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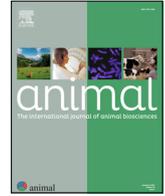
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Quantitative relationships between ingested and intestinal flows of linoleic and alpha-linolenic acids, body weight and milk performance in mid-lactation dairy cows



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ABSTRACT

Linoleic acid (**LA**) and alpha-linolenic acid (**ALA**) are essential fatty acids found in variable quantities in ruminant feedstuffs. Revision of French feed unit systems in 2018 has proposed the reassessment of energy requirements through a between-experiment approach expressing metabolisable energy supply as a function of the energy expenditures for maintenance and production, with these expenditures that reflect homeorhetic regulations. Based on the same approach, LA and ALA intake can be related to animal characteristics (i.e., BW) reflecting maintenance expenditures and secretion characteristics (i.e., milk yield, milk fat content and contents of LA and ALA in milk fat). Therefore, the objective of this work was to analyse the between-experiment relationships between ingested, duodenal, or absorbed flows of LA and ALA, BW and milk LA and ALA secretion by meta-analysis in mid-lactation dairy cows. These relationships were analysed using LA and ALA subsets of 96 and 99 experiments, respectively. Between-experiment regressions of daily flows of ingested, duodenal or absorbed LA and ALA on BW and milk LA and ALA flows were studied, with statistical unit defined as the mean of within-experiment treatments. For LA, the BW-associated coefficient was 0.019 (± 0.0034) g absorbed LA/d per kg BW and milk LA secretion-associated coefficient was 0.70 (± 0.081) g absorbed LA/g of LA secreted into milk. For ALA, the BW-associated coefficient was 0.0058 (± 0.00093) g absorbed ALA/d per kg BW and milk ALA secretion-associated coefficient was 0.57 (± 0.097) g absorbed ALA/g of ALA secreted into milk. When coding the diets as either control or milk fat depression diets, the BW-associated coefficient for LA was 0.017 (± 0.0032) g absorbed LA/d per kg BW for both diets. For milk fat depression diets, milk LA secretion-associated coefficient was 1.02 (± 0.119) g absorbed LA/g of LA secreted into milk, whereas it was 0.70 (± 0.075) g absorbed LA/g of LA secreted into milk for control diets. Significant BW and milk performance coefficients were obtained in all LA and ALA equations, allowing the calculation of ingested and intestinal flows of LA and ALA based on measured BW, milk fat yield and milk fat content of LA and ALA. The relationships between ingested and intestinal flows of LA and ALA, BW and milk performance obtained in the present work could be integrated into renewed feed unit systems for energy and protein in dairy cows.

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Implications

Essential fatty acids serve major body functions and regulatory processes, and are secreted into cow's milk. Essential fatty acid requirements have not been quantified in dairy cows though deficiencies could exist for immunity and reproduction functions. We analysed the relationships between ingested, duodenal or

absorbed flows of essential fatty acids, BW and milk essential fatty acid flows. These relationships, combined with renewed feed unit systems for energy and protein, could be used to improve feed ration formulation in dairy cows.

Introduction

Linoleic acid (**LA**, C18:2n-6) and alpha-linolenic acid (**ALA**, C18:3n-3) are essential fatty acids (**EFA**) for growth and normal cell function. Mammals cannot endogenously synthesise LA and

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ALA; so these EFA have to be provided by diet. Burr and colleagues demonstrated the essentiality of LA and ALA in rats fed fat-free diets in a series of papers in the 1930s (Burr et al., 1932). These EFA undergo desaturation and elongation into the long-chain fatty acid (FA) arachidonic acid (C20:4n-6), eicosapentaenoic acid (C20:5n-3) and docosahexaenoic acid (C22:6n-3) (Sprecher et al., 1995). These EFA and their long-chain FA products have three main roles: as core components of brain and retina cell membranes (Alessandri et al., 2004), as precursors of eicosanoids which are involved in inflammation, cardiac physiology, platelet aggregation and reproduction (Harizi et al., 2008), and as regulators of gene expression (Nakamura et al., 2004). No requirements for LA and ALA have been published in ruminants though Mattos and Palmquist (1977) estimated in dairy cows an availability of LA above requirements for milk LA secretion (i.e., availability for maintenance) of 244 mg absorbed LA/d per kg BW^{0.75}, which constitutes an estimate of maintenance LA requirements. In pre-weaned calves, Garcia et al. (2015) determined an optimal dietary LA supply of 0.2–0.3 g/d per kg BW^{0.75} (3–5 g/d) and an optimal dietary ALA supply of 0.02–0.04 g/d per kg BW^{0.75} (0.3–0.6 g/d) based on growth performance and immune responses during the first 30 days of life. The absence of external signs of deficiency under typical ruminant diets has been associated with an efficient conservation of EFA by ruminants (Palmquist, 2010), which is illustrated in vitro by the greater amounts of EFA esterified into lower-turnover phospholipid class than into triglyceride class by bovine muscle explants (Caldari-Torres et al., 2016). However, as genetic selection for milk yield over the past decades has widened the gap between nutrient expenditures and intake (Opsomer, 2015), it is likely that EFA partitioning between functions has been modified, with utilization shifting towards the mammary gland and away from reproduction and immunity. This hypothesis is consistent with numerous studies showing that diets supplemented with n-3 and n-6 FA lead to an improvement in immune function and reproduction in periparturient cows (Dirandeh et al., 2013; Moallem, 2018). All these findings underline the need to study ingested, duodenal and absorbed flows of LA and ALA according to animal characteristics and milk performance in mid-lactation dairy cows.

For the revision of feed unit systems by the National Research Institute for Agriculture, Food and the Environment (INRA) (INRA, 2018a; 2018b), energy requirements were reassessed using a between-experiment approach expressing metabolisable energy supply as a function of the energy expenditures for maintenance and production. In the between-experiment approach, one observation is the mean of all the treatments per experiment, assuming that the variance across experiments results mainly from differences in animal contexts or level of homeorhesis, independently of the dietary factors tested within-experiment (INRA, 2018b). Based on a similar approach, this work aims to analyse through a meta-analysis the between-experiment relationships between ingested, duodenal, or absorbed flows of LA and ALA, BW and milk LA and ALA flows.

Material and methods

Data identification and screening

The AGrum database (IDDN.FR.001.510032.000.R.C.2011.000.1 0300) aggregates publications from 1970 to 2019 based on in vivo trials studying FA intake, dairy performance and milk FA profiles of cows in response to dietary strategies such as lipid supplementation (saturated or unsaturated FA as seed, oil, fat or free FA), forage type, and forage-to-concentrate ratio. Statistical unit of the AGrum database is the published mean of an experimental

treatment within a trial. To study the relationships between input flows of LA and ALA (i.e., ingested, duodenal, or absorbed flows of LA and ALA), BW and milk LA and ALA secretion flows, all flows were expressed in a common unit (g/d). We screened the AGrum database (Fig. 1) to select papers enabling the calculation of both LA or ALA intake and LA or ALA secretion into milk and reporting BW. Duodenal and absorbed flows of LA and ALA were calculated using empirical prediction models (Prado et al., 2019). As the equations had initially been built in bovine (beef and dairy cows) and ovine species, they were reassessed for dairy cows only (Table 1 and Supplementary Material S1).

