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ORIGINAL ARTICLE

Methodology for the In Vivo Measurement of the Δ^9-Desaturation of Myristic, Palmitic, and Stearic Acids in Lactating Dairy Cattle

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Abstract There is limited methodology available to quantitatively assess the activity of the Δ^9-desaturase enzyme in vivo without chemically inhibiting the enzyme or using radioactively labeled substrates. The objective of these experiments was to develop methodology to determine the incorporation and desaturation of ^{13}C-labeled fatty acids into milk lipids. In a preliminary experiment, 3.7 g [1-^{13}C]myristic acid ([1-^{13}C]14:0), 19.5 g [1-^{13}C] palmitic acid ([1-^{13}C]16:0), 20.0 g [1-^{13}C]stearic acid ([1-^{13}C]18:0) were combined and infused into the duodenum of a cow over 24 h. In a following experiment, 5.0 g [1-^{13}C]14:0, 40.0 g [1-^{13}C]16:0, and 50.0 g [1-^{13}C]18:0 were infused into the abomasums of separate cows as a bolus over 20 min or continuously over 24 h. Milk fat was extracted using chloroform:methanol. Fatty acids were methylated, and fatty acid methyl esters (FAME) were converted to dimethyl disulfide derivatives (DMDS). The FAME and DMDS were analyzed by gas chromatography mass spectrometry. In the preliminary experiment, ^{13}C enrichment in 14:0 but not 16:0 or 18:0 was observed. When dosage amounts were increased in the following experiment, peak enrichments from the bolus infusion were observed at 8 h. Enrichments for continuous infusion peaked at 16 h for 14:0 and 18:0, and at 24 h for 16:0. The Δ^9-desaturase products of these fatty acids were estimated to be 90% of *cis*-9 14:1, 50% of *cis*-9 16:1, and 59% of *cis*-9 18:1. This study demonstrates that ^{13}C-labeled fatty acids may be utilized in vivo to measure the activity of the Δ^9-desaturase enzyme.

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Abbreviations

CE	Cholesterol ester
CLA	Conjugated linoleic acid
DM	Dry matter
DMDS	Dimethyl disulfide
E	Enrichment
FAME	Fatty acid methyl ester
MDG	Mono and diacylglycerols
MUFA	Monounsaturated fatty acids
[1-^{13}C]14:0	[1-^{13}C]myristic acid
NEFA	Non-esterified fatty acids
[1-^{13}C]16:0	[1-^{13}C]palmitic acid
PL	Phospholipid
PUFA	Polyunsaturated fatty acids
SFA	Saturated fatty acids
[1-^{13}C]18:0	[1-^{13}C] stearic acid
TTR	Tracer to tracee ratio
TG	Triacylglycerol
VA	Vaccenic acid

Introduction

Impact of the ruminant diet on the fatty acid profile of milk fat and total fat production is an active area of research. Academic and industry recommendations for "ideal" milk fatty acids [1] included changing the fatty acid profile from the typical 5% polyunsaturated fatty acids (PUFA)

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including n-3 fatty acids, 70% saturated fatty acids (SFA), and 25% monounsaturated fatty acids (MUFA) to 10% PUFA, up to 8% SFA with the remainder (>80%) being MUFA. Some reasons for altering the fatty acid profile are to benefit consumer health by decreasing the content of saturated and *trans* fatty acids, and increasing the content of mono and polyunsaturated fatty acids, which are generally considered healthier for the consumer [2]. Included in the mono and polyunsaturated groups of fatty acids are the products of the Δ^9-desaturase enzyme, *cis*-9 mono and diunsaturated fatty acids, such as *cis*-9, *trans*-11 18:2 (conjugated linoleic acid; CLA).

One mechanism for specifically altering the *cis*-9 monounsaturated and *cis*-9, *trans*-11 CLA content of milk fat is via the Δ^9-desaturase enzyme. The Δ^9-desaturase enzyme is present in many tissues within the cow and is very active in the mammary gland inserting a double bond at carbon 9 into saturated fatty acids [3]. The Δ^9-desaturase enzyme is important in maintaining liquidity of the milk fat globule [4]. This enzyme uses myristic (14:0), palmitic (16:0) and stearic acid (18:0) as substrates for its actions creating myristoleic (14:1), palmitoleic (16:1) and oleic (18:1) acid, respectively [5]. Additionally, *cis*-9, *trans*-11 CLA can be made from *trans*-vaccenic acid (*trans*-11 18:1; VA) by the action of the Δ^9-desaturase in the mammary gland [6, 7]. The substrate product pairs of this enzyme represent approximately 75% of the total fatty acids in milk fat [8]. Thus, the Δ^9-desaturase enzyme is a very important determinant for the fatty acid content of milk fat.

