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Sulfur Intake, Excretion, and Ruminal Hydrogen Sulfide Concentrations in Lambs Fed Increasing Concentrations of Distillers Dried Grains with Solubles

B. W. Neville[1,2], G. P. Lardy[1], K. K. Karges[3], L.A. Kirschten[1], and C. S. Schauer[4]

[1] *Department of Animal Sciences; North Dakota State University; Fargo, ND 58108*

[2] *Corresponding author: bryan.neville@ndsu.edu; Present address: Central Grasslands Research Extension Center, 4824 48th Ave SE, Streeter, ND, 58483*

[3] *Dakota Gold Research Association, Sioux Falls, SD 57104*

[4] *Hettinger Research Extension Center, North Dakota State University, Hettinger, ND 58639*

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Summary

The objective of this research was to evaluate the effects of increasing dietary concentration of DDGS on S intake, excretion, and ruminal H_2S gas concentrations in lambs. Sixteen wether lambs (36.7 kg ± 2.3 kg) were utilized in a completely randomized design. Treatments were based on increasing concentrations of DDGS in the final finishing diet and included: 1) 0 percent DDGS, 2) 20 percent DDGS, 3) 40 percent DDGS, and 4) 60 percent DDGS. Ruminal H_2S concentrations were measured weekly via rumen puncture as lambs were adapted to their respective finishing diets. Feed, water, feces, and urine were collected over a 10 d collection period. Hydrogen sulfide gas concentrations did not differ ($P \geq 0.24$) until d 7 when lambs fed increasing concentrations of DDGS had a linear increase ($P = 0.009$) in ruminal H_2S concentrations. Linear increases ($P < 0.001$) in ruminal H_2S concentrations were also observed on d 14, d 28, and d 35 in lambs fed increasing concentrations of DDGS. Dietary DDGS inclusion did not affect DMI (1.37 ± 0.07 kg·hd^{-1}·d^{-1}; $P = 0.25$). Sulfur intake from feed and water, as well as S excretion in feces and urine increased linearly ($P \leq 0.009$) with increasing DDGS inclusion. Sulfur retention increased linearly ($P = 0.02$) with increasing inclusion of DDGS, although this does not reflect losses due to H_2S. Increasing concentration of DDGS in the diet did not result in the occurrence of PEM. This research suggests that lambs excrete substantial amounts of S from DDGS and that water intake and urinary output increase with increasing S intake.

Key Words: Distillers Dried Grains with Solubles, Lambs, Polioencephalomalacia, Sulfur, Water Intake

Introduction

Feeding increased concentrations of distillers dried grains with solubles (DDGS) to ruminants has been avoided, due to risks of S toxicity and concerns about animal performance. High S diets can cause polioencephalomalacia (PEM) in ruminants (Gould, 1998). However, research has demonstrated that lambs fed 60 percent DDGS did not develop PEM (Neville et al., 2010a) and performed similarly to those fed lesser concentrations of DDGS (Schauer et al., 2008). The data reported by Schauer et al. (2008) and Neville et al. (2010a) provide an opportunity for increased utilization of DDGS in lamb-finishing rations. However, this research stands in contrast to other findings in lambs (Low et al., 1996) and beef cattle (Zinn et al., 1999; Lamm et al., 2010), which characterize dietary S as a primary cause of PEM. The recommendations outlined by NRC (2005) list 0.3 percent S as the maximum tolerable level for ruminants consuming high-concentrate diets. Elucidating the mechanism by which lambs fed 0.7 percent S did not develop PEM (Neville et al., 2010a) is important to the livestock industry and could potentially increase the utilization of DDGS in lamb finishing rations.

Feed and water are the two sources of dietary S. Sulfur is primarily excreted as sulfate in the urine or as organic S in feces (Underwood and Suttle, 1999) or eructated as hydrogen sulfide (H_2S; Dougherty et al., 1965). Research exploring how animals adapt to excess S concentrations is limited in the literature and additional research is warranted.

We hypothesized that feeding increased concentrations of DDGS would alter intake and excretion patterns in lambs. Further, we hypothesized that feeding increased concentrations of DDGS would increase ruminal H_2S concentrations. The objective of this study was to evaluate the effects of increasing dietary concentration of DDGS on S intake, excretion, and ruminal H_2S gas concentrations in lambs.

