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Jhones O. Sarturi
University of Nebraska-Lincoln

Terry J. Klopfenstein
University of Nebraska-Lincoln, tklopfenstein1@unl.edu

Kelsey M. Rolfe Rolfe
University of Nebraska-Lincoln, krolfe2@unl.edu

Crystal C. D. Buckner
University of Nebraska-Lincoln, cbuckner2@unl.edu

Matt K. Luebbe
University of Nebraska-Lincoln, mluebbe2@unl.edu

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Jhones O. Sarturi
Galen E. Erickson
Terry J. Klopfenstein
Kelsey M. Rolfe
Crystal C. D. Buckner
Matthew K. Luebbe[1]

Summary

The relationship between ruminal degradable sulfur intake (RDSI) and ruminal hydrogen sulfide concentration ([H_2S]) as well as ruminal parameters were evaluated. Steers were fed diets containing organic, inorganic, and wet distiller grains with solubles (WDGS) sources of sulfur, as well as a control diet. A laboratory procedure was developed to measure RDS of ingredients. RDSI explained 65% of [H_2S] variation, whereas total sulfur intake and ruminal pH, individually, explained 29 and 12%, respectively. Availability of sulfur for ruminal reduction is more important than total sulfur in the diet.

Introduction

Sulfur (S) availability for ruminal fermentation can be variable depending on degradability in the rumen. Variation in ruminal hydrogen sulfide gas concentration ([H_2S]) may be better predicted by measuring ruminal degradable sulfur intake (RDSI) instead of only total S intake. Therefore, the objectives of this study are: 1) determine the relationship between RDSI and [H_2S], as well as other ruminal parameters; and 2) develop a laboratory procedure to measure ruminal S degradability.

Procedure

Diets, Feeding and Experimental Design

Five ruminally cannulated crossbred beef steers (1,209 ± 102 lb BW) were assigned randomly to one of the five treatments in a 5x5 Latin square design. Steers were fed once daily for *ad libitum* intake through five periods of 21 days each. Each of the five periods consisted of a 14-day adaptation to the diet followed by a 7 day collection period. Diets (Table 1) were formulated to provide: organic source of S (S amino acids from corn gluten meal) at two levels of inclusion; inorganic source of S (ammonium sulfate), as well as a control diet (dry-rolled corn base). A diet containing wet distillers with solubles (WDGS) was also used since this co-product contains both organic and inorganic sources of S.

Ruminal Degradable Sulfur (RDS) Coefficients

Initially, RDS of the diets were estimated (calculated) based on two assumptions: 1) inorganic sources of S are 100% available for ruminal reduction to sulfide; 2) organic sources of S (S amino acids) are available for ruminal fermentation similar to protein that is ruminally degraded (DIP). These generalizations do not account for the inorganic and organic sources of S that are incorporated into the bacterial mass, and are not available to be reduced to sulfide by sulfate-reducing bacteria, since the bacterial CP leaves the rumen. Other sources of S present in feedstuffs with unknown degradability characteristics, such as sulfolipids, glutathione, β-thioglucose, succinyl-CoA, and CoA, are considered 100% available for ruminal reduction. To measure degradability coefficients of S, an IVDMD study was performed. Ingredients (1.5 g of DM), were incubated (26 hours) in triplicate with 75 mL of ruminal fluid collected from heifers (n = 2; BW = 705 lb; fed 60% corn based diet) and 75 mL of McDougall's Buffer. After incubation, bottles were cooled in ice, centrifuged (18,623 x *g*; 20 min; 4ºC), decanted, and the precipitate was dried at 100ºC and analyzed for S. The RDS (% of DM) coefficients were obtained by the following equation:

RDS = {1 – [(g of S in the residue – g of S in the blank)/g of S in the original sample]}*100

Measured RDS coefficients from ingredients utilized in this study were used to adjust values of RDSI, and this correction is noted in the results by the word "measured."

