Effects of esterification, saturation, and amount of fatty acids infused into the rumen or abomasum in lactating dairy cows

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Effects of esterification, saturation, and amount of fatty acids infused into the rumen or abomasum in lactating dairy cows

Noah B. Litherland,[1†] A. Denise Beaulieu,[1‡] Christopher K. Reynolds,[2] and James K. Drackley[1*]

[1]Department of Animal Sciences, University of Illinois, Urbana, IL 61801

[2]School of Agriculture, Policy and Development, University of Reading, RG6 6AR, Berkshire, UK.

*Corresponding author: J. K. Drackley, University of Illinois, Department of Animal Sciences, 260 Animal Sciences Laboratory, Urbana, IL 61801; Phone: 217-244-3157, e-mail: drackley@illinois.edu

[†]Current address: Vita Plus Corp., Madison, WI 53725.

‡Current address: Prairie Swine Centre, Saskatoon, Sask. S7H 5N9 Canada.

ABSTRACT

Our objective was to determine the effects of chemical structure, amount, and site of infusion of long-chain fatty acids (LCFA) in lactating dairy cows. Six multiparous Holstein cows were used in a 6 × 6 Latin square design with 21-d periods. During d 1 to 14, 250 g/d of LCFA and during d 15 to 21, 500 g/d of LCFA were infused continuously into either the rumen or abomasum. Treatments were 1) Control (CONT); 200 g/d of meat solubles plus 12 g/d of Tween 80 in 10 L of water, administered half in the rumen and half in abomasum; 2) control plus mostly saturated LCFA into the abomasum (SFAA); 3) control plus mostly saturated LCFA into the rumen (SFAR); 4) control plus soy (mostly unsaturated LCFA) free fatty acids (FFA) into the abomasum (UFAA); 5) control plus soy triglycerides (TG) into the abomasum (TGA); and 6) control plus soy TG into the rumen (TGR). The first 10 d of each period were for adaptation and washout from the previous treatment. The diet consisted of 30% (dry matter basis) corn silage, 20% alfalfa silage, and 50% concentrate. Cows infused with UFAA had lower dry matter intake and milk yield than those infused with SFAA or TGA and reductions were greater at the higher infusion amount. Milk fat yield was decreased by UFAA relative to other treatments. Unsaturated LCFA decreased milk fat yield more than saturated LCFA. All LCFA treatments decreased short- and medium-chain FA in milk relative to CONT, with greatest decreases for UFAA. Apparent total tract digestibilities of nutrient fractions were decreased by UFAA compared with TGA and SFAA and tended to be lower at the higher infusion amount. Apparent digestibility of total fatty acids (FA) was greater for SFAR than for SFAA. Plasma glucagon-like peptide-1 was greater for cows infused with UFAA than SFAA or TGA and increased at the higher amount. Plasma cholecystokinin was greater for cows infused with LCFA compared with CONT. Postruminal unsaturated FFA reduced intake and digestibility of nutrients and FA

compared with postruminal TG infusion; saturated FA did not decrease dry matter intake or disrupt nutrient digestion. Glucagon-like peptide-1 may be involved in regulation of feed intake by long-chain fatty acids.

Key words: long-chain fatty acid, postruminal infusion, gut hormone, digestibility

1 | INTRODUCTION

Despite many years of research on supplemental fats, factors that limit the use of fat for dairy cows are not completely understood. Nutritional strategies for dairy cows must balance the high energy requirement necessitated by copious milk production while at the same time taking advantage of the rumen's ability to utilize large amounts of forage. Limited amounts of rapidly fermentable carbohydrates can be fed to high producing dairy cattle and still maintain optimal rumen function. Supplementing fat to lactating cow diets offers an opportunity to increase the energy density of the diet without excessive use of rapidly fermentable carbohydrates. The use of supplemental fat, however, has been burdened with complicating factors such as decreased nutrient intake, impaired rumen fermentation and nutrient digestibility, and decreased milk production and milk component contents (Palmquist & Jenkins, 2017). Questions remain unanswered pertaining to the effects of amount of fat, degree of saturation and esterification, and the interaction of fat with digestive processes within different portions of the digestive tract of lactating dairy cows (e.g. Loften et al., 2014; de Souza, Prom, & Lock, 2020).

Previous data have shown that abomasal infusion of unsaturated, but not saturated, long-chain fatty acids (**LCFA**) decreases dry matter intake (**DMI**) in lactating cows (Drackley, Klusmeyer, Trusk, & Clark, 1992; Christensen, Drackley, LaCount, & Clark, 1994; Bremmer, Ruppert, Clark, & Drackley, 1998). We determined that the degree of esterification may

influence the effects of fat source on DMI and digestibility (Litherland et al., 2005). These data indicate that chemical structure of fat affects the physiological responses of dairy cows. Our previous research (Benson, Reynolds, Humphries, Rutter, & Beever, 2001; Litherland et al., 2005; Relling & Reynolds, 2007) determined that postruminal infusion or feeding unsaturated free fatty acids (**FFA**) increase the release of glucagon-like peptide-1 (7-36) amide (**GLP-1**), presumably by activating receptors in the upper duodenum (Drackley et al., 1992; Bremmer et al., 1998). Because hydrolysis of triglycerides postruminally occurs in the jejunum, FFA are produced distally to FA-sensing mechanisms. These sensors are located in endocrine cells in the proximal duodenum and control the release of GLP-1 and cholecystokinin-8 (**CCK**), which decrease feed intake in a number of species when fat is fed (Knapper, Heath, Fletcher, Morgan, and Marks, 1995; Reidelberger et al., 1994; Turton et al., 1995; Walsh, 1994). In contrast to our results for GLP-1, CCK was not increased by unsaturated LCFA infused into the abomasum as either FFA or triglycerides (**TG**; Benson et al., 2001; Litherland et al., 2005). However, plasma concentration of CCK was increased by feeding rumen-inert fats to lactating dairy cows and the response was less for fats higher in SFA (Relling & Reynolds, 2007).

If GLP-1 is a hormone that plays a physiological role in regulating DMI when unsaturated FFA reach the small intestine, abomasal infusion of saturated FFA, which do not decrease DMI, should not increase GLP-1. Effects of amount and chemical form of LCFA on circulating concentrations of GLP-1 and CCK have not been determined. Our objectives, therefore, were to 1) define the effects of chemical form, amount, and site of administration of LCFA on DMI, production, and metabolism in lactating dairy cows within a single experiment, and 2) to determine the association between these factors and circulating concentrations of the gut hormones CCK and GLP-1. Our hypothesis was that increasing amounts of unsaturated FFA

infused postruminally would more potently inhibit DMI than equivalent amounts of saturated FA or unsaturated TG infused ruminally or postruminally, and that these changes would be accompanied by corresponding differences in concentrations of GLP-1, but not CCK.

2 | MATERIALS AND METHODS

2.1 | Housing and Management

The University of Illinois Institutional Animal Care and Use Committee approved all procedures. Multiparous Holstein cows (n = 6) averaging 77 days in milk at the start of the experiment were housed in individual tie-stalls and tethered so they could stand and lie freely. Cows were bedded with straw on top of rubber-filled mattresses and allowed to exercise daily in a dry lot from 0630 to 0800 h. Cows were milked twice daily at 0500 and 1700 h. Feed was provided in individual mangers and water was available from individual water cups at all times. The diet contained alfalfa silage, corn silage, ground shelled corn, soybean meal, minerals, and vitamins (Table 1) and was formulated to meet or exceed nutrient requirements for dairy cows based on body weight (**BW**) and predicted milk yield and composition (NRC, 2001). The diet was fed as a total mixed ration (**TMR**) for ad libitum daily intake twice daily at 0800 and 1600 h with refusals removed before the 0800 h feeding.

2.2 | Experimental Design and Treatments

The experimental design was a 6 × 6 Latin square with 21-d periods. Cows were assigned randomly to sequences of 6 treatments (Table 2) balanced for carry-over effects. Research staff were not blinded to treatments. Treatments were continuous abomasal infusions of carrier or carrier plus LCFA infused at either 250 or 500 g/d. Treatments were 1) control (**CONT**): 200 g/d of meat solubles (APC, Inc., Ames, IA) + 12 g/d of Tween 80 (Sigma Chemical Co., St. Louis

MO) in 10 L of tap water, administered half in the rumen and half in the abomasum; 2) saturated LCFA infused abomasally (**SFAA**): CONT + mostly saturated LCFA (prilled free LCFA from Energy Booster 100; Milk Specialties Co., Dundee, IL); 3) saturated LCFA infused ruminally (**SFAR**); 4) unsaturated LCFA infused abomasally (**UFAA**): CONT + mostly unsaturated free LCFA (Emery 618 soy fatty acid; Henkel Corporation, Emery Division, Cincinnati, OH); 5) unsaturated soy TG infused abomasally (**TGA**): CONT + soy oil (Archer Daniels Midland, Decatur, IL); and 6) unsaturated soy TG infused ruminally (**TGR**). The control infusate (meat solubles plus Tween 80) served as the carrier for the LCFA mixtures.

