Influence of thiamin supplementation on hydrogen sulfide gas concentrations in ruminants fed high sulfur diets

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The objective of this research was to evaluate the influence of thiamin supplementation on hydrogen sulfide gas concentration and ruminal pH in lambs fed high sulfur diets. Moderate levels of thiamin supplementation seem to decrease hydrogen sulfide concentrations. Our data suggests that changes in ruminal hydrogen sulfide concentration cannot be attributed solely to ruminal pH, and are likely affected by multiple factors which interact within the ruminal environment and in the animal.

Summary
The objective of this study was to evaluate the effect of increasing level of thiamin supplementation on ruminal gas cap hydrogen sulfide (H_2S) concentration and pH in lambs. Twenty crossbred lambs (84.5 ± 7.0 lb) were adapted over 28 d to a finishing diet consisting of (DM basis) 60% distillers dried grains with solubles, 21.4% corn, 15% alfalfa hay, and 3.6% supplement. Treatments diets differed in the amount of supplemental thiamin supplied; diets were formulated to provide: 1) **CON** (no supplemental thiamin), 2) **LOW** (50 mg·hd^{-1}·d^{-1} thiamin), 3) **MED** (100 mg·hd^{-1}·d^{-1} thiamin), 4) **HIGH** (150 mg·hd^{-1}·d^{-1} thiamin), or 5) **HIGH+S** (150 mg·hd^{-1}·d^{-1} thiamin with dietary S increased from 0.71% to 0.87% (DM basis) with the addition of dilute sulfuric acid to DDGS). Thiamin supplementation was based on an estimated daily DMI of 3 lb·hd^{-1}·d^{-1}. Hydrogen sulfide and rumen fluid pH were collected via rumen puncture on d -6, -3, 0, 3, 7, 10, 14, 17, 21, 24, 28, and 31. No differences in H_2S concentration ($P > 0.10$) between treatments were apparent until d 10, at which point lambs fed LOW had lower H_2S concentrations than all other treatments. Lambs fed HIGH had the greatest concentrations of H_2S on d 31 (7700 ppm H_2S; $P < 0.009$). Ruminal pH for lambs fed CON and MED were not different from d 0 throughout sampling ($P > 0.18$). Ruminal pH of LOW, HIGH, and HIGH+S groups decreased ($P < 0.03$) over time. Thiamin appears to influence ruminal H_2S concentrations, although the mechanism by which this occurred remains unknown. Changes in H_2S concentration cannot be attributed solely to ruminal pH, and are likely affected by multiple factors which interact within the ruminal environment and in the animal.

Introduction
One of the challenges with use of ethanol co-products is the potential for high dietary S levels. High S diets can cause polioencephalomalacia (**PEM**) in ruminants. Inclusion of large percentages of co-product feeds, like distillers dried grains with solubles (**DDGS**), in finishing

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rations has been avoided, in part, due to problems with PEM as well as concerns about optimal animal performance and carcass characteristics. Thiamin supplementation is one proposed method of reducing or preventing PEM in ruminant animals. The efficacy of thiamin supplementation in preventing PEM is likely impacted by the mechanisms by which PEM is caused (e.g. long-term thiamin deficiency or high hydrogen sulfide gas concentration). Further, the effect and dose of thiamin necessary to prevent such cases of PEM requires more investigation. Hydrogen sulfide gas, as previously mentioned has been implicated as a cause of PEM in ruminants. Both high sulfur feed (Niles et al., 2002) and water (Loneragan et al., 2005) sources can cause increases in H_2S production. Presently there is no published literature evaluating the effect of dietary thiamin concentrations on ruminal H_2S gas concentration. Therefore, our objective was to evaluate the effect of increasing level of thiamin supplementation on ruminal gas cap H_2S concentration and ruminal pH in lambs being adapted to a finishing diet containing 60% DDGS.

