Some Chemical and Physical Properties of a Slime from the Rumen of Cattle

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Previous work has shown the percentage of encapsulated bacteria increased when cattle were placed on a feed-lot bloat type diet (Jacobson et al., 1957) and a recent investigation has shown Streptococcus bovis and Peptostreptococcus elsdenii increases in numbers as the onset of bloat symptoms occurred in cases of feed-lot bloat (Gutierrez et al., 1959). Slime produced by ruminal microorganisms in grain bloat may increase the viscosity of the rumen fluid and thereby contribute to the entrapment of the fermentation gases in a stable foam. The froth interferes with the animal's normal belching mechanism (Dougherty, Habel, and Bond, 1958). Although bacterial polysaccharides have been suggested as possible sources of ruminal slime, quantitative data along these lines are not available. The work reported here concerns an attempt to isolate slime fractions from grain bloaters, and to give some of the main chemical and biological characteristics of the residues found.

MATERIALS AND METHODS

The methods for the maintenance and the feeding of the animals for the production of experimental bloat have been described (Gutierrez et al., 1959). All rumen samples were centrifuged for 1 hr at 5200 RCF to remove debris, protozoa, and most of the bacteria. Measurements of relative viscosity of the different samples of intact rumen fluid after centrifugation were made with an Ostwald viscosimeter at 25 C. The slime fraction from animals on the high grain diet was precipitated by the addition of two volumes of absolute ethanol to 20 ml of the centrifuged rumen fluid. The slimy fibrous residue rose to the surface of the liquid and could easily be harvested without contamination from other nonviscous precipitates which settled to the bottom. The slime mat was washed twice by suspending in distilled water, centrifuging, and discarding the supernatant. The phase microscopic examination (1455X magnification) of the freshly harvested slime showed a fine granular appearance with very few bacterial cells and no protozoa embedded in the slime. The mat was dried overnight at 60 C and weighed.

For further analysis of amino acids, carbohydrates, and nucleic acids, the dried residue was usually ground to a fine powder with a mortar and pestle. The slime fraction (100 mg) was autoclaved for 9 hr with 5 ml of 3 N H2SO4 at 120 C and 15 lb pressure. The hydrolyzate was neutralized with 2 N Ba(OH)2, centrifuged to remove the precipitate, and the supernatant evaporated at room temperature with a jet of air over the surface. The residue was taken up with 0.2 ml of water and 20 μg spotted on strips of Whatman no. 1 paper. Carbohydrates in the slime fraction were developed with butanol:acetic acid:water (40:11:19) (Lesley and Hochster, 1959) and sprayed with 1 per cent AgNO3 in acetone followed with 2 per cent NaOH in ethyl alcohol. Amino acids were analyzed by hydrolyzing 15 mg of the slime fraction in the same manner as the carbohydrate sample except that the final residue was taken up with 0.2 ml ethanol instead of water. The hydrolyzate for amino acid identification was spotted on large sheets of Whatman no. 1 paper and developed in the first direction with phenol:water (4:1) in an atmosphere of ammonia, and the organic layer of a mixture of n-butanol:acetic acid:H2O (4:1:5) was used in the second direction. In some of the experiments for the development of the amino acids, a mixture of n-butanol:methyl ethyl ketone:17 N ammonia:H2O (5:3:1:1) was substituted for phenol:water in the first direction (Wolfe, 1957).

For the chromatographic analysis of nucleic acids, 20 mg of the slime fraction were hydrolyzed in 5 ml of 2 per cent H2SO4 for 3 hr at 100 C. The hydrolyzate was evaporated to dryness and taken up with 0.5 ml ethanol and spotted on sheets of paper. The nucleic acid hydrolyzate was developed with n-butanol saturated with 10 per cent aqueous urea in the first direction followed by 5 per cent Na2HPO4 and isoamyl alcohol in equal layers (Carter, 1950). Nucleic acid derivatives and the free nitrogen bases were located by their fluorescence and ultraviolet absorption with a mineral light lamp' at 2537 Å and were identified from their RF values (Carter, 1950). Tests for deoxyribonucleic acid (DNA) were made using diphenylamine and cystine hydrochloride methods described by Dische (1930, 1944).

Residual carbohydrate in the crude slime fraction was determined by the anthrone reagent and compared colorimetrically to a glucose standard curve. Analysis

1 Ultra-Violet Products, Inc., San Gabriel, California.
of soluble polysaccharide in centrifuged rumen fluid was made after treatment with 5 per cent trichloroacetic acid by the method of Roe (1954).

Total nitrogen content of the slime fraction was obtained by Kjeldahl determinations. Ashing of the samples was made at 600 C for 2½ hr. Total moisture was determined by drying the slime fraction overnight at 60 C, followed by 5 hr at 100 C in a vacuum oven.

Total phosphorus in the slime residue was measured according to Munsey (1948).

**RESULTS**

Gram stained smears of 1:10 dilution of rumen contents removed from animals upon the onset of bloat symptoms were very similar to those reported in previous experiments (Gutierrez et al., 1959) (figure 1).

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*Figure 1.* A 1:10 dilution of rumen contents from bloated animal. Long chains of paired streptococci and other organisms. Gram stained. Original magnification 1091X.

*Figure 2.* A 1:10 dilution of rumen contents from bloated animal. Paired streptococci illustrating capsule formation. White's stain. Original magnification 1091X.

