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Factors associated with the content of mammary-synthesized fatty acids in milk fat: A meta-analysis

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ABSTRACT

Consumption of specific fatty acids (FA) that are synthesized in the mammary gland, namely de novo FA, has implications for human health. The objective of the present meta-analysis was to study the associations between milk fat content of de novo FA, with (1) diet composition, and (2) milk production and composition. Milk FA data from 96 peer-reviewed studies published between 1990 and 2016 that included 324 treatment means from 83 bovine experiments, 36 treatment means from 12 caprine experiments, and 40 treatment means from 12 ovine experiments were used in this analysis. Individual species models including the fixed effect of experiment were fitted using multiple regression to explain milk content of de novo FA as a function of diet composition and milk production and composition variables. We also evaluated replacing the effect of the experiment by the effect of the experiment nested in the laboratory at which the research had been conducted, and the effect of the laboratory. Butyric acid content in milk fat was positively but weakly related to dietary ether extract in does and ewes. Lauric, myristic, and palmitic acid contents in milk fat were negatively related to dietary ether extract in does and to a somewhat lesser extent in cows and ewes. The results confirm that the inclusion of lipids in the diet may not only affect the availability of preformed FA but also the profile of FA synthesized de novo in the mammary gland. Most of the variation in all prediction models was explained by the experiment or by the laboratory if the latter was included in the model. The ample variation in analytical methods reported by the different research groups suggests that differences in analytical protocols might explain a substantial proportion of the variation in de novo FA profile. A main conclusion of this study is the potential influence of differences in analytical procedures to explain the variation in de novo FA profile. Standardization of methods of FA analysis to improve reproducibility seems to be an aspect of importance to this area of research.
Key words: fatty acid, milk, de novo, meta-analysis

INTRODUCTION

Ruminant milk fatty acids (**FA**) originate from preformed sources (diet and mobilized adipose tissue) and from de novo synthesis in the mammary gland. Major substrates for de novo FA synthesis are acetate and BHB, whereas end products of de novo synthesis include milk FA ≤14 carbons in length, and about half of 16 carbon FA (Emery, 1973). Effects of milk FA synthesized de novo in the mammary gland on human health are related to FA chain length. Historically, the longest de novo synthesized FA [i.e., lauric (12:0), myristic (14:0), and palmitic (16:0) acids] have been identified as cholesterolemic FA in milk (Aro et al., 1997; Ashes et al., 1997), although more recent evidence (Praagman et al., 2016) has suggested that the effects of C12:0, C14:0, and C16:0 on the incidence of cardiovascular disease could differ. Due to the new insights of the effects on cardiovascular health, it is also of interest to explore and understand the variables that influence milk fat content of C12:0, C14:0, and C16:0 in the major dairy production species.

At the same time, a negative association between consumption of short- and medium-chain FA (C4:0 through C10:0) and the incidence of ischemic heart disease has been reported (Praagman et al., 2016). Thus, given that in general shorter de novo synthesized FA appear to be more beneficial to human health than the longer members C12:0, C14:0, and C16:0, it is of interest to understand the factors influencing the average length of de novo mammary-synthesized FA.

In particular, butyric acid (C4:0), the shortest member of milk FA, has been attributed to have nutraceu-

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tical properties exerted both on the gastrointestinal tract and on postabsorptive metabolism (Guilloteau et al., 2010a). Evidence suggests that C4:0 can prevent colorectal cancer, improve the immune and oxidative status, stimulate colonocyte proliferation, and be involved in trans-epithelial fluid and ion absorption (Hamer et al., 2008). At the postabsorptive level, C4:0 has been shown to decrease insulin resistance, inhibit cholesterol synthesis, increase energy expenditure and FA oxidation, stimulate lean tissue deposition, and inhibit body fat accretion (Canfora et al., 2015). In young animals, dietary administration of C4:0 has been shown to promote performance and health, as shown in piglets (Kotunia et al., 2004; Manzanilla et al., 2006) and calves (Guilloteau et al., 2009, 2010b; Gorka et al., 2011). Given the nutritional qualities of butyric acid, we studied the variables associated with butyric acid content in bovine, ovine, and caprine milk fat.

Coefficients of variation of 21.7, 26.8, 29.9, 29.4, 28.4, 16.4, and 17.4% were reported for concentration of C4:0, C6:0, C8:0, C10:0, C12:0, C14:0, and C16:0 in bovine milk fat, respectively (Moate et al., 2007), which suggests that there might be opportunities to alter their content in milk fat if factors that affect them are understood. Less has been investigated about the most influential factors on ovine and caprine milk fat composition.

Previous meta-analyses of bovine milk FA profile have studied associations to diet composition (Moate et al., 2008) and oilseed supplementation (Glasser et al., 2008; Leduc et al., 2017; Meignan et al., 2017). The objective of the present meta-analysis was to examine dietary and animal performance variables associated with milk fat content of C4:0, C12:0, C14:0, and C16:0, as well as the average length of de novo synthesized FA in the mammary gland (**AVDN**), and to extend previous work to ovine and caprine milk fat in addition to bovine. Also, in the present analysis we found that the experiment effect in the meta-regressions could be explained by the research group conducting the study, which led us to examine the association between milk FA profile and the laboratory methods of FA analysis.

MATERIALS AND METHODS

Database

We searched the pubmed.gov online database for peer-reviewed articles published until 2012 using the following Boolean string: (cow OR dairy OR ewe OR sheep OR goat OR doe) AND milk AND fatty acids. We then discarded (1) experiments conducted with Latin square or cross-over designs, which would have fewer animals and smaller standard error of the mean than randomized plots or randomized blocks; and (2) experiments not reporting butyrate, which was of primary interest in this work. Some scientific articles reporting bovine, ovine, or caprine milk FA profile and published in 2013, 2014, and 2015 were then added unsystematically. The final database of milk FA profile included 96 peer-reviewed studies published between 1990 and 2015 was compiled (Supplemental Tables S1, S2, S3, and S4 and Supplemental Database References; https://doi.org/10.3168/jds.2018-15157). The database included a total of 400 treatment means in 107 experiments. A total of 324 treatment means corresponded to 83 bovine experiments, 36 treatment means corresponded to 12 caprine experiments, and 40 treatment means corresponded to 12 ovine experiments. Means, standard deviations, and ranges for the regressors and response variables of the experiments in the present analysis are provided in Table 1.

Multiple Correlations

Multiple Pearson correlations were conducted among the content of C4:0, C6:0, C8:0, C10:0, C12:0, C14:0, and C16:0 in total milk FA by ruminant species.