Measurements and Statistical Analysis

Intakes were calculated based on DM offered after subtracting DM refused. Intake pattern was measured electronically since bunks were equipped with weigh cells coupled to a computer. On day 15, pH probes were calibrated to record ruminal pH each minute and were introduced through the cannula into the rumen, then removed on day 1 of the following period. Ruminal gas samples were collected on the last three days of each period, twice daily (8 and 13 hours post feeding), except for the first period when samples were collected on the last five days. A pipette was inserted through the ruminal cannula (cannula cap adapted to avoid gas exchanges during collection) and ruminal [H_2S] analyzed with a spectrophotometer. On day 21, ruminal fluid was collected through a manual vacuum pump at 9, 14, and 22 hours post feeding and frozen immediately to determine VFA molar proportions. Data were analyzed using the GLIMMIX procedures of SAS (SAS Inst., Inc., Cary, N.C.). Day was accounted as a repeated measure for ruminal pH, intake and [H_2S], as well as time for VFA data. Stepwise multiple regression analysis were performed to determine the effect of RDSI, total S intake, and ruminal pH measurements on ruminal [H_2S].

(Continued on next page)

Results

Intake, expressed as lb/day or % BW, was not different among treatments. However, steers fed inorganic S tended ($P = 0.12$) to decrease intake by 12% (Table 2). Greatest and least dietary total S and RDS ranged between 0.21 and 0.50, and 0.15 and 0.32% of DM, for Control and WDGS, respectively (Table 1). Ingredient RDS coefficient estimates from the *in vitro* study were predicted from DIP (% of CP). The DIP values were 50.7, 4.3, 30.2, and 45.0 for dry rolled corn (DRC), corn silage (CS), corn gluten meal (CGM), and WDGS, respectively. As a percentage of total S, RDS coefficient estimates were 45.0, 78.0, 40.0, and 70.8 for DRC, CS, CGM, and WDGS, respectively. Total S intake followed diet S concentrations (Table 1), being greater ($P < 0.01$) for steers fed WDGS followed by organic high, inorganic and organic low (not different), and the least for control diet. Calculated and measured RDSI were greater ($P < 0.01$) for steers fed WDGS followed by inorganic, organic high, organic low, and control diets (Table 2). Number of meals was not affected ($P = 0.23$) by sources of S. However, steers fed WDGS and inorganic diets spent 13% more time eating ($P < 0.01$) compared to other treatments. As DMI was not different, these two diets provided smaller rates of intake compared to other treatments (Table 2). Therefore, intake pattern appears to be related with RDSI, since rate of intake was slowed down when greater RDSI was observed.

There was an interaction ($P = 0.05$) between dietary treatment and time point of ruminal gas collection (Figure 1). Regardless of time of gas collection, similar [H_2S] was observed for steers fed inorganic and WDGS diets ($P = 0.28$), which were greater ($P \leq 0.05$) than other treatments. Greater [H_2S] at 8 hours post feeding compared to 13 hours was observed for steers fed organic high, organic low, and control diets ($P \leq 0.04$), regardless of dietary treatment. Greater RDSI for inorganic and

Table 1. Dietary treatments and nutrient composition of diets containing inorganic and organic sources of sulfur.

Ingredients, % DM	Control[1]	Inorg.	Org. High	Org. Low	WDGS
Dry-rolled corn	75.0	75.0	51.7	65.2	30.0
Corn silage	15.0	15.0	15.0	15.0	15.0
Corn gluten meal	—	—	23.3	9.8	—
WDGS	—	—	—	—	50.0
Molasses	5.0	5.0	5.0	5.0	—
Supplement[2]	5.0	5.0	5.0	5.0	5.0
Nutrient composition, % DM					
CP	12.5	12.5	23.9	15.1	19.5
Fat	3.9	3.9	3.5	3.8	7.6
NDF	14.5	14.5	14.6	14.6	22.9
Total sulfur offered	0.20	0.35	0.45	0.30	0.50
Total sulfur corrected for orts[3]	0.21	0.36	0.45	0.30	0.50
RDS (calculated)[4]	0.16	0.31	0.25	0.19	0.35
RDS (measured)[4]	0.15	0.30	0.21	0.17	0.32

[1]Treatment and S source: control – no extra S added; inorganic – extra S from ammonium sulfate; organic high and low – extra S from corn gluten meal; WDGS – no extra S.
[2]Supplements: Supplements were formulated to provide 30 g/ton of DM of Monensin, 90 mg/steer/day of Tylosin, and 150 mg/steer/day of Thiamine; control and inorganic had 27.3 and 17.7% urea, respectively; inorganic had 21.9% of ammonium sulfate.
[3]Corrected for orts – amount refused (orts) subtracted from amount offered. This correction was made only for total S, since orts were not analyzed for S degradability.
[4]RDS – ruminal degradable S: calculated denotes estimated based on DIP of ingredients, and measured denotes correction based on measured coefficients (*in vitro* study) of S degradability.