One approach that has been explored in the research of the Δ^9-desaturase enzyme is to genetically modify ruminant animals to increase the expression of the Δ^9-desaturase enzyme gene [9]. However, the use of genetically modified dairy cattle for milk production may not be well received by the consumer. Additionally, its implementation into large commercial production settings may be impractical. On the other hand, understanding the regulation of the Δ^9-desaturase enzyme may lead to new management strategies that would result in altered milk fatty acid profile.

Understanding and controlling the activity of the Δ^9-desaturase enzyme could lead to increased MUFA content resulting in an improved milk fatty acid profile. The use of in vivo measurements of the enzyme's activity in response to various dietary manipulations would provide information on the impact of diet and regulation of the enzyme. A long term goal would be to alter the Δ^9-desaturase enzyme activity using nutrients provided in the diet. The first step in this process is to develop an in vivo assay of Δ^9-desaturase enzyme activity. The objective of these preliminary studies was to determine the methodology necessary to use ^{13}C-labeled fatty acid substrates to measure the activity of the Δ^9-desaturase enzyme in vivo in lactating dairy cattle. While the experiments described only utilized one cow per fatty acid per infusion treatment, the data yielded have provided valuable information in the implementation of this methodology. For example, these data provided the methodology necessary to demonstrate the conversion of [1-^{13}C]vaccenic acid to *cis*-9, *trans*-11 CLA in vivo in lactating dairy cattle and women by our lab [6, 10]. Without the data from each of these cows, we would be limited in our attempts to develop new and innovative techniques.

Materials and Methods

The University of Idaho Animal Care and Use Committee preapproved all of the procedures involving cows. All cows were housed in tie-stalls and had ad libitum access to feed and water. Dietary dry matter (DM) was ~64% and the ingredients were alfalfa silage, barley silage, whole cottonseed, alfalfa hay, and a concentrate pellet. The diet contained 18.1% crude protein, 26.8% neutral detergent fiber, 20.4% acid detergent fiber, and 6.1% ether extract on a DM basis.

In the preliminary experiment, one multiparous Holstein cow received 3.7 g [1-^{13}C]myristic acid ([1-^{13}C]14:0), 19.5 g [1-^{13}C]palmitic acid ([1-^{13}C]16:0), and 20.0 g [1-^{13}C]stearic acid ([1-^{13}C]18:0) delivered in a semi-continuous manner every 3 h over a 24 h period via a duodenal cannula. The cow was milked every 6 h for 36 h prior to the infusion, and every 6 h for 60 h after the initial infusion. At 60 h post infusion, the cow was returned to the milking herd, and milk samples were taken in the parlor every 12 h for 24 h. Blood samples were taken via a jugular catheter at each milking and infusion time. This frequent collection of milk and blood served as the template for determining adequate ^{13}C labeled fatty acid dosage and sample times in the subsequent experiment.

Three primiparous ruminally cannulated Holstein cows (~150 days in milk, consumed (mean ± SE) 18.3 ± 0.8 kg DM, and produced 35.1 ± 0.8 kg of milk per day with 3.5 ± 0.1% fat) each received infusions of different ^{13}C-labeled fatty acids. Infusion lines were passed through the rumen cannula and secured in the abomasum with a rubber flange. The fatty acids were delivered as a bolus infusion over 20 min or continuous infusion over 24 h. Infusion periods were separated by a 7-day washout period. The free fatty acids (5.0 g of [1-^{13}C]myristic acid, 40.0 g of [1-^{13}C]palmitic acid, or 50.0 g of [1-^{13}C]stearic acid (Isotec, Miamisburg, OH, USA)) were converted to potassium salts and combined with 5 or 15 L of water for the bolus or continuous infusion, respectively. For the bolus infusion period, the cows were milked every 6 h for 24 h prior to the infusion, and every 4 h for 48 h after the

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infusion began. At 48 h post infusion, the cows were returned to the milking herd, and milk samples were taken in the parlor every 12 h for 24 h. Prior to each machine milking and every 2 h for 48 h after the infusion began, additional milk samples were taken by hand. Blood samples were taken via a jugular catheter at each hand milking and at times 0.25, 0.5, 0.75, 1, 1.5, 2.5, 3, 3.5, 5, and 7 h after initiation of infusion. Fecal samples were also taken at each hand milking time. For the continuous infusion period, milk and fecal samples were taken as in the bolus period. Blood samples were taken at the hand milking times. Heparinized blood samples (10,000 U/L) were centrifuged for 20 min at 1,000×*g* at 4 °C, and the plasma was collected. All plasma, milk, and fecal samples were stored at –20 °C.

