Effects of corn feeding reduced-fat distillers grains with or without monensin on nitrogen, phosphorus, and sulfur utilization and excretion in dairy cows

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ABSTRACT

This study investigated effects of high inclusion of reduced-fat corn distillers grains with solubles (RFDG) with or without monensin on utilization and excretion of dietary N, P, and S. The experiment was conducted for 11 wk (2-wk diet adaptation, 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) a control diet (CON); (2) CON with RFDG included at 28.8% (dry matter basis) by replacing soybean meal, soyhulls, and supplemental fat and phosphorus (DG); and (3) DG with monensin (Rumensin; Elanco Animal Health, Greenfield, IN) supplemented at a rate of 20 mg/kg of DM offered (DGMon). Contrasts were used to compare CON versus DG and DG versus DGMon. Inclusion of RFDG at 28.8% of dietary DM replacing mainly soybean meal did not change crude protein content (17.6% on a DM basis) but decreased rumen-degradable protein and increased rumen-undegradable protein. In addition, the DG diets increased P (0.48 vs. 0.36%) and S concentrations (0.41 vs. 0.21%; DM basis) compared with the CON diet. As a result, DG versus CON decreased plasma and milk urea N concentrations and urinary N excretion. However, the increase in P concentration when feeding the DG versus CON diet to lactating cows increased P intake, plasma P concentration, and urinary and fecal P excretion without affecting milk P secretion. Intake of S was greater for cows fed the DG versus CON diet, resulting in greater plasma total S and sulfate concentration and urinary and fecal S excretion. However, milk S secretion was not affected by DG compared with CON. Monensin supplementation to the DG diet did not affect N intake, utilization, and excretion except that apparent N digestibility was lower compared with DG. In addition, feeding the DGMon diet did not affect P and S utilization and excretion compared with

DG. The study suggests that inclusion of high RFDG in a ration by replacing mainly soybean meal altered N, P, and S utilization and excretion, but monensin supplementation to a high-RFDG diet, overall, had minimal effects on N, P, and S utilization and excretion in lactating dairy cows.

Key words: reduced-fat distillers grains, monensin, dairy cows

INTRODUCTION

Ethanol production continues to increase, resulting in increased production of corn dried distillers grain with solubles (DDGS; Renewable Fuels Association, 2017), which has been widely used as an animal feed. More recently, DDGS with less fat content, reduced-fat DDGS (RFDG), has increased in availability and can be used as an animal feed. According to multiple studies with dairy cows, up to 30% (DM basis) RFDG can be included in dairy rations without negatively affecting DMI, milk yield, and milk composition (Mjoun et al., 2010b; Castillo-Lopez et al., 2014; Ramirez-Ramirez et al., 2016). However, due to the high concentration of certain nutrients in RFDG, such as RUP, P, and S, feeding a diet with high RFDG may cause environmental concerns by increasing the excretion of those nutrients. However, studies about utilization and excretion of N, P, and S when dairy cows are fed high-RFDG diets are scarce (N) or not available (P and S).

An in situ and in vitro study by Mjoun et al. (2010a) indicated that intestinal digestibility and total digestible (i.e., RDP plus digested RUP) is less for RFDG compared with soybean meal (SBM); therefore, N utilization for milk and urinary N excretion could decrease and fecal N excretion increase when feeding RFDG versus SBM. Furthermore, the concentration of P and S in SBM is about 0.7 and 0.4%, respectively (NRC, 2001), whereas the P and S content of RFDG is 0.8 to 0.9% and 0.7 to 1.2%, respectively (DuFour, 2017). Therefore, increased dietary inclusion of RFDG can increase dietary P and S content, resulting in increased excretion of P and S. Inclusion of RFDG at about 30% of diet DM increased dietary P content from 0.36 to

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0.52% (Castillo-Lopez et al., 2014) and S content from 0.25 to 0.53% (Ramirez-Ramirez et al., 2016). Manure with high P concentration has potential for P losses (e.g., runoff) when the manure is applied to the field (Hanrahan et al., 2009). Manure with high S concentration can negatively affect public perception and human and animal health (e.g., odor and toxicity of S-containing gas to animals and humans; Guidotti, 2010). Due to increased dietary S content with high-RFDG diets, S excretion can increase, potentially resulting in increased hydrogen sulfide (H_2S) emissions from manure, especially when manure is mixed and pumped out (Andriamanohiarisoamanana et al., 2015). However, excretion of P and S has been rarely considered when the inclusion rate of RFDG in a ration is determined.

