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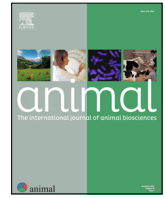
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## Predicted essential fatty acid intakes for a group of dairy cows also apply at individual animal level



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### ABSTRACT

The ruminant requirements for essential fatty acids (EFAs), particularly linoleic acid (LA) and alpha-linolenic acid (ALA), have not been fully determined, although evidence suggests that an adequate supply of polyunsaturated fatty acids (FAs) could improve immunity and reproduction in transition cows. In previous studies, we predicted EFA intake for a group of cows based on animal characteristics and milk EFA secretions. However, to support precision livestock feeding, we need to match the nutrient requirements and intakes of each cow as closely as possible. Our group-level predictions may not be accurate enough to estimate the EFA intake of an individual cow, due to inter-individual variations in EFA digestion and metabolism related to differences in feed intake, intake patterns, and the composition and functioning of the rumen microbiota. To address this issue, here we set out to establish specific equations that predict EFA intake for an individual cow based on the difference (i.e. the residuals) between observed EFA intake and the predicted EFA intake based on our group-level equations. We studied a database of individual dairy cows (26 experiments; 503 datapoints from three research teams) and we predicted the residuals from (1) dietary and animal-related factors (i.e. full predictions) and (2) animal-related factors only (i.e. field predictions), which are considered more field-amenable. The variance of predicted LA and log ALA intake was explained to 68% by observed LA intake and 66% by observed log ALA intake, respectively. The residuals of LA intake were predicted by dietary ALA content, total FA intake, BW, milk yield and fat content in full predictions, and by BW, feeding level, milk yield and fat content, and sum of milk C4:0 to C14:0 FA in field predictions. The log residuals of ALA intake were predicted by dietary NDF and total FA contents, NDF intake, BW, milk protein, LA and ALA contents, and fat yield in full predictions, and by BW, DM intake, milk LA and ALA contents, and fat yield in field predictions. The field predictions showed a moderate loss of accuracy compared to full predictions based on RMSE of prediction (from 38 to 54 g/d for LA and from 0.090 to 0.12 log (g/d) for ALA). This work is the first to predict the EFA intake of an individual cow based on previously established group-level predictions of EFA intake adjusted for dietary and animal-related factors.

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### Implications

We previously predicted essential fatty acid intakes for a group of dairy cows according to body weight and milk essential fatty acid secretion. The ability to identify individual variations in essential fatty acid intakes in response to dietary and animal-related variables could help formulate feed rations in order to supply adequate amounts of essential fatty acids for periods of specific need,

such as reproductive periods or depressed immune phases, or periods of negative energy balance.

### Introduction

Ruminants are thought to efficiently conserve essential fatty acids (EFAs), particularly the dietary linoleic acid (LA, C18:2n-6) and alpha-linolenic acid (ALA, C18:3n-3), as they do not show external signs of deficiency under typical diets (Palmquist, 2010). However, high-yielding dairy cows have high nutrient requirements, particularly at onset of lactation, which exposes them to

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severe negative nutrient balance, metabolic diseases, and impaired immune and reproductive performance, which can be partly reversed by an appropriate supply of polyunsaturated fatty acids (FAs) (Sordillo, 2016; Moallem, 2018). Denis et al. (2022) predicted the amount of EFA ingested by a group of cows based on the flows of EFA directed towards maintenance functions and the secretion of EFA in milk. However, cows that share the same breed, parity and lactation stage and are reared in a similar environment (nutrition, management, and housing) nevertheless show large inter-individual variations in their performance response to diets. This could be related to inter-individual differences in (1) genetic potential for milk secretion, (2) degrees of physiological imbalance in relation to homeorhetic and homeostatic regulations (Ingvarsen and Friggens, 2005), (3) feeding patterns (Rumphorst et al., 2022), (4) rumen microbial populations associated with different levels of production of biohydrogenation (BH) intermediates and resulting impacts on milk fat content (Zhang et al., 2023), and (5) the efficiency of transfer of intestinal EFA to milk fat (Moallem et al., 2012). Taken together, these observations point to inter-individual variations in EFA digestion and metabolism that need to be accounted for to individualise daily adjustment of EFA supply to the nutritional requirements of each individual in a herd. Nevertheless, to our knowledge, no study has quantified individual variations in the relationships between EFA intake, BW and milk EFA secretion. In an effort to address this gap, we hypothesised that the cow-group-level equations (Denis et al., 2022) estimating EFA intake from BW and milk EFA yield can also predict EFA intake at an individual-cow level in order to precisely optimise livestock feeding. The objective of this work is to establish specific prediction equations of EFA intake for an individual cow, based on predicting the difference (i.e. the residuals) between observed EFA intake and predicted EFA intake based on group-level equations (Denis et al., 2022). The residuals are predicted from (1) dietary and animal-related factors (i.e. 'full' predictions) and (2) animal-related factors only (i.e. 'field' predictions) which are considered more field-amenable.

