



## Models to predict milk fat concentration and yield of lactating dairy cows: A meta-analysis

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### ABSTRACT

Few models have attempted to predict total milk fat because of its high variation among and within herds. The objective of this meta-analysis was to develop models to predict milk fat concentration and yield of lactating dairy cows. Data from 158 studies consisting of 658 treatments from 2,843 animals were used. Data from several feed databases were used to calculate dietary nutrients when dietary nutrient composition was not reported. Digested intake (DI, g/d) of each fatty acid (FA; C12:0, C14:0, C16:0, C16:1, C18:0, C18:1 *cis*, C18:1 *trans*, C18:2, C18:3) and absorbed amounts (g/d) of each AA (Arg, His, Ile, Leu, Lys, Met, Phe, Thr, Trp, Val) were calculated and used as candidate variables in the models. A multi-model inference method was used to fit a large set of mixed models with study as the random effect, and the best models were selected based on Akaike's information criterion corrected for sample size and evaluated further. Observed milk fat concentration (MFC) ranged from 2.26 to 4.78%, and milk fat yield (MFY) ranged from 0.488 to 1.787 kg/d among studies. Dietary levels of forage, starch, and total FA (dry matter basis) averaged  $50.8 \pm 10.3\%$  (mean  $\pm$  standard deviation),  $27.5 \pm 7.0\%$ , and  $3.4 \pm 1.3\%$ , respectively. The MFC was positively correlated with dietary forage (0.294) and negatively associated with dietary starch (−0.286). The DI of C18:2 (g/d) was more negatively correlated with MFC (−0.313) than that of the other FA. The best variables for predicting MFC were days in milk, FA-free dry matter intake, forage, starch, DI of C18:2, DI of C18:3, and absorbed Met, His, and Trp. The best predictor variables for MFY were FA-free dry matter intake, days in milk, absorbed Met and Ile, and intakes of digested C16:0 and C18:3. This model had a root mean square error of 14.1% and concordance correlation coefficient of 0.81. Surprisingly, DI of C18:3 was positively related to milk fat, and this relationship

was consistently observed among models. The models developed can be used as a practical tool for predicting milk fat of dairy cows, while recognizing that additional factors are likely to also affect fat yield.

**Key words:** milk fat, palmitic acid, methionine, meta-analysis, multi-model inference

### INTRODUCTION

Milk fat is a major source of energy and essential fatty acids, and bioactive lipids are related to the quality of dairy products (Michalski et al., 2002; Bauman and Griinari, 2003; Wiking et al., 2004) and the value of milk (Stoop et al., 2008). Milk fat production can vary widely among herds and animals, and understanding milk fat variation at the farm level is a challenge for many dairy scientists and nutritionists.

A large number of studies have been published on factors that affect milk composition, such as genetics (Andersson-Eklund and Rendel, 1993; Abdallah and McDaniel, 2000; Stoop et al., 2008), animal health (Boerhout et al., 2016; Albaaj et al., 2017), diet composition (Bauman and Griinari, 2003; Stoop et al., 2008; Caccamo et al., 2012), diet particle size (Caccamo et al., 2014; McCarthy et al., 2018), dietary fat source (Jensen, 2002), feed additives (Jensen, 2002; Kozerski et al., 2017), management (Kuhn et al., 2006), lactation stage, season, and time of day (Stoop et al., 2008).

Milk fat synthesis in ruminants is mediated by variation in substrate supply, de novo synthesis, and use of preformed fatty acids (FA). Short-chain milk FA (C4 to C14) are primarily synthesized de novo by mammary epithelial cells, C16 is both synthesized and derived from blood supplies, and C18 and longer are derived solely from blood (Palmquist, 2006). De novo synthesis of milk FA <C16 and a portion of C16 is influenced by dietary concentrations of C16 and modifiers of mammary gland FA synthesis (Bauman and Griinari, 2003). More recently, there is evidence that some AA could stimulate milk fat synthesis in the mammary gland of gilts via protein kinase (AKT), the signaling pathway that regulates cell growth and protein translation (mTOR),

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and the transcription factor that regulates the metabolism of fatty acids and triglycerides (SREBP1; Che et al., 2019). Thus, some AA that stimulate mTOR may also affect milk fat synthesis.

Milk fat has increased over the past decades due to the increased demand for milk fat for cheese and butter production. As a result, dairy farmers have used genetic and nutritional strategies to achieve higher milk fat (USDA, 2021). Most of the US milk payment system has also changed to component-based pricing, which rewards greater fat production. Consequently, many dairy nutritionists are using different feed technologies such as fat supplements with high concentrations of a specific FA to manipulate milk fat. Dorea and Armenitano (2017) indicated that the increase in milk fat with C16:0 supplementation was due solely to increased milk C16:0 output, with no changes in the total mass yield of C14 or shorter-chain FA. Nutrition plays a key role in milk fat synthesis; therefore, empirical modeling using published data would be useful to better understand milk fat biology and its manipulation.

Milk fat yield (MFY) is influenced by both milk production and milk fat concentration (MFC); because of its economic importance for dairy farmers, we hypothesized that intakes of specific nutrients, diet composition, and animal characteristics were the primary drivers of total MFY. The objective of this study was to develop models to predict the MFC and MFY of lactating dairy cows from dietary components and DIM.

## MATERIALS AND METHODS

### Data Collection

Institutional Animal Care and Use Committee or equivalent approval was not required because this study used existing data available in the literature, and no animal procedures were conducted.

Data used for the meta-analysis were collected from publications indexed in PubMed, Web of Knowledge, Scopus, and Google Scholar subject to the following inclusion criteria: (1) the work was conducted with dairy cattle; (2) complete descriptions of all treatments were provided; (3) milk fat data were reported; (4) milk production and DMI data were reported; (5) complete diet composition was reported or could be reconstructed from the information provided; (6) DIM data were reported; and (7) the number of animals represented in each treatment mean was reported. The final data search was completed in 2019 and the search terms included the following: milk fat, milk fat yield, milk fat

percentage, milk composition, dairy cows, dairy cattle, lactating cows, lactational performance, and combinations of those terms.

First, titles and abstracts were screened based on the inclusion criteria, and then full texts were evaluated. Search and application of the exclusion criteria resulted in selection of 158 studies (published in peer-reviewed journals) containing 658 treatment means representing results from 2,843 dairy cows which were used for development and evaluation of the milk fat prediction models. A list of the studies is provided in Supplemental File S1 (<https://vtechworks.lib.vt.edu/handle/10919/110841>). Data from selected studies were collated in a data set within an Excel workbook (Microsoft Corp.) and analyzed to verify biological coherence.

### Calculations

The NRC (2001) and Tran et al. (2020) feed databases were used for estimating dietary nutrients when the dietary ingredient composition was not available from studies. The NRC (2001) data were only used when data were not available from the Tran et al. (2020) library. The FA and AA composition for each ingredient were taken from the publications or estimated from the Cornell Net Carbohydrate and Protein System (Higgs et al., 2015) feed library. Dietary intakes of all nutrients available in the feed libraries were calculated, including C12:0, C14:0, C16:0, C16:1, C18:0, C18:1 *cis*, C18:1 *trans*, C18:2, and C18:3 intakes. When dietary nutrient concentrations or intakes were reported, they were compared with those calculated by summation of the ingredient nutrients, and deviations between reported and predicted were used to bias adjust the corresponding nutrient concentrations of all dietary ingredients used in the study proportional to their contribution as described previously (Li et al., 2018; Fleming et al., 2019b). Most studies in the overall data set reported BW (about 85%), but some did not, in which case, we estimated BW based on parity.

Previously derived coefficients for total-tract, apparent digestibility of total FA for each dietary fat source (Daley et al., 2020) were used to calculate digested intakes of total FA. For AA, the diet composition and DMI were used to calculate the intakes of each AA (Arg, His, Ile, Leu, Lys, Met, Phe, Thr, Val). Absorbed (Abs) amounts of each AA (g/d) were calculated as described by Fleming et al. (2019a,b). If not reported, MFY was calculated as milk fat percentage  $\div$  100  $\times$  milk yield. To reduce the correlation between DMI and FA intake, FA-free DMI was calculated as DMI (kg/d) – total FA intake (kg/d).

### Candidate Predictor Variables

Candidate variables included in the global model were DIM, BW, FA-free DMI, FA-free digested energy (DE) intake (Mcal/d), diet composition [forage (% of DM), NDF (% of DM), starch, (% of DM), RDP (% of DM)], digested intake (DI, g/d) of each fatty acid (C12:0, C14:0, C16:0, C16:1, C18:0, C18:1 *cis*, C18:1 *trans*, C18:2, C18:3), and Abs amounts (g/d) of each AA (Arg, His, Ile, Leu, Lys, Met, Phe, Thr, Trp, Val). If not reported, RDP was calculated using values of CP, insoluble protein degradation rates (Kd), and rates of passage (Kp) from NASEM (2021). Fatty acid-free DE intake was calculated as FA-free DMI (kg/d) × DE of the diet (Mcal/kg of DM). If DE was not reported, we adopted DE described by NASEM (2021). A fixed digestibility coefficient was used for each fat source to calculate DI from FA intake. The following apparent total-tract FA digestibility coefficients were used: 0.73 for low-FA feeds and oilseeds, 0.70 for oils, 0.63 for blended triglyceride, 0.68 for tallow triglyceride, 0.61 for SFA-enriched tallow triglycerides, 0.44 for extensively saturated triglycerides, 0.76 for Ca salts of palm fatty acid, 0.69 for SFA-enriched tallow fatty acid, 0.73 for palmitic acid ~85% of C16:0, 0.31 for palmitic or stearic acid >90% (Daley et al., 2020). Most studies did not describe the use of monensin; consequently, we did not evaluate the effect of monensin on milk fat.

