Effect of Increasing Monensin Concentration on the Performance of Lactating Dairy Cows Fed Contemporary Diets

A. C. Benoit[1], P. A. LaPierre[1], G. D. Mechor[2], D. M. Barbano[3], and M. E. Van Amburgh[1]
[1]Department of Animal Science, Cornell University, Ithaca, NY 14853
[2]Elanco Animal Health, Greenfield, IN, 46140
[3]Department of Food Science, Cornell University, Ithaca, NY 14853

Introduction

Monensin is a carboxylic polyether ionophore produced naturally by *Streptomyces cinnamonensis* and fed to dairy cattle to alter rumen microbial population and fermentation by reducing gram-positive bacteria and enhancing gram-negative metabolism (McGuffey et al., 2001; Vasquez et al., 2021). This shift in microbial population increases propionate production in coordination with the disposal of H_2 due to reduced methane production (Russell and Strobel, 1989; Fellner et al., 1997). Through these changes, feed efficiency improves because of the increased availability of propionate for glucose production in the liver that can be used by the mammary gland to increase milk production (Ipharraguerre and Clark, 2003; Duffield et al, 2008b). Although the mode of action of monensin is well understood, treatment effects reported in previous studies have been inconsistent (Phipps et al., 2000; Dubuc et al., 2009; McCarthy et al., 2015). A meta-analysis by Duffield et al. (2008b) reported a 0.7 kg/d increase in milk production and a 0.3 kg/d reduction in dry matter intake (DMI) across monensin studies, but treatment effects were influenced by stage of lactation, diet type, and dose level.

Although monensin is associated with improved feed efficiency, negative effects on milk fat production and synthesis have been previously reported. Monensin altered the content of saturated and unsaturated fatty acids (FA) in ruminal fermenters through inhibition of biohydrogenation (Fellner et al., 1997), thus it is hypothesized that the mode by which monensin decreases milk fat is through an accumulation of conjugated FA in the rumen that inhibit milk fat synthesis (Alzahal et al., 2008; Baumgard et al., 2000). More recently, the effect of monensin on milk fat production was greatest in studies that fed diets high in unsaturated FA (Alzahal et al., 2008; He et al., 2012), and a reduction in milk fat synthesis was predicted to be caused by an accumulation of long chain FA in the rumen that inhibit de novo FA synthesis (Dubuc et al., 2009). Further, monensin in high starch diets has been associated with a decrease in milk fat production due to a reduction in biohydrogenation caused by monensin and high levels of rumen fermentable starch that decrease ruminal pH (Bradford and Allen, 2004; Van Amburgh et al., 2008). And more recently, Akins et al. (2014) reported a numerical decrease in milk fat content with monensin feeding in average starch (27%) diets, but not in reduced starch (21%) diets.

Using diet formulation systems such as Cornell Net Carbohydrate Protein System (CNCPS), nutritionists can monitor rumen unsaturated FA load (RUFAL), dietary fat, starch, and NDF content to help minimize diet induced milk fat depression, and therefore understand how to optimize the use of monensin in lactating dairy cows. Previous studies

that reported a decrease in milk fat production with monensin feeding were performed decades ago when dietary nutrients in dairy diets were not as well understood as they are today, and more recent monensin studies have reported no effect on milk fat production (Akins et al., 2014; Hagen et al., 2015; Vasquez et al., 2021).

The FDA has approved the use of monensin in lactating dairy cattle diets at levels of 11 g/ton to 22 g/ton (DM basis), but recently, few studies have been conducted evaluating lactation performance at various monensin concentrations using more contemporary diets formulated with refined nutrient requirements and supplies. Therefore, the amount of monensin in the diet needed to effect milk production and composition, intake, and shifts in milk FA profile is of interest. The objective of this study was to evaluate increasing dietary monensin (Rumensin, Elanco Animal Health, Greenfield, IN) concentration on milk performance, milk FA profile, and production efficiencies (component-corrected milk/ DMI) in lactating dairy cows fed contemporary diets. We hypothesized milk performance and feed efficiency would improve with increasing levels of dietary monensin with no negative effects on milk component yield or shifts in FA profile.

Materials and Methods

Experimental Design and Treatments

The experiment was conducted from September to December 2020 at the Cornell University Ruminant Center (Harford, NY), and all procedures were approved by Cornell University Animal Care and Use Committee. One-hundred ninety-two cows (120 ± 50 DIM; mean ± standard deviation) were stratified by parity, DIM, and pre-trial milk production, and assigned to 1 of 12 pens housing 16 cows per pen (12 multiparous and 4 primiparous) in a 91-day longitudinal study with a 29 day covariate and 62 day experimental period. All cows were fed 11 g/ton (DM basis) monensin for the adaptation and covariate period. Following the covariate period, pens were randomly assigned 1 of 4 treatment diets stratified by milk performance and BW data collected in the covariate period. Cattle were housed in freestall pens with 16 headlocks and sand-bedded stalls, and had free access to feed, water, and bedding. Cows were milked three times daily at 0700h, 1500h, and 2300h in a double-16 parallel parlor. Feed was delivered once daily as a TMR at 0600h ad libitum to allow for 5% refusals.

Diets were formulated to meet or exceed nutrient demands for high producing lactating dairy cows using CNCPS (v6.55; Van Amburgh et al., 2015). Methionine and lysine were balanced using the latest information on requirements and supply as generated in the studies of LaPierre et al. (2020) where amino acid requirements are described on a gram per unit of ME basis (Higgs and Van Amburgh, 2016). For diet formulation, the methionine requirement was set at 1.19 g methionine per Mcal ME and lysine was set at 3.21 g per Mcal ME (or 2.7 times the grams methionine). All diets consisted of (DM basis) 34.9 % corn silage, 19.4 % grass haylage, 18 % corn meal, 6.8 % soybean meal, and 21 % pre-mix containing monensin (Purina Animal Nutrition, Caledonia, NY; Table 1). Treatments were 0 g/ton monensin (CON), 11 g/ton monensin

(R11), 14.5 g/ton monensin (R14.5), and 18 g/ton monensin (R18) on a DM basis, and monensin intake was formulated to be 305 mg/d, 404 mg/d, and 515 mg/d for R11, R14.5, R18, respectively.

