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Determining Optimal Nonlinear Regression Models for Studying the Kinetics of Fatty Acid Ruminal Biohydrogenation In Vitro

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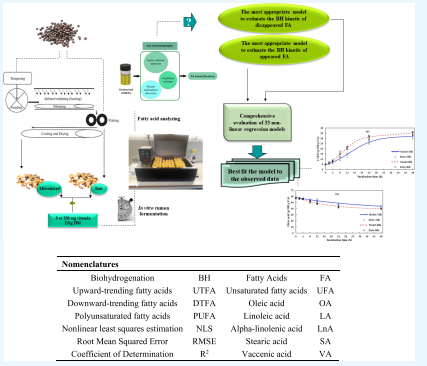
ABSTRACT: The accurate estimation of in vitro ruminal biohydrogenation (BH) kinetics of fatty acids (FA) allows for a more accurate understanding of their dynamics and develop targeted strategies to enhance desirable FA bypass. This study comprises a comprehensive evaluation of 33 nonlinear regression models to determine the most suitable model for accurately estimating the in vitro BH kinetics of individual FA. The data set utilized in the present research originates from a recent investigation on the effects of micronization and vitamin E on the in vitro ruminal BH of rapeseed. For the nonlinear regression analysis, data comprising FA concentrations (expressed as g FA/100 g FA) at the conclusion of 2, 4, 8, 12, 24, and 48 h incubation periods were employed. The evaluation of nonlinear regression models focused on identifying the ideal model based on criteria including the highest R^2 value, the lowest RMSE value, and statistically significant coefficients. The results pinpoint the Gompertz model as an effective choice for estimating the in vitro ruminal BH kinetics of upward-trending fatty acids, including intermediate unsaturated fatty acids and saturated end FA. Additionally, the first-order kinetic model of Ørskov and McDonald emerges as the preferred model for investigating the BH kinetics of downward-trending fatty acids, including oleic acid, linoleic acid, and alpha-linolenic acid. In summary, this rigorous evaluation led to the identification of the most appropriate model, one that not only exhibited an exceptional fit to the data but also provided profound insights into the intricate relationships between predictors and the dynamic behavior of FA. The established nonlinear regression models will serve as invaluable tools for future research investigating FA biohydrogenation kinetics.



1. INTRODUCTION

The addition of oilseeds to ruminant diets provides a valuable source of unsaturated fatty acids (UFAs) for animals. These UFAs not only serve as a potential energy source[1] but also perform various functional roles[2−4] and contribute to the improvement of the FA profile in dairy products.[5] Nevertheless, the UFAs ingested by animals undergo extensive processes of hydrolysis, isomerization, and BH in the rumen due to the action of rumen microorganisms.[6] Ruminal BH and isomerization lead to a reduction in the proportion of UFA and the emergence of stearic acid (SA) and other UFAs, such as conjugated linoleic acid isomers (CLA) and trans 18:1 isomers.[7] Consequently, the profile of FA that reaches the small intestine of ruminants differs from the composition of those initially consumed.[6]

The consumption of saturated FAs has been limited by nutritional guidelines, with a significant proportion originating from animal products such as meat and milk.[5] Evidence suggests that beneficial cardiovascular, anticarcinogenic, and anti-inflammatory effects on human health can be attributed to UFA and their BH intermediates.[8,9] Therefore, the processes of microbial BH of UFA in the rumen might aid in the development of strategies for producing animal products with beneficial effects on human nutrition. The most common approach for producing UFA-enriched animal products involves the supplementation of ruminants' diet with oilseeds, which are a rich source of UFAs.[5] In order to optimize the availability of UFAs for absorption at the duodenum, protection of dietary mono- and polyunsaturated fatty acids (PUFAs) from ruminal BH is of paramount importance. Notably, the application of heat treatment to oilseeds has been substantiated as a potent strategy to shield dietary PUFAs from the impacts of ruminal BH.[10,11] The reduction of ruminal BH of UFA in oilseeds through the process of micronization as a heat treatment has led to an increase in the intermediate FA content, particularly VA and CLA, in animal products, especially milk fat.[12−14] Dairy products serve as a primary

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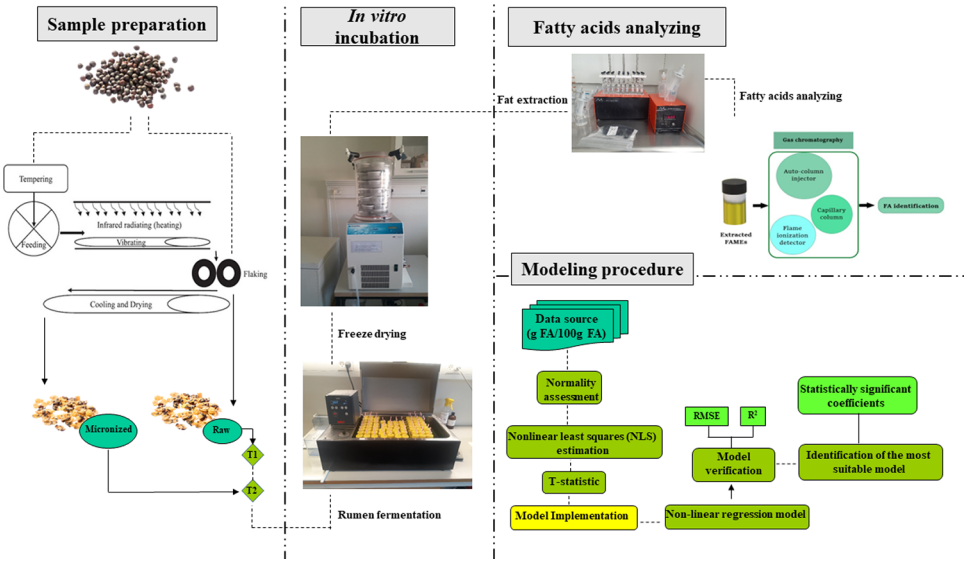

Figure 1. Experimental and modeling processes for estimating the in vitro BH kinetics of FA.

source of CLA in human diets.[15] In recent years, there has been a growing interest in the levels of CLA in human diets due to the potential health benefits associated with it.[16] Studies have reported the anticarcinogenic properties of CLA in rodent mammary and colon cancer models as well as in vitro models of human melanoma, colorectal cancer, and breast cancer. Additionally, the potential beneficial effects on the body composition and immune function have also been documented.[17]

While animal performance can be influenced by the absorbed FA, predicting the quantitative flow of individual isomers remains challenging. Therefore, accurate estimation of the ruminal BH kinetics of various processed oilseeds is an essential prerequisite for quantifying the relationships between dietary FA and the FA profile observed in animal products. A comprehensive evaluation of the efficacy inherent in diverse methods utilized to shield UFA from the intricate landscape of ruminal metabolism necessitates a meticulous exploration of the kinetics governing BH of FA within the rumen. The modeling of ruminal BH kinetics is considered a beneficial tool for characterizing the impact of diet, processing methods, microorganism ecology, and ruminal factors on the process of fatty acid BH, as well as for postulating potential ruminal BH pathways.[7] The use of mathematical models enables accurate predictions and saves time by testing the best model with a high accuracy and low error. In most studies, nonlinear regression models are employed to examine UFA kinetics during ruminal BH.[18−23] The parameters in nonlinear models often possess a biologically meaningful interpretation, based on the units and definitions associated with them.[24−26] This interoperability renders nonlinear models valuable for gaining insights into biological processes. It should be noted that nonlinear regression models also encounter limitations. Nonlinear models are generally less flexible than linear models, making model selection crucial. Additionally, as there is no analytical solution for parameter estimation in nonlinear models, numerical methods must be employed. This introduces challenges such as checking the convergence of the algorithm and selecting appropriate starting values for the parameters.[24] Overall, nonlinear regression models can serve as appropriate tools for determining the kinetic behavior of FA.[27,28]

The first-order kinetics have been previously utilized to estimate the ruminal BH kinetics of UFA from different sources.[20,29,30] In a study conducted by Enjalbert et al.,[21] the in vitro ruminal BH kinetics of disappearing UFA (mg/100 g DM) in canola seeds were estimated by employing an exponential model developed by Ørskov and McDonald.[31] There are limited studies in which the BH kinetics of intermediate FA have been estimated. Ribeiro et al.[7] used the first-order kinetic model of Ørskov and McDonald[31] to estimate the fractional rates of VA appearance and disappearance. Moate et al.,[32] in their research, harnessed the Michaelis−Menten model to elucidate the rate of LA disappearance in vitro, alongside the emergence of vaccenic acid (VA) and stearic acid (SA), when varying doses of LA (320, 650, and 970 mg/L) were subjected to incubation in a rumen fluid. Notably, the same model was applied to both the groups of FA that disappeared and those that appeared. In a parallel endeavor, Lashkari et al.[9] adeptly utilized the Michaelis−Menten model to quantitatively capture the in vitro BH kinetics encompassing both the disappearance and appearance of FA (mg/100 g DM) in flaxseeds. Building upon this foundation, Vargas et al.[33] ingeniously employed a multicompartmental model to delineate the in vitro BH kinetics of the disappearing UFA (mg/100 g DM). Furthermore, the kinetics of VA appearance were meticulously calculated through the adept fitting of the Gompertz model.[34]