### Statistical Analysis

All analyses were conducted using R (version 3.5.1; <https://www.r-project.org/>). Descriptive statistics, preliminary plots, and correlations analyses were performed using the packages psych, ggplot2, cowplot, and ggcorrplot, respectively. Values more than 3 standard deviations (SD) from the mean or with studentized residuals >3.5 (absolute value) were removed as putative outliers. All equations were fit to the observed data using the lmer function of the lme4 package of R (Bates et al., 2015) with the study effect (PubID) specified as a random effect on the overall mean (intercept; St-Pierre, 2001). The linear and quadratic terms and interactions among variables were tested. Observed data were weighted by the square root of the number of observations in each treatment.

The global mixed model for MFC and MFY was

$$Y = \mathbf{X}\beta + \mathbf{Z}\gamma + \epsilon,$$

where  $Y$  is the dependent variable [MFC (%) or MFY (g/d)], and  $\mathbf{X}$  is the design matrix for fixed effects;  $\mathbf{X}$  has dimension  $n \times p$ , where  $n$  is the number of observations in the data set and  $p$  is the number of fixed-effect

parameters in the model;  $\beta$  is the vector of regression coefficients for fixed effects;  $\mathbf{Z}$  is the design matrix for the random effects with the vector of random effects  $\gamma$ ; and  $\epsilon$  is the error vector.

The multi-model inference was performed with the MuMIn package (Barton, 2011). The objective of multi-model inference is to examine all possible models and identify the best set of models based on information criteria, such as Akaike's information criterion (AIC; Burnham and Anderson, 2002; Grueber et al., 2011). This approach is used as an alternative to the traditional null hypothesis test; therefore,  $P$ -values were not used for variable selection. In the first phase of this approach, the global mixed model was fitted with candidate variables and subsets of models with different combinations of fixed effect terms were developed. Only submodels with independent variables that had variance inflation factor (VIF) values <10 were selected to reduce problems with multicollinearity among predictor variables. Moreover, we used FA-free DMI (DMI – total FA intake) as a potential predictor for both MFC and MFY instead of DMI. Subtraction of total FA intake from DMI removes the mathematical collinearity when FA is included in both terms. This helps ensure that FA and DMI parameters are uniquely defined and not correlated.

In the second phase, models that were complex versions of simpler models [more variables but similar AIC corrected for small sample size (AIC<sub>C</sub>; Hurvich and Tsai, 1989)] or that were biologically implausible were removed from the candidate model data set. The best models were selected from the remainder based on their AIC<sub>C</sub> values. These were screened to determine which had a greater probability of being inferior to the global model using the ANOVA procedure.  $P < 0.05$  was considered significant.

The reduced set of candidate models were evaluated for root mean square error (RMSE), mean bias, slope bias, concordance correlation coefficient (CCC; Lin, 1989), and coefficient of determination (R<sup>2</sup>). A repeated cross-evaluation was also performed (repeated 500 times, 85% training data and 15% test data) to estimate model reliability and stability based on the variance in the parameter estimates, RMSE, and CCC when subsets of the data were excluded from the training set.

## RESULTS

### Database

Nutrient intakes, diet composition, and animal performance data are presented in Table 1. Mean (± SD) DMI and milk yields were 21.3 ± 3.4 and 32.4 ± 6.9

kg/d, respectively. Mean diet composition was  $50.8 \pm 10.3\%$  forage,  $27.5 \pm 7.0\%$  starch, and  $3.4 \pm 1.3\%$  FA, and DIM ranged from 36 to 344 d. The correlations among MFC and MFY and the independent variables are shown in Figure 1.

As expected, MFC was positively correlated with dietary forage ( $r = 0.294$ ,  $P < 0.01$ ) and negatively

correlated with dietary starch ( $r = -0.286$ ,  $P < 0.01$ ; Figure 1A). Also, MFC was positively correlated only with DI of C12:0 and C18:3. The DI of C18:2 had a higher negative correlation with MFC ( $-0.313$ ,  $P < 0.01$ ) than did that of other FA (Figure 1B). Overall, the association of MFC with absorbed AA was weak ( $r < 0.21$ ; Figure 1C).

**Table 1.** Summary of the database used for estimating the milk fat model (n = 158 studies)

Variable	n	Mean	SD	Minimum	Maximum
<b>Intake</b>					
DMI, kg/d	658	21.4	3.4	9.1	31.8
Fatty acid (FA)-free DMI, kg/d	658	20.6	3.2	8.7	30.7
Digested energy (DE) intake, Mcal/d	658	66.5	11.1	28.6	100.5
FA-free DE intake, Mcal/d	658	61.5	10.1	26.1	93.2
FA intake, g/d	658	731	310	218	1,635
<b>Intakes of FA, g/d</b>					
C12:0	658	12	9	1	45
C14:0	658	9	7	1	38
C16:0	658	146	95	40	635
C16:1	658	7	8	1	43
C18:0	658	36	48	4	604
C18:1 <i>cis</i>	658	162	101	15	507
C18:1 <i>trans</i>	658	5	7	0	43
C18:2	658	268	111	69	727
C18:3	658	67	28	17	167
Other FA	658	24	17	7	123
C18:1 <i>cis</i> + C18:2 + C18:3	658	497	188	153	1,144
<b>Intakes of digested (DI) FA, g/d</b>					
DI of C12:0	658	9	6	0.5	33
DI of C14:0	658	6	5	1	26
DI of C16:0	658	106	69	29	482
DI of C16:1	658	5	5	1	30
DI of C18:0	658	24	26	3	196
DI of C18:1 <i>cis</i>	658	117	72	11	351
DI of C18:1 <i>trans</i>	658	3	5	0.01	29
DI of C18:2	658	195	81	51	532
DI of C18:3	658	49	20	12	122
DI of other FA	658	17	12	5	87
<b>Individual intakes of absorbed (Abs) AA, g/d</b>					
Abs Arg	658	113	24	37	181
Abs His	658	51	11	17	103
Abs Ile	658	124	23	46	191
Abs Leu	658	193	43	75	395
Abs Lys	658	162	29	57	248
Abs Met	658	49	10	19	83
Abs Phe	658	123	24	44	203
Abs Thr	658	112	20	42	176
Abs Trp	658	28	5	9	43
Abs Val	658	133	25	49	225
<b>Diet composition</b>					
Crude fat, % of DM	658	4.2	1.3	2.25	7.78
FA, % of DM	658	3.4	1.3	1.50	7.83
Forage, % of DM	658	50.8	10.3	9.61	86.23
Starch, % of DM	658	27.5	7.0	9.86	43.82
CP, % of DM	658	16.7	2.4	7.71	24.38
RDP, % of DM	658	11.5	1.7	5.10	17.17
<b>Animal performance</b>					
Milk, kg/d	658	32.4	6.9	13.0	53.8
Milk fat, %	658	3.56	0.42	2.26	4.78
Milk fat, g/d	658	1,143	236	488	1,787
Milk protein, %	654	3.09	0.21	2.57	3.90
Milk lactose, %	348	4.78	0.16	4.13	5.34
BW, kg	658	604	47	476	768
DIM, d	658	136	56	36	344



Milk fat yield had a strong positive relationship with FA-free DMI ( $r = 0.70$ ) and a weak positive relationship with BW ( $r = 0.32$ ); MFY and DIM were negatively associated ( $r = -0.14$ ). Overall, the correlation coefficients between MFY and DI of FA were weak ( $r < 0.36$ ), but correlations for individual Abs AA and MFY were moderate and ranged from 0.50 to 0.60 (Figure 1F).

The correlations between concentrations of dietary forage and C18:3 or DI of C18:3 were weak ( $r < 0.19$ ). The DI of C16:1 had a very strong positive relationship with DI of C18:0 (0.83) and C18:1 *trans* (0.70). In addition, DI of C18:0 showed a strong positive relationship with DI of C18:1 *cis* plus *trans* ( $r = 0.79$ ). The DI of C18:2 had a moderate positive correlation with DI of C18:1 *cis* ( $r = 0.52$ ). In the present work, dietary starch interactions with DI of FA were tested and removed from the model if found to be nonsignificant ( $P > 0.05$ ).

For initial data exploration, regression plots of adjusted Y values from the mixed model analysis (St-Pierre, 2001) were created to visually assess the dependent variable versus independent variables (Figures 2 and 3). Days in milk, forage, and DI of C12:0 and C18:3 had positive effects on MFC ( $P < 0.05$ ). However, DI of C18:2 and C14:0, starch, FA-free DE intake, and FA-free DMI reduced MFC ( $P < 0.05$ ). For MFY, only starch was not significant ( $P = 0.18$ ).

### Global Models

The global models included all potential candidate variables based on our hypothesis. These included individual intakes of digested FA (C12:0, C14:0, C16:0, C16:1, C18:0, C18:1 *cis*, C18:1 *trans*, C18:2, C18:3, other FA, g/d); absorbed AA (Arg, His, Ile, Leu, Lys, Met, Phe, Thr, Trp, Val; g/d); FA-free DMI (kg/d); dietary content (% of DM) of starch, forage, and RDP; BW (kg); and DIM (d). The combination of those variables yielded  $2^{26} = 67,108,864$  candidate models to evaluate. Handling such a large matrix was found to be impossible in R. Therefore, the global models (containing all potential predictors) for both MFC and MFY were reduced based on backward selection (threshold  $P < 0.10$ ) to 11 and 14 candidate variables, respectively, resulting in 1,280 and 10,240 possible models to be fitted for MFC and MFY, respectively.