Milk and plasma lipids were processed as previously described by Mosley et al. [10]. Freeze-dried ground fecal samples were methylated in a two-step procedure using methanolic-HCl and sodium methoxide [11]. The fatty acid methyl esters (FAME) (<1 mg) of milk, plasma lipid fractions (triacylglycerols (TG), cholesterol esters (CE), mono and diacylglycerols (MDG), phospholipids (PL), and non-esterified fatty acids (NEFA)), and feces were converted to dimethyl disulfide (DMDS) derivatives, and the FAME and DMDS derivatives were analyzed by GC and GC–MS as previously described [6].

Data Analysis

For saturated fatty acids, the tracer to tracee ratio (TTR) was calculated from the mass abundance of the molecular ion (M) and M + 1 fragments using the equation TTR = (M + 1)/M. For monounsaturated fatty acids, the TTR was calculated from analysis of the DMDS of FAME. The DMDS derivatives produce distinctive spectral fragments that are indicative of the double bond position when analyzed by mass spectrometry. The TTR was calculated from the mass abundance of the ^{12}C and ^{13}C fragments (mass fragments 217 and 218) using the equation TTR = ^{13}C/^{12}C. In order to account for the natural levels of ^{13}C, the mean TTR of samples taken before the infusion was subtracted from the TTR of all samples. Therefore, enrichment (E) of the fatty acid with ^{13}C at each sample period was calculated as (TTR – mean $TTR_{\text{prior to infusion}}$) × 100. The calculated E was adjusted for spectrum skew [12].

Data from the enrichment of milk fatty acids in the bolus and continuous infusions were used to calculate an estimate of the desaturation of the ^{13}C-labeled saturated fatty acids. Trend lines were fit to the observed data from each cow. Area under each curve was calculated and used to determine the percent of the substrate fatty acid desaturated, as previously described [6]. Statistical analyses were not performed on data from either experiment because there was only one observation per time per treatment per fatty acid. Therefore, the data will only be described.

Results

Milk production parameters were not affected by the duodenal or abomasal infusion of ^{13}C-labeled fatty acids. For the preliminary experiment (Fig. 1), average enrichment pre-infusion was 0% for 14:0, 16:0, 18:0, and the Δ^9-desaturase products of these fatty acids (data not shown) in milk fat. Enrichment was only detected in 14:0 and 16:0 in milk fat. Enrichment values did not increase for 18:0 or any of the Δ^9-desaturase products of these fatty acids in milk fat due to inadequate dosage of the ^{13}C-labeled fatty acids. Similar results were observed when plasma lipid was analyzed (Fig. 1).

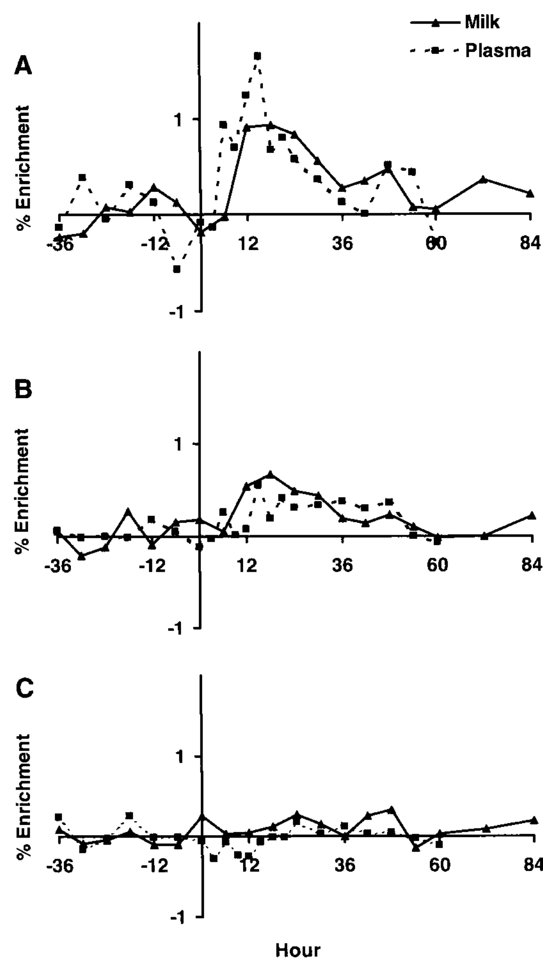

Fig. 1 Enrichment of ^{13}C in **a** myristic, **b** palmitic, and **c** stearic acid of milk and plasma from one lactating cow receiving a semi-continuous duodenal dose of 3.7 g [1-^{13}C]myristic acid, 19.5 g [1-^{13}C]palmitic acid, and 20.0 g [1-^{13}C]stearic acid. Infusions started at hour zero

