ R^2 = 0.92.$$

This relation shows that the FA_{mic}-to-N_{mic} ratio was close to 1 for diets poor in FA, but greater than 1.4 for diets providing more than 40 g FA/kg DM, which reflects the high ability of bacteria to absorb and store intracellularly or adsorb dietary FA. The increased FA content in bacteria

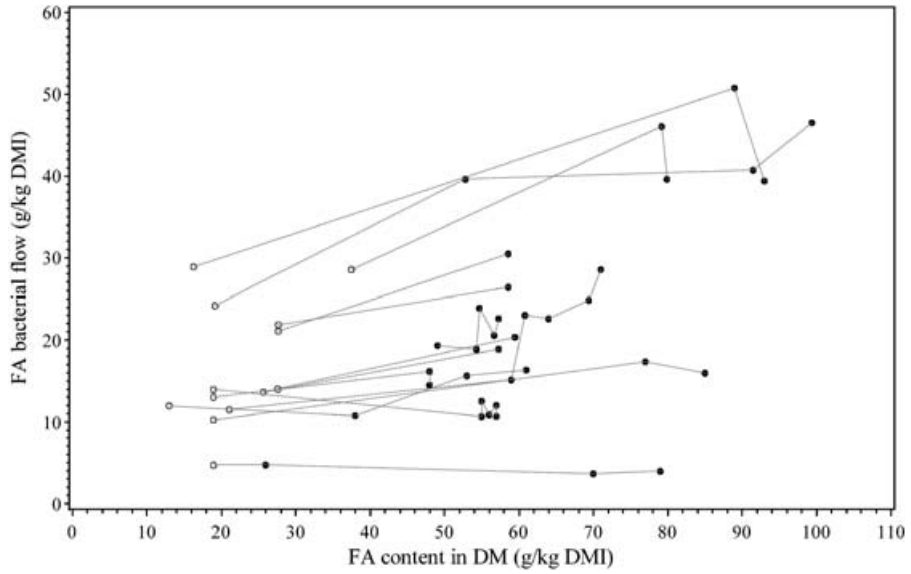

Figure 5 Influence of dietary fatty acid (FA) content in dry matter intake (DMI) on the bacterial flow of FA to the duodenum in ruminants fed control (○) or lipid-supplemented diets (●). Each point corresponds to an experimental treatment, and the lines link treatments from the same experiment.

associated with dietary FA may stem from either physical adsorption on the cell surface, incorporation of feed FA or increased lipid synthesis leading to FA being deposited in the cell walls or, more presumably, in intracellular lipid droplets (Bauchart *et al.*, 1990). Quantitatively speaking, since FA could not be removed by washing bacteria cells with hexane or sodium hydroxide (Harfoot *et al.*, 1974), direct incorporation is a potentially highly significant process in microbial lipid metabolism. Moreover, it is generally assumed that *de novo* synthesis of bacterial lipids decreases when the amount of exogenous lipids increases (Doreau and Ferlay, 1994). Indeed, ruminal bacteria can reduce the *de novo* synthesis of FA in high-fat diets both *in vitro* (Demeyer *et al.*, 1978) and *in vivo* (Czerkawski *et al.*, 1975), which suggests a preferential utilization of preformed FA rather than synthesis of FA, which could be considered as an energy-sparing process since direct incorporation of FA demands less energy than synthesis (Demeyer and Van Nevel, 1995).