It is important to consider that only candidate variables that were significant ( $P < 0.10$ ) in the backward selection were discussed and used in the global models. Although they were not well explored, we evaluated forage and NDF as candidate variables for backward selection; forage had the lowest significance, and thus it was selected for use in the global model. We recognize that forage NDF and nonforage NDF may be better

predictors for de novo FA than forage or NDF; therefore, they should be evaluated in further studies due to their relevance to ration formulation and MFC.

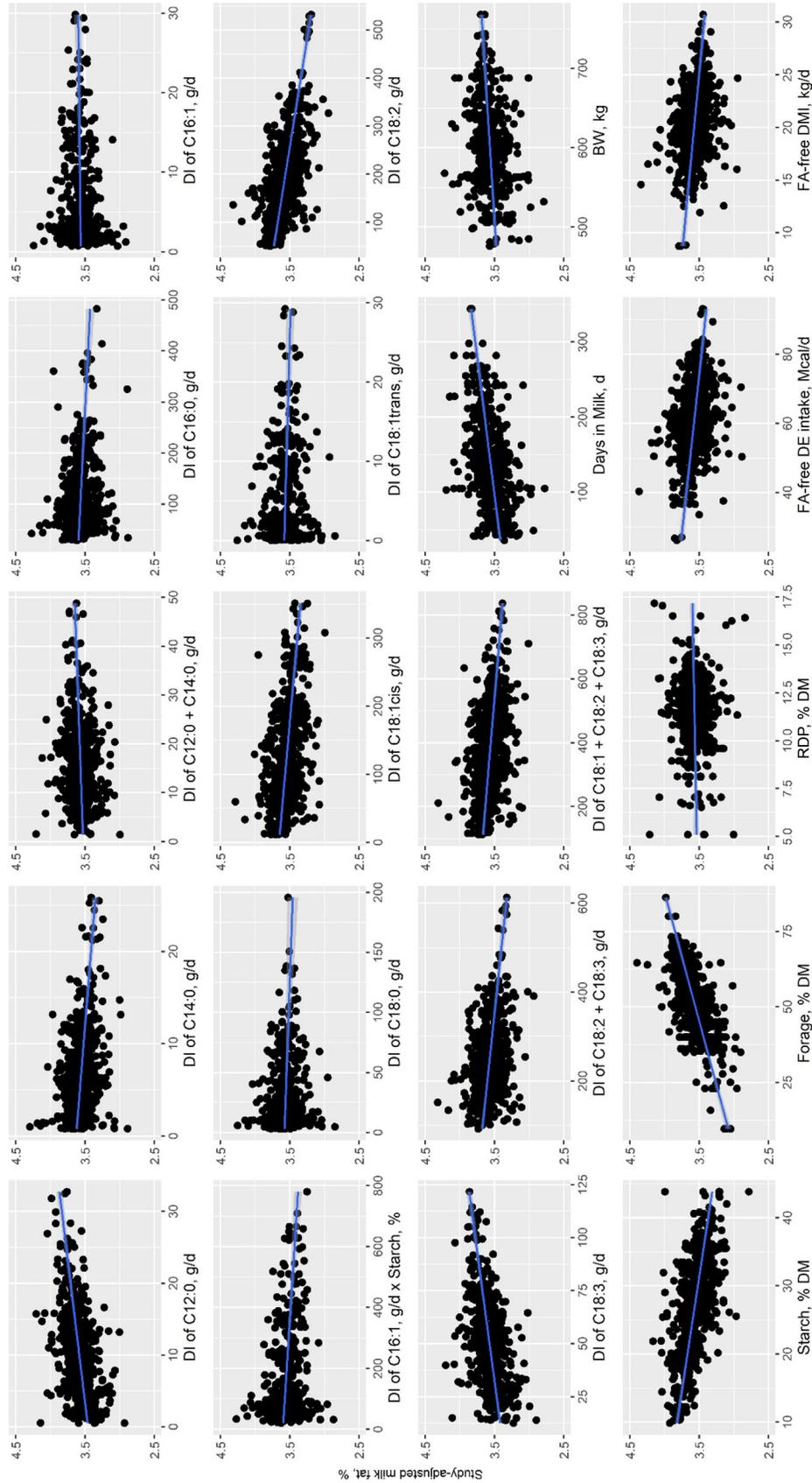
For MFC, interactions between starch and C18:1 *cis* or *trans*, C18:2, and C18:3 were not significant ( $P > 0.05$ ) and therefore removed from the global model. The global model did not contain DI of C16:0 ( $P = 0.52$ ), nor did it include BW as an independent variable ( $P = 0.75$ ); therefore, models containing BW were not selected. We detected a negative interaction between DI of C16:1 and dietary starch concentration on MFC in the global model ( $P = 0.04$ ). In addition to digested FA intakes (C18:2 and C18:3), DIM, FA-free DMI, forage concentration, and Abs His, Met, and Trp were or tended to be significant ( $P < 0.10$ , Supplemental Table S1; <https://vtechworks.lib.vt.edu/handle/10919/110841>).

As in the MFC model, interactions between starch and C18:1 *cis* or *trans*, C18:2, and C18:3 were not observed in the global MFY model ( $P > 0.05$ ). When the global model for MFY was fitted, containing all candidate predictors, DIM, FA-free DMI, DI of C16:0, and Abs Met were significant ( $P < 0.05$ ; Supplemental Table S1). Models containing DI of C12:0 and C14:0 were not selected in the backward selection ( $P > 0.80$ ); therefore, models containing these variables were not included in the global model.

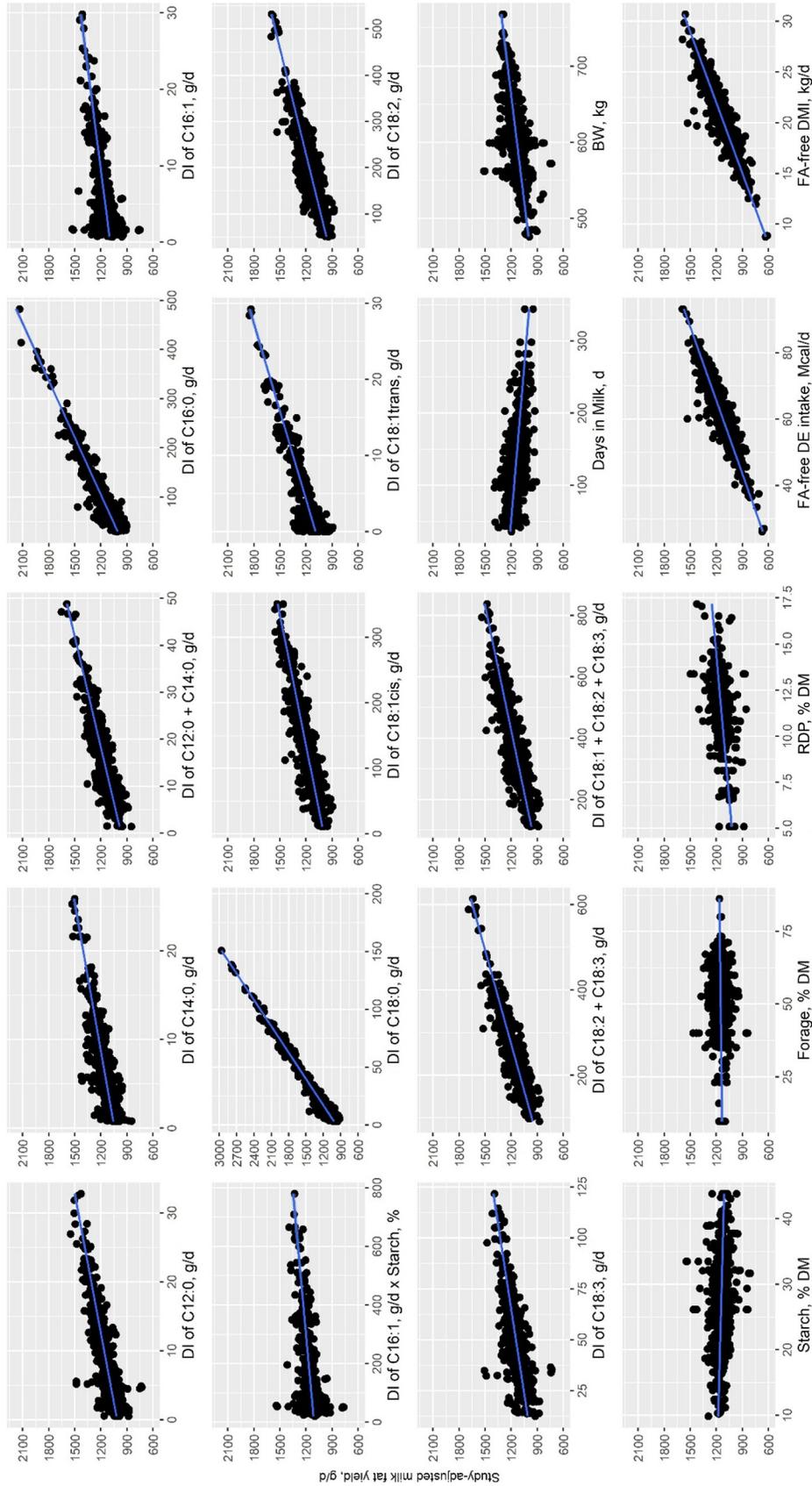
### Models for MFC

A summary of the solutions derived from the global MFC model is presented in Supplemental Table S2 (<https://vtechworks.lib.vt.edu/handle/10919/110841>). A negative term for DI of C18:2 was observed among all candidate models. The slope values for DI of C16:1 *cis* varied from negative to positive values, but for DI of C18:3, the slope was consistently positive across all candidate models.