Table 1. Ingredient composition of experimental diets

	Diet[1]			
Ingredient, % of DM	CON	R11	R14.5	R18
Corn silage	34.9	34.9	34.9	34.9
Grass haylage	19.4	19.4	19.4	19.4
Corn meal	18.0	18.0	18.0	18.0
Soybean meal	6.81	6.81	6.81	6.81
SoyPass[2]	5.83	5.83	5.83	5.83
Citrus pulp	4.49	4.49	4.49	4.49
Wheat middlings	4.49	4.49	4.49	4.49
Dextrose	1.60	1.60	1.60	1.60
Bloodmeal	1.00	1.00	1.00	1.00
Berga fat F100[3]	0.60	0.60	0.60	0.60
Energy Booster 100[4]	0.60	0.60	0.60	0.60
Ground limestone	0.54	0.54	0.54	0.54
Min AD[5]	0.45	0.45	0.45	0.45
Sodium bicarbonate	0.42	0.42	0.42	0.42
White salt	0.27	0.27	0.27	0.27
Vitamin and mineral mix[6]	0.22	0.22	0.22	0.22
Magnesium oxide	0.11	0.11	0.11	0.11
Smartamine M[7]	0.10	0.10	0.10	0.10
Smartamine ML[7]	0.10	0.10	0.10	0.10
Levucell SC[8]	0.05	0.05	0.05	0.05
Rumensin 90[9]	-	0.006	0.008	0.01

[1]CON = 0 g/ton monensin, R11 = 13 g/ton monensin, R14.5 = 15.8 g/ton monensin, R18 = 19.3 g/ton monensin.
[2]Lignotech USA, Rothschild, WI.
[3]Berg + Schmidt America LLC, Libertyville, IL.
[4]Milk specialties, Eden Prairie, MN.
[5]Calcium (22%) and magnesium (12%) supplement (Min-AD, Winnemucca, NV).
[6]Contained (DM basis) 27.4% Ca; 223 ppm Fe; 24,997 ppm Zn; 5,765 ppm Cu; 18,473 ppm Mn; 134.5 ppm Se; 568 ppm Co; 568 ppm I; 2021 KIU/kg vitamin A; 562 KIU/kg vitamin D; 9660 IU/kg vitamin E)
[7]Adisseo Inc, Alpharetta, GA.
[8]Lallemand Inc, Milwaukee, WI.
[9]Monensin, 90.7 g/lb. (Elanco Animal Health, Greenfield, IN).

Forages and TMR were sampled twice weekly, composited, and sent to Cumberland Valley Analytical Services (Waynesboro, PA) once per week for nutrient analysis. Additionally, FA profile was determined on TMR samples. Grains were sampled once weekly, and a 4 wk composite was sent once monthly for chemical analysis. Grain mixes were sent for determination of monensin concentration upon delivery of a new batch (Eurofins Food Chemistry Testing US, Inc, Greenfield, IN). Feed DM was determined twice weekly for diet adjustment and calculation of DMI. Pen level intake was obtained daily using Feedwatch (Valley Agricultural Software, Tulare, CA), and determined using observations of feed offered and feed refused.

Milk production was recorded at every milking (Delpro, DeLaval Inc, Kanas City, MO) and milk samples were taken at 3 consecutive milk sessions once weekly during the last two weeks of the covariate period and every week of the experimental period. Samples were analyzed for fat, true protein, anhydrous lactose, and MUN using a FTIR spectrophotometer (Lactoscope model FTA, Delta Instruments, Drachten, the Netherlands) at the Department of Food Science at Cornell University (Ithaca, NY). De novo, mixed-origin, and preformed FA were analyzed by FTIR on all milk samples according to PLS prediction models described by Woolpert et al. (2016) and calibration was carried out using gas-liquid chromatography reference chemistry described by Wojciechowski and Barbano (2016). The same calibration set was used for milk components and FA analysis with concentrations ranging from 0.05 to 1.4 g/100g milk de novo FA, 0.08 to 2.2 g/100g milk mixed FA, and 0.06 to 1.9 g/100g milk preformed FA. In addition, FA chain length (mean carbon number per FA) and unsaturation (double bonds per FA) were measured as previously described by Wojciechowski and Barbano (2016). Body weight (BW) was obtained once weekly following the 1500h milk session as well as body condition score (BCS) using a 5-point scale according to Wildman et al. (1982). Blood samples were collected once weekly via the coccygeal vein into tubes containing sodium heparin. Samples were centrifuged (3,000 × *g* for 20 min at 4˚C), and plasma was harvested and frozen at -20 ˚C for urea nitrogen analysis (No. 640, Sigma-Aldrich, St. Louis, MO). Finally, rumination time (minutes per day) was obtained from cows with a pre-existing Smartbow ear tag (Zoetis, Parsippanny, NJ; CON: n = 34, R11: n = 38, R14.5: n = 42, and R18: n = 42).

Statistical Analysis

All data, excluding BCS, were analyzed through SAS version 9.4 (SAS Institute Inc., Cary, NC) using PROC MIXED and LSMEAN statements to compare treatment means. When individual cow variables with covariate structure and repeated weekly measurements (milk production, milk composition and FA profile, BW, rumination, and PUN) were analyzed, pen was the experimental unit and cow was the observational unit as previously described by Fessenden et al. (2020) and Bellow et al. (2016), and the following model was used:

$$Y_{ijklm} = \mu + T_i + W_j + TW_{ij} + P_{k:i} + B_{l:k:i} + BX_{lik} + \varepsilon_{ikklm},$$

where Y_{ijklm} = dependent variable, μ = overall mean, T_i = fixed effect of treatment i, W_j = fixed effect of week j, TW_{ij} = fixed interaction of treatment i and week j, $P_{k:l}$ = random effect of pen k within treatment i, $B_{l:k:i}$ = random effect of cow within pen k within treatment i, BX_{lik} = the covariate adjustment for each cow, and ε_{ikklm} = residual error. An auto-regressive structure [AR(1)] was used to analyze repeated measurements with cow in pen within treatment. For pen level variables (DMI and production efficiencies), a random effect of pen within treatment was used. Three cows did not complete the experiment due to health issues (1 and 2 cows from R14.5 and CON, respectively). The BW data from wk 6 to 9 of the experimental period were removed from statistical analysis due to scale malfunctions during extreme cold weather conditions, with wk 5 BW was used as final BW to determine BW change. Degrees of freedom were determined using Kenward-Roger option and least square means were adjusted by Tukey method for multiple comparison tests. Body condition score data was analyzed using a non-parametric analysis (PROC NPAR1WAY) with treatment as the classification variable. Statistical significance was reported as $P \leq 0.05$ and tendencies as $0.05 < P \leq 0.10$.

