Continuous 11-week feeding of reduced-fat distillers grains with and without monensin reduces lactation performance of dairy cows

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ABSTRACT

This study investigated the effects of continuous feeding of high inclusion of reduced-fat corn distillers grains with solubles with and without monensin on dry matter intake (DMI), production, milk fatty acid profile, and plasma AA profile in lactating cows. The experiment was conducted for 12 wk (1-wk covariate, 2-wk diet adaptation, and 9-wk experimental period of data collection) with 36 Holstein cows in a randomized complete block design. Cows were blocked by parity, days in milk, and milk yield and assigned to the following diets: (1) control (CON), (2) CON with reduced-fat corn distillers grains with solubles included at 28.8% (dry matter basis) replacing soybean meal, soyhulls, and supplemental fat (DG), and (3) DG with monensin (Rumensin; Elanco Animal Health, Greenfield, IN) supplemented at a rate of 20 mg/kg of DM offered (DGMon). Orthogonal contrasts were used to compare CON versus DG and DGMon and to compare DG versus DGMon. Milk yield was not affected (40.3 vs. 40.8 kg/d) by DG and DGMon compared with CON. However, for DG and DGMon compared with CON, decreased DMI (24.9 vs. 26.4 kg/d), milk fat yield (1.12 vs. 1.55 kg/d), milk protein yield (1.24 vs. 1.32 kg/d), and energy-corrected milk yield (37.7 vs. 43.5 kg/d) were observed. Feeding DGMon compared with DG did not affect DMI (24.4 vs. 25.4 kg/d) and milk yield (39.2 vs. 41.3 kg/d) but decreased milk fat yield (1.08 vs. 1.23 kg/d), milk protein yield (1.20 vs. 1.28 kg/d), and energy-corrected milk yield (36.0 vs. 39.4 kg/d). Interactions between treatment and week for DMI, milk fat yield, and energy-corrected milk indicate that production responses to DG and DGMon versus CON were decreased over the experimental period. Cows fed DG and DGMon had increased milk fat concentration

of *trans*-10,*cis*-12 18:2, *trans*-10 18:1, and long-chain (>16C) and polyunsaturated fatty acids and decreased short-chain (<16C) and odd- and branched-chain fatty acids compared with CON. No difference was observed between DG and DGMon in milk fatty acid profile. In the current study, feeding a high-DG diet did not sustain DMI and production, and supplementing monensin to a high-DG diet further decreased DMI and production.

Key words: reduced-fat distillers grains, monensin, performance, dairy cow

INTRODUCTION

In past decades, the push for alternative fuel sources has resulted in an increase in ethanol production, primarily from corn, subsequently increasing production of co-products (Renewable Fuels Association, 2017). The most common co-product is corn dried distillers grains with solubles (DDGS). Because of their low cost, feeding a diet containing high DDGS to dairy cows can be economical (Ranathunga et al., 2010). However, concerns over protein quality, particularly Lys deficiency, arise when DDGS are substituted for soybean meal (SBM; Paz et al., 2013; Paz and Kononoff, 2014) due to low Lys content of corn products (NRC, 2001). Additionally, because traditional DDGS contain high concentrations of fat and PUFA, milk fat depression has occurred in various studies when DDGS are fed at 30% of diet DM (Benchaar et al., 2013; Ramirez-Ramirez et al., 2016). Recently, the ethanol industry has adapted methods to extract oil from the co-product stream (Majoni et al., 2011). This extraction decreases crude fat of DDGS by 40 to 60%, resulting in production of reduced-fat DDGS (RFDG) with a crude fat of 5.5 to 7.5% (DM basis; DuFour, 2017). Although a risk of Lys deficiency with high-RFDG diets still exists, RFDG are suggested to decrease the risk of milk fat depression compared with DDGS (Ramirez-Ramirez et al., 2016).

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Previous studies indicated that RFDG can be included in a ration for lactating cows up to 30% of dietary DM and maintain or increase DMI, milk yield, and milk fat and protein yield (Castillo-Lopez et al., 2014; Ramirez-Ramirez et al., 2016). However, these studies were short-term experiments (Latin square designs). Production responses to dietary modification can be different according to the experimental length and designs. For example, recovery from milk fat depression can take 2 to 3 wk (Weiss, 2012; Rico and Harvatine, 2013). This suggests that using 3 wk per period in a Latin square design may not be sufficient to evaluate milk fat depression caused by dietary modification. In agreement, Benefield et al. (2009) indicated that treatment sequence can influence milk fat production in a Latin square design. In addition, Hristov and Giallongo (2014) suggested that longer-term studies (e.g., 10-wk randomized complete block design) are needed to assess production responses to dietary protein modification because labile AA might compensate for short-term nutritional deficiencies. A recent meta-analysis by Zanton (2016) also indicated that production responses to changing dietary protein content differed between continuous and change-over experimental designs. Therefore, a continuous longer-term study might be necessary for accurate evaluation of production responses to high-RFDG diets. To our knowledge, very few studies were conducted with RFDG in the long term. A continuous 12-wk study reported that feeding RFDG at 20% of diet DM replacing SBM and soyhulls did not negatively affect milk protein and fat production of lactating cows (Mjoun et al., 2010a). In this study, however, the fat content of RFDG was only 3.5% (DM basis), which does not represent current RFDG (5.5–7.5% fat). Therefore, evaluation of production responses to a high-RFDG (about 30% of DM) ration in a continuous longer-term study is needed.