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In the subsequent experiment, in milk fat the ^{13}C enrichments from the bolus infusion peaked at 8 h and then declined until not detected after 24 h (Fig. 2). Enrichments for the 24 h continuous infusion reached a plateau from about 12–30 h after initiation of the infusion and then declined. Enrichment was detected in Δ^9-desaturase products for both bolus and continuous infusions, with enrichment patterns similar to the saturated substrate. Milk samples taken by hand prior to machine milking for both bolus and continuous abomasal infusions of fatty acids yielded data similar to that from milk fat obtained during machine milking (data not shown). Using area under the curve analysis and FAME data (Table 1), the percent of the product originating from the substrate for the bolus and continuous infusions, respectively, was calculated as 90 and 92% for myristoleic, 51 and 56% for palmitoleic, and 74 and 43% for oleic acid, resulting in 5.2 and 7.0% of myristic acid, 2.6 and 2.5% of palmitic acid, and 60.3 and 48.7% of stearic acid being desaturated by the Δ^9-desaturase enzyme.

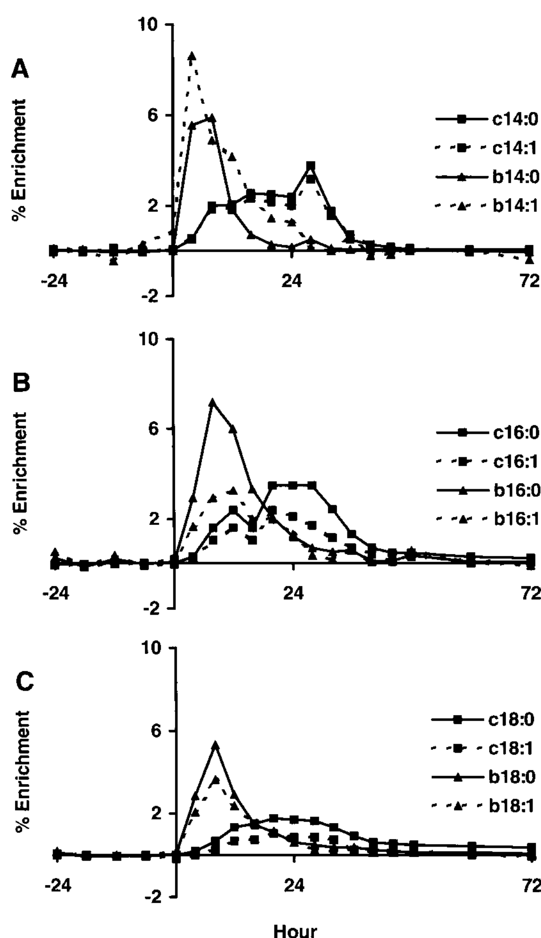

Fig. 2 Enrichment of ^{13}C in myristic acid (14:0; A), myristoleic acid (14:1; A), palmitic acid (16:0; B), palmitoleic acid (16:1; B), stearic acid (18:0; C) and oleic acid (18:1; C) of milk during either a bolus (b) or continuous (c) abomasal infusion of (**a**) 5 g [1-^{13}C]myristic acid, (**b**) 40 g [1-^{13}C]palmitic acid, or (**c**) 50 g [1-^{13}C]stearic acid. Bolus infusions were delivered within 20 min while continuous infusions were delivered over 24 h. Infusions started at hour zero. Each *panel* represents data from a single cow

Table 1 Fatty acid composition of milk fat

Fatty acid	Infusion		
	Preliminary	Bolus	Continuous
	g/100 g fatty acids		
4:0	3.3 ± 0.12	2.8 ± 0.2	2.6 ± 0.2
6:0	2.3 ± 0.11	1.5 ± 0.2	1.6 ± 0.2
8:0	1.1 ± 0.06	0.8 ± 0.2	0.9 ± 0.2
10:0	2.3 ± 0.16	1.6 ± 0.5	1.9 ± 0.5
12:0	2.4 ± 0.16	1.7 ± 0.5	2.2 ± 0.6
13:0	0.1 ± 0.01	0.1 ± 0.01	0.1 ± 0.02
14:0	9.2 ± 0.42	7.4 ± 1.2	8.6 ± 1.2
cis-9 14:1	0.7 ± 0.06	0.5 ± 0.1	0.6 ± 0.1
15:0	0.7 ± 0.02	0.7 ± 0.1	0.8 ± 0.1
16:0	29.5 ± 0.68	26.4 ± 1.2	26.9 ± 1.6
17:0	1.0 ± 0.07	1.2 ± 0.2	1.2 ± 0.1
cis-9 16:1	0.5 ± 0.02	0.7 ± 0.1	0.6 ± 0.1
18:0	16.2 ± 0.69	14.9 ± 1.9	13.3 ± 1.02
trans-18:1[a]	4.2 ± 0.21	3.0 ± 0.3	2.8 ± 0.2
cis-9 18:1	18.4 ± 0.85	27.8 ± 2.2	26.8 ± 3.3
cis-18:1[b]	2.4 ± 0.19	1.6 ± 0.1	1.5 ± 0.1
cis-9, *cis*-12 18:2	2.6 ± 0.45	2.9 ± 0.3	2.7 ± 0.3
cis-9, *trans*-11 18:2	0.3 ± 0.02	0.4 ± 0.04	0.4 ± 0.1
cis-9, *cis*-12, *cis*-15 18:3	0.3 ± 0.03	0.4 ± 0.04	0.4 ± 0.1
20:0	0.1 ± 0.01	0.1 ± 0.03	0.1 ± 0.01
Others	2.3 ± 0.09	3.7 ± 0.2	3.8 ± 0.2