Data eligibility and inclusion

In order to study accurate flows of EFA, treatments providing an estimate of DM intake (DMI) with no direct measurement (trials at pasture) were removed from the database. Treatments including post-ruminal infusions of lipids or FA were excluded, as the infused FA escaped ruminal biohydrogenation (RBH). We also removed any treatments that included factors known to decrease RBH of unsaturated FA, such as additives protecting FA against microbial activity (Fig. 1). Diets using tannins were therefore removed, as tannins have been shown to alter the bacteria of the rumen microbiota and globally the RBH of FA (Vasta et al., 2019). Treatments containing ionophore antibiotics, such as monensin, were also excluded as they have been associated with a limitation of the complete RBH of unsaturated FA (AlZahal et al., 2008; Ishlak et al., 2015). Treatments containing fatty acyl amides or FA encapsulated in a protein-formaldehyde coating were removed because they are designed to protect unsaturated FA from RBH (Gulati et al., 2005; Jenkins and Bridges, 2007). The treatments using calcium salts of FA were not discarded from the analysis, as these rumen-protected fats provide inconsistent and limited rumen protection responses (Jenkins and Bridges, 2007; Gadeyne et al., 2017). Likewise, the factors known to reduce plasma FA uptake by the mammary gland, and either decrease or increase de novo lipogenesis of FA in the mammary gland were discarded (Fig. 1). Treatments using diets supplemented with trans-10, cis-12 conjugated linoleic acid were therefore removed as these supplements were associated with a dose-dependent reduction in milk fat content and milk fat yield as a result of a decrease in de novo lipogenesis in the mammary gland and plasma uptake of preformed FA by the mammary gland (Gervais et al., 2005). Treatments supplemented with acetate or butyrate were excluded since short-chain FA are precursors of milk fat synthesis and can stimulate de novo lipogenesis by the mammary gland (Maxin et al., 2011). One experiment was removed due to milk ALA yield that was greater than the corresponding intake (Whitlock et al., 2003). Experiments using precalving supplementation of oils or seeds were excluded because postpartum mobilization of adipose tissue EFA, at least LA (Lerch et al., 2015), may bias the relationships between input EFA flows, BW and milk EFA flows. Finally, any dietary treatments using marine-source lipid supplements such as fish oils, fish or algae meals, whether fed in free or protected form, were also excluded as they potentially reduce LA and ALA transfers from diet to milk. To confirm this hypothesis, we extracted a subset of 17 experiments that contained at least one treatment supplemented with marine-source products (MARINE) and one treatment free of any marine-source product (CONTROL) (Supplementary Table S1 and Supplementary Material S2). We then ran within-experiment regressions between milk LA or ALA secretion and ingested LA or ALA flows using linear mixed-effects models in R with the “lmerTest” package (version 3.1-3). First, the marine effect (CONTROL versus MARINE; fixed effect) and the experiment effect (17 experiments; random effect; St-Pierre, 2001) were tested on LA or ALA intake. Then, milk LA or ALA yield was studied as a function

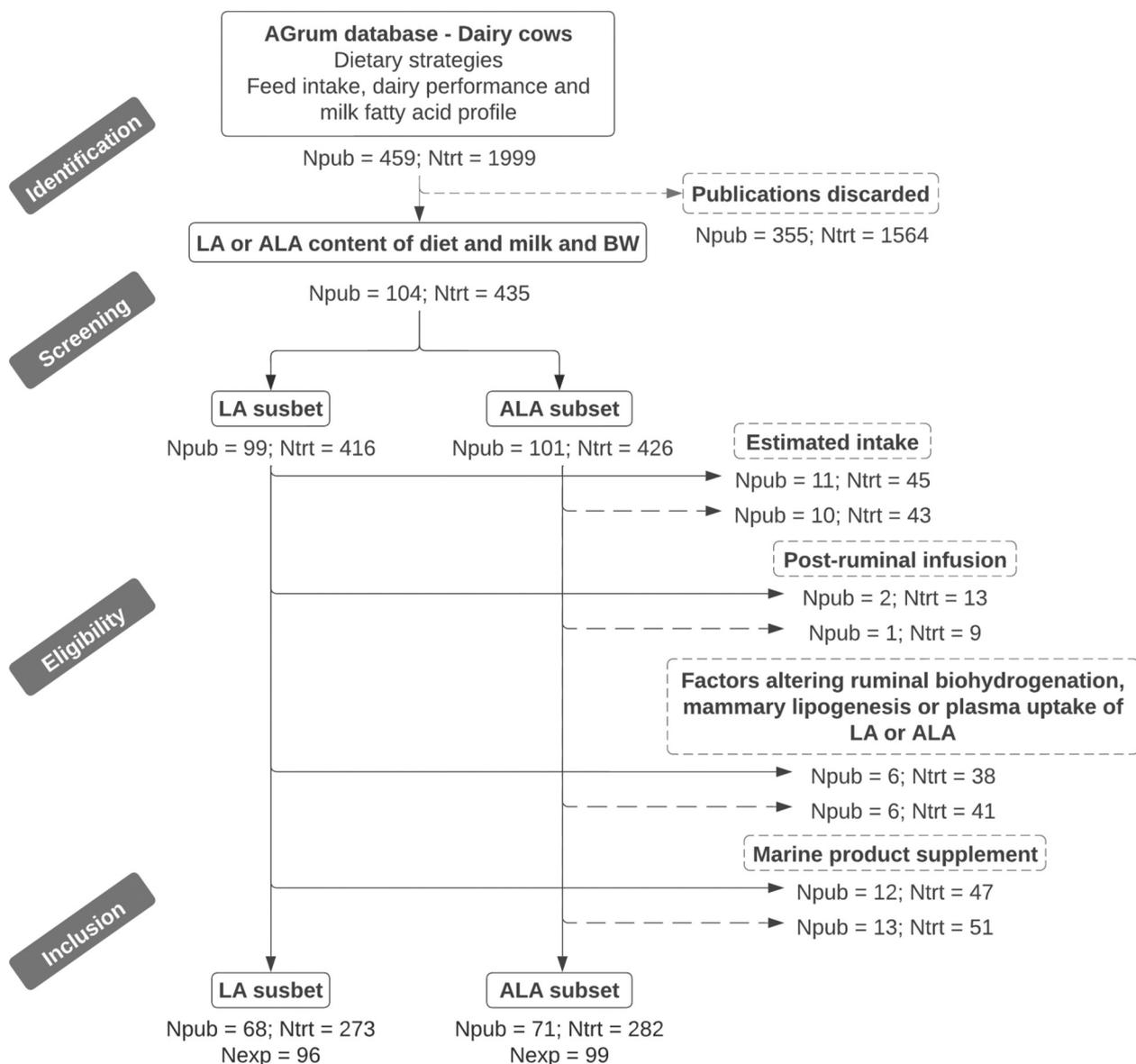


Fig. 1. PRISMA flow diagram describing the data selection process resulting in linoleic and alpha-linolenic acid datasets used for the development and evaluation of the models in dairy cows (LA = linoleic acid, ALA = alpha-linolenic acid, Npub = number of publications, Nex = number of experiments and Ntrt = number of treatments).