## Material and methods

### Database construction

The database was created using individual cow data from in vivo trials led by the INRAE-UMRH (Theix, France), Ghent University Laboratory for Animal Nutrition and Animal Product Quality (Ghent, Belgium), and Université Laval Département des Sciences Animales (Québec, Canada). These trials quantified cow daily FA intakes, dairy performance and milk FA profiles in response to various dietary strategies such as lipid supplementation (source and form), forage type, concentrate type (i.e. starch- or fibre-rich), forage-to-concentrate ratio, and metabolisable protein supply. The statistical unit of the database is the individual cow data on a given diet measured at a given timepoint within an experiment. All the databased experiments and treatments adhered to the identification, screening, eligibility and inclusion criteria defined by Denis et al. (2022). Briefly, the included data made it possible to calculate both LA or ALA intake and milk LA or ALA secretion together with reported BW. All data on individual cows during the first seven days in milk (DIM) were removed from the database, as colostrum has a significantly different fat composition compared to milk (McGrath et al., 2016). The input variables of the database were associated with diet composition (i.e. percent dietary concentrate, NDF, CP, total FA, LA and ALA content), animal characteristics (i.e. DIM and BW), nutrient intake (i.e. DM intake (DMI), feeding level (i.e. DMI-to-BW ratio)), and dairy performance (i.e. milk yield, milk fat, protein, and lactose content and yield, milk

LA and ALA content and yield, and sum of milk even-chain C4:0 to C14:0 FA). The database included 26 experiments, 70 treatments and 503 datapoints: 384 datapoints (from 19 experiments) from Université Laval, 67 datapoints (from three experiments) from INRAE-UMRH, and 52 datapoints (from four experiments) from Ghent University. Details of the published studies included in the database can be found in Supplementary Table S1 and Supplementary Material S1. The database includes five experiments already considered in Denis et al. (2022) (Ferlay et al., 2010; Saliba et al., 2014; Leduc et al., 2017, Supplementary Material S1).

### Calculations and coding

The LA and ALA intakes and milk secretion flows were calculated as described in Denis et al. (2022). In order to evaluate how the group-level equations from Denis et al. (2022) could serve to estimate LA (Eq. (1)) and ALA (Eq. (2)) intake at an individual-cow level, we applied these equations to individual data and then calculated the difference between the observed and predicted values, which we define as the 'residuals':

$$\text{LA intake (g/d)} = 0.13 (\pm 0.051) \times \text{BW (kg)} + 8.4 (\pm 1.23) \times \text{LA milk (g/d)} \quad (1)$$

$$\log(\text{ALA intake (g/d)}) = 0.0015 (\pm 0.00019) \times \text{BW (kg)} + 1.3 (\pm 0.17) \times \log(\text{ALA milk (g/d)}) \quad (2)$$

### Statistical analyses

#### Model development

All statistical analyses were performed using R version 4.0.2 (R Foundation for Statistical Computing, Vienna, Austria). We aimed to predict the residuals via the variables related to diet, animal characteristics, nutrient intake and dairy performance in order to improve the group-level estimates of LA intake and log ALA intake for an individual-cow level. Significant Pearson correlations ( $P < 0.05$ ) between the residuals and all the variables in the database were determined and visualised using the `corrplot` function (`corrplot` package version 0.90 in R). We then tested and compared two approaches for predicting the residuals.

First, we tested all the variables that had significant Pearson correlations ( $P < 0.05$ ) with the residuals (except dietary LA content in the prediction of LA residuals, and dietary ALA content in the prediction of ALA residuals) in the linear mixed-effects model (built using the `lmer` function of the `lmerTest` package version 3.1-3 in R) of prediction of the residuals with experiment added as a random effect (Eq. (3)).

$$\text{residual}_{ij} = \mu + \beta_1 P_{1,ij} + \beta_2 P_{2,ij} + \dots + \beta_n P_{n,ij} + E_i + \varepsilon_{ij} \quad (3)$$

where  $\text{residual}_{ij}$  = observed intake<sub>ij</sub> – predicted intake<sub>ij</sub>,  $\mu$  is the overall mean,  $P_{1,ij} \dots P_{n,ij}$  are the predictors considered as fixed effects,  $E_i$  is the random experiment effect, and  $\varepsilon_{ij}$  is the residual error of the model, with  $i$  the experiment and  $j$  the observation. The predictors were selected based on their variance inflation factors (VIFs; using the `vif` function of `car` package version 3.0-11 in R) within the models (Eq. (3)) in order to avoid potential multicollinearity in model development. A  $\text{VIF} < 10$  was used to retain candidate predictors of the residuals (St-Pierre and Glamocic, 2000). Finally, we applied a backward elimination of the non-significant effects in the linear mixed-effects models using the `step` function (`lmerTest` package version 3.1-3 in R) in order to obtain a complex final prediction model of the residuals (i.e. full predictions).

Second, we tested only the animal-related variables (i.e. DIM, BW, DMI, feeding level, milk yield, milk fat, protein and lactose content and yield, milk LA and ALA content, and the sum of milk C4:0 to C14:0 FA) that had significant Pearson correlations ( $P < 0.05$ ) with the residuals in the model (Eq. (3)), which were selected as described above to obtain a simpler final model for predicting the residuals (i.e. field predictions). This second approach was used to predict the residuals via more field-amenable variables in order to adjust the group-level equations estimating EFA intake. The overall quality of the models developed was assessed based on their adjusted  $R^2$  and the RMSE. We calculated the corrected predicted LA intake and log ALA intake based on the initial LA intake and log ALA intake predictions obtained from Denis et al. (2022) and the predicted residuals, and we then assessed the improvement over initial predictions.

#### Model evaluation

The predictions of LA and ALA residuals were evaluated using an external validation process involving random initial splitting of observations within each experiment in the database into a training dataset for model development and a test dataset for model evaluation (using the caret package version 6.0-88 in R) using a training-to-test dataset size ratio of 70/30. The two datasets resulting from the split were compared by ANOVA on diet variables, animal characteristics, nutrient intake and dairy performance variables and on the residuals to ensure homogeneity between the training dataset and the test dataset. We assessed how each model performed in terms of accurately predicting the residuals based on the adjusted  $R^2$  of validation and the RMSE of prediction.

