

Enhancing milk component yields in lactating dairy cows and effects of monensin on the performance of lactating dairy cows fed contemporary diets

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INTRODUCTION

In many milk markets across the U.S., milk volume is being managed while milk components are in high demand. This puts more pressure on nutritionists to develop diets that optimize milk fat and protein yields which means several factors related to diet composition, and source of nutrients need to be evaluated. Also, this requires diets that optimize rumen function and efficiency and make sure all requirements are met. There are several dietary factors that positively impact milk components, and this paper will briefly touch on a few of them and then describe a study where the concept of a "modern diet" was evaluated in the context of different levels of monensin and the effects on milk components.

The primary factor impacting the ability to optimize rumen function and provide substrates for milk components and maintain rumen health is digestible aNDFom, especially in the form of forage fiber. When working to evaluate diets and models, the factor accounting for most of the variation in amino acid supply is digestible aNDFom (Higgs et al., 2015) and this is intuitive given the nature and function of the rumen. One of the most limiting factors in diet formulation can be digestible aNDFom or a combination of highly digestible fiber with a low inventory, so the opportunity for the high digestibility forage cannot be used to optimize the diets. Acetate from fiber digestion is one of the primary energy sources for the cow and can account for approximately 30% of the energy supply and is also the primary source for de novo fatty synthesis in the gland. There are many studies describing the role of acetate in milk fat synthesis and reducing equivalents necessary for elongation of milk fat (Bauman et al., 1970; Van Soest, 1994; Shephard and Combs, 1998; Palmquist, 2007; and Maxin et al., 2011) thus, providing highly digestible aNDFom is one of the primary dietary means to ensure high acetate production and also formulate diets that are first limiting on total aNDFom intake and not negatively impacted by uNDF levels. From the calculations of Mertens (2009) concerning intake capacity of aNDFom, the general expected levels of aNDFom intake for high cows just post-peak are approximately 1.2% to 1.30% of body weight (BW). Since those guidelines were published, and with the ability to directly measure and estimate the uNDF of the forages and byproducts, we have data suggesting that the upper limits to intake and rumen fill as suggested by Mertens are consistent with high digestibility forages and that uNDF in forages can be high enough to be first limiting dry matter intake (DMI) (Cotanch et al, 2014; Van Amburgh et al., 2018). From data generated at Miner Institute, University of Bologna and Cornell University using rumen emptying and intake experiments, total aNDFom intake can reach about 1.5% of BW with good digestibility and some legume containing diets and that translates into approximately 1.3% BW of rumen fill. Intake of uNDFom with high forage diets is about 0.4% BW and rumen mass is about 0.48% to 0.62%. Therefore, to optimize rumen function, and acetate production, when available, diets need to be formulated with the largest digestible pool of

aNFDom to ensure adequate DMI and fiber digestion for acetate yield keeping the relative limits to DMI in mind.

In high quality pastures, the typical NFC fractions formulated for in North America are reversed with large water and ethanol soluble carbohydrates and lower starch. This suggests that the ruminant is more adapted to high sugar than high starch diets and from a component basis this makes sense as sugars will ferment to butyrate, a fat precursor (Oba et al., 2015 and Penner et al., 2011). In addition, sugar fermentation improves rumen pH by enhancing bacterial and protozoal yield (Penner et al., 2009; Dineen et al., 2020) and enhancing rumen energy status and epithelial transport of acids and glucose (Penner et al., 2011). Increased butyrate production can enhance milk fat percentage and yield and enhance fiber digestibility (Broderick et al., 2008). Data from Hoover (1987) and Hoover and Miller-Webster (1998) would suggest that in TMR fed cattle, rumen function is optimized at sugar levels of 6% to 8% dry matter in the diet. This would provide more substrates for milk fat synthesis, greater microbial yield for milk protein synthesis and enhance fiber digestion which would improve DMI, substrates and microbial yield.