Values represent a mean of all samples taken in each experimental infusion period. Data for preliminary infusion are mean ± SD from 20 measurements taken from one cow. Data for bolus and continuous infusions are mean ± SD from 33 measurements taken from each of three cows during each infusion

[a] *trans*-18:1 sum of all *trans*-18:1 isomers

[b] *cis*-18:1 sum of all *cis*-18:1 isomers excluding *cis*-9

Furthermore, ^{13}C enrichment was observed in 14:0, 16:0, and 18:0 in the TG, CE, NEFA, and PL of plasma in the bolus infusion. Between 1.5 and 3 h post-infusion, maximal enrichments for TG, CE, NEFA, PL, and total plasma 14:0 were 81.1, 2.6, 3.8, 15.4, and 33.5%, respectively. Between 2 and 3 h post-infusion, maximal enrichments for TG, CE, NEFA, PL, and total plasma 16:0 were 92.6, 5.2, 5.2, 1.4, and 9.0%, respectively. Maximal enrichments for TG, CE, NEFA, PL, and total plasma 18:0 were also detected between 2 and 3 h post-infusion (26.5, 9.1, 7.5, 0.4, and 3.3%, respectively). Maximal enrichments detected in the continuous infusions occurred at later times than was observed in the bolus period. For example,

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maximal enrichment of TG, CE, NEFA, PL, and total plasma 14:0 occurred between 8 and 24 h post-infusion (13.4, 2.2, 1.2, 3.0, and 5.1%, respectively), between 20 and 24 h post-infusion for 16:0 (6.2, 0.3, 0.5, 1.8, and 1.8%, respectively), and again between 8 and 24 h post-infusion for 18:0 (2.4, 2.0, 0.2, 0.6, and 0.9%, respectively). However, enrichment was not observed in the MDG fractions for either the bolus or continuous infusions. Unlike the enrichment of the infused saturated fatty acids in plasma lipid fractions, the Δ^9-desaturase products of these fatty acids were not enriched at any time with ^{13}C in any plasma lipid fraction (data not shown).

In fecal samples (Fig. 3), ^{13}C enrichments from the bolus infusion were maximal from 8 to 10 h and then declined until not detected after about 20 h. The ^{13}C enrichments from the continuous infusion reached a plateau from about 16 to 30 h and then began to decline. No enrichment was detected for any of the Δ^9-desaturase products of these fatty acids in fecal lipid during either the bolus or continuous infusions (data not shown). The ratio of the area under the bolus and continuous enrichment curves was 0.43, 0.56, and 0.57 for myristic, palmitic, and stearic acids, respectively.

