Effects of haylage and monensin supplementation on performance, carcass characteristics, and ruminal metabolism of feedlot cattle fed diets containing 60% dried distillers grains

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Effects of haylage and monensin supplementation on performance, carcass characteristics, and ruminal metabolism of feedlot cattle fed diets containing 60% dried distillers grains[1]

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ABSTRACT: The objectives of this research were to determine the interaction of monensin and haylage supplementation for steers fed 60% dried distillers grains (DDGS) on 1) mineral status, performance, and carcass characteristics, and on 2) ruminal pH, H_2S, and short-chain fatty acid concentrations. In Exp. 1, Angus-cross steers (n = 168; BW = 277 ± 67 kg) were blocked by BW and allotted in a 2 × 2 factorial arrangement of treatments to 24 pens. Dietary treatments were 1) 0 mg of monensin/kg of diet + 0% haylage, 2) 33 mg of monensin/kg of diet + 0% haylage, 3) 0 mg of monensin/kg of diet + 10% haylage, and 4) 33 mg of monensin/kg of diet + 10% haylage. The remainder of the diet was 60% DDGS, 10% corn silage, 15% supplement, and corn (either 5 or 15%) on a DM basis. When supplemented with 0 mg of monensin/kg of diet, added haylage increased ADG by 5.7%, whereas when supplemented with 33 mg of monensin/kg of diet, added haylage increased ADG by 13% ($P < 0.01$). No interactions of monensin and haylage were observed for DMI or G:F ($P \geq 0.36$). Haylage inclusion increased ($P < 0.01$) DMI and decreased ($P < 0.01$) G:F. No interactions ($P > 0.05$) on plasma mineral concentrations were observed; however, over time, plasma Cu concentrations decreased ($P < 0.01$), whereas plasma ceruloplasmin and S concentrations increased ($P < 0.01$). There were no treatment effects ($P \geq 0.08$) on carcass characteristics. Cattle fed the 60% DDGS diets benefitted from increased dietary forage, and the effects of monensin and forage were additive for ADG and final BW. In Exp. 2, ruminally fistulated steers (n = 8; BW = 346 ± 34 kg) were used in a replicated 4 × 4 Latin square design and were randomly assigned to the diets used in Exp. 1. Haylage inclusion increased ruminal pH from 1.5 through 12 h postfeeding, and the effects of monensin supplementation were additive ($P < 0.05$). From 1.5 through 9 h postfeeding, steers fed 33 mg of monensin/kg of diet tended to have reduced ($P \leq 0.10$) concentrations of H_2S when compared with steers fed 0 mg of monensin/kg of diet. Acetate:propionate ratios at 6 h postfeeding were 0.94, 0.93, 1.29, and 1.35 for diets 1 to 4, respectively ($P < 0.01$); total lactate was decreased regardless of treatment (range: 0.94 to 1.42 μmol/mL). Sulfuric acid in DDGS, not ruminal short-chain fatty acids, may be responsible for the low rumen pH observed and may influence the maximum inclusion of DDGS in cattle diets. Monensin supplementation decreased H_2S concentration and may decrease the risk of polioencephalomalacia for cattle fed high-DDGS diets.

Key words: dried distillers grains, feedlot cattle, monensin, sulfur

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INTRODUCTION

Strategies to reduce negative consequences of high dietary inclusion of distillers grains (**DG**) are lacking. Increasing DG in the diet of feedlot cattle results in increased concentrations of S and a greater risk for polioencephalomalacia (**PEM**; Gould et al., 1991, 2002; Gould, 1998; Loneragan et al., 1998). The production of H_2S gas in the rumen is thought to be the primary cause of PEM. Gould (1998) identified 4 factors that may alter H_2S in the rumen gas cap: generation of H_2S in the rumen fluid, pH, eructation, and absorption of H_2S across the rumen wall. Generation of H_2S may be increased when ruminal pH is low because low pH values represent an increase in free hydrogen ions (H^+) available to form H_2S (Gould et al., 1997; Gould, 1998; Kung et al., 2000). Ionophores, such as monensin, increase trace mineral absorption (Starnes, et al., 1984). Ionophores also decrease the available H^+ because of

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their actions on acetogenic bacteria (Chen and Wolin, 1979; Bergen and Bates, 1984). The effects of monensin on H_2S production have been studied in vitro, but results are conflicting (Kung et al., 2000; Quinn et al., 2009). Additional dietary forage also attenuates low ruminal pH because of increased salivary buffering (Owens et al., 1998). No information exists on the effects of monensin and added dietary forage on H_2S production in vivo. We hypothesized that increasing rumen pH, through haylage addition and including ionophores in feedlot diets containing increased amounts of dried DG with solubles (**DDGS**), would decrease ruminal H_2S production, improve trace mineral status, and improve performance (ADG and G:F). The objectives of this study were to determine the effects of monensin, haylage, and their interactions in steers fed 60% DDGS diets on 1) mineral status and feedlot performance, and on 2) ruminal pH, concentration of H_2S in the rumen gas, and concentrations of S^{2-} and short-chain fatty acids (**SCFA**) in the rumen liquid.

MATERIALS AND METHODS

All animal procedures were approved by the Agricultural Animal Care and Use Committee of The Ohio State University and followed guidelines recommended in the Guide for the Care and Use of Agricultural Animals in Agricultural Research and Teaching (FASS, 2010).