Materials and Methods

All animal care and handling procedures were approved by the North Dakota State University Animal Care and Use Committee prior to the initiation of the research.

Animals and Treatments.

Sixteen western, white-faced Rambouillet wether lambs (36.7 kg ± 2.3 kg) were utilized in a completely random design to evaluate the effects of increasing dietary concentration of DDGS on S intake, excretion, and ruminal H_2S gas concentrations in lambs. Treatments were based on increasing concentrations of DDGS in the final finishing diet and included: 1) 0 percent DDGS, 2) 20 percent DDGS, 3) 40 percent DDGS, and 4) 60 percent DDGS. Distillers dried grains with solubles contained 27.45 percent CP, 33.65 percent NDF, 7.57 percent ADF, 0.10 percent Ca, 1.03 percent P, and 0.97 percent S. Lambs were vaccinated for clostridial disease (Covexin 8, Schering-Plough, Kenilworth, N.J.) two weeks prior to weaning, at weaning, and again at the initiation of the study. Additionally, lambs were treated for coccidiosis beginning at weaning for 10 d with Corid (9.6 percent Amprolium, Merial, Ltd., Duluth, Ga.). The diets fed from weaning to the initiation of the study are presented in Table 1 and did not contain DDGS. Treatment diets were formulated to meet or exceed CP requirements; NE was formulated for a 40 kg lamb gaining 400 g/d (NRC, 2007; Table 2). The dietary treatments were formulated to provide minimum Ca to P ratio of 1.5:1, with copper sulfate (0.002 percent, DM basis) and ammonium chloride (0.5 percent, DM basis) added to all diets to aid in the prevention of copper deficiency and urinary calculi, respectively. Thiamine was included in all diets to provide 150 mg/lamb daily using a predicted DMI of 1.36 kg.

Ruminal Hydrogen Sulfide Sampling

Ruminal H_2S gas concentrations were measured weekly via rumen puncture, as lambs were adapted from a medium-concentrate diet to their respective high-concentrate finishing rations. On d 0, lambs began the dietary adaptation period, which increased the concentrate portion of the diet to 85 percent over 28 d (Table 3). Hydrogen sulfide measurements were collected on d -7, d 0, d 7, d 14, d 21, d 28, and d 35 of the adaptation period. Ruminal fluid was also collected via rumenocentesis at the same time ruminal gas-cap samples were collected. Ruminal pH was determined immediately with a combination electrode (model 2000 pH/temperature meter; VWR Scientific Products, West Chester, Pa.). Ruminal H_2S and fluid samples were collected 4 h after feed was offered.

Procedures for ruminal gas-cap sampling were adapted from those of Gould et al. (1997). In order to obtain ruminal gas-cap samples, wool was shorn from a 15 cm by 15 cm area of the animal's left side immediately posterior to the 13th rib. Shearing was done with surgical clippers with care taken to remove all wool. After shearing, this area was scrubbed and disinfected with alternating isopropyl alcohol and Betadine scrubs. In order to accomplish multiple samples while maintaining the integrity of the rumen gas, two separate portions of the sampling apparatus were developed (Neville et al., 2010a). The first portion included the 7.6 cm 12-gauge needle, which was connected to a 20-cm (4.75 mm diam.) tubing (Tygon ®, S-50-HL Class VI) via a Luer-lock connection. The second portion of the sampling apparatus included a 140 mL catheter-tip

Table 1. Diets fed to lambs prior to initiation of research diets (%, DM basis).

Ingredient	Weaning	2 wk Post-Wean	4 wk Post-Wean	6 wk Post-Wean
Creep Pellet[1]	100	50	25	--
Alfalfa	--	15	20	20
Dry Rolled Corn	--	20	30	50
Barley	--	15	25	30

[1] Creep pellet contained: 16% CP, 3.5% crude fat, 12% crude fiber, 1% Ca, 0.55% P, 0.5% salt, 0.2 mg/kg Se, 5,730 IU/kg vitamin A, 573 IU/kg vitamin D, 22 IU/kg vitamin E, and 50g/ton chlortetracycline.

Table 2. Ingredient and nutritional composition of lamb diets.