Procedures

Twenty western white-face wether lambs (84.5 ± 7.0 lb) were sampled during the adaptation period (receiving ration to a final finishing ration). Adaptation was accomplished by increasing the amount of concentrate on a weekly basis; adaptation diets are outlined in (Table 1). The final finishing diet was balanced to contain 60% DDGS (DM basis; Table 2). Treatments diets differed in the amount of supplemental thiamin supplied; diets were formulated to provide: 1) **CON** (no supplemental thiamin), 2) **LOW** (50 $mg \cdot hd^{-1} \cdot d^{-1}$ thiamin), 3) **MED** (100 $mg \cdot hd^{-1} \cdot d^{-1}$ thiamin), 4) **HIGH** (150 $mg \cdot hd^{-1} \cdot d^{-1}$ thiamin), or 5) **HIGH+S** (150 $mg \cdot hd^{-1} \cdot d^{-1}$ thiamin with dietary S increased from 0.71% to 0.87% (DM basis) with the addition of dilute sulfuric acid to DDGS). Thiamin supplementation was based on an estimated daily DMI of 3 $lb \cdot hd^{-1} \cdot d^{-1}$. Feed was offered daily on an ad libitum basis with refusals collected and weighed weekly.

Sampling for ruminal H_2S was conducted on 12 occasions beginning 6 d prior to initiation of treatment diets. Gas cap samples from these lambs were collected on d -6, -4, 0, 3, 7, 10, 14, 17, 21, 24, 28, and 31 of the feeding period. Hydrogen sulfide gas measured on H_2S detector tubes (GASTEC©, Kanagawa, Japan). Ruminal fluid was also collected at the same time for determination of rumen fluid pH.

Results

The influence of hydrogen sulfide gas on incidence of PEM in ruminants could be impacted by the way H_2S concentration changes during adaptation to finishing rations. In the present study, no differences in H_2S concentration between treatments ($P > 0.10$; Table 3) were apparent until d 10, at which point lambs fed LOW had lower H_2S concentrations than all other treatments. At this point in adaptation the amount of roughage included in the diet had not changed although the inclusion of DDGS had increased from 0 to 29% of dietary DM. Those lambs fed the HIGH treatment diet showed the most dramatic increases in ruminal H_2S concentration; on d 21 of adaptation dietary hay was decreased from 35 to 25% and DDGS increased from 40 to 50% of dietary DM. Over the course of the next 3 d ruminal H_2S concentration increased by over 3000 ppm, and within 7 d had increased by 4700 ppm H_2S.

While the hydrogen sulfide concentrations in our lambs did not reach the levels in steers reported by Niles et al. (2002), our peak concentrations were above those reported by Loneragan et al. (2005); both of these studies had steers with positive cases of PEM. These results indicate that the concentration of H_2S required to cause symptoms of PEM may vary depending on species.

Of further interest is the way H_2S concentration in lambs fed HIGH+S changed over adaptation. Specifically, on days 7, 14, and 21 the concentration of H_2S was greater in HIGH+S than HIGH; however, after 3 d of adaptation (d 10, 17, 24) the concentration of ruminal H_2S from HIGH+S was lower or equal to that found in HIGH fed lambs.

There are multiple factors that influence the conversion of dietary S into H_2S in the rumen during adaptation. Among these are decreases in ruminal fluid pH, increases in the proportion of sulfur reducing bacteria, and increases in dietary S. In our study, ruminal pH did not differ among treatments ($P = 0.13$) at any time point (data not shown). Lambs fed CON and MED were not different from d 0 throughout sampling ($P > 0.18$). However, ruminal pH of LOW, HIGH, and HIGH+S groups did decrease ($P < 0.03$) over time. Decreases in ruminal pH may also impact incidence of PEM by other means.

Our research suggests that thiamin may influence ruminal H_2S concentrations, but we did not investigate the fate of the H_2S. Further, our data suggests that changes in ruminal hydrogen sulfide concentration cannot be attributed solely to ruminal pH, and are likely affected by multiple factors which interact within the ruminal environment and in the animal.