As previously stated, a limited selection of nonlinear regression models has been employed to estimate the kinetic parameters for both the disappeared and appeared FA. One problem associated with previous studies is that they have usually failed to separate the estimation of BH kinetics of the appeared from disappeared FA. These two subsets exhibit distinct behaviors during the BH process, rendering the application of a uniform nonlinear regression model inappropriate. The use of separate models enables a more precise representation of BH kinetics, yielding valuable insights into the intricate dynamics of UFA, saturated FA, and intermediate FA involved in rumen BH. Significantly, the systematic evaluation of nonlinear models in terms of their competence

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Table 1. Mathematical Models to Estimate the In Vitro BH Kinetics of FA in Raw- or Micronized-Flaked Rapeseeds

symbol	form	model	name
LE 0	$y = \left(\frac{b_1 \times (1 - \exp(-b_2 x))}{\left(1 + \exp\left(\log\frac{1}{b_3}\right) - b_2 x\right)}\right)$	M1	logistic exponential without LAG
LE LAG	$y = \left(\frac{b_1 \times (1 - \exp(-b_2 \times (x - b_3)))}{\left(1 + \exp\left(\log\left(\frac{1}{b_4}\right) - b_2 \times (x - b_3)\right)\right)}\right)$	M2	logistic exponential with LAG
EXP0	$y = (b_1 \times (1 - \exp(b_2 x)))$	M3	exponential model without LAG
EXP Lag	$y = (b_1 \times (1 - \exp(b_2) \times (x - b_3)))$	M4	exponential model with LAG
GOM	$y = (b_1 \times \exp(-1 \times \exp(1 - b_2) \times (x - b_3)))$	M5	Gompertz model
LOG	$y = \left(\frac{b_1}{(1 + \exp(2 + b_2 \times (b_3 - x)))}\right)$	M6	logistic model
GM	$y = (b_1 \times (1 - \exp(-b_2 \times (x - b_3) - b_4 \times (x^{0.5} - b_3^{0.5}))))$	M7	generalization of Mitscherlich
MM	$y = \left(\frac{b_1 \times x^{b_2}}{(x^{b_2} + b_3^{b_2})}\right)$	M8	Michaelis−Menten
MMM	$y = \left(\frac{b_1 \times x^{b_2}}{(x^{b_2} + b_3)}\right)$	M9	modified MM
TPEXP	$y = (b_1 \times (1 - \exp(-b_2 \times (x - b_3))) + b_4 \times (1 - \exp(b_5 \times (x - b_3))))$	M10	two-pool exponential
TPLOG	$y = \left(\frac{b_1}{(1 + \exp(2 - 4 \times b_2 \times (x - b_3)))}\right) + \left(\frac{b_4}{(1 + \exp(2 - 4 \times b_5 \times (x - b_3)))}\right)$	M11	two-pool logistic
MGOM	$y = \left(b_1 \times \exp\left(-1 \times \exp\left(\left(\frac{b_2 \times 2.7183}{b_1}\right) \times (b_3 - x) + 1\right)\right)\right)$	M12	modified Gompertz model
LOG2	$y = \left(\frac{b_1}{(1 + b_2 \times \exp(b_3 - x))}\right)$	M13	logistic model
GOM2	$y = (b_1 \times \exp(-b_2 \times \exp(-b_3 \times x)))$	M14	Gompertz model
RCD	$y = \left(\frac{b_1}{(1 + b_2 \times \exp(-b_3 \times x))^{(1/b_4)}}\right)$	M15	Richard model
DSM	$y = \left(\frac{b_1}{(1 + \exp(-(b_2 + b_3 \times x + b_4 \times x^2 + b_5 \times x^3)))}\right)$	M16	double-sigmoid model
MLM	$y = \left(\frac{b_1 \times (1 - \exp(-b_2 \times x)) + b_3}{(1 + \exp(-b_4 \times (x - b_5)))}\right)$	M17	monomolecular logistic model
CRM	$y = (b_1 \times (1 - b_2 \times \exp(-b_3 \times x))^{(1/(b_4))})$	M18	Chapman−Richard model
ELM	$y = \left(\frac{b_1}{(b_2 \times \log(1 + \exp(b_2 \times (x - x \times b_3))))}\right)$	M19	exponential linear model
ELM	$y = \left(b_1 \times \log\left(\exp\left(\frac{b_2 \times (x - b_3)}{b_4}\right) + \exp\left(\frac{b_5 \times (x - b_6)}{b_7}\right)\right) + b_6\right)$	M20	exponential linear model
CN	$y = \frac{b_1}{(1 + (b_2 \times x)^{(-b_3)})}$	M21	Cone model
CNT	$y = \left(b_1 \times \left(\frac{1 - b_2}{(b_3 \times x + b_2 - 1)}\right)\right)$	M22	Contois model
FZH	$y = b_1 \times (1 - \exp(-b_2 \times x)^{b_3})$	M23	Fitzhugh model
FRA_1	$y = b_1 \times (1 - \exp(-b_2 \times (x - c) - b_3 \times (\mathrm{sqrt}(x) - \mathrm{sqrt}(b_4))))$	$M_1$24	France model
FRA2	$y = \frac{(b_1 \times (1 - \exp(-b_2 \times x)))}{(1 - b_3 \times \exp(-b_2 \times x))}$	$M_2$24	

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Table 1. continued

symbol	form	model	name
SCH_1	$y = (b_1^{b_2} + (b_3^{b_2} - b_1^{b_2}) \times \left(\frac{(1 - \exp(-b_4 \times (x - b_5)))}{(1 - \exp(-b_4 \times (b_6 - b_5)))}\right)^{(1/b_2)}$	$M_1$25	Schnute model
SCH_2	$y = ((b_1) + b_2 \times \exp(b_3 \times x))^{b_4})$	$M_2$25	
MON_0	$y = \left(\frac{b_1 \times b_2 \times x}{(b_2 \times x + 1)}\right)$	M26	Monod model without LAG
MON_{LAG}	$y = \left(\frac{b_1 \times b_2 \times (x - b_3)}{(b_2 \times (x - b_3) + 1)}\right)$	M27	Monod model with LAG
OPG	$y = b_1 \times \exp(-1 \times \exp(1 + b_2 \times \exp 1 \times (b_3 - x)))$	M28	one-pool Gompertz function
TPG	$y = b_1 \times \exp(-1 \times \exp(1 + b_2 \times (b_3 - x))) + b_4 \times \exp(-1 \times \exp(1 + b_5 \times (b_6 - x)))$	M29	two-pool Gompertz function
FOØM1	$y = b_1 + b_2 \times (\exp(-b_3 \times (x - b_4)))$	M30	first-order kinetic model of Ørskov and McDonald
FOØM2	$y = b_1 + b_2 \times (1 - \exp(-b_3 \times x))$	M31	
MAX1	$y = \left(b_1 + b_2 \times \exp\left(\frac{-x}{b_3}\right)\right)$	M32	Maxwell 1
MAX2	$y = b_1 + b_2 \times \exp\left(\frac{-x}{b_3}\right) + b_4 \times \exp\left(\frac{-x}{b_5}\right)$	M33	Maxwell 2

in accommodating BH data constitutes a noticeable gap in current research. Our underlying hypothesis proposes the potential existence of two distinct models, each aptly capturing the rate and extent of FA disappearance and appearance. Therefore, the main objective of this study was to comprehensively evaluate 33 nonlinear regression models, ultimately selecting the most suitable models to illustrate the changes in FA concentration throughout the in vitro rumen incubation of both raw- and micronized-flaked rapeseeds. Through this comprehensive exploration, our work aims to bridge this critical research gap, thus advancing our comprehension of BH kinetics in processed oilseeds.

2. MATERIALS AND METHODS

2.1. Data Collection and Experimental Setup. The data utilized in this study were derived from our recent research that examined the effect of micronization and vitamin E on the in vitro ruminal BH of rapeseeds.

The survey of experimental steps and modeling procedures is illustrated in Figure 1. In brief, full-fat rapeseeds of the Neptune variety (*Brassica napus*), harvested in June 2021, were divided into two batches. One batch was soaked in 5.0% water (w/w) an hour before micronization, with intermittent mixing every 15 min. Micronization was carried out using a gas-fired ceramic micronizer (Faravardaneh Ferdowsi Mashhad, Mashhad, Iran) at a wavelength of 2.8 μm. The rapeseed monolayers were conveyed on a vibrating conveyor positioned 12 cm below an infrared radiation source, reaching a surface temperature of 130 °C upon excitation. Immediately, the micronized rapeseeds were flaked by passing them between two rotating rollers with a gap distance of 0.50 mm. Nonmicronized rapeseeds were similarly flaked without prior micronization.