The stability of parameter estimates across models is useful for identifying parameters that are not well supported by the data or that have collinearity problems. Smaller parameter CV estimates among runs indicate those parameters that are well defined by the data and are not sensitive to the presence or absence of other variables in the model. The importance of terms is also indicated by the number of top model solutions that contain them. For example, the starch term was in 82% of the solutions, whereas the interaction of DI of 16:1 and starch was in only 28% of the solutions. Thus, regardless of what other terms were in the model, starch was present. The intercept and forage terms had coefficients of variation (CV) of <15%, indicating they were relatively stable estimates. The DI of C16:1 and Abs His had very large CV (>70%), and thus those



**Figure 2.** Relationship between study-adjusted milk fat concentration (%) and linear terms of candidate predictor variables. Each data point represents an individual treatment mean included in the meta-analysis. DI = digested intake; DE = digested energy; FA = fatty acid.



**Figure 3.** Relationship between study-adjusted milk fat yield and linear terms of candidate variables. Each data point represents an individual treatment mean included in the meta-analysis. DI = digested intake; DE = digested energy; FA = fatty acid.

**Table 2.** Parameter estimates, SE, and evaluations for the final milk fat models

Predictor	Model 1: Milk fat, %			Model 2: Milk fat, g/d		
	Estimate	SE	<i>P</i> -value	Estimate	SE	<i>P</i> -value
Intercept	3.58	0.19	<0.01	452.9	57.1	<0.001
DIM, d	5.91e-04	3.28e-04	0.07	-1.42	0.13	<0.001
Fatty acid-free DMI, kg/d	-3.30e-02	8.17e-03	<0.01	24.52	3.04	<0.001
Forage, % of DM	5.22e-03	1.66e-03	<0.01			
Starch, % of DM	-5.99e-03	2.77e-03	0.03			
DI <sup>1</sup> of C16:0, g/d				0.407	0.078	<0.001
DI of C18:2, g/d	-4.25e-04	2.42e-04	0.08			
DI of C18:3, g/d	3.19e-03	1.05e-03	<0.01	1.804	0.359	<0.001
Abs <sup>2</sup> His, g/d	-6.81e-03	2.38e-03	<0.01			
Abs Ile, g/d				1.458	0.360	<0.001
Abs Met, g/d	4.26e-03	1.38e-03	<0.01	1.341	0.532	0.012
Abs Trp, g/d	1.96e-02	5.82e-03	<0.01			
Evaluation on data <sup>3</sup>						
AIC <sub>C</sub>		14			7,870	
Observed mean		3.56			1,143.2	
Predicted mean		3.56			1,143.7	
RMSE, <sup>4</sup> % or g/d		0.381			161.1	
RMSE, <sup>4</sup> % of mean		4.2			4.8	
RMSE, <sup>4</sup> % of mean		10.7			14.1	
Cb		0.993			0.999	
r		0.935			0.973	
CCC		0.928			0.972	
CCC <sup>4</sup>		0.282			0.806	
Mean bias, % of MSE		0			0	
Slope bias, % of MSE		1.6			0.6	
Residual error		98.4			99.4	

<sup>1</sup>DI = digested intake.

<sup>2</sup>Abs = absorbed.

<sup>3</sup>AIC<sub>C</sub> = Akaike's information criterion (Akaike, 1973) corrected for small sample size (Hurvich and Tsai, 1989); RMSE = root mean square error; Cb = bias correction factor (accuracy); r = correlation coefficient (precision); CCC = concordance correlation coefficient (Lin, 1989); MSE = mean squared error.

<sup>4</sup>Unadjusted value.

estimates are likely to be very poorly defined. Absorbed Met, DI of C18:2, and DI of C18:3 had moderate CV (<33%), suggesting that estimates of those variables may be defined with moderate precision. Dietary concentrations of forage and starch, FA-free DMI, and DIM were consistently used as predictor variables across the set of candidate models.

The best models were selected from those solution matrices based on a combination of biological coherence and AIC<sub>C</sub>. As previously stated, *P*-values were not used for selection of independent variables. However, because the AIC<sub>C</sub> calculation has a penalty for the number of parameters in the model, the selection process inherently discriminates against variables that do not explain significant variance.

Parameter estimates and model performance evaluations for the best MFC model are presented in Table 2. The selected model was not significantly different (*P* > 0.05) from the global model (Supplemental Table S1). There was negligible mean and slope bias, the unadjusted RMSE was 10.7% of the mean observed value, and the CCC was 0.93. The independent variables

remaining in the final model consistently had low CV results. Days in milk, FA-free DMI, forage, starch, and DI of C18:2 and C18:3, as well as Abs His, Met, and Trp remained in the model.

The cross-evaluation results for the best MFC model are presented in Table 3, and plots of observed, predicted, and residual values are presented in Figure 4. The CV for the parameter estimates were all moderate, ranging from 34 to 61%. All terms were significant except for DI of C18:2, which had a mean (SD) *P*-value of 0.11 (0.08). This term also had the greatest CV, 61%, across models, indicating that the precision of the parameter estimate was not high; however, as pointed out previously, the presence of the term in the model resulted in improved AIC<sub>C</sub>, and thus it is justified given the full data set.

### Models for Predicting MFY

A summary of the MFY solutions is presented in Supplemental Table S3 (<https://vtechworks.lib.vt.edu/handle/10919/110841>). Forage, RDP, starch, and DI of

**Table 3.** Results for repeated cross-evaluation (n = 500) for models developed for estimating milk fat (%) in lactating dairy cows

Predictor	Model 1: Milk fat, %							
	Estimate		SE		CV		P-value	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Intercept	3.45	0.09	0.20	0.003	6	0	<0.001	<0.01
DIM, d	7.56e-04	1.21e-04	3.57e-04	1.01e-05	48	8	0.04	0.03
Fatty acid-free DMI, kg/d	-2.62e-02	3.89e-03	8.80e-03	1.82e-04	34	5	0.01	0.01
Forage, % of DM	5.16e-03	8.97e-04	1.77e-03	4.29e-05	35	6	0.01	0.01
Starch, % of DM	-5.83e-03	1.11e-03	2.90e-03	9.08e-05	52	11	0.06	0.05
DI <sup>1</sup> of C18:2, g/d	-4.44e-04	9.60e-05	2.58e-04	1.20e-05	61	16	0.11	0.08
DI of C18:3, g/d	2.86e-03	5.65e-04	1.15e-03	3.89e-05	42	9	0.02	0.03
Abs <sup>2</sup> His, g/d	-6.21e-03	1.35e-03	2.57e-03	1.87e-04	46	24	0.04	0.09
Abs Met, g/d	3.91e-03	5.88e-04	1.53e-03	5.32e-05	40	6	0.02	0.02
Abs Trp, g/d	1.86e-02	3.67e-03	6.29e-03	4.63e-04	36	11	0.02	0.04
Evaluation								
Average RMSE <sup>3</sup>	0.380 ± 0.027							

<sup>1</sup>DI = digested intake.

<sup>2</sup>Absorbed.

<sup>3</sup>Root mean square error.

C18:0 had CV >100%, indicating very low precision of the parameter estimates. However, DIM, FA-free DMI, and DI of C16:0 had the highest precision (CV <14%), with parameters varying between -1.82 and -1.37, 22.33 and 40.63, and 0.24 and 0.46, respectively (Supplemental Table S3).

A consistent positive effect of DI of C16:0 on MFY was observed across all candidate models, resulting in a low CV (13%). The slopes for DI of C18:0, C18:1 *cis*, and C18:2 ranged between -0.23 and 0.63, -0.21 and 0.45, and -0.05 and 0.49, respectively, resulting in large CV (>70%). This variation was reflected in greater AIC<sub>C</sub> values; thus, models containing those terms were not selected. Rumen-degradable protein was a driver of MFY in the second-best model (AIC<sub>C</sub> = 7,870), with a slope value of 3.89 ± 4.07 (SE), but it had a large CV in both sets of candidate models and cross-evaluation (>100%). Absorbed Lys was included in the global model, and parameters varied between 0.42 and 2.23, but its CV was greater than that of Abs Met (49 vs. 29%; Supplemental Table S3).

The best final MFY model retained DIM, FA-free DMI, DI of C16:0 and C18:3, and Abs Met and Ile as predictors and had the smallest CV parameter (average of 20%) with low AIC<sub>C</sub> values (~7,870) and the best fit compared with the best candidate models. The unadjusted RMSE was 14.1% compared with 10.7% for the MFC model. However, the latter would have to be paired with a milk volume prediction to allow prediction of MFY, which is the output required for a milk value assessment. Thus, although the MFC model appears to be more precise, it is not as useful as the MFY prediction. The MFY prediction had negligible slope and mean bias and an unadjusted CCC of 0.81.