Monensin is a commonly used feed additive in the dairy industry and has been shown to increase N utilization efficiency (Plaizier et al., 2000; Ruiz et al., 2001; Martineau et al., 2007). Increased apparent absorption and retention of certain minerals, including P, were also observed when monensin was fed to beef cattle and sheep (Starnes et al., 1984; Greene et al., 1986). Furthermore, monensin can alter ruminal S metabolism by altering rumen microbial communities. As an example, monensin supplementation to a diet containing DDGS in beef cattle decreased ruminal H_2S in some (Felix and Loerch, 2011) but not all studies (Felix et al., 2012). Therefore, monensin supplementation to a high-RFDG diet has potential to alter N, P, and S utilization and excretion in dairy cows. Although monensin and RFDG are a widely used feed additive and ingredient for dairy cows, few studies have examined N, P, and S utilization in terms of environmental impacts.

The objectives of our study were to assess the effect of feeding RFDG with or without monensin on N, P, and S utilization and excretion in dairy cattle. Our hypotheses were (1) feeding RFDG decreases urinary N excretion but increases fecal and urinary P and S excretion, and (2) monensin supplementation to a high-RFDG diet will decrease N and P excretion and alter S utilization and excretion.

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 the experiment.

The experiment was carried out at the Ohio Agricultural Research and Development Dairy Center (Wooster, OH). Information about animals, experimental design, and dietary treatments were described in de-

tail in our previous study (Morris et al., 2018). Briefly, 36 (9 primiparous and 27 multiparous) Holstein cows (average $\pm$ SD at the beginning of the trial: milk yield = 44.2 ± 8.1 kg/d; DIM = 80 ± 31 d; BW = 675 ± 74 kg) were blocked into groups of 3 ($n = 12$) by parity, DIM, and milk yield. Cows in each block were randomly allocated to 1 of 3 treatment diets (a randomized complete block design): (1) control diet (**CON**; a typical lactating cow ration; Table 1); (2) the CON with RFDG included at 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**). For the DG diets (i.e., DG and DGMon), RFDG fully replaced SBM, soyhulls, and supplemental fat and calcium phosphate. All diets were formulated to be isoenergetic and isonitrogenous and to meet or exceed nutrient requirements according to NRC (2001). Supplemental P (i.e., calcium phosphate) was removed from the DG diets to minimize P overfeeding. Although this resulted in a decrease in dietary Ca content in the DG diets (Table 1), Ca supply exceeded NRC (2001) requirements. Further information about dietary composition is available in our previous study (Morris et al., 2018). The experiment consisted of a diet adaptation period for 2 wk and a 9-wk experimental period (wk 1 through 9 refers to the 9-wk experimental period). During the first week of the diet adaptation, the inclusion rate of monensin for DGMon was half of the target rate (i.e., 10 mg/kg of DM) and it was increased to 20 mg/kg of DM at the start of wk 2. Cows were housed in individual tiestalls with free access to water and milked twice a day. All diets were prepared daily and fed as TMR once a day with a target refusal rate of 5%.

Sample Collection

Animals were sampled during wk 1, 5, and 9. During the first 3 d of sampling week, spot fecal and urine samples (from the rectum and by massaging the vulva, respectively) were collected every 9 h, yielding 8 samples representing every 3 h in a 24-h period. Samples were composited on an equal volume basis during collection and frozen (-20°C) between sampling events until later analysis. Urine samples were acidified with 2 M HCl to maintain a pH < 3.0 . During fecal and urine sampling (first 3 d of wk 1, 5, and 9), feeds offered and refused were monitored, sampled, and composited and feed ingredients; refusal samples were collected for the 3 d and composited by week for feeds and by cow and week for refusals (on a weight basis). In addition, individual cows were weighed for 2 consecutive days in wk 1, 5, and 9 (4 to 6 h after feeding). Therefore, feed