The experimental substrates underwent incubation within culture vials containing rumen fluid obtained from three ruminally cannulated heifers, as described by Petersen and Jensen.[35] These heifers, housed in the experimental barn at Aarhus University in Denmark, were fed a diet composed of 4.0 kg of grass hay, 2.0 kg of barley straw, and 2.8 kg of concentrate consisting of barley, soybean meal, rapeseed meal, oats, sugar beet molasses, as well as a vitamin and mineral premix at levels of 400, 100, 30, 400, 30, and 40 g/kg dry matter, respectively. It is worth noting that the incubation was performed once, with each treatment comprising six replicates for each incubation time (resulting in two observations for each individual heifer). Samples were incubated for durations of 0, 2, 4, 8, 12, 24, and 48 h, after which they were frozen at −20 °C, subjected to freeze-drying, and stored at −20 °C until FA analysis.

To determine the FA composition, the extracted FA methyl esters were analyzed by using a gas chromatograph (Hewlett-Packard 6890 series, Agilent Technologies, Palo Alto, CA, USA) equipped with an automatic column injector (Hewlett-Packard 7673). A capillary column with an inner diameter of 60 m × 0.32 mm and a 0.25 μm film thickness (OmegawaxTM 320; Supelco 4-293-415, Sigma-Aldrich) was employed, along with a flame ionization detector. Identification of FA was achieved by comparing the retention times to those of external standards (GLC 68C, Nu-Prep-Check, Elysian, MN, USA).

2.2. Nonlinear Regression Analysis. In the pursuit of understanding the dynamic behavior of various FA, our study ventures into the intricate kinetics of oleic acid (OA), linoleic acid (LA), alpha-linolenic acid (LnA), SA, VA, and trans-10 C18:1. These FA are the focal points of our investigation, as we aim to comprehensively comprehend their behavior under diverse experimental conditions. Within this ensemble of FA, we encounter both DTFA (OA, LA, and LnA) and UTFA (SA, VA, and trans-10 C18:1), each showing unique patterns of appearance and transformation, making them intriguing subjects for our study. To conduct our nonlinear regression analysis, we utilized data comprising FA concentrations (expressed in g FA/100 g FA) in the culture tubes at the culmination of a 48 h incubation period. Before embarking on this analysis, it was imperative to ensure the data set's conformity with the assumptions of normality. Thus, the data underwent a rigorous normality assessment through the Shapiro−Wilk test. This crucial step ensured the validity and reliability of our subsequent nonlinear regression modeling, providing a solid foundation for the exploration of FA kinetics under various experimental scenarios.

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In the realm of FA kinetic research, a noticeable trend has emerged: a preference for a limited set of regression models primarily focused on data fitting rather than comprehensive analysis. Many studies lack the depth needed to fully understand the intricacies of the FA behavior. In response, our study has undertaken the task of creating an extensive repository of nonlinear regression models scattered across various research works. These models exhibit the potential for a close fit with the complex behaviors of FA. Our approach distinguishes itself by providing a thorough evaluation of this expansive model library, a resource that can benefit other researchers in this field. To facilitate knowledge sharing, we have included practical MATLAB code implementations of these models, empowering researchers to effectively utilize them and deepen our collective understanding of FA kinetics.

In our research, powerful tools in the form of parametric nonlinear regression models are harnessed to unravel the complex relationships between continuous response variables, specifically the dynamics of FA, and a single continuous predictor variable, incubation time (h). These models are constructed following the fundamental equation:

$$y = f(X, \beta) + \varepsilon \tag{1}$$

In this context, the observed FA measurements are represented by the vector "y", with dimensions $n \times 1$. To predict the corresponding values of 'y,' a function 'f' is employed, which takes into account both the predictor matrix 'X' and the vector of unknown parameters 'β,' as illustrated in Table 1. The predictor matrix, denoted as incubation time (h) 'X' has dimensions $n \times p$, with each row corresponding to an observation and each column representing a predictor. On the other hand, the vector β has dimensions $p \times 1$ and encompasses the parameters that require estimation. Additionally, the vector ε possesses dimensions $n \times 1$ and comprises independent and identically distributed random disturbances.

Nonlinear least-squares estimation (NLS) is a statistical technique used to estimate the parameters of a nonlinear model by minimizing the sum of the squares of the differences between the observed and predicted values. The goal is to find the parameter values that best fit the model to the observed data.

The fundamental equation for NLS can be expressed as follows:

$$\min_{\beta} \sum_{i=1}^{n} [y_i - f(x_i, \beta)]^2 \tag{2}$$

where $\min_{\beta}$ represents the minimization process with respect to the parameter vector β, n is the number of observations, y_i is the observed response for the ith observation, x_i is the vector of predictor variables associated with the ith observation, and $f(x_i, \beta)$ is the nonlinear model function that relates the predictors x_i and the parameter vector β to the predicted response y_i.

The objective is to find the values of the parameter vector β that minimizes the sum of squared residuals, which are the differences between the observed y_i and the predicted $f(x_i, \beta)$ values. The NLS estimation process often involves an iterative approach because the equations involved in the minimization process are nonlinear. A simplified outline of the steps involved is as follows:

1. Initialization: Start by assigning initial values to the parameter vector β.
2. Iteration: Begin an iterative process aimed at improving the parameter estimates. In each iteration, the algorithm calculates the predicted values $f(x_i, \beta)$ for each observation and computes the residuals $y_i - f(x_i, \beta)$.
3. Update Parameters: Adjust the parameter values β in a way that reduces the sum of squared residuals. This adjustment typically involves a gradient-based optimization algorithm, such as the Gauss−Newton method or the Levenberg−Marquardt algorithm. These methods adjust the parameter values in the direction that minimizes the objective function.
4. Convergence Check: After each iteration, check for convergence. Convergence is typically achieved when the change in the parameter estimates or the change in the objective function falls below a predefined threshold.
5. Repeat Iterations: Continue iterating until convergence is reached or a maximum number of iterations is reached.
6. Parameter Estimates: The final estimates of the parameter vector β are obtained when the convergence criterion is met.

To evaluate the significance of nonlinear regression coefficients, several key steps are followed. First, the model parameters, including coefficients (β), are estimated using a method like NLS. Then, the standard errors of these coefficients ($\mathrm{SE}(\beta_i)$), which measure their uncertainty, are calculated. The t-statistic (t_i) for each coefficient is computed by dividing the estimated coefficient by its standard error (eq 3). The next step involves calculating the p-value associated with each t-statistic, representing the probability of observing such a statistic under the null hypothesis that the coefficient is zero. By comparing p-values to a chosen significance level (α), often 0.05, one can determine if a coefficient is statistically significant. If $p \leq \alpha$, the null hypothesis is rejected, signifying significance; otherwise, it fails to be rejected. Significant coefficients indicate influential predictors, while nonsignificant ones suggest less contribution to the model. In summary, the evaluation of coefficient significance involves the estimation of parameters, the computation of standard errors, the generation of t-statistics and p-values, and the comparison to a significance threshold, enabling meaningful inferences to be drawn about the regression model.

$$t_i = \frac{\beta_i}{\mathrm{SE}(\beta_i)} \tag{3}$$

The process of determining the optimal coefficients for the regression models presented in Table 1 was carried out within the MATLAB software environment, utilizing two indispensable functions: 'fitnlm' and 'fit'. These functions constitute the essential components of MATLAB's modeling and optimization toolkit, playing a pivotal role in the parameter optimization process. To employ these functions effectively, the following steps were undertaken. Initially, the coefficients for all 33 nonlinear regression models were computed using the 'fit' function, with the subsequent calculation of their corresponding RMSE and R^2 values. It is worth noting that determining the suitable lower and upper bounds for the coefficient ranges of nonlinear regression models often necessitates an iterative process, drawing upon prior experience and insights from previous research endeavors. In the final phase, following the preliminary model screening, the significance of the nonlinear regression coefficients was

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assessed using the 'fitnlm' function, allowing for a rigorous evaluation of the models' coefficient significance. The implementation of the nonlinear regression (NLR) model using MATLAB is depicted in Figure 2. The methodology

```
k = 2;
t = Time{ k }; g = Y{ k };
% Selection model
SM = 1;
[ M, SP ] =  SelectNlModel( SM );
opts = statset( 'Display' , 'off' , 'TolFun' , 1e-200 , 'RobustWgtFun' , 'fair');
Mfit = fitnlm( t , g , M , SP , 'Options' , opts );
[ pv , yci ] = predict( Mfit , t );
dv = g;
Cofe   = Mfit.Coefficients.Estimate';
PV     = Mfit.Coefficients.pValue';
StdCof = Mfit.Coefficients.SE;
R2     = [ Mfit.Rsquared.Ordinary Mfit.Rsquared.Adjusted ];
```

Figure 2. Pseudocode implementation of the NLR model using MATLAB.

employed in this study to establish the initial values of the nonlinear regression coefficients was meticulously designed to ensure the robustness of our research. Given the substantial impact of these initial values on the convergence process of the NLR method, a comprehensive approach was adopted. The coefficients obtained from prior research studies in the field were considered as a starting point and laid the groundwork for the investigation. Furthermore, a trial-and-error approach was employed to refine the initial values. Through systematic experimentation and analysis, the coefficients were iteratively adjusted, taking into account various factors including data characteristics, model complexity, and desired convergence properties. This iterative process enabled fine-tuning of the initial values and optimization of their appropriateness for the specific research objectives. By integrating these approaches, the aim was to strike a harmonious balance between leveraging the existing knowledge and tailoring the initial values to align with the unique requirements of the study. Paramount importance is placed on the transparency and reproducibility of the methodology as efforts are made to contribute to the scientific community and facilitate future research endeavors.