It should also be emphasized that the high variability of FA_{mic} may be explained by inter-experiment differences in the quantification of the bacterial flows to the duodenum. These differences may arise from methodological considerations (microbial marker, digesta flow marker and frequency of duodenal sampling relatively to feed distribution, see Materials and Methods, and Harvatine and Allen (2006) for a discussion of these methodological considerations) and/or biochemical differences in lipid metabolism between bacterial species. First, the values for total FA content in bacteria do not distinguish FA from bacterial synthesis and FA of dietary origin that are adsorbed on the cell wall or included in the cytoplasm. Second, it is well known that there are important differences in metabolic activities and FA composition between LAB and SAB (Bauchart *et al.*, 1990), whose relative proportions in the ruminal samples depend on the sampling procedure used to harvest these two fractions (Legay-Carmier and Bauchart, 1989). Moreover, it remains to establish whether the separation procedures isolated protozoa from bacteria, because protozoa may also contribute to the duodenal flow of microbial FA. Yanez-Ruiz *et al.* (2006) reported that the protozoa flow of FA may contribute to approximately 15% of the duodenal flow of total FA, but their contribution may vary between 2% (for C17:0) and 20% for C16:0 and C18:0, respectively. Third, SAB and LAB bacteria may have different responses to dietary manipulation. Legay-Carmier (1989) reported an increase in the total FA content of LAB from ruminal samples of cows fed oilseed, whereas SAB were not affected. Similarly, Vlaeminck *et al.* (2006) reported that a decrease in forage-to-concentrate ratio led to a three-fold higher increase in the bacterial concentration of trans-10 C18:1 in LAB compared with SAB. Consequently, the relationship between bacterial synthesis of FA and FA_{int} needs to be assessed in detail, taking into account the specific bacteria species forming the SAB or LAB groups.

Apparent absorption of FA in the small intestine

Absorbed flows of total FA. Flows of apparently absorbed FA (FA_{abs}, g/kg DMI) and FA with 12, 14, 16 and 18 C units (Appendix 1) were studied in trials providing flows of FA measured between the duodenum and terminal ileum. Absorbed flow of each FA was predicted from their respective duodenal flows (Equation (9) to Equation (13) in Table 4), with the constraint of a null intercept in the models because preliminary analysis indicated that intercepts were not significant. Apparent small intestine digestibility (%) of total FA and FA with 12 to 18 C units was calculated as a proportion of their respective duodenal flows (Appendix 1). The data set contained 30

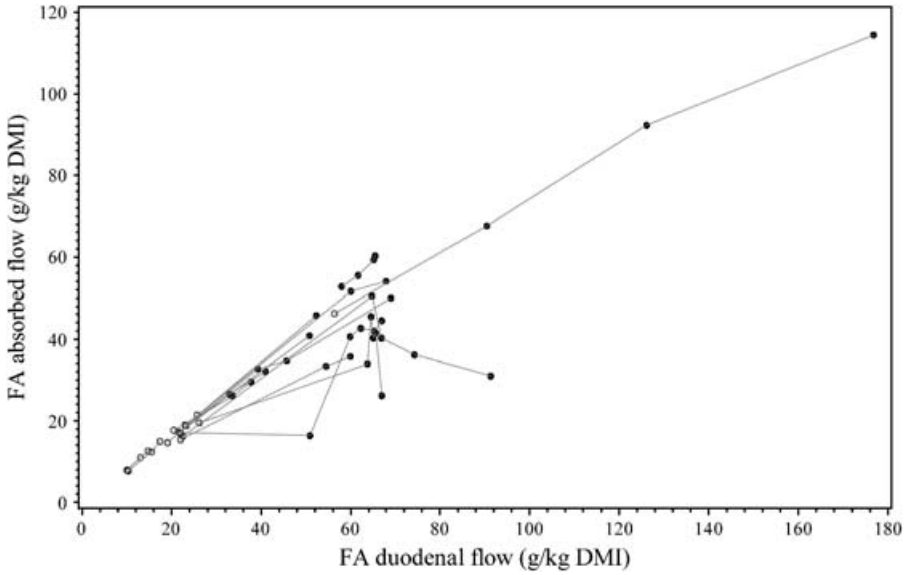

Figure 6 Apparent absorption of fatty acids (FA) in the small intestine as a function of duodenal flow of FA in ruminants fed control (○) or lipid-supplemented diets (●). Each point corresponds to an experimental treatment, and the lines link treatments from the same experiment.

experiments (71 treatments including data with complete forage diets).