Table 1. Dietary ingredients and chemical composition (% of DM)¹

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	—	—
Calcium phosphate	0.23	—	—
Mineral/vitamin mix ⁴	1.73	1.73	1.73
Monensin ⁵	—	—	0.01
Chemical composition (average $\pm$ SD)			
CP	17.6 $\pm$ 0.7	17.7 $\pm$ 0.3	17.4 $\pm$ 0.1
RDP ⁶	10.7	9.9	9.8
RUP ⁶	7.0	7.8	7.6
Ca	0.72 $\pm$ 0.02	0.61 $\pm$ 0.01	0.61 $\pm$ 0.03
P	0.36 $\pm$ 0.01	0.48 $\pm$ 0.02	0.47 $\pm$ 0.01
Mg	0.22 $\pm$ 0.01	0.25 $\pm$ 0.01	0.24 $\pm$ 0.00
K	1.64 $\pm$ 0.04	1.45 $\pm$ 0.04	1.44 $\pm$ 0.03
S	0.21 $\pm$ 0.01	0.41 $\pm$ 0.01	0.41 $\pm$ 0.02
Na	0.30 $\pm$ 0.03	0.38 $\pm$ 0.05	0.43 $\pm$ 0.07
Cl	0.81 $\pm$ 0.01	0.77 $\pm$ 0.09	0.80 $\pm$ 0.09

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

²RFDG = reduced-fat distillers grains (POET Nutrition Inc., Sioux Falls, SD).

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

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

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

⁶Estimated according to NRC (2001).

intakes and BW of individual animals observed during the sampling week were used for calculations of nutrient utilization and excretion.

Milk samples were collected for 4 consecutive milkings on d 2 and 3 of each sampling week (wk 1, 5, and 9), composited by cow and week and frozen at -20°C until later analysis. Blood samples were collected approximately 4 h after feeding during d 4 and 5 in wk 1, 5, and 9 into evacuated lithium heparin tubes by coccygeal venipuncture. Plasma was immediately separated by centrifugation at $3,000 \times g$ for 15 min at 4°C and frozen at -20°C until later analysis.

Sample Analysis

Silage, refusals, and fecal samples were dried (55°C for 72 h) and ground to pass a 1-mm screen (Wiley Mill; Arthur H. Thomas Co., Philadelphia, PA). Dried and ground feed, refusals, and fecal samples were analyzed for analytical DM (AOAC International, 2000; method 934.01; 100°C overnight), ash (AOAC International, 2000; method 942.05; 600°C overnight), N (Elemental Analyzer; Flash 2000; Thermo Fisher Sci-

entific, Waltham, MA), NDF (Ankom200 Fiber Analyzer; Ankom Technology Corp., Fairport, NY), and minerals (Service Testing and Research Laboratory, Ohio Agricultural Research and Development Center, Wooster, OH) using inductively coupled plasma emission spectroscopy (Isaac and Johnson, 1985) after microwave digestion with nitric acid (Jones et al., 1991). In addition, all feeds, refusals, and feces were analyzed for indigestible NDF as described by Lee et al. (2012). Briefly, samples were weighed in quadruplicate into filter bags (25- μm pore size; Ankom Technology Corp.) and incubated in 2 cannulated, lactating Holstein cows for 12 d. After incubation, bags were rinsed until water ran clear and analyzed for NDF (Ankom200 Fiber Analyzer; Ankom Technology Corp.). Fecal excretion was estimated using indigestible NDF intake and fecal content.

Urine samples were analyzed for N (Flash 2000; Thermo Fisher Scientific), creatinine (kit 500701; Cayman Chemical, Ann Arbor, MI), urea N (UUN; kit UR107; Randox Laboratories Ltd., Crumlin, United Kingdom), and minerals (Service Testing and Research Laboratory, OARDC, Wooster, OH). Urine excretion

was estimated based on urinary creatinine concentration assuming a creatinine excretion rate of 29.0 mg/kg of BW (Valadares et al., 1999).

Plasma was analyzed for plasma urea N (PUN; kit UR107; Randox Laboratories Ltd.), total minerals as for urine, and inorganic P (kit 0830–125; Stanbio Laboratories, Boerne, TX). Plasma sulfate was determined using ion chromatography (Service Testing and Research Laboratory, OARDC, Wooster, OH) after protein precipitation with acetonitrile.

Milk samples were analyzed for N (Flash 2000; Thermo Fisher Scientific), MUN (Skalar SAN Plus segmented flow analyzer, Skalar Inc., Norcross, GA) by DHI Cooperative Inc. (Columbus, OH), and minerals as described for urine.