Table 2. Intake and intake pattern, ruminal pH and VFA profile from steers fed diets containing inorganic and organic sources of sulfur.

Variables	Control[1]	Inorg.	Org. High	Org. Low	WDGS	SEM	*P*-values Treat
			Intake				
DMI, lb/day	24.3	21.4	24.5	24.8	23.5	2.22	0.12
DMI, % BW	2.01	1.81	2.02	2.04	1.94	0.13	0.22
S intake, g/day	22.2^{d}	37.8^{c}	48.7^{b}	33.7^{c}	55.9^{a}	3.62	< 0.01
RDS intake[2] (calculated), g/day	16.4^{e}	32.3^{b}	26.8^{c}	20.7^{d}	38.6^{a}	2.46	< 0.01
RDS intake[2] (measured), g/day	15.6^{e}	31.5^{b}	22.9^{c}	18.6^{d}	36.2^{a}	2.88	< 0.01
			Intake pattern				
Time eating, hours/day	9.6^{cd}	10.5^{ab}	9.9^{bc}	8.9^{d}	11.2^{a}	0.84	< 0.01
Number of meals, n/d	5.3	5.4	4.8	4.6	5.2	0.37	0.23
Rate of intake, %/ hours	11.9^{b}	11.3^{c}	12.7^{a}	13.1^{a}	10.7^{d}	1.45	< 0.01
			Ruminal pH				
Average	5.65^{a}	5.30^{b}	5.46^{b}	5.71^{a}	5.67^{a}	0.07	< 0.01
Variance	0.30^{a}	0.21^{b}	0.22^{b}	0.28^{a}	0.25^{ab}	0.02	0.05
Area < 5.6, min*pH/day	184^{c}	461^{a}	293^{b}	150^{c}	116c	60	< 0.01
			Volatile fatty acids, mMol/100 mMol of total VFA				
Acetate	49.1^{ab}	46.1^{c}	51.0^{a}	48.0^{bc}	50.0^{ab}	1.33	0.01
Propionate	28.0^{b}	35.1^{a}	29.2	30.9^{b}	30.5^{b}	1.75	0.02
A:P ratio	1.87^{a}	1.34^{b}	1.78^{a}	1.75^{a}	1.74^{a}	0.15	0.02
Butyrate	17.4	14.7	13.4	15.1	13.8	1.61	0.14
Total, mMol/mL	131.5^{a}	133.8^{a}	120.9^{bc}	119.2^{c}	130.6^{ab}	5.87	0.06

[1]Treatment and S source: control — no extra S added; inorganic — extra S from ammonium sulfate; organic high and low — extra S from corn gluten meal; WDGS — no extra S.
[2]Calculated — denotes ruminal degradable S intake (RDSI) estimated based on DIP of ingredients; measured — denotes RDSI corrected for S degradability coefficients measured (*in vitro* study) from ingredients.

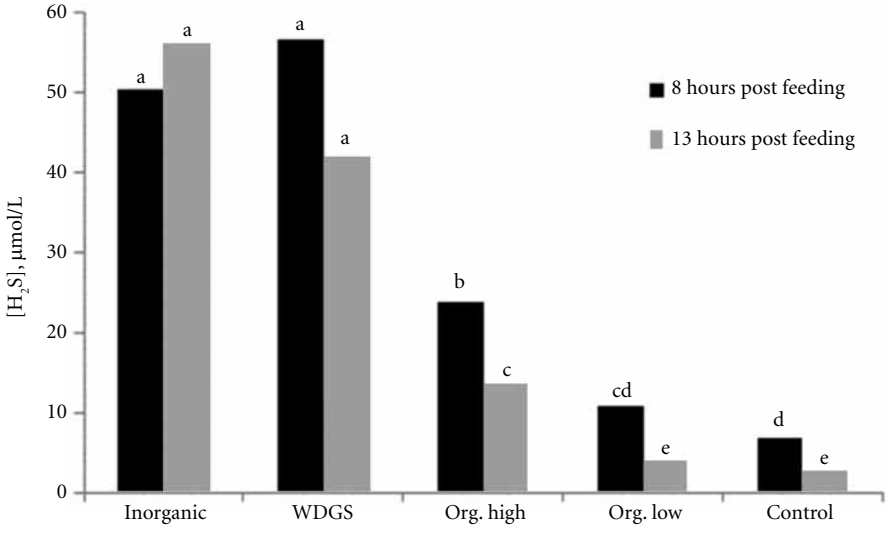

Figure 1. Ruminal hydrogen sulfide gas concentration ([H_2S]), µmol/L.