Regressions

The responses of interest were C4:0, C12:0, C14:0, and C16:0 (all in mg/100 mg of total FA), and AVDN (expressed in average carbon atoms per FA molecule). Contents of C4:0, C12:0, C14:0, and C16:0 were expressed in terms of FA profile (g/100 g of total FA excluding unidentified FA) rather than as absolute content in milk because the long-term objective of this area of research is regarded as generating knowledge about the factors that relate to the content of de novo FA in milk fat rather than total content of each de novo FA in milk, which would be very strongly influenced by milk fat content. Average chain length of saturated de novo synthesized FA was calculated as the sum of milk content of each SFA $\leq$14 carbon atoms multiplied by its number of carbon atoms, plus half of C16:0 content multiplied by 16, all divided by the total amount of SFA of 14 carbon atoms or less, plus half of 16:0:

$$\text{AVDN} = \left[\sum_{i=4}^{i=14}\left(\text{C}i{:}0\times i\right)+\text{C16:0}\times{}'16\div{}'2\right]\Bigg/\left(\sum_{i=4}^{i=14}\text{C}i{:}0+\text{C16:0}\div 2\right),$$

where Ci:0 is the content of SFA i with i carbons in total milk FA, multiplied by its number of carbons i.

Regressions were conducted separately within each species to identify different independent variables as-

sociated with the responses of interest; because no study in our database included experiments comparing species, a species term in the model would have been co-linear with the experiment effect.

In each regression by species, regressors were grouped as follows:

(1) diet composition variables [CP, NDF, ether extract (**EE**), and concentrate, all expressed as %DM]; and
(2) production variables [milk production (kg), and fat, protein, and lactose content in milk (g/100 g)].

We did not attempt to model the ruminant species effect (bovine, caprine, or ovine) as it was 100% co-linear with the experiment effect (i.e., no controlled experiments in our database compared species under the same conditions). However, we conducted a principal component analysis of the content of C4:0, C6:0, C8:0, C10:0, C12:0, C14:0, and C16:0 in total milk FA, labeling each treatment mean by species.

Likewise, we did not model the effects of genetic and physiological variables such as breed, DIM, number of calving, or milking frequency on response variables because few controlled experiments evaluated the effects of those variables on milk FA profile; hence, genetic and physiological variables were largely co-linear with the experiment effect.

The fixed effect of the experiment was included in all models. The experiment was included as a fixed, rather than as a random, effect, because the main objective of the analysis was to understand the observed variation in de novo FA profile, rather than predicting the outcomes of new experiments. Linear and quadratic terms of all quantitative regressors were initially available for inclusion. Models were fitted using a backward stepwise multiple regression with a threshold to leave the model of $P \geq 0.05$ and with the experiment effect included as a locked in term. If a quadratic term was significant, its corresponding linear term was left in the model. Partial R^2 were calculated from sequential type I sums of squares with the experiment effect appearing previous to all other effects in all models. Treatment means were weighted by the reciprocal of their standard errors scaled to 1 (Sauvant et al., 2008). The reciprocal of the standard errors of AVDN were calculated as the reciprocal of the mean standard errors of C4:0, C6:0, C8:0, C10:0, C12:0, C14:0, and C16:0.

When EE was significant in the diet composition models for bovine milk FA, the effect of the FA composition of the dietary EE fraction was further analyzed. A stepwise mixed backward regression was conducted, including the experiment as a fixed effect and the dietary percentage in the DM of C16:0, C18:0, and C18:1 (all isomers reported), C18:2 (all isomers reported), and C18:3 (all isomers reported). Linear and quadratic terms of the content of each C16:0, C18:0, C18:1, C18:2,

Table 1. Multiple Pearson correlation coefficients among contents of individual fatty acids in bovine, caprine, and ovine milk total fatty acids[1]

	Fatty acid					
Item	C4:0	C6:0	C8:0	C10:0	C12:0	C14:0
Bovine						
C6:0	0.80***					
C8:0	0.61***	0.79***				
C10:0	0.54***	0.72***	0.70***			
C12:0	0.38***	0.55***	0.56***	0.82***		
C14:0	0.17**	0.35***	0.41***	0.69***	0.83***	
C16:0	0.018	0.17**	0.23***	0.31***	0.48***	0.60***
Caprine						
C6:0	0.85***					
C8:0	0.62***	0.85***				
C10:0	0.65***	0.84***	0.86***			
C12:0	0.36*	0.38*	0.24	0.58***		
C14:0	0.058	−0.013	−0.12	0.17	0.76***	
C16:0	−0.16	−0.36*	−0.44**	−0.17	0.55***	0.75***
Ovine						
C6:0	0.52***					
C8:0	0.057	0.87***				
C10:0	0.031	0.68***	0.76***			
C12:0	−0.25	0.52***	0.76***	0.93***		
C14:0	−0.37*	0.14	0.38*	0.60***	0.77***	
C16:0	−0.34*	−0.025	0.20	0.30†	0.52***	0.83***

[1]C4:0, butyric acid; C12:0, lauric acid; C14:0, myristic acid; C16:0, palmitic acid.
***$P < 0.001$; **$P < 0.01$; *$P < 0.05$; †$P < 0.10$.

and C18:3 in total dietary FA were initially available for inclusion, and the P value to leave the model was set at $P \geq 0.05$. This analysis was conducted only in the bovine species due to the limited number of data available on dietary FA composition for the caprine and ovine species. Dietary contents of main FA C:16:0, C18:0, and C18:1 (total isomers), C18:2 (total isomers), and C18:3 (total isomers) in the bovine species are shown in Supplemental Table S5 (https://doi.org/10.3168/jds.2018-15157).

Effects of the Laboratory and Analytical Methods

All models reported include the partial R^2 of the experiment effect. The laboratory (i.e., location and research group that conducted each experiment) was also included in the database. To understand how much of the experiment effect could be ascribed to the laboratory where the experiment had been conducted, we conducted a second regression for each response variable, in which the experiment effect was replaced by the effect of the experiment nested in the laboratory, the fixed effect of the laboratory, in that order, and all significant regressors included in each of the models previously fitted. Sequential type I sums of squares were then obtained and the partial R^2 of the experiment nested in the laboratory and of the laboratory effect were calculated and compared, to understand to what extent the experiment effect was associated with the laboratory at which the experiment had been conducted.

An association between a response variable and the laboratory at which the experiment was conducted can be due to common biological factors linked to the location, such as animal, diet, climate, and so on, or to technical factors associated with the laboratory, such as the analytical methods employed. To understand how much of the laboratory effect could be associated with variation in methods of chemical analysis of FA, backward stepwise regression was used to build models using variables of the different steps of sample preparation for FA analyses: centrifugation (yes or no), freeze-drying (yes or no), filtration (yes or no), type of solvent used in extraction (methanol, chloroform, water, ammonium, diethyl ether, petrol ether, hexane, hydrochloric acid, n-pentane, isooctane, ethanol, n-propanol, isopropanol, or combinations of more than one), methylation method (in acid, in base, or both), and sample purification using thin layer chromatography (yes or no). These regressions conducted to study the effect of sample preparation procedures did not include the experiment or laboratory effects. Because extraction was most often conducted using more than one solvent, the different solvents were included in the regressions as yes or no dummy variables (Supplemental Table S6; https://doi.org/10.3168/jds.2018-15157). Probability to leave the model was set at $P \geq 0.05$.