Following a thorough evaluation of the 33 regression models, the model selection process aimed to identify the optimal model for accurately capturing the dynamic behavior of FA. This selection relied on two crucial criteria: the root-mean-squared error (RMSE) and the coefficient of determination (R^2), which assess the model's ability to align with the observed data. A lower RMSE indicates a more precise fit, signifying a close agreement between the model's predictions and actual data. Conversely, a higher R^2 value suggests a stronger correlation between the model's predictions and observed values, highlighting its robust explanatory power. Additionally, the significance of the regression model coefficients plays a vital role. Acceptable RMSE and R^2 values are insufficient if some model parameters lack significance. Therefore, the ideal model combines the highest R^2 value, the lowest RMSE value, and the statistically significant coefficients. This comprehensive assessment led to the identification of the most suitable model, one that not only provided an outstanding fit to the data but also offered profound insights into the intricate relationships between the predictors and the dynamic behavior of FA.

$$\mathrm{RMSE} = \sqrt{\frac{1}{n}\sum_{i=1}^{n}(\hat{y}_i - y_i)^2} \tag{4}$$

$$R^2 = 1 - \frac{\sum_{i=1}^{n}(\hat{y}_i - y_i)^2}{\sum_{i=1}^{n}(y_i - \bar{y})^2} \tag{5}$$

where n is the number of observations, $\hat{y}_i$, y_i represent the predicted and observed values of the response variable for observation 'i', respectively, and $\bar{y}$ is the mean of the observed values of the response variable.

Table 2. Assessment of Nonlinear Regression Models using RMSE and R^2 for C18:0, C18:1 Trans-11, and C18:1 Trans-10 in Raw- and Micronized-flaked Rapeseeds[a]

			C18:0		C18:1 trans-11		C18:1 trans-10	
model	name	criteria	MR	RR	MR	RR	MR	RR
M15	Richard (RCD)	RMSE	0.97	1.16	0.04	0.06	0.01	0.01
		R^2	0.99	0.99	0.98	0.97	0.98	0.97
M11	two-pool logistic (TPLOG)	RMSE	1.07	1.14	0.04	0.06	0.01	0.01
		R^2	0.99	0.99	0.98	0.97	0.98	0.97
M6	logistic model(LOG)	RMSE	1.11	1.39	0.05	0.07	0.01	0.01
		R^2	0.99	0.99	0.98	0.96	0.98	0.97
M5	Gompertz model (GOM)	RMSE	1.20	1.17	0.04	0.07	0.01	0.01
		R^2	0.99	0.99	0.99	0.97	0.98	0.97
M14	Gompertz model (GOM2)	RMSE	1.20	1.17	0.04	0.07	0.01	0.01
		R^2	0.99	0.99	0.98	0.97	0.97	0.97
M12	modified Gompertz model (MGOM)	RMSE	1.20	1.17	0.04	0.07	0.01	0.01
		R^2	0.99	0.99	0.98	0.97	0.97	0.97
M17	monomolecular logistic model (MLM)	RMSE	1.55	1.49	0.04	0.06	0.01	0.01
		R^2	0.98	0.98	0.98	0.97	0.97	0.97
M18	Chapman−Richard model (CRM)	RMSE	1.62	1.53	0.05	0.07	0.01	0.01
		R^2	0.97	0.97	0.98	0.97	0.97	0.97

[a]Note: Treatments encompass raw- and micronized-flaked rapeseeds denoted as RR and MR, respectively. Evaluation criteria: coefficient of determination (R^2) and the root-mean-square error (RMSE).

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Table 3. Comprehensive Assessment of Coefficient Values from Four Selected Models for Predicting Changes in the Vehavior of UTFA[a]

models	UTFA	treatment	coefficients		
			a	*b*	*c*
Gompertz model(GOM)M5	C18:0	MR	33.43** ± 0.72	0.08** ± 0.01	−2.67** ± 0.49
		RR	35.68** ± 0.56	0.11** ± 0.01	−2.03** ± 0.37
	C18:1 trans-11	MR	0.90** ± 0.02	0.12** ± 0.01	3.83** ± 0.45
		RR	1.00** ± 0.02	0.17** ± 0.01	2.27** ± 0.36
	C18:1 trans-10	MR	0.24** ± 0.01	0.14** ± 0.01	3.46** ± 0.52
		RR	0.18** ± 0.01	0.18** ± 0.01	1.11** ± 0.42
modified Gompertz model(MGOM1)M12	C18:0	MR	33.43** ± 0.72	1.04** ± 0.04	−2.67** ± 0.49
		RR	35.68** ± 0.56	1.49** ± 0.06	−2.03** ± 0.37
	C18:1 trans-11	MR	0.90** ± 0.02	0.04** ± 0.01	3.83** ± 0.45
		RR	1.00** ± 0.02	0.06** ± 0.01	2.27** ± 0.36
	C18:1 trans-10	MR	0.24** ± 0.01	0.01** ± 0.01	3.46** ± 0.52
		RR	0.18** ± 0.01	0.01** ± 0.01	1.11** ± 0.42
Gompertz model(GOM2)M14	C18:0	MR	33.43** ± 0.72	2.17** ± 0.07	0.08** ± 0.01
		RR	35.68** ± 0.56	2.16** ± 0.07	0.11** ± 0.01
	C18:1 trans-11	MR	0.90** ± 0.02	4.32** ± 0.34	0.12** ± 0.01
		RR	1.00** ± 0.02	3.97** ± 0.31	0.17** ± 0.01
	C18:1 trans-10	MR	0.24** ± 0.01	4.49** ± 0.49	0.14** ± 0.01
		RR	0.18** ± 0.01	3.34** ± 0.30	0.18** ± 0.01
logistic model(LOG)M6	C18:0	MR	32.05** ± 0.52	0.15** ± 0.01	0.00** ± 0.00
		RR	34.51** ± 0.47	0.21** ± 0.01	0.00** ± 0.00
	C18:1 trans-11	MR	0.81** ± 0.01	0.27** ± 0.02	5.72** ± 0.31
		RR	0.96** ± 0.01	0.31** ± 0.02	3.79** ± 0.36
	C18:1 trans-10	MR	0.23** ± 0.01	0.27** ± 0.02	4.93** ± 0.51
		RR	0.17** ± 0.01	0.31** ± 0.02	2.34** ± 0.39

[a]Note: Treatments encompass raw- and micronized-flaked rapeseeds denoted as RR and MR, respectively. Parameters denoted as "*a*" stand for maximum cumulative appearance of fatty acids (g/100 g FA), "*b*" for rate constant (h^{-1}), and "*c*" for the lag phase (h).

3. RESULTS AND DISCUSSION

The FA concentrations over time can either increase or decrease, contingent upon the distinct BH pathways. Consequently, the outcomes have been organized under two primary sections: models capable of elucidating the decline of initial FA, termed "DTFA", and models delineating the emergence of intermediates and saturated terminal FA, referred to as "UTFA".

The evaluation process involved comparing the experimental data with the model-predicted data, utilizing the prediction accuracy criterion (RMSE) for assessing the model prediction errors and data fit criterion (R^2) for the goodness of fit. To ensure content coherence, the presentation of results for all 33 nonlinear regression models was avoided. Instead, focus was placed on the selection of initial models, chosen based on their ability to capture the essence of UTFA or DTFA behavior.

3.1. Upward-Trending Fatty Acids. *3.1.1. Evaluating and Selecting the Best-Fitted Nonlinear Regression Model for Appeared UTFA.* As previously mentioned, a total of 33 nonlinear regression models was scrutinized to discern the optimal models for projecting the in vitro ruminal BH kinetics of SA, VA, and trans-10 C18:1 appearances in both raw- and micronized-flaked rapeseeds (as shown in Table 1). It is important to highlight that these models were devised for both raw- and micronized-flaked rapeseeds and for each individual FA.