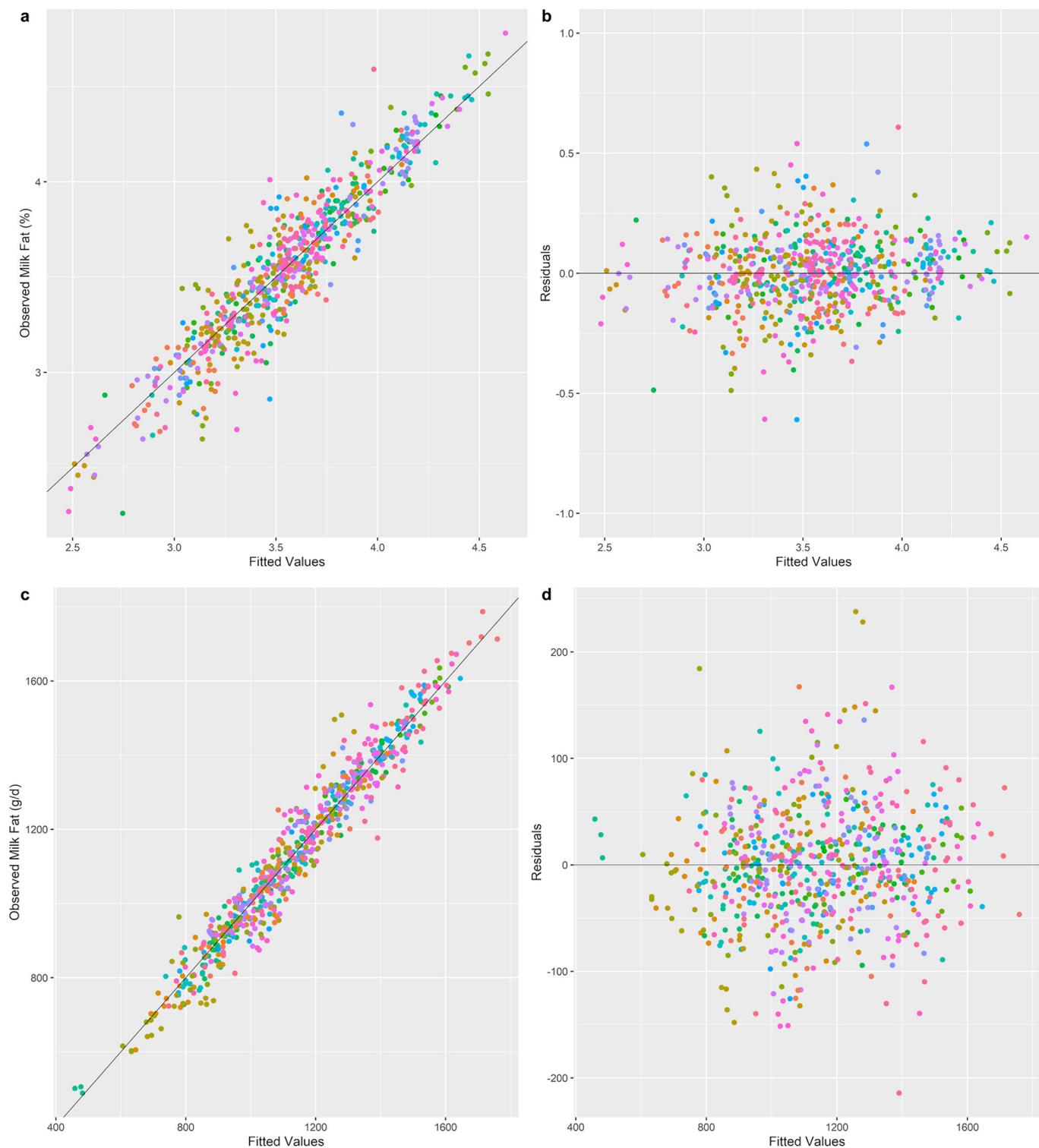
Cross-evaluations showed that all terms except Abs Met were well defined by the data (Table 4). Absorbed Met had a moderate CV of 63%, resulting in a mean (SD) *P*-value of 0.12 (0.10), but all models containing this term (n = 2,774) had positive slopes ranging from 1.1 to 4.03 (Supplemental Table S3). Plots of observed and predicted values and residuals versus predicted values are presented in Figures 4 and 5.

The cows were fed mostly with group of feeds classified as energy source, plant protein, and forages. Among forages, legumes were fed more than grasses, and silages were more frequent than hay (Figure 6A and B).

The total FA intake (g/d) or total FA concentration of the diets (% of DM) positively affected both milk production and MFY (*P* < 0.05) but not MFC (*P* > 0.05) (Supplemental Tables S4 and S5; <https://vtechworks.lib.vt.edu/handle/10919/110841>). Although DI of C16:0 had a positive slope for MFC, it only significantly increased milk yield and MFY (*P* < 0.05; Supplemental Table S6; <https://vtechworks.lib.vt.edu/handle/10919/110841>). Milk production, MFY, and MFC have increased over the past several decades, with increased rates from 2015 (Figure 7), indicating that nutrition, management, and genetics are improving the milk fat of lactating cows. In Table 5, it is possible to identify that grass or legume forage and grain crop forage were the major source of C18:3 in dairy cow diets.

## DISCUSSION

The main goal of this work was to develop models to estimate MFC and MFY mostly from diet intake and



**Figure 4.** Observed versus predicted (panels a and c) and residuals versus predicted (panels b and d) values for milk fat concentration (panels a and b) and milk fat yield (panels c and d). Each point represents a treatment mean ( $n = 658$ ), and studies are represented by color.

composition. Milk fat yield is an economically important component for the milk payment system, and it is expected that the value of milk fat will continue to

increase. Diet composition has been shown to change MFC and composition in dairy cows, and manipulation of the amount and composition of milk fat has been

**Table 4.** Results for repeated cross-evaluation (n = 500) of models developed for estimating milk fat yield (g/d) in lactating dairy cows

Predictor	Model 2: Milk fat yield, g/d							
	Estimate		SE		CV		P-value	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Intercept	382.6	25.6	60.7	0.9	16	1	<0.001	<0.001
DIM, d	-1.31	0.09	0.14	0.00	11	1	<0.001	<0.001
Fatty acid-free DMI, kg/d	27.59	1.36	3.23	0.06	12	1	<0.001	<0.001
DI <sup>1</sup> of C16:0, g/d	0.39	0.03	0.08	0.00	21	2	<0.001	<0.001
DI of C18:3, g/d	1.75	0.16	0.39	0.01	22	2	<0.001	<0.001
Abs <sup>2</sup> Met, g/d	1.01	0.28	0.58	0.02	63	21	0.116	0.102
Abs Ile, g/d	1.55	0.20	0.40	0.01	26	3	0.001	0.001
Evaluation Average RMSE <sup>3</sup>	165 ± 11.7							

<sup>1</sup>DI = digested intake.

<sup>2</sup>Absorbed.

<sup>3</sup>Root mean square error.

an important strategy for the improvement of dairy products for human nutrition. Therefore, identification of the major factors driving milk fat production and the ability to predict those effects will allow dairy producers and their nutritionists to manipulate both to achieve greater economic returns by producing milk with the desired composition.

Regression equations are used to predict the response for a given predictor. In our framework, we evaluated the VIF in all candidate models; VIF is a measure of the amount of multicollinearity (correlation between predictor variables) in a set of multiple regression variables, and our models had VIF <10. Therefore, we did not expect to have correlation among independent variables in our final models when a set of different feed ingredients is used. We also evaluated the stability of parameter estimates across candidate models. Where collinearity is a problem, the presence or absence of collinear variables in the model will affect estimates of the variables it is correlated with. When present, the parameter estimates for the collinear terms will generally vary considerably across models, which is an indication of a collinearity problem.

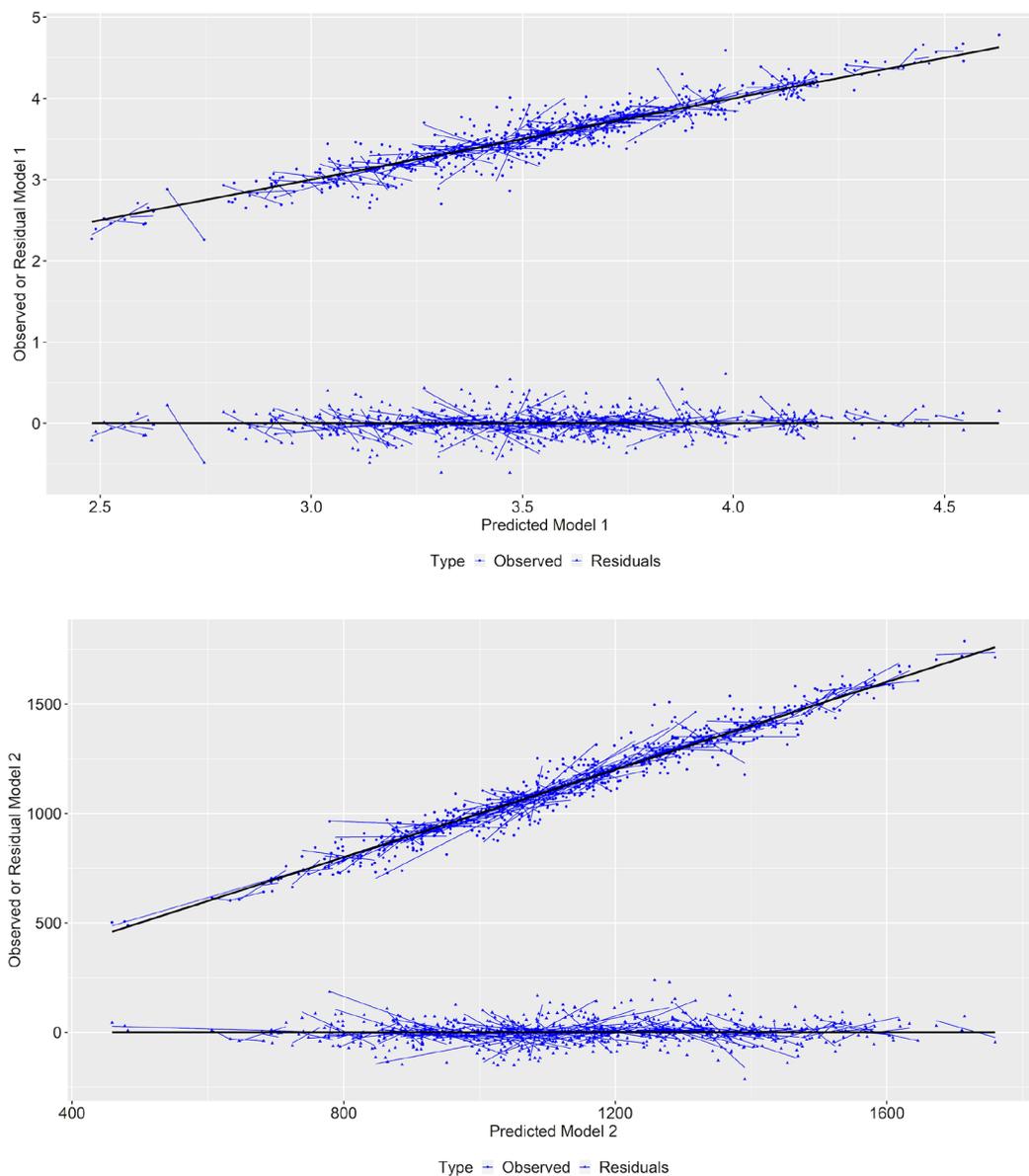
It is important to consider that the effects of some variables on milk fat are unclear, but they might be due to direct causation (e.g., a positive effect of C18:3 on milk fat yield) or indirect causation (e.g., C18:3 could indicate a high-alfalfa diet with an unknown variable not evaluated in this study, such as water-soluble carbohydrates or residual OM). Although the use of feed type in models is desired to understand the effect of feed ingredients on milk fat, we would need to develop one model for each feed, considering its level in the diet (low, moderate, high), which would be impractical. Therefore, we suggest that future studies include feed categories grouped by the ruminal availability of