Analyses of Residuals, Outliers, and Influential Treatment Means

We examined homoscedasticity through residual against predicted plots, and the assumption of residuals normality through residual normality plots. Outliers were identified as those treatment means whose absolute value of studentized residuals was greater than $t_{N-k-1,\,0.95}$, with k being the number of parameters and N the number of treatment means. Influential treatment means were identified as those with a leverage value larger than $2k/N$ (Belsey et al., 1980). Experiments containing outliers or influential treatment means (or both) were deleted one at a time and regressions refitted in their absence. If the conclusions of the analysis changed after the deletion of experiments containing outliers or influential treatment means (significant effects became nonsignificant or vice versa, or the direction of the response changed), the results are presented and discussed both with and without the experiments containing the outliers or influential observations (or both).

The software JMP 12.2.0 (SAS Institute Inc., Cary, NC) was used for all statistical analyses.

RESULTS

Multiple Correlations Between Milk Fatty Acids

Associations between de novo synthesized FA varied somewhat depending on the ruminant species. In cows almost all the novo synthesized FA associated positively with each other, whereas in goats C16:0 was negatively associated with C6:0 and C8:0. In sheep, negative associations were observed between C4:0 and C12:0, C14:0, and C16:0 (Table 1). In all species, FA differing in 1 or 2 pairs of carbon generally exhibited the greatest degree of association.

Association Between Milk Fatty Acid Profile and Ruminant Species

Cow milk fat differed from sheep and goat because of greater content of C6:0, C8:0, C10:0, and C12:0 in the latter (Figure 1).

Factors Related to Butyric Acid Content in Milk Fat

In the cow, butyric acid content in milk total FA was not explained by any of the diet composition variables

Table 2. Regression models relating diet composition and performance variables with butyric acid (C4:0) content in bovine, caprine, and ovine milk fat (mg/100 mg of fatty acids)

Type of variables	Species	First model including the experiment (Exp) effect[1]	Partial R^2 (R^2_p) of model including the laboratory (Lab) effect[2]	
			Exp(Lab)[1]	Lab[2]
Diet composition	Bovine	$y = 3.53$ (±0.041; $P < 0.001$) + Exp ($P < 0.001$; $R^2_p = 0.90$); $R^2 = 0.90$ ($P < 0.001$); n = 180 (46 experiments)	0.32	0.58
	Caprine	$y = 2.07$ (±0.077; $P < 0.001$) + Exp ($P < 0.001$; $R^2_p = 0.95$) + 0.078 (±0.019; $P < 0.001$; $R^2_p = 0.025$) ether extract; $R^2 = 0.98$ ($P < 0.001$); n = 25 (6 experiments)	0.024	0.93
	Ovine	$y = 3.65$ (±0.11; $P < 0.001$) + Exp ($P < 0.001$; $R^2_p = 0.87$) + 0.068 (±0.023; $P = 0.008$; $R^2_p = 0.035$) ether extract; $R^2 = 0.91$ ($P < 0.001$); n = 33 (9 experiments)	0.38	0.49
Performance	Bovine	$y = 4.94$ (±0.90) + Exp ($P < 0.001$; $R^2_p = 0.97$) + 0.024 (±0.0063; $P < 0.001$; $R^2_p = 0.000011$) milk (kg/d) + 0.41 (±0.045; $P < 0.001$; $R^2_p = 0.0085$) %fat – 0.079 (±0.027; $P = 0.004$; $R^2_p = 0.00020$) (%fat – 3.88)^2 – 0.83 (±0.20; $P < 0.001$; $R^2_p = 0.0020$) %lactose; $R^2 = 0.98$ ($P < 0.001$); n = 212 (55 experiments)	0.18	0.79
	Caprine	$y = 2.22$ (±0.059; $P < 0.001$) + Exp ($P < 0.001$; $R^2_p = 0.86$); $R^2 = 0.86$ ($P < 0.001$); n = 30 (10 experiments)	0.022	0.84
	Ovine	$y = 3.90$ (±0.066; $P < 0.001$) + Exp ($P < 0.001$; $R^2_p = 0.87$); $R^2 = 0.87$ ($P < 0.001$); n = 25 (6 experiments)	0.18	0.69

[1]Model including the experiment but not the laboratory effect.

[2]Model including the effect of the experiment nested in the laboratory and the effect of the laboratory.

evaluated, but was positively related to dietary EE in does ($P < 0.001$) and ewes ($P = 0.008$; Table 2).

Butyric acid content in the bovine total milk FA was positively associated with milk production ($P < 0.001$), negatively associated with lactose content in milk ($P < 0.001$), and had a positive quadratic relationship with milk fat content (with a theoretical maximum at 6.47% ($P = 0.004$; Table 2). Butyric acid content in total milk FA was not associated with milk production or composition in the caprine or ovine species (Table 2).

In all 3 species, more than 85% of variation in butyric acid content in milk total FA was not explained by dietary and performance variables but by the experiment effect. However, the experiment lost most of its importance as an explanatory variable with the inclusion of the laboratory in the model (Table 2). Twelve different variables related to sample preparation explained together 35% of variation in the measured content of butyric acid in milk total FA. The 3 most important explanatory variables were extraction with n-pentane, hexane, and hydrochloric acid (not shown). Extraction with n-pentane and hexane was associated with more butyric acid in total FA, and conversely, extraction with hydrochloric acid was associated with less butyric acid in total FA (Table 3).

Factors Related to Lauric Acid Content in Milk Fat

The content of lauric acid in milk total FA was negatively associated with dietary EE both in the bovine and in the caprine ($P < 0.001$; Table 4). Lauric acid content in bovine total milk FA decreased linearly as dietary palmitic ($P < 0.005$) and stearic acid increased

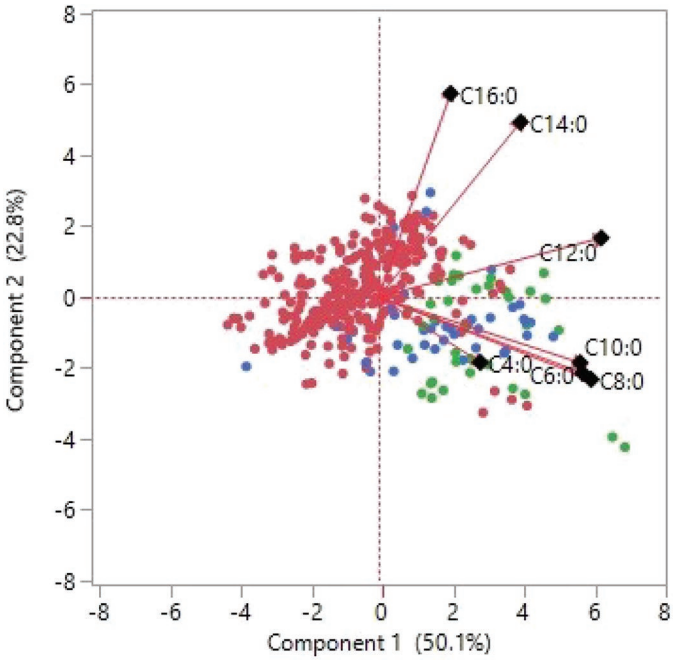

Figure 1. Principal components of the main de novo milk fatty acids butyric acid (C4:0), caproic acid (C6:0), caprylic acid (C8:0), capric acid (C10:0), lauric acid (C12:0), myristic acid (C14:0), and palmitic acid (C16:0). Red dots depict cow milk fat treatment means, blue dots depict sheep milk, and green dots depict goat milk.