Table 1. Adaptation diets fed to lambs (% DM Basis)

	Arrival d -6	Step 1 d 0	Step 2 d 7	Step 3 d 14	Step 4 d 21	Step 5 d 28
Ingredient, %						
Alfalfa Hay	46.00	46.00	46.00	35.00	25.00	15.00
Corn	50.38	35.88	21.38	21.38	21.38	21.38
DDGS	0.00	14.50	29.00	40.00	50.00	60.00
Supplement[1]	3.62	3.62	3.62	3.62	3.62	3.62

[1]Supplement contained: (% of total diet DM) 0.5% ammonium chloride, 2.25% limestone, 0.085% lasalocid, 0.78% trace mineral, 0.002% copper sulfate, and were formulated to provide one of four levels of thiamin (0, 50, 100, or 150 $mg \cdot hd^{-1} \cdot d^{-1}$).

Table 2. Ingredient and nutritional composition (DM basis) of final finishing rations fed to lambs

	Treatments[1]				
Item	CON	LOW	MED	HIGH	HIGH+S
Ingredient,%					
Alfalfa Hay	15.00	15.00	15.00	15.00	15.00
Corn	21.38	21.38	21.38	21.38	21.38
DDGS	60.00	60.00	60.00	60.00	60.00
Supplement[2]	3.62	3.62	3.62	3.62	3.62
Nutrient[3]					
CP, %	23.3	23.6	23.4	22.7	23.5
ADF, %	10.8	11.0	11.6	11.6	11.3
S, %	0.76	0.69	0.75	0.71	0.87
Ca, %	1.55	1.42	1.65	1.66	1.77
P, %	0.79	0.81	0.92	0.91	0.87
Thiamin[4]	0	50	100	150	150

[1] Treatments: CON (no supplemental thiamin), LOW (50 $mg \cdot hd^{-1} \cdot d^{-1}$ thiamin), MED (100 $mg \cdot hd^{-1} \cdot d^{-1}$ thiamin), HIGH (150 $mg \cdot hd^{-1} \cdot d^{-1}$ thiamin), and HIGH+S (150 $mg \cdot hd^{-1} \cdot d^{-1}$ thiamin with 0.87% S).
[2] Supplement (% total diet): 0.5% Ammonium chloride, 2.25% limestone, 0.085% Lasalocid, 0.78% Sheep Mineral 12 (Hubbard Feeds, Mankato MN), 0.002% Copper sulfate, and either 0, 0.004, 0.007, or 0.11% thiamin mononitrate.
[3] Laboratory analysis of nutrient concentration.
[4] Formulated level (ppm), thiamin inclusion in diet calculated based on an estimated DMI of 3.0 $lb \cdot hd^{-1} \cdot d^{-1}$.

Table 3. Influence of thiamin and sulfur level on hydrogen sulfide production in lambs fed a 60% DDGS based finishing diet

Treatment[1,2]				
CON	LOW	MED	HIGH	HIGH+S
0.0	0.0	0.0	190.6	75.0
66.7	0.0	112.5	25.0	28.1
71.5	0.0	146.9	71.9	93.8
531.3	375.0	310.5	737.5	475.0
778.1	575.0	759.4	1237.5	1350.0
2200.0[a]	887.5[b]	2200.0[a]	2453.1[a]	2378.1[a]
2390.6[a]	1087.5[b]	1875.0[a]	1906.3[a]	2015.6[a]
2852.6[a]	1418.8[b]	2609.4[a]	2406.3[ab]	2406.3[ab]
3312.5[a]	1531.3[c]	2328.1[abc]	1958.2[bc]	3140.6[ab]
2062.5[a]	3287.5[b]	3275.0[b]	4991.6[c]	3046.9[ab]
4687.5[a]	2662.5[b]	2906.3[b]	6657.8[c]	4390.6[a]
5687.5[a]	2650.0[b]	3843.8[c]	7701.3[d]	4859.4[ac]

[1]Treatments: CON (no supplemental thiamin), LOW (50 $mg \cdot hd^{-1} \cdot d^{-1}$ thiamin), MED (100 $mg \cdot hd^{-1} \cdot d^{-1}$ thiamin), HIGH (150 $mg \cdot hd^{-1} \cdot d^{-1}$ thiamin), and HIGH+S (150 $mg \cdot hd^{-1} \cdot d^{-1}$ thiamin with 0.87% S).
[2] When tube measurement was below 100ppm tube was considered to read 0.
[abc] Means with different superscripts within a row differ $P < 0.10$.

Literature Cited

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