The saturated LCFA infusate contained predominantly C16:0 and C18:0 and was utilized because in previous experiments at our location (Drackley et al., 1992; Christensen et al., 1994; Bremmer et al., 1998) it did not decrease DMI or milk production when infused abomasally. Unprotected unsaturated FFA from soy were not infused into the rumen because they would be rapidly biohydrogenated by rumen microbes, similar to the TGR treatment. Infusion of TG into the abomasum and rumen served to evaluate degree of esterification of LCFA when compared with unsaturated LCFA of a similar profile infused into the abomasum. The fatty acid (**FA**) profiles of the soy TG and soy FFA were designed to be similar to test the effect of FFA compared with esterified LCFA. Moreover, we postulated that infusion of TG into the rumen would result in mostly saturated FFA entering the small intestine, similar to the SFAR treatment. Because TG of mostly saturated LCFA are poorly digested in the rumen or small intestine (Elliott, Drackley, Beaulieu, Aldrich, and Merchen, 1999), we did not include such a treatment. Our predictions, therefore, were that: 1) saturated LCFA infused into the rumen or abomasum would not reduce DMI and should increase milk production; 2) soy TG infused into the abomasum would decrease DMI and milk production, whereas infusion of soy TG into the

rumen would negatively affect rumen function; 3) unsaturated FFA from soy infused into the abomasum would decrease DMI and milk production to a greater extent than soy TG in both the rumen and abomasum; and 4) these effects would be more pronounced when the amount of LCFA increased.

Each period was divided into two parts as repeated measures; during d 1 to 14 of each period cows were infused with 250 g/d of LCFA and from d 15 to 21 cows were infused with 500 g/d of LCFA. The first 10 d of each period served as adaptation and washout phases. By design, infusion amount was confounded with time. For TG treatments, the amount of TG infused was 277.5 g/d during d 1 to 14 and 555 g/d during d 15 to 21 for each period to account for the weight of glycerol and equalize amounts of LCFA infused among treatments. Treatments were designed to be isoenergetic and equal in glycerol content by infusing 27.5 and 55 g/d of glycerol during d 1 to 14 and 15 to 21, respectively, for UFAA, SFAA, and SFAR treatments to account for the extra energy in TG treatments. Due to an error, however, only 5 and 10 g/d of glycerol was included in the UFAA, SFAA, and SFAR treatments during d 1 to 14 and 15 to 21, respectively. This decreased gross energy intake by 404 and 808 kJ, respectively, in those treatments, equating to only 0.15 to 0.20% of gross energy intake.

Infusion solutions were prepared fresh daily. Meat solubles (200 g/d) and Tween 80 (12 g/d) were weighed and added to 10 L of hot (50 to 60°C) tap water while being stirred vigorously (Lightnin mixer; Mixer Equipment Co., Rochester, NY). Prior to weighing, soy FFA and TG were briefly warmed on a hot plate to facilitate flow and improve mixing. Saturated LCFA were weighed and then melted in 1 L of water while being stirred on a hot plate; the melted solution then was added to the carrier to a final volume of 10 L. Individual infusate

156 solutions were added slowly to the solution of meat solubles with vigorous stirring for 10 to 15
157 min before placement on magnetic stir plates, and were continuously mixed during infusions.
158 The homogenous solutions were infused through an infusion line that was passed through
159 a hole in the plug of the ruminal cannula and terminating in the rumen (for ruminal infusions) or
160 anchored into the abomasum with a rubber flange (for abomasal infusions). Infusion tubing was
161 Tygon fuel and lubricant tubing (4.8 mm; Cole-Parmer Instrument Company, Vernon Hills, IL)
162 attached to a polypropylene bottle as described by Drackley et al. (1992). Tubing placement was
163 monitored daily. Infusions occurred over 20 to 22 h/d via peristaltic pumps (Harvard Apparatus,
164 South Natick, MA). Infusions of 250 g/d for the first 2 wk of each 21-d period allowed for
165 measurements at a lower LCFA amount and also allowed cows to adjust to abomasal tubing
166 placement and infusion of treatments. During the entire period the amounts of meat solubles and
167 Tween 80 infused remained constant. The CONT infusate was delivered in equal 5-L proportions
168 simultaneously into the rumen and the abomasum to serve as a control for both rumen and
169 abomasal infusion of treatments. Use of meat solubles and Tween 80 as an LCFA carrier has
170 been shown previously to have no measurable positive or negative effects on cows (Christensen
171 et al., 1994).

172 ***2.3 | Feed and Feed Refusals***

173 Feed DM offered and orts were measured and recorded daily to allow calculation of
174 DMI. Samples of individual feed ingredients, TMR, and orts from individual cows were
175 collected daily and pooled on an equal dry weight basis on d 11 to 14 and d 18 to 21 of each
176 period. Orts were weighed daily before the 1600-h feeding. Samples of individual feedstuffs,
177 TMR, and orts were dried at 55°C in an oven for 72 h and then ground in a Wiley mill (Arthur H.
178 Thomas, Philadelphia, PA) through a 1-mm screen. Composite samples were analyzed for

179 contents of dry matter (**DM**; AOAC, 1990), organic matter (**OM**; 600°C for 8 h), Kjeldahl N
180 (AOAC, 1990), neutral detergent fiber (**NDF**) using heat stable α-amylase (Thermamyl 120L;
181 Novo Nordisk Biochem, Franklinton, NC) and sodium sulfite (Van Soest, Robertson, & Lewis,
182 1991), acid detergent fiber (**ADF**; Van Soest et al., 1991), FA by gas chromatography of fatty
183 acid methyl esters (**FAME**; Sukhija & Palmquist, 1988), and energy by bomb calorimetry (1261
184 Isoperibol Oxygen Bomb Calorimeter; Parr Instrument Co., Moline, IL).

185 ***2.4 | Milk Production and Composition***

186 Milk weights were recorded at each milking. Milk was sampled at each milking from
187 each cow on d 12 to 14 and d 19 to 21 of each period. During each collection period average
188 daily milk production was calculated for each cow. Milk samples from each cow were brought to
189 room temperature, composited daily in proportion to milk yield at each milking, preserved with
190 2-bromo-2-nitropropane-1,3 diol, and stored at 4°C. Milk samples were analyzed by Dairy Lab
191 Services (Dubuque, IA) for contents of fat, protein, urea N, lactose, and solids-not-fat by infrared
192 procedures (Foss 4000; Foss North America, Eden Prairie, MN). A separate aliquot of the
193 composited milk samples was frozen (-20°C) for determination of FA composition of milk fat.
194 Milk for analysis of FA was subsequently thawed and centrifuged to separate milk fat. Milk fat
195 was transferred into a clean test tube, weighed, and FA were methylated using the procedures of
196 Sukhija & Palmquist (1988). The resultant FAME were separated on a Shimadzu 17A gas
197 chromatograph equipped with an AOC 20i auto sampler, a flame ionization detector, and a
198 Supelco (Bellefonte, PA) 2380 fused silica capillary column (100 m × 0.25 mm i.d., 0.2 μm
199 phase film; Supelco Bellefonte, PA). Column conditions for determination of milk FA were as
200 follows. The initial temperature of 70°C was maintained for 4 min; the oven temperature was
201 increased at a rate of 8°C/min to 180°C where it was held for 7 min, then ramped at 5°C/min to

220°C. Total run time was 37 min. The carrier gas was He at a flow rate of 21 cm/s and the split ratio was 80:1. Fatty acids were identified by comparison of their retention times with those of known standards (Nu-Chek Prep, Elysian, MN; Matreya, Pleasant Gap, PA).