Table 3. Regression models relating the contents of butyric, lauric, myristic, and palmitic acids in milk fat (mg/100 mg of fatty acids) with steps of sample preparation for fatty acid analysis[1]

Response	Final model[2]
Butyric acid	$y = 3.57$ (±0.30; $P < 0.001$) – 0.31 (±0.072; $P < 0.001$; $R^2_p = 0.063$) centrifugation(no) – 0.18 (±0.078; $P = 0.019$; $R^2_p = 0.0082$) chloroform(no) – 0.34 (±0.099; $P < 0.001$; $R^2_p = 0.0037$) ethanol(no) + 0.62 (±0.16; $P < 0.001$; $R^2_p = 0.050$) hydrochloric acid – 0.69 (±0.23; $P = 0.003$; $R^2_p = 0.030$) isooctane(no) + 0.31 (±0.061; $P = 0.003$; $R^2_p = 0.053$) acid methylation(no); n = 400; $R^2 = 0.37$ ($P < 0.001$)
Lauric acid	$y = 2.85$ (±0.60; $P < 0.001$) + 0.25 (±0.072; $P < 0.001$; $R^2_p = 0.046$) chloroform(no) – 0.21 (±0.092; $P = 0.024$; $R^2_p = 0.0045$) water(no) + 0.68 (±0.10; $P < 0.001$; $R^2_p = 0.0058$) ethanol(no) – 0.62 (±0.072; $P < 0.001$; $R^2_p = 0.11$) diethyl ether(no) + 1.20 (±0.54; $P = 0.027$; $R^2_p = 0.0084$) petrol ether(no) – 1.19 (±0.23; $P < 0.001$; $R^2_p = 0.057$) hydrochloric acid(no) + 0.28 (±0.14; $P = 0.014$; $R^2_p = 0.0087$) propanol(no); n = 400; $R^2 = 0.27$ ($P < 0.001$)
Myristic acid	$y = 10.9$ (±0.19; $P < 0.001$) – 1.40 (±0.51; $P = 0.007$; $R^2_p = 0.0015$) methanol(no) + 1.46 (±0.52; $P = 0.005$; $R^2_p = 0.020$) chloroform(no) – 0.60 (±0.17; $P = 0.001$; $R^2_p = 0.030$); n = 400; $R^2 = 0.046$ ($P < 0.001$)
Palmitic acid	$y = 31.7$ (±1.24; $P < 0.001$) – 0.85 (±0.33; $P = 0.001$; $R^2_p = 0.037$) methanol(no) – 1.53 (±0.44; $P < 0.001$; $R^2_p = 0.019$) water(no) – 1.99 (±0.39; $P < 0.001$; $R^2_p = 0.057$) ethanol(no) + 2.84 (±1.13; $P = 0.012$; ; $R^2_p = 0.0013$) hydrochloric acid(no) – 2.01 (±0.46; $P < 0.001$; $R^2_p = 0.025$) isooctane – 0.73 (±0.27; $P = 0.007$; $R^2_p = 0.048$) acid methylation(no) + 2.11 (±0.59; $P < 0.001$; $R^2_p = 0.022$) base methylation(no) – 1.17 (±0.55; $P = 0.03$; $R^2_p = 0.0094$) thin layer chromatography (no); n = 400; $R^2 = 0.15$ ($P < 0.001$)
Average chain length of de novo synthesized fatty acids	$y = 12.9$ (±0.29; $P < 0.001$) + 0.13 (±0.032; $P < 0.001$; $R^2_p = 0.21$) centrifugation(no) + 0.14 (±0.036; $P = 0.018$; $R^2_p = 0.0082$) freeze-drying(no) – 0.44 (±0.13; $P < 0.001$; $R^2_p = 0.0070$) methanol(no) + 0.40 (±0.13; $P = 0.003$) chloroform(no) – 0.23 (±0.049; $P < 0.001$) ethanol(no) + 0.26 (±0.033; $P < 0.001$; $R^2_p = 0.098$) diethyl ether(no) – 0.91 (±0.27; $P < 0.001$; $R^2_p = 0.014$) petrol ether(no) + 0.51 (±0.096; $P < 0.001$; $R^2_p = 0.034$) hydrochloric acid(no) – 0.16 (±0.025; $P < 0.001$; $R^2_p = 0.065$) acid methylation(no); n = 400; $R^2 = 0.42$ ($P < 0.001$)

[1]Including all 3 ruminant species studied.

[2]R^2_p = partial R^2 for each regressor. (no) indicates that the procedure described for the corresponding regressor coefficient was not conducted for preparing the sample.

Table 4. Regression models relating diet composition and performance variables with lauric acid (C12:0) content in bovine, caprine, and ovine milk fat (mg/100 mg of fatty acids)

			Partial R^2 (R^2_p) of model including the laboratory (Lab) effect[2]	
Type of variables	Species	First model including the experiment (Exp) effect[1]	Exp(Lab)[1]	Lab[2]
Diet composition	Bovine	$y = 6.16$ (±0.62; $P < 0.001$) + Exp ($P < 0.001$; $R^2_p = 0.48$) − 0.036 (±0.016; $P = 0.029$; $R^2_p = 0.0049$) NDF − 0.38 (±0.042; $P < 0.001$; $R^2_p = 13.9$) ether extract; $R^2 = 0.78$; n = 180 (46 experiments)	0.22	0.14
	Caprine	$y = 6.48$ (±0.24; $P < 0.001$) + Exp ($P = 0.005$; $R^2_p = 0.26$) – 0.49 ether extract (±0.061; $P < 0.001$; $R^2_p = 0.59$); $R^2 = 0.85$ ($P < 0.001$); n = 25 (6 experiments)	0.011	0.25
	Ovine	$y = 6.54$ (±0.90; $P < 0.001$) + Exp ($P < 0.001$; $R^2_p = 0.33$) + 0.015 (±0.012; $P = 0.20$; $R^2_p = 0.023$) %concentrate – 0.0011 (±0.00028; $P = 0.001$; $R^2_p = 0.27$) (%concentrate – 66.3)^2; $R^2 = 0.62$ ($P < 0.001$); n = 33 (9 experiments)	0.15	0.18
Performance	Bovine	$y = -3.48$ (±1.11; $P = 0.002$) + Exp ($P < 0.001$; $R^2_p = 0.67$) + 0.33 (±0.13; $P = 0.010$; $R^2_p = 0.017$) %fat – 0.40 (±0.092; $P < 0.001$; $R^2_p = 0.043$) (%fat – 3.73)^2 + 1.74 (±0.36; $P < 0.001$; $R^2_p = 0.025$) %protein – 2.26 (±0.64; $P < 0.001$; $R^2_p = 0.019$) (%protein – 3.20)^2; $R^2 = 0.77$ ($P < 0.001$); n = 212 (55 experiments)	0.17	0.49
	Caprine	$y = 4.53$ (±0.14; $P < 0.001$) + Exp ($P = 0.25$; $R^2_p = 0.39$); $R^2 = 0.39$ ($P = 0.25$); n = 30 (10 experiments)	0.0086	0.38
	Ovine	$y = -15.1$ (±4.21; $P = 0.002$) + Exp ($P < 0.001$; $R^2_p = 0.44$) + 3.88 (±0.85; $P < 0.001$; $R^2_p = 0.31$) %protein; $R^2 = 0.75$ ($P < 0.001$); n = 25 (6 experiments)	0.12	0.33

[1]Model including the experiment but not the laboratory effect.