Exp. 1

Animals and Diets. A total of 168 Angus-cross steers (average initial BW = 276 ± 64 kg) were used at the Beef Research Station of the Ohio Agricultural Research and Development Center from October 2009 to May 2010. Steers were weighed on 2 consecutive days to determine initial BW and were blocked by BW into 3 blocks (small, medium, and large). Steers were then allotted within block to 24 pens (7 steers/pen). Pens (5.4 × 5.4 m) were constructed of metal gates and cables on concrete slatted floors in an open-sided barn and provided 81 cm of bunk space per steer. Treatments were arranged in a 2 × 2 factorial of monensin (Elanco Animal Health, Greenfield, IN) and haylage. Pens within block were randomly allotted to 1 of 4 dietary treatments: 1) 0 mg of monensin/kg of diet + 0% haylage inclusion, 2) 0 mg of monensin/kg of diet + 10% haylage inclusion, 3) 33 mg of monensin/kg of diet + 0% haylage inclusion, and 4) 33 mg of monensin/kg of diet + 10% haylage inclusion. Haylage was the first-cutting alfalfa harvest in mid to full bloom, with a chop length of 2.0 to 2.5 cm. The remainder of the diet was 10% corn silage, 60% DDGS, corn (5 or 15%), and 15% supplement (DM basis; Table 1). The composited sample of DDGS for the trial contained 0.73% S. The aforementioned diets were fed ad libitum until cattle within a block were deemed by visual appraisal to possess 1.2 cm of backfat. Therefore, days on feed was the same for all treatments (average = 159 d). Feed was delivered once daily at 1100 h. Feed samples were collected every 2 wk and were composited at the end of the trial for nutrient analysis.

Steers were implanted with Synovex S (Fort Dodge Animal Health, Overland Park, KS) at the start of the trial (d 0) and were reimplanted on d 81. Steers were weighed every 14 d during the experiment and were then weighed on 2 consecutive days at the end of the experiment to determine final BW before slaughter.

Sampling and Analysis. Composited feed samples were freeze-dried (Freeze Dryer 8, Labconco Corporation, Kansas City, MO) and then ground using a Wiley mill (1-mm screen; Arthur H. Thomas, Philadelphia, PA). All samples were analyzed for DM (24 h at 100°C). All freeze-dried samples were subjected to perchloric acid digestion and inductively coupled plasma atomic emission spectroscopy (**ICP**) analysis of complete minerals (method 975.03; AOAC, 1988). Feed samples were analyzed for ADF and NDF (using Ankom Technology methods 5 and 6, respectively; Ankom^{200} Fiber Analyzer, Ankom Technology, Fairport, NY), CP (macro-Kjeldahl N × 6.25), and fat (using the ether extract method; Ankom Technology).

On d 0, 81, and 139, blood samples (20 mL) from each steer were collected in 2 Monoject collection tubes (Tyco Healthcare Group LP, Mansfield, MA), 1 containing EDTA (0.07 mL of a 15% solution) and 1 containing no additive. Blood samples were centrifuged at 1,760 × *g* for 20 min at 25°C. Plasma samples, collected from tubes containing EDTA, were frozen at −20°C for subsequent analysis of trace minerals and S by ICP (method 975.03; AOAC, 1988). Serum samples, collected from tubes containing no additive, were taken back to the laboratory immediately after collection for subsequent ceruloplasmin analysis by spectophotometry (Genesys 10-S, Thermo Fisher Scientific, Waltham, MA) using a colorimetric assay (Houchin, 1958).

On d −1, liver biopsies were taken from 16 steers (2 steers randomly selected from each pen in the medium weight block) as described by Miles et al. (2001). Biopsied samples were freeze-dried (Freeze Dryer 8, Labconco) and were ground in a food processor, which was cleaned with ethanol between each sample. Liver samples were then analyzed for major elements by ICP (method 975.03; AOAC, 1988). Liver samples were also collected postmortem from the same steers that were biopsied on d −1, prepared as with the biopsied samples, and analyzed for major elements by ICP.

Three animals had to be removed from the study; 1 of these was removed because of PEM symptoms, and the other 2 were removed for nonmetabolic reasons. All steers were killed at Tucker Packing (Orrville, OH). Carcass data to determine USDA quality and yield grades were collected on all animals.

Statistical Analysis. The experimental design for this study was a randomized complete block design with subsampling and repeated measures. Statistical data were analyzed using the MIXED procedure (SAS

Table 1. Composition (%, DM basis) of diets fed in Exp. 1 and 2

Item	0% Haylage		10% Haylage	
	0 mg of monensin/ kg of diet	33 mg of monensin/ kg of diet	0 mg of monensin/ kg of diet	33 mg of monensin/ kg of diet
DDGS[1]	60	60	60	60
Rolled corn	15	15	5	5
Corn silage	10	10	10	10
Alfalfa haylage	—	—	10	10
Supplement	15	15	15	15
Ground corn	11.165	11.146	11.165	11.146
Limestone	2.790	2.790	2.790	2.790
Trace mineral salt[2]	0.457	0.457	0.457	0.457
Vitamin A, 30,000 IU/g	0.009	0.009	0.009	0.009
Vitamin D, 3,000 IU/g	0.009	0.009	0.009	0.009
Vitamin E	0.027	0.027	0.027	0.027
Selenium	0.046	0.046	0.046	0.046
Monensin[3]	0.000	0.019	0.000	0.019
Tylosin[4]	0.046	0.046	0.046	0.046
Animal-vegetable fat	0.450	0.450	0.450	0.450
Tribasic copper chloride[5]	0.001	0.001	0.001	0.001
Analyzed composition				
NDF, %	29.41	29.72	33.49	33.80
ADF, %	9.97	9.70	14.25	13.98
CP, %	19.92	20.10	21.22	21.40
Ether extract, %	7.52	7.33	7.25	7.06
S, %	0.50	0.50	0.51	0.51
NE_m, Mcal/kg	2.08	2.08	1.99	1.99
NE_g, Mcal/kg	1.42	1.42	1.35	1.35

[1]Dried distillers grains with solubles.