Results and Discussion

Ingredient composition and chemical analysis of the diets are in Table 1 and 2, respectively, and chemical analysis of the forages and concentrate mixes are in Table 3. The analyzed monensin concentration for all treatment pre-mixes, on a DM basis, are as follows: CON = 0 g/ton monensin, R11 = 13 g/ton monensin, R14.5 = 15.8 g/ton monensin, and R18 = 19.3 g/ton monensin. The actual monensin intake was 0, 384, 465, and 589 mg/d for CON, R11, R14.5, and R18, respectively. Lactation performance results are in Table 4. We observed a numerical increase in DMI in the R18 group compared to CON, R11, and R14.5 (27.7 vs. 26.9, 26.8, and 26.7 kg/d, respectively). Monensin treatment tended to have a quadratic effect on DMI ($P = 0.10$) where R11 and R14.5 had slightly decreased DMI compared to CON, but DMI increased in the R18 group. This finding is not consistent with previous studies as increasing dietary monensin has been associated with either no change or a slight decrease in DMI (Akins et al., 2014; Hagen et al., 2015), although Recktenwald et al. (2014) reported a trend for increased DMI in cows fed monensin compared to none in diets high and low in starch and protein content. Milk yield was not affected by monensin treatment in agreement with experiments of Alzahal et al. (2008) and Hagen et al. (2015) (Table 4). The lack of an adaptation period for the CON group following the covariate diet of 11 g/ton monensin was predicted to decrease the ability to detect treatment effects because we observed a decrease in milk yield in the CON group compared to all monensin treated groups from wk 4 to 9 (data not shown) indicating cows were still adjusting to the removal of monensin in the beginning 3 wk of the experimental period. This is consistent with lactose production data as we observed a decrease in lactose yield in the CON group compared to all monensin treated groups following wk 3 of the experimental period (data not shown). In agreement, Akins et al. (2014) reported an increase in milk yield in cows fed monensin from wk 4 to 12, but not from wk 1 to 3, suggesting cows were still adapting to monensin changes in the diet.

Table 2. Analyzed nutrient composition (mean ± SD) of experimental diets

	Diet[1]			
Item	CON	R11	R14.5	R18
DM, % as-fed	43.4 ± 1.5	44.0 ± 1.2	43.5 ± 1.3	44.1 ± 1.4
CP, % of DM	15.3 ± 0.3	14.9 ± 0.6	15.0 ± 0.6	15.4 ± 0.6
ADF, % of DM	19.4 ± 1.6	20.4 ± 1.6	19.7 ± 1.0	18.8 ± 1.4
aNDF, % of DM	32.0 ± 1.4	32.8 ± 0.9	31.7 ± 1.1	31.3 ± 1.7
Sugars, % of DM	5.7 ± 0.3	5.7 ± 0.7	5.8 ± 0.2	5.9 ± 0.4
Starch, % of DM	25.6 ± 1.6	24.9 ± 1.0	25.3 ± 0.9	26.2 ± 1.2
Ether extract, % of DM	4.4 ± 0.2	4.2 ± 0.3	4.4 ± 0.2	4.2 ± 0.3
Ash, % of DM	7.2 ± 0.3	7.0 ± 0.3	7.1 ± 0.4	7.1 ± 0.3
NFC, % of DM	43.7 ± 1.2	43.7 ± 0.9	44.5 ± 1.6	44.6 ± 1.4
NSC, % of DM	31.3 ± 1.5	30.5 ± 1.1	31.1 ± 0.8	32.1 ± 1.1
ME, Mcal/kg[2]	2.7	2.7	2.7	2.7
FA, % of DM				
Total	3.56 ± 0.31	3.47 ± 0.11	3.73 ± 0.27	3.78 ± 0.28
16:0	1.12 ± 0.13	1.04 ± 0.03	1.14 ± 0.11	1.19 ± 0.10
18:0	0.33 ± 0.05	0.31 ± 0.03	0.33 ± 0.06	0.35 ± 0.05
18:1 *cis*-9	0.50 ± 0.07	0.49 ± 0.02	0.53 ± 0.05	0.54 ± 0.06
18:2 *cis*-9, *cis*-12	1.13 ± 0.08	1.11 ± 0.05	1.20 ± 0.07	1.20 ± 0.07
18:3 *cis*-9, *cis*-12, *cis*-15	0.31 ± 0.04	0.34 ± 0.02	0.33 ± 0.04	0.32 ± 0.03
RUFAL[3]	1.94	1.94	2.06	2.06

[1]CON = 0 g/ton monensin, R11 = 13 g/ton monensin, R14.5 = 15.8 g/ton monensin, R18 = 19.3 g/ton monensin.
[2]Predicted using the Cornell Net Carbohydrate and Protein System v6.5 (Van Amburgh et al., 2015).
[3]Rumen unsaturated fatty acid load = 18:1 + 18:2 + 18:3 from the chromatographic analysis of the diets.

Table 3. Nutrient analysis (mean ± SD) of diet ingredients

Item	Corn Silage	Grass Haylage	CON Mix	R11 Mix	R14.5 Mix	R18 Mix
DM, % as-fed	29.3 ± 0.7	39.5 ± 4.0	90.5 ± 0.3	90.7 ± 0.9	90.5 ± 0.4	90.4 ± 0.3
CP, % of DM	7.5 ± 0.4	15.7 ± 0.7	21.9 ± 0.5	23.9 ± 1.9	21.2 ± 1.3	22.4 ± 1.5
ADF, % of DM	24.1 ± 1.1	34.4 ± 1.4	14.7 ± 2.0	14.0 ± 2.6	14.8 ± 2.9	14.3 ± 2.4
aNDF, % of DM	39.7 ± 1.7	52.0 ± 1.9	22.7 ± 3.3	22.1 ± 3.6	23.4 ± 3.5	22.5 ± 2.2
Sugars, % of DM	0.4 ± 0.2	3.4 ± 0.6	17.5 ± 0.9	16.2 ± 2.0	18.3 ± 0.9	18.4 ± 1.0
Starch, % of DM	34.5 ± 1.6	1.4 ± 0.3	5.0 ± 0.7	5.3 ± 3.9	5.4 ± 1.7	5.8 ± 3.3
Ether extract, % of DM	3.2 ± 0.1	3.7 ± 0.3	6.7 ± 0.9	5.6 ± 1.3	6.8 ± 1.7	6.9 ± 2.2
Ash, % of DM	3.4 ± 0.3	8.5 ± 0.5	13.0 ± 1.9	12.1 ± 2.5	12.8 ± 0.3	13.2 ± 1.2
NFC, % of DM	46.8 ± 1.4	23.1 ± 1.4	38.8 ± 2.0	35.1 ± 2.5	39.6 ± 3.1	40.4 ± 1.5
NSC, % of DM	34.9 ± 1.6	4.8 ± 0.6	22.5 ± 0.7	21.5 ± 3.6	23.7 ± 1.4	24.2 ± 2.5

[1]CON = 0 g/ton monensin, R11 = 13 g/ton monensin, R14.5 = 15.8 g/ton monensin, R18 = 19.3 g/ton monensin.