[2]Model including the effect of the experiment nested in the laboratory and the effect of the laboratory.

($P = 0.001$; Supplemental Table S7; https://doi.org/10.3168/jds.2018-15157).

In the ovine, the content of lauric acid in milk total FA was quadratically associated with dietary concentrate with a theoretical maximum at 59% ($P = 0.013$; Table 4), although the relationship became negative and linear ($P = 0.020$) if the study by Gomez-Cortes et al. (2009), which had influential treatment means, was removed (not shown).

In the bovine, a quadratic association was observed between milk fat ($P < 0.001$) and milk protein ($P < 0.001$) with lauric acid content in milk total FA (Table 4). Content of lauric acid in milk total FA was not associated with the milk composition variables studied in the caprine ($P > 0.05$), and was positively associated with milk protein percentage in the ovine ($P < 0.001$).

In all 3 species, a large percentage of the model variation was accounted for by the experiment effect. However, the percentage of the model variation accounted for by the experiment greatly decreased if the laboratory was introduced in the model (Table 4). Eight sample preparation variables explained 27% of variation in measured content of lauric acid in milk total FA (Table 3), the most important of which were extraction with diethyl ether and ethanol (not shown).

Factors Related to Myristic Acid Content in Milk Fat

In the bovine ($P < 0.001$), caprine ($P < 0.001$), and ovine ($P = 0.011$), a negative association was observed between the content of myristic acid in milk total FA and dietary EE, the regression coefficients being numerically similar in the bovine and caprine (Table 5). Quadratic decreases occurred in C14:0 in total bovine milk FA to dietary increases in C16:0 ($P = 0.011$), C18:1 ($P = 0.015$), and C18:2 ($P = 0.010$; Supplemental Table S7; https://doi.org/10.3168/jds.2018-15157).

In bovine milk, there was a quadratic relationship between the content of myristic acid in total FA and milk fat ($P < 0.001$) and protein ($P < 0.001$) contents, with a maximum at 4.25% fat and 3.35% protein (Table 5). The content of myristic acid in total FA in caprine and ovine milk was not explained by any of the milk composition variables examined ($P > 0.10$; Table 5).

A large percentage of the myristic acid model's variation was explained by the experiment; and in the bovine and caprine, most of the variation was explained by the laboratory effect, if the laboratory was included in the model (Table 5). Extraction with methanol ($P = 0.015$) and chloroform ($P = 0.009$), and acid methylation ($P = 0.001$), together explained 4.6% of variation in measured myristic acid content in milk FA (Table 3).

Factors Related to Palmitic Acid Content in Milk Fat

In bovine milk, a quadratic relationship was present between the content of palmitic acid in milk total FA and the percentage of concentrate in the diet ($P < 0.001$) and a negative linear relationship ($P < 0.001$) with dietary EE (Table 6). Content of C16:0 in total

Table 5. Regression models relating diet composition and performance variables with myristic acid (C14:0) content in bovine, caprine, and ovine milk fat (mg/100 mg of fatty acid)

			Partial R^2 (R^2_p) of model including the laboratory (Lab) effect[2]	
Type of variables	Species	First model including the experiment (Exp) effect[1]	Exp(Lab)[1]	Lab[2]
Diet composition	Bovine	$y = 14.0$ (±0.40; $P < 0.001$) + Exp ($P < 0.001$; $R^2_p = 0.39$) − 0.80 (±0.080; $P < 0.001$; $R^2_p = 0.16$) ether extract; $R^2 = 0.79$; n = 180 (46 experiments)	0.15	0.49
	Caprine	$y = 12.9$ (±0.28; $P < 0.001$) + Exp ($P = 0.015$; $R^2_p = 0.44$) – 0.79 ether extract (±0.072; $P < 0.001$; $R^2_p = 0.49$); $R^2 = 0.93$ ($P < 0.001$); n = 25 (6 experiments)	0.0040	0.43
	Ovine	$y = 11.6$ (±0.49; $P < 0.001$) + Exp ($P = 0.003$; $R^2_p = 0.46$) – 0.31 (±0.11; $P = 0.011$; $R^2_p = 0.16$) ether extract; $R^2 = 0.66$ ($P = 0.004$); n = 33 (9 experiments)	0.44	0.020
Performance	Bovine	$y = 0.033$ (±2.19; $P = 0.99$) + Exp ($P < 0.001$; $R^2_p = 0.61$) + 0.46 (±0.26; $P = 0.075$; $R^2_p = 0.011$) %fat – 0.98 (±0.19; $P < 0.001$; $R^2_p = 0.080$) (%fat – 3.79)^2 + 2.81 (±0.71; $P < 0.001$; $R^2_p = 0.020$) %protein – 4.18 (±1.34; $P < 0.001$; $R^2_p = 0.017$) (%protein – 3.22)^2; $R^2 = 0.74$ ($P < 0.001$); n = 202 (55 experiments)	0.12	0.49
	Caprine	$y = 9.93$ (±0.20; $P < 0.001$) + Exp ($P < 0.001$; $R^2_p = 0.71$); $R^2 = 0.71$ ($P < 0.001$); n = 30 (10 experiments)	0.0018	0.71
	Ovine	$y = 10.2$ (±0.21; $P < 0.001$) + Exp ($P = 0.032$; $R^2_p = 0.50$); $R^2 = 0.50$ ($P = 0.032$); n = 25 (6 experiments)	0.47	0.028

[1]Model including the experiment but not the laboratory effect.

[2]Model including the effect of the experiment nested in the laboratory and the effect of the laboratory.

FA in bovine milk had a quadratic relationship with dietary C16:0 with a minimum at 1.58% DM ($P < 0.001$; Supplemental Table S7; https://doi.org/10.3168/jds.2018-15157).

The content of palmitic acid in caprine milk total FA was negatively related to dietary EE ($P < 0.001$; Table 6). Content of palmitic acid in total FA in ovine milk was quadratically related to dietary content of concentrate ($P < 0.001$), CP ($P = 0.019$), NDF ($P = 0.029$), and EE ($P = 0.034$; Table 6). However, after removing experiment 2 of the study by Addis et al. (2005) from the analysis, the linear ($P = 0.70$) or quadratic ($P = 0.56$) terms of CP were not related to palmitic acid in total FA in ovine milk (not shown).

In bovine milk, a quadratic relationship was present between palmitic acid content in milk FA and milk fat ($P < 0.001$) and protein ($P < 0.001$) content, with a maximum at 4.3% fat and 3.3% protein. No relationship was present between the content of palmitic acid in total FA in caprine ($P > 0.10$) or ovine ($P > 0.10$) milk and the milk composition variables examined (Table 6).