Table 1
Within-experiment prediction equations of duodenal and absorbed linoleic and alpha-linolenic acid flows in dairy cows based on the reassessment of equations of Prado et al. (2019).¹

Equation	Prediction equation	Nobs	Nexp	AIC	BIC	R ²	RMSE	RSR
1	Duodenal LA (g/kg DMI) = 0.024 (±0.0027) × PCO + 0.037 (±0.0102) × Diet LA (g/kg DMI)	75	25	95.0	106.6	0.88	0.19	0.27
2	Duodenal ALA (g/kg DMI) = 0.22 (±0.045) + 0.035 (±0.0067) × Diet ALA (g/kg DMI)	70	24	-18.8	-7.5	0.94	0.10	0.20
3	Absorbed LA (g/kg DMI) = -0.14 (±0.059) + 0.90 (±0.045) × Duodenal LA (g/kg DMI)	25	9	-31.9	-27.1	0.99	0.049	0.076
4	Absorbed ALA (g/kg DMI) = 0.79 (±0.035) × Duodenal ALA (g/kg DMI)	20	8	-63.0	-60.0	0.99	0.024	0.068

Abbreviations: LA = linoleic acid; ALA = alpha-linolenic acid; DMI = DM intake; PCO = percentage of concentrate in diet (% of DM); Nobs = number of observations; Nex = number of experiments; AIC = Akaike Information Criterion; BIC = Bayesian Information Criterion; R² = adjusted R²; RMSE is expressed in g/kg of DMI; RSR = RMSE-to-standard deviation ratio.

¹ Prado et al. (2019).

of LA or ALA intake with the marine (fixed) and experiment (random) effects that were tested on both the slope and the intercept of this relationship (St-Pierre, 2001). This data selection process led us to the final LA subset (number of publications = 68; number of experiments = 96; number of treatments = 273) and ALA subset (number of publications = 71; number of experiments = 99; number of treatments = 282) (Fig. 1). Details of the publications

included in these LA and ALA subsets can be found in [Supplementary Table S2](#) and [Supplementary Material S3](#).

Calculations and coding

The variables of interest were ingested, duodenal and absorbed flows, and milk secretion of EFA-all on a daily basis. For each EFA,

the intake (g/d) was calculated by multiplying diet EFA content (g/g of total FA) by dietary total FA content (g/kg of DM) and DMI (kg/d). For experiments providing diet EFA content in g/kg of DM, diet EFA content was directly multiplied by DMI to obtain EFA intake. Milk EFA yield (g/d) was calculated by multiplying milk EFA content (g/g of total FA) by milk fat yield (g/d) and by a coefficient of 0.933, which is the FA content of total milk lipids (Glasser et al., 2007). The milk fat depression (MFD) diets were coded in the database, and the other diets were coded as control (CTL) diets. The MFD diets were considered as the diets that alter fermentation in the rumen, leading to the release of specific RBH intermediates such as trans-10, cis-12 conjugated linoleic acid which is known to reduce both lipogenesis in the mammary gland and plasma uptake of preformed FA by the mammary gland (Gervais et al., 2005; Harvatine et al., 2009). Treatments were coded as MFD when milk fat content and milk fat yield decreased by more than 15% compared to CTL with no variation in milk yield, and there was an associated high content of milk fat trans-10, cis-12 conjugated linoleic acid (>0.025 g/100 g of total FA) or trans-10 C18:1 (>5 g/100 g of total FA) or both (Shingfield et al., 2010; Leskinen et al., 2019).

Statistical analyses

Models developed

In accordance with INRA approach (INRA, 2018b), we defined an 'experiment' within a trial as all treatments linked by one experimental factor, using only the treatments retained at the end of the selection process (Fig. 1). For each variable, the mean of all the treatments per experiment was calculated in order to study between-experiment regressions, with the assumption that animals have the same characteristics across treatments. In the between-experiment approach, it is assumed that the variability among experiments is not due to differences among rations (treatments) that reflect homeostasis and laws of response to changes in diet composition, but to differences among animals that reflect homeorhesis and that can be expressed according to BW, physiological stage, and dairy performance (INRA, 2018b). Consequently, between-experiment regressions of daily ingested, duodenal and absorbed EFA flows (EFA_{input flow}) on BW and daily milk EFA secretion flow (EFA_{output flow}) were studied according to the following equation:

$$\text{EFA}_{\text{input flow},i} = \beta_1 \times \text{BW}_i + \beta_2 \times \text{EFA}_{\text{output flow},i} + \varepsilon_i$$

where β_1 (g of EFA input flow/d per kg BW) is the coefficient for the BW_i , β_2 (g of EFA input flow/g of EFA output flow) is the coefficient for the $\text{EFA}_{\text{output flow},i}$ and ε_i is the residual error of the model ($i = 1, \dots, n$ experiments). All models were coded in R (version 4.0.2; R Foundation for Statistical Computing, Vienna, Austria) using the `lm` function in the "stats" package (version 4.0.2). The residuals of the models were regressed against the main potential quantitative interfering factors of the database to determine how these factors could modify the relationships between input EFA flows, BW and milk EFA flows (Sauvant et al., 2020). These factors were related to diet composition (forage-to-concentrate ratio, NDF, CP, starch, total FA), animal characteristics (BW, days in milk (DIM), DMI, milk yield), and milk composition (concentrations and yields of fat, protein and lactose). The qualitative interfering factor "type of diet" (CTL versus MFD diet) was tested on the residuals by ANOVA in R using the "stats" package (version 4.0.2), and when tests were significant, the influence on the coefficients of the models was assessed. The overall quality of the regression models developed was assessed based on the adjusted R^2 and the RMSE of the models.