2.5 | Ruminal Fermentation Characteristics

Ruminal fluid was collected from each cow through the ruminal cannula from 4 sites in the rumen approximately 50 to 60 cm lateral and ventral from the rumen cannula and combined to produce a pooled sample on d 12 and 19 of each period. Samples were taken prior to feeding and at 2, 4, 6, 8, 10, and 12 h after feeding. A glass electrode was used to determine pH of the ruminal fluid immediately after sampling. After measurement of pH, a subsample of 50 mL was acidified to pH < 2.0 with 18 *M* H_2SO_4 and frozen at -20°C for later analysis. After thawing, ruminal fluid samples were centrifuged at 20,000 × *g* for 20 min at 4°C. A 4-mL aliquot of the supernatant was diluted with 25% metaphosphoric acid (4:1 ratio). Samples were frozen overnight, thawed, and centrifuged at 11,000 × *g* for 10 min at 4°C. Subsamples then were used to determine volatile fatty acids (**VFA**) concentrations with a gas chromatograph (model 5890 Series II; Hewlett-Packard, Avondale, PA) equipped with a 1.8-m glass column packed with 10% SP 1200/1% H_3PO_4 on 80/100 chromosorb W AW (Supelco, 1975). Nitrogen was the carrier gas and the temperatures of the injector port and column were 175°C and 125°C, respectively. Ruminal fluid NH_3 N concentration was determined according to the procedures outlined by Chaney & Marbach (1962) as modified by Cotta & Russell (1982).

2.6 | Apparent Total Tract Nutrient Digestibilities

Chromic oxide (Cr_2O_3) was used as an indigestible marker to assess the passage of digesta to fecal excretion. Cows were dosed with 10 g of Cr_2O_3 in gelatin capsules via the ruminal cannula twice daily at 0800 and 1700 h on d 7 to 21 of each period to determine

apparent digestibility of nutrients in the total gastrointestinal tract. Fecal grab samples were collected at 12-h intervals during d 11 to 14 and again during d 18 to 21 of each period so that digestibilities could be calculated for each LCFA infusion amount. Subsamples were pooled on an equal wet weight basis into one sample for each cow for each digestibility period at each infusion amount. Pooled samples were dried at 55°C in plastic containers and then ground through a 1-mm screen in a Wiley mill (Arthur H. Thomas). Fecal samples were analyzed for contents of DM, OM, crude protein (**CP**), ADF, NDF, FA, and energy as described for feeds. Chromium content of fecal samples was determined by atomic absorption spectroscopy (air plus acetylene flame; Perkin-Elmer, Norwalk, CT) after preparation of samples by the procedure of Willams, David, & Iismaa (1962). The Cr_2O_3 marker was used to calculate apparent total tract digestibilities of DM, OM, CP, ADF, NDF, FA, and energy.

2.7 | Blood Sampling and Analyses

On d 13 and 20 of each period, blood was collected from the coccygeal vein or artery into evacuated tubes (Vacutainer; Becton Dickinson Vacutainer Systems USA, Rutherford, NJ) containing heparin or EDTA once before the a.m. feeding and at 3, 6, and 9 h after feeding. Blood was placed on ice immediately after collection. Blood was centrifuged at 14,000 × *g* for 15 min at 4°C to obtain plasma. Plasma was stored frozen (-20°C) until analysis for concentrations of nonesterified fatty acids (**NEFA**; NEFA-C kit; Wako Chemicals, Dallas, TX), glucose (kit 315; Sigma Chemical Co., St. Louis MO), urea N (kit number 640; Sigma Chemical Co.), and total protein (total protein reagent number 541-2 and protein standard number 540-10; Sigma Chemical Co., St. Louis MO). Plasma concentrations of CCK-8 and GLP-1 (7-36 amide) were determined using double antibody radioimmunoassay as described by Benson & Reynolds

(2001). Throughout the text CCK-8 is denoted as CCK and GLP-1 (7-36 amide) is denoted as GLP-1.

2.8 | Statistical Analysis

Data obtained during treatment infusions for each cow in each period were subjected to ANOVA by using the MIXED procedure (Littell, Milliken, Stroup, & Wolfinger, 1996) of SAS version 9.3 (SAS Institute, Cary, NC, USA) for a Latin square design with repeated measures. Model fixed effects included period, treatment (fat type), amount of infusion, time (for repeated effects), and the interactions among these effects. Cow within period was designated as a random effect and was the experimental unit. Several covariance matrices were tested and the one that provided the lowest Akaike Information Criterion was used in the model, which was autoregressive order 1. Orthogonal contrasts were used to separate differences among treatments. Contrast comparisons were: 1) CONT vs. all lipid treatments, 2) saturated vs. unsaturated FA infused abomasally (SFAA vs. UFAA, 3) unsaturated triglyceride vs. saturated FA infused abomasally (TGA vs. SFAA), 4) unsaturated triglyceride vs. unsaturated FA infused abomasally (TGA vs. UFAA), and 5) unsaturated triglyceride vs. saturated FA infused ruminally (TGR vs. SFAR). Contrasts for interactions of each treatment contrast with amount of infusion also were evaluated. Model residuals were examined for normality and homoscedasticity. The experimental power for an effect size of 2 kg/d difference in DMI was 0.80. Least squares means are presented in the tables. Significance was declared at $P < 0.05$. During wk 2 of period 3, the cow receiving TGR developed acute mastitis. Data for this cow from periods 3, 4, 5, and 6 were excluded from the analysis. The largest standard error of the mean is reported throughout. All data are available upon request.

3 | RESULTS

3.1 | Nutrient Intake

Infusions of UFAA into the abomasum of lactating dairy cows decreased ($P < 0.05$) DMI compared with infusion of TGA and SFAA for both 250 and 500 g/d infusion amounts, with the interaction of UFAA vs. TGA and SFAA × amount indicating that the depression in DMI by UFAA was greater when 500 g/d was infused (Table 3). No other treatment contrasts were significant for DMI. Intakes of OM, total FA, dietary FA, total CP, ADF, NDF, and gross energy followed patterns similar to DMI (Table 3).

3.2 | Milk Production and Composition

Milk production (Table 4) was decreased ($P < 0.05$) by UFAA infusion compared with TGA and SFAA infusion. Increasing the amount of UFAA infused decreased ($P < 0.05$) milk production more than increasing the amount of TGA and SFAA. Milk production decreased as TGA infusion increased from 250 to 500 g/d, but remained constant for TGR.

Milk fat content (Table 4) was higher ($P < 0.05$) for cows infused with SFAA or SFAR than milk fat content of cows infused with TGA. Milk fat content was higher ($P < 0.05$) during SFAR infusion than during SFAA infusion. Additionally, milk fat content was higher ($P < 0.05$) during UFAA infusion than during TGA infusion at the 500 g/d infusion amount. Milk fat yields were lower ($P < 0.05$) when cows were infused with UFAA than when they were infused with TGA or SFAA (Table 4). Cows infused with SFA produced greater ($P < 0.05$) amounts of milk fat than did cows infused with TG ($P < 0.05$). Yields of 3.5% FCM were greater ($P < 0.05$) for TGA than for UFAA, with the difference greater ($P < 0.05$) at the 500 g/d amount (Table 4.4). Yields of 3.5% FCM were greatest from infusion of 250 g/d of SFAR. Yield of 3.5% FCM

292 increased as infusion of SFAA increased, however, 3.5% FCM decreased with increasing
293 amount of SFAR infused ($P < 0.05$).

294 Milk CP content (Table 4) was lower ($P < 0.05$) for cows infused with UFAA than for
295 TGA or SFAA. There was a trend ($P = 0.06$) for higher CP content in milk from cows infused
296 with TGA compared with UFAA. Similar to milk CP content, milk CP yield (Table 4) was
297 greater ($P < 0.05$) when cows were infused with TGA and SFAA than UFAA. Milk CP yield
298 also was greater ($P < 0.05$) when cows were infused with TGA than SFAA at the 500 g/d
299 infusion amount.

300 ***3.3 | Composition of Milk Fat***

301 Results from milk FA analysis (Table 5) indicate that the milk FA profile was altered
302 predictably by either abomasal or ruminal infusion of LCFA. Milk FA profile tended to be
303 modified according to the FA profile of the infusate. Infusion of SFAA resulted in increased
304 percentage of $C_{4:0}$ and $C_{6:0}$ in milk fat when compared with UFAA. Percentages of $C_{8:0}$, $C_{10:0}$,
305 and $C_{12:0}$ decreased ($P < 0.05$) with infusion of LCFA compared with CONT and UFAA. The
306 percentage of $C_{14:0}$ was greater ($P < 0.05$) when TGA and SFAA were infused compared with
307 UFAA, with differences increasing at the higher infusion amount ($P < 0.05$). The percentage of
308 $C_{14:0}$ was decreased by lipid infusion compared with CONT ($P < 0.05$). The percentage of $C_{14:1}$
309 was lower ($P < 0.05$) when UFAA was infused compared to TGA and SFAA. Percentage of $C_{15:0}$
310 was greater ($P < 0.05$) with infusion of CONT compared with lipid infusion at both lipid
311 amounts ($P < 0.05$).

