THE INFLUENCE OF FEED AND FEEDING UPON THE RUMINAL GAS FORMATION XI. HYDROGEN SULFIDE IN THE RUNEN GAS

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XI. HYDROGEN SULFIDE IN THE RUNEN GAS

By

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(*Received, February 28, 1962*)

Introduction

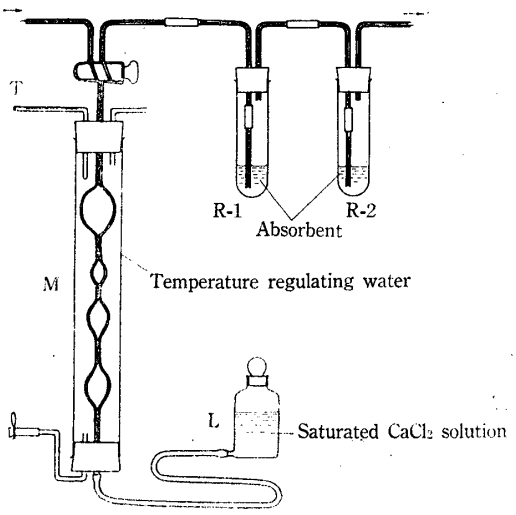
The fermentation in the rumen brings about massive quantities of gas. The main components of the rumen gas are always carbon dioxide, methane, and nitrogen with small amounts of oxygen and hydrogen. Other gases, notably hydrogen sulfide and carbon monoxide have been detected in the rumen under certain circumstances (1). Dougherty (2) noticed that hydrogen sulfide or carbon monoxide, injected into the rumen, caused a distinct paralysis of the organ when sufficient concentrations were reached. Block *et al.* (3) have demonstrated that labelled sulfate fed to the goat is rapidly incorporated into the cystine and methionine of the milk protein, but in others, Lewis (4) has proved the reduction of sulfate to hydrogen sulfide in the rumen of the sheep. Thomas *et al.* (5) reported that the normal concentration of sulfate in grass is the order of 0.3 per cent sulfate S in the dry matter, rising under certain circumstances to 1 per cent. It has been found that the juice of certain legumes, such as Ladino clover or alfalfa, administered to cattle by means of a stomach tube, can cause bloat and also prevent muscular movements, such as produce belching. Some believe that death in bloat may be due to the absorption of poisonous compounds produced in the rumen fermentation, or possibly to carbon monoxide or hydrogen sulfide, which may be formed in small amounts (6).

But, the cause of death in bloat is not definitely known. Many theories of bloat have been proposed, but none of them have been found to be fully satisfactory (7, 8). So the present work has been restricted to confirming the influence of grass feeding on the content of hydrogen sulfide in the rumen gas.

Experimental

1. Apparatus and reagents for microestimation of hydrogen sulfide.

The technique employed is essentially the same as that described by St. Lorant (9). Figure 1 shows the apparatus for measuring the volume of sample gas and the reaction tubes. The sample is aspired into the measuring tube (M) which is caliblated at 40 *ml*, 50 *ml*, 70 *ml*, and 100 *ml*. Then the known volume of the sample is expelled gently into the reaction tubes (R—1 and R—2) which contain 5 *ml* absorbing solution for hydrogen sulfide. Reagents are prepared according to Johnson and Nishita (10).



T : thermometer
M : volume measuring tube
L : leveling bottle
R : reaction tube

Fig. 1. The apparatus for the measurement of hydrogen sulfide in the rumen gas.

Standard sulfate solution. Prepare a stock solution containing about 2.44 g sodium sulfate per liter. The accurate value of sulfur concentration is determined by iodometry. Dilute the stock solution with pure water which have been deionised with ion exchange resin, as required for the working solutions of the desired concentrations.

Absorbing solution. Dissolve 50 g of zinc acetate dihydrate and 12 g of sodium acetate trihydrate in pure water. Make up to 1 liter volume and filter.

Color developer I (C-1). To 25 g of ferric ammonium sulfate add 5 *ml* of concentrated sulfuric acid and 195 *ml* of pure water.