Evaluation of the models

The models were evaluated using three different methods: external validation, leave-one-out cross-validation and

10-times-repeated 5-fold cross-validation. These methods were run together and compared for each model. First, an external validation was performed by randomly splitting each initial dataset into a training dataset for model development and a test dataset for model evaluation with the "caret" package in R (version 6.0-88) using training-to-test datasets size ratio of 70/30. In accordance with the statistical unit used in this approach (mean value of treatments per experiment), the splitting of the dataset was performed by experiment. The two datasets resulting from the splitting were compared by ANOVA on the main variables of interest (diet composition, animal characteristics, milk composition, ingested, duodenal and absorbed EFA flows, milk EFA flow, EFA content in diet and milk) to ensure homogeneity between the training and test datasets. Then, leave-one-out cross-validation was performed with the "caret" package in R using the "LOOCV" method. Finally, 10-times-repeated 5-fold cross-validation was run with the "caret" package in R using the "repeatedcv" method with $k = 5$ and number of repetitions = 10. The performance of each model to accurately relate input flows of LA and ALA to BW and milk LA and ALA flows was assessed with several model evaluation metrics. For both external validation and cross-validation, the adjusted R^2 validation and the root mean square of prediction error were calculated. For external validation, errors in central tendency, errors due to regression, and errors due to disturbances (Bibby and Toutenburg, 1977) were also calculated as the three components of the mean square of prediction error. Finally, we determined the root mean square of prediction error-to-mean ratio as it represents the rate of prediction error. The root mean square of prediction error-to-mean ratio was calculated as the root mean square of prediction error divided by the mean of observed values.

Results

Meta-design and statistical description of the subsets

The descriptive statistics of the main variables of LA and ALA subsets are presented in Tables 2 and 3 for the training and test subsets and in Supplementary Tables S3 and S4 for the whole subsets. Mean duration of trials included in either LA subset or ALA subset or both subsets was 80 d, and the experimental design was mainly Latin Square (62% of trials) (Supplementary Table S2). Dietary LA content (% of DM) was not correlated to dietary ALA content (% of DM) in both the LA ($P = 0.717$) and the ALA ($P = 0.664$) subsets (Supplementary Fig. S1). Milk LA content (% of total FA) was not correlated to milk ALA content (% of total FA) in both the LA ($P = 0.179$) and the ALA ($P = 0.185$) subsets (Supplementary Fig. S2).

Relationships between input flows of essential fatty acids, BW and milk essential fatty acid flows

Models for linoleic acid flows

The models used to study the relationships between input LA flows, BW and milk LA flows are presented in Table 4. Models on the whole dataset (1a–6a) are shown first, followed by models on the training dataset (1b–6b) and then the different validation methods. Significant BW-associated coefficients and milk LA secretion-associated coefficients were obtained for all ingested, duodenal and absorbed LA flow models (equations 1b, 3b and 5b). The residuals of the ingested flow model (1b) were positively correlated with dietary starch and total FA and negatively correlated with milk fat content and sum of milk C4:0–C14:0 FA (Supplementary Table S5). The residuals of the duodenal (3b) and absorbed (5b) flow models were positively correlated with concentrate level, dietary starch and total FA, and negatively correlated

Table 2
Between-experiment descriptive statistics of the different variables of the linoleic acid datasets used for the development and evaluation of the models in dairy cows.

Variable	Training dataset for models development					Test dataset for models evaluation				
	Nexp	Mean	SD	Min	Max	Nexp	Mean	SD	Min	Max
Animal characteristics										
BW, kg	70	633	80	391	755	26	653	58	462	749
DIM, d	70	125	60	1	267	26	108	41	53	213
Diet composition										
Concentrate, % of DM	70	45.1	12.0	11.2	65.0	26	47.2	12.4	11.2	74.2
Total FA, % of DM	68	3.8	1.4	1.5	7.7	26	3.7	1.3	1.7	6.9
CP, % of DM	66	16.5	1.7	13.1	20.3	24	16.5	1.7	13.3	19.8
NDF, % of DM	67	35.3	6.1	25.0	59.7	25	36.3	8.0	19.3	59.7
Starch, % of DM	51	20.7	6.7	1.5	33.8	15	21.0	11.6	5.5	49.8
NE _L , MJ/kg of DM	18	6.7	0.4	5.9	7.5	7	6.7	0.4	6.3	7.1
LA, % of DM	70	1.27	0.58	0.29	2.78	26	1.32	0.61	0.44	2.73
ALA, % of DM	70	0.59	0.56	0.11	2.83	26	0.52	0.59	0.02	2.26
Intake										
DMI, kg/d	70	21.8	4.2	14.2	32.2	26	22.7	3.8	16.2	29.6
LA intake, g/d	70	285.1	152.5	46.5	673.5	26	300.9	141.3	79.0	615.8
ALA intake, g/d	70	116.1	100.9	21.8	557.8	26	106.1	108.1	5.9	420.1
Duodenal LA, g/d	70	34.8	13.7	7.6	60.1	26	37.3	12.5	7.8	58.4
Absorbed LA, g/d	70	28.3	11.8	4.6	49.8	26	30.4	10.9	4.6	48.5
Milk nutrient content and yield										
Milk yield, kg/d	70	31.3	9.6	12.7	50.2	26	32.4	7.9	17.2	47.1
Fat, %	70	3.75	0.55	2.59	5.22	26	3.82	0.64	2.43	5.30
Fat, g/d	70	1 131	331	380	2 150	26	1 200	286	685	1 653
Protein, %	65	3.21	0.23	2.66	4.00	24	3.23	0.25	2.83	4.02
Protein, g/d	65	995	293	390	1 550	24	1 054	240	626	1 468
Lactose, %	58	4.73	0.24	4.08	5.25	22	4.75	0.21	4.34	5.24
Lactose, g/d	58	1 531	476	530	2 485	22	1 567	411	786	2 293
LA, % of total FA	70	2.20	0.77	0.85	4.38	26	2.36	1.04	1.07	5.04
LA, g/d	70	23.9	11.8	5.9	60.2	26	26.5	12.8	9.5	58.1
ALA, % of total FA	70	0.56	0.24	0.20	1.29	26	0.48	0.25	0.12	1.36
ALA, g/d	70	5.7	2.6	1.4	12.7	26	5.1	2.3	0.7	11.4

Abbreviations: DIM = days in milk; FA = fatty acids; NE_L = net energy for lactation; LA = linoleic acid; ALA = alpha-linolenic acid; DMI = DM intake; Nexp = number of experiments; Min = minimum; Max = maximum.

Table 3
Between-experiment descriptive statistics of the different variables of the alpha-linolenic acid datasets used for the development and evaluation of the models in dairy cows.