## Results

#### Statistical description of the database and initial fitting of the group-level equations at individual level on the training dataset

Descriptive statistics for the main variables in the database are presented in Table 1 for the training and test subsets and in Supplementary Table S2 for the whole database. In the training subset, BW ranged from 481 to 916 kg, LA intake ranged from 37 to 960 g/d, milk LA yield ranged from 4 to 47 g/d, ALA intake ranged from 16 to 824 g/d, and milk ALA yield ranged from 1 to 25 g/d. The individual efficiencies for diet-to-milk LA transfer (i.e. milk LA yield-to-LA intake ratio) varied from 2 to 37%, with a mean of 8%. The individual efficiencies for diet-to-milk ALA transfer (i.e. milk ALA yield-to-ALA intake ratio) varied from 1 to 20%, with a mean of 6%. The more transfer-efficient animals were characterised by an overestimation of LA intake and log ALA intake, while the less transfer-efficient animals were characterised by an underestimation of LA intake and log ALA intake.

In the training subset, both predicted LA intake and log ALA intake showed a linear relationship with observed LA intake and log ALA intake, respectively (Table 2). Adjusted  $R^2$  was 0.68 for the LA model and 0.66 for the ALA model, and RMSE expressed as a percentage of the observed mean was 16% for the LA model and 9% for the ALA model.

#### Development of prediction models for the residuals of linoleic acid intake on the training dataset

##### Correlations between the residuals of linoleic acid intake and predictors on the training dataset

The residuals of LA intake were significantly ( $P < 0.05$ ) correlated with dietary LA content ( $r = 0.91$ ), total FA intake ( $r = 0.81$ ), dietary total FA content ( $r = 0.69$ ), nitrogen intake ( $r = 0.44$ ), dietary

CP ( $r = 0.39$ ), ALA content ( $r = 0.31$ ), NDF ( $r = -0.23$ ), and percent concentrate ( $r = 0.21$ ). The residuals of LA intake were also significantly ( $P < 0.05$ ) correlated with feeding level ( $r = 0.36$ ), sum of milk C4:0 to C14:0 FA ( $r = -0.28$ ), milk fat content ( $r = -0.24$ ), DMI ( $r = 0.24$ ), milk lactose yield ( $r = 0.21$ ), BW ( $r = -0.21$ ), milk lactose content ( $r = 0.20$ ), milk yield ( $r = 0.17$ ), and milk protein yield ( $r = 0.15$ ) (Supplementary Fig. S1).

##### Full predictions of the residuals of linoleic acid intake

A selection process was run on the variables that significantly correlated with LA residuals in the training dataset. The process led to a final residual prediction model in which the predictors were dietary ALA content, total FA intake, BW, milk yield, and milk fat content (Eq. (1); Table 3). All predictors had a VIF  $< 3$ . The residuals were predicted with an accuracy of 30 g/d (i.e. RMSE) in the training dataset and 38 g/d (i.e. RMSE of prediction) in the test dataset (Eq. (1); Table 3). Correcting the group-level estimate of LA intake using the predicted residuals led to a significant improvement in the prediction of LA intake at individual-cow level in the test dataset (Fig. 1).

##### Field predictions of the residuals of linoleic acid intake

The variable selection process led to a final residual prediction model in which the predictors were BW, feeding level, milk yield, milk fat content, and sum of milk C4:0 to C14:0 FA (Eq. (2); Table 3). All predictors had a VIF  $< 2$ . The residuals were predicted with an accuracy of 45 g/d in the training dataset and 54 g/d in the test dataset (Eq. (2); Table 3). Correcting the group-level estimate of LA intake using the predicted residuals led to a significant improvement in the prediction of LA intake at individual-cow level in the test dataset (Fig. 1).

#### Development of prediction models for the residuals of log alpha-linolenic acid intake on the training dataset

##### Correlations between the residuals of log alpha-linolenic acid intake and predictors on the training dataset

The residuals of log ALA intake were significantly ( $P < 0.05$ ) correlated with dietary ALA content ( $r = 0.50$ ), total FA content ( $r = 0.38$ ), NDF content ( $r = 0.32$ ), total FA intake ( $r = 0.29$ ), dietary LA content ( $r = 0.26$ ) and CP content ( $r = 0.11$ ), and NDF intake ( $r = 0.11$ ). The residuals of log ALA intake were also significantly ( $P < 0.05$ ) correlated with milk fat yield ( $r = -0.48$ ), milk yield ( $r = -0.43$ ), milk LA content ( $r = -0.41$ ), milk lactose yield ( $r = -0.40$ ), milk protein yield ( $r = -0.38$ ), DIM ( $r = 0.36$ ), milk protein content ( $r = 0.30$ ), BW ( $r = -0.28$ ), DMI ( $r = -0.20$ ), and milk ALA content ( $r = -0.15$ ) (Supplementary Fig. S2).

##### Full predictions of the residuals of log alpha-linolenic acid intake

A selection process was run on the variables that significantly correlated with ALA residuals in the training dataset. The process led to a final residual prediction model in which the predictors were dietary NDF content, total FA content, NDF intake, BW, milk protein content, LA content, ALA content and milk fat yield (Eq. (3); Table 3). All predictors had a VIF  $< 2$ . The residuals were predicted with an accuracy of 0.072 log (g/d) in the training dataset and 0.090 log (g/d) in the test dataset (Eq. (3); Table 3). Correcting the group-level estimate of log ALA intake using the predicted residuals led to a significant improvement in the prediction of log ALA intake at individual-cow level in the test dataset (Fig. 2).