Additionally, the experimental period for Akins et al. (2014) was 3 wk longer than the current study, allowing for greater detection of monensin effects on milk yield over time.

No significant treatment effects were observed for milk fat concentration or yield; however, milk fat percentage increased numerically with increasing monensin concentration (4.60, 4.67, 4.71, and 4.66 for CON, R11, R14.5, and R18 respectively; Table 4). The numerical increase in milk fat was most likely an effect of monensin on de novo FA synthesis as there was a linear increase ($P < 0.05$; Table 5) in de novo and mixed fat content with increasing levels of monensin. Previous research has shown monensin decreases milk fat concentration with increasing monensin levels (Dubuc et al., 2009; Duffield et al., 2008b), while others (Martinez et al., 2009; McCarthy et al., 2018) have reported no effect on milk fat. More recently, monensin has been shown to interact with other dietary factors such as starch content and unsaturated oils to reduce milk fat, rather than causing milk fat depression independently (McCarthy et al., 2018). Van Amburgh et al. (2008) also reported monensin diets high in starch content and unsaturated oil might have a stepwise negative effect on milk fat production, whereas rumen unsaturated FA increase, the risk of milk fat depression increases with monensin. In the current study, monensin concentration had no negative effect on milk fat production, rather milk fat content increased with monensin treatment due to the change in de novo and preformed fat synthesis. This finding is consistent with the expected increase in propionate production which would provide more energy for productive functions in the gland (Prange et al., 1978; Van Maanen, et al., 1978).

Milk FA profile results are in Table 5. The de novo and mixed FA concentration linearly increased in cattle fed monensin compared to CON but yields were not significantly different ($P = 0.21$) although there was a trend for a linear increase in both de novo ($P < 0.06$) and mixed FA (0.09). Both Duffield et al. (2008b) and Alzahal et al. (2008) reported a significant decrease in de novo FA concentration per total FA with monensin treatment, so the results of this experiment are not consistent with previous observations. The mixed FA yield and percent of total FA did not differ among treatment groups ($P < 0.10$), but mixed FA content linearly increased compared to CON ($P = 0.02$). The preformed FA concentration and yield were not different among treatment groups nor was preformed FA as a percentage of total FA. Alzahal et al. (2008) also found monensin treatment had no effect on preformed concentrations as a function of total FA. There was a trend for C16 concentration and yield tended to be greater ($P = 0.09$) with a significant linear effect of monensin consistent with the mixed FA results. The C18 and *cis*-9 C18:1 concentration and yield were not affected by monensin treatment. The biohydrogenation of oleic acid to stearic acid is achieved by gram-negative bacteria (Alzahal et al., 2008; Harfoot and Hazelwood, 1988) who, unlike gram-positive bacteria, are not inhibited by monensin treatment, therefore, this theory might explain the lack of treatment effects on stearic and oleic acid in the current study. The level of unsaturation of FA decreased with increasing monensin levels and was likely due to the level of de novo and mixed FA contents of the milk across treatments ($P = 0.01$; Table 5). All monensin treated groups approached a tendency for a reduction in FA chain length compared to CON ($P = 0.11$, 0.14, and 0.16 for R11, R14.5, R18, respectively) likely due to an increase in de novo synthesis in the monensin treated groups. Alzahal et al. (2008) and Fellner et al. (1997)

suggest monensin has a role in inhibiting ruminal biohydrogenation which would reduce milk fat synthesis, but in the current study, the milk fat concentration levels, de novo FA levels, and FA unsaturation suggests that monensin treatment enhanced biohydrogenation in the rumen or had some effect on FA synthesis. An alternative observation is that monensin did not impact biohydrogenation and the increased concentration of saturated FA was related to the increase in de novo and mixed FAs which would dilute out the unsaturated FA given the level of milk fat yield. We did not measure other C18:1 or C18:2 isomers that would have given more insight into the effect of monensin on biohydrogenation, although the high levels of fat production and the reduction in FA unsaturation in monensin fed cows suggest monensin did not play a role in inhibiting biohydrogenation or milk fat synthesis in the current study.

The increase in de novo and mixed FA synthesis and yield in mid- to late lactation dairy cattle was an interesting and exciting observation and one that is not well documented. The increase in de novo and mixed FA through the feeding of monensin could be due to a couple different substrate supplies. Monensin is known to increase the supply of propionate and under certain conditions, propionate can be part of an initiation sequence where synthesis of acyl chains from carbon atoms could potentially lead to incorporation into chain elongation of FA (Palmquist, 2007). In addition, with increased propionate, there will be greater glucose and capacity for reducing equivalents which means increased NADPH +H supply which would allow for an increase in the FA synthase reaction allowing for production and elongation of FA. The protein sparing effect of monensin could increase the supply of certain amino acids, including the branched chain amino acids and their conversion to branched chain volatile FA and these could serve as precursors for chain elongation for chain lengths less than 16 carbons (Massart-Leen et al., 1981; Ha and Lindsay, 1990; Liu et al., 2018). Diets were not formulated to contain high quantities of fat, thus it is possible that with lower exogenous FA, there was less competition for certain enzymes related to glycerol production and utilization, but de novo FA synthesis could be increased. Finally, it is also possible, that some of the fat content and yield was related to the supply of methionine and lysine. In the current study, the methionine and lysine were supplied at what we believe are closer to the true requirements and, with the DMI observed, the metabolizable methionine level was approximately 85 g/d and the lysine levels were approximately ≥225 g/d, levels much higher than typically fed. This data would suggest that overcoming the limitation of at least two essential amino acids (EAA) allowed for greater milk fat synthesis in these cows. There is emerging data to suggest there is a link between mTOR signaling, EAA, and the regulation of milk fat synthesis (Li et al., 2016; Nichols et al., 2020).