312 The percentage of $C_{16:0}$ in milk fat ranged from 31.1 to 21.1 and was greatest when
313 SFAA or TGA were infused and smallest when UFAA was infused (Table 5). The larger
314 proportion of $C_{16:0}$ in milk fat when SFAA or SFAR were infused resulted from the larger

amount of $C_{16:0}$ infused (Table 2) compared with that contained in UFAA. Infusion of saturated LCFA (SFAA and SFAR) resulted in greater ($P < 0.05$) percentages of $C_{16:0}$ than did infusion of unsaturated LCFA (TGA and UFAA); increasing amounts of SFA infused increased $C_{16:0}$ whereas increasing amounts of TG decreased $C_{16:0.}$ Percentages of $C_{16:1}$ were greater for TGR than TGA infusion and were greater ($P < 0.05$) when saturated LCFA (SFAA and SFAR) were infused than when unsaturated LCFA (TGA and TGR) were infused. Percentages of $C_{17:0}$ and $C_{17:1}$ in milk fat were not affected by treatment.

Infusion of TGA compared with UFAA resulted in a greater ($P < 0.05$) percentage of $C_{18:0}$ in milk fat, with the difference being greater at the higher infusion amount. The percentage of $C_{18:0}$ in milk fat from cows infused with UFAA was greater than that from cows infused with SFAA. Milk fat content of *cis*-$C_{18:1}$ was greater for infusion of UFAA than SFAA as the amount infused increased. Infusion of unsaturated LCFA into the abomasum caused a considerable increase in $C_{18:2}$ content in milk fat compared with infusion of saturated LCFA. This response was due to the large amount of $C_{18:2}$ (54%) infused in UFAA and TGA. The TGR infusion resulted in percentages of $C_{18:2}$ similar to that of CONT, presumably because of the almost complete biohydrogenation of $C_{18:2}$ by rumen microbes. The percentage of $C_{18:2}$ in milk was greater for UFAA infusion than for TGA infusion, with differences larger as amount infused increased. Similar to $C_{18:2,}$ percentage of $C_{18:3}$ was increased by UFAA. Percentage of milk fat $C_{18:3}$ was greater ($P < 0.05$) for UFAA than SFAA and greater for lipid infusion vs. CONT because $C_{18:3}$ from TGR was likely biohydrogenated by rumen microbes. Milk fat content of *cis*-9, *trans*-11 CLA was increased ($P < 0.05$) by TGR infusion compared with SFAR.

3.4 | Ruminal Fermentation and Total Tract Digestibilities

The pH of rumen fluid was lower for SFAA compared to UFAA at the 250 g/d infusion amount (Table 6). Ruminal fluid concentrations of NH_3N and total VFA were not different among treatments, although total VFA concentrations decreased in UFAA vs. SFAA infused cows. Molar percentages of acetate were higher ($P < 0.05$) when cows were infused with UFAA compared with TGA. Molar percentage of propionate and the acetate to propionate ratio in rumen fluid were greater ($P < 0.05$) for CONT compared with lipid infusion. Molar percentages of isobutyrate and isovalerate were not different among treatments. Molar proportions of valerate were greater ($P < 0.05$) for cows infused with lipid compared with CONT. Molar proportions of butyrate and isovalerate in ruminal fluid were not different among treatments.

Apparent total tract digestibilities of DM, OM, CP, NDF, and energy were decreased ($P < 0.05$) by infusion of UFAA compared with TGA and SFAA (Table 8). Depressions in apparent digestibilities of DM, OM, CP, and energy by UFAA tended to be greater ($P < 0.10$) at higher infusion of UFAA. Because digestibilities did not differ appreciably among most treatments, comparisons among treatments for the quantities of DM, OM, CP, ADF, NDF, and energy digested in the total gastrointestinal tract (data not shown) followed patterns similar to those for DMI (Table 3).

Apparent digestibilities of total FA, total C_{16} FA, and total C_{18} FA (Table 7) were decreased ($P < 0.05$) by infusion of UFAA compared with TGA and SFAA, with the differences being greater at the higher infusion amount. Total FA digestibilities were higher for SFAR vs. TGR. Digestibilities of C_{18} FA were higher ($P < 0.05$) for infusion of TGA and SFAA than UFAA.

3.5 | Blood Metabolites

The concentration of glucose in plasma was not different among treatment contrasts (Table 8). Total protein was higher ($P < 0.05$) for UFAA vs. SFAA. Plasma urea was higher ($P < 0.05$) in cows infused with lipid vs. control and lower for SFAA vs. UFAA. The concentration of NEFA in plasma was increased ($P < 0.05$) by infusion of UFAA compared with infusion of TGA and SFAA, with the difference between treatments being greater at the higher infusion amount.

3.6 | Gut Hormones in Plasma

Plasma concentrations of GLP-1 were increased ($P < 0.05$) by infusion of UFAA compared with infusion of TGA an SFAA, with the difference between UFAA and TGA being greater at the higher infusion amount (Table 8). Infusion of UFAA at 500 g/d produced the highest GLP-1 response of any treatment. Concentrations of GLP-1 in plasma for all other LCFA treatments were numerically higher, but the contrast of CONT versus lipid treatments was not significant. In agreement with the lack of effect on DMI, there was no difference in GLP-1 concentration between infusions of saturated FA and unsaturated TG. Plasma concentration of GLP-1 increased ($P < 0.05$) with increasing amount of lipid infused for all lipid treatments, suggesting that amount of lipid present in the small intestine may play a role in the GLP-1 response.

Plasma CCK concentrations were greater for cows infused with LCFA than during CONT infusions ($P < 0.05$). There was a tendency ($P = 0.06$) for higher CCK in SFAA vs. TGA. Overall, plasma CCK concentrations increased ($P < 0.05$) at the higher infusion amount.

4 | DISCUSSION

4.1 | Nutrient Intake

Infusion of UFAA decreased DMI compared with SFAA and TGA. At the 500 g/d dose, the decreases were 43% and 37% compared with SFAA and TGA, respectively. Our results agree with previous studies (Drackley et al., 1992; Christensen et al., 1994; Bremmer et al., 1998), in which abomasal infusion of mostly unsaturated LCFA significantly decreased DMI compared with infusion of mostly saturated LCFA. In a previous experiment (Drackley et al., 1992), infusion of 700 g/d of mostly unsaturated free LCFA caused cows to go off feed, decrease milk production, and develop diarrhea. Similar results were observed for 3 out of the 6 cows used in this study when 500 g/d of UFA was infused.

Our results agree with other studies in which unsaturated LCFA have been supplied postruminally. Gagliostro & Chilliard (1991) reported a decrease in DMI by infusion of rapeseed oil into the duodenum of dairy cows. Abomasal infusion of increasing amounts of unsaturated FFA from canola oil elicited a linear decrease in DMI (LaCount, Drackley, Laesch, & Clark, 1994). Increasing dietary amounts of calcium salts of LCFA (3, 6, and 9 % of total dietary DM) decreased DMI when amounts were greater than 3 % of DM (Choi & Palmquist, 1996). Abomasal infusion of a mixture of rapeseed and sunflower oils supplying predominantly unsaturated LCFA significantly reduced DMI in both early and midlactation dairy cows (Benson et al., 2001). When rumen-inert fats were fed to midlactation dairy cows (Relling & Reynolds, 2007) DMI was decreased but the effect tended to be less for mostly SFA compared to a high MUFA or high PUFA fat sources.

Abomasally infused TG were less potent inhibitors of DMI than FFA in the current study, in agreement with data from our previous study (Litherland et al., 2005). Duodenal infusion of oleic acid in rats decreased food intake in a dose-dependent manner, but infusion of triolein was 4 times less potent than oleic acid in the suppression of intake (Woltman, Castellanos, &

406 Reidelberger, 1995). Data from that study showed that hydrolysis of TG to FFA was necessary
407 for the inhibition of food intake in rats. Duodenal infusion of rapeseed oil TG (1100 and 700 g/d)
408 decreased DMI (2.6 and 1.8 kg/d) in midlactation cows (Gagliostro & Chilliard, 1991), but the
409 decrease was much less than the effect of smaller amounts of unsaturated FFA administered into
410 the abomasum (Drackley et al., 1992; Christensen et al., 1994; Bremmer et al., 1998). The
411 considerably greater decreases in DMI in our studies compared with the French study
412 (Gagliostro & Chilliard, 1991) may be attributed to the form of LCFA reaching the intestine as
413 postulated by Bremmer et al. (1998). Thus, FFA reaching the upper duodenum may stimulate the
414 release of CCK or GLP-1 that may suppress DMI. Duodenal infusions of TG may not have
415 triggered the LCFA-specific sensing mechanisms because hydrolysis of TG to FFA and glycerol
416 occurs in the jejunum distal to the greatest area of FFA-sensing receptors in the duodenum.
417 Therefore, esterified FA would bypass most of the inhibitory mechanisms activated by FFA.