In the whole database, mean ± s.d. FA_{abs} was 33.2 ± 19.0 g/kg DMI and mean ± s.d. apparent small intestine digestibility of FA_{duo} was 74.4 ± 12.9%. When trials with an intra-experiment SD lower than 2.5 (0.75 × $SDFA_{int}$) for FA_{duo} were excluded, the relationship between FA_{abs} and FA_{duo} was curvilinear (Figure 6 and Table 4), which indicated possible limitation of FA absorption with increasing flows of FA_{duo}. This limitation is probably related to the low intestinal digestibility of C18:0 (see below and Glasser *et al*., 2008). The interaction between FA_{duo} and added lipid was significant: hydrogenated tallows (−0.23 g FA_{abs}/kg DMI) and rapeseed (−0.21, regardless of technological treatment) had lower coefficients than control diets.

For complete forage diets, mean ± s.d. FA_{abs} was 15.0 ± 1.25 g/kg DMI ($N_{trt} = 15$) and mean ± s.d. apparent small intestine digestibility was 83.9 ± 1.4%. Although the variation in FA_{duo} (10 to 27 g/kg DMI) was low, the relationship between FA_{abs} and FA_{duo} was significant and linear with a coefficient of 0.86 ± 0.02 ($N_{exp} = 5$; $N_{trt} = 15$; RMSE = 0.52). There were no forage type or process effects.

For fish oils, only Scollan *et al*. (2001) provided data on FA absorption as determined by ileal measurements, giving a mean ± s.d. FA_{abs} of 56.5 ± 5.27 g/kg DMI ($N_{trt} = 2$), and a mean ± s.d. apparent small intestine digestibility of 91.5 ± 0.71% ($N_{trt} = 2$).

Based on experiments that simultaneously determined bacterial flows of FA (FA_{mic}) and intestinal absorption of FA (FA_{abs}), it was possible to estimate apparent intestinal digestibility for FA of bacterial origin and FA of dietary and endogenous origin ($FA_{duo} - FA_{mic}$). We used a multiple regression model of FA_{abs} with flow of bacterial origin and flow of dietary and endogenous origin as independent variables to obtain estimated absorption coefficients for both groups of FA. The inter-experiment relation was:

$$FA_{abs} = 1.24(\pm 4.66) + 0.90(\pm 0.23) \times FA_{mic} + 0.46(\pm 0.08) \times (FA_{duo} - FA_{mic}),\ N_{trt} = 22,\ RMSE = 5.3.$$

This relation had a non-significant intercept, which is logical because endogenous FA and FA of dietary origin were pooled and assigned the same digestibility coefficient. These coefficients suggested a tendency for higher apparent absorption for bacterial FA than dietary or endogenous FA.

Absorbed flows of FA with 12, 14, 16 and 18 C units. The absorbed flows of FA with 12, 14, 16 or 18 C units are presented in Appendix 1. The flows of apparently absorbed FA in relation to their respective duodenal flows are presented in Table 4. All linear coefficients were significant. There was a trend towards a quadratic response of $C12_{abs}$ to $C12_{duo}$ ($P < 0.10$), due to two data from Scollan *et al*. (2001) that gave high $C12_{abs}$ values (0.30 to 0.60 g/kg DMI). When these data were eliminated, the relationship was linear, with no lipid source or lipid treatment effect. The calculated coefficient was very similar to the calculated mean ± s.d. apparent small intestine digestibility of $C12_{duo}$, i.e. 66.9 ± 11.5%. For $C14_{abs}$, heated soybean seeds (−0.43) and hydrogenated tallow (−0.15) tended to have a lower coefficient ($P < 0.01$ and $P < 0.12$) than control diets; however, the calculated coefficient for $C14_{duo}$ was close to the mean apparent small intestine digestibility of $C14_{duo}$, i.e. 66.7 ± 17.4%. Also for $C16_{abs}$, hydrogenated tallow had a significantly lower coefficient (−0.29) than the control diet, and the mean ± s.d. apparent small intestine digestibility of $C16_{duo}$ was 76.7 ± 11.9%. The mean ± s.d. apparent small intestine digestibility of $C18_{duo}$ was 75.4 ± 14.8%. As observed for total FA (see above), the model obtained to predict intestinal absorption of C18 had