##### Field predictions of the residuals of log alpha-linolenic acid intake

The variable selection process led to a final residual prediction model in which the predictors were BW, DMI, milk LA content, milk ALA content, and milk fat yield (Eq. (4); Table 3). All predictors had a VIF  $< 2$ . The residuals were predicted with an accuracy

**Table 1**  
Descriptive statistics of the different variables of the training and test datasets used for the development and evaluation of the models in dairy cows.

| Variable                               | Training dataset for model development |       |       |       |       | Test dataset for model evaluation |       |       |       |       |
|--|--|-------|-------|-------|-------|-----------------------------------|-------|-------|-------|-------|
|  | n                                      | Mean  | SD    | Min   | Max   | n                                 | Mean  | SD    | Min   | Max   |
| <b>Animal characteristics</b>          |  |       |       |       |       |                                   |       |       |       |       |
| BW, kg                                 | 365                                    | 666   | 75    | 481   | 916   | 138                               | 664   | 73    | 485   | 884   |
| DIM, d                                 | 365                                    | 126   | 73    | 9     | 392   | 138                               | 126   | 68    | 8     | 300   |
| <b>Diet composition</b>                |  |       |       |       |       |                                   |       |       |       |       |
| Concentrate, % of DM                   | 365                                    | 41.2  | 13.2  | 13.2  | 70.0  | 138                               | 41.2  | 13.0  | 13.6  | 70.0  |
| NDF, % of DM                           | 365                                    | 34.9  | 6.7   | 21.1  | 55.3  | 138                               | 34.9  | 6.5   | 20.4  | 56.3  |
| CP, % of DM                            | 365                                    | 15.4  | 1.9   | 10.8  | 24.7  | 138                               | 15.2  | 1.7   | 10.7  | 21.0  |
| Total FA, % of DM                      | 365                                    | 3.1   | 1.7   | 0.8   | 10.1  | 138                               | 3.1   | 1.6   | 0.8   | 9.4   |
| LA, % of DM                            | 365                                    | 1.16  | 0.60  | 0.20  | 3.04  | 138                               | 1.17  | 0.60  | 0.20  | 3.04  |
| ALA, % of DM                           | 365                                    | 0.59  | 0.70  | 0.10  | 4.04  | 138                               | 0.57  | 0.63  | 0.10  | 3.69  |
| <b>Nutrient intake</b>                 |  |       |       |       |       |                                   |       |       |       |       |
| DMI, kg/d                              | 365                                    | 23.3  | 4.3   | 9.9   | 36.2  | 138                               | 23.1  | 4.2   | 12.9  | 34.9  |
| Feeding level, kg DM/d per kg BW       | 365                                    | 0.035 | 0.007 | 0.014 | 0.055 | 138                               | 0.035 | 0.007 | 0.019 | 0.051 |
| LA intake, g/d                         | 365                                    | 274.1 | 163.4 | 36.5  | 960.2 | 138                               | 274.0 | 160.1 | 41.4  | 862.1 |
| ALA intake, g/d                        | 365                                    | 128.1 | 134.6 | 16.1  | 823.5 | 138                               | 124.4 | 127.4 | 16.8  | 818.3 |
| <b>Milk nutrient content and yield</b> |  |       |       |       |       |                                   |       |       |       |       |
| Milk yield, kg/d                       | 365                                    | 32.2  | 8.6   | 9.4   | 56.3  | 138                               | 32.1  | 8.0   | 7.7   | 50.2  |
| Fat, %                                 | 365                                    | 3.85  | 0.55  | 1.93  | 5.20  | 138                               | 3.86  | 0.56  | 2.32  | 5.68  |
| Fat, g/d                               | 365                                    | 1 226 | 317   | 415   | 2 238 | 138                               | 1 228 | 308   | 292   | 1 972 |
| Protein, %                             | 365                                    | 3.19  | 0.31  | 2.30  | 4.18  | 138                               | 3.17  | 0.28  | 2.59  | 3.98  |
| Protein, g/d                           | 365                                    | 1 015 | 239   | 352   | 1 661 | 138                               | 1 009 | 228   | 289   | 1 488 |
| Lactose, %                             | 365                                    | 4.69  | 0.27  | 3.69  | 5.60  | 138                               | 4.67  | 0.28  | 3.60  | 5.44  |
| Lactose, g/d                           | 365                                    | 1 514 | 412   | 396   | 2 676 | 138                               | 1 506 | 390   | 301   | 2 273 |
| LA, % of total FA                      | 365                                    | 1.67  | 0.51  | 0.60  | 3.23  | 138                               | 1.63  | 0.49  | 0.75  | 3.21  |
| LA, g/d                                | 365                                    | 19.5  | 8.7   | 3.9   | 47.0  | 138                               | 19.2  | 8.5   | 3.7   | 50.4  |
| ALA, % of total FA                     | 365                                    | 0.46  | 0.25  | 0.11  | 2.22  | 138                               | 0.46  | 0.31  | 0.14  | 1.97  |
| ALA, g/d                               | 365                                    | 5.1   | 2.8   | 1.0   | 25.1  | 138                               | 5.1   | 3.2   | 0.7   | 23.1  |
| C4:0-C14:0 FA, % of total FA           | 365                                    | 24.63 | 3.96  | 12.00 | 34.82 | 138                               | 24.72 | 3.94  | 11.37 | 32.96 |

Abbreviations: DIM = days in milk; FA = fatty acids; LA = linoleic acid; ALA = alpha-linolenic acid; DMI = DM intake; C4:0-C14:0 FA = sum of milk even-chain C4:0 to C14:0 fatty acids; n = number of observations; Min = minimum; Max = maximum.