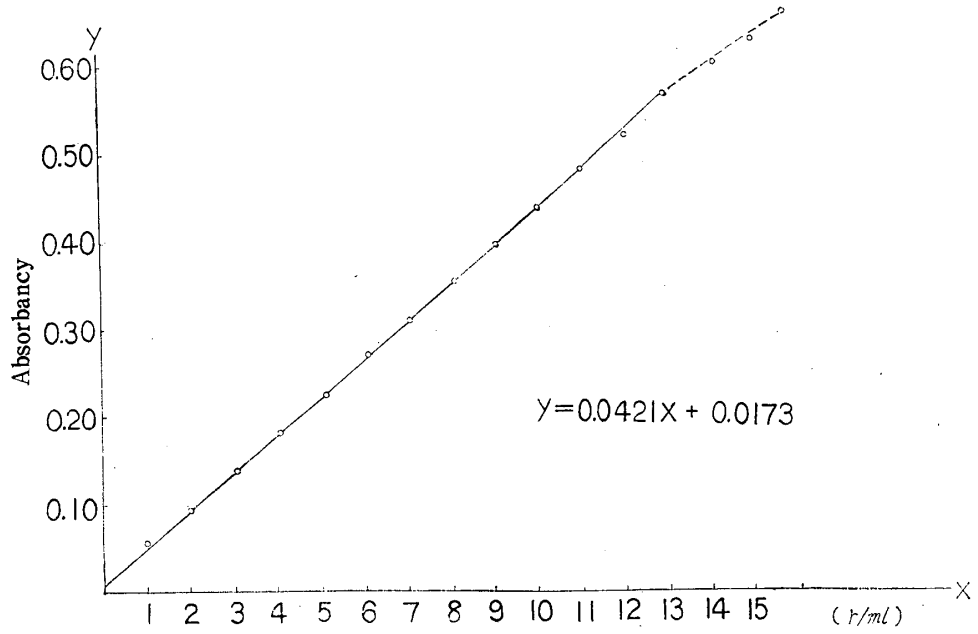
Color developer II (C-2). Add 400 *ml* of concentrated sulfric acid to 1500 *ml* of pure water. After cooling, dissolve 2 g of *p*-aminodimethylaniline sulfate and make up to 2 liter volume.

After the absorption of hydrogen sulfide, the contents of R-1 and R-2 are mixed and 1 *ml* of C-1 and 5 *ml* of C-2 are added. Stop tightly and shake the mixed solution. One hour later, the hydrogen sulfide is determined photometrically as methylene blue.

The linear relationship between the amount of hydrogen sulfide and the absorbancy of the resulting methylene blue solution were obtained in the 1 to 12γ/*ml* of sulfur. This relation is shown in Figure 2.

Volume of hydrogen sulfide can be caluculated as follows:

1 γ of S $\equiv$ 0.0007 *ml* of H_2S at Normal State



Erma Model IV, Photoelectric photometer, wave length 670 mμ

Fig. 2. Absorbancy of methylene blue solution as a function of sulfur taken through the procedure.

2. Determination of hydrogen sulfide in the rumen gas.

An intact female goat, weighing 35 kg, was fed with the diet shown in Table 1 and confined to a pen during the control period. But, beside the regular feeding time, the goat was put to leguminous grass from 9:00 a.m. to noon

Table 1. Feeding regime

Period	Time	Feeds
Experimental	9:00—12:00	Pasturing on leguminous grass
Sep. 10—Oct. 10	13:00	Wheat bran 150 g+barley 150 g+hay 300 g
1959	16:00	Hay 300 g
Control	13:00	Wheat bran 150 g+barley 150 g+hay 500 g
Oct. 11—Nov. 20	16:00	Hay 300 g
1959		

Table 2. Percentage composition of feeds used in the experiment

	Wheat bran	Barley	Hay
Moisture	15.26	14.17	6.59
Crude protein	15.61	11.08	1.75
Crude fat	4.59	2.20	2.49
Nitrogen free extract	52.30	64.67	43.04
Crude fiber	8.41	5.32	38.63
Ash	4.28	2.56	7.23

every morning and on the other hand the quantity of hay was reduced during the experimental period. Feeding regime and proximate analysis of feeds are given in Table 1 and Table 2. The goat was in good health during both periods and took the whole feeds which were given in the pen. Samples of rumen gas were collected at 8:30 a.m., 10:00 a.m., 11:30 a.m., 1:00 p.m., 1:30 p.m., 2:30 p.m., 4:00 p.m., 5:30 p.m., 6:00 p.m., 7:00 p.m., and 8:00 p.m.. But, the number of collection times per day was kept under twice and the estimation of hydrogen sulfide content at each designated time, were repeated thrice during both periods. The results are shown in Table 3.

Table 3. Hydrogen sulfide content of the rumen gas (volume per cent)