A large percentage of the palmitic acid models' variation was explained by the experiment, and in bovine and caprine milk, most of the variation accounted for by the experiment was accounted for by the laboratory if the laboratory was included in the model (Table 6). Six sample preparation variables were associated with the measured content of palmitic acid in milk FA (Table 3). Extraction with ethanol was the most important explanatory variable associated with measured palmitic acid in milk FA (not shown). Content of palmitic was positively related to extraction with water ($P < 0.001$; Table 3).

Factors Related to the Average Chain Length of Mammary-Synthesized Fatty Acids

In the bovine, AVDN was negatively associated with dietary CP ($P = 0.004$) and EE ($P < 0.001$; Table 7) but was not explained by the dietary content of individual FA (not shown). The AVDN was strongly negatively associated with EE in the caprine ($P < 0.001$). None of the diet composition variables evaluated were associated with AVDN in the ovine (Table 7).

In the bovine, a quadratic relationship was present between AVDN and milk production with a maximum at 34.1 kg/d ($P = 0.002$), and a positive quadratic relationship was present with milk protein content, peaking at 3.33% ($P = 0.011$; Table 7). In the caprine, a negative relationship was observed between AVDN

Table 6. Regression models relating diet composition and performance variables with palmitic acid (C16:0) content in bovine, caprine, and ovine milk fat (mg/100 mg of fatty acids)

			Partial R^2 (R^2_p) of model including the laboratory (Lab) effect[2]	
Type of variables	Species	First model including the experiment (Exp) effect[1]	Exp(Lab)[1]	Lab[2]
Diet composition	Bovine	$y = 44.9$ (±2.37; $P < 0.001$) + Exp ($P < 0.001$; $R^2_p = 0.71$) − 0.16 (±0.043; $P < 0.001$; $R^2_p = 0.0013$) %concentrate − 0.0048 (±0.0014; $P < 0.001$; $R^2_p = 0.012$) (%concentrate − 43.9)^2 − 2.12 (±0.20; $P < 0.001$; $R^2_p = 0.13$) ether extract; $R^2 = 0.85$; n = 180 (46 experiments)		
	Caprine	$y = 39.3$ (±1.52; $P < 0.001$) + Exp ($P = 0.010$; $R^2_p = 0.23$) − 3.00 (±0.39; $P < 0.001$; $R^2_p = 0.60$) ether extract; $R^2 = 0.83$ ($P < 0.001$); n = 25 (6 experiments)	0.0071	0.22
	Ovine	$y = 34.7$ (±5.68; $P < 0.001$) + Exp ($P < 0.001$; $R^2_p = 0.42$) + 0.048 (±0.021; $P = 0.034$; $R^2_p = 0.038$) % concentrate + 0.0023 (±0.00048; $P < 0.001$; $R^2_p = 0.0056$) (%concentrate − 63.0)^2 + 0.21 (±0.14; $P = 0.17$; $R^2_p = 0.027$) CP − 0.040 (±0.015; $P = 0.019$; $R^2_p = 0.036$) (CP − 17.1)^2 − 0.55 (±0.20; $P = 0.014$; $R^2_p = 0.010$) NDF + 0.026 (±0.011; $P = 0.029$; $R^2_p = 0.10$) (NDF − 31.0)^2 − 1.36 (±0.16; $P < 0.001$; $R^2_p = 0.28$) ether extract + 0.18 (±0.075; $P = 0.034$; $R^2_p = 0.023$) (ether extract − 4.47)^2; $R^2 = 0.94$ ($P < 0.001$); n = 33 (9 experiments)	0.36	0.062
Performance	Bovine	$y = 1.96$ (±5.64; $P = 0.73$) + Exp ($P < 0.001$; $R^2_p = 0.72$) + 3.59 (±0.66; $P < 0.001$; $R^2_p = 0.037$) %fat − 1.31 (±0.50; $P = 0.010$; $R^2_p = 0.029$) (%fat − 3.77)^2 + 3.87 (±1.82; $P = 0.035$; $R^2_p = 0.0016$) %protein − 10.7 (±3.26; $P = 0.001$; $R^2_p = 0.014$) (%protein − 3.18)^2; $R^2 = 0.82$ ($P < 0.001$); n = 202 (55 experiments)	0.18	0.55
	Caprine	$y = 26.4$ (±0.67; $P < 0.001$) + Exp ($P = 0.011$; $R^2_p = 0.60$); $R^2 = 0.60$ ($P < 0.001$); n = 30 (10 experiments)	0.0032	0.60
	Ovine	$y = 23.0$ (±0.42; $P < 0.001$) + Exp ($P = 0.061$; $R^2_p = 0.46$); $R^2 = 0.46$ ($P = 0.061$); n = 25 (6 experiments)	0.40	0.054

[1]Model including the experiment but not the laboratory effect.

[2]Model including the effect of the experiment nested in the laboratory and the effect of the laboratory.

and milk production ($P = 0.012$), and a quadratic relationship was observed with milk fat concentration with a minimum at 4.43% ($P = 0.015$). In the ovine, no relationship was observed between AVDN and milk production or composition (Table 7). Across all 3 species, AVDN was negatively associated with total milk FA content of C4:0 ($P < 0.001$), C6:0 ($P < 0.001$), C8:0 ($P < 0.001$), C10:0 ($P < 0.001$), and C12:0 ($P < 0.001$), and positively correlated with C16:0 ($P < 0.001$; not shown).

Most of the variation in AVDN was not explained by dietary or performance variables but by the experiment, and in turn, variation explained by the experiment was largely due to the laboratory (Table 7). Thirteen sample preparation-related variables explained 42% of variation in AVDN (Table 3), the most important one being extraction with diethyl ether (not shown).

DISCUSSION

Relationships Between De Novo Synthesized Fatty Acids

It is perhaps not surprising that associations among contents of C4:0 through C14:0 in milk total FA were generally positive in the bovine, because milk FA with 14 carbons or shorter are synthesized de novo in the mammary gland (Emery, 1973). The positive associations among FA synthesized de novo in the bovine mammary gland found herein agree with the previous meta-analysis by Moate et al. (2007) conducted on the bovine species. In the present meta-analysis, we extended those observations to the ovine and caprine species. The caprine was generally similar to the bovine except that C16:0 was negatively associated with C6:0 and C8:0. In the ovine, negative associations were present between C4:0 with C14:0 and C16:0. The notion of C4:0 to C14:0 FA being synthesized de novo in the mammary gland with C16:0 being a mixed FA partly taken up from circulation was provided by experiments with dairy cows (Emery, 1973; Grummer, 1991), but has not been verified for the ovine and caprine species. If some of the C14:0 was taken up from circulation in the ovine, this could perhaps explain its negative association with C4:0 in this species.