Item	Diet[1]			
	0% DDGS	20% DDGS	40% DDGS	60% DDGS
	DM basis			
Ingredient, %				
Alfalfa Hay	15.00	15.00	15.00	15.00
Dry Rolled Corn	81.38	61.38	41.38	21.38
DDGS[2]	0.00	20.00	40.00	60.00
Ammonium Chloride	0.5	0.5	0.5	0.5
Limestone	2.25	2.25	2.25	2.25
Lasalocid[3]	0.085	0.085	0.085	0.085
TM package[4]	0.78	0.78	0.78	0.78
Copper Sulfate	0.002	0.002	0.002	0.002
Thiamine	0.011	0.011	0.011	0.011
Nutrient composition (analyzed)				
CP, %	14.0	19.4	22.0	24.7
NDF, %	23.7	27.6	30.6	31.8
ADF, %	10.1	11.0	11.1	11.5
S, %	0.22	0.52	0.70	0.84
Ca, %	1.72	1.64	1.35	1.16
P, %	0.50	0.65	0.77	0.81
Cu, mg/kg	19	19	15	17
Zn, mg/kg	59	95	90	73
Thiamine[5], mg/kg	70.8	67.2	55.5	51.5

[1] Diets were balanced to meet or exceed requirements set by (NRC, 2007). Treatments based on distillers dried grains with solubles inclusion: 1) 0% DDGS, 2) 20% DDGS), 3) 40% DDGS, 4) 60%DDGS.
[2] Distillers dried grains with solubles.
[3] Lasalocid (Bovatec 68, Alpharma Inc., Fort Lee, NJ).
[4] Trace Mineral (TM) package contained: 11.7% Ca, 10.0% P, 14% salt, 0.1% K, 0.1% Mg, 20 mg/kg Co, 100 mg/kg I, 2,450 mg/kg Mn, 50 mg/kg Se, 2,700 mg/kg Zn, 661,500 IU/kg Vitamin A, 66,150 IU/kg Vitamin D3, and 1,320 IU/kg Vitamin E.
[5] Formulated based on estimated feed intake of 1.36 $kg \cdot hd^{-1} \cdot d^{-1}$, with a target of 150 mg/lamb daily intake of thiamine.

syringe (Monoject, Sherwood Medical, Ballymoney, N. Ireland), which was connected to an 8-cm (4.75 mm diam.) portion of tubing via Luer-lock connection. The two portions were then connected or disconnected through Luer-lock connections with ratchet tubing clamps utilized on both sides of the Luer-lock connectors. After the needle was introduced through the skin and into the rumen gas cap, a 120 mL sample (approximately) of ruminal gas was drawn into the syringe. The first of two syringes was then disconnected and a second filled in the same manner. Hydrogen sulfide gas detector tubes (Gastec©, Kanagawa, Japan) were connected to a volumetric, gas-sampling pump and a volume (100 mL) was drawn through the detector tube to acquire a measurement of ruminal gas-cap H_2S. At each sampling point duplicate measurements were taken from each lamb, and the average of the two samples was used for any calculations. If the detector tube failed to reach 100 ppm H_2S (the lowest detectable concentration recommended by the manufacturer) the reading was recorded as a zero and 'zero' used for all mean calculations. Following gas and fluid sampling, the needle was removed, and the sampling site was sprayed with a 10-percent iodine solution. Lambs were then given injections of penicillin (3 mL/d; Pro-Pen-G, Bimeda Inc., LeSueur, Minn.) for three consecutive d following sampling to prevent peritonitis. Ruminal H_2S concentrations were converted from parts per million to grams per cubic meter H_2S through the following equation: $H_2S\ (g/m^3) = [(H_2S\ (ppm) \times 139.06)/1000000]$ assuming standard temperature and pressure values (Neville et al., 2010a).

