Quin’s Oval and Other Microbiota in the Rumens of Molasses-Fed Sheep

JOHN L. VICINI,1 WILLIAM J. BRULLA,1 CARL L. DAVIS,1 AND MARVIN P. BRYANT1,2*

Departments of Animal Sciences1 and Microbiology,2 University of Illinois, Urbana, Illinois 61801

Received 26 September 1986/Accepted 10 March 1987

Two rumen-cannulated wether sheep were fed a diet containing 1 kg of a liquid-molasses mixture, 80 g of soybean oil meal, and 100 g of chopped wheat straw once a day. In 6 weeks and thereafter, the microbiota adapted such that Quin’s oval, a very large bacterium, was present in huge numbers (1.3 × 10^16 and 1.3 × 10^18 ml^-1 after 73 days). Direct microscopic counts were also done on small bacteria, moderate-sized Selenomonas spp., and small Entodinium spp., which were the only protozoa seen. After the necessary dilution of rumen contents to make the microbial cells visible, Quin’s ovals were seen to be much smaller in sheep 1 than in sheep 2. Most-probable-number estimates indicated that Methanobrevibacter spp. were present at 10^7 ml^-1, Methanosarcina spp. were present at 10^5 ml^-1, and Eubacterium limosum-like bacteria were present at 10^3 ml^-1. In the adapted sheep, the dry portion of the diet was rapidly consumed, but the molasses mixture was consumed over a 9- to 10-h period. Volatile fatty acids in the rumen were present in very low amounts just prior to feeding and were much higher during the consumption of the diet, with about a 1:1 molar ratio of propionate to acetate between 1 and 9 h after feeding. Data were obtained on hourly feed consumption, levels of volatile fatty acids, and pH. The results are discussed relative to the microbial volume in the rumen and previous results with sheep fed similar diets in Australia, where Methanosarcina sp. was a dominant bacterium, and in Illinois, where E. limosum was the dominant rumen bacterium.

In a previous study in Australia in which sheep were fed a mainly liquid-molasses diet, large numbers (6 × 10^9/ml, direct count) of Methanosarcina spp. were found in the rumen (13). A large amount of CO₂ was produced from [14C]acetate in the rumen of these animals, and acetate was by far the major volatile fatty acid (VFA) found. In an attempt to repeat these results in Illinois, Gentner et al. (2) found little if any Methanosarcina spp. Instead, Eubacterium limosum, which utilizes various sugars, amino acids, lactate, methanol, H₂CO₃, and CO as energy sources, was found as the dominant microbe. Products in the rumen reflected this finding in that acetate, large amounts of butyrate, and 2-methyl butyrate, but very little propionate, were present. Because of the vast differences in microbiota found in the above two studies, a third study with the same diet is reported here, with the main difference being that a third lot of sugarcane molasses and a third pair of wether sheep were used. Huge volumes of the very large bacterium, Quin’s oval (1, 6, 8, 12, 15), were found. The major VFA found in the rumen were propionate and acetate, with smaller amounts of butyrate.

MATERIALS AND METHODS

Two rumen-cannulated wether sheep weighing about 40 kg were fed a diet containing 1 kg of a liquid-molasses mixture, 80 g of soybean oil meal, and 100 g of chopped wheat straw once a day (2, 13). The molasses mixture contained 41.3 g of blackstrap (sugarcane) molasses, 1.36 g of urea, 230 g of trace mineral salts, 340 g of dicalcium phosphate, 230 g of NaCl, and 1 kg of added water. Rumen contents were sampled from four sites to obtain as representative a composite sample as possible 6 h after feed was first offered. About weekly, wet mounts of samples, diluted 10-fold, were studied with the phase-contrast microscope and with the regular light microscope, with Gram iodine solution added. After the microbiota stabilized, quantitative cell counts of the microbiota were done with samples fixed and suitably diluted in the methyl green-Formalin-saline solution of Ogimoto and Imai (7). The Petroff-Hauser counting chamber was used to count bacteria, and the Levy chamber was used to count ciliate protozoa.

Well after the microbiota stabilized with huge numbers of Quin’s ovals, feed intake was measured, and rumen samples were analyzed for pH and VFA. These samples were taken at 0 h (24 h) and hourly through 11 h after the sheep were fed. Quantitative three-tube, most-probable-number (MPN) estimates were done for methanol- and H₂CO₃-using bacteria with the anaerobic methods and culture media described by Gentner et al. (2). VFA, pH, methane, and H₂ analyses were also those of Gentner et al. (2).