Monensin is a feed additive commonly fed to dairy cattle, and a meta-analysis by Duffield et al. (2008b) indicated that monensin increased production efficiency, milk yield, and milk protein yield by 2.5, 2.3, and 1.9%, respectively, partly due to increased total-tract N digestibility (Ruiz et al., 2001; Martineau et al., 2007). However, Duffield et al. (2008b) observed a decrease in milk fat concentration when monensin was fed to dairy cows, and milk fat depression was exacerbated with monensin when dietary PUFA content increased (AlZahal et al., 2008). Although RFDG and monensin are widely included in dairy rations, the effects of the combination of RFDG and monensin on milk fat yields have not been studied. Therefore, the objective of this experiment was to determine the 11-wk continuous production responses of lactating dairy cows fed a

diet with high RFDG, fully replacing SBM, with and without monensin. The hypothesis was that a diet with about 30% RFDG (DM basis) will negatively affect milk fat yields in the long term and that the addition of monensin to a high-RFDG diet may exacerbate milk fat depression.

MATERIALS AND METHODS

Animals and Treatments

Animal care and procedures were approved by The Ohio State University Institutional Animal Care and Use Committee. The cows used were not fed monensin for at least 6 mo before starting the experiment.

The experiment was carried out at the Ohio Agricultural Research and Development Dairy Center (Wooster, OH) in a randomized complete block design using 36 Holstein cows (9 primiparous and 27 multiparous; average $\pm$ SD at the beginning of the trial: milk yield, 44.2 ± 8.1 kg/d; DIM, 80 ± 31 ; BW, 675 ± 74 kg). Cows were blocked by parity, DIM, and milk yield, and cows in each block were randomly allocated to 1 of 3 treatment diets: (1) a lactating cow ration not containing RFDG or monensin (control diet; **CON**; Table 1); (2) the CON diet containing RFDG (28.8% of diet DM) replacing SBM, soyhulls, and supplemental fat (**DG**); and (3) the DG diet with monensin (Rumensin; Elanco Animal Health, Greenfield, IN) supplemented at a rate of 20 mg/kg of DM offered (**DGMon**). All diets were formulated to be isonitrogenous and isoenergetic and meet or exceed nutrient requirements according to NRC (2001).

The experiment was conducted for 12 wk (1-wk covariate period, 2-wk diet adaptation period, and 9-wk experimental period; wk 1 through 9 refers to the 9-wk experimental period from here on). To allow the rumen microbiota to adapt to monensin, the inclusion rate of monensin for DGMon was half of the target rate (i.e., 10 mg/kg of DM) during the first week of the diet adaptation and increased to 20 mg/kg of DM at the start of wk 2. Prior to the experiment, all cows had received the lactating herd ration [% of DM: corn silage, 38.5; alfalfa silage, 8.8; corn grain, 24.5; SBM, 10.0; cottonseed, 8.8; AminoPlus (Ag Processing Inc., Omaha, NE), 3.1; soyhulls, 3.1; animal-vegetable fat, 0.4; minerals and vitamins, 2.8] since calving, and the same diet was fed to all cows during the covariate period. Approximate nutrient composition for the covariate diet was (% of DM) CP, 17.1; NDF, 29.3; and ether extract, 5.2. Cows were housed in individual tiestalls with free access to water and fed TMR once daily with a target refusal rate of 5%.

Table 1. Chemical composition of feed ingredients¹

Item, % of DM	RFDG ²	Corn silage	Alfalfa silage	Alfalfa hay
DM, % as is	88.4	34.8	33.6	91.4
CP	34.4	9.1	20.3	19.4
ADF	12.3	23.0	33.7	35.2
NDF	27.5	36.0	38.7	40.3
Starch ³	—	28.3	1.1	2.0
Ether extract	7.4	3.8	3.5	1.9
Ash	6.2	4.2	10.0	9.8
Ca	0.03	0.26	1.27	1.11
P	0.91	0.26	0.31	0.28
Mg	0.35	0.18	0.28	0.28
K	1.20	1.42	2.96	2.76
S	0.92	0.14	0.32	0.28

¹Forages (n = 3) and RFDG (n = 2).²Reduced-fat corn dried distillers grains with solubles (Poet Nutrition Inc., Sioux Falls, SD).³Starch for RFDG was not determined.

Measurements, Sampling, and Laboratory Analyses

Feed intake of individual cows (feed offered and refused) was measured daily. Cows were milked twice daily, and all milk weights were recorded throughout the entire experiment. Individual cows were weighed for 2 consecutive days before the start of the trial and during wk 1, 5, and 9 (4–6 h after feeding). Consecutive measurements were averaged, and BW change (**BWC**) per day was calculated.

Weekly samples of silages were analyzed for DM (100°C for 24 h), and as-fed amounts were adjusted for moisture changes. Refusal samples were collected for 3 consecutive days, composited, and analyzed for DM (100°C for 24 h) during wk 1, 5, and 9. Individual feeds were also collected for 3 consecutive days during wk 1, 5, and 9 and composited by week. Silages were dried at 55°C for 72 h and ground to pass a 1-mm screen (Wiley mill; Arthur A. Thomas Co., Philadelphia, PA). All feed samples were submitted to Rock River Laboratory Inc. (Watertown, WI) for chemical analysis of N (method 990.03; AOAC International, 2000), NDF (with heat-stable α -amylase and sodium sulfite; Goering and Van Soest, 1970), ADF (Goering and Van Soest, 1970), ether extract with diethyl ether (method 920.39; AOAC International, 2000), ash (942.05; AOAC International, 2000), and starch (Schalla et al., 2012) and reported on a DM basis. Feeds were also analyzed for minerals by Service Testing and Research Laboratory (Ohio Agricultural Research and Development Center, Wooster) using inductively coupled plasma emission spectroscopy (Isaac and Johnson, 1985) after microwave digestion with nitric acid (Jones et al., 1991). All diet samples were analyzed for long-chain fatty acids (Weiss and Wyatt, 2003). The feeds collected from wk 9 were submitted to the University of

Missouri–Columbia Agricultural Experiment Station Chemical Laboratory for complete AA profile (method 982.30; AOAC International, 2000).