linear and quadratic coefficients, and the linear coefficients for hydrogenated tallow (−0.32) and rapeseed (−0.31) were significantly lower than that of control diets. This significant quadratic effect indicated a saturation of the intestinal digestibility of C18, which is exclusively due to the limited intestinal absorption of stearic acid (Glasser *et al.*, 2008). Possibly, hydrogenated tallow may have low intestinal digestibility due to incomplete ruminal lipolysis, and low digestibility of FA from rapeseed may be related to their passing intact to the faeces. Moreover, the lower absorption of C14 (a trend), C16 and C18 from hydrogenated tallow was probably due to the increased flow of esterified FA at the ileum, given that these FA are less soluble in micelles (Pantoja *et al.*, 1996; Elliott *et al.*, 1999). Based on the database, mean apparent intestinal digestibility increased with increasing chain length from C12 to C16 and was similar for C16 and C18, although the absorption of C18 was a quadratic function of its duodenal flow.

With fish-oil-containing or complete forage diets, there were insufficient data (fish oils: $N_{trt} = 2$, Scollan *et al.*, 2001; forage: $N_{trt} = 5$, Lee *et al.*, 2003) to study the intestinal digestibility of individual FAs in relation to duodenal flow.

General discussion

The synthesis of published data through meta-analysis is increasingly common in the area of animal nutrition and has been shown to be a useful tool for obtaining precise predictions of response of a given phenomenon to quantitative or qualitative diet variations (Sauvant *et al.*, 2005). However, one of the main drawbacks of this approach is that the matrix of data is far from complete, which implies that multivariate analysis is not feasible. Ideally, the data should have included different levels of FA_{int} for each source of added lipid and for each technological treatment applied to the different lipid sources. Moreover, some fat supplements are under-represented in the database, and thus their contribution to the patterns of response may be biased or confounded with experiment effects. For example, diets supplemented with vegetable oils were common in the database, whereas complete forage diets or diets with fish oils were rare. This is detrimental to the renewed interest for grazing systems or the potential use of fish oils in ruminant nutrition. Moreover, the choice to include experiments based on their s.d. for the variance–covariance analysis eliminated part of the data, with a subsequent loss of biological variation: e.g. most of the effects of technological treatments are studied with iso-lipidic diets, which were not retained with the criterion we selected. This is probably the reason why few effects of technological treatments were found to be significant beyond the variation they induced through FA levels. Consequently, our results indicate that the first determinant of duodenal or absorbed flows of total FA was the level of FA_{int}. For prediction of total FA flows, intra- and inter-experiment relationships were nearly the same, suggesting that the prediction of these flows was probably not biased. However, it should be emphasized that high FA_{int} increased the variability of the flows, and this could have been taken into account by using logarithmic transformation. We did not use this approach because the obtained coefficients would have lost all biological and practical significance.

Despite the increasing variability in response to digestive flows to increased FA_{int} and the model we used (random effect for experiment), the RMSEs of the models were rather low and the coefficients of the equations generally had low s.d. Due to the hypothesis linked to the mixed models, the variability of the adjusted coefficients is higher than that of coefficients obtained by fixed-effect models (St-Pierre, 2001). Despite the potential bias discussed above (see duodenal flows of bacterial FA), these equations may be used to predict bacterial FA (that resulted from FA synthesis, incorporation into bacterial cells or adsorption on the cell wall) and duodenal flow of total FA of dietary origin, for both practical (feed formulation) and scientific purposes, i.e. to estimate FA flows as an input for metabolic models of FA. However, more research is needed to more clearly determine whether the flows of these FA groups differed in terms of intestinal digestibility in order to build a unit system to accurately predict flows of digestible FA in the intestine. More specifically, there is a clear need to obtain more data on microbial FA metabolism, as well as FA flows for complete forage diets and diets including fish oils. Moreover, interactions with other non-lipid components of the diet have to be quantified in more detail as they can determine duodenal flows of specific FA, particularly trans-FA or CLA (Glasser *et al.*, 2008). Finally, before a fully applicable unit system can be obtained, a clear definition of animal needs for specific FA has to be determined.