Table 4. Effect of increasing dietary monensin concentration on lactation performance

	Diet[1]					*P*-value[2]			
Item	CON	R11	R14.5	R18	SEM	Linear	Quad	Trt	Trt x Wk
Days in milk[3]	190	168	193	184	7.2	-	-	-	-
Monensin, mg/d	0	384	465	589	-	-	-	-	-
DMI, kg/d	26.9	26.8	26.7	27.7	0.31	0.29	0.09	0.22	< 0.01
Milk, kg/d	39.3	39.9	39.7	39.6	0.34	0.48	0.38	0.69	< 0.01
Fat, %	4.60	4.67	4.71	4.66	0.04	0.16	0.40	0.38	0.16
Fat, kg/d	1.79	1.83	1.85	1.83	0.02	0.15	0.52	0.40	< 0.01
Protein, %	3.35	3.37	3.36	3.39	0.02	0.15	0.89	0.41	< 0.01
Protein, kg/d	1.30	1.33	1.33	1.33	0.01	0.13	0.46	0.41	< 0.01
Lactose, %	4.63	4.65	4.63	4.63	0.01	0.98	0.27	0.51	< 0.01
Lactose, kg/d	1.82	1.85	1.84	1.84	0.02	0.34	0.50	0.71	< 0.01
MUN, mg/dL	8.96[a]	10.24[b]	9.61[ab]	9.52[ab]	0.28	0.12	0.04	0.05	< 0.01
PUN, mg/dL	9.11	9.13	9.04	8.89	0.17	0.42	0.42	0.72	< 0.01
ECM[4], kg/d	46.0	46.9	47.1	46.8	0.50	0.17	0.47	0.46	< 0.01
3.5% FCM[5], kg/d	46.0	46.9	47.2	46.8	0.53	0.19	0.51	0.49	< 0.01
SCM[5], kg/d	42.5	43.3	43.5	43.2	0.46	0.17	0.41	0.42	< 0.01
BW, kg	692	691	694	693	2.1	0.74	0.67	0.83	0.26
BW change, kg/d	0.16	0.27	0.16	0.44	0.09	0.07	0.33	0.08	-
BCS[6]	2.93	2.93	3.04	2.93	0.40	-	-	-	< 0.01
Rumination, min/d	647	645	639	641	6.2	0.40	0.91	0.77	0.01

[a-b]Means within a row differ with different superscripts ($P < 0.05$).

[1]CON = 0 g/ton monensin, R11 = 13 g/ton monensin, R14.5 = 15.8 g/ton monensin, R18 = 19.3 g/ton monensin

[2]Week effect for all estimates ($P < 0.01$).

[3]Average of experimental period.

[4]Calculated according to Tyrell and Reid (1965).

[5]Calculated according to NRC (2001).

[6]Largest standard deviation of treatment means.

Table 5. Effect of increasing dietary monensin concentration on de novo, mixed, and preformed fatty acid production

	Diet[1]					*P*-value[2]			
Item	CON	R11	R14.5	R18	SEM	Linear	Quad	Trt	Trt x Wk
Total FA, g/100 g milk	4.33	4.39	4.43	4.37	0.04	0.22	0.34	0.41	0.31
De novo[3]									
g/100 g milk	1.13	1.16	1.17	1.16	0.01	0.05	0.32	0.17	0.35
g/d	438	452	458	454	6.3	0.06	0.46	0.21	0.06
g/100 g FA	26.1	26.4	26.2	26.3	0.11	0.24	0.54	0.41	< 0.01
Mixed[4]									
g/100 g milk	1.85	1.88	1.91	1.90	0.02	0.02	0.79	0.10	0.07
g/d	720	737	753	746	11.8	0.09	0.76	0.28	< 0.01
g/100 g FA	42.8	42.9	43.0	43.1	0.18	0.25	0.66	0.64	< 0.01
Preformed[5]									
g/100 g milk	1.34	1.35	1.36	1.33	0.02	0.95	0.27	0.61	< 0.01
g/d	520	527	533	521	7.1	0.61	0.28	0.54	< 0.01
g/100 g FA	31.0	30.7	30.8	30.6	0.21	0.15	0.98	0.46	< 0.01
Chain length	14.57	14.54	14.54	14.54	0.01	0.02	0.27	0.08	< 0.01
Level of unsaturation	0.235[a]	0.231[ab]	0.227[b]	0.227[b]	0.002	<0.01	0.94	0.01	< 0.01
Fatty acids									
16:0, g/100 g milk	1.79[y]	1.81[xy]	1.85[x]	1.83[xy]	0.02	0.02	0.74	0.09	0.07
16:0, g/d	695[y]	712[xy]	728[x]	720[xy]	9.6	0.02	0.67	0.09	< 0.01
18:0, g/100 g milk	0.36	0.36	0.37	0.36	0.01	0.80	0.33	0.60	< 0.01
18:0, g/d	140	142	145	141	2.3	0.35	0.26	0.32	< 0.01
18:1 *cis*-9, g/100 g milk	0.79	0.79	0.79	0.78	0.01	0.91	0.59	0.86	< 0.01
18:1 *cis*-9, g/d	305	308	311	306	4.0	0.57	0.42	0.66	< 0.01

[a-b]Means within a row differ with different superscripts ($P < 0.05$).
[1]CON = 0 g/ton monensin, R11 = 13 g/ton monensin, R14.5 = 15.8 g/ton monensin, R18 = 19.3 g/ton monensin.
[2]Week effect for all estimates ($P < 0.01$).
[3]C4 to C14 (Barbano and Melilli, 2016).
[4]C16, C16:1, and C17.
[5]Greater than or equal to C18.

There is a strong correlation between true protein yield and de novo FA content of milk (Barbano et al. 2019), demonstrating an integrated outcome of metabolism and the metabolic signaling related to nutrient supply (Lobley, 2007; Rius et al., 2010). Milk protein concentration and yield were unaffected by monensin treatment (P = 0.41; Table 4), however, milk protein content and yield were both high, and paralleled the de novo and mixed FA yields again likely due to some effects of the level of EAA fed in this study. Milk protein responses to monensin treatment have been inconsistent in many studies where some have reported a decrease (Akins et al., 2014; Martinez et al., 2009), no effect (Alzahal et al., 2008; McCarthy et al., 2015), or an increase in protein content with monensin feeding (Van Amburgh et al., 2008). A meta-analysis by Duffield et al. (2008b) found monensin reduced milk protein concentration but increased milk protein yield suggesting dilution effect might be a factor as monensin increases milk production (Alzahal et al., 2008; Ipharraguerre & Clark, 2003). Given the previously described protein sparing effect of monensin on ruminal feed digestion (Poos et al., 1979; Chen and Russell, 1991; Ruiz et al, 2001), under certain conditions it is possible when feeding monensin that more feed protein can escape fermentation and flow to the small intestine, which would provide more amino acids independent of any microbial yield effects. That outcome, combined with a shift in propionate production (Prange et al., 1978; Van Maanen, et al., 1978), could possibly result in an enhancement of milk protein yield. The milk lactose concentration and yield did not differ among treatment groups (P = 0.51 and P = 0.71, respectively; Table 4). In agreement with the current study, Akins et al. (2014) and Hagen et al. (2015) found monensin had no effect on milk lactose concentration.