Statistical Analysis

One cow on the DG treatment was removed from the trial at the end of wk 8 due to a displaced abomasum. Data were analyzed in R (v3.4.1) using the linear and nonlinear mixed effects models (v3.1–131) package (Pinheiro et al., 2017). All data were analyzed using an autoregressive first-order covariance structure selected according to lowest Bayesian information criterion. The model included the fixed effects of treatment, week, and the interaction of treatment and week, and the random effect of block, cow within block, and error. Significant interactions of dietary treatments by week were presented when overall dietary effects were significant. Residuals for all variables were visually analyzed for homogeneity and normality and all were found to meet the assumptions of ANOVA. All data including graphs are presented as least squares means $\pm$ largest standard error of the mean, unless noted otherwise. Contrasts were used to evaluate the effects of RFDG and monensin. Significance was declared at $P \leq 0.05$ and trends are discussed at $0.05 < P \leq 0.10$.

RESULTS AND DISCUSSION

The RFDG used in the current experiment contained 34.4% CP, 27.5% NDF, 7.4% ether extract, 0.91% P, and 0.92% S on a DM basis. Although CP content was similar between diets, estimated RDP content (NRC, 2001) was lower and RUP was greater for DG and DGMon compared with CON (Table 1). Additionally, although supplemental P was removed from the DG diets, P content was greater (0.48 vs. 0.36%) for DG and DGMon compared with CON. Inclusion of RFDG at 28.8% of diet DM increased (0.41 vs. 0.21%) dietary S content compared with CON. In agreement, increased RUP, P, and S in diets containing high RFDG have

been observed (Castillo-Lopez et al., 2014; Ramirez-Ramirez et al., 2016).

Fecal and Urine Outputs

Fecal output (DM basis) did not differ ($P \geq 0.13$) among treatments. However, feeding DG compared with CON decreased ($P = 0.04$) urine outputs by 15% without a difference between DG and DGMon (Table 2). We found no changes in fecal output when cows were fed diets containing up to 30% (DM basis) RFDG (Castillo-Lopez et al., 2014); however, a decrease in urinary output for cows fed DG versus CON was not expected. High inclusion of wheat (Li et al., 2014) and corn DDGS (Salim et al., 2012) in a ration increased urinary excretion in beef cattle. Li et al. (2014) demonstrated that increases in dietary DDGS can increase water consumption and, thus, increase urinary excretion. In the current study, although certain dietary minerals, such as P, S, and Na, were increased, dietary K concentration decreased. Dietary K concentration has been known to increase urine output (Weiss et al., 2009; Eriksson and Rustas, 2014). Furthermore, MUN was greater for cows fed CON than RFDG, and MUN is positively correlated with urine output (Nennich et al., 2006).

Nitrogen Utilization and Excretion

Intake of N did not differ among treatments (Table 3). Fecal N excretion, fecal N excretion as a proportion of N intake, and apparent N digestibility (Table 4) were not different ($P \geq 0.65$) for cows fed DG compared with CON. However, urinary N excretion decreased ($P \leq 0.04$) by 9%, whereas retained N tended to be greater (28 vs. 3 g/d; $P = 0.07$) for the DG diet versus CON. Urinary urea N excretion decreased ($P = 0.03$) by 12% for when feeding DG compared with CON. Milk urea N and PUN concentrations were also lower ($P < 0.01$; Table 5) in cows fed DG compared with CON. The DGMon diet did not affect N excretion and secretion except that DGMon decreased ($P = 0.04$) fecal N as a proportion of N intake (i.e., increased apparent N digestibility) compared with DG.

An increase in fecal N excretion was expected because of high RUP of RFDG protein compared with SBM protein. However, in situ and in vitro results by Mjoun et al. (2010a) indicated that total digestible protein (rumen and small intestine) between RFDG and SBM was similar (95 and 99%, respectively) in spite of different RUP (32 vs. 60%, respectively). This may have resulted in minimal effects on apparent total-tract N digestibility in cows fed either the DG or CON diet.