Weekly milk samples (a.m. and p.m.) were collected and analyzed for milk fat, protein, lactose (B2000 infrared analyzer, Bentley Instruments, Chaska, MN) by DHI Cooperative Inc. (Columbus, OH). Additionally, p.m. milk samples were collected during wk 1, 5, and 9, and within 24 h the milk fat layer was separated by centrifugation ($20,000 \times g$ for 30 min at 4°C) and frozen until later analysis. To determine milk fatty acid profile, a 2-step methylation procedure (Jenkins, 2000) with separation by GLC using a CP-SIL88 capillary column (100 m $\times$ 0.25 mm $\times$ 0.2- μ m film thickness; Varian Inc., Palo Alto, CA) was used.

Blood samples were collected into evacuated lithium heparin tubes by coccygeal venipuncture approximately 4 h after feeding during d 4 and 5 of wk 1, 5, and 9 of the experimental period. Plasma was immediately separated by centrifugation at $1,500 \times g$ for 15 min and frozen at –20°C until later analysis. Plasma samples for cows fed CON and DG diets from 9 blocks out of 12 were submitted for free AA analysis to the University of Missouri–Columbia Agricultural Experiment Station Chemical Laboratory (Deyl et al., 1986; Fekkes, 1996).

Statistical Analysis

One cow receiving the DG treatment was removed from the trial at the end of wk 8 due to a displaced abomasum. Data from the diet adaptation period (first 2 wk on experimental diets) were not used in the statistical analysis. Daily measurements (DMI, milk yield) were averaged by cow and week. For covariates, milk yield and DMI recorded daily during the 1-wk covariate period were averaged by cow. Because milk composition

of cows was monitored once per week, data collected in 2 wk before the covariate period were also included and averaged (total 3 wk) due to potential daily variability. During the 3 wk, all animals received the same herd diet as described previously. Data were analyzed in R (version 3.4.1) using the linear and nonlinear mixed effects models package (Pinheiro et al., 2017). All data except BW change (compound symmetry; lowest Bayesian information criterion) were analyzed using an autoregressive first order covariance structure (lowest Bayesian information criterion). The following model for production, milk composition, and BW was used:

$$Y_{ijk} = \mu + \text{Cov} + B_i + \tau_j + B\tau_{ij} + W_k + \tau W_{jk} + e_{ijk},$$

where Y_{ijk} is the dependent variable, μ is the overall mean, Cov is the corresponding covariate variables, B_i is the block, τ_j is the j th treatment, $B\tau_{ij}$ is the block $\times$ treatment interaction, W_k is the experiment week (wk 1 to 9), and τW_{jk} is the treatment $\times$ week interaction with the error term e_{ijk} . Treatment, week, the interaction of treatment and week, and corresponding covariate were fixed effects, and block and block $\times$ treatment and error were random effects. The same model was used for milk fatty acid profile and BW change, except the covariate was not included. Plasma AA were analyzed by the same model, except week and covariate were removed and the random term was block. Residuals for all variables were visually analyzed for homogeneity and normality, and all were found to meet the assumptions of ANOVA except for milk CLA *trans*-10, *cis*-12 concentration. The large amount of 0 values for CLA *trans*-10, *cis*-12 resulted in heteroscedastic residuals; however, because of the large difference between CON versus DG and DGMon, interpretation of results was not affected. All data including graphs are presented as least squares means (covariate adjusted where applicable) $\pm$ largest standard error of the mean unless noted otherwise. Orthogonal contrasts were used to evaluate the effects of RFDG (CON vs. DG and DGMon) and monensin (DG vs. DGMon). When a significant ($P < 0.05$) treatment $\times$ week interaction was present, the main effect of treatment within each week was examined. Significance was declared at $P \leq 0.05$, and trends are discussed at $P \leq 0.10$.

RESULTS AND DISCUSSION

The fat content of our RFDG ($7.4 \pm 1.6\%$ of DM; Table 1) is in the range reported in a recent RFDG survey study (5.5–7.5%; DuFour, 2017). Although the dose of monensin (20 mg/kg of DM or ~ 500 mg/d) used in the current study may be higher than the quantity

typically used in experiments (Duffield et al., 2008a), it is still within the maximum allowable dose (up to 22 mg/kg) and was chosen to maximize effects on rumen microbial populations. Feeding monensin at around 450 mg/d as top-dress (Van der Werf et al., 1998; Phipps et al., 2000) or mixed in TMR (24 mg/kg of DM; Bell et al., 2006) had minimal effects on DMI and milk component yield in individual studies. As a result of replacing SBM, soyhulls, and fat in the CON diet with RFDG in the DG and DGMon diets, the composition of CP, NDF, fat, starch, and NE_L was similar across diets (Table 2). Compared with CON, the inclusion of RFDG resulted in a shift in long-chain fatty acids from saturated to unsaturated. This shift is typical with high inclusion of RFDG (Ramirez-Ramirez et al., 2016). Dietary concentration of Leu and Met increased for DG versus CON by 22 and 20%, respectively, whereas concentration of Lys, Arg, and Ile decreased by 34, 36, and 14%, respectively (Table 3).

Cows receiving DG and DGMon tended to have lower DMI (24.9 vs. 26.4 kg/d; $P = 0.08$) compared with CON (Table 4). A decrease in DMI was not observed in previous studies in which dairy cattle were fed 30% RFDG diets (Mjoun et al., 2010b; Castillo-Lopez et al., 2014; Foth et al., 2015; Ramirez-Ramirez et al., 2016). The discrepancy between the current study and previous studies could have occurred due to a difference in experimental length. The studies by Castillo-Lopez et al. (2014) and Ramirez-Ramirez et al. (2016) were conducted in the short term (3 wk per period in a Latin square design); therefore, differences in DMI might not have been realized. Indeed, an interaction between treatment and week ($P < 0.01$) occurred in the current study such that the difference in DMI between treatments became greater as the experiment progressed and DMI among treatments was not different until week 7 (Figure 1). However, in a 12-wk randomized complete block design by Mjoun et al. (2010a), no difference was observed in DMI between control and 20% RFDG diets. This discrepancy in DMI can be associated with the source of RFDG used. Compared with the current study, Mjoun et al. (2010a) used RFDG containing much lower fat (3.5 vs. 7.4%). Therefore, dietary PUFA content was lower in their RFDG diet, and it was reported that increases in dietary PUFA (specifically 18:2) decreased DMI (He et al., 2012).