[2]Included 95% NaCl; 0.35% Zn, as ZnO; 0.28% Mn, as MnO_2; 0.175% Fe, as $FeCO_3$; 0.040% Cu, as Cu_2O; 0.007% I, as $Ca_5(IO_6)_2$; and 0.007% Co, as $CoCO_3$.

[3]Rumensin 80, 176 g/kg (Elanco Animal Health, Greenfield, IN).

[4]Tylan 10, 22 g/kg (Elanco Animal Health).

[5]Included supplemental Cu to bring the dietary Cu to 15 mg/kg (Micronutrients TBCC, Heritage Technologies LLC, Indianapolis, IN).

Inst. Inc., Cary, NC). The model used for the performance and carcass data was

$$Y_{ijkl} = \mu + b_i + p_j + F_k + R_l + (FR)_{kl} + e_{ijkl},$$

where Y_{ijkl} is the observation; μ is the response variable; b_i is the random effect of block; p_j is the random effect of pen; F_k is the fixed effect of forage inclusion; R_l is the fixed effect of monensin inclusion; $(FR)_{kl}$ is the fixed effect of the interaction of forage and monensin inclusion; and e_{ijkl} is the experimental error. Pen was the experimental unit. To evaluate repeated measures for plasma trace minerals and ceruloplasmin, the model below was used:

$$Y_{ijklm} = \mu + b_i + p_j + F_k + R_l + (FR)_{kl} + T_m + (TF)_{mk} + (TR)_{ml} + (TFR)_{mkl} + e_{ijklm},$$

where the errors $\sim {}_{id}N(0, \Sigma)$, and where Y_{ijklm} is the observation; μ is the response variable; b_i is the random effect of block (df = 2); p_j is the random effect of pen (df = 23); F_k is the fixed effect of forage inclusion (df = 1); R_l is the fixed effect of monensin inclusion (df = 1); $(FR)_{kl}$ is the fixed effect of the interaction of forage and monensin inclusion (df = 1); T_m is the fixed effect of repeated of time of collection (df = 4); $(TF)_{mk}$ is the fixed effect of the interaction of time and forage inclusion (df = 4); $(TR)_{ml}$ is the fixed effect of the interaction of time and monensin inclusion (df = 4); $(TFR)_{mkl}$ is the fixed effect of the interaction of time and forage and monensin inclusion (df = 4); and e_{ijklm} is the experimental error (df = 14).

Exp. 2

Animals and Diets. Eight Angus-cross, ruminally fistulated steers (starting BW = 347 ± 29 kg) were placed on trial February 15, 2010, at the Ohio Agricultural Research and Development Center feedlot in Wooster. Steers were randomly allotted to the 4 dietary treatments from Exp. 1 (Table 1). Diets were fed ad libitum in a replicated 4 × 4 Latin square design. Dietary treatments were randomly allotted to steers in each period, with the restriction that no steer was fed the same diet twice.

Cattle were housed in individual pens on slatted concrete floors and fed daily at 0800 h. Dietary ingredient samples were collected at the beginning of each period to determine DM adjustments. Diet and orts samples were collected and weighed on the day before and the day of collection. A sample of orts and 0.45 kg of each

diet component were collected for later analysis of NDF, ADF, CP, ether extract, and minerals, as described for feed samples in Exp. 1. Each period consisted of a 14-d feeding phase followed by 1 d of rumen collection at 0, 1.5, 3, 6, and 9 h for H_2S gas and at 0, 1.5, 3, 6, 9, 12, and 18 h for liquid S^{2-} and pH determination.

Sampling. Rumen gas was sampled though the cannula cap via puncture with a 10-gauge needle. The H_2S gas was collected at 0, 1.5, 3, 6, and 9 h, and concentration was measured via H_2S precision gas detector tubes (No. 120SF, Sensidyne, Clearwater, FL) attached to a calibrated gas detection pump (Model AP-20S, Sensidyne). The concentration of H_2S was read from the tube by the same individual for each sampling. This sampling technique was validated by Gould et al. (1997).

Rumen fluid samples were strained through 4 layers of cheesecloth for pH, S^{2-}, and SCFA measurements. Measurements of pH and liquid S^{2-} were taken at 0, 1.5, 3, 6, 9, 12, and 18 h. A dual electrode meter was used to measure pH and S^{2-} (Accumet Excel XL25 dual-channel pH/ion meter, Fisher Scientific, Hampton, NH), and samples were measured within 2 min of collection. To measure pH, the electrode (Accument pH/ATC polypropylene body liquid-filled combination electrode with Ag/AgCl reference with BNC mini connector; Fisher Scientific) was simply submersed in unadulterated rumen fluid. To measure liquid S^{2-}, 25 mL of rumen liquid was mixed with 25 mL of sulfide antioxidant buffer (SAOB, Cat. No. 13-641-882, Fisher Scientific) to stabilize the sulfur ions. The samples were shaken vigorously and then S^{2-} was measured via sulfide electrode (Accumet silver/sulfide combination electrode, Fisher Scientific).

Samples for SCFA were taken at 0 and 6 h to represent SCFA concentration before feeding and at peak fermentation. Samples were initially prepared by mixing 10 mL of H_3PO_4 with 50 to 75 mL of rumen fluid, and then adding water to match the volume of rumen fluid used. This mixture was placed in the refrigerator for 2 d and mixed several times per day by shaking. On the third day, the samples were removed from the refrigerator, and approximately 40 mL of sample was poured into centrifuge tubes and centrifuged at 45,000 $\times$ *g* at 25°C for 20 min. The supernatant was filtered through a 0.45-μm filter. A 1.0-mL quantity of the filtered sample was then pipetted in to a gas chromatography vial with 0.1 mL of 2-ethyl butyrate as the internal standard. The gas chromatography vials were stored at −20°C until analyzed with a gas chromatograph (Model 5890A, Hewlett-Packard, Palo Alto, CA) for VFA. Lactic acid was analyzed using a colorimetric method (Boehringer Mannheim Test-Combination D-Lactic Acid/L-Lactic Acid, RBiopharm AG, Darmstadt, Germany) on a plate reader spectrophotometer (Multiskan MCC, Thermo Electron Corporation, Fisher Scientific).