Variable	Training dataset for models development					Test dataset for models evaluation				
	Nexp	Mean	SD	Min	Max	Nexp	Mean	SD	Min	Max
Animal characteristics										
BW, kg	72	636	83	391	755	27	644	50	542	741
DIM, d	72	121	55	1	267	27	126	60	61	249
Diet composition										
Concentrate, % of DM	72	46.3	12.4	11.2	74.2	27	44.1	10.9	14.9	59.8
Total FA, % of DM	72	3.7	1.3	1.5	7.1	25	4.0	1.4	2.3	7.7
CP, % of DM	68	16.5	1.7	13.1	20.3	25	16.5	1.6	13.1	18.8
NDF, % of DM	69	35.6	7.1	19.3	59.7	26	36.1	4.6	29.2	45.6
Starch, % of DM	50	20.6	8.6	1.5	49.8	19	20.0	6.1	9.4	29.2
NE _L , MJ/kg of DM	19	6.7	0.4	5.9	7.5	4	6.7	0.4	6.3	7.1
LA, % of DM	70	1.26	0.60	0.29	2.78	27	1.34	0.53	0.37	2.68
ALA, % of DM	72	0.57	0.53	0.07	2.27	27	0.67	0.72	0.12	2.83
Intake										
DMI, kg/d	72	21.8	4.1	14.2	32.2	27	21.7	3.8	14.3	28.7
LA intake, g/d	70	283.9	155.0	46.5	673.5	27	296.8	135.7	62.7	615.8
ALA intake, g/d	72	114.5	95.2	10.7	420.1	27	130.4	127.4	28.8	557.8
Duodenal ALA, g/d	72	8.8	3.2	3.9	19.1	27	9.3	4.1	5.0	23.9
Absorbed ALA, g/d	72	7.0	2.5	3.1	15.1	27	7.4	3.3	4.0	18.9
Milk nutrient content and yield										
Milk yield, kg/d	72	31.7	9.2	13.1	49.0	27	30.5	8.7	12.7	50.2
Fat, %	72	3.81	0.58	2.43	5.30	27	3.66	0.55	2.54	4.50
Fat, g/d	72	1 157	306	534	2 150	27	1 101	336	380	1 653
Protein, %	65	3.24	0.26	2.66	4.02	27	3.16	0.17	2.82	3.50
Protein, g/d	65	1 024	283	455	1 550	27	949	261	390	1 470
Lactose, %	59	4.73	0.19	4.08	5.13	24	4.74	0.30	4.18	5.25
Lactose, g/d	59	1 540	461	620	2 360	24	1 477	440	530	2 485
LA, % of total FA	67	2.20	0.82	0.85	4.38	27	2.35	0.95	0.92	5.04
LA, g/d	67	24.4	12.0	6.4	60.2	27	24.7	13.0	5.9	58.1
ALA, % of total FA	72	0.54	0.23	0.12	1.36	27	0.54	0.27	0.20	1.26
ALA, g/d	72	5.6	2.6	0.7	12.7	27	5.2	2.4	1.4	10.2

Abbreviations: DIM = days in milk; FA = fatty acids; NE_L = net energy for lactation; LA = linoleic acid; ALA = alpha-linolenic acid; DMI = DM intake; Nexp = number of experiments; Min = minimum; Max = maximum.

Table 4

Between-experiment regressions of daily ingested, duodenal, or absorbed linoleic acid flows as a function of BW and milk flows of linoleic acid in dairy cows.

Model development						Model validation								
Equation	Dataset	Equation	Nexp	R ²	RMSE	Method	Dataset	Nexp	R ²	RMSPE	ECT	ER	ED	RMR
1a	Whole	LA intake (g/d) = 0.17 (±0.043) × BW (kg) + 7.3 (±1.01) × LA milk (g/d)	96	0.88	110.0	LOOCV			0.43	112.2				
2a	Whole	CTL DIET: LA intake (g/d) = 0.13 (±0.038) × BW (kg) + 7.4 (±0.87) × LA milk (g/d) MFD DIET: LA intake (g/d) = 0.13 (±0.038) × BW (kg) + 13.8 (±1.42) × LA milk (g/d)	96	0.91	94.3	RepeatedCV			0.48	110.9				
						LOOCV			0.56	98.2				
3a	Whole	LA duodenum (g/d) = 0.027 (±0.0032) × BW (kg) + 0.75 (±0.075) × LA milk (g/d)	96	0.95	8.1	LOOCV			0.61	8.3				
						RepeatedCV			0.66	8.2				
4a	Whole	CTL DIET: LA duodenum (g/d) = 0.025 (±0.0030) × BW (kg) + 0.75 (±0.070) × LA milk (g/d) MFD DIET: LA duodenum (g/d) = 0.025 (±0.0030) × BW (kg) + 1.1 (±0.11) × LA milk (g/d)	96	0.96	7.6	LOOCV			0.65	7.8				
						RepeatedCV			0.69	7.7				
5a	Whole	LA absorbed (g/d) = 0.021 (±0.0028) × BW (kg) + 0.64 (±0.066) × LA milk (g/d)	96	0.95	7.2	LOOCV			0.60	7.3				
						RepeatedCV			0.64	7.2				
6a	Whole	CTL DIET: LA absorbed (g/d) = 0.019 (±0.0027) × BW (kg) + 0.65 (±0.061) × LA milk (g/d) MFD DIET: LA absorbed (g/d) = 0.019 (±0.0027) × BW (kg) + 0.95 (±0.100) × LA milk (g/d)	96	0.95	6.6	LOOCV			0.64	6.9				
						RepeatedCV			0.69	6.8				
1b	Training	LA intake (g/d) = 0.13 (±0.051) × BW (kg) + 8.4 (±1.23) × LA milk (g/d)	70	0.88	108.3	External	Test	26	0.33	117.1	2.52	5.50	91.98	0.40
2b	Training	CTL DIET: LA intake (g/d) = 0.099 (±0.0453) × BW (kg) + 8.4 (±1.07) × LA milk (g/d) MFD DIET: LA intake (g/d) = 0.099 (±0.0453) × BW (kg) + 14.7 (±1.70) × LA milk (g/d)	70	0.91	93.4	External	Test	26	0.53	98.9	5.26	5.09	89.66	0.34
3b	Training	LA duodenum (g/d) = 0.025 (±0.0038) × BW (kg) + 0.81 (±0.092) × LA milk (g/d)	70	0.95	8.1	External	Test	26	0.55	8.3	1.69	3.24	95.07	0.23
4b	Training	CTL DIET: LA duodenum (g/d) = 0.023 (±0.0036) × BW (kg) + 0.81 (±0.086) × LA milk (g/d) MFD DIET: LA duodenum (g/d) = 0.023 (±0.0036) × BW (kg) + 1.2 (±0.14) × LA milk (g/d)	70	0.96	7.5	External	Test	26	0.60	7.9	2.78	4.64	92.58	0.22
5b	Training	LA absorbed (g/d) = 0.019 (±0.0034) × BW (kg) + 0.70 (±0.081) × LA milk (g/d)	70	0.94	7.1	External	Test	26	0.53	7.4	1.50	3.40	95.10	0.25
6b	Training	CTL DIET: LA absorbed (g/d) = 0.017 (±0.0032) × BW (kg) + 0.70 (±0.075) × LA milk (g/d) MFD DIET: LA absorbed (g/d) = 0.017 (±0.0032) × BW (kg) + 1.02 (±0.119) × LA milk (g/d)	70	0.95	6.5	External	Test	26	0.59	7.0	2.58	4.76	92.66	0.23

Abbreviations: Nexp = number of experiments; R² = adjusted R²; RMSE is expressed in g/d; RMSPE = root mean square of prediction error (g/d); ECT = errors in central tendency; ER = errors due to regression; ED = errors due to disturbances; ECT, ER and ED are expressed in % of MSPE; RMR = RMSPE-to-mean ratio; LOOCV = leave-one-out cross-validation; RepeatedCV = 10-times-repeated 5-fold cross-validation; LA = linoleic acid; CTL = control diets; MFD = milk fat depression diets.