FA (protected, unprotected, partially protected) and their fatty acid profile as independent variables in the model. Moreover, we need more experimental studies evaluating different levels of feed ingredients or fat supplements within the same study.

We did not evaluate other important factors that may affect milk fat yield or concentration, such as animal genetics, environmental factors, and cows' milk production level (low- vs. high-producing cows). Lower- and higher-producing cows may respond differently to fat supplements (Rico et al., 2014a; Piantoni et al., 2015; Western et al., 2020), but this was not included in the models. Our work is an initial investigation that used a large database for a better understanding of milk fat biology in dairy cows, which is a complex system influenced by many variables and their interactions.

### Predicting MFC

Our work showed that the best predictors for MFC were DIM, FA-free DMI, dietary forage and starch concentrations, DI of C18:2 and C18:3, and Abs Met, Trp, and His. On average, the cows used in the selected studies were in mid lactation (about 136 DIM), producing milk containing nearly  $3.6 \pm 0.42\%$  (SD) milk fat. This value is representative of MFC of Holstein dairy cows, where MFC is generally high at the start of lactation, decreasing until 60 DIM, and increasing from lactation peak until the end of lactation (Stanton et al., 1992). The dilution effect of milk yield on MFC explains the different predictors for the MFC and MFY models (Hanigan et al., 2008). Milk yield is an additional determinant of MFC, and it is driven by other substrates such as glucose (Xiao and Cant, 2005) and  $\alpha$ -lactalbumin (Ebner and Schanbacher, 1974; Hanigan and Baldwin, 1994). Thus, the MFC equation inher-



**Figure 5.** Study-adjusted values for observed or residual versus predicted values for milk fat concentration (model 1) and milk fat yield (model 2). Each point represents a treatment mean ( $n = 658$ ), and regression for each study is shown in blue.

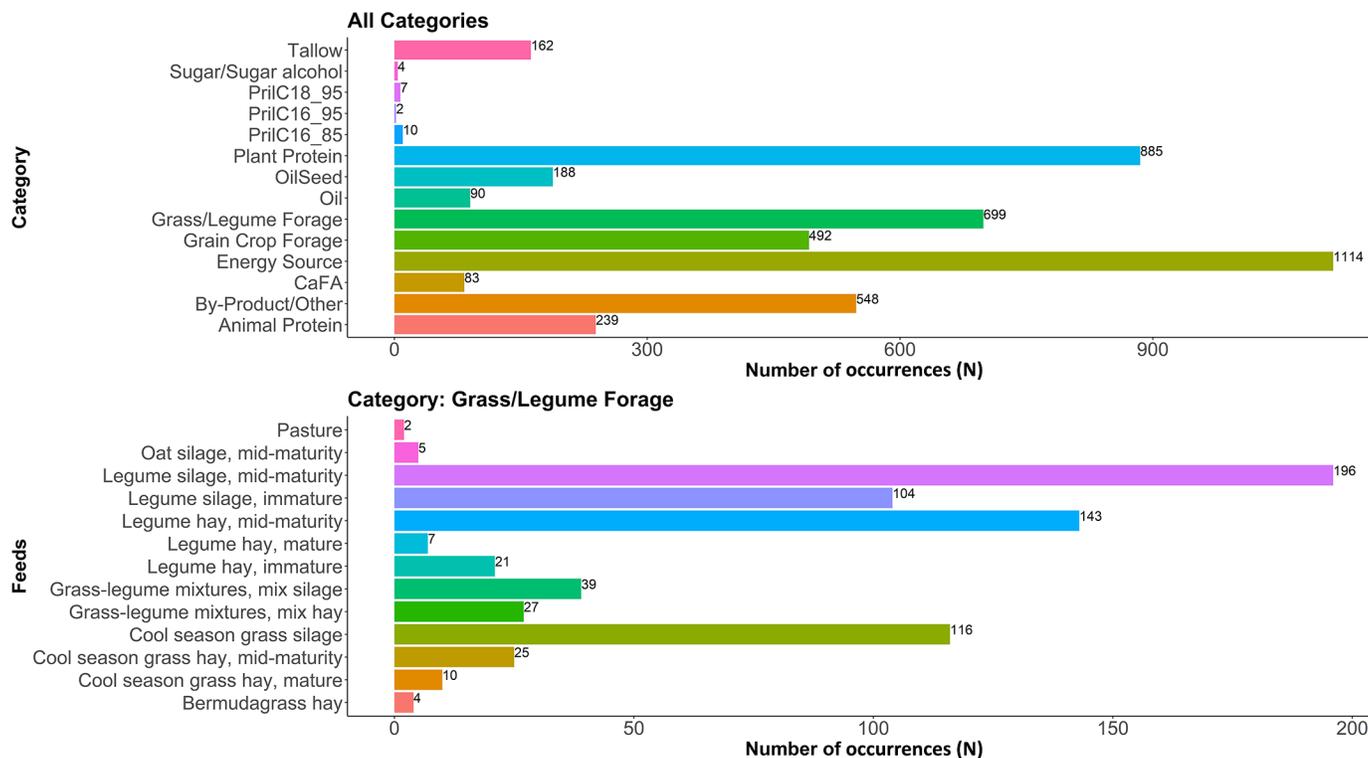
ently considers influencers of milk volume, whereas the MFY equation does not. This result is consistent with observations in Maxin et al. (2011) of different responses to dietary nutrients among MFC and MFY.

Days in milk is a non-nutritional factor that consistently affected MFC in dairy cows. The coefficient for DIM and the correlation between DIM and MFC as well as the slopes of all candidate models were positive. This represents an increase in MFC as lactation progresses, which is associated with a reduction in milk yield after peak lactation. As the average DIM in the observed data was 136, the data almost exclusively represented

post-peak performance, which is consistent with the linear increase in MFC. This relationship may explain the negative genetic correlation between milk yield and MFC ( $-0.20$  from Tabler and Touchberry, 1959).

Animal BW was not a significant predictor of MFC in dairy cows, but changes in BCS could indicate the cow's fat mobilization status, which may increase the milk fat from body tissues. This variable should be considered a potential predictor of MFC in further research, mainly for early-lactation cows.

Recent work suggested that milk fat and protein prices in the local market should be considered when