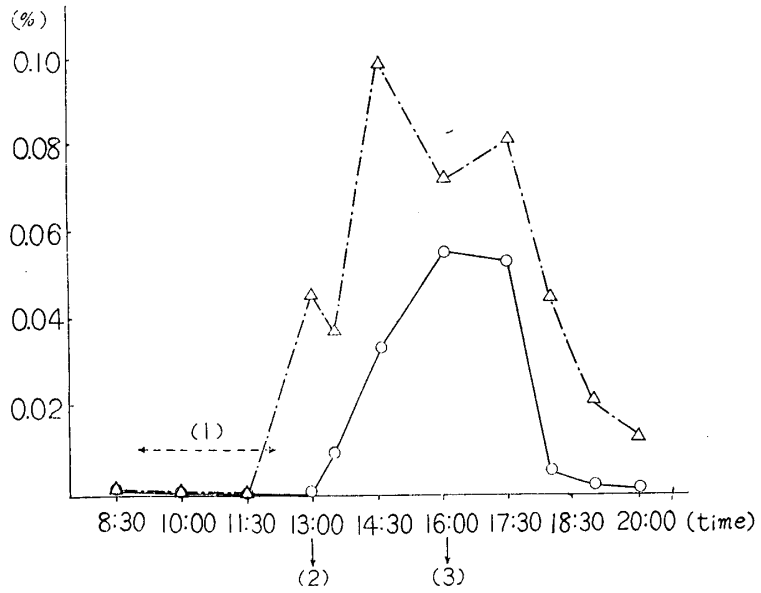
Time	Experimental	Control	Significance
8:30	0.001±0.001	0.001±0.004	−
10:00	0.001±0.000	0.000±0.000	−
11:30	0.000±0.000	0.000±0.000	−
13:00	0.046±0.040	0.000±0.000	++
13:30	0.038±0.014	0.009±0.008	++
14:30	0.100±0.048	0.033±0.018	+
16:00	0.072±0.036	0.055±0.001	−
17:30	0.082±0.012	0.053±0.003	++
18:00	0.045±0.001	0.005±0.002	++
19:00	0.021±0.023	0.002±0.002	−
20:00	0.013±0.003	0.001±0.002	++
Mean value	0.042±0.035	0.014±0.021	++

++ : Difference means significant at 5 per cent level.
+ : Difference means significant at 10 per cent level.

The mean value of 33 estimations during the experimental period was 0.042±0.035 per cent and that of the same time estimations in the control period was 0.014±0.021 per cent. The difference between the two was statistically significant. The variation of hydrogen sulfide content during the day time are summarized in Figure 3. This figure shows the effect of pasturing upon the hydrogen sulfide production in the rumen.

3. Determination of hydrogen sulfide in the rumen liquor.

In addition to the estimation of hydrogen sulfide in the rumen gas, detections of hydrogen sulfide in the rumen liquor were carried out. A fisturated goat was used for this experiment and the same dietary program as shown in Table 1 was adopted. Rumen liquor was collected through a catheter, which was inserted into the ventral of the rumen. After centrifugation of the samples for 10 minutes at the rate of 3,000 rpm, the hydrogen sulfide content in the supernatant was determined by the St. Lorant method (9). Rumen liquors were collected at 10:00 a.m., 11:30 a.m., 1:00 p.m., 2:00 p.m., and 3:30 p.m..



△—·—·△ Experimental period, ○—○ Control period
(1) Grazing time, (2) Stall-feeding time, (3) Stall-feeding time
Fig. 3. Variation of hydrogen sulfide contents in the rumen gas.

Determination of hydrogen sulfide in the rumen liquor at each designated times was repeated three times. Occasionally the rumen liquor contained a trace of hydrogen sulfide, but we were unable to measure the values photometrically during both periods.

Discussion

Olson (11) noticed that when animal bloat, there was a considerable increase in hydrogen sulfide gas, as compared to animals on dry feed. Max Kleiber *et al.* (12) measured the hydrogen sulfide content of the rumen gas of bloated and non-bloated cows on alfalfa pasture and found, on the average of 26 measurements, 0.11 ± 0.01 per cent hydrogen sulfide. But, they did not take the lapse of time after feeding into consideration when they compared the hydrogen sulfide contents of bloated and non-bloated animals. As a matter of course, hydrogen sulfide content in the rumen gas may vary with the lapse of time after feeding. In our experiment, the highest peak of hydrogen sulfide content appeared at two or four hours after feeding. Comparing the experimental period with the control period, it is evident that grass feeding may promote the hydrogen sulfide production in the rumen.

It should be noticed that hydrogen sulfide is very highly toxic and the threshold limit of allowance for human health is decided under 0.002 per cent (13). Collapse, coma, and death from respiratory failure may come within a few seconds after one or two inspirations and even low concentrations may produce irritation of mucous membranes (12). This work, therefore, calls for

fresh experiments to investigate the relation between hydrogen sulfide content and bloat.

Summary

1. The variation of hydrogen sulfide contents in the rumen gas in the day time were examined. The content were very little before feeding and markedly increased after feeding, ranging from 0.000 per cent to 0.148 per cent during the experimental period (grazing in the morning and stall-feeding in the afternoon) and from 0.000 per cent to 0.062 per cent in the control period (stall-feeding, without fresh grass).

2. The mean value of 33 estimations during the experimental period was 0.042 $\pm$0.035 per cent and that of the same times estimations in the control period was 0.014$\pm$0.021 per cent. Though the contents of hydrogen sulfide in the rumen gas were always very little, it was evident that pasturing on grass promoted the production of hydrogen sulfide gas in the rumen.

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