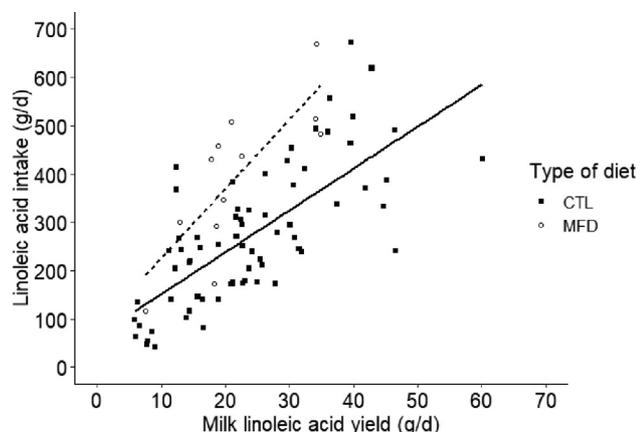


Fig. 2. Between-experiment regressions of linoleic acid intake (g/d) in function of milk linoleic acid yield (g/d) according to the type of diet (control (CTL) (solid line) or milk fat depression (MFD) (dashed line) diet) (one dot corresponds to a mean of treatments of a same experiment) in dairy cows.

with dietary NDF and milk fat content (Supplementary Table S5). Between models 1b and 2b, 3b and 4b, and 5b and 6b, the variable “type of diet” (CTL (Nexp = 59) versus MFD (Nexp = 11) diet) was tested on both BW-associated coefficient and milk LA secretion-associated coefficient. Similarly, significant BW-associated coefficients and milk LA secretion-associated coefficients were obtained for all ingested, duodenal and absorbed LA flow models, for both CTL and MFD diets (equations 2b, 4b and 6b). For all these models 2b, 4b and 6b, BW-associated coefficients were not different between CTL and MFD diets, while milk LA secretion-associated coefficients were always higher for MFD diets compared to CTL diets (14.7 versus 8.4, 1.2 versus 0.81 and 1.02 versus 0.70 g of input LA flow/g of output LA flow for ingested, duodenal and absorbed flow models, respectively) (Fig. 2). The residuals of the ingested flow model (2b) were positively correlated with dietary starch and total FA (Supplementary Table S5). The residuals of the duodenal (4b) and absorbed (6b) flow models were positively correlated with concentrate level, dietary starch and total FA, and

negatively correlated with dietary NDF (Supplementary Table S5). Residuals were not correlated with DIM for any of the models.

Models for alpha-linolenic acid flows

The models used to study the relationships between input ALA flows, BW and milk ALA flows are presented in Table 5. Models on the whole dataset (1c–3c) are shown first, followed by models on the training dataset (1d–3d) and then the different validation methods. Since data of ALA intake (g/d) were not normally distributed, we log₁₀-transformed this variable to obtain normality. Significant BW-associated coefficients and milk ALA secretion-associated coefficients were obtained for all ingested, duodenal and absorbed ALA flow models (equations 1d, 2d and 3d). The residuals of the ingested flow model (1d) were negatively correlated with DMI, milk yield, milk fat, protein and lactose yields (Supplementary Table S6). The residuals of the duodenal (2d) and absorbed (3d) flow models were positively correlated with dietary total FA and negatively correlated with milk protein and lactose yields and sum of milk C4:0–C14:0 FA (Supplementary Table S6). Residuals were not correlated with DIM for any of the models.

Assessments of model quality

For all models, coefficients were stable between the whole datasets and the training datasets. Moreover, adjusted R² and RMSE were similar between the whole and training datasets, indicating a similar share of the data variance explained by the models and a similar error between models. The share of the data variance explained by the models was higher for LA models compared to ALA models based on adjusted R² validation, though the training and test datasets used very similar numbers of data. For all LA and ALA models, the root mean square of prediction error was very similar to the RMSE of the models developed. For all LA and ALA models, errors due to disturbances accounted for the largest share of the mean square of prediction error (90–95% of mean square of prediction error for LA models and 74–94% of mean square of prediction error for ALA models), indicating that errors were mainly due to weak correlations between observed and fitted values.

Table 5
Between-experiment regressions of daily ingested, duodenal, or absorbed alpha-linolenic acid flows as a function of BW and milk flows of alpha-linolenic acid in dairy cows.

Model development			Model validation											
Equation	Dataset	Equation	Nexp	R ²	RMSE	Method	Dataset	Nexp	R ²	RMSPE	ECT	ER	ED	RMR
1c	Whole	log(ALA intake (g/d)) = 0.0016 (±0.00017) × BW (kg) + 1.2 (±0.15) × log(ALA milk (g/d))	99	0.97	0.34	LOOCV RepeatedCV			0.28 0.32	0.29 0.29				
2c	Whole	ALA duodenum (g/d) = 0.0077 (±0.00111) × BW (kg) + 0.73 (±0.118) × ALA milk (g/d)	99	0.90	3.0	LOOCV RepeatedCV			0.19 0.27	3.1 3.0				
3c	Whole	ALA absorbed (g/d) = 0.0060 (±0.00088) × BW (kg) + 0.57 (±0.094) × ALA milk (g/d)	99	0.90	2.4	LOOCV RepeatedCV			0.19 0.27	2.4 2.4				
1d	Training	log(ALA intake (g/d)) = 0.0015 (±0.00019) × BW (kg) + 1.3 (±0.17) × log(ALA milk (g/d))	72	0.97	0.33	External	Test	27	0.22	0.36	8.72	17.19	74.09	0.18
2d	Training	ALA duodenum (g/d) = 0.0074 (±0.00118) × BW (kg) + 0.72 (±0.122) × ALA milk (g/d)	72	0.91	2.8	External	Test	27	0.21	3.7	5.78	0.00	94.22	0.40
3d	Training	ALA absorbed (g/d) = 0.0058 (±0.00093) × BW (kg) + 0.57 (±0.097) × ALA milk (g/d)	72	0.91	2.2	External	Test	27	0.21	2.9	5.78	0.00	94.22	0.40

Nexp = number of experiments; log = log base 10; R² = adjusted R²; RMSE is expressed in log (g/d) for models 1c and 1d, and g/d for all other models; RMSPE = root mean square of prediction error (log (g/d) for models 1c and 1d, and g/d for all other models); ECT, ER and ED are expressed in % of MSPE; RMR = RMSPE-to-mean ratio; LOOCV = leave-one-out cross-validation; RepeatedCV = 10-times-repeated 5-fold cross-validation; ALA = alpha-linolenic acid.