Sulfur Intake and Excretion

On d 35, lambs were placed into metabolism crates and adapted to the crates for 10 d. Following adaptation, lambs were fitted with fecal-collection bags. Samples of feed, water, feces, and urine were collected over a 10 d period at 0700 each d. Feed intake was recorded daily, with daily adjustments made to target ad libitum intake (10-percent feed remaining). Ort samples were collected, weighed, and dried before being composited on an equal weight basis (10 g/d) within lamb for laboratory analysis. Water was provided twice daily. Water intake was calculated by subtracting any unconsumed water measured (volume) from water offered. Daily water samples were collected and frozen (-20°C) before being composited for laboratory analysis of water sulfates. Water was analyzed for sulfate (93 mg/L) by a commercial laboratory (Stearns DHIA, Sauk Centre, Minn.). Fecal bags were emptied daily, total feces weighed and 10-percent wet weight added to a composite sample, which was frozen (-20°C) for later analysis. Plastic buckets (3.78 L) were placed beneath false-bottom metabolism crates to facilitate collection of urine. Urine buckets were acidified with 150 mL hydrochloric acid (50 percent w/v) to inhibit microbial growth and prevent volatilization. Urine output was filtered through four layers of cheesecloth before volume (mL) and weight (g) were recorded; a 10 percent subsample of urine weight was composited and frozen for later analysis.

Laboratory Analysis

Feed and ort samples were dried using a forced-air oven (55°C; The Grieve Corporation, Round Lake, Il.) for 48 h. Dried samples were ground using a Wiley Mill (Arthur H. Thomas Co., Philadelphia, Pa.) to pass a 2 mm screen. Feed samples were analyzed for DM, ash, N, P; and Ca, Cu, and Zn (methods 934.01, 942.05, 2001.11, 965.17; and 968.08 respectively; AOAC, 2010). Concentrations of NDF (Van Soest et al., 1991; as modified by Ankom Tech-

Table 3. Adaptation diets (%, DM basis) fed to lambs on d 0 - 28.

	Diet				
	Step 1	Step 2	Step 3	Step 4	Step 5
	d				
Ingredient	0	7	14	21	28
0% DDGS					
Alfalfa Hay	46	46	35	25	15.0
Dry Rolled Corn	50.4	50.4	61.4	71.4	81.4
DDGS[1]	0	0	0	0	0
Supplement[2]	3.6	3.6	3.6	3.6	3.6
S (%, DM basis)	0.24	0.23	0.25	0.20	0.22
20% DDGS					
Alfalfa Hay	46	46	35	25	15
Dry Rolled Corn	50.4	45.4	51.4	56.4	61.4
DDGS[1]	0	5	10	15	20
Supplement[2]	3.6	3.6	3.6	3.6	3.6
S (%, DM basis)	0.31	0.35	0.41	0.39	0.52
40% DDGS					
Alfalfa Hay	46	46	35	25	15
Dry Rolled Corn	50.4	40.4	41.4	41.4	41.4
DDGS[1]	0	10	20	30	40
Supplement[2]	3.6	3.6	3.6	3.6	3.6
S (%, DM basis)	0.39	0.45	0.60	0.59	0.70
60% DDGS					
Alfalfa Hay	46	46	35	25	15
Dry Rolled Corn	50.4	35.4	31.4	26.4	21.4
DDGS[1]	0	15	30	45	60
Supplement[2]	3.6	3.6	3.6	3.6	3.6
S (%, DM basis)	0.41	0.60	0.69	0.70	0.84

[1] Distillers dried grains with solubles.
[2] Supplement contained (% total diet): 0.5% ammonium chloride, 2.25% limestone, 0.085% Lasalocid (Bovatec 68), 0.78% trace mineral, 0.002% copper sulfate, and 150 mg·hd^{-1}·d^{-1} thiamine.

nology, Fairport, N.Y.) and ADF (Goering and Van Soest, 1970, as modified by Ankom Technology, Fairport, N.Y.) were determined using an Ankom 200 Fiber Analyzer (Ankom Technology, Fairport, N.Y.)) without sodium sulfite, with amylase, and without ash corrections as sequentials. Sulfur and thiamine were analyzed by Inductively Coupled Argon Plasma and AOAC procedure 942.23/HPLC, respectively, by a commercial laboratory (Midwest Laboratories, Omaha, Neb.).

Statistical Analysis

Hydrogen sulfide gas and pH data were analyzed utilizing the repeated measures analysis in the Mixed Procedures of SAS (SAS Inst. Inc., Cary, N.C.) with *P*-values ≤ 0.05 considered significant. Treatment, day, and the treatment by day interaction were evaluated. The covariate structure used was autoregressive [AR(1)]. Other structures were tested; however autoregressive was the best fit based on fit statistics. Sulfur intake and excretion data were analyzed as a completely randomized design using the Mixed procedures of SAS with lamb serving as the experimental unit. The model included treatment. Linear and quadratic contrasts were used to evaluate the effect of increasing concentration of DDGS inclusion. Significance was declared at $P \leq 0.05$.