**Table 2**  
Initial fitting of the group-level equations of prediction of linoleic acid intake and log alpha-linolenic acid intake of Denis et al. (2022) at an individual level on the training dataset in dairy cows.

| Equation | Fitting equation <sup>1</sup>  | Nobs | Nexp | R <sup>2</sup> | RMSE (%) |
|----------|--|------|------|----------------|----------|
| 1        | Predicted LA intake (g/d) = 186.7 (±13.82) + 0.27 (±0.037) × observed LA intake (g/d)              | 365  | 26   | 0.68           | 16       |
| 2        | Predicted log (ALA intake (g/d)) = 0.21 (±0.108) + 0.85 (±0.050) × observed log (ALA intake (g/d)) | 365  | 26   | 0.66           | 9        |

Abbreviations: LA = linoleic acid; log = log base 10; ALA = alpha-linolenic acid; Nobs = number of observations; Nexp = number of experiments; R<sup>2</sup> = adjusted R<sup>2</sup>; RMSE is expressed as a percentage of the observed mean.

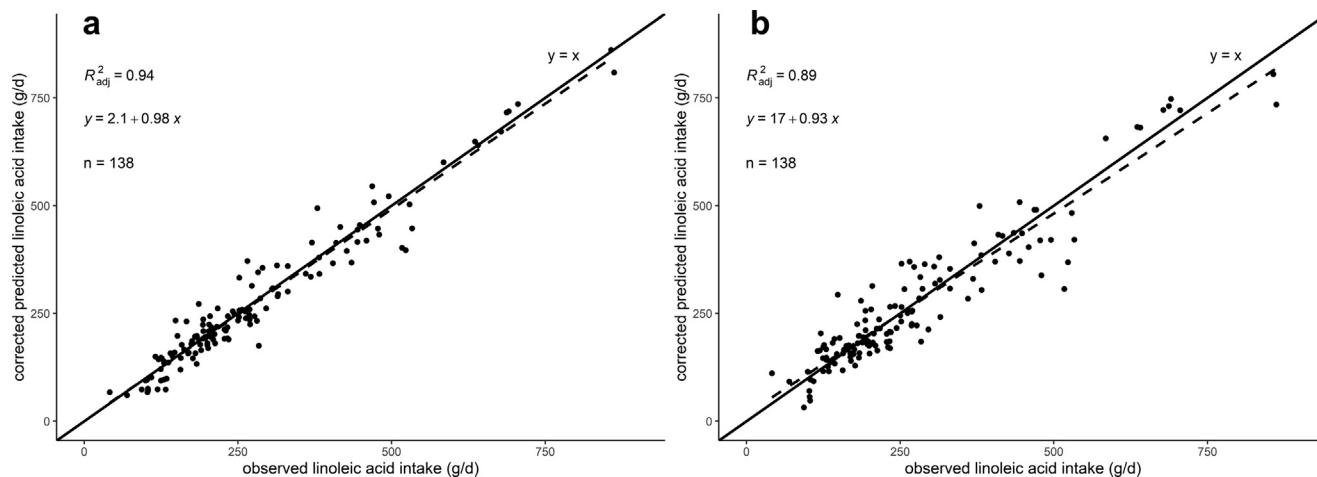
<sup>1</sup> Linear mixed-effects models including the random effect of experiment.

**Table 3**  
Prediction models of the residuals (i.e. difference between observed linoleic acid intake and predicted linoleic acid intake by equations from Denis et al. (2022) and difference between observed log alpha-linolenic acid intake and predicted log alpha-linolenic acid intake by equations from Denis et al. (2022)) in dairy cows.