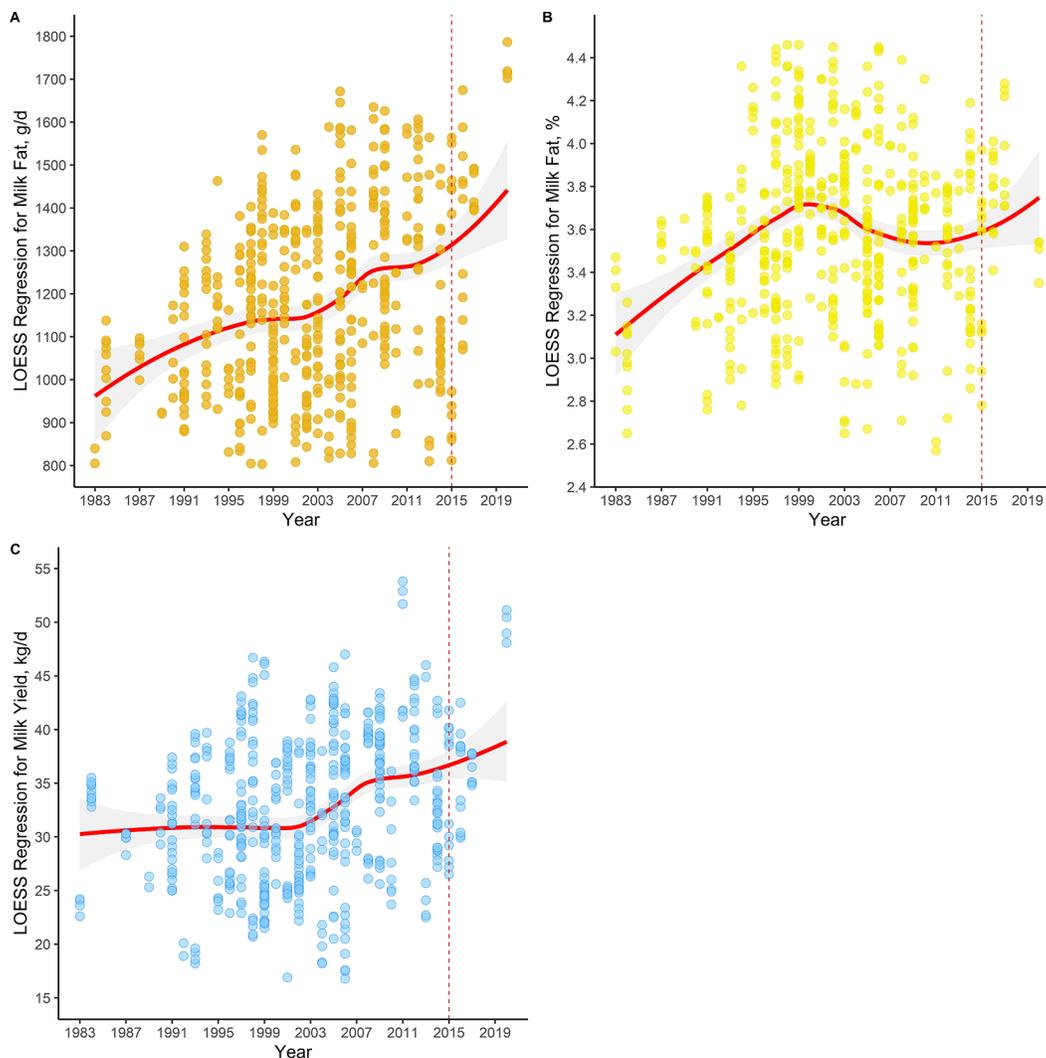
**Figure 6.** Category of feeds used in the experimental diets for dairy cows. (A) Each bar represents a feed category and its frequency in the database; (B) types of feeds in the category “grass/legume forage.”

evaluating a breed for a given farm system (Edwards et al., 2019). Large genetic variation in milk fat composition associated with the fat percentage within a dairy cattle population was previously identified in Dutch Holstein cows (Schennink et al., 2007); thus, the variation within the population likely contributes to the imprecision of some parameter estimates. Our work accounted for the random effect of the study on the intercept, so differences in farm management, genotypes, and other factors not identified were modeled as random effects in the analysis. It is important to consider that high-milk-fat breeds such as Jersey were very poorly represented in the data set used for model derivation.

We used FA-free DMI (DMI – total FA intake) as a potential predictor for both MFC and MFY. Both models included this term in the best equation, and VIF was <5 for all terms in the models, indicating that the approach avoided the potential collinearity problem. A quadratic effect of MFC with an increase in FA-free DMI is expected because highly digested diets produce more propionate in the rumen and milk production but also decrease DMI. Thus, DMI is related to milk production and limited by the physical and chemical characteristics of the diet (Allen, 2000; NRC, 2001).

However, in our work, the quadratic term for FA-free DMI on MFC was not significant, which could be associated with the digestibility of the forage used in those studies. Biologically, increasing FA-free DMI would be expected to result in greater milk volume, which would dilute milk fat until it reached a plateau. However, that depends on the substrates consumed from the diet.

Dietary starch and forage concentrations were significant predictors in the best MFC models but not in the best MFY model. Increasing dietary starch favors an increased yield of propionate from ruminal fermentation (Sutton et al., 2003) and greater starch flow to the intestine (Gregorini et al., 2015). Both stimulate insulin secretion and contribute to glucose supply, which can contribute to lactose production, driving milk volume and diluting milk fat (Mackle et al., 1999; Molento et al., 2002). This was reflected in the negative coefficient for MFC. Ruminal forage fermentation favors the production of acetate and  $\beta$ -hydroxybutyrate, which are major precursors of de novo FA synthesis in the mammary gland in ruminants (Palmquist, 2006), and this possible substrate effect is reflected in the positive coefficient for dietary forage content. Our work used forage as a potential predictor of MFC due to a limitation of reported NDF or physically effective (pe)NDF data



**Figure 7.** Effect of year of publication on milk fat and milk production. Each point represents a treatment mean. LOESS = locally estimated scatterplot smoothing.

**Table 5.** Feed category, total fatty acids (FA, % of DM), and FA profile (% of total FA) from the experimental diets used to develop models for estimating milk fat in lactating dairy cows

Category	N	Total FA		C16:0		C18:0		C18:1		C18:2		C18:3	
		Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Animal protein	239	3.5	3.0	24.4	3.3	17.0	6.3	25.5	8.3	10.3	5.8	0.5	0.3
By-product/other	548	4.1	3.9	18.8	4.8	4.0	3.0	18.1	4.7	48.6	7.9	6.8	4.9
Ca salts of fatty acids	83	85.1	5.4	49.0	9.5	4.0	0.8	34.4	6.7	6.8	1.3	0.2	0.0
Energy source	1,114	2.6	1.7	16.5	3.7	2.1	0.7	20.7	6.3	54.9	3.3	3.3	2.2
Grain crop forage	492	2.3	0.5	19.4	6.5	2.5	0.5	17.3	4.9	43.7	10.5	10.6	10.8
Grass/legume forage	699	1.9	0.4	19.8	3.3	3.1	0.8	2.5	0.6	18.1	3.4	39.9	4.8
Oil	90	86.7	8.9	14.0	9.3	7.9	5.4	46.0	10.6	19.6	12.6	4.0	3.4
Oilseed	188	17.4	3.0	17.2	6.2	3.4	1.0	19.5	4.0	53.4	6.2	5.0	8.3
Plant protein	885	2.7	6.4	15.5	3.1	3.6	1.0	20.4	11.1	49.0	9.2	8.7	5.5
Prill C16:0 (85%)	10	88.4	5.8	0	0	0	0	0	0	0	0	0	0
Prill C16:0 (95%)	2	76.0	0.0	0	0	0	0	0	0	0	0	0	0
Prill C18:0 (95%)	7	89.7	13.3	0	0	95	0	0	0	0	0	0	0
Sugar/sugar alcohol	4	5.2	0.0	0	0	0	0	0	0	0	0	0	0
Tallow	162	88.5	6.9	24.4	0	17.9	0	41.6	0	1.1	0	0.5	0

across the majority of studies. Moreover, high correlations ( $r > 0.60$ ) were found between forage and NDF, as well as forage and peNDF.

Models containing DI of C12:0 or C14:0 terms were not selected. Our work suggests different responses to DI of C12:0 and C14:0 on MFC, where basal levels of C12:0 had a positive coefficient, whereas dietary levels of C14:0 had varying effects on MFC. The effects of basal levels of C12:0 ( $12 \pm 8.6$  g/d; mean  $\pm$  SD) and C14:0 ( $9 \pm 6.5$  g/d) on MFC are still unclear because most studies have evaluated higher levels (above 200 g/d) of medium-chain FA in diets for cows.

Milk fat concentrate has been used as an indicator of milk fat depression in dairy cows (Davis and Brown, 1970; Koch and Lascano, 2018; Baldin et al., 2019). Milk fat depression is described as a reduction of milk fat yield without altering yields of milk or protein (Harvatine et al., 2009). This reduction in milk fat is primarily due to inhibition of milk fat synthesis in the mammary gland of dairy cows by intermediates, including *trans*-10,*cis*-12 CLA (Baumgard et al., 2000). High dietary fermentable carbohydrate (Maxin et al., 2011), high rumen UFA load (C18:1 + C18:2 + C18:3; Mannai et al., 2016), and a reduced forage particle size (Koch and Lascano, 2018) have been shown to contribute to milk fat depression. In the present work, the best model for predicting MFC (the model with the lowest AIC<sub>C</sub> value and CV) included dietary forage, DI of C18:2, and DI of C18:3.

Although Mannai et al. (2016) found a negative effect of rumen UFA load (RUFAL, C18:1 + C18:2 + C18:3 FA) on MFC, our work showed different results when these FA were analyzed separately. When DI of individual FA was considered in the global model, models that included DI of C18:1 ( $n = 40,960$  fitted models) had an overall negative slope ( $-0.01\%$ ), but the coefficients varied from  $-0.08$  to  $0.04\%$  (CV = 181%), contributing minimally to AIC<sub>C</sub>; therefore, DI of C18:1 was not selected. Our results indicate that dietary C18:1 *cis* may not reduce MFC for all diets, and its effects depend on other factors not identified in the present study. The coefficients for DI of C18:2 were consistently negative across all candidate models (average  $-0.06\% \pm 0.02\%$ , SD,  $n = 353$  fitted models) and in the best model ( $-0.04\%$ ). This result is in accordance with previous studies (Mannai et al., 2016; Dorea and Armentano, 2017) reporting a greater negative effect of C18:2 on MFC than of C18:1 and C18:0.

In our database, there was a slightly positive effect of DI of C18:3 on MFC ( $0.003 \pm 0.001$ , SE), which was consistent across all candidate models. However, Mannai et al. (2016) observed a negative effect of C18:3 on MFC. In the present work, averages for dietary concentration and intake of C18:3 were 0.32% of total FA

( $67 \pm 28$  g/d, SD), respectively. This value is close to the value of 0.38% reported by Mannai et al. (2016). A limitation of our study is that we did not try to model potential matrix effects of different feed sources. For example, the C18:2 supply is generally associated with a high-corn silage diet and C18:3 with a high-haylage or high-alfalfa diet. Feed matrix effects and some interactions among nutrients and the matrix; that is, fiber and starch digestion rates or extents, and residual OM composition, were not explored in our work.