Statistical Analysis. The experimental design was a replicated 4 × 4 Latin square with a 2 × 2 factorial arrangement of treatments to measure the effects of haylage inclusion and monensin inclusion on H_2S concentration, liquid S^{2-}, pH, and SCFA of growing beef steers. Repeated measures were used to analyze rumen sulfide, pH, and SCFA because these data represent a single animal on a given day. Statistical data were analyzed using the MIXED procedure (SAS Inst. Inc., Cary, NC). The model used was

$$Y_{ijklmn} = \mu + S_i + c_{j(i)} + p_k + F_l + R_m + (FR)_{lm} + T_n + (TF)_{nl} + (TR)_{nm} + (TFR)_{nlm} + c_{i(n)} + e_{ijklmn},$$

where Y_{ijklmn} is the observation; μ is the response variable; S_i is the fixed effect of square (df = 1); $c_{j(i)}$ is the random effect of calf nested within square (df = 6); p_k is the random effect of period (df = 3); F_l is the fixed effect of forage inclusion (df = 1); R_m is the fixed effect of monensin inclusion (df = 1); $(FR)_{lm}$ is the fixed effect of the interaction of forage and monensin inclusion (df = 1); T_n is the fixed effect of repeated of time of collection (df = 4); $(TF)_{nl}$ is the fixed effect of time and forage inclusion (df = 4); $(TR)_{nm}$ is the fixed effect of time and monensin inclusion (df = 4); $(TFR)_{nlm}$ is the fixed effect of time and forage and monensin inclusion (df = 4); $c_{i(n)}$ is the random effect of calf nested within time (df = 28); and e_{ijklmn} is the experimental error (df = 14).

RESULTS AND DISCUSSION

Exp. 1

There were interactions ($P < 0.03$) of monensin and haylage on final BW and ADG (Table 2). When steers were supplemented with 0 mg of monensin/kg of diet, added haylage increased ADG by 5.7%, whereas when steers were supplemented with 33 mg of monensin/kg of diet, added haylage increased ADG by 13% (interaction; $P < 0.01$). Dry matter intake and G:F were affected ($P < 0.01$) only by the main effect of haylage; 10% added haylage resulted in a 19% increase in DMI and a 9% decrease in G:F. Zinn et al. (1994) fed monensin with 10 or 20% forage but did not find any interaction on the growth and performance of feedlot cattle. Goodrich et al. (1984) compiled research from more than 16,000 cattle and found that the optimal energy density to observe a monensin response was 1.37 Mcal/kg of NE_g. The calculated NE_g in our diets were similar to this optimum (1.42 Mcal/kg for the 0% haylage diets, and 1.35 Mcal/kg in the 10% haylage diets). The interaction ($P < 0.01$) of monensin and haylage that was observed for ADG indicates that monensin did not improve ADG when 0% added haylage was fed, but it increased ADG when 10% haylage was added to the diet. Rumen conditions in steers fed the 0% haylage diets may not have been conducive for a monensin response to occur.

There were no effects ($P > 0.05$) of treatment on serum ceruloplasmin or plasma mineral concentration

Table 2. Feedlot growth, intake, and efficiency of cattle fed 60% dried distillers grains with solubles (DDGS) diets with or without haylage and monensin in Exp. 1

	0% Haylage		10% Haylage			*P*-value		
Item	0 mg of monensin/ kg of diet	33 mg of monensin/ kg of diet	0 mg of monensin/ kg of diet	33 mg of monensin/ kg of diet	SE	Monensin	Haylage	M × H[1]
Animals, n (pens)	41 (6)	41 (6)	41 (6)	42 (6)	—	—	—	—
Initial BW, kg	275	276	276	276	22.8	0.32	0.77	0.39
Final BW, kg	526[c]	520[c]	540[b]	553[a]	26.5	0.39	<0.01	0.03
ADG, kg	1.57[c]	1.54[c]	1.66[b]	1.74[a]	0.02	0.27	<0.01	0.01
DMI, kg	8.48	8.31	9.92	10.06	0.33	0.92	<0.01	0.36
G:F	0.187	0.186	0.167	0.174	0.01	0.20	<0.01	0.12

[a–c]Within a row, means without a common superscript differ ($P < 0.05$).

[1]M × H = monensin (Rumensin 80, Elanco Animal Health, Greenfield, IN) × haylage interaction.

(Table 3). However, there was an effect ($P < 0.01$) of time on ceruloplasmin, S, and Cu concentrations. Over time, S increased in the plasma, whereas Cu decreased. Suttle (1974) found that S additions to the diet, as methionine and as Na_2SO_4, decreased plasma Cu concentrations over time. However, Suttle (1974) saw a similar response for ceruloplasmin and Cu, whereas we found an inverse response. Ceruloplasmin is the predominant protein to which Cu is bound in circulation, and it has been reported previously to follow plasma Cu concentrations (Gengelbach et al., 1994).

Liver minerals were not altered ($P > 0.05$) because of treatment (Table 4). We had hypothesized that monensin would increase mineral absorption based on previous studies (Elsasser, 1984; Kirk et al., 1985; Spears, 1990; Salles et al., 2008). The lack of difference in mineral concentration does not imply that a difference in mineral absorption did not occur because mineral retention was not measured in this study.