Table 2. Effects of feeding reduced-fat corn distillers grains (RFDG) with or without monensin on DMI and feces, urine, and milk outputs (kg/d)¹

Item	Diet ^{2,3}			SEM	<i>P</i> -value ⁴	
	CON	DG	DGMon		DG	Mon
DMI	26.0	25.4	24.4	0.89	0.57	0.39
Milk	40.6	42.3	38.5	2.21	0.37	0.051
Feces (DM basis)	7.6	8.1	7.3	0.34	0.34	0.13
Urine	32.5	27.7	29.7	1.76	0.04	0.39

¹During wk 1, 5, and 9 sampling collection periods.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 SE of treatment mean is shown.⁴DG = CON vs. DG; Mon = DG vs. DGMon.

In agreement with our results, no differences in fecal N outputs were observed with 25% (DM basis) wet distillers grains or 28.8% RFDG in dairy rations (Gehman and Kononoff, 2010; Foth et al., 2015). Decreases in urinary N and UUN excretion for DG compared with CON occurred due to lower ruminal degradation of RFDG protein versus SBM protein (Mjoun et al., 2010a), which is in line with decreased MUN and PUN for DG compared with CON in the current study. Because UUN is the primary contributor to ammonia emissions from manure (Bussink and Oenema, 1998; Thomsen, 2000), feeding cows a diet with high RFDG replacing SBM can potentially decrease ammonia volatilization from manure. Compared with DG, DGMon did not affect most measures of N utilization and excretion. However, apparent total-tract N digestibility increased

slightly (74.0 vs. 72.3; Table 4) with DGMon versus DG. Increased apparent N digestibility with monensin supplementation has often been reported in other studies (Plaizier et al., 2000; Ruiz et al., 2001; Martineau et al., 2007). Monensin inhibits AA-fermenting bacteria in the rumen (Chen and Russell, 1989), and thus increases dietary protein entering the small intestine. If dietary protein is more digestible than microbial protein, increases in dietary protein entering the small intestine should increase total-tract N digestibility (Martineau et al., 2007).

Interactions of treatment by week for urinary N and UUN excretion and PUN ($P \leq 0.02$) were observed (Figure 1). The interactions occurred because these variables were similar among treatments in wk 1 and the differences in these among treatments became larger as

Table 3. Effects of feeding reduced-fat corn distillers grains (RFDG) with or without monensin on N utilization and excretion

Item	Diet ^{1,2}			SEM	<i>P</i> -value ³	
	CON	DG	DGMon		DG	Mon
N intake, g/d	722	718	684	26.4	0.92	0.35
N secretion and excretion, g/d						
Feces	192	200	181	9.1	0.53	0.15
Urine ⁴	304	277	270	12.2	0.03	0.56
Urea N ⁴	207	183	174	10.7	0.03	0.45
Urinary urea N/total N, %	68	66	64	1.3	0.22	0.24
Total excreta	497	477	451	18.8	0.34	0.21
Milk	223	214	205	9.2	0.32	0.33
Retained	3	28	30	10.4	0.07	0.86
As proportion of N intake, %						
Feces	26.6	27.7	26.1	0.52	0.15	0.04
Urine	42.1	38.7	39.5	1.38	0.09	0.67
Total excreta	68.6	66.4	65.7	1.25	0.21	0.66
Milk	30.8	29.9	30.0	1.03	0.44	0.89
Retained	0.5	3.7	4.3	1.62	0.13	0.79
MUN, mg/dL ⁴	15.1	11.7	10.7	0.6	<0.01	0.23

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 SE of treatment mean is shown.³DG = CON vs. DG; Mon = DG vs. DGMon.⁴Treatment $\times$ week interaction ($P \leq 0.02$).

Table 4. Effects of feeding reduced-fat corn distillers grains with solubles (RFDG) with or without monensin on apparent total-tract digestibility (%)

Item	Diet ^{1,2}			SEM	P-value ³	
	CON	DG	DGMon		DG	Mon
DM	70.8	68.3	70.1	0.56	0.01	0.02
OM	71.5	69.0	70.7	0.56	<0.01	0.04
NDF ⁴	53.5	47.4	50.4	0.93	<0.01	0.03
N	73.4	72.3	73.9	0.52	0.15	0.04
P ⁴	49.7	43.9	44.7	2.14	0.06	0.78
S	58.0	74.7	75.1	0.48	<0.01	0.51

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 SE of treatment mean is shown.