Glasser et al. (2008) evaluated a database of 145 experiments that used oilseed supplements and did not observe negative effects of protected (encapsulated oils or formaldehyde-treated) C18:2 and C18:3 intake on MFC. Moreover, a meta-analysis reported that duodenal infusion of long-chain FA from animals or plants increased MFC, but these FA were mostly SFA or MUFA (Maxin et al., 2011). Therefore, the source and total supply of individual FA of both the basal diet and supplements should be considered in further studies evaluating milk fat in dairy cows. The positive effect of C18:3 on both MFY and MFC remains unclear, but we must note that our work did not consider the modification of C18:3 in the rumen. We also did not observe significant interactions between dietary starch concentration and DI of C18:1, C18:2, or C18:3 on MFC, which may be due to acceptable levels of dietary starch or low levels or protected forms of PUFA.

Methionine is typically a limiting AA relative to milk protein synthesis in lactating dairy cows (Broderick et al., 1970; NRC, 2001; Schwab and Broderick, 2017). High-producing dairy cows (average  $44.1 \pm 4.5$  kg of milk/d; mean  $\pm$  SD) supplemented with a Met analog [2-hydroxy-4-(methylthio)butanoate; HMTBa] had increased MFC and MFY, but that was not observed in low-producing cows (average  $31.4 \pm 4.3$  kg of milk/d; mean  $\pm$  SD) (Baldin et al., 2018). Thus, the effect of Met on milk fat may be restricted to high-producing cows fed high-fermentable carbohydrate and high-UFA diets. The response may be mediated through pathways that decrease absorption of intermediates from ruminal FA biohydrogenation, which can reduce milk fat synthesis in the mammary gland (Baldin et al., 2018). Thus, supplemental Met may be alleviating a shortage of choline, which supports milk fat synthesis (Sharma and Erdman, 1988).

Isoleucine and Leu have also been shown to regulate mTOR signaling (Appuhamy et al., 2012), resulting in increased rates of protein synthesis (Arriola Apelo et al., 2014; Zhao et al., 2019). Although it is not fully understood, SREBP1 activity is also regulated by the mTOR pathway (Li et al., 2014), as is expression of some of the lipogenic genes (Li et al., 2016); thus, the regulation of mTOR by some of the essential AA

may explain the link between AA supply and milk fat concentrations. Diets deficient in Ile or Val have been observed to result in decreased BW and body fat mass, reduced intake, and increased energy expenditure in mice (Du et al., 2012), indicating a positive association between Ile and Val supply and fat synthesis in tissues. However, absorbed Val did not significantly alter MFC in the present work. Dietary deficiency of His in diets adequate in MP, Met, and Lys has been associated with negative effects on DMI, yields of milk, protein, and ECM in lactating dairy cows, indicating that His is an important AA that affects the yields of milk and protein (Giallongo et al., 2017). As MFC is affected by milk volume and protected His has the potential to increase milk production (Morris and Kononoff, 2020), the negative effect of His on MFC could be a dilution effect because of its positive effect on milk volume.

### Predicting Milk Fat Yield

The best model for predicting MFY included the variables DIM, FA-free DMI, DI of C16:0 and C18:3, as well as Abs Met and Ile. This model was fitted using data from cows ranging from 36 to 344 DIM, where it is expected that a 1-unit increase in DIM reduces MFY by 1.4 g/d. This effect is associated with animal physiology, where the number of mammary cells, and thus the synthetic capacity, declines with increasing DIM (Wilde et al., 1986), leading to decreased milk component yields (Stanton et al., 1992). In terms of DMI, the regression coefficient for FA-free DMI was positive, and the correlation between FA-free DMI and MFY was strong ( $r > 0.7$ ). This relationship presumably reflects the provision of energy and de novo fat precursors in the FA-free DMI.

Identifying the effects of individual FA on MFY is challenging due to the moderately high correlations among digested FA supplies (Figure 1) and the limited number of studies that have evaluated the effects of single, purified forms of FA (Loften et al., 2014). This is a particular problem for C18:1 FA, which had relatively high correlations with most other FA.

Palmitic acid is a major FA in bovine milk; its level can vary from 22 to 35% of milk fat, depending on animal and feed factors, and it is a significant contributor to variation in milk FA composition (Jensen, 2002). Previous studies showed that cows supplemented with fat supplements enriched with C16:0 increased de novo FA synthesis in the mammary gland, which was associated with increased MFY (Rico et al., 2014b; Mathews et al., 2016; Western et al., 2020). In the present work, DI of C16:0 was used as a predictor of MFY in all the best candidate models, where a 1-unit increase of C16:0

resulted in a 0.24 to 0.46 g/d increase in MFY. Positive effects of C16:0 supplementation on MFY have been reported (Mannai et al., 2016), and the effect presumably reflects the use of C16:0 as a FA for milk fat triacylglycerol synthesis in the mammary gland of lactating cows. Moreover, studies have indicated that dietary C16:0 increased milk C16:0 yield without changing the synthesis of milk short-chain FA (<C16; Stoffel et al., 2015; Dorea and Armentano, 2017).

Similar to the MFC model, C18:3 DI also resulted in positive effects on MFY. It is unclear whether this positive effect was caused by direct or indirect factors on milk fat synthesis or the low levels of C18:3 in basal diets for cows. It is important to note that the lack of terms for the other UFA would be expected to contribute to predictions of MFD, as any increase in the supply of those FA would not elicit an increase in MFY.

The cross-evaluation of the best model resulted in moderate to low CV for both C16:0 and C18:3 DI (about 22%), indicating a strong relationship in the data. Sources of rumen-protected fats have been used as a strategy to partly protect UFA from biohydrogenation by rumen microorganisms. These methods to partly protect UFA in the rumen have been described by different studies and include calcium salts of FA, encapsulation, extrusion, heat treatment, and formaldehyde-treated seeds (Moallem, 2018). Partly protected FA are often provided in enriched forms, which increases the orthogonal power of those treatments, yielding more reliable parameter estimates.

In the present work, the average level for dietary C18:3 was 0.32% of DM, which was low compared with another meta-analysis, which reported a value of 0.63% of DM (Dorea and Armentano, 2017). In terms of FA intake, cows ingested an average of 67 g/d of C18:3, and 73% of total FA was predicted to be digested (Daley et al., 2020), which resulted in an intake of 49 g/d of digested C18:3. However, the amount of C18:3 in the diet that escapes biohydrogenation in the rumen was not considered in our model, and the amount of available UFA for postabsorptive use can vary depending on dietary fat sources and its availability in the rumen. When intakes of digested FA were evaluated individually (Supplemental Table S6), C18:3 significantly increased milk yield, MFC, and MFY, indicating that its effect on MFY was due to increased MFC and milk production. Although DI of C18:3 (about  $67 \pm 28$  g/d; SD) affected milk fat positively, the specific cause for this increase is not clear. Moreover, this fact might be due to its association with other variables not evaluated in this work, such as feed ingredient levels, types of lipids (triglycerides, free FA, phospholipids, galactolipids) in feeds, and other nutrients, such as water-soluble carbohydrates, in forages.

Estimating the biohydrogenation level of dietary C18 in the rumen is an issue in FA modeling; a practical model was developed by Moate et al. (2004), and that model has been widely used to predict biohydrogenation rates for PUFA in the rumen. However, the model did not consider all feed sources (only 27 feeds for lipolysis) or the potential effects of varying rates of passage on biohydrogenation (Moate et al., 2004). Further work is needed to include biohydrogenation rates in high- and low-producing dairy cows and their effects on milk fat. Also, it is necessary to consider that the effect of C18:3 could be a marker of different diet ingredients, which was not evaluated in this study.

The positive effects of Abs Ile and Met on MFY argue more strongly for an mTOR effect on milk fat synthesis, as Ile is not a methyl donor, nor does it supply significant carbon to the acetyl-CoA pool to explain the enhanced rates of fat synthesis. Additional work is needed to explore the potential effect of Ile signaling on milk fat synthesis, as the value of supplementing Ile and Met would derive not solely from enhanced milk protein production, but also from milk fat production. The results showed that Abs Met and Ile are drivers of milk fat synthesis in the mammary gland of dairy cows.

Overall, model predictions should be interpreted with caution because non-nutritional factors such as genetics, season, and milk production level, as well as complex interactions among feed nutrients or feed additives, may also affect MFY. Currently, efforts are being made to understand the relationship between de novo FA and MFC. A recent meta-analysis showed that MFC is positively associated with de novo FA (Matamoros et al., 2020). Thus, further work to improve milk fat models should consider the effect of dietary components, lactation period, and production level on de novo FA and consequent increases in both MFC and MFY.

## CONCLUSIONS

Our results indicated that DI of C18:2 was the best FA for predicting MFC in dairy cows, whereas DI of C16:0 was the best FA for predicting MFY. We observed inconsistent MFY responses to DI of C18:0, C18:1, and C18:2, possibly due to collinearity with other factors or interactions that were not captured in the models. The DI of C18:3 resulted in positive responses in both MFC and MFY, suggesting that this FA is not inhibitory in its pure form within the range of intakes represented by the data, and it is possible that it was actually slightly deficient in lactating dairy cows. Absorbed Met and IL were positively related with MFY, but only Abs Met was positively related with MFC. The models developed can be used as practical tools for predicting milk

fat in dairy cows. Further research should evaluate the potential effects of breed, environment, management, and other nutritional factors.

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