The identification of the double-sigmoid model producing the poorest prediction results underscores the critical importance of meticulously selecting appropriate models for predicting UTFA's BH kinetics The fitting outcomes of the models demonstrated that out of the 33 models, only 8 exhibited superior ability in estimating the kinetic parameters for SA, VA, and C18:1 trans-10 appearances. Table 2 presents the R^2 and RMSE values for the eight selected models pertaining to SA, VA, and C18:1 trans-10 appearances. Models with lower RMSE and higher R^2 values can predict FA appearances more closely to the observed values. The highest R^2 values ranging from 0.96 to 0.99, coupled with the lowest RMSE values spanning from 1.16 to 1.62 across all FA, signify robust concordance between alterations in UTFA concentration and model predictions. Our analysis did not unveil a significant performance difference among the eight selected models for SA, VA, and C18:1 trans-10 appearances in raw- and micronized-flaked rapeseeds. Despite some models exhibiting suitable R^2 and RMSE values, their coefficients were not statistically significant. TPLOG, RCD, MLM, and CRM models displayed subpar performance in elucidating changes in the UTFA concentration compared to other models. In contrast, GOM, MGOM, GOM2, and LOG were pinpointed as the best models due to the significant coefficients that they exhibited. It is important to note that this issue has often been inadequately addressed in numerous preceding studies.[7,21,32,36]

Table 3 displays the values of model coefficients (*a*, *b*, and *c*), along with their corresponding standard deviation values and the significance outcomes, for different UTFA in both raw- and micronized-flaked rapeseeds using the four selected models. The negative values of the c parameter observed for the GOM and MGOM models hold no biological meaning, raising doubts about the utility of such models for estimating ruminal BH kinetics. Considering the significance of coefficients, their relational simplicity, and ease of application

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in calculations, the GOM2 (M14) model emerged as the recommended choice for investigating UTFA's BH kinetics from these four selected models. The predictive performance of model coefficients mirrored patterns similar to the observed values, signifying the accuracy of the M14 model. This enhances its practicality across diverse experimental methodologies. However, it is worth highlighting that the final model selection should also factor in the specific needs of the application and the inherent characteristics of the analyzed data.

3.1.2. Investigation of the Kinetic Behavior of Upward-Trending Fatty Acids. The investigation of the behavior of UTFA was conducted using the chosen model (M14). Model M14 accurately estimated the kinetic behavior of SA, VA, and C18:1 trans-10 over time (Figure 3), falling within the 95% prediction interval of the in vitro ruminal BH data. These estimations highlight the proficiency of the M14 model in elucidating the relationship between observed and predicted values, effectively describing the appearance kinetics of UTFA. While a few measured concentrations of SA, VA, and C18:1 trans-10 slightly diverged from the predicted values, the overall outcomes were deemed satisfactory. Additionally, the coefficient of determination corroborated the precision of the selected model (M14).

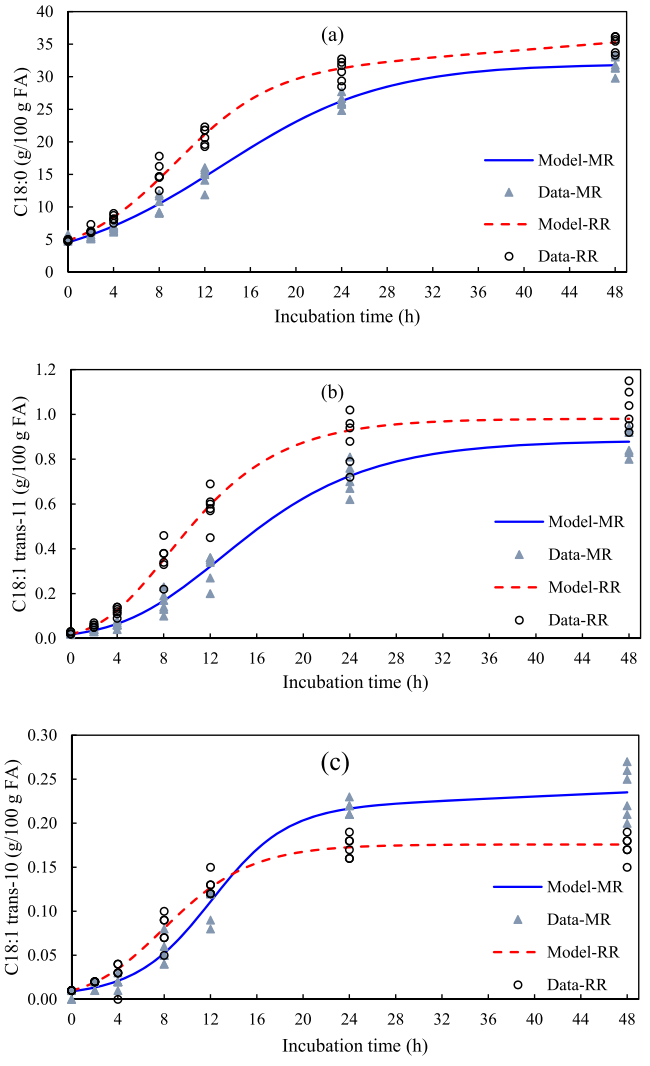

Figure 3. Nonlinear regression modeling of the dynamic behavior of UTFA, including C18:0 (a), C18:1 trans-11 (b), and C18:1 trans-10 (c), during incubation. Treatments encompass raw- and micronized-flaked rapeseeds denoted as RR and MR, respectively.

The modeling results consistently demonstrated that micronized-flaked rapeseed consistently showed lower SA production over time compared with raw-flaked rapeseed (Figure 3a). At 2, 4, 8, 12, 24, and 48 h of incubation, the appearance of SA decreased in micronized-flaked rapeseed as opposed to raw-flaked rapeseed. Additionally, raw-flaked rapeseeds achieved a steady state of appearance sooner and maintained a consistent level over time. Specifically, raw-flaked rapeseed attained a stable appearance state after approximately 20 h, while micronized-flaked rapeseed required around 35 h to reach a comparable level of production. A higher production of SA observed for raw rapeseed suggests a more complete BH. We speculated that micronization might affect the bacteria responsible for the final BH step, resulting in a decrease in SA appearance. In agreement with our results, Troegeler-Meynadier et al.[10] reported a decrease in SA appearance by the heat processing of soybean seeds. Saturated fatty acids (SFA) are perceived to be less healthy than UFA. Human nutritional guidelines have advised decreasing the consumption of SFA which originate from animal products.[5] Our results demonstrate that micronization can decrease the appearance of SA in the rumen, especially in growing or high-producing animals that have a shorter retention time of feed particles in the rumen, and thus decrease its concentration in animal products. As illustrated in Figure 3b, the formation of VA in micronized-flaked rapeseed consistently remained approximately 10% lower over time when compared to raw-flaked rapeseed. At 4, 8, 12, 24, and 48 h after incubation, the appearance of VA decreased in micronized-flaked rapeseed in comparison to raw-flaked rapeseed. Furthermore, raw-flaked rapeseed attained a stable and constant value earlier than micronized-flaked rapeseed, requiring approximately 24 and 34 h, respectively, to reach a steady state for this particular FA. The appearance of C18:1 trans-10 exhibited a distinct behavior compared to SA and VA (Figure 3c). At 12 h after incubation, the C18:1 trans-10 appearance was lower in micronized-flaked rapeseed than in raw-flaked rapeseed. However, at 24 and 48 h after incubation, the C18:1 trans-10 appearance increased in micronized-flaked rapeseed compared to raw-flaked rapeseed. Both micronized- and raw-flaked rapeseeds reached a stable state after approximately 24 h, with the micronized-flaked rapeseed producing 20% more C18:1 trans-10 than raw-flaked rapeseed. As stated by Privé et al.,[37] heated oilseeds can contain some products that favor bacteria producing trans-10 isomers and inhibit the activity and/or growth of bacteria producing trans-11 isomers. In line with our findings, Kaleem et al.[38] reported that heated oilseeds increased trans-10 isomers and decreased trans-11 isomers. In addition, the lower appearance of SA and VA in micronized-flaked rapeseed might be due to an inhibition at the second and/or third reactions of linoleic acid BH. Consistent with our results Lashkari et al.[11] observed a decrease in the appearance of SA and VA by heating partly defatted flaxseed.

3.1.3. Investigation of Upward-Trending Fatty Acid Production Rates. Nonlinear regression models and their first derivatives can be utilized to derive functions that describe the rate of change in diverse processes. These functions offer valuable insights into the fundamental mechanisms governing these processes and enable predictions of their temporal behavior. The capacity to deduce such functions constitutes a

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crucial tool across numerous scientific domains, aiding researchers in comprehending intricate phenomena and devising efficacious interventions to tackle them.