Although monensin supplementation to DG (i.e., DGMon) decreased DMI by 1 kg compared with DG, this was not statistically different ($P = 0.29$). Decreased DMI by monensin was reported in a meta-analysis by Duffield et al. (2008b), and numerical decreases in DMI were observed when monensin was fed to dairy cows (Ramanzin et al., 1997; Oelker et al., 2009). It is also

important to note that the difference in DMI between DG and DGMon became larger as the experiment progressed. Indeed, further analysis indicated that DMI for DGMon in wk 8 and 9 tended to be lower ($P \leq 0.08$; data not shown) compared with DG, suggesting the importance of continuous observation.

Compared with CON, milk yield was not affected ($P = 0.58$) by DG and DGMon, although DMI tended

Table 2. Ingredient and chemical composition of diets¹ (% of DM, unless noted)

Item	CON	DG	DGMon
Ingredient			
Corn silage	41.6	41.6	41.6
Alfalfa silage	9.7	9.7	9.7
Alfalfa hay	5.0	5.0	5.0
RFDG ²	—	28.8	28.8
Corn grain, ground	12.9	13.2	13.2
Soybean meal 48%	15.1	—	—
Soyhulls	12.3	—	—
Fat ³	1.34	—	—
Dicalcium phosphate	0.23	—	—
Mineral-vitamin mix ⁴	1.73	1.73	1.73
Monensin ⁵	—	—	0.01
Chemical composition			
CP	17.6	17.7	17.4
ADF	22.6	19.1	18.9
NDF	30.5	31.0	30.4
Starch	20.4	21.6	21.7
Ether extract	4.2	4.7	4.7
LCFA, % of LCFA	3.8	4.6	4.7
16:0	18.6	14.7	14.8
18:0	12.6	2.5	2.7
18:1 <i>cis</i> -9	12.8	18.4	18.5
18:2	32.7	43.6	43.1
18:3	4.6	4.4	4.4
Others ²	18.7	16.4	16.6
UFA	53.0	68.5	68.0
PUFA	38.0	48.7	48.1
SFA	36.4	22.2	22.5
Ash	7.1	6.4	6.5
Ca	0.72	0.61	0.61
P	0.36	0.48	0.47
Mg	0.22	0.25	0.24
K	1.64	1.45	1.44
S	0.21	0.41	0.41
DCAD ⁷	192	65	73
NE _L , ⁸ Mcal/kg	1.57	1.59	1.59

¹CON = control diet; DG = diet containing 28.8% RFDG (DM basis); DGMon = DG with monensin at 20 mg/kg of DM.

²Reduced-fat distillers grains (Poet Nutrition Inc., Sioux Falls, SD).

³Energy Booster 100 (Hubbard Feeds, Mankato, MN).

⁴Contained per kilogram of premix: 488 g of trace mineral salt, 377 g of limestone, 1,331 mg of zinc (Zinpro 120; Zinpro Corp., Eden Prairie, MN), 559 mg of copper (from copper sulfate), 13 mg of selenium (from sodium selenate), 1,269 kIU of vitamin E, 253 kIU of vitamin A, and 53 kIU of vitamin D.

⁵Monensin (Rumensin 90; Elanco Animal Health, Greenfield, IN) replaced corn grain.

⁶Long-chain fatty acids.

⁷DCAD = Na + K - Cl - S.

⁸Estimated using NRC (2001).

Table 3. Amino acid composition (% of CP) of diets^{1,2}

Item	CON	DG
Total EAA	44.8	40.7
Arginine	5.5	3.5
Histidine	2.5	2.4
Isoleucine	4.9	4.2
Leucine	8.7	10.6
Lysine	6.0	3.8
Methionine	1.5	1.8
Phenylalanine	5.2	4.9
Threonine	3.9	3.6
Tryptophan	1.2	0.8
Valine	5.4	5.2

¹CON = control diet; DG = diet containing 28.8% reduced-fat distillers grains (DM basis).

²Feed samples of only CON and DG collected in wk 9 were used for AA analysis.

to decrease. Similarly, several studies have reported no effect of feeding 20 to 30% RFDG (DM basis) on milk yield (Mjoun et al., 2010a,b; Castillo-Lopez et al., 2014). However, DGMon versus DG numerically decreased ($P = 0.102$) milk yield by 2 kg/d. Although a meta-analysis by Duffield et al. (2008b) indicated that the inclusion of monensin can increase milk yield by 2.3%, this is not always observed (Ramanzin et al., 1997; AlZahal et al., 2008; He et al., 2012). Yields of 3.5% FCM and ECM were decreased ($P < 0.01$) for DG and DGMon compared with CON and for DGMon compared with DG. The interaction between treatment and week was significant for 3.5% FCM ($P = 0.047$) and tended to be for ECM ($P = 0.08$; Figure 1). These interactions indicate a gradual decrease in 3.5% FCM and ECM for DG and DGMon as the experiment progressed, whereas CON remained relatively constant (Figure 1). The decrease in 3.5% FCM and ECM with DG and DGMon was driven by a decrease in milk fat and protein yields (see later discussion). The decreases in milk fat and protein yields resulted in an overall 6% decrease in feed efficiency ($P = 0.04$), calculated as ECM per kilogram of DMI, for DG and DGMon compared with CON. Mjoun et al. (2010b) reported a linear increase in ECM per kilogram of DMI, driven by an increase in milk fat yield, when RFDG were increased from 0 to 30% of diet DM. However, as previously discussed, RFDG containing much lower fat (3.5% of DM) was used in the aforementioned experiment, which did not negatively affect DMI, milk yield, and milk composition. Monensin supplementation to DG (DGMon) did not affect ($P = 0.21$) ECM per kilogram of DMI compared with DG. Monensin typically increases feed efficiency (i.e., milk energy yield/energy intake) via an increase in milk protein yield and a decrease in DMI (Duffield et al., 2008b); however, no effect of monensin