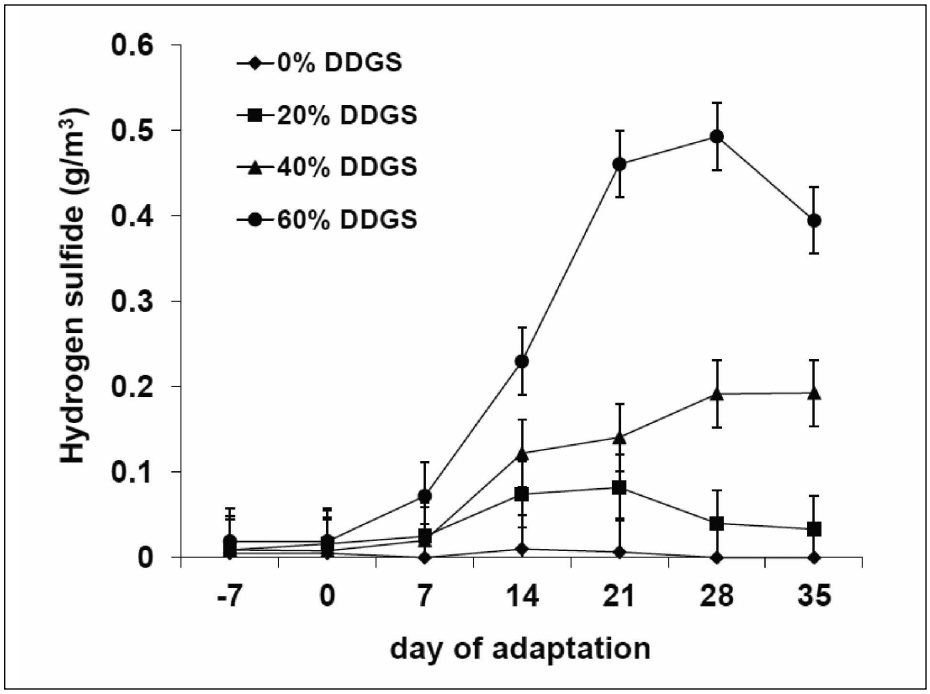
Results and Discussion

Ruminal pH and Hydrogen Sulfide Concentration

Hydrogen sulfide gas concentration was affected by treatment, day, and a day by treatment interaction ($P < 0.001$; Figure 1). Hydrogen sulfide gas concentrations did not differ ($P \geq 0.24$) until d 7 when lambs fed increasing concentrations of DDGS had a linear increase ($P = 0.009$) in ruminal H_2S concentrations. Linear increases ($P < 0.001$) in ruminal H_2S concentrations were also observed on d 14, d 28, and d 35 in lambs fed increasing concentrations of DDGS. A quadratic increase ($P < 0.001$) in ruminal H_2S concentration was observed on d 21. Ruminal pH (data not shown) was not affected by a day x treatment interaction ($P = 0.65$) or by treatment ($P = 0.32$), but decreased ($P < 0.001$) across the adaptation phase from 5.82 (d -7) to 5.33 (d 35).

Lambs fed 60-percent DDGS in the present study had ruminal H_2S concentrations nearly half of those reported by Neville et al. (2010a) in finishing lambs fed diets similar in dietary and nutrient composition. Water sulfate concentrations were 74 mg/L and 93 mg/L for Neville et al. (2010a) and the present study, respectively, so it is unlikely differences in water sulfate contributed greatly to the differences between the studies. Dietary S concentrations for the 60-percent DDGS treatment in the two studies were 0.71 percent and 0.84 percent S for Neville et al. (2010a) and the present study, respectively. Given that dietary S concentrations (from feed and water) as well as feeding regimen and dietary adaptation were similar for both studies, the differences in H_2S concentrations between the two studies may be a result of differences in sulfate reducing bacteria population in the rumen. Another potential explanation could be differences in form of S in the diet. While we did not measure the various forms of S (amino acids, sulfate, etc.) for each diet, differences in S form may be occurring. Further, the current study also shows decreases in ruminal pH coincide with increasing ruminal H_2S concentrations, which supports previous research. Gould (1998) suggested that sulfide in rumen fluid, ruminal fluid pH, frequency of eructation, and absorption of sulfide through

Figure 1. Influence of increasing concentrations (g/m^3) of distillers dried grains with solubles DDGS on ruminal H_2S concentrations in lambs. *P*-values for effect of treatment (*P < 0.001*), day (*P < 0.001*), and treatment by day interaction (*P < 0.001*). Treatment diets were based on increasing the concentration of DDGS (0, 20, 40, or 60% of dietary dry matter). Concentrations of H_2S gas measured via rumenocentesis in H_2S detector tubes (Gastec, Kanagawa, Japan).



the rumen mucosa may explain differences in ruminal H_2S concentrations.