The first-order derivative of the M14 model was employed to compute the kinetics of UTFA. Figure 4 illustrates that the

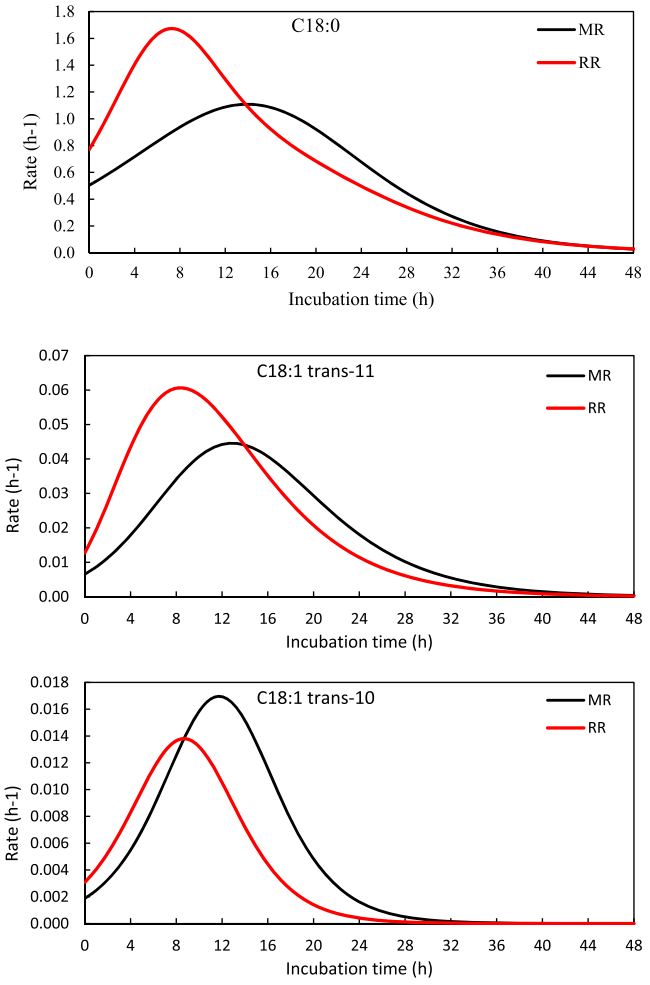

Figure 4. Rates of appearance for UTFA, encompassing C18:0, C18:1 trans-11, and C18:1 trans-10 during incubation. Treatments encompass raw- and micronized-flaked rapeseeds denoted as RR and MR, respectively.

rate of SA appearance noticeably decreased over time for both raw- and micronized-flaked rapeseeds. Within the initial 12 h of incubation, the appearance rate of SA was lower in micronized-flaked rapeseed compared to raw-flaked rapeseed. At the 24 h mark of incubation, the appearance rate of SA increased in micronized-flaked rapeseed. Similar patterns were observed for the rate of VA appearance (Figure 4), albeit the incline in the appearance rate of SA and VA at the outset of incubation was significantly steeper in raw-flaked rapeseeds than in micronized-flaked rapeseeds. Within the initial 8 h of incubation, the appearance rate of C18:1 trans-10 decreased due to micronization (Figure 4). At the 12 and 24 h marks of incubation, a decrease in the appearance rate of C18:1 trans-10 was observed in raw-flaked rapeseed. The rate of SA is influenced by the BH rate of C18:1 trans-10, and VA in micronized-flaked rapeseed. The heat treatment can cause the isomerization of cis-9 isomers of OA into trans-10 and trans-11 isomers.[37] In the rumen BH pathway, C18:1 trans-10 and C18:1 trans-11 are readily hydrogenated by rumen microbes.[10] As a result, C18:1 trans-10 and C18:1 trans-11 isomers referentially convert into C18:0, following their rate of hydrogenation. Therefore, in heated oilseeds, the rate of C18:0 is influenced by the biohydrogenation rates of C18:1 trans-10 and C18:1 trans-11. The decrease in the appearance rate of VA and the increase in the appearance rate of C18:1 trans-10 might be linked to the impact of micronization on the microbiota ecosystem. The alternation of the microbiota ecosystem and/or the inhibition of the reductase activity of ruminal microbes[11] might be responsible for the delay in the appearance of SA, VA, and C18:1 trans-10 in micronized- compared to raw-flaked rapeseed. These findings offer insightful perspectives into the dynamic behavior of FA production and emphasize the importance of vigilant monitoring of their concentrations over time.

3.2. Downward-Trending Fatty Acids. *3.2.1. Evaluation and Selection of the Best-Fitted Nonlinear Regression Model for Disappeared DTFA.* The investigation of the downward-trending FA (DTFA) necessitates the utilization of dedicated nonlinear regression models designed for their unique dynamics. In accordance with Table 1, a comprehensive assessment of 33 nonlinear regression models was conducted for both raw- and micronized-flaked rapeseeds. This evaluation aimed to model the behavior of three distinct FA: OA, LA, and LnA, across various incubation times. The outcomes of the selected models are presented in Table 4. The evaluation results highlight discernible differences in the capabilities of these chosen nonlinear regression models. Notably, the R^2 values ranged from 0.88 to 0.98, while the RMSE values ranged from 0.3 to 2.48. These variations emphasize the need for a more comprehensive evaluation of the six selected models to determine the most suitable final model. It is important to acknowledge that the R^2 and RMSE results suggest that these six models exhibit a commendable ability to elucidate the intricate nature of the DTFA behavior. They can serve as primary candidates for further investigation and selection. However, it is imperative to undertake additional investigations to arrive at the ultimate model, a topic that will be explored in subsequent discussions.

Continuing the evaluation of the regression model validity, it becomes imperative to scrutinize the significance of regression coefficients. While a model might yield acceptable R^2 and RMSE values, the presence of nonsignificant regression coefficients can be deemed a flaw, undermining its overall reliability. In light of this, this study employed the significance results of all coefficients extracted from the selected regression models presented in Table 4. Among the initial six models, only three emerged as viable contenders: ELM, FOØM2, and MAX1. This selection was guided by the exclusion of the other three models, each of which harbored at least one non-significant coefficient. Table 4 provides a comprehensive illustration of the significance evaluation results for the coefficients within the nonlinear regression model, accompanied by their respective standard deviation values. This comprehensive evaluation spans two treatments and three distinct FA. Evidently, all regression coefficients hold significance at the 1% level of significance. From this subset of the three remaining regression models, a final choice must be made. While the performance discrepancies among these three models are minimal, the FOØM2 model gains prominence owing to its mathematical formulation, simplicity, and user-friendliness. This model aligns with all previously

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Table 4. Evaluation of Nonlinear Regression Models Based on RMSE and R^2 for OA, LA, and LnA in Raw- and Micronized-Flaked Rapeseeds[a]

model	name	criteria	OA		LA		LnA	
			MR	RR	MR	RR	MR	RR
M19	exponential linear model (ELM)	RMSE	1.27	2.48	0.98	1.1	0.51	0.54
		R^2	0.93	0.88	0.95	0.94	0.96	0.95
M28	one-pool Gompertz function (OPG)	RMSE	1.25	2.24	0.96	1.15	0.52	0.52
		R^2	0.93	0.88	0.96	0.92	0.96	0.95
M29	two-pool Gompertz function (TPG)	RMSE	0.85	1.01	0.96	0.56	0.52	0.52
		R^2	0.97	0.98	0.96	0.98	0.96	0.95
M31	first-order kinetic model of Ørskov and McDonald (FOØM2)	RMSE	1.07	1.25	0.9	0.66	0.52	0.38
		R^2	0.95	0.96	0.96	0.97	0.95	0.97
M32	Maxwell 1 (MAX1)	RMSE	1.07	1.25	0.9	0.66	0.53	0.38
		R^2	0.95	0.96	0.96	0.97	0.95	0.97
M33	Maxwell 2 (MAX2)	RMSE	0.82	1.13	0.56	0.57	0.3	0.29
		R^2	0.97	0.97	0.98	0.98	0.98	0.98

[a]Note: OA, oleic acid; LA, linoleic acid; and LnA, alpha-linolenic acid. Treatments: raw- and micronized-flaked rapeseeds denoted as RR and MR, respectively. Evaluation criteria: coefficient of determination (R^2) and ro ot-mean-square error (RMSE).