Table 4. Effects of feeding reduced-fat corn distillers grains (RFDG) with and without monensin on intake, production, and BW

Item ¹	Diet ^{2,3}			SEM	P-value ⁴	
	CON	DG	DGMon		RFDG	MON
DMI, ⁵ kg/d	26.4	25.4	24.4	0.7	0.08	0.29
Milk yield, kg/d	40.8	41.3	39.2	1.0	0.58	0.102
3.5% FCM, ^{5,6} kg/d	42.8	38.1	34.5	1.1	<0.01	<0.01
ECM, ^{6,7} kg/d	43.5	39.4	36.0	1.1	<0.01	<0.01
ECM/kg of DMI	1.63	1.57	1.50	0.04	0.04	0.21
Fat, ⁵ %	3.81	3.00	2.77	0.11	<0.01	0.12
Fat, ⁵ kg/d	1.55	1.23	1.08	0.05	<0.01	0.02
Protein, %	3.26	3.11	3.06	0.03	<0.01	0.30
Protein, kg/d	1.32	1.28	1.20	0.04	<0.01	0.01
Lactose, %	4.78	4.84	4.83	0.02	0.07	0.84
Lactose, kg/d	1.95	2.02	1.91	0.06	0.89	0.09
BW, ⁵ kg	707	703	705	5	0.55	0.77
BW change, kg/d	0.8	0.6	0.6	0.15	0.13	0.96

¹Main effect of week was significant for all variables ($P < 0.05$).

²CON = control diet; DG = diet containing 28.8% RFDG (DM basis); DGMon = DG with monensin at 20 mg/kg of DM.

³Least squares means; largest standard error of treatment mean is shown.

⁴RFDG = CON vs. the average of DG and DGMon; MON = DG vs. DGMon.

⁵Treatment $\times$ week interaction ($P \leq 0.05$).

⁶3.5% FCM = $[0.432 \times \text{milk yield (kg)}] + [16.216 \times \text{fat (kg)}]$; ECM = $[0.327 \times \text{milk yield (kg)}] + [12.95 \times \text{fat (kg)}] + [7.65 \times \text{true protein (kg)}]$ (Tyrrell and Reid, 1965).

⁷Treatment $\times$ week interaction ($P = 0.09$).

on feed efficiency is often reported in individual studies (Ramanzin et al., 1997; Oelker et al., 2009; Mathew et al., 2011). Furthermore, milk fat and protein production was negatively affected by monensin (see later discussion).

Compared with CON, the concentration and yield of milk fat with cows fed DG and DGMon decreased ($P < 0.01$) 24 and 25%, respectively. Milk fat responses to high-RFDG diets in previous studies have been variable. Feeding RFDG at about 30% of DM did not affect (Castillo-Lopez et al., 2014; Foth et al., 2015; Ramirez-Ramirez et al., 2016) or increased (Mjoun et al., 2010b) milk fat concentration and yield. In the current experiment, milk fat depression was clearly observed for DG and DGMon compared with CON. Depressed milk fat could be attributed to 28% greater PUFA content in the DG and DGMon diets compared with the CON diet (Table 2). Compared with CON, increases in PUFA intake of cows fed the DG and DGMon diets likely altered rumen microbiota and biohydrogenation (Rico et al., 2015) causing milk fat depression, which is supported by changes in milk fatty acid profile (see later discussion). However, despite the greater PUFA intake for RFDG diets versus the control in studies by Castillo-Lopez et al. (2014), Foth et al. (2015), and Ramirez-Ramirez et al. (2016), milk fat depression was not observed. The discrepancy between these studies and the current study occurred as follows: (1) different

dietary ingredients replaced with RFDG and (2) difference in experimental design (i.e., short term vs. long term). Ramirez-Ramirez et al. (2015) observed milk fat depression in a 20% DDGS diet when replacing soyhulls with corn grain at 8.5% of the diet, which increased dietary starch content from 19.4 to 25.6% and decreased NDF from 40.0 to 33.0% (DM basis). Milk fat depression can occur with diets high in readily fermentable carbohydrates (e.g., starch) and low in fiber content (Rico and Harvatine, 2013). In the studies in which milk fat depression was not observed when feeding high-RFDG diets, a portion of ground corn in the control diet was replaced with RFDG, and soyhulls were maintained or increased in the 30% RFDG diets (Castillo-Lopez et al., 2014; Foth et al., 2015; Ramirez-Ramirez et al., 2016). In our experiment, dietary proportion of corn grain was almost the same and soyhulls were fully replaced, resulting in similar starch and NDF concentrations across diets. However, because the starch and NDF contents (21.6 and 30.7%, respectively) were typical for lactating cow rations, replacing corn grain or soyhulls with RFDG was likely not the sole factor causing the discrepancy in milk fat responses to RFDG between our study and those in the literature. Differences in experimental designs may also explain the discrepancy. Most studies feeding high-RFDG diets were conducted in the short term (i.e., Latin square designs). Generally, in a Latin square design, production responses to

dietary modification are determined for 7 d after 14 d of dietary adaptation in each period for an experiment. However, the presence of carry-over effects in milk fat concentration up to 3 wk has been reported (Weiss, 2012), and Rico and Harvatine (2013) reported that reduced milk fat concentration and yield induced by low NDF and high fat and starch (29.5, 6.9, and 27.0% of DM, respectively) was not fully recovered until d 19 and 15, respectively, after feeding a control diet. This suggests that a 14-d diet adaptation and 7-d observation for milk fat may not be sufficient to detect clear

differences among dietary treatments. In agreement, we observed an interaction between treatment and week ($P < 0.01$; Figure 1), suggesting that differences in milk fat concentration and yield between the CON and DG diets became larger over time. Because we observed differences in milk fat concentration and yield in wk 1 (the first week after the 2-wk diet adaptation; $P < 0.01$), potential carryover effects may have masked milk fat depression affected by dietary treatments, especially in short-term experiments with Latin square designs (Castillo-Lopez et al., 2014; Ramirez-Ramirez et al.,