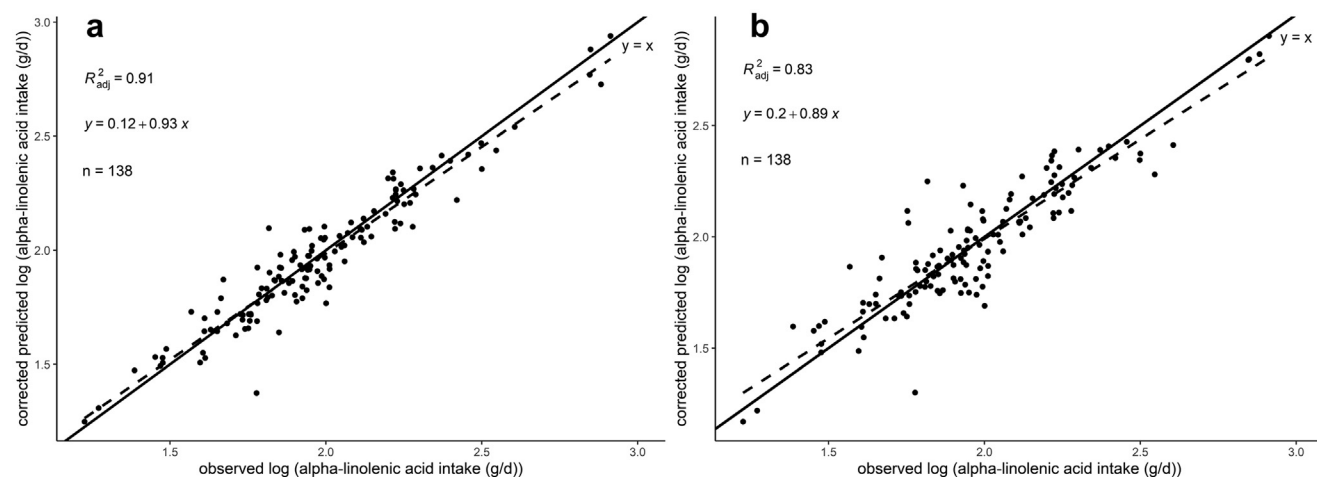
| No. | Equation <sup>1</sup>  | Model development |                |       |         |         | Model evaluation |                               |       |
|-----|--|-------------------|----------------|-------|---------|---------|------------------|-------------------------------|-------|
|     |  | n                 | R <sup>2</sup> | RMSE  | AIC     | BIC     | n                | R <sup>2</sup> <sub>val</sub> | RMSEP |
| 1   | residual = 204.7 (±28.43) – 72.1 (±6.63) × diet ALA% + 0.36 (±0.015) × total FA intake – 0.17 (±0.027) × BW – 5.4 (±0.33) × milk yield – 33.9 (±3.88) × milk fat%  | 365               | 0.95           | 30.19 | 3 659.4 | 3 690.6 | 138              | 0.93                          | 38.15 |
| 2   | residual = 36.5 (±51.53) + 0.16 (±0.045) × BW + 7 489.6 (±760.58) × feeding level – 4.8 (±0.53) × milk yield – 42.5 (±5.79) × milk fat% – 3.9 (±0.94) × milk C4-C14%   | 365               | 0.89           | 45.20 | 3 942.5 | 3 973.7 | 138              | 0.86                          | 53.93 |
| 3   | residual = 1.4 (±0.11) – 0.0090 (±0.00153) × NDF% + 0.13 (±0.0065) × diet total FA% + 0.053 (±0.0047) × NDF intake – 0.0014 (±0.000070) × BW + 0.058 (±0.0182) × milk protein% – 0.14 (±0.015) × milk LA% – 0.58 (±0.025) × milk ALA% – 0.00046 (±0.000020) × milk fat yield | 365               | 0.93           | 0.072 | –656.4  | –613.5  | 138              | 0.91                          | 0.090 |
| 4   | residual = 1.6 (±0.086) – 0.0015 (±0.00010) × BW + 0.021 (±0.0024) × DMI – 0.20 (±0.022) × milk LA% – 0.26 (±0.029) × milk ALA% – 0.00047 (±0.000028) × milk fat yield   | 365               | 0.86           | 0.11  | –410.2  | –379.0  | 138              | 0.83                          | 0.12  |

Abbreviations: residual = difference between observed linoleic acid intake and predicted linoleic acid intake by equation from Denis et al. (2022) in equations nos. 1 and 2 (expressed in g/d) and difference between observed log alpha-linolenic acid intake and predicted log alpha-linolenic acid intake by equation from Denis et al. (2022) in equations nos. 3 and 4 (expressed in log (g/d)); in equations nos. 1 and 3, all variables were initially included and in equations nos. 2 and 4, only animal-related variables were initially included; diet ALA% = dietary alpha-linolenic acid content (%DM); total FA intake = total fatty acid intake (g/d); BW in kg; milk yield in kg/d; milk fat% = milk fat content (%); feeding level = DM intake-to-BW ratio in kg DM/d per kg BW; milk C4-C14% = sum of milk even-chain C4:0 to C14:0 fatty acids (% of total fatty acids); NDF % = dietary NDF (%DM); diet total FA% = dietary total fatty acid content (%DM); NDF intake in kg/d; milk protein% = milk protein content (%); milk LA% = milk linoleic acid content (% of total fatty acids); milk ALA% = milk alpha-linolenic acid content (% of total fatty acids); milk fat yield in g/d; DMI = DM intake (kg/d); n = number of observations; R<sup>2</sup> = adjusted R<sup>2</sup>; RMSE is expressed in g/d in equations nos. 1 and 2 and in log (g/d) in equations nos. 3 and 4; AIC = Akaike Information Criterion; BIC = Bayesian Information Criterion; R<sup>2</sup><sub>val</sub> = adjusted R<sup>2</sup> of validation; RMSEP = RMSE of prediction expressed in g/d in equations nos. 1 and 2 and in log (g/d) in equations nos. 3 and 4.

<sup>1</sup> Linear mixed-effects models including the random effect of experiment.



**Fig. 1.** Group-level estimate of linoleic acid intake by Denis et al. (2022) corrected by the predicted residuals (a, equation no. 1 of Table 3; b, equation no. 2 of Table 3) according to observed linoleic acid intake in the test dataset in dairy cows. Abbreviations:  $R^2_{adj}$  = adjusted  $R^2$ .



**Fig. 2.** Group-level estimate of log alpha-linolenic acid intake by Denis et al. (2022) corrected by the predicted residuals (a, equation no. 3 of Table 3; b, equation no. 4 of Table 3) according to observed log alpha-linolenic acid intake in the test dataset in dairy cows. Abbreviations:  $R^2_{adj}$  = adjusted  $R^2$ .

of 0.11 log (g/d) in the training dataset and 0.12 log (g/d) in the test dataset (Eq. (4); Table 3). Correcting the group-level estimate of log ALA intake using the predicted residuals led to a significant improvement in the prediction of log ALA intake at individual-cow level in the test dataset (Fig. 2).