³DG = CON vs. DG; Mon = DG vs. DGMon.

⁴Treatment × week interaction ($P = 0.03$).

the experiment progressed (wk 5 and 9), which was not expected and is difficult to explain. The second batch of RFDG for DG and DGMon was used from wk 5 to 9. Although CP contents of RFDG between the first and second batch were almost identical (34.22 vs. 34.60%), differences in processing method could have affected rumen protein property (e.g., RDP and RUP) and may have contributed to the treatment by week interactions. Additionally, microbial degradation of rumen protein and postabsorptive AA catabolism can contribute to urea production (Nousiainen et al., 2004). Therefore, protein catabolism pre- or postabsorption might change as lactation progressed (Larsen et al., 2015). However, milk protein production remained consistent throughout the experiment, which is shown in our companion study (Morris et al., 2018).

Phosphorus Utilization and Excretion

Intake of P increased (122.6 vs. 93.3 g/d; $P < 0.01$) in cows fed DG compared with CON, resulting in increased ($P < 0.01$) fecal excretion and a tendency (P

= 0.054) for increased urinary P excretion (Table 6). However, milk P secretion did not differ ($P = 0.58$), resulting in decreased milk P as a proportion of P intake (32.4 vs. 43.3%; $P < 0.01$) for DG compared with CON. Feeding the DG diet compared with CON tended ($P = 0.06$) to decrease (43.9 vs. 49.7) apparent total-tract P digestibility (Table 4), but increased ($P < 0.01$) plasma total and inorganic P concentrations (Table 5). Monensin supplementation to the DG diet did not alter dietary P utilization and excretion.

Approximately 80% of the increased P intake by feeding the DG diet versus CON was partitioned toward fecal excretion. Feces are the primary route of dietary P excretion (Morse et al., 1992). A meta-analysis by Alvarez-Fuentes et al. (2016) showed a clear positive relationship between P intake and fecal P output in dairy cows. Increases in urinary P excretion for DG versus CON were also expected. Although the primary route of P excretion is feces, urinary P excretion increases when P intake exceeds requirements for maintenance and production (Knowlton and Herbein, 2002; Suttle and Underwood, 2010). Although plasma P concentra-

Table 5. Effects of feeding reduced-fat corn distillers grains with solubles (RFDG) with or without monensin on plasma urea, P, and S concentrations (mg/dL)

Item	Diet ^{1,2}			SEM	P-value ³	
	CON	DG	DGMon		DG	Mon
Urea-N ⁴	23.7	21.0	21.2	0.62	<0.01	0.83
Total P	15.3	18.3	18.0	0.69	<0.01	0.71
Inorganic P	6.6	7.8	8.2	0.18	<0.01	0.102
Total S	110.2	115.7	109.8	1.71	0.03	0.02
Sulfate	3.5	4.5	4.2	0.20	<0.01	0.21

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 SE of treatment mean is shown.

³DG = CON vs. DG; Mon = DG vs. DGMon.

⁴Treatment × week interaction ($P < 0.01$).

Figure 1. Nitrogen excretion and utilization during the 9-wk experimental period with control (CON), 28.8% reduced-fat distillers grains (POET Nutrition Inc., Sioux Falls, SD; DG), or DG with monensin (DGMon) diets (Trt = overall diet effect; Trt $\times$ Wk = interaction of Trt by week). Means are LSM with error bars indicating SEM.

tion is not an appropriate indicator for P status of dairy cows, an increase in plasma inorganic P concentration and urinary P excretion generally occur (Knowlton and Herbein, 2002; Zhang et al., 2016) with increased P intake. Therefore, feeding a diet with RFDG at 28.8% (DM basis) can significantly increase manure P concentration, even though supplemental mineral P is removed from the ration, causing potential concerns of high P supply with losses from manure after field application (Ebeling et al., 2002). No difference in milk P secretion among treatments is in agreement with Knowlton and Herbein (2002). Shennan and Peaker (2000) suggested that milk P secretion is highly associated with lactose production, because inorganic P is generated during lactose synthesis and is subsequently sequestered in milk. In the current study, milk lactose yield was similar between DG versus CON (Morris et al., 2018).