Figure 1. Production responses during the 9-wk experimental period with control (CON), 28.8% reduced-fat corn distillers grains (DG), or DG with monensin at 20 mg/kg of DM (DGMon) diets. Interaction of treatment $\times$ week was significant for all, except ECM tended to interact ($P = 0.08$) and milk yield had no interaction ($P = 0.53$). Asterisks indicate significance of overall dietary effect (* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$). Means are covariate-adjusted LSM and error bars indicate SEM.

2016). However, because we did not compare experimental designs, future studies that directly compare milk fat responses to dietary modification between continuous and crossover experimental design are required.

Milk fat concentration was unchanged ($P = 0.12$) for DGMon compared with DG; however, milk fat yield decreased ($P = 0.02$) by 12% (Table 4). Milk fat responses to monensin supplementation have been variable. Although a meta-analysis by Duffield et al. (2008b) indicated that monensin decreased milk fat concentration and did not affect milk fat yield, both outcomes displayed heterogeneity and thus indicate large differences in effect of monensin on milk fat concentration and yield. The high dose (20 mg/kg of DM) of monensin used in the current experiment may have contributed to the further decrease in milk fat production. Feeding monensin at 22 to 24 mg/kg of diet DM induced milk fat depression in high-oil diets (Bell et al., 2006; AlZahal et al., 2008). It was suggested that monensin decreases milk fat production via an inhibition of bacteria involved in biohydrogenation (Weimer et al., 2008) and consequently increases flow of bioactive fatty acids to the small intestine, specifically *trans*-10,*cis*-12 18:2, causing milk fat depression. Although a numerical decrease in milk fat concentration (2.77 vs. 3.00%; $P = 0.12$) was observed for DGMon versus DG, the addition of monensin to DG likely did not exacerbate the alteration of biohydrogenation and rumen fermentation according to milk fatty acid profile (see later discussion). Therefore, reduced milk fat yield for DGMon compared with DG was caused by a numerical decrease in milk yield and milk fat concentration.

It is worth noting that the fat content for the first and second (fed starting at wk 6) batches of RFDG was 6.2 and 8.5% (DM basis), respectively, which resulted in a dietary fat content in the DG and DGMon diets of 4.5, 4.9, and 4.8% for wk 1, 5, and 9, respectively (data not shown). The slightly higher fat content in the DG and DGMon diets after wk 6 may have contributed to the treatment $\times$ week interaction ($P < 0.01$) for milk fat concentration and yield. However, in the current study, the rate of decrease in milk fat concentration and yield for DG and DGMon remained constant; therefore, changes in fat content of RFDG were likely not the major factor explaining progressive milk fat depression. Rather, increasing alteration of the rumen environment (supported by shifts in milk fatty acid profile as the experiment progressed; see later discussion) explains the gradual decrease in milk fat concentration and yield for DG and DGMon versus CON.

Milk protein concentration and yield were 6 and 5% lower ($P < 0.01$), respectively, for the DG and DGMon diets compared with CON, and DGMon decreased ($P < 0.01$) milk protein yield by 6% compared with DG.

This indicates that the DG diets in the current experiment decreased milk protein yield via reduced milk protein concentration with high inclusion of RFDG fully replacing SBM in the diet, and the addition of monensin to the DG diet exacerbated the decrease in milk protein yield, likely by numerically decreasing milk yield. Rations with a large proportion of RFDG may become deficient in Lys for lactating cows because of low Lys content in corn by-products (NRC, 2001). In the current study, when RFDG fully replaced SBM, dietary Lys content decreased by 37% (Table 3) and plasma Lys decreased by 34% (Table 5). Using actual production and milk composition, the estimated duodenal supply of Lys was 237, 198, and 190 g/d or 6.3, 5.2, and 5.3% of MP for the CON, DG, and DGMon diets, respectively, according to NRC (2001). Because supply of Lys was below the NRC (2001) recommendation of 6.6% of MP, milk protein depression may have occurred for the DG and DGMon diets due to Lys deficiency. Yet, in a recent study, adding rumen-protected Lys to a 15 or 30% RFDG diet (DM basis; 16.7% CP) did not affect milk protein yield and only marginally increased milk protein concentration (Paz and Kononoff, 2014). In this study, signs of AA deficiency (e.g., depressed milk protein concentration and yield) were not observed in their non-Lys-supplemented RFDG diets. Therefore, more studies for high-RFDG diets are needed to clarify Lys deficiency in relation to milk protein production. Because of the complexity of AA utilization and requirements for optimal milk protein synthesis (Arriola Apelo et al., 2014), other AA besides or in conjunction with Lys may have been limiting milk protein production in high-producing cows. In our experiment, com-

Table 5. Effects of feeding reduced-fat corn distillers grains (RFDG) on plasma AA composition ($\mu\text{g/mL}$) of a subset of cows ($n = 9$) fed treatment diets during wk 9¹

Item	Diet ^{2,3}		SEM	P-value ³
	CON	DG		
Total EAA	147.23	139.17	6.56	0.41
Arginine	14.62	11.59	0.91	0.05
Histidine	9.21	9.38	0.58	0.83
Isoleucine	19.11	15.32	1.01	0.03
Leucine	23.19	33.09	1.80	<0.01
Lysine	12.00	7.95	0.67	<0.01
Methionine	3.53	3.76	0.23	0.51
Phenylalanine	7.21	7.57	0.38	0.53
Threonine	13.87	10.47	0.67	0.01
Tryptophan	6.33	5.81	0.20	0.08
Valine	38.16	34.22	1.88	0.18

¹Plasma AA composition for DG with monensin at 20 mg/kg of DM (DGMon diet) was not determined.