Although non-significant, ECM, FCM, and SCM all increased with monensin treatment compared to CON likely from the increase in milk component production in the monensin fed groups (Table 4). Previously, experiments by He et al. (2012) and Martinez et al. (2009) found monensin had no significant effect on component corrected milk yield. We observed an average 7 kg/d increase in ECM and FCM yield compared to actual milk yield across all treatment groups, and a 3.5 kg/d increase in SCM yield, again likely a result of the diet formulation of higher EAA levels, modest fat levels and strong rumen fermentation conditions. The CON group tended (P = 0.09) to have greater feed efficiency (actual milk/DMI) and R11 and R14.5 were significantly greater than R18 (P = 0.02 and P = 0.04, respectively) than R18 treatment due to the increased DMI of the cows on the R18 treatment (Table 6). However, there was a quadratic effect on ECM/DMI, FCM/DMI, and SCM/DMI by monensin treatment due to the level of DMI in the R18 treatment (Table 6). A couple of factors impacting the ability to identify differences in production efficiency are the numerical increase in DMI of the cows on the R18 treatment and the re-adjustment to the treatment diet following the covariate period as previously outlined. Although non-significant, the 0.8 kg difference in DMI of the cows on the R18 treatment obscured the typical outcome of enhanced feed efficiency at that level of monensin intake (Akins et al., 2014; Hagen et al., 2015), and likely more relevant, the re-adjustment to the CON diet from the covariate period appeared to impact treatment effects on milk yield. In the current study, monensin had no effect on estimated diet energy while Akins et al. (2014) and Hagen et al. (2015) reported an increase in estimated diet energy in cows fed 18 g/ton monensin compared to no monensin.

Milk urea nitrogen concentration was significantly greater in R11 compared to CON (P = 0.04), but not different in R14.5 or R18 (Table 4). Martinez et al. (2009) found monensin had no effect on MUN while Akins et al. (2014) reported an increase in MUN with monensin treatment. Additionally, McCarthy et al. (2015) reported significantly higher MUN values in early lactation cows who were fed diets top-dressed with monensin. Plasma urea nitrogen was unaffected by monensin treatment, although a meta-analysis (Duffield et al., 2008a) reported blood, plasma, and serum concentration increased with monensin treatment (Table 4). Recktenwald et al. (2014) suggests monensin plays a role in retaining urea N in the blood as they observed higher PUN values and larger plasma N pools with monensin treatment; however, that was not observed in the current study. The R11 and R18 treatment groups had a nonsignificant increase in BW compared to CON with R18 approaching a tendency to be greater (P = 0.11), although this observation warrants the recognition that wk 5 BW data is used to determine final BW due to an error with the scale (Table 4). In a previous study, Phipps et al. (2000) reported a significant increase in BW change with increasing levels of monensin. In the current study, BCS was not significantly different among treatment groups. This data suggests cows with few nutritional limitations will partition as much energy and nutrients towards milk production and away from BW and BCS gain even in later lactation as many of these cows were greater than 200 DIM while on treatment and not gaining appreciable amounts of weight or BCS. This observation requires further study and suggests BW accumulation in later lactation might be partially due to inadequate nutrient supply for milk and component yield, thus nutrients are retained in the tissue at a greater rate. Monensin treatment had no effect on rumination time and the values were quite high indicating good rumen health (Table 4).

Table 6. Effect of increasing dietary monensin concentration on milk production efficiency

	Diet[1]					*P*-value[2]			
Item	CON	R11	R14.5	R18	SEM	Linear	Quad	Trt	Trt x wk
Milk/DMI	1.47[a]	1.48[a]	1.48[a]	1.42[b]	0.01	0.11	< 0.01	0.02	< 0.01
ECM/DMI	1.71	1.74	1.76	1.69	0.02	0.63	0.04	0.13	0.13
3.5% FCM/DMI	1.71	1.74	1.76	1.70	0.02	0.66	0.04	0.13	0.12
SCM/DMI	1.58	1.61	1.62	1.56	0.02	0.71	0.03	0.10	0.09
Estimated diet energy[3]	1.64	1.65	1.65	1.68	0.02	0.34	0.49	0.62	-

[a-b]Means within a row differ with different superscripts (P < 0.05).
[1]CON = 0 g/ton monensin, R11 = 13 g/ton monensin, R14.5 = 15.8 g/ton monensin, R18 = 19.3 g/ton monensin.
[2]Week effect for all estimates (P < 0.01).
[3]Estimated diet energy content = [0.08 × BW, kg$^{0.75}$ + BW change, kg/d × 5.34 + milk, kg × (0.0929 × milk fat, % + 0.0563 × milk protein, % + 0.0395 × milk lactose, %)]/DMI, kg (NRC, 2001).

Conclusion

Overall, the milk and component yield of these mid- to late lactation cattle was high and unprecedented suggesting the conditions of evaluating monensin feeding in cattle fed more contemporary diets was achieved. Increasing the supply of monensin had no significant effects on milk yield, DMI, or production efficiencies; however, some of that lack of difference is likely due to shift from a covariate period with monensin feeding to a control diet where monensin was removed and an inadequate adjustment period. We observed a positive response to monensin treatment with linear increases in de novo and mixed FA concentration which resulted in enhanced milk fat yield. This indicates monensin can be fed at higher concentrations to achieve high milk component yields in lactating cows fed contemporary diets optimized for component yield, and more research is warranted to understand the relationship between monensin and ruminal FA synthesis, especially the de novo and mixed FA.

References

Akins, M. S., K. L. Perfield, H. B. Green, S. J. Bertics, and R. D. Shaver. 2014. Effect of monensin in lactating dairy cow diets at 2 starch concentrations. J Dairy Sci.97:917-929. http://dx.doi.org/10.3168/jds.2013-6756.