There were no interactions ($P > 0.10$) of haylage and monensin on carcass characteristics except for HCW ($P = 0.08$; Table 5). There was an increase in HCW ($P = 0.002$) with haylage inclusion, which led to a trend for increased dressing percentage ($P = 0.07$) and was reflected in the larger ($P = 0.05$) LM area of those carcasses as well. These responses can be explained by the above-mentioned increase in final BW of the steers that were fed haylage.

Exp. 2

Fistulated steers fed 10% added haylage diets had increased ($P < 0.01$) DMI compared with those fed no additional haylage (Table 6). Consumption of individual minerals followed the DMI response.

There were no monensin × haylage interactions ($P > 0.13$) for H_2S concentration (Table 7); therefore, the main effects are discussed. There was a trend ($P \leq$

Table 3. Plasma ceruloplasmin and mineral concentrations after 81 and 139 d on feed in steers consuming 60% dried distillers grains with solubles (DDGS) diets with or without haylage and monensin in Exp. 1

	0% Haylage		10% Haylage			*P*-value		
Item[1]	0 mg of monensin/ kg of diet	33 mg of monensin/ kg of diet	0 mg of monensin/ kg of diet	33 mg of monensin/ kg of diet	SE	Monensin	Haylage	M × H[2]
d 81								
Ceruloplasmin,[3] mg/100 mL	6.21	5.90	5.95	8.30	0.87	0.24	0.21	0.14
S,[2] μg/mL	810.8	807.3	810.4	792.3	12.2	0.37	0.52	0.66
Cu,[2] μg/mL	0.99	0.91	0.95	0.98	0.06	0.56	0.80	0.61
Fe, μg/mL	2.32	2.27	2.05	2.30	0.21	0.54	0.46	0.63
Zn, μg/mL	1.27	1.33	1.27	1.25	0.05	0.71	0.48	0.76
d 139								
Ceruloplasmin,[3] mg/100 mL	8.09	7.13	9.11	9.79	0.84	0.87	0.03	0.12
S,[2] μg/mL	917.2	922.9	915.4	929.8	12.2	0.40	0.83	0.83
Cu,[2] μg/mL	0.44	0.43	0.44	0.40	0.06	0.68	0.72	0.93
Fe, μg/mL	2.16	2.32	2.10	2.43	0.21	0.14	0.87	0.47
Zn, μg/mL	1.31	1.34	1.34	1.38	0.05	0.50	0.47	0.80

[1]n = 18 animals (6 pens) per treatment.

[2]M × H = monensin (Rumensin 80, Elanco Animal Health, Greenfield, IN) × haylage interaction.

[3]$P < 0.01$ for the main effect of time.

Table 4. Concentrations of liver minerals (DM basis) in steers consuming 60% dried distillers grains with solubles (DDGS) with or without haylage and monensin in Exp. 1

	0% Haylage		10% Haylage			*P*-value		
Item,[1,2] mg/kg	0 mg of monensin/ kg of diet	33 mg of monensin/ kg of diet	0 mg of monensin/ kg of diet	33 mg of monensin/ kg of diet	SE	Monensin	Haylage	M × H[3]
S	6,238.1	6,339.9	6,454.2	6,404.1	133.5	0.85	0.33	0.58
Cu	185.62	182.69	244.09	234.55	28.26	0.81	0.06	0.91
Fe	192.24	166.62	198.43	215.46	15.41	0.78	0.10	0.19
Zn	125.2	127.12	117.98	126.41	6.25	0.42	0.50	0.60
Mn	13.20	13.17	12.61	13.14	0.80	0.73	0.65	0.76

[1]n = 4 animals (2 pens) per treatment.
[2]Values presented are from samples taken at slaughter, and d −1 biopsies were used as a covariate in the statistical analysis.
[3]M × H = monensin (Rumensin 80, Elanco Animal Health, Greenfield, IN) × haylage interaction.

0.10) for monensin supplementation to decrease H_2S concentration beginning at 1.5 h after feeding and continuing through all collections, up to 9 h after feeding, regardless of haylage inclusion. The H_2S response was not associated with S intake because cattle fed monensin and haylage consumed 17.2% more S than cattle fed monensin with no additional haylage (Table 6). Monensin inhibits acetogenic bacteria, decreasing the production of their byproduct, H^+ (Chen and Wolin, 1979; Bergen and Bates, 1984). In vitro studies on the effect of monensin on H_2S production have been inconclusive. Some researchers suggest no change in H_2S production with monensin in vitro (Quinn et al., 2009; Smith et al., 2010), whereas Kung et al. (2000) found an increase in in vitro H_2S production with monensin additions to a high-S diet. These in vitro experiments were carried out under highly buffered conditions and may not be applicable to in vivo conditions where rumen pH is low and H^+ ions are abundant. We had hypothesized that the decrease in H^+ from monensin supplementation and from the increased pH with haylage inclusion (because of salivary buffering) would cause an additive response to decrease rumen H_2S concentrations. Although this interaction did not occur, the substantial reduction in H_2S concentration attributable to monensin supplementation from 1.5 to 9 h postfeeding suggests a possible efficacy for PEM risk reduction. The only response in H_2S concentration attributable to haylage inclusion was a trend for a reduction ($P = 0.08$) at 1.5 h postfeeding. Gould et al. (1997) found that H_2S exceeding 2,000

Table 5. Carcass characteristics of steers consuming 60% dried distillers grains with solubles (DDGS) diets with or without haylage and monensin in Exp. 1