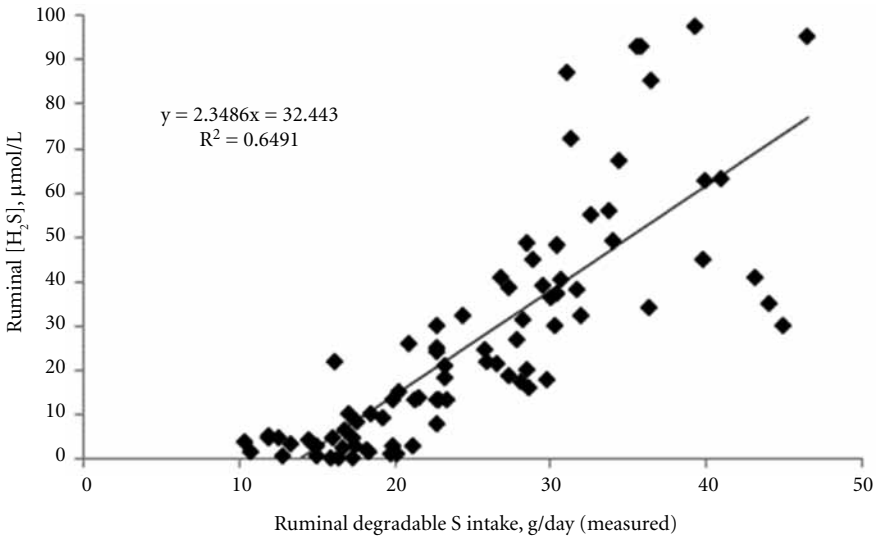

Figure 2. Regression between ruminal hydrogen sulfide gas concentration ([H_2S]) and ruminal degradable S intake (RDSI). Measuared denotes RDSI corrected for S degradability coefficients measured *in vitro*. A linear relationship ($P < 0.01$) where RDSI explained 65% of [H_2S] variation (quadratic relationship; $P = 0.69$).

WDGS diets matches with greater [H_2S] observed for these two treatments. Even though organic high and WDGS diets had similar total S concentration (0.45 and 0.50%, respectively), WDGS diet provided more RDS (0.32 vs. 0.21%), and therefore more [H_2S] was observed for this treatment. The same concept can be used to explain the similar [H_2S] for steers fed inorganic and WDGS, since both diets had similar concentration of RDS, even though WDGS diet had more total S.

Approximately 65% of [H_2S] variation was explained (linear; $P < 0.01$) by RDSI (Figure 2), whereas total S intake was able to explain only 29% of the variation in [H_2S] ($P < 0.01$). Average of ruminal pH was negatively related with [H_2S], however it accounted for only 12% of [H_2S] variation (linear, $P < 0.01$).

The only difference between control and inorganic diets was the presence of ammonium sulfate added to inorganic diet supplement. Lower average ruminal pH ($P < 0.01$), greater area of pH < 5.6 ($P < 0.01$) and less pH variance ($P = 0.05$) were observed for steers fed inorganic diet compared to control. Lower acetate ($P = 0.01$), greater propionate molar proportions ($P = 0.02$), and a lower A:P ratio ($P = 0.02$) were observed for steers fed the inorganic diet compared to control. This may explain why dietary S decreased DMI in performance study (*2011 Nebraska Beef Cattle Report,* p. 62) at a greater magnitude compared with ADG, since greater propionate molar proportion supports a greater energetic value compared to acetate.

Source of S plays an important role on ruminal S utilization. Availability of S for ruminal fermentation is more important than total S in the diet, since variation in [H_2S] is better explained by RDSI than total S intake. Coefficients of RDS for individual ingredients can be well predicted by the *in vitro* procedure proposed. Ruminal [H_2S] may modulate intake pattern.

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[1]Jhones O. Sarturi, graduate student; Kelsey M. Rolfe and Crystal C. D. Buckner, research technicians; Matthew K. Luebbe, former assistant professor, University of Nebraska–Lincoln (UNL) Panhandle Research and Extension Center, Scottsbluff, Neb.; Galen E. Erickson and Terry J. Klopfenstein, professors, UNL Department of Animal Science, Lincoln, Neb.