Counter to our hypothesis, the addition of monensin to the DG diet (i.e., DGMon) did not affect P utilization and excretion compared with DG. Previously, monensin decreased fecal excretion of P via increased apparent digestibility and increased P retention in growing beef cattle (Starnes et al., 1984) or sheep (Greene et al., 1986). In these experiments, dietary P concentrations (0.45% of dietary DM) and monensin dosage rates (0.80 and 0.56 mg/kg of BW, respectively) were similar to the current study (0.45% and 0.68 mg/kg of BW, respectively). The reason for the discrepancy between the current study and the previous studies is not known. However, the route of P excretion changes from feces to urine when diets are switched from high fiber to high concentrate (Spears and Weiss, 2014). Indeed, previous studies used high-concentrate diets for steers (74% in diet DM) and sheep (71% of diet DM)

Table 6. Effects of feeding reduced-fat corn distillers grains (RFDG) with or without monensin on P and S excretion

Item	Diet ^{1,2}			SEM	<i>P</i> -value ³	
	CON	DG	DGMon		DG	Mon
Phosphorus						
Intake, g/d ⁴	93.3	122.6	113.8	4.28	<0.01	0.11
Secretion and excretion, g/d						
Feces ⁴	46.9	69.3	64.4	3.96	<0.01	0.36
Urine ⁴	0.3	4.5	7.0	1.44	0.054	0.24
Total excreta ⁴	47.2	74.2	71.3	3.29	<0.01	0.50
Milk	40.4	39.3	36.4	2.02	0.58	0.13
Retained	5.7	9.0	6.1	1.26	0.04	0.07
As proportion of P intake, %						
Feces	50.3	56.1	55.3	2.14	0.06	0.78
Urine ⁴	0.4	3.8	6.5	1.38	0.09	0.18
Total excreta ⁴	50.6	60.3	61.8	1.45	<0.01	0.41
Milk ⁴	43.3	32.4	32.1	1.56	<0.01	0.87
Retained	6.1	7.4	6.1	1.15	0.35	0.34
Sulfur						
Intake, g/d ⁴	54.1	104.5	98.7	3.57	<0.01	0.22
Secretion and excretion, g/d						
Feces	22.8	26.6	24.9	1.19	0.02	0.29
Urine ⁴	17.3	63.1	60.3	2.02	<0.01	0.29
Total excreta ⁴	40.0	89.6	85.2	2.99	<0.01	0.24
Milk	13.4	13.5	12.7	0.61	0.84	0.17
Retained	0.7	1.4	0.7	1.43	0.71	0.70
As proportion of S intake, %						
Feces	42.0	25.3	24.9	0.48	<0.01	0.51
Urine ⁴	31.9	60.5	61.4	1.62	<0.01	0.69
Total excreta ⁴	74.0	85.9	86.3	1.58	<0.01	0.82
Milk	24.7	12.9	13.0	0.53	<0.01	0.96
Retained	1.3	1.2	0.7	1.89	0.97	0.81

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 SE of treatment mean is shown.

³DG = CON vs. DG; Mon = DG vs. DGMon.

⁴Treatment × week interaction ($P < 0.03$).

compared with 44% in the current study. Therefore, the interaction of monensin with dietary P on absorption and utilization might be dependent upon types of diet. More studies are required to confirm the effects of monensin in relation to dietary forage-to-concentrate ratios on P utilization and retention.

Interactions of treatment by week for P intake, fecal P excretion (Figure 2), and urinary P excretion ($P \leq 0.02$) were observed. The interactions occurred due to large decreases in these variables in wk 9 for DGMon. The decreases in wk 9 for DGMon were a consequence of decreased P intake. Intake of P for DG and DGMon decreased gradually compared with CON as the experiment progressed (treatment × wk, $P < 0.01$; Figure 2), where the degree of decreases in P intake was greater for DGMon compared with DG.

Sulfur Utilization and Excretion

Compared with CON, feeding cows the DG diet increased ($P \leq 0.02$) S intake and fecal and urinary

excretion by 93, 17, and 265%, respectively (Table 6). Urinary S excretion as proportion of S intake also increased ($P < 0.01$) for cows fed DG compared with CON. As a result, total excretion of S was greater (89.6 vs. 40.0 g/d; $P < 0.01$) for cows fed DG versus CON. However, milk S secretion did not differ ($P = 0.84$), and as a result, milk S efficiency (milk S secretion as proportion of S intake) decreased ($P < 0.01$) for cows fed the DG compared with CON diet. Apparent total-tract S digestibility increased (74.7 vs. 58.0%; $P < 0.01$) for cows fed the DG compared with CON diet (Table 4). Furthermore, plasma total S increased (115.7 vs. 110.2 mg/dL), and plasma sulfate (4.5 vs. 3.5 mg/dL) was greater ($P \leq 0.03$) for DG versus CON (Table 5). However, monensin supplementation to the DG diet did not affect S utilization and excretion in the current study.