Discussion

While EFA requirements have already been published at least partially in some non-ruminant livestock animals (LA and ALA requirements for preruminant calves (Garcia et al., 2015) and LA requirements for sows (National Research Council (NRC), 2012), there is only one paper reporting estimated LA requirements for dairy cows (Mattos and Palmquist, 1977). This is probably related to the difficulty involved in quantifying the requirements due to the complexity of lipid metabolism in ruminants (Mattos and Palmquist, 1977). This metabolism is characterised by coexisting ruminal processes of (1) ruminal lipolysis, (2) extensive RBH of dietary unsaturated free FA by ruminal microorganisms leading to mainly saturated and unsaturated FA and 'by-pass' FA, and (3) de novo synthesis of FA by the microorganisms. All these processes lead to mixed flows of both dietary 'by-pass' FA, saturated and unsaturated FA originating from RBH, and microbial FA to the intestine. Here, the absence of correlation between LA and ALA contents in diet and in milk allowed a separate approach between the two EFA for the study of the relationships between input flows of each EFA, BW and milk secretion of each EFA, which raises prospects for diet formulation to reach target LA or ALA contents in milk fat.

Between-experiment approach: methodological proof and limitations

The methodology used for revising INRA feed unit systems for energy (INRA, 2018b) was based on a between-experiment approach that reflects homeorhetic regulations and a factorial decomposition of energy requirements into maintenance and productive requirements. Based on this methodology, we analysed, through a meta-analysis, the between-experiment relationships between ingested, duodenal, or absorbed flows of EFA, BW and milk EFA secretion. This strategy allows the quantification of the variability linked to differences among animal contexts that reflect homeorthesis levels and is appropriate to study input EFA flows according to animal characteristics (i.e., BW) and performance (i.e., milk EFA secretion) (INRA, 2018b). However, the between-experiment approach raises two major limitations. First, the statistical unit is the mean of experimental treatments per experiment, which strongly reduced the number of available data (from 273 treatments down to 96 experiments in the LA subset and from 282 treatments down to 99 experiments in the ALA subset), which may be a limit for performing external validation. Here, the number of data was particularly limited for the qualitative variable "type of diet", which was unbalanced as there are more published trials studying milk FA profile in response to dietary strategies than trials studying diet-induced MFD. Second, the between-experiment approach offers no possibility to account for the variability within each experiment, which may therefore leave a major share of the variability within each model unexplained.

Interpretation of the coefficients relating input flows of essential fatty acids to BW and milk essential fatty acid flows

Our factorial approach has also been used by Mattos and Palmquist (1977), who split absorbed flows of LA into milk LA secretion and absorbed flow of LA available for maintenance functions. Consequently, we can compare our BW-associated coefficient to their absorbed flow of LA available for maintenance functions. Mattos and Palmquist (1977) estimated absorbed flows of LA from [1-¹⁴C] LA injected intravenously or placed into the omasal canal of five cows. Based on Mattos and Palmquist (1977) published individual cow available LA and BW, we calculate 0.051 g of absorbed LA available for maintenance/d per kg BW.

Using mean milk LA yield (23.9 g/d), mean BW (633 kg) and mean calculated absorbed LA flow (28.8 g/d, equation 5b) of the present study, we calculate 0.0077 g of absorbed LA available for maintenance/d per kg BW. The discrepancy between the estimates of the two studies could be explained by the very low LA RBH calculated by Mattos and Palmquist (68.1%), in contrast with the mean LA RBH (86.8%) calculated from the reassessment of the duodenal LA flows (g/kg DMI) for dairy cows only (Table 1). Moreover, the digestibility used in Mattos and Palmquist (1977) was 0.93, which is higher than the mean digestibility coefficient of 0.79 obtained after reassessing the absorbed LA flows (g/kg DMI) for dairy cows only (Table 1). Methodological limitations in Mattos and Palmquist (1977) could also explain the difference in results, as (1) only five animals were used, (2) the gas-chromatography column used for FA analysis was likely far less powerful at accurately separating isomers than the columns available nowadays, and (3) the use of three different methods to assess absorbed LA flows could have created variability in the quantification of flows among cows. Methodological limitations in reassessing Prado et al. (2019) models for dairy cows could also explain the difference in results. Indeed, the number of available data for dairy cows only was lower than in Prado et al. (2019) who used data of bovine (beef and dairy cows) and ovine species.

In the publications used for building the models, BW variations were almost never reported, and as we related input EFA flows to BW and milk EFA flows, we can wonder if BW and milk EFA flows account for variations in body reserves, as cows were mostly in mid-lactation (mean DIM = 123 d in training LA and ALA subsets) and probably replenished body reserves. Variations in body reserves could be encompassed in either the BW-associated coefficient or milk EFA secretion-associated coefficient. As the BW used in the models was often measured at the beginning of experiments (74 and 76% of treatments in LA and ALA datasets, respectively), it is likely that the BW-associated coefficient does not account for the variations in body reserves. Moreover, if experiments are carried out over short periods, then BW variations are likely to remain low, and so the variations in body reserves are probably encompassed in the milk EFA secretion-associated coefficient, as observed with milk LA secretion-associated coefficients, which were higher for MFD compared to CTL diets. Thus, variations in BW or net energy balance would be valuable data to help improving the accuracy of the relationships between input flows of LA and ALA, BW and milk LA and ALA flows, and to determine to what extent variations in body reserves contribute to input flows of LA and ALA. Taking into consideration body condition score variations of 1.3 points (on a 1–5 body condition score scale) from peak to end of lactation, where a body condition score variation of one point (on a 1–5 body condition score scale) corresponds to 40 kg body fat (Komaragiri and Erdman, 1997), we can calculate a 52 kg body fat replenishment during the last 28 weeks of lactation, which is equivalent to 265 g/d of body fat deposition. With mean adipose tissue contents of 1.5% of total FA for LA and 0.3% of total FA for ALA in mid-lactation cows (Hiller et al., 2013; Brzozowska et al., 2018), daily fat deposition would represent 4 g/d of LA and 0.8 g/d of ALA. With the hypothesis of 80%-efficient transfer from absorbed EFA flows to EFA deposition in adipose tissue (mean conversion efficiency of metabolisable energy to body reserves; INRA, 2018b), this would lead to a flow for replenishing body fat reserves of 5 g of absorbed LA/d and 1 g of absorbed ALA/d. Using mean calculated absorbed LA flow of 28.8 g/d (equation 5b, Table 4, with mean BW = 633 kg and mean milk LA yield = 23.9 g/d), variations in body reserves would represent 17% of absorbed LA flow. Using mean calculated absorbed ALA flow of 6.9 g/d (equation 3d, Table 5, with mean BW = 636 kg and mean milk ALA yield = 5.6 g/d), variations in body reserves would represent 14% of absorbed ALA flow.