AlZahal, O., N. E. Odongo, T. Mutsvangwa, M. M. Or-Rashid, T. F. Duffield, R. Bagg, P. Dick, G. Vessie, and B. W. McBride. 2008. Effects of monensin and dietary soybean oil on milk fat percentage and milk fatty acid profile in lactating dairy cows. J Dairy Sci. 91:1166-1174. http://dx.doi.org/10.3168/jds.2013-0232.

Barbano, D. M., H. Dann, A. Pape, C. Melilli, and R. Grant. 2019. Herd management milk analysis: Jersey versus Holstein and between lab agreement of results. Proc. Cornell Nutr. Conf., Syracuse, NY. https://hdl.handle.net/1813/67038.

Barbano, D. M. and C. Melilli. 2016. New milk analysis technologies to improve dairy cattle performance. Proc. Cornell. Nutr. Conf. Syracuse, NY. https://ecommons.cornell.edu/bitstream/handle/1813/44747/3Barbano_manuscrt.pdf?sequence=1

Baumgard, L. H., B. A. Corl, D. A. Dwyer, A. Sæbø, and D. E. Bauman. 2000. Identification of the conjugated linoleic acid isomer that inhibit milk fat synthesis. Am. J. Physiol. 278:R179-R184.

Bello, N. M., M. Kramer, R. J. Tempelman, W. W. Stroup, N. R. St-Pierre, B. A. Craig, L. J. Young, and E. E. Gbur. 2016. Short communication: On recognizing the proper experimental unit in animal studies in the dairy sciences. J. Dairy. Sci. 99: 8871 8879. https://doi.org/10.3168/jds.2016-11516.

Bradford, B. J. and M. S. Allen. 2004. Milk fat responses to a change in diet fermentability vary by production level in dairy cattle. J. Dairy Sci. 87:3800-3807. https://doi.org/10.3168/jds.S0022-0302(04)73519-5.

Chen, G. J. and J. B. Russell. 1991. Effect of monensin and a protonophore on protein degradation, peptide accumulation, and deamination by mixed ruminal microorganisms in vitro. J. Anim. Sci. 69:2196-2203. https://doi.org/10.2527/1991.6952196x

Dubuc, J., D. DuTremblay, M. Brodeur, T. Duffield, R. Bagg, J. Baril, and L. DesCôteaux. 2009. A randomized herd-level field study of dietary interactions with monensin on
milk fat percentage in dairy cows. J Dairy Sci. 92:777-781. https://doi.org/10.3168/jds.2008-1658.

Duffield T. F., Rabiee A. R., and I. J. Lean. 2008a. A meta-analysis of the impact of monensin in lactating dairy cattle. Part 1. Metabolic effects. J. Dairy Sci. 91:1334-1346. https://doi.org/10.3168/jds.2007-0607.

Duffield T. F., Rabiee A. R., and I. J. Lean. 2008b. A meta-analysis of the impact of monensin in lactating dairy cattle. Part 2. Production effects. J. Dairy Sci. 91:1347-1360. https://doi.org/10.3168/jds.2007-0608.

Fellner, V., F. D. Sauer, and J.K.G. Kramer. 1997. Effect of nigericin, monensin, and tetronasin on biohydrogenation in continuous flow-through ruminal fermenters. J Dairy Sci. 80: 921-928.

Fessenden, S. W., D. A. Ross, E. Block, and M. E. Van Amburgh. 2020. Comparison of milk production, intake, and total-tract nutrient digestion in lactating dairy cattle fed diets containing either wheat middlings and urea, commercial fermentation by-product, or rumen-protected soybean meal. J Dairy Sci. 103:5090-5101. https://doi.org/10.3168/jds.2019-17744.

Ha, J.K., Lindsay, R.C. 1990. Method for the quantitative analysis of volatile free and Total branched-chain fatty acids in cheese and milk fat. J. Dairy Sci. 73, 1988–1999.

Hagen, A., R. Martin, and R. D. Shaver. 2015. Effect of dietary monensin supplementation and amino acid balancing on lactation performance by dairy cows. Prof. Ani. Sci. 31:278-283. http://dx.doi/10.15232/pas.2015-01391.

Harfoot, C. G., and G. P. Hazelwood. 1998. Lipid metabolism in the rumen. Page 285 in The Rumen Microbial Ecosystem. P. N. Hobson, ed. Elsevier Sci. Publi., London, UK.

He, M., K. L. Perfield, H. B. Green, and L. E. Armentano. 2012. Effect of dietary fat blend enriched in oleic or linoleic acid and monensin supplementation on dairy cattle performance, milk fatty acid profiles, and milk fat depression. J Dairy Sci. 95:1447-1461. http://dx.doi.org/10.3168/jds.2011-4635.

Higgs R. J. and M. E. Van Amburgh. 2016. Evolution of the CNCPS: development of v7. Pp. 125-146. Proc. Cornell Nutr. Conference. Syracuse, NY. Cornell University, Ithaca, NY. https://hdl.handle.net/1813/44752

Ipharraguerre, I. R. and J. H. Clark. 2003. Usefulness of ionophores for lactating dairy cows: A review. Anim. Feed Sci. Technol. 106:39-57.

LaPierre, P. A., S. Fredin, D. A Ross, and M. E. Van Amburgh. 2020. Impact of starch and energy on amino acid requirements of lactating dairy cattle. Proc. Cornell Nutr. Conf., Ithaca, NY. https://hdl.handle.net/1813/72897

Li, Shanshan, Afshin Hosseini, Marina Danes, Carolina Jacometo, Jianxin Liu and Juan J. Loor. 2016. Essential amino acid ratios and mTOR affect lipogenic gene networks and miRNA expression in bovine mammary epithelial cells. J. Anim. Sci. and Biotech. 7:44 DOI 10.1186/s40104-016-0104-x