Sulfur Intake and Excretion

In our study, level of dietary DDGS inclusion did not affect DMI (1.37 ± 0.07 kg·hd^{-1}·d^{-1}; *P* = 0.25; Table 4). Zinn et al. (1997) reported that increasing levels of ammonium sulfate affected DMI and ADG in feedlot cattle. Kandylis (1984) also reported a number of studies in both beef cattle and lambs that demonstrated DMI was reduced when feeding 0.3 percent to 1.2 percent dietary S from either inorganic and organic sources. Qi et al. (1993) reported that DMI of growing goats peaked when dietary S was 0.2 percent. The present study contradicts these findings in that increasing S from DDGS did not result in decreased intake when dietary S exceeded 0.22 percent. However, the source of S (calcium sulfate vs. DDGS), as well as the range of S concentration evaluated, likely influenced these findings and explain in part differences between Qi et al. (1993) and the present study. Another possible explanation for these discrepancies is differences in dietary ingredients. Most importantly, Qi et al. (1993) included 1.5 percent urea-N which resulted in a N:S ratio of 10:1. This is the recommended ratio for lambs (NRC, 2007). The 10:1 ratio is recommended to ensure enough S is present to allow for microbial production of S-amino acids when urea-N is included in the diet. In the present study, our N:S ratios were 10:1, 6:1, 5:1, and 4.7:1 for the 0, 20, 40, and 60 percent DDGS diets, respectively. Zinn et al. (1997) also indicated differences in ruminal and total tract availability of S may influence animal performance. The present study, along with results of Schauer et al. (2008), indicate diets which include up to 60-percent DDGS (percent, DM basis) do not result in reduced DMI or growth performance in growing and finishing lambs. Decreases in ruminal and intestinal motility (Bird, 1972; Kandylis, 1984) could explain the decreased DMI observed with increasing DDGS inclusion in other studies. Loneragan et al. (2001) hypothesized that either decreased gut motility or hepatic injury may reduce animal performance. Liver function was not assessed in the present study; therefore it is possible that liver metabolism could have been impacted. Data from a concurrent project (Neville et al., 2010b) found no liver abscess in steers fed increasing concentrations of DDGS (dietary S levels > 0.6 percent S). However, presence of liver abscesses may be dependent on rate of dietary adaptation and use of antimicrobial compounds such as tylosin (Nagaraja and Chengappa, 1998; Vasconcelos and Galyean, 2008) and should not be viewed entirely as an indicator of liver function.

Sulfur intake from feed and water, as well as S excretion in feces and urine increased linearly (*P* ≤ *0.009*) with increasing DDGS in the diet. Lambs fed 60 percent DDGS had water intakes 54 percent greater than those fed no DDGS (*P < 0.01*). Increased water intake resulted in an increase of 3-fold in urine volume and a 4.8-fold increase in urinary S excretion (*P < 0.01*) compared to lambs fed no DDGS. Multiple factors could be responsible for the increased water intake, including sulfur, sodium, or nitrogen content of the DDGS. Given the water intake and urine output data, ad libitum access to low-sulfate water may be key to increasing S tolerance when high amounts of DDGS are fed to growing and finishing lambs. Sulfur is primarily excreted as sulfate in the urine or as organic S in feces (Underwood and Suttle, 1999). Sulfur retention increased linearly (*P = 0.004*) with increasing inclusion of DDGS in finishing diets. Actual S balance is not reported as the total volume of eructated H_2S gas was not measured. Digestibility of S did not differ (*P = 0.62*) with S digestibility equaling 44.6 percent, 46.1 percent, 36.8 percent, and 45.0 percent for 0 percent, 20 percent, 40 percent, and 60 percent DDGS diets, respectively. As stated earlier, we did not measure the volume of gas eructated, but it is likely that substantial amounts of S were also excreted via eructation. Further research is needed to quantify S excretion via H_2S gas by eructation. The present study serves as another example of the need to quantify H_2S lost via eructation, and more importantly, with respect to H_2S toxicity, it underscores the need to quantify H_2S inhalation after eructation.