418 ***4.2 | Milk production and Composition***

419 Infusion of UFAA at 500 g/d decreased milk yield compared with other treatments.
420 Similar results have been observed previously with dairy cows in other studies (Gagliostro &
421 Chilliard, 1991; Drackley et al., 1992; Christensen et al., 1994; Bremmer et al., 1998) in which
422 postruminal infusion of mostly unsaturated LCFA significantly decreased milk production as a
423 result of decreased DM and energy intakes. The decreased DMI (10.5 kg/d) would account for
424 enough NE_L to produce 22.7 kg of 4% fat-corrected milk, so the actual decrease in milk (13.2
425 kg/d) was more than accounted for by the decreased DMI.

426 The increase in milk fat content when UFAA was infused was likely due to the marked
427 decrease in volume of milk secreted with less change in milk fat secretion. The lower milk fat
428 content when TGR was infused compared to SFAR may be due to the amount of intermediates

with *trans*-10 double bonds such as *trans*-10, *cis*-12 conjugated linoleic acid (CLA) produced during rumen bacterial biohydrogenation of unsaturated LCFA (Palmquist & Jenkins, 2017). Similar to the observations by Bremmer et al. (1998), in the current study milk CP was not greatly different among treatments, which may be due to adequate CP in the diet (17.5%) and the addition of 200 g/d of meat solubles infused into either the abomasum or rumen. Changes in milk CP content may have been due to reductions in DMI, which would reduce dietary CP intake.

4.3 | Milk Fat Composition

Alterations of milk fat composition were largely as might be predicted based on the LCFA composition of the infusate. Milk CLA can result from incomplete biohydrogenation of dietary polyunsaturated LCFA, predominantly linoleic acid, in the rumen (Harfoot, 1978; Tanaka & Shigeno, 1976). The biohydrogenation of linoleic acid begins with isomerization mainly to *cis*-9 *trans*-11 $C_{18:2}$, followed by hydrogenation to *trans*-11 $C_{18:1}$. Milk CLA also arises from desaturation of *trans*-11 C18:1 to CLA by mammary Δ-9 desaturase (Baumgard, Corl, Dwyer, Saebo, & Bauman, 2000)**.** Dietary supply of $C_{18:2}$ and manipulation of rumen pH can alter ruminal biohydrogenation (Kalscheur, Teter, Piperova, & Erdman, 1997; Romo, Casper, Erdman, & Teter, 1996; Griinari et al., 1998). In general these data agree with data from previous experiments in which ruminally protected fat was fed to lactating dairy cows (Schauff & Clark, 1989, 1992; Elliott, Overton, & Drackley, 1994) or when fat was infused postruminally in lactating dairy cows (Gagliostro & Chilliard, 1991; Christensen et al., 1994; Bremmer et al., 1998).

4.4 | Ruminal Fermentation and Total Tract Digestibilities

As expected no major differences were observed in ruminal fermentation. The subtle changes that were observed were unlikely to explain the differences in DMI or milk yield. Values were in expected ranges for cows past peak production and fed a diet such as we fed.

Our data showing decreased digestibility with infusion of UFAA differ from those of Drackley et al. (1992) and Christensen et al. (1994), in which infused unsaturated LCFA into the abomasum of lactating dairy cows did not affect digestibilities of DM, OM, ADF, NDF, and energy. Part of the difference may be attributable to the slightly greater infusion amount (500 g/d) in the present study compared with 450 g/d in previous studies. Accompanying the pronounced reduction in DMI, some of the cows infused with UFAA developed diarrhea, which may be associated with decreased intestinal digestibility. High incidence of diarrhea was observed after duodenal infusions of 200 to 500 g/d of FFA from soy oil (Chilliard, Gagliostro, Flechet, Lefaivre, & Sebastian, 1991). Alternatively, results from the current study might be explained by a potential effect of UFAA on fiber digestion in the large intestine. In the current study, other lipid sources infused abomasally or ruminally had no effect on apparent total tract digestibilities.

Results from previous studies that infused unsaturated FA into the abomasum showed no difference or a numerical increase in FA digestibility (Drackley et al., 1992; Christensen et al., 1994; Bremmer et al., 1998). Differences in FA digestibility observed in the current study likely were due to reduced DMI for UFAA as well as possible poor recovery of digestive marker due to diarrhea at the higher treatment amount.

Higher FA digestibility for ruminal infusions of SFA may be due to greater dispersion and increased attachment of FA to feed particles and thus their greater subsequent digestion in the small intestine compared with SFAA. Digestibility of FA for SFAA was similar to that

observed in previous infusion studies (Drackley et al., 1992; Christensen et al., 1994; Bremmer et al., 1998) as well as studies in which SFA were fed (Western, de Souza, & Lock, 2020).

Intestinal digestibility of unsaturated FA has been reported to be greater than intestinal digestibility of saturated FA (Wu, Ohajuruka, & Palmquist, 1991). We calculated by difference the apparent digestibility of the infused FA (Table 7). Digestibilities of FA in the basal diet agree with previous studies (Bremmer et al., 1998; Western et al., 2020). Digestibility of SFAR was greater than that of SFAA, with digestibility of SFAR higher than expected. The digestibility of SFAA was lower than most of the other treatments, which agrees with data where SFA were fed (Western et al., 2020). Digestibility of UFAA was lower than that of TGA and decreased at the higher level of infusion. However, digestibility of TGA was greater than that of TGR, which presumably would have been mostly hydrogenated in the rumen so that greater amounts of UFA would have reached the small intestine for TGA.

4.5 | Blood Metabolites

The lack of effects of LCFA infusion on plasma metabolite concentrations agrees with other studies in which fat was infused into the abomasum (Drackley et al., 1992; Christensen et al., 1994) and where fat was fed (Palmquist & Conrad, 1978; Grummer & Carroll, 1991; Choi & Palmquist, 1996). Increased plasma concentration of NEFA in cows infused with UFAA suggests that the reduction in DMI due to UFAA infusion caused mobilization of lipid reserves. Plasma NEFA concentrations during infusion of UFAA were similar to those reported in other studies (Drackley et al., 1992; LaCount et al., 1994; Christensen et al., 1994; Bremmer et al., 1998; Choi, Palmquist, & Allen, 2000). Changes in all plasma metabolites were within physiologically normal ranges.

4.6 | Gut Hormones in Plasma

Hormones or other signals elicited due to interaction of unsaturated LCFA with intestinal endocrine cells, such as K (releasing glucose-dependent insulinotropic polypeptide [**GIP**]) and I cells (releasing CCK) concentrated in the duodenum, and L cells concentrated in the distal small intestine, might at least in part be the cause for reductions in DMI when lipids are fed or abomasally infused. Studies using rodents as a model suggest that hormones such as CCK and GLP-1 may be significant regulators of food intake (Moran, Ameglio, Schwartz, & Mchugh, 1992; Holzer, Turkelson, Soloman, & Raybould, 1994; Woltman et al., 1995; Schwartz, Whitney, Skoglund, Castonguay, & Moran, 1999). Intestinal release of GIP, CCK, and GLP-1 respond to the inflow of nutrients through direct and indirect effects on secretory cells. In the case of GLP-1 secretion from L cells in the distal intestine, FA stimulate secretion through direct effects via the L cell membrane receptors, or indirect effects via GIP secreted from the K cells and subsequent vagal and GIP effects on the L cells (Lim & Brubaker, 2006). In addition, CCK released from the I cells in response to increased FA concentrations in the duodenum has been shown to stimulate GLP-1 release from L cells through direct effects (Gutierrez-Aguilar & Woods, 2011). In this respect, the presence of FFA in the duodenum may have greater effects on GLP-1 secretion through increased CCK and GIP secretion and vagal responses.

Plasma concentrations of GLP-1 for CONT, UFAA, and TGA closely resembled those observed previously when UFAA and TGA were infused at increasing amounts of 0, 200, 400, and 600 g/d (Litherland et al., 2005). Additionally, GLP-1 concentration in plasma in the current study was similar to that observed with infusion of 400 g/d of mostly unsaturated TG (Benson & Reynolds. 2001).