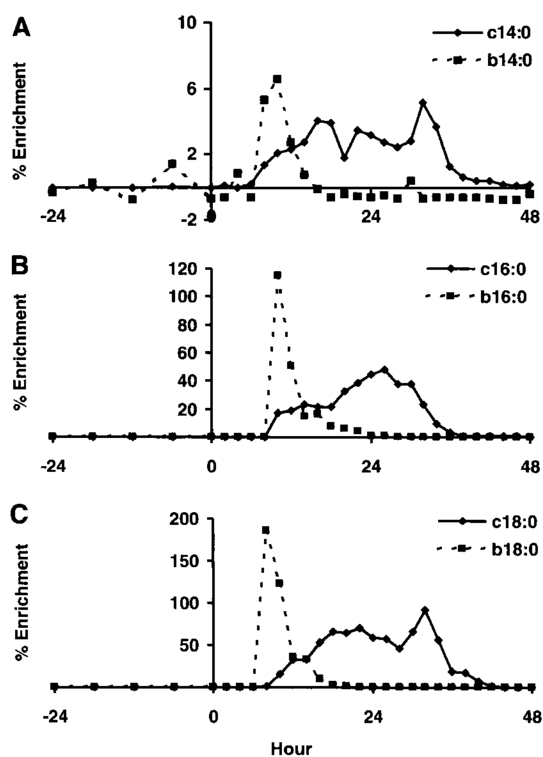

Fig. 3 Enrichment of ^{13}C in myristic acid (14:0; A), palmitic acid (16:0; B), and stearic acid (18:0; C) of feces during either a bolus (b) or continuous (c) abomasal infusion of (**a**) 5 g [1-^{13}C]myristic acid, (**b**) 40 g [1-^{13}C]palmitic acid, or (**c**) 50 g [1-^{13}C]stearic acid. Bolus infusions were delivered within 20 min while continuous infusions were delivered over 24 h. Infusions started at hour zero. Each *panel* represents data from a single cow

Discussion

Industry and academic recommendations for altering the profile of milk fatty acids included decreasing SFA and increasing unsaturated fatty acids, MUFA and PUFA [1]. While the recommendations may be dated, there is still strong desire to enrich milk with MUFA while reducing SFA [13]. For example, conversion of SFA to MUFA in milk fat would be beneficial to the human consumer by reducing their risk of coronary heart disease [14]. Understanding and controlling the activity of the Δ^9-desaturase enzyme would lead to increased MUFA, thereby improving the milk fatty acid profile.

The Δ^9-desaturase enzyme is present in many tissues within the cow and inserts a double bond at carbon 9 into saturated fatty acids [3]. The enzyme in bovine tissues occurs primarily in mammary [15] and adipose tissue [16, 17] while rates of desaturation in liver are quite low [18]. Variability in desaturase activity between tissues may be explained somewhat by dietary [19] and breed differences [20]. Our data agree that the majority of *cis*-9 desaturation of fatty acids occurs primarily in the mammary gland within 24 h of absorption. However, the lack of detection of ^{13}C in the plasma of Δ^9-desaturase products indicates that tissues other than the mammary gland do not contribute to their synthesis. However, the lower activity of the Δ^9-desaturase enzyme in tissues other than mammary and the rate of turnover of individual fatty acids between lipid pools may simply be beyond the detection limits of the assay. It may be possible to detect these changes using continuous infusions longer than 24 h with higher doses of the ^{13}C tracer.

It is also interesting to note the differences in maximal enrichment detected in the various plasma lipid fractions. In our initial experiment, we only measured total plasma enrichment (Fig. 1). It was evident that the enrichments achieved in milk fatty acids were not possible unless lipid classes within the total plasma had enrichment that exceeded that which was measured in milk. For example, we labeled only a small lipid pool in the plasma and larger unlabeled lipid pools essentially diluted the total enrichment values. This is supported by our second experiment where the TG pool contained the greatest maximal enrichment regardless of infusion type. As we would not be able to attain enrichments in the final pool (milk fatty acids) that was greater than our starting material (the plasma lipid pool utilized by the mammary gland to extract fatty acids), our data indicate that the TG pool is the primary pool utilized for delivering preformed fatty acids to the mammary gland. More research is needed for further analysis of fatty acid transfer between the various plasma lipid classes and the mammary gland. This type of data could be used to determine the actual contribution of each

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plasma lipid class to the fatty acids extracted for milk fat synthesis by the mammary gland.

This study utilized ^{13}C-labeled fatty acids to develop an in vivo assay of Δ^9-desaturase enzyme activity in lactating dairy cattle. Typically, enzyme assays utilize a radiolabeled substrate and microsomal preparations from collected tissue in an in vitro system [16, 21]. The conditions used to isolate microsomal protein for in vitro assays and the detection limits associated with these methods may inhibit one's ability to quantitatively determine the conversion of substrate to product as it relates to in vivo metabolism. For example, in vitro estimates of mammary desaturation of 18:0 are only ~31% [21] while in vivo calculations provide an estimate of ~52% [22]. The development of an in vivo assay (i.e., utilizing metabolic tracer methodology coupled with measurements of gene expression changes) may prove useful when quantitatively determining treatment effects on the Δ^9-desaturase enzyme while examining all of the fatty acid substrates simultaneously. Similar concepts for the in vitro assay are used for the in vivo assay, without using a radioactive fatty acid and with minimal disruption of the physiological state of the animal. The stably labeled fatty acid is used as a metabolic tracer, preferably with a dose that does not alter the fatty acid pools within the animal. Additionally, the stable tracer may be detected in the blood and milk. This labeling of two pools allows for identification of desaturation occurring in the mammary gland versus the rest of the body tissues. A drawback of this method is that liver, adipose, and other tissues cannot be distinguished from each other.