Liu, Q., Q. Liu, C. Wang, G. Guo, W. J. Huo, S. L. Zhang, C. X. Pei, Y. L. Zhang and H. Wang.2018. Effects of branched-chain volatile fatty acids on lactation performance

and mRNA expression of genes related to fatty acid synthesis in mammary gland of dairy cows. Animal. 12:2071-2079. doi:10.1017/S1751731118000113
Lobley, G. 2007. Protein-energy interactions: horizontal aspects. In Energy and Protein Metabolism and Nutrition. EAAP Publ. 124, Pp 445-461. Editor: I. Ortigues-Marty. Wageningen Academic Publ.
Martinez, C. M., Y.-H. Chung, V. A. Ishler. K. W. Bailey and G. A. Varga. 2009. Effects of dietary forage level and monensin on lactation performance, digestibility and fecal excretion of nutrients, and efficiency of feed nitrogen utilization of Holstein dairy cows. J. Dairy Sci. 92:3211-3221. https://doi.org/10.3168/jds.2008-1306.
Massart-Leen, A.M., DePooter, H., Decloedt, M., Schamp, N. 1981. Composition and variability of the branched-chain fatty acid fraction in the milk of goats and cows. Lipids. 16:286–292.
McCarthy, M. M., T. Yasui, C. M. Ryan, G. D. Mechor, and T. R. Overton. 2015. Performance of early-lactation dairy cows as affected by dietary starch and monensin supplementation. J. Dairy Sci. 98:3335-3350. http://dx.doi.org/10.3168/jds.2014-8820.
McCarthy, M. M., T. R. Overton, G. D. Mechor, D. E. Bauman, T. C. Jenkins, and D. V. Nydam. 2018. Short communication: Field study to investigate the associations between herd-level risk factors for milk fat depression and bulk tank milk fat percent in dairy herds feeding monensin. J Dairy Sci. 101:3118-3125. https://doi.org/10.3168/jds.2017-13932.
McGuffey, R. K., L. F. Richardson, and J. I. D. Wilkinson. 2001. Ionophores for dairy cattle: Current status and future outlook. J. Dairy Sci. 84:E194-E203.
National Research Council. Nutrient Requirements of Dairy Cattle: 2001. National Academies Press; 2001 Feb 9.
Nichols, Kelly, André Bannink, Jurgen van Baal and Jan Dijkstra. 2020. Impact of post ruminally infused macronutrients on bovine mammary gland expression of genes involved in fatty acid synthesis, energy metabolism, and protein synthesis measured in RNA isolated from milk fat. J. Anim. Sci and Biotech. 11:53. https://doi.org/10.1186/s40104-020-00456-z
Prange, R.W., C.L. Davis, and J.H. Clark. 1978. Propionate production in the rumen of Holstein steers fed either a control or monensin supplemented diet. J. Anim. Sci. 46:1120-1124.
Phipps, R. H., J. I. D. Wilkinson, L. J. Jonkert, M. Tarrant, A. K. Jones, and A. Hodge. 2000. Effect of monensin on milk production of Holstein-Friesian dairy cows. J Dairy Sci. 83:2789-2794.
Poos, M. I., T. L. Hanson, and T. J. Klopfenstein. 1979. Monensin effects on diet digestibility, ruminal bypass and microbial protein synthesis. J. Anim. Sci. 48:1516-1524. https://doi.org/10.2527/jas1979.4861516x
Recktenwald, E. B., D. A. Ross, S. W. Fessenden, C. J. Wall, and M. E. Van Amburgh. 2014. Urea-N recycling in lactating dairy cows fed diets with 2 different levels of dietary crude protein and starch with or without monensin. J Dairy Sci. 97:1611-1622. http://dx.doi.org/10.3168/jds.2013-7162.
Rius, A. G., J. A. D. R. N. Appuhamy, J. Cyriac, D. Kirovski, O. Becvar, J. Escobar, M. L. McGilliard, B. J. Bequette, R. M. Akers, and M. D. Hanigan. 2010. Regulation of

protein synthesis in mammary glands of lactating dairy cows by starch and amino acids. J. Dairy Sci. 93:3114-3127. doi: 10.3168/jds.2009-2743.

Ruiz. R., G. L.Albrecht, L. O. Tedeschi, J. B. Russell, D. G. Fox. 2001. Effect of monensin On the performance and nitrogen utilization of lactating dairy cows consuming fresh forage. J. Dairy Sci. 84:1717–1727. https://doi.org/10.3168/jds.S0022-0302(01)74607-3.

Russell, J. B. and H. J. Strobel. 1989. Effect of ionophores on ruminal fermentation. App. And Environ. Micro. 55:1-6.

Tyrell, H. F., and J. T. Reid. 1965. Prediction of the energy value of cow's milk. J. Dairy. Sci. 48:1215-1223. https://doi.org/10.3168/jds.S0022-0302(65)88430.2.

Van Amburgh. M. E., J. L. Capper, G. D. Mechor, and D. E. Bauman. 2008. Rumensin and milk fat production. Pages 99-112 in Proc. Cornell Nutrition Conf., Syracuse, NY

Van Amburgh. M. E., E. A. Collao-Saenz, R. J. Higgs, D. A. Ross, E. B. Recktenwald, E. Raffrenato, L. E. Chase, T. R. Overton, J. K. Mills, and A. Foskolos. 2015. The Cornell Net Carbohydrate and Protein System: Updates to the model and evaluation of version 6.5. J. Dairy. Sci. 98: 6361-6380. https:// doi.org/10.3168/jds.2015-9378.

Van Maanen, R. W., J. H. Herbein, A. D. McGilliard, J.W. Young. 1978. Effects of monensin on in vivo rumen propionate production and blood glucose kinetics in cattle. J. Nutr. 108:1002-1007. https://doi.org/10.1093/jn/108.6.1002.

Vasquez, J. A., M. M. McCarthy, B. F. Richards, K. L. Perfield, D. B. Carlson, A. L. Lock, and J. K. Drackley. 2021. Effects of prepartum diets varying in dietary energy density and monensin on early-lactation performance in dairy cows. J. Dairy Sci. 104:2881-2895. https://doi.org/10.3168/jds.2020-19414.

Wildman, E. E., G. M. Jones, P. E. Wagner, R. L. Boman, H. F. Troutt, and T. N. Lesch. 1982. A Dairy Cow Body Condition Scoring System and Its Relationship to Selected Production Characteristics. J. Dairy. Sci. 65:495–501. https://doi.org/10.3168/jds.S0022-0302(82)82223-6.

Wojciechowski, K. L., D. M. Barbano. 2016. Prediction of fatty acid chain length and unsaturation of milk fat by mid-infrared milk analysis. J. Dairy Sci. 99: 8561-8570. http://doi.org/10.3168/jds.2016-11248.

Woolpert, M. E., H. M. Dann, K. W. Cotanch, C. Melilli, L. E. Chase, R. J. Grant, and D. M. Barbano. 2016. Management, nutrition, and lactation performance are related to bulk tank milk de novo fatty acid concentration on northeastern US dairy farms. J. Dairy Sci. 99:8486-8497. https://doi.org/10.3168/jds.2016-10998.