The markedly increased concentration of plasma GLP-1 associated with decreased DMI during infusion of UFAA, coupled with modest increases of GLP-1 during infusion of other lipid

519 sources where DMI was not altered appreciably, is consistent with a possible role of GLP-1 in
520 feed intake regulation. In nonruminants, GLP-1 has been shown to have numerous effects that
521 may impact appetite and DMI, including appetite suppressing effects on the hypothalamus,
522 reduced gut motility, and increased insulin synthesis and secretion (Holst, 2000; Lim &
523 Brubaker, 2006; Gutierrez-Aguilar & Woods, 2011). Changes in circulating concentrations of
524 GLP-1 in response to abomasal or ruminal infusions of LCFA in the present study, as well as
525 previous studies (Benson & Reynolds, 2001; Litherland et al., 2005; Relling & Reynolds, 2008)
526 indicate that GLP-1 secretion is responsive to LCFA supply to the small intestine in lactating
527 dairy cows. Given the repeatability of these findings, we suggest that GLP-1 is at least one of the
528 mediators of DMI when LCFA are infused. Some studies with dietary LCFA have confirmed this
529 relationship (Relling & Reynolds, 2007; Bradford, Harvatine, & Allen, 2008) whereas others
530 have not (Fukomori et al., 2012; Zapata, Salehi, Ambrose & Chelikani, 2015; Hu, Yin, Lin, Yan,
531 & Wang, 2015).

532 Values reported here are lower than those previously reported (Choi & Palmquist, 1996;
533 Benson & Reynolds, 2001; Litherland et al., 2005), perhaps as a result of extended storage of
534 plasma samples at -20°C despite the addition of aprotinin as a trypsin and related protease
535 inhibitor prior to freezing. Plasma concentrations of CCK for the current study are comparable
536 with those of Furuse et al. (1991), in which plasma CCK concentrations ranged from 5 to 7
537 pmol/L.

538 Nicholson & Omer (1983) suggested that unsaturated LCFA might increase release of
539 CCK, which may act to decrease DMI by reducing reticuloruminal motility. Plasma CCK
540 concentration in dairy cows was first reported by Furuse et al. (1991). Abomasal infusion of 400
541 g/d of mostly unsaturated LCFA significantly reduced DMI, but did not affect splanchnic

metabolism or arterial concentration of CCK (Benson & Reynolds, 2001). In an earlier study, high fat diets containing calcium salts of LCFA fed to dairy cattle linearly decreased DMI and linearly increased plasma CCK in a sample taken before feeding, but the effect was not maintained after feeding (Choi & Palmquist, 1996). Additionally, administration of a CCK-8 antagonist (MK-239) to heifers fed a high fat diet increased DMI by 92% compared to vehicle injection during a 2 h period after feeding (Choi et al., 2000), but there was no effect of MK-239 on total daily DMI. Although CCK was increased by LCFA administration in our study, the lack of relationship to differences in DMI casts doubt on its role as an important mediator of differences in DMI when fats are infused. Relling & Reynolds (2008) reached a similar conclusion after infusing soybean oil into the abomasum. However, effects of CCK on DMI may be mediated through effects on GLP-1 secretion that are modulated by other direct effects of FFA on L cells in the distal small intestine. In this regard, others have shown increased CCK when LCFA were fed and DMI decreased (Harvatine & Allen, 2005; Relling & Reynolds, 2007; Bradford et al., 2008; Hu et al., 2015).

5 | CONCLUSIONS

Results from this study confirmed that unsaturated FFA reaching the small intestine decrease DMI in lactating dairy cows, as determined previously. Abomasal and ruminal infusion of mostly saturated FFA did not affect nutrient intake, digestibility of nutrients, or milk yield. Abomasal infusion of unsaturated TG did not depress DMI to the extent of abomasal infusion of unsaturated FFA. Infusion of 500 g/d of UFAA decreased milk yield by 11.7 kg/d per cow compared with infusion of TGA. The decrease in milk yield was due to decreased nutrient intakes and apparent digestibility of nutrients because infusion of UFAA did not greatly affect

ruminal characteristics. These data suggest that the degree of saturation, degree of esterification, and the amount of LCFA passing to the small intestine all may play important roles in the responses of dairy cows to supplemental fats. Plasma concentrations of GLP-1 were increased by infusion of UFAA compared with TGA and this increase was greater at the higher infusion amount. This increase in the concentration of GLP-1 coincided with the decrease in DMI. Plasma concentration of CCK increased with LCFA supply, but did not appear to be associated with amount, profile, or site of administration of LCFA or with DMI in this experiment. Thus, GLP-1 is more likely to have a major role in control of DMI by dietary fat than is CCK.

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CONFLICT OF INTEREST

The authors have no conflict of interest.

ANIMAL WELFARE STATEMENT

The authors confirm that the ethical policies of the journal, as noted on the journal's author guidelines page, have been adhered to and the appropriate ethical review committee approval has been received. The authors confirm that they have followed EU standards for the protection of animals used for scientific purposes.

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TABLE 1 Ingredients and nutrient composition of the TMR (DM basis)[1].

Composition	(%)
Ingredient	
Alfalfa silage	20.00
Corn silage	30.00
Soybean hulls	4.50
Ground shelled corn	27.00
Soybean meal	16.00
Sodium chloride	0.20
Mineral and vitamin mixture[2]	0.20
Limestone	0.74
Dicalcium phosphate	0.40
Magnesium oxide	0.21
Sodium bicarbonate	0.75
Nutrient	
DM	66.67
OM	91.97
CP	17.51
NDF	30.33
ADF	21.40
Total FA[3]	2.43
Gross energy,[4] MJ/kg	17.99
NE_L,[5] MJ/kg	6.69

[1] Mean of samples from each period (n = 6). All nutrient values are analyzed from sampled feeds.

[2] Contains: 5.0% Mg, 7.5% K, 10.0% S, 3.0% Zn, 3.0 % Mn, 2.0% Fe, 0.5% Cu, 0.025% I, 0.015% Se, 0.004% Co, 2200 IU of vitamin A/g, 660 IU vitamin D_3/g, and 8 IU of vitamin E/g.

[3] Fatty acid content of the diet; does not include FA from infusates.

[4] Gross energy content of the diet; does not include gross energy from infusates.

[5] Calculated from NRC (2001). Does not include infusates.

TABLE 2 Fatty acid (FA) composition of infusates and dietary ingredients.

FA	Infusate[1]				Dietary ingredients		
	CONT	SFAA, SFAR	UFAA	TGA, TGR	Haylage	Corn silage	Concentrate
	----------(g/100 g of FA)----------						
$C_{12:0}$	0.03	0.20	ND[2]	ND	1.75	2.91	4.11
$C_{14:0}$	2.61	2.07	0.10	0.08	0.87	0.23	8.85
$C_{16:0}$	3.46	41.08	7.93	10.40	19.06	17.50	13.82
$C_{16:1}$	3.50	0.51	0.12	0.09	1.75	0.42	0.31
$C_{17:0}$	0.01	1.44	0.01	0.10	0.35	0.04	0.51
$C_{18:0}$	16.19	41.87	3.20	4.30	2.96	3.01	3.02
cis-$C_{18:1}$	37.08	13.3	22.37	23.60	2.78	20.39	19.32
$C_{18:2}$	7.24	2.2	54.13	52.40	15.74	46.34	39.27
$C_{18:3}$	ND	0.04	5.40	7.71	20.80	3.80	2.61
Total C_{16}	6.96	41.59	8.05	10.49	20.81	17.92	14.13
Total C_{18}	60.51	48.95	85.10	88.01	42.28	73.54	64.22
$C_{20:1}$	0.04	0.57	0.94	0.02	ND	0.45	0.22
$C_{22:0}$	ND	0.47	0.15	0.33	0.98	0.42	0.12
$C_{24:0}$	ND	ND	ND	0.11	1.20	0.34	0.27
Other FA	2.84	4.71	5.65	0.86	31.78	4.15	7.54

[1]CONT = Control (meat solubles and Tween 80); SFAA = Mostly saturated LCFA infused into the abomasum; SFAR = Mostly saturated LCFA infused into the rumen; UFAA = free FA from soybean oil; TGA = soybean oil infused into the abomasum; TGR = soybean oil infused into the rumen.

[2]Not detected.

TABLE 3 Least squares means for intakes of nutrients.