Reports of alterations in the synthesis of Δ^9-desaturase enzyme products generally focus on relative changes in some ratio of the milk fat concentrations of the product to substrate, also referred to as the desaturase index. The desaturase index does not provide a quantitative measurement of Δ^9-desaturase enzyme activity, it only serves as a proxy measurement. In order to estimate the actual enzyme activity, one approach is to assume that 100% of myristoleic acid is derived from the Δ^9-desaturase enzyme and then chemically inhibit the enzyme, again assuming 100% efficiency [7]. Alternatively, estimates of Δ^9-desaturase enzyme activity may be based on the duodenal flow of the Δ^9-desaturase substrates and milk fat composition of the products and substrates [23] or arterio-venous differences in the Δ^9-desaturase enzyme products and substrates [22].

Our data show that ~90% of myristoleic and ~50% of palmitoleic acids are synthesized by the Δ^9-desaturase enzyme. We are unaware of any in vivo estimates for the synthesis of myristoleic or palmitoleic acids in the lactating bovine. Previously, the desaturation of stearic to oleic acid was calculated to be 52% based on arterio–venous differences [22]. Our data show that during a continuous infusion, ~49% of the stearic acid pool is desaturated, yielding ~43% of the oleic acid in the milk fat. However, during the bolus infusion, the conversion was increased to ~60%, yielding ~74% of the oleic acid. It is likely that the 50 g bolus dose of [1-^{13}C]18:0 increased the typical quantity of stearic acid available to be absorbed from the intestine. This is supported by differences in the label recovered in the feces. In all instances, approximately two times more label was recovered in the feces during the continuous infusions. The ^{13}C enrichment of stearic acid in milk was most affected. One possible explanation is the preferential use of specific fatty acids for various metabolic functions. Another consideration would be differences in the digestibilities of the various fatty acids, as shorter chain fatty acids are considered more digestible [24]. We did not evaluate these types of factors, however, it is an area for future investigations utilizing ^{13}C-labeled fatty acid methodology. Despite these effects due to type of infusion, the methodology does yield similar results when compared to other data, as demonstrated in our infusion of [1-^{13}C]vaccenic acid in lactating dairy cows [6].

The ^{13}C enrichment detected is dependent on the dose of ^{13}C-labeled fatty acid. In the preliminary experiment, the dose of ^{13}C-labeled fatty acids delivered was underestimated because the dose was initially calculated based on the daily output of each fatty acid in milk. The ^{13}C enrichment was only detected in 16:0 and 18:0 when the dose was about 12% of the milk output of both substrate and product (Table 2). The 14:0 dose was acceptable in both experiments. However, the higher dose used in experiment 2 was needed to detect the ^{13}C enrichment in desaturase products. The comparison of bolus versus continuous administration of the ^{13}C-labeled fatty acids also showed a relationship between dose, maximum enrichment detected, and route of administration. While both types of infusion provide information about the utilization of the ^{13}C-labeled fatty acids in vivo, the bolus infusion resulted in greater ^{13}C enrichment values for the same mass of fatty acids delivered continuously over 24 h. Because of the expense of ^{13}C-labeled fatty acids and the analytical

Table 2 Dose of ^{13}C-labeled fatty acids based on milk fatty acid output

Fatty acid	Preliminary experiment			Bolus versus continuous experiment		
	Dose (g)	Output (g)	%	Dose (g)	Output (g)	%
14:0 + *cis*-9 14:1	3.7	120	3.1	5.0	98	5.1
16:0 + *cis*-9 16:1	19.5	370	5.4	40.0	321	12.5
18:0 + *cis*-9 18:1	20.0	420	4.8	50.0	476	10.5

%, dose as a percent of output

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techniques utilized, the ability to maximize ^{13}C enrichment with minimal input is desirable.

This study demonstrates that ^{13}C-labeled fatty acids may be utilized in vivo to measure the activity of the Δ^9-desaturase enzyme. Doses of the ^{13}C-labeled fatty acids are dependent on the production level of the animal and preliminary measurements of milk fat would be warranted for determination of an appropriate dosage level. This methodology will provide a powerful tool in assessing dietary impacts on Δ^9-desaturase enzyme activity. In the future, this in vivo assay of enzyme activity may be coupled with other techniques such as gene expression analyses in order to provide more insight on the impact of treatments on the Δ^9-desaturase enzyme.