Finally, LA and ALA flows directed towards gestation and foetus development were considered negligible in modelling input flows of LA and ALA compared to BW and milk LA and ALA secretion terms due to the very limited transfer of EFA through the ruminant placenta (Elphick et al., 1979).

Relationships between input flows of essential fatty acids, BW and milk essential fatty acid flows and metabolism of essential fatty acids in dairy cows

Globally, the residuals of LA models (1b, 3b, and 5b; Table 4) were positively correlated with dietary starch and total FA, and negatively correlated with milk fat content and sum of milk C4:0-C14:0 FA, indicating that ingested, duodenal and absorbed flows of LA were underestimated for high dietary starch and high dietary total FA and overestimated for high milk fat content and high sum of milk C4:0-C14:0 FA. These parameters are strongly associated with the MFD syndrome mainly observed when feeding diets that are (1) high in rapidly fermentable carbohydrates and low in effective fibre, supplemented or not with polyunsaturated FA, or (2) supplemented with marine oils regardless of the level of effective fibre (Bauman and Griinari, 2003). These two types of diets inducing MFD lead to a decrease in milk fat content and in lipogenesis in the mammary gland as evidenced by the reduction in milk content of de novo-synthesised FA (short- and medium-chain milk C4:0-C14:0 FA). The milk LA secretion-associated coefficient for the ingested, duodenal and absorbed flow models was higher for MFD than for CTL diets. On one side, given that the BW-associated coefficient was not affected by the type of diet, this could indicate a priority for LA flow to safeguard a certain amount of absorbed LA for the cow's basal functions. On the other side, the increase in milk LA secretion-associated coefficient with MFD diets could be related to a reduction in plasma uptake of LA by the mammary gland and storage of the remaining absorbed LA (total absorbed LA – (LA for maintenance + LA secretion into milk)) into body reserves (mainly in adipose tissues and probably, to a lesser extent, in muscles). Thus, the milk LA secretion-associated coefficient in MFD diets could contain both milk LA secretion and LA stored in body reserves (adipose tissues and muscles) during body reconstitution phases (mean DIM = 125 d in training LA subset). Indeed, MFD diets have been shown to reduce de novo lipogenesis in the mammary gland, plasma uptake of preformed FA by the mammary gland, and stearoyl-CoA desaturase activity in the mammary gland (Baumgard et al., 2001; 2002). Short-term abomasal infusion of trans-10, cis-12 conjugated linoleic acid, which partly simulates MFD in dairy cows, was associated with an alteration of energy partitioning towards a strong decrease in milk energy output and a moderate reduction in DMI resulting in net excess energy. This energy status was associated with an upregulation of adipose tissue genes involved in lipid synthesis (FA synthase), uptake (lipoprotein lipase), desaturation (stearoyl-CoA desaturase) and transport (FA-binding protein) (Harvatine et al., 2009).

Towards an improvement in the variables modelled

In both the LA and ALA models, errors due to disturbances accounted for the largest part of the mean square of prediction error, indicating that errors were mainly due to weak correlations between observed and fitted values. This weak correlation could be explained by the lack of precision and the uncertainties related to the variables modelled (BW, milk LA or ALA secretion, and ingested, duodenal and absorbed LA or ALA flows). The BW used in the models was often measured at the beginning of experiments (data found in most publications), and this does not always reflect BW at sampling time. Moreover, most papers fail to specify how BW was measured (beginning, middle or end of the experiment;

morning or evening measure; single or repeated measure). There is uncertainty around the measure of milk fat content due to day-to-day variation and the method of analysis (mid-IR spectroscopy). Furthermore, there is uncertainty around the measure of milk LA and ALA content, since different methods, protocols and apparatuses are used to analyse milk FA (Ungerfeld et al., 2019). Indeed, the gas-chromatography column is a crucial factor for accurate separation of FA isomers, and it is likely that some publications report co-elution of ALA (e.g., with cis-11 C20:1 or cis-9 C20:1) or LA (e.g., with cis-9, cis-15 C18:2) instead of true ALA or LA. Similarly to milk FA analysis, different methods, protocols and apparatuses are used across laboratories to determine feed FA profile, which has been shown to be subjected to common analytical errors (Jenkins, 2010) that can result in uncertainties around LA and ALA intake. Finally, uncertainties around duodenal and absorbed flows of LA and ALA exist since these flows are obtained from prediction equations (see the above-mentioned limitations in reassessing Prado et al. (2019) models), which implies that our present models are based on either measured data (ingested flow models) or calculated data (duodenal and absorbed flow models). Ideally, models based on measured data only could lead to more precise relationships between EFA flows, but there are scarce few published trials measuring both duodenal (or absorbed) flows of EFA and their secretion into milk.

As both LA and ALA datasets are mostly based on lipid-supplemented diets, it is likely that cows are overfed LA and ALA, and that they oxidise the excess of LA and ALA as general energy sources. Ideally, the inclusion of diets with very low or almost depleted LA or ALA content could allow more accurate relationships between EFA flows, as these EFA are less prone to oxidation. However, these types of diets can be difficult to implement, as they need to be almost depleted of cereals, oilseeds and forages. The lack of publications studying low LA and ALA content in diets of dairy cows constitutes also a limit for estimating LA and ALA requirements. Consequently, there is a need for future research to design specific feeding trials with low LA and ALA content in diets.

Conclusion

This study was based on a meta-analysis applied to a large dataset of published data from nutrition-trial experiments measuring diet and milk content of LA or ALA in mid-lactation dairy cows. The between-experiment regression approach has been used in accordance with the approach proposed by INRA feed unit systems (2018) in renewing energy requirements. We analysed between-experiment relationships between ingested, duodenal, or absorbed flows of LA and ALA, BW and milk LA and ALA flows in mid-lactation dairy cows. These relationships could be integrated into the renewed INRA feed unit systems for energy and protein, and could be used to improve feed ration formulation in dairy cows.

Supplementary material

Supplementary material to this article can be found online at <https://doi.org/10.1016/j.animal.2022.100661>.

Ethics approval

Not applicable.

Data and model availability statement

The data and models were not deposited in an official repository. The data and models that support the study findings are available from the authors upon request.

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Declaration of interest

None.

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