	0% Haylage		10% Haylage			*P*-value		
Item	0 mg of monensin/ kg of diet	33 mg of monensin/ kg of diet	0 mg of monensin/ kg of diet	33 mg of monensin/ kg of diet	SE	Monensin	Haylage	M × H[1]
Animals, n (pens)	41 (6)	41 (6)	41 (6)	42 (6)	—	—	—	—
HCW, kg	320	314	331	339	23.95	0.84	<0.01	0.08
Dressing percent	60.8	60.3	61.1	61.0	1.56	0.31	0.07	0.52
Backfat, cm	1.24	1.24	1.29	1.33	0.24	0.72	0.18	0.72
KPH	2.00	2.15	2.18	2.18	0.23	0.47	0.30	0.47
LM area, cm^2	76.12	73.91	76.70	79.30	3.51	0.89	0.05	0.12
QG[2]	4.82	4.75	5.12	5.02	0.17	0.55	0.07	0.90
Marbling score[3]	533	525	556	545	21.00	0.48	0.14	0.92
YG[4]	3.02	3.12	3.17	3.15	0.32	0.68	0.37	0.57
Select, %	33.7	38.5	12.3	23.8	9.36	0.40	0.09	0.72
Low Choice, %	54.0	47.2	68.2	54.8	9.05	0.30	0.26	0.72
Average Choice, %	7.6	14.3	17.1	16.7	7.25	0.49	0.20	0.43
High Choice, %	4.8	0.0	0.0	4.8	2.66	1.00	1.00	0.10
Prime, %	0.0	0.0	2.4	0.0	1.19	0.34	0.34	0.34
YG1, %	4.8	0.0	0.0	2.4	2.66	0.62	0.62	1.00
YG2, %	40.9	49.6	43.7	35.7	23.75	0.97	0.54	0.37
YG3, %	44.1	33.7	36.5	52.4	19.57	0.76	0.55	0.19
YG4, %	10.3	19.9	16.7	9.5	8.93	0.74	0.84	0.19

[1]M × H = monensin (Rumensin 80, Elanco Animal Health, Greenfield, IN) × haylage interaction.
[2]QG = USDA quality grade. The scale for the USDA QG was 4 = Select, 5 = Low Choice, and 6 = Average Choice.
[3]The scale for marbling score was 400 = slight, 500 = small, 600 = moderate.
[4]YG = USDA yield grade.

Table 6. Daily DMI and mineral intake of steers consuming 60% dried distillers grains with solubles (DDGS) diets with or without haylage and monensin in Exp. 2

	0% Haylage		10% Haylage			*P*-value		
Item[1]	0 mg of monensin/ kg of diet	33 mg of monensin/ kg of diet	0 mg of monensin/ kg of diet	33 mg of monensin/ kg of diet	SE	Monensin	Haylage	M × H[2]
DMI, kg	8.91	9.38	10.08	10.29	0.33	0.13	<0.01	0.70
DMI, g/d (DM basis)								
P	45.31	48.12	51.90	52.53	1.88	0.19	0.01	0.58
K	73.91	79.40	105.48	105.03	4.74	0.54	<0.01	0.55
Ca	96.21	98.76	117.08	123.45	3.71	0.09	<0.01	0.62
Mg	22.67	24.50	27.00	26.98	1.22	0.38	<0.01	0.47
S	42.30	43.09	47.64	50.49	1.53	0.06	<0.01	0.50
Cu	0.14	0.13	0.16	0.15	0.01	0.33	0.04	0.88
Mn	0.27	0.32	0.34	0.39	0.02	0.02	<0.01	0.97
Mo	0.01	0.01	0.01	0.01	0.00	0.26	<0.01	0.88
Na	29.66	29.52	34.29	31.45	1.58	0.30	0.03	0.41
Zn	0.65	0.66	0.73	0.74	0.03	0.48	<0.01	0.97

[1]n = 8 animals per treatment.
[2]M × H = monensin (Rumensin 80, Elanco Animal Health, Greenfield, IN) × haylage interaction.

mg/L in the rumen preceded the onset of PEM. None of the cattle in Exp. 2 suffered from PEM; however, these animals were slowly adapted to high-S diets before the initiation of the trial. Further in vivo investigation based on this initial finding is warranted.

Similar trends were expected for S^{2-} concentration based on previous research that suggests S in the rumen liquid is maintained at equilibrium with S in the gas cap (Gould et al., 1997; Loneragan et al., 1998). Monensin supplementation decreased ($P < 0.09$) S^{2-}

Table 7. Rumen variables for steers fed 60% dried distillers grains with solubles (DDGS) diets with or without haylage and monensin in Exp. 2

	0% Haylage		10% Haylage			*P*-value		
Item[1]	0 mg of monensin/ kg of diet	33 mg of monensin/ kg of diet	0 mg of monensin/ kg of diet	33 mg of monensin/ kg of diet	SE	Monensin	Haylage	M × H[2]
H_2S gas,[3,4] mg/L								
0	1,520	1,041	460	786	521	0.88	0.32	0.58
1.5	4,168	2,658	2,626	1,764	780	0.10	0.08	0.13
3	4,335	2,505	3,144	2,145	861	0.08	0.32	0.25
6	3,722	2,378	3,765	2,092	775	0.04	0.86	0.21
9	4,168	2,955	3,649	1,761	977	0.09	0.33	0.29
S^{2-} fluid,[3,4] mg/L								
0	8.30	11.40	6.80	8.51	3.38	0.44	0.48	0.75
1.5	18.92	8.08	9.03	8.01	3.43	0.07	0.12	0.07
3	15.89[a]	7.19[b]	10.62[ab]	7.71[b]	2.37	0.01	0.25	0.03
6	11.67	6.48	10.05	6.93	2.20	0.03	0.75	0.18
9	15.24	7.86	11.28	8.92	2.30	0.02	0.46	0.08
12	11.58	5.62	10.31	7.50	2.19	0.02	0.87	0.13
18	11.48	7.16	9.24	7.69	2.05	0.09	0.61	0.33
pH[3]								
0	6.10[b]	6.30[ab]	6.60[a]	6.54[a]	0.14	0.58	0.01	0.04
1.5	5.16[b]	5.35[ab]	5.60[a]	5.73[a]	0.15	0.24	0.01	0.03
3	5.07[b]	5.16[b]	5.50[a]	5.52[a]	0.13	0.61	<0.01	0.02
6	5.02	5.12	5.42	5.43	0.16	0.73	0.02	0.13
9	4.91[b]	5.05[b]	5.37[a]	5.50[a]	0.15	0.31	<0.01	0.02
12	4.79[b]	4.98[ab]	5.24[a]	5.21[a]	0.13	0.51	0.01	0.04
18	5.43[b]	5.60[b]	5.99[a]	5.68[ab]	0.12	0.52	0.01	0.01