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References

1. O'Donnell JA (1989) Milk fat technologies and markets: a summary of the Wisconsin milk marketing board 1988 roundtable. J Dairy Sci 72:3109–3115
2. Hu FB, Manson JE, Willett WC (2001) Types of dietary fat and risk of coronary heart disease: a critical review. J Am Coll Nutr 20:5–19
3. Christie WW (1981) The effects of diet and other factors on the lipid composition of ruminant tissues and milk. In: Lipid metabolism in ruminant animals. Pergamon Press, Oxford, pp 193–226
4. Timmen H, Patton S (1988) Milk fat globules: fatty acid composition, size and in vivo regulation of fat liquidity. Lipids 23:685–689
5. Ntambi JM (1999) Regulation of stearoyl-CoA desaturase by polyunsaturated fatty acids and cholesterol. J Lipid Res 40:1549–1558
6. Mosley EE, Shafii B, Moate PJ, McGuire MA (2006a) Conjugated linoleic acid (*cis*-9, *trans*-11 CLA) is synthesized directly from vaccenic acid in lactating dairy cattle. J Nutr 136:570–575
7. Griinari JM, Corl BA, Lacy SH, Chouinard PY, Nurmela KVV, Bauman DE (2000) Conjugated linoleic acid is synthesized endogenously in lactating dairy cows by Δ^9-desaturase. J Nutr 130:2285–2291
8. Jensen RG (2002) The composition of bovine milk lipids: January 1995 to December 2000. J Dairy Sci 85:295–350
9. Reh WA, Maga EA, Collette NMB, Moyer A, Conrad-Brink JS, Taylor SJ, DePeters EJ, Oppenheim S., Rowe JD, BonDurant RH, Anderson GB, Murray JD (2004) Hot topic: using a stearoyl-CoA desaturase transgene to alter milk fatty acid composition. J Dairy Sci 87:3510–3514
10. Mosley EE, McGuire MK, Williams JE, McGuire MA (2006b) *cis*-9, *trans*-11 conjugated linoleic acid is synthesized from vaccenic acid in lactating women. J Nutr 136:2297–2301
11. Kramer JK, Fellner V, Dugan MER, Sauer FD, Mossoba MM, Yurawecz MP (1997) Evaluating acid and base catalysts in the methylation of milk and rumen fatty acids with special emphasis on conjugated dienes and total *trans* fatty acids. Lipids 32:1219–1228
12. Wolfe RR (1992) Radioactive and stable isotope tracers in biomedicine, principles and practice of kinetic analysis. Wiley, New York
13. Hillbrick G, Augustin MA (2002) Milkfat characteristics and functionality: opportunities for improvement. Aust J Dairy Technol 57:45–51
14. German JB, Dillard CJ (2004) Saturated fats: what dietary intake? Am J Clin Nutr 80:550–559
15. McDonald TM, Kinsella JE (1973) Stearyl-CoA desaturase of bovine mammary microsomes. Arch Biochem Biophys 156:223–231
16. St John LC, Lunt DK, Smith SB (1991) Fatty acid elongation and desaturation enzyme activities of bovine liver and subcutaneous adipose tissue microsomes. J Anim Sci 69:1064–1073
17. Yang A, Larsen TW, Smith SB, Tume RK (1999) Δ^9-desaturase activity in bovine subcutaneous adipose tissue of different fatty acid composition. Lipids 34:971–978
18. Bell AW (1981) Lipid metabolism in liver and selected tissues and in the whole body of ruminant animals. In: Lipid metabolism in ruminant animals. Pergamon Press, Oxford, pp 363–410
19. Chang JHP, Lunt DK, Smith SB (1992) Fatty acid composition and fatty acid elongase and stearoyl-CoA desaturase activities in tissues of steers fed high oleate sunflower seed. J Nutr 122:2074–2080
20. Siebert BD, Pitchford WS, Kruk ZA, Kuchel H, Deland MPB, Bottema CDK (2003) Differences in Δ^9-desaturase activity between Jersey and Limousin-sired cattle. Lipids 38:539–543
21. Kinsella JE (1972) Stearyl CoA as a precursor of oleic acid and glycerolipids in mammary microsomes from lactating bovine: possible regulatory step in milk triglyceride synthesis. Lipids 7:349–355
22. Enjalbert F, Nicot M, Bayourthe C, Moncoulon R (1998) Duodenal infusions of palmitic, stearic or oleic acids differently affect mammary gland metabolism of fatty acids in lactating dairy cows. J Nutr 128:1525–1532
23. Piperova LS, Sampugna J, Teter BB, Kalscheur KF, Yurawecz MP, Ku Y, Morehouse KM, Erdman RA (2002) Duodenal and milk *trans* octadecenoic acid and conjugated linoleic acid (CLA) isomers indicate that postabsorptive synthesis is the predominant source of *cis*-9-containing CLA in lactating dairy cows. J Nutr 132:1235–1241
24. Steele W, Moore JH (1968) The digestibility coefficients of myristic, palmitic, and stearic acids in the diet of sheep. J Dairy Res 35:371–376

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