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

B.W. Neville[1], C.S. Schauer[2] and G.P. Lardy[1]

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 that interact within the ruminal environment and in the animal.

Summary

The objective of this study was to evaluate the effect of increasing levels of thiamin supplementation on ruminal gas cap hydrogen sulfide (H_2S) concentration and pH in lambs. Twenty crossbred lambs (84.5 ± 7 pounds) were adapted in 28 days to a finishing diet consisting of (dry-matter [DM] basis) 60 percent dried distillers grains with solubles, 21.4 percent corn, 15 percent alfalfa hay and 3.6 percent supplement. Treatment diets differed in the amount of supplemental thiamin supplied; diets were formulated to provide: 1) CON (no supplemental thiamin), 2) LOW (50 milligrams per head per day [$mg \cdot hd^{-1} \cdot d^{-1}$] 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 sulfur [S] increased from 0.71 percent to 0.87 percent (DM basis) with the addition of dilute sulfuric acid to dried distillers grains with solubles [DDGS]). Thiamin supplementation was based on an estimated daily dry-matter intake (DMI) of 3 $lb \cdot hd^{-1} \cdot d^{-1}$.

Hydrogen sulfide and rumen fluid pH were collected via rumen puncture on day minus 6, minus 3, 0, 3, 7, 10, 14, 17, 21, 24, 28 and 31. No differences in H_2S concentration ($P > 0.10$) among treatments were apparent until day 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 day 31 (7,700 parts per million [ppm] H_2S; $P < 0.009$). Ruminal pH for lambs fed CON and MED were not different from day 0 throughout sampling ($P > 0.18$). Ruminal pH of LOW, HIGH and HIGH+S groups decreased ($P < 0.03$) through 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 likely are affected by multiple factors that 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, such as dried distillers grains with solubles (DDGS), in finishing 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 likely is impacted by the mechanisms by which PEM is caused (for example, long-term thiamin deficiency or high hydrogen sulfide gas concentration). Further, the

*Partial support for this research and dried distillers grains with solubles were provided by Poet Nutrition, Sioux Falls, S.D. Disclaimer: Any opinions, findings, conclusions or recommendations expressed in this publication are those of the author(s) and do not necessarily reflect the view of Poet Nutrition.

[1]North Dakota State University, Department of Animal Sciences, Fargo, N.D.

[2]Hettinger Research Extension Center, Hettinger, N.D.

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. No published literature that evaluates the effect of dietary thiamin concentrations on ruminal H_2S gas concentration is available. 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 percent DDGS.

Procedures

Twenty western white-face wether lambs (84.5 ± 7 pounds) 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 percent DDGS (DM basis; Table 2). Treatment 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 percent to 0.87 percent [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 six days prior to initiation of treatment diets. Gas cap samples from these lambs were collected on day minus 6, minus 3, 0, 3, 7, 10, 14, 17, 21, 24, 28 and 31 of the feeding period. Hydrogen sulfide gas was measured on H_2S detector tubes (GASTEC©, Kanagawa, Japan). Ruminal fluid also was collected at the same time for determination of rumen fluid pH.

Results and Discussion

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 among treatments ($P > 0.10$; Table 3) were apparent until day 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

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

	Arrival day -6	Step 1 day 0	Step 2 day 7	Step 3 day 14	Step 4 day 21	Step 5 day 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 and 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, Minn.), 0.002% copper sulfate and 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 concentration 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	1,237.5	1,350.0
2,200.0[a]	887.5[b]	2,200.0[a]	2,453.1[a]	2,378.1[a]
2,390.6[a]	1,087.5[b]	1,875.0[a]	1,906.3[a]	2,015.6[a]
2,852.6[a]	1,418.8[b]	2,609.4[a]	2,406.3[ab]	2,406.3[ab]
3,312.5[a]	1,531.3[c]	2,328.1[abc]	1,958.2[bc]	3,140.6[ab]
2,062.5[a]	3,287.5[b]	3,275.0[b]	4,991.6[c]	3,046.9[ab]
4,687.5[a]	2,662.5[b]	2,906.3[b]	6,657.8[c]	4,390.6[a]
5,687.5[a]	2,650.0[b]	3,843.8[c]	7,701.3[d]	4,859.4[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 100 ppm, tube was considered to read 0.
[abc] Means with different superscripts within a row differ $P < 0.10$.

although the inclusion of DDGS had increased from 0 percent to 29 percent of dietary DM. Those lambs fed the HIGH treatment diet showed the most dramatic increases in ruminal H_2S concentration; on day 21 of adaptation, dietary hay was decreased from 35 percent to 25 percent and DDGS increased from 40 percent to 50 percent of dietary DM. During the course of the next three days, ruminal H_2S concentration increased by more than 3,000 ppm and within seven days had increased by 4,700 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 the H_2S concentration in lambs fed HIGH+S changed during adaptation. Specifically, on days 7, 14 and 21, the concentration of H_2S was greater in HIGH+S than HIGH; however, after three days of adaptation (days 10, 17, 24) the concentration of ruminal H_2S from HIGH+S was lower or equal to that found in HIGH fed lambs.

Multiple factors 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 day 0 throughout sampling ($P > 0.18$). However, ruminal pH of LOW, HIGH and HIGH+S groups did decrease ($P < 0.03$) through time. Decreases in ruminal pH also may 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 likely are affected by multiple factors that interact within the ruminal environment and in the animal.

Literature Cited

Loneragan, G, D. Gould, J. Wagner, F. Garry and M. Thoren. 2005. The magnitude and patterns of ruminal hydrogen sulfide production, blood thiamin concentration, and mean pulmonary arterial pressure in feedlot steers consuming water of different sulfate concentrations. The Bovine Practitioner. 39:16-22.

Niles, G.A., S. Morgan, W.C. Edwards and D. Lalman. 2002. Effects of dietary sulfur concentrations on the incidence and pathology of polioencephalomalacia in weaned beef calves. Vet. Human Toxicol. 44(2):70-72.