	Treatments[1]												
	CONT		SFAA		SFAR		UFAA		TGA		TGR		
Variable	0	0	250	500	250	500	250	500	250	500	250	500	SEM
Voluntary DMI, kg/d[a,b,c,d]	24.2	24.4	24.0	24.3	24.3	22.3	21.2	13.9	22.3	22.2	23.7	23.0	1.9
DM infused, g/d	160.2	160.2	410.2	660.2	410.2	660.2	410.2	660.2	437.7	715.2	437.7	715.2	…
Total DMI[a,b,c,d]	24.3	24.7	24.4	24.9	24.7	22.9	21.6	14.5	22.7	22.9	24.1	23.7	1.9
OM, kg/d[a,b,c,d]	22.5	22.7	22.5	23.0	22.7	21.1	19.9	13.4	20.9	21.1	22.2	21.8	1.8
Total FA,[2] g/d[a,b,c,d,e,f]	669	703	919	1188	938	1120	851	903	877	1142	932	1154	57
Dietary FA, g/d[a,b,c,d]	664	698	674	705	694	636	597	398	623	638	677	650	57
Infused FA, g/d	5	5	247	490	247	490	255	505	254	504	254	504	…
Total CP,[3] kg/d[a,b,c,d]	4.2	4.2	4.3	4.3	4.3	3.8	3.8	2.4	4.0	3.8	4.2	4.0	0.3
ADF, kg/d[a,b,c,d]	4.2	4.7	4.2	4.7	4.4	4.3	3.9	3.0	3.9	4.2	4.3	4.6	0.3
NDF, kg/d[a,b,c,d]	6.7	7.3	6.9	7.3	7.0	6.7	6.0	4.2	6.2	6.7	6.8	7.1	0.6
Voluntary gross energy intake[4], MJ/d[a,b,c,d]	436.8	441.8	443.5	456.5	446.8	418.4	390.8	266.5	410.4	415.5	435.6	437.6	36.0
Infused gross energy intake, MJ/d	0	0	7.9	15.9	7.9	15.9	7.9	16.3	9.2	18.0	9.2	18.0	…
Total gross energy, MJ/d[a,b,c,d]	436.8	441.8	451.4	472.0	454.4	433.5	398.3	281.6	419.2	433.0	444.8	455.2	34.3

[1]CONT = Control (meat solubles and Tween 80); SFAA = mostly saturated LCFA infused into the abomasum; SFAR = mostly saturated LCFA infused into the rumen; UFAA = free FA from soybean oil infused into the abomasum; TGA = soybean oil infused into the abomasum; TGR = soybean oil infused into the rumen.

[2]Intakes of total FA; includes FA from diet and infusate.

[3]Includes CP from diet.

[4]Gross energy from diet.

[a]SFAA vs. UFAA, $P < 0.05$.

[b]SFAA vs. UFAA × amount, $P < 0.05$.

[c]TGA vs. UFAA, $P < 0.05$.

[d]TGA vs. UFAA × amount, $P < 0.05$.

[e]CONT vs. lipid infusion, $P < 0.05$.

[f]CONT vs. lipid infusion × amount, $P < 0.05$.

TABLE 4 Least squares means for yield and composition of milk.

	Treatments[1]												
	CONT		SFAA		SFAR		UFAA		TGA		TGR		
Variable	0	0	250	500	250	500	250	500	250	500	250	500	SEM
Milk, kg/d[a,b,c,d,e,f]	31.7	34.1	32.9	33.5	33.9	30.9	32.9	19.6	34.2	31.3	33.2	34.0	2.4
Fat, %[h,f,g]	3.3	3.4	3.0	3.5	3.5	3.7	2.8	3.8	3.2	3.2	3.0	2.8	0.25
Fat, kg/d[a,b, d, e, h]	1.0	1.2	1.0	1.2	1.2	1.1	0.9	0.7	1.1	1.0	1.0	0.9	0.13
3.5 % FCM,[2] kg[a,b, c,d,e,f]	31.0	34.2	30.7	33.5	34.2	31.9	29.6	20.2	32.9	30.2	30.7	30.2	3.0
CP, %[f,h,]	3.1	3.2	2.9	3.2	3.2	3.1	2.7	3.3	3.2	3.1	3.0	2.9	0.19
CP, kg/d[a,b,d,e,f]	1.0	1.1	0.9	1.0	1.1	0.9	0.9	0.6	1.1	1.0	1.0	0.9	0.09

[1]CONT = Control (meat solubles and Tween 80); SFAA = mostly saturated LCFA infused into the abomasum; SFAR = mostly saturated LCFA infused into the rumen; UFAA = free FA from soybean oil infused into the abomasum; TGA = soybean oil infused into the abomasum; TGR = soybean oil infused into the rumen.

[2]3.5% FCM = 0.4324 (kg milk) + 16.216 (kg fat).

[a]SFAA vs. UFAA, $P < 0.05$.

[b]TGA vs. UFAA, $P < 0.05$.

[c]CONT vs. Fat × amount, $P < 0.05$.

[d]SFAA vs. UFAA × amount, $P < 0.05$.

[e]TGA vs SFAA × amount, $P < 0.05$.

[f]TGA vs. UFAA × amount, $P < 0.05$.

[g]TGA vs. SFAA, $P < 0.05$.

[h]TGR vs. SFAR, $P < 0.05$.

TABLE 5 Least squares means for proportions of individual fatty acids (FA) in milk.

	Treatment[1]												
	CONT		SFAA		SFAR		UFAA		TGA		TGR		
FA	0	0	250	500	250	500	250	500	250	500	250	500	SEM
	(g/100 g of FA)												
$C_{4:0}^{a,b}$	4.03	3.63	3.96	3.89	3.87	3.69	3.54	3.10	3.61	3.30	3.64	3.88	0.17
$C_{6:0}^{a}$	2.68	2.46	2.55	2.40	2.49	2.27	2.17	1.77	2.22	2.02	2.39	2.33	0.16
$C_{8:0}^{a,c}$	1.63	1.48	1.47	1.34	1.42	1.27	1.24	0.96	1.31	1.66	1.39	1.30	0.12
$C_{10:0}^{a,c}$	3.71	3.46	3.26	2.91	3.06	2.75	2.74	2.06	2.90	2.59	3.05	2.73	0.29
$C_{12:0}^{b,c}$	4.29	4.13	3.80	3.41	3.48	3.14	3.10	2.18	3.32	2.91	3.50	3.10	0.31
$C_{14:0}^{a,b,c}$	11.67	11.62	11.28	10.67	10.57	9.77	9.58	6.81	10.04	8.53	10.76	9.91	0.53
$C_{14:1}^{a,b,c}$	1.20	1.30	1.30	1.27	1.19	0.95	0.95	0.50	0.95	0.69	1.19	1.17	0.11
$C_{15:0}^{a,c,d,e,f}$	1.10	1.25	1.12	1.06	1.08	0.83	0.90	0.50	0.96	0.78	1.04	0.95	0.08
$C_{16:0}^{a,b,c,e,f,g,h,i}$	28.54	29.70	30.77	31.18	29.86	31.11	23.82	21.09	24.29	22.34	26.62	23.64	0.76
$C_{16:1}^{a,b,c,g}$	2.03	2.20	2.15	2.21	2.16	2.12	1.70	1.36	1.44	1.28	1.97	1.66	0.17
$C_{17:0}^{c,f}$	0.66	0.75	0.56	0.69	0.55	0.58	0.62	0.52	0.56	0.58	0.55	0.44	0.07
$C_{17:1}$	0.13	0.20	0.01	0.24	0.14	0.14	0.14	0.15	.013	0.14	0.14	0.11	0.04
$C_{18:0}^{a,c,d,j}$	9.04	8.19	8.30	8.39	8.97	10.11	9.95	7.99	9.43	10.47	9.94	10.20	0.58
trans-$C_{18:1}^{b,j}$	0.79	0.60	0.61	0.91	0.71	0.96	1.20	0.73	1.02	2.68	1.10	2.46	0.56
cis-$C_{18:1}^{a,j}$	19.49	19.61	19.64	20.51	20.70	21.36	21.70	23.92	21.20	20.35	20.77	21.84	1.37
trans-$C_{18:2}^{i}$	0.19	0.22	0.17	0.18	0.20	0.10	0.17	0.10	0.16	0.16	0.18	0.25	0.03
cis-$C_{18:2}^{a,b,c,d,f,h,j}$	2.51	2.63	2.79	2.69	3.32	2.53	8.55	18.38	8.05	11.98	2.91	2.43	0.80
$C_{20:0}$	0.08	0.09	0.08	0.08	0.11	0.11	0.10	0.07	0.11	0.12	0.10	0.15	0.02
$C_{20:1}$	0.17	0.09	0.07	0.13	0.09	0.10	0.13	0.09	0.09	0.02	0.07	0.08	0.07
$C_{18:3}^{a,b,c}$	0.03	0.04	0.46	0.38	0.54	0.38	0.94	1.49	1.12	1.55	0.38	0.34	0.17
CLA9,11^{c,g,i,j}	0.31	0.31	0.32	0.27	0.31	0.21	0.48	0.22	0.38	0.36	0.60	1.30	0.07
Other FA	5.72	3.05	5.33	5.19	5.18	5.87	6.28	6.01	6.83	5.50	7.10	9.73	---

[1]CONT = Control (meat solubles and Tween 80); SFAA = mostly saturated LCFA infused into the abomasum; SFAR = mostly saturated LCFA infused into the rumen; UFAA = free FA from soybean oil infused into the abomasum; TGA = soybean oil infused into the abomasum; TGR = soybean oil infused into the rumen.