²CON = control diet; DG = diet containing 28.8% RFDG (DM basis).

³Least squares means; largest standard error of treatment mean is shown.

pared with CON, DG decreased dietary Ile content by 14% and plasma Ile concentration by 20%. Extraction of Ile by the mammary gland increased when diets with 20 to 30% RFDG (DM basis) were fed (Mjoun et al., 2010a; Paz and Kononoff, 2014). Furthermore, removal of Ile from culture medium with mammary tissue slices decreased protein synthesis rate in vitro (Appuhamy et al., 2012). Moreover, in a study with beef steers fed at maintenance, increasing DDGS concentration in the diet decreased duodenal supply of bacterial protein (Castillo-Lopez et al., 2013), an enriched source of Ile (Sok et al., 2017). Therefore, in the current study, Lys or Ile in the RFDG diets were potentially limiting, causing milk protein depression.

In contrast to expectations, milk protein concentration and yield did not differ by treatment and week ($P > 0.41$), indicating rapid milk protein depression when RFDG fully replaced SBM in the ration. In previous continuous studies (9- to 12-wk randomized complete block design), interactions between treatment and week for milk protein production were not observed when dietary protein or certain AA supplies were deficient (Lee et al., 2012a,b; Giallongo et al., 2016). Although milk protein depression occurred rapidly after feeding the DG diets in the current study, recovery from reduced milk protein concentration and yield might not occur rapidly after switching to a control diet, as is similar with milk fat recovery from depression (Rico and

Table 6. Effects of feeding reduced-fat corn distillers grains (RFDG) with and without monensin on milk fatty acid profile during the experimental period (% of total fatty acids)

Fatty acid	Diet ^{1,2}			SEM	<i>P</i> -value ³	
	CON	DG	DGMon		RFDG	MON
4:0	2.93	2.99	2.80	0.14	0.81	0.27
6:0 ⁴	2.35	2.07	1.88	0.11	<0.01	0.20
8:0 ⁴	1.30	1.07	0.95	0.06	<0.01	0.18
10:0 ⁴	3.14	2.38	2.12	0.14	<0.01	0.20
12:0 ⁴	3.71	2.79	2.54	0.13	<0.01	0.18
<i>iso</i> 13:0	0.03	0.02	0.02	0.01	<0.01	0.97
<i>ante</i> 13:0 ⁴	0.09	0.07	0.07	0.01	<0.01	0.60
13:0 ⁴	0.16	0.10	0.09	0.01	<0.01	0.42
<i>iso</i> 14:0	0.09	0.07	0.06	0.01	0.01	0.47
14:0 ⁴	12.00	9.58	9.45	0.21	<0.01	0.67
<i>iso</i> 15:0	0.24	0.16	0.15	0.01	<0.01	0.77
<i>ante</i> 15:0 ⁴	0.55	0.44	0.43	0.01	<0.01	0.52
15:0 ⁴	1.39	0.94	0.93	0.04	<0.01	0.93
<i>iso</i> 16:0 ⁴	0.22	0.18	0.18	0.01	0.02	0.99
16:0 ⁴	33.91	25.16	26.18	0.57	<0.01	0.20
<i>iso</i> 17:0	0.41	0.38	0.4	0.01	0.11	0.22
17:0 ⁴	0.86	0.57	0.57	0.01	<0.01	0.72
18:0 ⁴	9.84	12.09	11.35	0.38	<0.01	0.18
<i>trans</i> -6,8 18:1 ⁴	0.27	0.76	0.88	0.07	<0.01	0.19
<i>trans</i> -9 18:1 ⁴	0.22	0.68	0.76	0.07	<0.01	0.38
<i>trans</i> -10 18:1 ⁴	0.47	2.47	3.15	0.37	<0.01	0.16
<i>trans</i> -11 18:1	0.75	1.28	1.19	0.08	<0.01	0.41
<i>trans</i> -12 18:1 ⁴	0.42	0.61	0.62	0.02	<0.01	0.60
<i>cis</i> -9 18:1 ⁴	17.86	23.87	23.77	0.45	<0.01	0.88
<i>cis</i> -11 18:1	0.53	0.63	0.66	0.04	0.01	0.59
18:2 ⁴	2.13	4.61	4.26	0.18	<0.01	0.16
18:3 ⁴	0.40	0.40	0.39	0.01	0.89	0.58
<i>cis</i> -9, <i>trans</i> -11 18:2 ⁴	0.36	0.71	0.68	0.03	<0.01	0.42
<i>trans</i> -10, <i>cis</i> -12 18:2 ⁴	0.002	0.032	0.033	0.004	<0.01	0.78
<16C ⁴	29.03	23.63	22.62	0.66	<0.01	0.28
>16C ⁴	34.84	49.43	49.08	0.89	<0.01	0.78
Total SFA	73.30	61.20	60.26	1.10	<0.01	0.55
Total PUFA ⁴	2.89	5.79	5.41	0.20	<0.01	0.19
OBCFA ^{4,5}	4.24	3.08	3.09	0.06	<0.01	0.90

¹CON = control diet; DG = diet containing 28.8% RFDG (DM basis); DGMon = DG with monensin at 20 mg/kg of DM.