Increased S excretion with increasing S intake for cows fed DG versus CON was expected. Although S intake increased both fecal and urinary S excretion, the degree of increase in urinary S excretion was consider-

Figure 2. Phosphorus and sulfur excretion and utilization during the 9-wk experimental period with control (CON), 28.8% reduced-fat distillers grains (POET Nutrition Inc., Sioux Falls, SD; DG), or DG with monensin (DGMon) diets (Trt = overall diet effect; Trt $\times$ Wk = interaction of Trt by week). Treatment by week interactions were also observed for other variables about P and S utilization and excretion but not presented because trends were similar to current graphs. Means are LSM with error bars indicating SEM.

ably greater than fecal S excretion. An increase in S intake has been reported to increase urinary S excretion in beef cattle fed high-DDGS diets (Spiehs and Varel, 2009; Salim et al., 2012; Li et al., 2014) and in dairy cows fed supplemental inorganic S (MgSO_4 ; Tebbe et al., 2018). However, milk S secretion was not affected by treatments, in agreement with Tebbe et al. (2018), who found increased dietary S content by inclusion of MgSO_4 did not affect milk S secretion. This suggests that mammary uptake of S is likely independent of plasma S status.

We observed no effects of adding monensin to the DG diet except that total plasma S concentration decreased (109.8 vs. 115.7 mg/dL; $P = 0.02$). Plasma total S concentration can be affected by several factors, such as S intake, S absorption in the rumen (inorganic

S) and small intestine (microbial S), and inhaling H_2S produced from the rumen. We expected monensin to alter rumen microbial populations and possibly decrease ruminal H_2S production (Felix and Loerch, 2011). Although microbial populations were not examined, a lack of difference in milk fatty acid profile (Morris et al., 2018) indicates minimal effects of monensin on altering microbial population and fermentation in the rumen.

A treatment $\times$ week interaction ($P < 0.01$) for urinary S excretion occurred where DGMon decreased urinary S excretion in wk 9 compared with DG. The decreases in urinary S excretion for DGMon versus DG in wk 9 can be also explained by gradual decreases (treatment $\times$ week, $P < 0.01$; Figure 2) in S intake as the experiment progressed.

It is worth noting that apparent total-tract NDF digestibility decreased ($P < 0.01$) for cows fed the DG versus CON diet (47.4 vs. 53.5; Table 4). Feeding a diet with 2.6% sulfate negatively affected rumen microbial growth and fermentation (van Zijderveld et al., 2010). In an in vitro study, increased S decreased cellulose digestion (Hubbert et al., 1958). Additionally, the source of fiber likely contributed to the difference in NDF digestibility between CON and DG. In the current study, the major difference in fiber sources originated from RFDG replacing soyhulls in CON for DG. Firkins (1997) reported the ruminal digestibility of NDF for DDGS and soyhulls were 71.6 and 90.7%, respectively.

CONCLUSIONS

Inclusion of RFDG at 28.8% of diet DM by replacing mainly SBM in a dairy ration did not affect dietary CP content and total N intake. However, feeding a high-RFDG diet decreased urinary N excretion without differences in fecal N excretion. The RFDG diet increased dietary concentrations of dietary P and S contents, resulting in increases in P and S excretion in feces and urine. Supplementation of monensin to a high-RFDG diet did not have major effects on N, P, and S utilization and excretion compared with the RFDG diet without monensin. However, an increase in N digestibility was observed for cows fed a high-RFDG diet with monensin. The current study suggests that feeding RFDG at 28.8% (DM basis) as a replacement for mainly SBM can decrease urinary N excretion, potentially lowering ammonia emissions from manure; however, large increases in fecal and urinary P and S excretion with the high-RFDG diet may increase environmental concerns (e.g., P runoff and H₂S emissions from manure).

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