Table 5. Comprehensive Assessment of Coefficient Values from Three Selected Models for Predicting Changes in the Behavior of DTFA[a]

models	DTFA	treatment	coefficients		
			a	*b*	*c*
exponential linear model (ELM)M19	OA	MR	267** ± 0.01	3.21** ± 0.02	1.00** ± 0.01
		RR	322** ± 0.01	3.94** ± 0.04	1.00** ± 0.01
	LA	MR	155** ± 0.01	6.24** ± 0.10	1.00** ± 0.01
		RR	267** ± 0.01	11.04** ± 0.19	1.00** ± 0.01
	LnA	MR	488** ± 0.07	41.55** ± 0.83	1.00** ± 0.01
		RR	431** ± 0.06	37.89** ± 0.69	1.00** ± 0.01
first-order kinetic model of Ørskov and McDonald (FOØM2)M31	OA	MR	58.36** ± 0.33	−20.14** ± 2.42	−0.03** ± 0.01
		RR	58.54** ± 0.49	−20.54** ± 1.06	−0.05** ± 0.01
	LA	MR	17.64** ± 0.30	−15.61** ± 0.98	−0.04** ± 0.01
		RR	17.43** ± 0.22	−12.58** ± 0.42	−0.06** ± 0.01
	LnA	MR	8.23** ± 0.17	−8.18** ± 0.51	−0.04** ± 0.01
		RR	8.12** ± 0.13	−6.78** ± 0.22	−0.07** ± 0.01
Maxwell 1 (MAX1)M32	OA	MR	−38.22** ± 2.57	−20.14** ± 2.42	−38.38** ± 8.49
		RR	−38.00** ± 1.13	−20.54** ± 1.06	−19.05** ± 2.55
	LA	MR	−2.03** ± 1.07	−15.61** ± 0.98	−25.02** ± 3.65
		RR	−4.85** ± 0.43	−12.58** ± 0.42	−16.37** ± 1.44
	LnA	MR	−0.07** ± 0.60	−8.15** ± 0.54	−23.67** ± 3.83
		RR	−1.36** ± 0.23	−6.76** ± 0.23	−14.92** ± 1.37

[a]Note: OA, oleic acid; LA, linoleic acid; LnA, alpha-linolenic acid. Treatments: raw- and micronized-flaked rapeseeds denoted as RR and MR, respectively. Parameters denoted as "*a*" stand for the maximum cumulative disappearance of fatty acids (g/100 g FA), "*b*" for rate constant (h^{-1}), and "*c*" for the lag phase (h).

established criteria and stands out as easy to implement (Table 5). Consequently, the subsequent progression of the results hinges on the application of the FOØM2 model. Furthermore, it is important to emphasize that employing a singular model for comparing diverse acid behaviors is advantageous. Such an approach allows for direct comparisons of coefficient magnitudes among different acids, offering insights into their relative impacts. It is worth noting that utilizing models with varying numbers of coefficients undermines this comparative capability.

3.2.2. Investigation of the Kinetic Behavior of Downward-Trending Fatty Acids. The behavior of downward-trending DTFA was investigated by using the selected model (M31). Model M31 effectively estimated the kinetic behavior of OA, LA, and LnA over time (Figure 5), consistently falling within the 95% prediction interval of the ruminal BH data observed in vitro. These estimates reflect the capabilities of the M14 model in elucidating the relationship between the observed and predicted values, describing the disappearance kinetics of DTFA. Although a few measured concentrations of OA, LA, and LnA exhibited slight deviations from the predicted values, the overall outcomes were deemed acceptable. Furthermore, the coefficient of determination affirmed the accuracy of the selected model (M31). The modeling outcomes revealed that micronized-flaked rapeseed consistently exhibited higher OA concentrations over time compared to raw-flaked rapeseed, implying a reduction in the ruminal disappearance of OA due to micronization (Figure 5a). At 4, 8, 12, 24, and 48 h of incubation, the disappearance of OA decreased in micronized-flaked rapeseed compared to raw-flaked rapeseed, with a more pronounced difference as the incubation time increased. In contrast, the disappearance of LA displayed a distinct behavior

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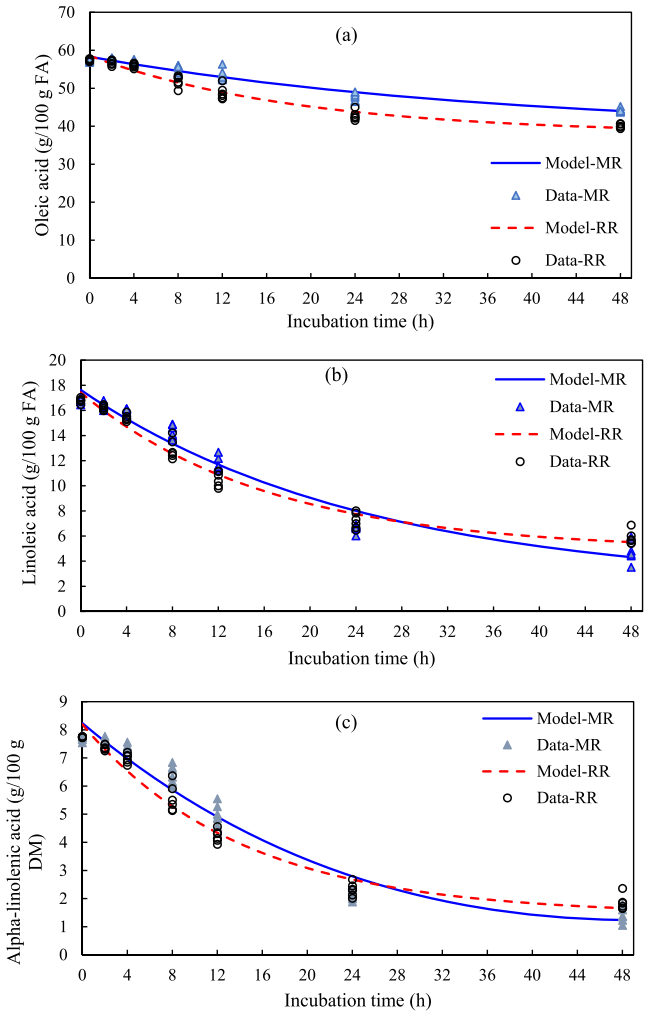

Figure 5. Nonlinear regression modeling of the dynamic behavior of DTFA including oleic acid (a), linoleic acid (b), and linolenic acid (c) during incubation. Treatments encompass raw- and micronized-flaked rapeseeds, denoted as RR and MR, respectively.

compared to OA (Figure 5b). The concentration of LA in raw- and micronized-flaked rapeseed exhibited an inverse relationship before the first 12 h, after which this relationship reversed. Although the disappearance of LA decreased during the initial 12 h of incubation for micronized-flaked rapeseed, raw flaked-rapeseed showed a higher concentration of LA shortly after 12 h. Similar trends were observed for the disappearance of LnA (Figure 5c). As illustrated, at 2, 4, 8, and 12 h of incubation, the disappearance of LnA was lower in micronized-flaked rapeseed compared to raw-flaked rapeseed. Conversely, the concentration of LnA decreased in micronized-flaked rapeseed after approximately 32 h. It seems that the reduced BH of LA, LnA, and OA observed in micronized-flaked rapeseed results from a reduced rate of rumen lipolysis due to protein denaturation in micronized seeds. It has been proven that heat treatments can denature the protein matrix surrounding the fat droplets and, therefore, protect UFA from rumen BH.[13,39] Consistent with our findings, Gonthier at al.[13] reported a reduction in BH of LA and LnA by the micronization of flaxseeds. The lower disappearance of LA and LnA in micronized-flaked rapeseed can confirm the reduction observed for the appearance of SA and VA.

3.2.3. Investigation of Downward-Trending Fatty Acid Disappearance Rates. The first-order derivative of the M31 model was employed to calculate the kinetic disappearance of DTFA. Figure 6 illustrates that the disappearance rate of OA

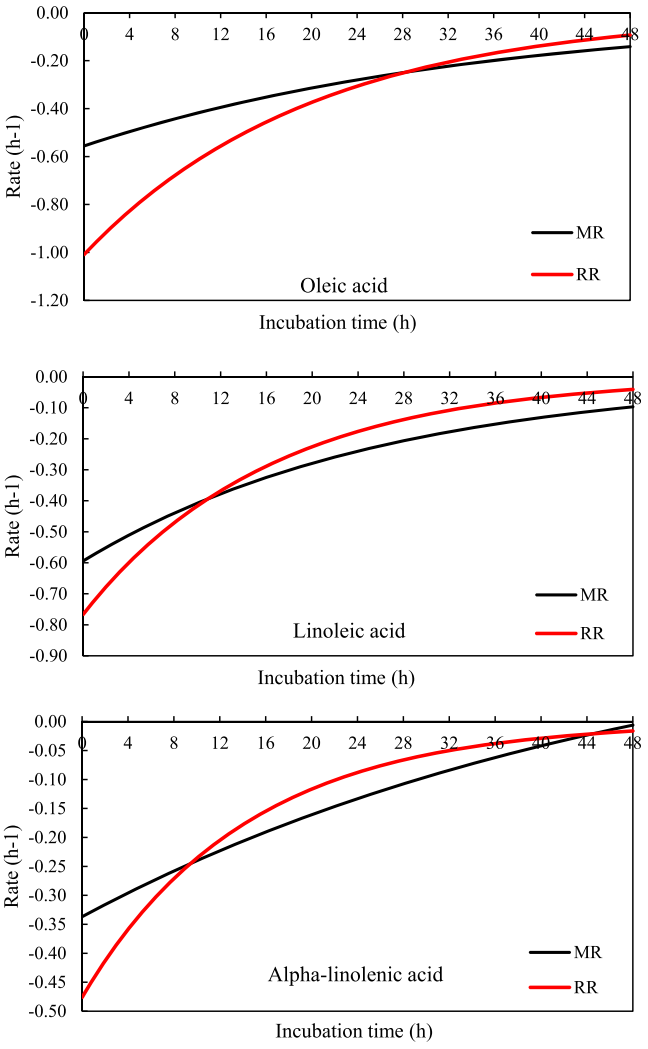

Figure 6. Rates of disappearance for DTFA encompassing oleic acid, linoleic acid, and alpha-linolenic acid during incubation. Treatments encompass raw- and micronized-flaked rapeseeds denoted as RR and MR, respectively.