[a,b]Within a row, means without a common superscript differ ($P < 0.05$).
[1]n = 8 animals per treatment.
[2]M × H = monensin (Rumensin 80, Elanco Animal Health, Greenfield, IN) × haylage interaction.
[3]$P < 0.05$ for the effect of time.
[4]$P < 0.05$ for the effect of monensin × time.

concentration at 6, 12, and 18 h postfeeding. There was an interaction ($P = 0.03$) for liquid S^{2-} at 3 h after feeding, with trends ($P < 0.08$) at 1.5 and 9 h. The effect of monensin supplementation on reducing S^{2-} concentration was greater for the 0% haylage diet than for diets contained 10% haylage. Sulfide in the rumen liquid can be absorbed rapidly across the rumen wall and detoxified by the blood (Kandylis, 1984) and liver (Anderson, 1956). Gould (1998) suggested that decreasing rumen pH should decrease the proportion of S in the liquid vs. the gas; however, in our data, this trend was not seen, perhaps because ruminal pH and S^{2-} concentration in our steers were already very low.

The effects of monensin supplementation and 10% haylage inclusion to increase rumen pH were additive at 0, 1.5, 3, 9, and 12 h postfeeding (interaction: $P \leq 0.04$). At 6 h postfeeding, haylage inclusion increased ($P = 0.02$) rumen pH. Vanness et al. (2009) hypothesized that increasing rumen pH would decrease H_2S production by decreasing the source of H^+, but they were unable to validate this hypothesis in vivo. In our samples, rumen pH was highest in steers fed haylage ($P < 0.05$), yet the decrease we observed in H_2S was primarily due to monensin, not to haylage inclusion.

A rapid postfeeding decrease in rumen pH occurred and, at 1.5 h postfeeding, rumen pH had fallen by approximately 1 pH unit for all treatments. The pH continued to decline for up to 12 h after feeding. Because of this rapid decline in pH observed at 1.5 h, we speculated that rumen acidity was not likely caused by SCFA produced during rumen fermentation. The titratable acidity of DDGS was analyzed in the laboratory to determine if acid present in the DDGS could have caused the low rumen pH observed. A 20-g sample of DDGS (with S content of 0.74%) was mixed in 80 mL of tap water and pH was measured, as described above for ruminal pH determination. The DDGS solution had a pH of 3.76. The same procedure was repeated with cracked corn, and a pH of 5.80 was measured in that solution. Using McDougall's artificial saliva (McDougall, 1948), the DDGS solution was titrated to pH 6.5 to estimate the saliva necessary to buffer the acidity of DDGS. It took 142.5 mL of McDougall's saliva to buffer the 20-g sample. We calculated, based on the daily intake of DDGS in Exp. 2 (Table 6), that 37 L of saliva would be necessary merely to buffer the acidity contained in DDGS. Finally, a 20-g sample of DDGS was prepared as stated above, and the amount of 1 *M* NaOH needed to bring that sample to pH 7.0 was found to be 10.5 mL. From this information, we calculated that 525 mol of NaOH would be necessary to neutralize 1 kg of DDGS. If the acidity in DDGS came from H_2SO_4 (a standard treatment in the ethanol production industry; McAloon et al., 2000), then the titratable acidity in 1 kg of DDGS would represent 25.8 g of H_2SO_4. It is unknown if the acidity in DDGS was due solely to H_2SO_4, but it seems likely that the acidity contained in this feedstuff caused the low rumen pH values observed, not SCFA production in the rumen. Rather than relieving the ruminal acid load by replacing starch-containing grains, DDGS may increase the acid load because it may carry substantial quantities of H_2SO_4. The effects of this acidity on rumen and animal metabolism are unknown, but they may be of significant consequence. It is notable that rumen pH values were close to 5 or below from 3 to 9 h postfeeding when steers were fed diets without 10% haylage (10% corn silage served as the sole source of forage). These steers showed no signs of acute acidosis, and DMI was approximately 2.6% of BW (Table 7). However, rumen pH was above 5.2 when 10% haylage was included in the diet. This response may explain why steers in Exp. 1 that were fed diets with 10% haylage had a 19% increase in DMI and a 9% increase in ADG compared with those not fed supplemental haylage. These data suggest that additional forage supplementation of cattle fed increased dietary concentrations of DDGS may reduce the negative consequences of acid load on ADG.