## Discussion

*Main variables explaining the differences between the predicted and observed values for linoleic acid intake*

The residuals of LA intake were positively correlated with dietary total FA content and negatively correlated with milk fat content and sum of milk C4:0 to C14:0 FA, in accordance with Denis et al. (2022). These three variables are often associated with the milk fat depression syndrome that is observed when cows are fed diets high in rapidly-fermentable carbohydrates and low in effective fibre, supplemented or not with polyunsaturated FA (Bauman and Griinari, 2003). These diets cause the release of specific BH intermediates in the rumen, such as the trans-10, cis-12 conjugated linoleic acid that depresses milk fat content and yield, and in-milk de novo-synthesised and preformed FA (Baumgard et al., 2001; 2002). In addition, inter-individual variations in rumination

times (Andreen et al., 2020) and feeding patterns (e.g. sorting), such as preferential uptake of particles rich in rapidly fermentable carbohydrates at the beginning of the meal (Conte et al., 2018), could be associated with various buffering capacity and fermentation conditions in the rumen of individual cows. Finally, the coefficients of variation in milk LA yield (12%) and milk ALA yield (31%) in response to abomasal infusion of flaxseed oil in cows point to inter-individual variations in EFA metabolism (Moallem et al., 2012). The present study fits with these findings, as we found high inter-individual variability in the efficiency of diet-to-milk LA transfer. This could be explained by both extrinsic environmental factors (e.g. diet) and intrinsic animal factors (e.g. lactation stage, parity, genotype, and cow individuality). Although diet is the most influential lever for altering milk LA and ALA content, intrinsic individual variations could explain the variability in milk LA and ALA content related to their moderate heritabilities (Bobe et al., 2008; Garnsworthy et al., 2010; Pegolo et al., 2016). High inter-individual variability in the efficiency of diet-to-milk LA transfer could also be due to polymorphism in genes encoding mammary stearoyl-CoA desaturase and diacylglycerol O-acyltransferase 1 enzymes (Schennink et al., 2007; 2008), which modulate the incorporation of polyunsaturated FA such as LA and ALA in milk fat in order to ensure milk fluidity.

The residuals of LA intake were positively correlated with dietary CP content. A shortage of dietary nitrogen has been associated with a reduction in rumen concentrations of total bacteria and cellulolytic bacteria and microbial diversity in dairy cattle (Belanche et al., 2012). As most of the bacteria responsible for ruminal BH are cellulolytic bacteria (Buccioni et al., 2012), a reduced abundance of biohydrogenating bacteria, leading to lower-than-expected LA BH, could have led to an overestimation of LA intake.

Finally, the residuals of LA intake were positively correlated with feeding level. We would expect LA intake to be overestimated at high feeding levels. High feeding levels can increase digestive passage rate and thus decrease rumen retention and digestion time, and so consequently, we would expect a lower adherence of bacteria to feed-particle surfaces and a shorter time of exposure to microbial activity (especially lipolytic and biohydrogenating bacteria) (Buccioni et al., 2012).

#### *Main variables explaining the differences between the predicted and observed values for log alpha-linolenic acid intake*

The residuals of log ALA intake were negatively correlated with DMI, milk yield, milk fat yield, milk protein and lactose yield, in line with Denis et al. (2022). The prediction models developed here overestimate the log ALA intake of animals that have greater production potential (i.e. 36.5 kg/d for residuals < 1st quartile) but similar DMI (i.e. 23.9 kg/d for residuals < 1st quartile) and feeding levels (i.e. 0.034 kg of DM/d per kg BW for residuals < 1st quartile) to the mean observation in the dataset. Individual dairy cow genetic indexes serving as a proxy of milk production potential could be useful for improving ALA intake predictions. The positive correlation between the residuals of log ALA intake and DIM is consistent with the overestimation of log ALA intake for high milk yields, as milk yield decreases with the advancing lactation stage.

#### *Limitations of the study design*

The structure of the database carries certain limitations that warrant caution when applying the models. The number of individual data in the study was limited due to the need for complete observations (each variable needed to be filled in) to build the prediction models of the residuals. Consequently, some variables could not be modelled, such as dietary starch content and intake (only 292 complete data out of 503) or BW variations and energy balance which are very rarely reported in published studies. All the 26 experiments used Holstein-breed cows (except one that used both Holstein and Montbéliarde-breed cows; Ferlay et al., 2010). Consequently, our predictions may not be valid for other breeds, as is also the case for the group-level predictions of LA and ALA intake in Denis et al. (2022). Even though half of the experiments had a Latin square or crossover design and half had a randomised complete block design, almost all the 26 experiments used experimental periods that lasted between 21 and 35 days (except one that used an experimental period of 119 days; Guyader et al., 2016), and data were collected during three to seven days, working to the assumption that rumen ecosystem and lipid metabolism remain stable. The studies in the database mostly focused on the mid-lactation period (i.e. mean of 126 DIM), as it is the period for which milk FA data is the most abundant (i.e. steady-state of milk production and composition variables). However, we are aware that EFA and their derivatives are involved in important physiological functions for the onset of lactation, and so it is paramount for further experimental work to study this onset-of-lactation stage.