Species Differences

Toral et al. (2015) compared the milk FA profile of cows and does fed 3 different diets and found that species differences generally interacted with the diet. In agreement with their results, in the present analysis doe milk generally contained more C8:0, C10:0, and C12:0, and was similar or lower in C4:0, C14:0, and C16:0 (Figure 1). Caution about the present comparison among species shown in Figure 1 is advised because

Table 7. Regression models relating diet composition and performance variables with the average mammary-synthesized fatty acid chain length (carbon atoms per molecule) in bovine, caprine, and ovine milk fat

			Partial R^2 (R^2_p) of model including the laboratory (Lab) effect[2]	
Type of variables	Species	First model including the experiment (Exp) effect[1]	Exp(Lab)[1]	Lab[2]
Diet composition	Bovine	$y = 13.4$ (±0.21; $P < 0.001$) + Exp ($P < 0.001$; $R^2_p = 0.87$) – 0.034 (±0.011; $P = 0.004$; $R^2_p = 0.0077$) CP – 0.035 (±0.014; $P = 0.018$; $R^2_p = 0.0051$) ether extract; $R^2 = 0.88$ ($P < 0.001$); n = 180 (46 experiments)	0.26	0.61
	Caprine	$y = 12.8$ (±0.095; $P < 0.001$) + Exp ($P = 0.026$; $R^2_p = 0.59$) – 0.016 (±0.024; $P < 0.001$; $R^2_p = 0.29$) ether extract; $R^2 = 0.89$ ($P < 0.001$); n = 25 (6 experiments)	0.014	0.58
	Ovine	$y = 11.8$ (±0.026; $P < 0.001$) + Exp ($P < 0.001$; $R^2_p = 0.68$); $R^2 = 0.68$ ($P < 0.001$); n = 33 (9 experiments)	0.34	0.34
Performance	Bovine	$y = 12.8$ (±0.38; $P < 0.001$) + Exp ($P < 0.001$; $R^2_p = 0.94$) – 0.0081 (±0.0047; $P = 0.084$; $R^2_p = 0.0016$) milk (kg/d) – 0.0011 (±0.00035; $P = 0.002$; $R^2_p = 0.0037$) [milk (kg/d) – 30.4]^2 + 0.090 (±0.092; $P = 0.33$; $R^2_p = 0.000012$) %protein – 0.35 (±0.14; $P = 0.011$; $R^2_p = 0.0022$) (%protein – 3.20)^2; $R^2 = 0.95$ ($P < 0.001$); n = 212 (55 experiments)	0.18	0.76
	Caprine	$y = 13.0$ (±0.68; $P < 0.001$) + Exp ($P < 0.001$; $R^2_p = 0.64$) – 0.50 (±0.18; $P = 0.012$; $R^2_p = 0.11$) milk (kg/d) – 0.018 (±0.11; $P = 0.88$; $R^2_p = 0.0096$) %fat + 0.19 (±0.072; $P = 0.015$; $R^2_p = 0.072$) (%fat – 4.38)^2; $R^2 = 0.83$ ($P < 0.001$); n = 30 (10 experiments)	0.0054	0.63
	Ovine	$y = 11.9$ (±0.032; $P < 0.001$) + Exp ($P = 0.004$; $R^2_p = 0.62$); $R^2 = 0.62$ ($P = 0.004$); n = 25 (6 experiments)	0.20	0.43

[1]Model including the experiment but not the laboratory effect.

[2]Model including the effect of the experiment nested in the laboratory and the effect of the laboratory.

results for cows, goats, and sheep were reported by different published experiments.

Dietary Factors and De Novo Milk Fatty Acids

Dietary EE was positively associated with milk butyrate in does and sheep, and negatively associated with the longest de novo synthesized milk FA, C12:0, C14:0, and C16:0, and with AVDN in cows and does. Interestingly, the use of lipid supplements in doe diets commonly results in increased milk fat secretion (Chilliard et al., 2007), and in particular linseed oil increased milk fat and milk C4:0 content (Chilliard et al., 2003), in agreement with the present results. It should be mentioned that some fats supplemented to ruminants as energy sources (i.e., "ruminally protected fats") may have little effects on rumen fermentation (Jenkins and Harvatine, 2014).

The bulk of milk FA is esterified as triacylglycerides (**TAG**; Pereira, 2014). In ruminants, synthesis of FA in the mammary gland is terminated by a transacylation reaction (Hansen and Knudsen, 1987b). It is possible that chain length of de novo FA is the result of the competition between the rates of FA synthesis versus their activation to acyl-CoA and subsequent esterification in TAG. In that regard, one or more dietary FA, or their biohydrogenation intermediates absorbed postruminally, could inhibit more FA synthases than acyltransferases, resulting in shorter de novo synthesized FA in milk (Harvatine et al., 2009). In contradiction with that line of thought, however, Bernard et al. (2017) found that FA synthase had greater activity in the mammary gland of does compared with cows, whereas glycerol-3-phosphate dehydrogenase followed the opposite pattern, even though supplementation with fish oil affected the proportions of C6:0 through C12:0 in milk fat of cows but not in does, whereas supplementation with sunflower oil and starch equally decreased them in both species (Toral et al., 2015).

Milk fat depression (**MFD**) has been described in cows, ewes, and does, and is characterized by a reduction in milk fat synthesis, without changes in milk yield or other milk components. During MFD, a synchronized reduction of milk de novo synthesis of FA mediated by rumen biohydrogenation intermediates, mainly *trans*-10, *cis*-12 CLA, occurs, and transfer of preformed FA to milk fat is reduced to a lesser extent (Bauman et al., 2008). However, in MFD the synthesis of de novo FA of all chain lengths is inhibited through a concerted inhibition of all enzymes involved in FA synthesis (Bauman et al., 2008, 2011), rather than FA synthases only. Moreover, during MFD, synthesis of milk C4:0 through C10:0 is affected more than C14:0 or C16:0 (Baumgard et al., 2002); thus, the negative association we observed between milk fat content of C12:0, C14:0, and C16:0, and AVDN, and the positive association between sheep and goats milk fat C4:0 with dietary EE with dietary EE in the present analysis might not correspond to classical MFD.

Dietary Fatty Acids and De Novo Milk Fatty Acids

We found a quadratic association between dietary and milk fat C16:0, with an initial decrease in milk fat C16:0 in the low range of dietary C16:0 followed by an increase. With C16:0 being a mixed FA partly taken up from circulation and partly synthesized de novo in the mammary gland, the observed quadratic response may be the outcome of 2 opposite responses, a positive effect of dietary C16:0 on milk fat C16:0 at low dietary concentrations of C16:0 mediated by direct incorporation of absorbed C16:0 from dietary origin, and a negative effect mediated by inhibition of milk fat synthesis by C16:0. The negative effect would prevail with low dietary C16:0 and the positive effect would become more important as dietary C16:0 is augmented. Enjalbert et al. (1998) reported that duodenal infusion of C16:0 did not affect total FA synthesized de novo or the content of C16:0 in milk fat, but decreased the average chain length of de novo synthesized FA through increasing the incorporation into milk of C4:0 taken up from blood circulation. Mathews et al. (2016) also found that dietary C16:0 supplementation stimulated the proportion of C4:0 in milk fat, but to a lesser extent than C16:0 incorporation. Piantoni et al. (2013) found no effects of supplementary C16:0 on milk fat C4:0, a decrease in the proportion of C6:0 through C14:0, and an increase in C16:0 in milk total FA. In their meta-analysis, Moate et al. (2008) identified absorbed C16:0 as a significant variable to predict milk C16:0 and C16:1, but not any other de novo FA or the sum of all de novo synthesized FA. Interestingly, Hansen and Knudsen (1987a) found that the addition of C16:0 to in vitro grown bovine mammary epithelial cells strongly stimulated incorporation of C4:0 into TAG, slightly stimulated de novo synthesis of C16:0 and its incorporation into TAG, and did not affect synthesis of C6:0 through C14:0. Also, in various studies reviewed by Loften et al. (2014), dietary supplementation of C16:0 to dairy cows decreased milk fat concentration of C4:0 to C14:0, while supplemented C16:0 presented a net apparent transfer to milk of up to 20%.