²Least squares means; largest standard error of treatment mean is shown.

³RFDG = CON vs. the average of DG and DGMon; MON = DG vs. DGMon.

⁴Treatment × week interaction ($P \leq 0.05$).

⁵Odd- and branched-chain fatty acids = *iso* 13:0 + *ante* 13:0 + 13:0 + *iso* 14:0 + *iso* 15:0 + *ante* 15:0 + 15:0 + *iso* 16:0 + *iso* 17:0 + 17:0 + 17:1.

Harvatine, 2013). If this is the case, it would partially explain why no decreases in milk protein production in studies using Latin square designs have been reported (Castillo-Lopez et al., 2014; Ramirez-Ramirez et al., 2016). Furthermore, these studies used a basal diet with dietary CP greater than 18.0% (DM basis); thus, actual supply of potentially limiting AA (i.e., Lys or Ile) was likely sufficient to maintain milk protein production. Further studies are needed to examine depression or recovering rate of milk protein after dietary modification.

Cows fed the DG and DGMon versus the CON diet had decreased ($P < 0.01$) concentrations of milk fatty acids <16 C, SFA, and odd- and branched-chain fatty

acids (**OBCFA**) and increased ($P < 0.01$) concentrations of fatty acids >16 C, MUFA, and PUFA (Table 6). Similar changes in milk fatty acid profile have been observed when milk fat depression occurs (Shingfield et al., 2006; AlZahal et al., 2008; Ramirez-Ramirez et al., 2015). Also, increased dietary 18:2 content in the DG and DGMon diets compared with CON may have increased milk MUFA and PUFA concentrations (He et al., 2012). The decrease in most OBCFA has occurred previously when RFDG was included at 30% of diet DM (Ramirez-Ramirez et al., 2016) or when corn oil and corn grain were added to a 20% DDGS diet (Ramirez-Ramirez et al., 2015). Because most OBCFA

Figure 2. Response in milk fatty acids (% of total fatty acids) during the 9-wk experimental period with control (CON), 28.8% reduced-fat corn distillers grains (DG), or DG with monensin at 20 mg/kg of DM (DGMon) diets. The interaction of treatment $\times$ week was significant ($P \leq 0.02$) for all responses. The overall dietary effect was significant at all weeks ($P \leq 0.01$). Means are LSM and error bars indicate SEM.

originate from de novo synthesis by rumen bacteria, it has been proposed as a biomarker for shifts in microbial profile (Fievez et al., 2012). All milk OBCFA except for *iso*-17:0 were negatively correlated with total dietary fatty acids (Vlaeminck et al., 2006). In agreement with our study, total milk OBCFA were decreased when supplemental linoleic acid was fed (Vlaeminck et al., 2006). The specific mechanism for the decrease in most OBCFA in the present study is not clear. However, we suggest that the DG and DGMon diets changed microbial population or rumen condition compared with the CON diet. It is known that rumen cellulolytic bacteria are enriched in *iso* fatty acids (Fievez et al., 2012), and because PUFA has been shown to be inhibitory to cellulolytic bacteria in vivo (Maia et al., 2007), their abundance may have been negatively affected. However, a difference in *iso*-17:0 between CON and DG was not observed. Vlaeminck et al. (2006) found that milk fat concentration of *iso*-17:0 was positively correlated with *trans*-monoene, which increased in the present study; however, the specific mechanism for this is unknown. The concentrations of *anteiso* fatty acids (*anteiso* 13:0 and 15:0) for DG and DGMon were lower than for CON, probably because dietary supply of Ile, the primary substrate for microbial synthesis of *anteiso* fatty acids (Vlaeminck et al., 2006), was 20% lower. Some milk odd-chain fatty acids are synthesized in the mammary gland from propionate (Vlaeminck et al., 2006). The inhibition of mammary lipogenesis (i.e., decrease in milk fatty acids <16C) in the current study might explain the decreases in milk odd-chain fatty acids for DG and DGMon versus CON.

Interactions between treatment and week occurred for most milk fatty acids. Selected interactions ($P \leq 0.02$) that support an increase in the severity of milk fat depression as the experiment progressed are shown in Figure 2. The responses include gradual increases in PUFA, >16C fatty acids, and *trans*-10,*cis*-12 18:2 and decreases in SFA, <16C, and OBCFA with DG and DGMon compared with CON. Similar gradual changes in milk fatty acid concentration have been noted in a previous study when milk fat depression occurred (Shingfield et al., 2006). An increase in the severity of milk fat depression may indicate an inability of rumen microbes to adapt to the challenges associated with DG and DGMon diets (i.e., PUFA). Gradual changes in milk fatty acid profile suggest that the rumen environment (i.e., milk fatty acid profile) did not reach a steady state for cows fed the DG and DGMon diets in our experiment. According to the gradual increase in severity of milk fat depression in this study with 9-wk continuous observation, we propose that continuous studies (8–10 wk) are better suited to determine

responses in milk fat production to diets containing RFDG.

CONCLUSIONS

Feeding RFDG at about 30% of diet DM by replacing mainly SBM decreased DMI, milk fat, and protein production compared with a control ration, and DMI and milk fat gradually decreased throughout the experiment. Supplementing monensin to a high-RFDG diet decreased milk fat yield. The decreased milk protein and plasma AA concentration with a high-RFDG diet suggest that RFDG diets might be deficient in Lys or Ile. Shifts in the milk fatty acid profile with a high RFDG suggest that the rumen environment was progressively affected by high inclusion of RFDG in a ration. These results suggest that a high-RFDG diet might not maintain DMI and milk fat production in an 11-wk continuous study and that future research on intake and milk fat response to significant diet manipulation may need continuous observation for at least 8 wk. No beneficial effect of monensin supplementation to a high-RFDG diet on production was found in the current study.

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