#### *Limitations of the experimental methods and data collection*

For most of the experiments, the milk and dietary FA profiles are based on one single analysis per cow per period from a pool of samples collected during three to seven days at the end of each experimental period (i.e. milk FA profile considered stable), which means the analysis may lack representativity. Moreover, for milk FA analysis, there were differences in pooling methods used for morning and evening milking samples (i.e. pooling according to morning and evening milk yields or using the constant 60/40 (vol/vol) ratio). In addition, there were differences in feeding strategy across experiments, as animals were fed diets during measurement periods at either ad libitum intake or at 95% ad libitum intake. Finally, analysis of milk and dietary FA uses various methods, protocols and apparatuses, which may create variability in the collected data. In fact, milk FA analysis is known to be very sensitive to methodology and laboratory effects (Ungerfeld et al., 2019), whereas dietary FA analysis is exposed to common analytical errors (Jenkins, 2010).

#### *Limitations of the correlational analysis*

We reported significant correlations between LA or ALA residuals and individual variables that could perhaps reflect mutual correlations among independent variables. For example, ALA residuals were negatively correlated with milk yield and positively correlated with DIM, whereas milk yield correlated negatively with DIM ( $r = -0.59$ ). Conversely, LA residuals were positively correlated with feeding level and negatively correlated with the sum of milk C4:0 to C14:0 FA, whereas we would expect both correlations to have the same sign, given that the advancing lactation stage is associated with an increase in DMI and milk concentrations of de novo-synthesised FA (Samková et al., 2012; Bainbridge et al., 2016). Consequently, this difference in correlation signs could be explained by individual-animal milk responses to milk fat depression diets. This result shows that the correlations between LA or ALA residuals and individual variables do not always reflect mutual correlations among independent variables. When designing the prediction models of the residuals, our strategy of using VIF as the criterion for selecting predictors averted redundant contributions of independent variables to the prediction of the residuals, and thus ensured that the coefficients obtained are robust and stable.

#### *From full predictions to field predictions: towards field-ready application*

The full predictions are based on a large number of variables, some of which may be difficult to access routinely in the field, whereas the field predictions use variables that are readily measurable on the animal and generally more field-amenable. The field predictions showed moderate loss of accuracy compared to full predictions, based on the RMSE of prediction (from 38 to 54 g/d for LA and from 0.090 to 0.12 log (g/d) for ALA). Milk yield and fat content can be measured daily in robotic milking systems or obtained monthly from milk recording organisations. Similarly, BW can easily be measured by robotic milking systems or by automated walk-through scales at exit from milking parlours. The DMI is harder to capture. However, several studies have shown that DMI can be predicted using data from cow accelerometers together with data on feeding patterns (Carpinelli et al., 2019; Ding et al., 2022), or modelled using single-point-in-time data on variables that are easily measurable on-farm (e.g. cow descriptors, milk yield and composition) (Brown et al., 2022). The determination of individual milk FA content for research purposes uses gas-chromatography and high-performance liquid chromatography as

reference methods, both of which are time- and effort-intensive. However, in the last decade, mid-IR spectroscopy has emerged as a compellingly quick, easy, and cost-effective alternative to chromatography methods. Mid-IR spectroscopy has long been used by milk recording organisations to determine milk fat, protein and lactose contents, and the technique has recently been extended to the determination of individual FA concentrations in milk. Research has shown that mid-IR spectroscopy can accurately predict milk de novo-synthesised FA concentrations, but performs less well for predicting polyunsaturated FA, including LA and ALA, and thus warrants further research (Soyeurt et al., 2006; 2011; Ferrand-Calmels et al., 2014).

#### *Linking group-level predictions of intake to individual-level predictions of residuals*

In terms of translating this research into practice, we can now aim to define target concentrations of LA and ALA in milk to support quality-based milk payments, and predict LA and ALA intakes to support vital functions for cows. We therefore need to determine the right dietary concentrations of LA and ALA to ensure milk quality and meet cow EFA requirements. First, we could use our previous group-level equations (Denis et al., 2022) to assess herd-level mean LA and ALA intake using herd mean BW, mean milk fat yield and target milk LA and ALA concentrations. We could thus constitute a herd-level ration delivering the target dietary LA and ALA contents. Second, we could screen each individual animal, using our previous group-level equations (Denis et al., 2022) and the field predictions of the residuals reported here, to determine whether the herd-level ration meets the individual animal's LA and ALA intake requirements. If not, we could increase the quantity of LA or ALA supplied to that animal in order to reach its required LA or ALA intake. This approach is in line with the search for more efficient use of resources with the least feed-food competition. The next step in this research work could be large-scale validation of these individual predictions of LA and ALA intake on various types of diets.

## Conclusion

This study assessed the validity at individual-cow level of equations for estimating LA and ALA intake from BW and milk LA and ALA secretions that were initially developed at cow-group level. The differences between observed EFA intake and predicted EFA intake based on group-level equations were predicted from (1) dietary and animal-related factors (i.e. full predictions) and (2) animal-related factors only (i.e. field predictions) which are considered more field-amenable. A simpler yet accurate correction of the initial equations is possible by using the field predictions, although some variables are harder to capture than others (e.g. milk concentrations of LA and ALA or DMI). This work, linking group-level predictions of intake to individual predictions of the residuals, is the first research to predict LA and ALA intake at an individual animal level.

## Supplementary material

Supplementary material to this article can be found online at <https://doi.org/10.1016/j.animal.2023.101005>.

## Ethics approval

Not applicable.

## Data and model availability statement

The data and models were not deposited in an official repository. The models that support the study findings can be found in [Supplementary Material S2](#). The data that support the study findings are available from the authors upon request.

## Declaration of Generative AI and AI-assisted technologies in the writing process

The authors did not use any artificial intelligence-assisted technologies in the writing process.

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## Declaration of interest

None.

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