To our knowledge there are no published reports which detail or quantify the various forms of S contained within

Table 4. Intake, excretion, and sulfur balance of lambs fed increasing concentrations of distillers dried grains with solubles.

	Treatment[1]						*P*-Value[3]	
Item	0% DDGS	20% DDGS	40% DDGS	60% DDGS	SEM[2]	*P*-value	Linear	Quadratic
Intake								
Feed, kg	1.3	1.5	1.4	1.3	0.07	0.25	0.68	0.06
Water, L	3.1	3.5	3.7	4.8	0.28	0.006	<0.001	0.31
Excretion								
Fecal, kg	0.20	0.23	0.27	0.25	0.02	0.17	0.06	0.33
Urine, L	0.59	0.85	1.1	2.4	0.3	0.008	0.002	0.12
DMD, %[4]	84.73	84.40	81.31	80.69	1.0	0.03	0.005	0.88
Sulfur								
Intake								
Feed, mg	2,487.5	6,076.2	7,429.4	9,029.6	816.6	<0.001	<0.001	0.25
Water, mg	94.8	109.4	115.7	148.9	8.7	0.006	0.001	0.31
Total S, mg	2,582.4	6,185.6	7,545.1	9,178.4	815.8	<0.001	<0.001	0.25
Excretion								
Feces, mg	761.4	947.6	1,112.1	1,130.5	90.6	0.05	0.009	0.37
Urine, mg	674.9	2,370.8	3,236.0	3,945.1	268.8	<0.001	<0.001	0.09
Total S, mg	1,436.3	3,318.4	4,348.0	5,075.6	344.5	<0.001	<0.001	0.12
Retention, mg	1,146.1	2,867.2	3,197.1	4,102.8	568.0	0.02	0.004	0.49

[1] DDGS = Distillers dried grains with solubles.
[2] n = 4.
[3] *P*-value for linear and quadratic effects of increasing concentration of DDGS in diet.
[4] DMD = Dry matter digestibility.

DDGS. Quantifying proportions of the various forms of S will undoubtedly add to the current literature and assist in determination of mechanisms of S toxicity in the ruminant animal. Additionally, determining how digestibility or availability of S in its various forms influences S reduction and creation of H_2S gas within the rumen will further aid in the understanding of S-toxicity mechanisms.

Kandylis (1984) reported that H_2S present in the rumen may cause neurological or respiratory distress. As in our previous research (Neville et al., 2010a), we did not observe any outward clinical signs of PEM. Hydrogen sulfide is reported in two forms in the literature either concentration of H_2S in rumen gasses, or production of H_2S from ruminal fluid, as in *in vitro* studies. The present study reports H_2S in terms of concentration. The values for S retention in the present study give some indication of the quantity of H_2S excreted by the animal. However, it should be noted that H_2S data and S-metabolism data were collected at different time points, and changes in either aspect could alter interpretation. These data do not account for the use of S in production of wool, muscle tissue, or other protein (S-amino acid) production, which also impacts calculations of S retention.

Conclusions

Increasing concentration of DDGS in the diet increased S intake, excretion, and H_2S gas concentrations but did not result in any clinical signs of PEM. However, the length of the feeding study may not have been great enough to allow for PEM to occur. Additionally, the inclusion of thiamine in the treatment diets may have prevented the occurrence of PEM. The research reported here indicates S excretion increased with increasing dietary S concentrations and, in part, explains why S toxicity did not occur even though dietary S concentrations were well in excess of the NRC maximum tolerable level for ruminants. Continued efforts to quantify H_2S production will add to the body of knowledge regarding S metabolism and excretion. The present study, along with previous research at our institution, has demonstrated that feeding up to 60-percent, dietary DDGS concentrations is possible without affecting lamb health or performance. Defining the role of various S sources as determining factors in S tolerance is needed. Accounting for digestibility or availability of various S sources will facilitate a more appropriate definition of both maximum tolerable and toxic levels of S in future recommendations.

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