*Figure 3.* Spatula showing coiled slime fraction which was isolated from a bloating animal by the ethanol precipitation procedure.
Capsule formation of the large streptococci chains was demonstrated using the combined positive and negative staining technique of White (1947) (figure 2). The slime harvested at various times by ethanol precipitation from the rumen fluid of a bloating steer are presented in table 1. The amount of slime collected from the surface of the ethanol and rumen fluid mixture increased with the onset and severity of the bloat symptoms. The amount of residue harvested from 6 animals ranged from 4.2 to 5.5 per cent calculated on a wet weight basis. The material was extremely viscid and the fibrous-like threads could be wrapped around a spatula with ease (figure 3). A preliminary Biuret test on the dried slime gave a positive reaction, and Kjeldahl determinations for three different samples showed the slime contained between 33 and 35 per cent crude protein. After acid hydrolysis (see Materials and Methods section) and paper chromatography the following amino acids were shown to be present in significant amounts: leucine, isoleucine, phenylalanine, valine, tyrosine, proline, methionine, glycine, histidine, serine, threonine, arginine, and glutamic acid plus traces of aspartic acid.

A test for residual carbohydrate in the crude slime fraction with the anthrone reagent was positive and quantitative experiments on the slime showed the material contained 18 per cent carbohydrate (Roe, 1954). Paper chromatography of acid hydrolyzates of the slime which were sprayed with AgNO₃ and NaOH showed spots which had RF values similar to glucose and D-ribose. Using single dimension strips with NaHPO₄-isomyl alcohol solvents and AgNO₃ spray, the RF for the latter pentose was 0.87 (published value, 0.87; Carter, 1950). Chromatography of acid hydrolyzates for nucleic acid derivatives showed fluorescent and ultraviolet absorbing compounds which had RF values of thymine, cytosine, adenine, and guanine. One dimensional strips run with 5 per cent Na₂HPO₄-isomyl alcohol mixtures revealed a spot with RF similar to thymine deoxyriboside (Carter, 1950). Color tests for DNA in the slime fraction were positive. A suspension containing 500 μg per ml slime material gave a blue color with diphenylamine as described by Dische (1930) and a pink color with cysteine hydrochloride and H₂SO₄ (Dische, 1944). The ash content of the dried slime samples from different animals varied between

<table>
<thead>
<tr>
<th>Animal</th>
<th>Bloat Symptoms</th>
<th>Amount of Polysaccharide per 100 ml Rumen Fluid</th>
</tr>
</thead>
<tbody>
<tr>
<td>303</td>
<td>Severe</td>
<td>15</td>
</tr>
<tr>
<td>488</td>
<td>Severe</td>
<td>18</td>
</tr>
<tr>
<td>79</td>
<td>Severe</td>
<td>9</td>
</tr>
<tr>
<td>491</td>
<td>None</td>
<td>4.5</td>
</tr>
<tr>
<td>66</td>
<td>None</td>
<td>2.2</td>
</tr>
<tr>
<td>143</td>
<td>None</td>
<td>4.5</td>
</tr>
</tbody>
</table>

DISCUSSION

The amino acids, purine and pyrimidine bases, pentoses, and phosphorus content in the slime fraction indicated the nucleic acids and nucleoproteins produced by the microorganisms in the rumen reached a high level with the carbohydrate-rich rations which were fed in the current experiments. Significant amounts of the fibrous, viscid slime could not be harvested from animals fed hay or pasture diets. Deoxyribonucleic acid and to a lesser extent ribonucleic acid solutions have a viscous characteristic (Chargaff and Davidson,
1955) and the increase in nucleic acids found in the present experiments probably contributed to the increase in the viscosity of the rumen fluid. Increases in the viscosity of rumen fluid were well correlated with the onset of bloat symptoms. Bacterial populations have been reported to increase as bloat occurred on grain rations (Gutierrez et al., 1959), but information is lacking on the rates at which cells of the ruminal population lyse and release nucleic acids into the medium. Lysis of cells in cultures has been reported for ruminal species *Bacteroides succinogenes* (Bryant, Robinson, and Chu, 1959). The quantitative evidence at hand indicates bacterial slime production arising from nucleic acids and nucleoprotein probably plays a significant role in altering the viscosity of the rumen fluid. Glucose-containing polysaccharides may also be a contributing factor in viscosity changes.

The sequence of ruminal biochemical events which occurred in bloating animals on grain rations is not likely to be the same for animals bloating on legumes, although the general principle of foam production has been found to occur in both (Davis, Gutierrez, and Lindahl, 1960). The current findings in feed-lot bloat would indicate searches for compounds, either of plant or microbial origin, which have the capacity of increasing viscosities of fluids may be worthwhile in studies of legume bloat. There are indications that plant pectins, hemicelluloses and saponins may be implicated in pasture bloat (Conrad et al., 1959).

**SUMMARY**

The amount of slime isolated from the rumen of cattle increased with the onset and severity of symptoms of bloat. Quantitative analysis showed the slime to contain 18 per cent carbohydrate and 33 to 35 per cent crude protein. Fourteen amino acids were identified in acid hydrolyzates. Thymine, cytosine, adenine, and guanine were detected by chromatography. Correlation was not observed between amount of soluble polysaccharide and appearance of severity of bloat.

**REFERENCES**


Dische, Z. 1930 Über einige neue charakteristische Farbreaktionen der Thymonukleinsäure und eine Mikromethode zur Bestimmung derselben in tierischen Organen mit Hilfe dieser Reaktionen. Mikrochimica, 8, 4-32.