In conclusion, the approach used in this study has to be completed by other approaches to FA metabolism, such as mechanistic modelling or thermodynamic approaches to ruminal metabolism. More specifically, in numerous parts of this meta-analysis, we pointed out that changes in microbial populations (protozoa *v.* bacteria) and/or in specific bacterial populations (possibly cellulolytic *v.* starch degrading species) may account for changes in EMPS, BH and flow of FA_{duo}. Clearly, there is a need to increase knowledge on the relationships between the microbial populations and dietary factors that may alter them, to improve the prediction of absorbed flows of FA.

Other attempts to predict ruminal FA metabolism and FA absorption in dairy cows have been developed using the mechanistic approach of ruminal lipolysis of dietary fats, BH of FA, *de novo* production of FA in the rumen and intestinal digestion of FA in dairy cows (Moate *et al.*, 2004). Estimation of parameters of this model have been developed on a smaller data set (eight publications) than the current database and validated on eight other publications (36 diets) in the original publication, and further on a higher data set with 63 diets (Moate *et al.*, 2006). The results show that for a wide range of diets, this mechanistic approach can accurately predict the apparent absorption of dietary total long-chain FA, as well as BH of FA and

synthesis of FA by bacteria. Unfortunately, residual variation of the prediction of FA_{duo} from FA_{int} and FA_{abs} from FA_{duo}, respectively, was not provided, which prevents any direct comparison of the accuracy of the two approaches. One of the main assumptions of this model was an inhibition of bacterial FA synthesis in the rumen with increasing FA content, as a result of increased uptake of long-chain FA by microbes, which is consistent with our data. However, some differences with our data are related to the prediction of FA absorption estimated with long-chain FA flows to the duodenum and to the faeces, whereas we selected publications where FA absorption was only estimated on ileal determinations. Despite that difference, they obtained strong linear relationships between the flow of long-chain FA to the duodenum and intestinal absorption, with lower intestinal digestibility for tallow (especially when hydrogenated) and whole soybeans.

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Appendix 1

Mean ± s.d. values of variables studied in the whole database, and according to the animal species