Further, to optimize milk components, amino acid (AA) supplies need to be optimized to meet the requirements for milk volume, fat, and protein (Yoder et al, 2020; LaPierre et al., 2019). With more data, it appears meeting requirements for amino acids, especially methionine and lysine with some data on histidine, that energetic efficiency, not protein efficiency is increased. This concept of horizontal integration was discussed by Lobley, (2007) where he demonstrates that AA will be used to fill any pathway necessary to improve the energetic efficiency of the animal and that protein and energy are integrated in metabolism and not separate entities as we normally formulate for. To that point, data have been published integrating the AA requirements on both a metabolizable energy basis (Higgs and Van Amburgh, 2016) or a digestible energy basis (Lapierre et al., 2019). This approach makes amino acid formulation much more precise and estimated requirements tend to be higher than previously described. Modeling and cow response data from Higgs and Van Amburgh (2016) demonstrated the approach of describing AA requirements on a gram per megacalorie of metabolizable energy (ME) was a useful approach for defining more precise requirements for lactating dairy cattle. Recent work by LaPierre et al, (2019) suggests that when applying this approach using Cornell Net Carbohydrate Protein System (CNCPS) v6.55 (Van Amburgh et al., 2015) the values for methionine are 1.19 g/Mcal ME and for lysine is 3.21 g/Mcal ME based on energy corrected milk responses from lactating dairy cattle. Thus, to improve milk and milk component yield, those AA should be formulated close to those values.

Some feed additives such as yeasts and ionophores are shown to impact milk components and the efficiency of microbial activity in the rumen. 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 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 \pm 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.

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 (Delpo, 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 ($3,000 \times g$ 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, Parsippany, 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:

$$Y_{ijklm} = \mu + T_i + W_j + TW_{ij} + P_{k:i} + B_{l:k:i} + BX_{lik} + \epsilon_{ikk1m},$$

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 , $P_{k:I}$ = 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

Table 1. Ingredient composition of experimental diets

Ingredient, % of DM	Diet ¹			
	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

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

²Lignotech USA, Rothschild, WI.

³Berg + Schmidt America LLC, Libertyville, IL.

⁴Milk specialties, Eden Prairie, MN.

⁵Calcium (22%) and magnesium (12%) supplement (Min-AD, Winnemucca, NV).

⁶Contained (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)

⁷Adisseo Inc, Alpharetta, GA.

⁸Lallemand Inc, Milwaukee, WI.

⁹Monensin, 90.7 g/lb. (Elanco Animal Health, Greenfield, IN).

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. 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

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

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

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

²Predicted using the Cornell Net Carbohydrate and Protein System v6.5 (Van Amburgh et al., 2015).

³Rumen unsaturated fatty acid load = 18:1 + 18:2 + 18:3 from the chromatographic analysis of the diets.

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

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

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

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 ($P = 0.11, 0.14, \text{ and } 0.16$ 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.

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

Item	Diet ¹				SEM	P-value ²			
	CON	R11	R14.5	R18		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 ($P < 0.05$).

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

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

³Average of experimental period.

⁴Calculated according to Tyrell and Reid (1965).

⁵Calculated according to NRC (2001).

⁶Largest standard deviation of treatment means.

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

Item	Diet ¹				SEM	P-value ²			
	CON	R11	R14.5	R18		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 <i>cis</i> -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 <i>cis</i> -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 ($P < 0.05$).

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

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

³C4 to C14 (Barbano and Melilli, 2016).

⁴C16, C16:1, and C17.

⁵Greater than or equal to C18.

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).

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).

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

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

Item	Diet ¹				SEM	P-value ²			
	CON	R11	R14.5	R18		Linear	Quad	Trt	Trt x wk
Milk/DMI	1.47 ^a b	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	0.12	0.12
SCM/DMI	1.58	1.61	1.62	1.56	0.02	0.71	0.03	0.09	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 ($P < 0.05$).

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

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

³Estimated diet energy content = $[0.08 \times \text{BW, kg}^{0.75} + \text{BW change, kg/d} \times 5.34 + \text{milk, kg} \times (0.0929 \times \text{milk fat, \%} + 0.0563 \times \text{milk protein, \%} + 0.0395 \times \text{milk lactose, \%})] / \text{DMI, kg}$ (NRC, 2001).

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.

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