Supplementing dairy cows with C18:0 resulted in decreased concentration of C10:0 to C16:0 in milk fat (Loften et al., 2014). Piantoni et al. (2015) found lower milk fat C14:0 and C16:0 with C18:0 supplementation,

with no changes in concentration of shorter de novo FA (C4:0 through C10:0). When compared with C16:0 supplementation, C18:0 supplementation had lesser effects on milk C6:0 to C14:0 (Rico et al., 2014).

In bovine mammary epithelial cell cultures, Kadegowda et al. (2009) reported that C18:0 inhibited the expression of genes encoding for FA synthase, and of acetyl-CoA carboxylase, required for FA synthesis, while it stimulated the expression of the gene encoding for diacylglycerol *O*-acyltransferase homolog 1 and 1-acylglycerol-3-phosphate *O*-acyltransferase, enzymes required for TAG synthesis. On the contrary, Jacobs et al. (2013), when treating bovine mammary epithelial cells with C18:0 or C16:0, failed to observe changes in expression of acetyl-CoA carboxylase and FA synthase or in genes encoding for TAG synthesis enzymes. Cell culture data should be interpreted with caution, as many other variables affecting milk fat synthesis in a mammary gland cannot be reproduced in these experimental models; however, they provide clues to mechanisms involved in the regulation of milk FA profile. In this sense, dietary C18:0 may perhaps decrease milk C12:0, as shown in this meta-analysis, through mechanisms involving increased expression and abundance of enzymes involved in TAG assembly, which compete for acyl-CoA for elongation. Additionally, it has been hypothesized that to maintain milk fluidity when imbalances in dietary FA occur, such as increased dietary C18:0, an increase in stearoyl CoA desaturase activity may compensate for the decrease in de novo FA synthesis as a mechanism to maintain milk fluidity (Harvatine et al., 2009).

From the above discussion, it could be hypothesized that C18:0 may be involved in shortening FA synthesized de novo in the mammary gland through transcriptional regulation of FA synthase (inhibition) and acyltransferases (stimulation), and possibly through regulation of the thioesterase (FA synthase sub unit) activity. Following this logic, a profile of de novo synthesized FA richer in <C12:0 FA might perhaps be possible to obtain through increasing rumen outflow of C18:0 through (1) supplementing dietary C18:0, and (2) promoting complete rumen biohydrogenation of C18:3 and C18:2 to C18:0.

The implications could be that feeding oil sources rich in FA with 18 carbons or more and at the same time low in C16:0 might promote a milk fat richer in shorter FA. For example, Chichlowski et al. (2005) obtained a decrease in all de novo synthesized milk FA through ground canola seed supplementation, but the effect was more pronounced on C8:0 through C16:0 compared with C4:0 and C6:0. Similar results were reported by Bayourthe et al. (2000) also with canola seed and canola meal and oil.

Milk Components and De Novo Milk Fatty Acids

Associations between the contents of individual FA in milk fat and milk production and content of total fat, protein, and lactose were in general small or nonexistent, with the exception of C12:0 content in goat milk fat and milk protein (Table 4), which suggests that improvements in those variables may not necessarily affect milk fat FA profile.

Potential Importance of Analytical Aspects on De Novo Milk Fatty Acid Profile

One of the main findings of the present meta-analysis was that much of the variation in de novo FA profile that was explained by the experiment effect could be in turn explained by the laboratory at which the experiment had been conducted. This finding, tied to the ample variation in analytical protocols reported, suggested that much of the variation in the reported de novo FA profile might be explained by differences in analytical procedures.

Basically, sample preparation for FA analysis involves extraction, hydrolysis, and methylation procedures, which differ depending on the nature of the sample, laboratory equipment, and research group (Aldai et al., 2012). Also, before extraction, samples can be dried, centrifuged, filtered, or purified (or a combination of these) using thin layer chromatography. It can be seen from Supplemental Table S6 (https://doi.org/10.3168/jds.2018-15157) that in our database, there were differences in the methods used for drying, centrifugation, filtration, methylation, and purification, along with the fact that 13 different solvents were employed in the different experiments. This resulted in a total of 55 different sequence combinations of sample preparation procedures belonging to 107 experiments (i.e., an average of only 1.95 experiments per combination of procedures of sample preparation). As shown in Table 3, several steps and solvents involved in samples preparation for FA analysis accounted for much of the variation in milk de novo FA profile and AVDN in the database used for this meta-analysis. It can therefore be stated that laboratory methods for FA analysis preferred by each research group are highly influential on study results, and that the lack of standardization of FA analysis methods among research groups may lead to less consistent treatment effects.

De Novo Synthesized Fatty Acids and Human Health

When considering the possibilities of manipulating the profile of FA synthesized de novo in the mammary gland, it should be reminded that the effect of milk

de novo FA intake on human health is an area of active research with yet unanswered questions. However, evidence is growing that intake of some specific FA and of dairy fat (Mozaffarian et al., 2010) could play a protective role against metabolic diseases that where traditionally attributed to dairy fats. The meta-analysis by Mensink et al. (2003), although confirming a positive association between total and low-density lipoprotein cholesterol with all 3 C12:0, C14:0, and C16:0, also revealed a negative association between C12:0 and the ratio of total to high-density lipoprotein cholesterol. Praagman et al. (2016) found a negative relationship between the incidence of ischemic heart disease and the intake of C14:0, and no significant associations with C12:0 or C16:0. On the other hand, the Nurses' Health Study (Zong et al., 2016) reported a positive association between the proportion of dietary energy intake as C12:0, C14:0, and especially C16:0, and the risk of coronary heart disease.

It is important to take into account that the above observational studies cannot establish cause:effect relationships. Few controlled studies have been done about the effect of individual FA intake in humans. For example, Feltrin et al. (2007) found that a duodenal load of C12:0 reduced energy intake in human subjects.

CONCLUSIONS

The present analysis highlights the potential of dietary composition, in particular EE, to manipulate the profile of FA synthesized in the mammary gland of goats and to a lesser extent of cows and sheep. However, most of the variation in FA profile was explained by the laboratory at which the different experiments were conducted. An ample variation in analytical protocols was reported, and the present results particularly suggest the need to standardize laboratory methods among research groups for appropriate interpretation of results in this area.

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