increased over time, with a higher rate observed for raw-flaked rapeseed in comparison to micronized flaked-rapeseed. Furthermore, the slope of increasing the disappearance rate of OA was steeper for raw flaked-rapeseed than for micronized-flaked rapeseed over time. Similar patterns were identified for the disappearance rate of LA (Figure 6). Although the BH rate of LA disappearance was initially higher for micronized-flaked rapeseed than for raw flaked-rapeseed, this rate decreased with the increasing incubation time due to micronization. In contrast, the rate of FA disappearance for LnA exhibited a distinct behavior compared to OA and LA. At the 12 and 40 h marks of incubation, a decrease in the disappearance rate of LnA was observed in micronized-flaked rapeseed. It is well established that a high content of available UFA can be toxic to the function of rumen microorganisms, potentially more so than their BH intermediates.[40] Heat treatment can protect lipid droplets from rumen lipolysis by denaturing protein

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Table 6. Estimation of Ruminal Biohydrogenation Kinetics of Fatty Acids using Nonlinear Regression Models

objectives	FA type	selected model	reference
to identify the factors affecting the rates of lipolysis and BH in ruminal contents	LA and LnA	Ørskov and McDonald	20
to determine and compare in vitro ruminal biohydrogenation and bypass of linolenic and linoleic acids in timothy (Phleum pratense L.) harvested from different growth stages.	LA and LnA	Ørskov and McDonald	29
to ascertain the effects of extrusion of canola seeds on lag time and rate of BH of UFA	OA, LA, and LnA	Ørskov and McDonald	21
to quantify the effect of pH on BH rates	LA, LnA, and VA	Ørskov and McDonald	7
to study the effect of heat treatment on biohydrogenation of linoleic acid (LA) and linolenic acid (LNA) and formation of stearic acid (SA), cis-9, trans-11 conjugated LA (CLA), trans-10, cis-12 CLA, and trans-vaccenic acid (VA)	LA, LnA, and SA	Michaelis−Menten	11
to evaluate the effects of LA:LN ratio in lipid supplements on the rumen biohydrogenation kinetics of LA and LN, as well as on the trans-vaccenic acid (VA) production, using an in vitro system.	LA and LnA	a multicompartmental model	33
	VA	Gompertz model	
to develop an assay to assess in vivo rates, pathways, and extent of BH of oleic (OA), linoleic (LA), and α-linolenic acid (ALA).	OA, LA, and LnA	Ørskov and McDonald	42
to investigate the nature of the separate kinetic processes describing lipolysis and BH and to estimate the magnitude of the rate constants describing these processes	LA, SA, and VA	Michaelis−Menten kinetics	32
(1) to verify the reproducibility of the in vivo BH assay proposed by Baldin et al. (2018), (2) to directly compare the BH rates of individual UFA, and (3) to extend the analysis of BH using compartmental modeling.	OA, LA, and LnA	Ørskov and McDonald	42
to investigate in vitro rumen BH of LNA and LA, BH end product, and formation of CLA isomers from LNA and LA esterified to TG, PL, or CE.	LA, LnA, and SA	Michaelis−Menten	22
to comprehensively evaluate 33 nonlinear regression models, ultimately selecting the most suitable models to illustrate the changes in FA concentration throughout the in vitro rumen incubation of both raw- and micronized-flaked rapeseeds.	OA, LA, and LnA	Ørskov and McDonald	present study
	C18:1 trans-10, VA, and SA	Gompertz model	

barriers.[13] Therefore, to mitigate the toxic effect of OA, LA, and LnA, rumen BH occurred to a greater extent in raw-flaked rapeseed than in micronized ones. The lower rate and longer delay in the appearance of SA, VA, and C18:1 trans-10 occurred in micronized-flaked rapeseed, which showed the lower rate and longer delay in the disappearance of LA, LnA, and OA. The results indicated that micronization could serve as a practical approach to safeguard UFA against ruminal biohydrogenation, leading to an increase in the UFA bypass. Consequently, this approach could elevate the concentration of favorable UFA in dairy products.[39] By incorporating micronized-flaked rapeseed into the diets of ruminants, we could contribute to the enhancement of human health within society.

It should be acknowledged that these models were constructed based on in vitro data, and their performance might exhibit variability in in vivo or in situ methods. Thus, exercise of prudence is advised when extrapolating the outcomes of this study to alternative experimental circumstances. The outcomes of the present investigation can serve as a valuable foundation for forthcoming studies aimed at enhancing the precision of FA BH kinetic prediction across a broader spectrum of conditions.

3.3. Advances in the Estimation of Fatty Acid BH Kinetics. Table 6 displays the application of nonlinear regression models in estimating the ruminal BH kinetics of FA in various studies. The variability of UFA biohydrogenation, influenced by factors such as the FA source, oilseed type, UFA nature, and processing methods, underscores the significance of selecting suitable models that can quantitatively characterize the BH process. Baldwin et al.[41] proposed a dynamic model using a simple first-order kinetics to describe UFA BH dynamics in the rumen. However, the simplistic assumptions and aggregated nature of this dynamic model restrict the accuracy and scope of its predictions, considering the intricate pattern of ruminal BH. Troegeler-Meynadier et al.[30] estimated the kinetic parameters of UFA biohydrogenation by incorporating a fraction escaping BH into the exponential model of Ørskov and McDonald.[31] Similarly, Ribeiro et al.[7] introduced a dynamic model for in vitro BH of UFA in alfalfa. Their model encompasses a combination of lipolysis and BH, as it does not distinctly model the kinetics of lipolysis and BH. Within this model, first-order kinetics were employed to describe the net BH of individual UFA. Nevertheless, the model presented by Ribeiro et al.[7] lacks nonlinear rate constants necessary for accurately describing the potential accumulation of vaccenic acid (VA) in the presence of substrates with high concentrations of linoleic acid (LA) or alpha-linolenic acid (LnA). Lashkari et al.[22] emphasized the dose-independent nature of the kinetic constants in the Michaelis−Menten model, making it useful in estimating the kinetic parameters in samples with varying UFA concentrations. Furthermore, Lashkari et al.[11] demonstrated the effectiveness of the Michaelis−Menten model in predicting the BH kinetics of initial FA disappearance, as well as the emergence of intermediates and saturated FA.

However, the adoption of a regression model to predict the behavior of FAs with differing BH patterns may not possess the requisite competence to effectively accommodate the observed BH data. In this study, a comprehensive array of nonlinear regression models was examined to identify the models that accurately delineated the behavior of individual FAs, an essential consideration overlooked in prior research. The utilization of distinct models for a more precise representation of BH kinetics offers valuable insights into the complex dynamics of individual FAs implicated in the rumen BH.

4. CONCLUSIONS

In this study, we conducted a thorough investigation into the behavior of various FA, aiming to elucidate their in vitro ruminal BH kinetics. Leveraging the power of nonlinear regression models, we scrutinized thirty-three candidate

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models to identify the most fitting ones for our purpose. Our findings demonstrate that the Gompertz model (GOM2) emerges as a robust choice for accurately estimating the BH kinetics of UTFA, encompassing intermediates such as UFA and saturated end FA. Conversely, the first-order kinetic model of Ørskov and McDonald (FOØM2) stands out as the preferred model for investigating the BH kinetics of DTFA, specifically OA, LA, and LnA.

Beyond these model selections, our study extends its contribution by consolidating a comprehensive repository of nonlinear regression models scattered across various research sources. These models exhibit the potential for a close fit with the complex behaviors of FA, providing valuable tools for future research endeavors. The meticulous evaluation and selection process presented herein equip researchers with a dependable set of established nonlinear regression models for a precise determination of FA BH kinetics. This standardized approach promises enhanced precision and comparability across studies, offering a consistent framework for calculating FA BH kinetics in various experimental contexts.

In conclusion, our research not only advances our understanding of FA behavior but also provides a valuable resource for researchers in the field. We invite further exploration and utilization of these models to enhance the depth and accuracy of future investigations of ruminal BH kinetics.

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Notes

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NOMENCLATURES

BH	biohydrogenation
FA	fatty acids
UTFA	upward-trending fatty acids
UFA	unsaturated fatty acids
DTFA	downward-trending fatty acids
OA	oleic acid
PUFA	polyunsaturated fatty acids
LA	linoleic acid
NLS	nonlinear least-squares estimation
LnA	alpha-linolenic acid
RMSE	root-mean-square error
SA	stearic acid
R^2	coefficient of determination
VA	vaccenic acid

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