Variable studied*	Abbreviation (units)	Overall database	Sheep	Growing cattle	Dairy cows
Dry matter intake	DMI (g/kg body weight)	2.30 ± 0.69 [246]	1.77 ± 0.38 [43]	2.00 ± 0.37 [125]	3.06 ± 0.57 [78]
FA intake	FA_{int} (g FA/kg DMI)	46.0 ± 22.4 [303]	36.5 ± 25.4 [61]	51.0 ± 23.0 [97]	46.7 ± 19.3 [145]
Biohydrogenation of FA in the rumen	BH_{pc} (%)	74.6 ± 13.1 [249]	80.2 ± 11.4 [45]	72.0 ± 17.2 [86]	74.2 ± 10.3 [118]
Rumen pH	pH (upH)	6.19 ± 0.28 [196]	6.07 ± 0.25 [30]	6.25 ± 0.34 [71]	6.19 ± 0.24 [95]
OM truly digested in the rumen	RDOMt (% OM intake)	57.3 ± 10.1 [123]	63.1 ± 8.8 [15]	64.1 ± 7.8 [40]	52.1 ± 8.5 [68]
Efficiency of microbial protein synthesis	EMPS (gN/kg RDOMt)	25.4 ± 7.0 [183]	24.4 ± 3.6 [21]	23.7 ± 5.4 [75]	27.2 ± 8.4 [87]
Duodenal flow of FA					
Total FA	FA_{duo} (g FA/kg DMI)	47.9 ± 23.0 [297]	43.4 ± 32.2 [61]	53.6 ± 22.9 [97]	46.4 ± 17.6 [139]
C12	$C12_{duo}$ (g C12/kg DMI)	0.21 ± 0.20 [134]	0.23 ± 0.27 [39]	0.18 ± 0.10 [52]	0.22 ± 0.22 [43]
C14	$C14_{duo}$ (g C14/kg DMI)	0.76 ± 0.81 [173]	0.76 ± 1.23 [39]	0.78 ± 0.40 [64]	0.73 ± 0.82 [70]
C16	$C16_{duo}$ (g C16/kg DMI)	9.1 ± 5.4 [255]	7.4 ± 4.8 [48]	9.8 ± 5.0 [94]	9.2 ± 5.9 [113]
C18	$C18_{duo}$ g C18/kg DMI)	32.7 ± 18.9 [278]	27.1 ± 26.8 [54]	36.9 ± 16.6 [101]	31.6 ± 15.5 [123]
Microbial FA	FA_{mic} (g FA/kg DMI)	20.7 ± 10.6 [62]	27.8 ± 5.0 [5]	10.2 ± 4.3 [14]	23.3 ± 10.2 [43]
Proportion of microbial FA	FA_{mic}/FA_{duo} (%)	41.6 ± 19.4 [62]	31.4 ± 6.0 [5]	23.9 ± 14.6 [14]	48.4 ± 17.7 [43]
Ruminal balance of FA	FA_{bal} (g FA/kg DMI)	−1.6 ± 9.8 [297]	−4.9 ± 12.1 [61]	−3.1 ± 8.8 [97]	1.1 ± 8.6 [139]
Flow of absorbed FA					
Total FA	FA_{abs} (g FA/kg DMI)	33.2 ± 19.0 [71]	34.3 ± 17.3 [14]	33.3 ± 13.8 [25]	33.2 ± 11.3 [32]
C12	$C12_{abs}$ (g C12/kg DMI)	0.13 ± 0.12 [24]	–	0.15 ± 0.13 [17]	0.07 ± 0.04 [7]
C14	$C14_{abs}$ (g C14/kg DMI)	0.62 ± 0.35 [33]	–	0.63 ± 0.36 [17]	0.60 ± 0.34 [16]
C16	$C16_{abs}$ (g C16/kg DMI)	7.7 ± 4.0 [53]	10.8 ± 4.2 [4]	7.6 ± 4.3 [25]	7.2 ± 3.5 [24]
C18	$C18_{abs}$ (g C18/kg DMI)	20.5 ± 15.5 [62]	38.9 ± 13.8 [7]	24.6 ± 10.5 [25]	18.1 ± 11.3 [30]
Apparent intestinal digestibility of FA					
Total FA	FA_{dSI} (%)	74.4 ± 12.9 [71]	77.2 ± 8.1 [14]	75.8 ± 15.1 [25]	72.8 ± 10.6 [32]
C12	$C12_{dSI}$ (%)	66.9 ± 11.5 [24]	–	67.6 ± 11.3 [17]	65.1 ± 12.9 [7]
C14	$C14_{dSI}$ (%)	66.7 ± 17.5 [33]	–	63.9 ± 20.1 [17]	71.5 ± 10.6 [16]
C16	$C16_{dSI}$ (%)	76.7 ± 11.9 [53]	81.0 ± 3.1 [4]	77.6 ± 12.2 [25]	74.6 ± 12.3 [24]
C18	$C18_{dSI}$ (%)	75.4 ± 14.8 [62]	80.0 ± 10.1 [7]	75.9 ± 16.3 [25]	73.4 ± 12.0 [30]

Data in brackets are the number of experimental groups.
*FA = fatty acid; DMI = dry matter intake; OM = organic matter; RDOMt = OM truly digested in the rumen; Biohydrogenation of FA in the rumen = intake of double bonds (DB) − duodenal flow of DB expressed as a proportion of the DB intake; Ruminal balance of FA = total FA intake − duodenal flow of total FA; Flow of absorbed FA in the intestine (SI) = duodenal flow of FA − ileal flow of FA; Apparent intestinal digestibility = flow of absorbed FA/duodenal flow of FA × 100.