[a]SFAA vs. UFAA, $P < 0.05$.

[b]TGA vs. SFAA, $P < 0.05$.

[c]CONT vs. Fat, $P < 0.05$.

[d]TGA vs. UFAA, $P < 0.05$.

[e]CONT vs. Fat × amount, $P < 0.05$.

[f]SFAA vs. UFAA × amount, $P < 0.05$.

[g]TGR vs. SFAR, $P < 0.05$.

[h]TGA vs SFAA × amount, $P < 0.05$.

[i]TGR vs. SFAR × amount, $P < 0.05$.

[j]TGA vs. UFAA × amount, $P < 0.05$.

TABLE 6 Least squares means for ruminal characteristics.

	Treatment[1]												
	CONT		SFAA		SFAR		UFAA		TGA		TGR		
Variable	0	0	250	500	250	500	250	500	250	500	250	500	SEM
pH[a]	6.06	5.97	5.94	6.00	6.01	5.99	6.05	5.97	5.94	5.99	6.05	6.00	0.1
NH_3 N, mg/dl	21.5	21.9	20.2	19.1	19.2	19.8	20.6	21.0	19.2	20.3	22.0	20.7	1.6
Total VFA, m*M*	123.8	126.9	128.9	120.7	121.3	117.9	122.6	116.7	124.7	120.8	122.8	121.4	3.8
Acetate, mol/100 mol[b]	64.2	63.5	63.8	65.6	63.6	63.7	64.5	65.8	62.5	63.2	62.0	64.1	1.2
Propionate, mol/100 mol[c,]	19.4	22.2	20.1	19.9	21.5	21.3	20.3	20.1	21.3	21.4	22.2	21.2	1.0
Acetate:propionate[c]	3.3	2.9	3.2	3.3	3.0	3.1	3.2	3.2	2.9	3.0	2.9	3.0	0.2
Butyrate, mol/100 mol[b,d]	11.1	9.7	11.1	9.8	10.1	10.0	10.4	9.6	10.5	10.4	10.6	9.8	0.6
Isobutyrate, mol/100mol	1.4	1.1	1.1	1.1	1.1	1.1	1.1	1.0	1.5	1.1	1.2	1.0	0.2
Isovalerate, mol/100 mol	2.1	1.9	2.0	1.9	1.9	2.1	2.0	2.0	2.3	2.0	2.1	1.9	0.1
Valerate, mol/100 mol[b]	1.5	1.5	1.5	1.4	1.5	1.6	1.4	1.3	1.7	1.6	1.6	1.6	0.1

[1]CONT = Control (meat solubles and Tween 80); SFAA = mostly saturated LCFA infused into the abomasum; SFAR = mostly saturated LCFA infused into the rumen; UFAA = free FA from soybean oil infused into the abomausm; TGA = soybean oil infused into the abomasum; TGR = soybean oil infused into the rumen.

[a]SFAA vs. UFAA, $P < 0.05$.

[b]TGA vs. UFAA, $P < 0.05$.

[c]CONT vs. fat × amount, $P < 0.05$.

[d]TGA vs. SFAA, $P < 0.05$.

TABLE 7 Least squares means for apparent digestibilities of nutrients in the total digestive tract.

	Treatment[1]												
	CONT		SFAA		SFAR		UFAA		TGA		TGR		
Fraction	0	0	250	500	250	500	250	500	250	500	250	500	SEM
	----------(%)----------												
DM[a,b,c]	68.6	67.7	57.8	64.9	70.1	66.3	59.5	39.2	62.0	61.4	68.2	65.7	5.7
OM[a,b,c]	70.3	69.3	60.1	66.7	71.6	68.3	62.1	45.4	64.2	63.6	70.0	67.6	5.1
CP[a,b,c,d]	68.7	67.1	58.8	63.8	69.5	66.4	60.1	32.1	62.4	61.2	68.9	66.2	6.8
ADF[c,e]	41.1	46.1	20.9	41.3	45.7	40.8	25.0	21.3	24.4	31.4	42.5	44.2	8.5
NDF[a,b,c]	44.2	49.0	25.8	42.7	47.9	43.2	28.2	1.0	31.4	36.1	45.5	45.6	10.8
Energy[a,b,c]	65.7	64.2	55.4	61.5	68.1	64.5	57.0	31.3	60.2	58.6	66.2	63.0	6.7
Total FA[a,b,c,d,f,g,h]	79.4	76.7	73.7	73.2	83.3	79.8	75.4	57.2	76.5	80.5	75.3	74.9	3.5
Total C_{16} FA[a,b,c,d,e]	74.4	72.5	75.5	73.9	83.7	82.6	67.3	26.5	69.9	73.6	73.9	74.3	5.7
Total C_{18} FA[b,c,d]	83.4	80.3	75.6	73.9	85.2	79.9	79.6	63.0	80.3	84.1	77.0	76.1	3.4
Infused FA[2]	---	---	61.8	66.2	98.1	82.1	69.2	40.8	72.7	83.6	68.0	70.8	---

[1]CONT = Control (meat solubles and Tween 80); SFAA = mostly saturated LCFA infused into the abomasum; SFAR = mostly saturated LCFA infused into the rumen; UFAA = free FA from soybean oil infused into the abomasum; TGA = soybean oil infused into the abomasum; TGR = soybean oil infused into the rumen.

[2]Calculated from means, so no statistical analysis performed.

[a]SFAA vs. UFAA, $P < 0.05$.

[b]TGA vs. UFAA, $P < 0.05$.

[c]SFAA vs. UFAA × amount, $P < 0.05$.

[d]TGA vs. UFAA × amount, $P < 0.05$.

[e]TGA vs. SFAA, $P < 0.05$.

[f]CONT vs fat, $P < 0.05$.

[g]TGR vs. SFAR, $P < 0.05$.

[h]CONT vs. fat × amount, $P < 0.05$.

TABLE 8 Least squares means for concentrations of metabolites and gut hormones in plasma.

	Treatments[1]												
	CONT		SFAA		SFAR		UFAA		TGA		TGR		
Component	0	0	250	500	250	500	250	500	250	500	250	500	SEM
Glucose, mg/dL	63.0	68.2	69.9	67.2	64.0	70.6	68.9	65.8	67.0	69.5	68.8	70.4	6.3
NEFA, μeq/L[a,b,c,d]	91.0	103.9	69.1	76.7	95.1	157.7	99.2	274.7	94.3	109.3	86.1	91.5	42.6
Urea, mg/dL[e,f,g]	20.9	18.9	18.4	20.5	19.6	21.4	20.7	20.7	21.7	20.4	20.4	20.9	1.4
Total protein, g/dL[a]	8.6	8.8	8.4	8.4	8.5	9.0	8.9	8.7	8.9	8.8	8.3	8.5	0.3
GLP-1, pmol/mL[a,d,f]	0.025	0.024	0.033	0.034	0.027	0.034	0.034	0.052	0.028	0.030	0.027	0.030	0.005
CCK, pmol/L[e]	5.04	5.98	8.50	8.91	8.56	10.47	8.41	8.25	6.34	7.79	6.17	8.61	1.82

[1]CONT = Control (meat solubles and Tween 80); SFAA = mostly saturated LCFA infused into the abomasum; SFAR = mostly saturated LCFA infused into the rumen; UFAA = free FA from soybean oil infused into the abomasum; TGA = soybean oil infused into the abomasum; TGR = soybean oil infused into the rumen.

[a]SFAA vs. UFAA, $P < 0.05$.

[b]TGA vs. UFAA, $P < 0.05$.

[c]SFAA vs. UFAA× amount, $P < 0.05$.

[d]TGA vs. UFAA × amount, $P < 0.05$.

[e]CONT vs. Fat × amount, $P < 0.05$.

[f]SFAA vs. UFAA × amount, $P < 0.05$.

[g]TGA vs. SFAA × amount, $P < 0.05$.