There was an interaction ($P = 0.05$) of monensin and haylage on total VFA at 0 h because monensin did not alter VFA when fed with haylage; however, it decreased total VFA concentration when no additional haylage was fed (Table 8). There were no treatment effects ($P > 0.05$) on total VFA at 6 h after feeding. There were no treatment effects ($P > 0.05$) on acetate at 0 or 6 h postfeeding. Typically, monensin has been shown to increase propionate concentration relative to acetate (Richardson et al., 1976). However, in the present experiment, at 0 h, monensin decreased propionate concentration (19%) when no haylage was fed and increased propionate (6%) when 10% haylage was fed (interaction: $P < 0.01$). At 6 h postfeeding, monensin had little effect on propionate when no haylage was fed, but monensin supplementation decreased propionate when 10% haylage was fed (interaction: $P < 0.01$). A similar interaction ($P < 0.05$) was noted for butyrate at 6 h after feeding. At 0 h, monensin increased ($P < 0.01$) acetate:propionate (**A:P**) with 0% haylage but had little effect with 10% haylage (interaction: $P < 0.01$). The low rumen pH caused by these diets likely affected A:P. At 6 h postfeeding, A:P was below 1 for the 0% haylage diets and was only slightly above 1 for the 10% haylage diets. In addition, microscopic examination of rumen fluid from these steers showed they were completely defaunated (data not shown). The low rumen pH and the lack of protozoa suggest that little fiber fermentation could occur by cellulolytic populations of bacteria. This would explain the low acetate concentrations despite the greater ADF and NDF values in the high-DDGS diets.

Total lactate was low for all treatments and was not increased ($P > 0.10$) by supplemental monensin or haylage. Lactate is a strong SCFA and is largely responsible for causing acidosis in cattle fed high-concentrate diets (Wilson et al., 1975). Although the concentration of lactate was reduced in steers fed these diets, rumen pH was also extraordinarily low. Normal rumen fluid will contain anywhere between 1 and 20 m*M* lactate

Table 8. Short-chain fatty acid profiles of steers consuming 60% dried distillers grains with solubles (DDGS) diets with or without haylage and monensin in Exp. 2

	0% Haylage		10% Haylage			*P*-value		
Item[1]	0 mg of monensin/ kg of diet	33 mg of monensin/ kg of diet	0 mg of monensin/ kg of diet	33 mg of monensin/ kg of diet	SE	Monensin	Haylage	M × H[2]
Acetate,[3] m*M*								
0 h[4]	27.83	25.22	25.26	28.54	2.35	0.84	0.82	0.39
6 h	42.71	39.67	44.40	40.34	2.94	0.15	0.62	0.47
Propionate,[3] m*M*								
0 h	25.73[a]	20.96[ab]	14.35[c]	15.15[bc]	3.24	0.33	<0.01	<0.01
6 h	45.38[a]	44.60[a]	35.27[b]	30.41[b]	4.01	0.36	<0.01	<0.01
Isobutyrate, m*M*								
0 h	0.46	0.42	0.50	0.51	0.06	0.77	0.16	0.48
6 h	0.46	0.35	0.46	0.53	0.09	0.80	0.23	0.43
Butyrate,[3] m*M*								
0 h	5.34	4.63	5.02	4.96	0.89	0.49	1.00	0.83
6 h	9.13[b]	8.24[b]	12.12[a]	8.97[b]	1.23	0.05	0.07	0.04
Isovalerate,[3] m*M*								
0 h	0.84	0.60	0.72	0.83	0.19	0.61	0.66	0.48
6 h	0.76	0.36	0.70	0.67	0.22	0.22	0.45	0.33
Valerate,[3] m*M*								
0 h	1.79[b]	1.25[a]	1.22[a]	1.17[a]	0.28	0.10	0.02	0.01
6 h	3.62	3.19	3.33	3.19	0.38	0.33	0.62	0.69
Total VFA,[3] m*M*								
0 h	62.03[a]	53.12[ab]	47.01[b]	51.21[b]	5.55	0.53	0.03	0.05
6 h	102.43	96.45	96.31	84.27	6.90	0.11	0.10	0.16
A:P[5]								
0 h	1.22[b]	1.36[b]	2.08[a]	2.06[a]	0.21	0.61	<0.01	<0.01
6 h	0.94[b]	0.93[b]	1.29[a]	1.35[a]	0.18	0.74	<0.01	<0.01
Total lactate,[3] m*M*								
0 h	0.82	0.84	1.10	1.08	0.14	0.48	0.23	0.43
6 h	1.35	0.94	1.35	1.42	0.20	0.09	0.70	0.34

[a–c]Within a row, means without a common superscript differ ($P < 0.05$).

[1]n = 8 animals per treatment.

[2]M × H = monensin (Rumensin 80, Elanco Animal Health, Greenfield, IN) × haylage interaction.

[3]$P < 0.05$ for effect of time.

[4]Time postfeeding.

[5]Acetate:propionate.

(Møller et al., 1997). Wilson et al. (1975) found that the mean rumen lactic acid concentration from healthy cattle was 1.33 m*M*, and in cattle diagnosed with sudden death from lactic acidosis, the concentration was 3.67 m*M*. Huntington et al. (1981) found that wethers consuming 85% concentrate diets had a total lactic acid concentration between 6.85 and 11.29 m*M*. We conclude, based on the reduced total lactate found in the present experiment, that the acidity measured in DDGS was largely responsible for the low rumen pH seen when these 60% DDGS diets were fed. This acidity likely came from the H_2SO_4 used in ethanol processing.

Conclusions

Dried distillers grains with solubles can be included in cattle diets at 60% of the ration DM, but cattle feed intake and growth rate was improved with added forage in the diet. When fed with haylage, monensin increased final BW and ADG; however, when monensin was fed to steers that did not receive an additional 10% haylage, there was no effect on final BW or ADG. There were no effects of monensin or haylage supplementation on serum ceruloplasmin or plasma mineral concentrations. The substantial reduction in H_2S concentration from 1.5 to 9 h postfeeding attributable to monensin supplementation suggests a possible efficacy for PEM risk management. Cattle may experience very low rumen pH when increased DDGS diets are fed; however, low pH is not caused by lactic acid production during fermentation, but likely by the H_2SO_4 used to control fermentation during ethanol production. The effects of this acid load on ruminal and animal function may be substantial.

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