RESULTS AND DISCUSSION

Adaptation to the diet. The sheep readily adapted to the molasses diet from an alfalfa hay diet and consumed the dry portions within the first 15 to 20 min. The molasses mixture was completely consumed within 8 to 11 h. The sheep always appeared to be healthy and vigorous. Tenfold dilutions of rumen fluid from day 16 onward, from samples collected 6 h after feeding, were examined with the microscope. Dasytricha spp. and relatively large ophyro sclocids, but no Ophyrosclex spp. or Epidinium spp., were observed only in the day 16 samples. Relatively small Entodinium spp. persisted throughout the study and were quite dominant among the ciliates in the day 16 samples. Flagellates, mainly zoospores of anaerobic fungi, persisted through the day 22 samples but were not seen thereafter. Various morphotypes of small bacteria persisted throughout the study, and these included relatively small numbers of Treponema spp., mainly the large species reported to catabolize pectin (9, 16). Chain-forming, lancet, gram-positive streptococci or Methanobrevibacter spp. were seen only in
TABLE 1. Direct microscopic counts and calculated microbial volume for the microbiota in the 6-h rumen samples after sheep were fed the molasses diet for 73 days

<table>
<thead>
<tr>
<th>Microbe</th>
<th>Sheep 1</th>
<th>Sheep 2</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No. of bacteria ml⁻¹</td>
<td>Individual cell vol (µm³)</td>
</tr>
<tr>
<td>Quin's oval</td>
<td>1.11 ± 3.2 x 10¹⁰</td>
<td>28⁴</td>
</tr>
<tr>
<td>Selenomonas spp.</td>
<td>2.4 ± 0.8 x 10¹⁰</td>
<td>5⁴</td>
</tr>
<tr>
<td>Small bacteria</td>
<td>8.3 ± 3.6 x 10¹⁰</td>
<td>1⁴</td>
</tr>
<tr>
<td>Total bacteria</td>
<td>22.8 ± 10¹⁰</td>
<td>1.0</td>
</tr>
<tr>
<td>Entodinium spp.</td>
<td>1.0 ± 0.4 x 10⁵</td>
<td>10⁴</td>
</tr>
</tbody>
</table>

* Assuming the shape of Quin’s oval is an oblate spheroid. In the fixed and diluted samples from sheep 1, Quin’s ovals were 2.1 to 2.5 by 3.5 to 6.5 µm, with a mean of 2.35 by 4.82 µm. In samples from sheep 2, they were 2.9 to 3.5 by 5.3 to 6.5 µm, with a mean of 3.14 by 5.85 µm.

Selenomonas spp. included only those of moderate size.

Based on a cylinder 1.26 by 4 µm.

See reference 14.

the day 16 samples. Megasphaerlike gram-negative chain-forming cocci, often indicative of lactate as an extracellular intermediate, were present in relatively large numbers in the day 16 and day 22 samples and were last seen in the day 29 samples. Relatively large numbers of moderate-sized selenomonads, about 1 to 1.5 by 3 to 5 µm, persisted throughout the study.

Large numbers of large selenomonads (6, 7, 11), about 2.5 to 3.5 by 5 to 8 µm, with tumbling motility and usually filled with glycogenlike material, persisted as the bulk of the microbial volume through day 29. These organisms were completely replaced by Quin’s ovals as by far the dominant bacteria by day 46. The latter were first seen in the day 29 samples from both sheep. Most of the Quin’s ovals exhibited tumbling motility, were gram negative, were highly refractile under phase-contrast microscopy, and were filled with glycogenlike material.

The rumen contents by day 46 and thereafter consisted of a thick souplike liquid, brownish rust colored, with little visible particulate material. That of sheep 2 was less thick than that of sheep 1. This difference seemed to be related to relatively fewer Quin’s ovals and more moderate-size to small bacteria in sheep 2.

An unexpected finding, first noticed after the sheep had been on the diet for 46 days, was that Quin’s ovals from sheep 2 were larger than those from sheep 1.

Numbers and estimated volumes of certain groups of microbes after adaptation. The direct microscopic counts indicated 1.9 x 10¹¹ to 2.2 x 10¹¹ total bacteria ml⁻¹ (Table 1). These numbers are extremely high, especially when the number and size of Quin’s ovals are considered. This species was eight- to ninefold more numerous, and selenomonads and small bacteria were less numerous, in sheep 1 than in sheep 2 (Fig. 1). Entodinium spp. were present at about 10⁵ ml⁻¹.

The estimated volumes of the various bacterial groups (Table 1) are obviously in error, since total microbial volume per milliliter of rumen contents can be 10¹² µm³ only if the rumen fluid is completely filled with microorganisms. If it is assumed that the estimated volumes of organisms other than Quin’s ovals are correct and that the organisms completely filled the rumen fluid 6 h after the sheep were fed, the individual mean volume of Quin’s ovals would be only 7 µm³ in sheep 1 but 49.9 µm³ in sheep 2. The latter value seems closer to reality than the former. It seems probable that the volumes of the selenomonads and small bacteria were overestimated, especially in sheep 1, and that the dilution of rumen fluid necessary to make individual cells visible caused considerable swelling of cells, again more so in the rumen fluid of sheep 1 than in that of sheep 2. This swelling might be due to a decreased osmolality in the extracellular fluid on dilution. We have considerable confidence in the enumerations if not in the estimated volumes.

The fact remains that we have never before seen rumen fluid with anywhere near the density of microbiota seen in the present study. Flagellates in the hind gut of the termite, Zootermopsis sp., have approached this density (3; M. P. Bryant, unpublished observations).

MPN estimates of methanol- and H₂-CO₂-using bacteria. After the sheep had been on the diet for 73 days, there were 8.6 x 10⁵ (sheep 1) and 8.6 x 10⁶ (sheep 2) methanol users ml⁻¹, and 2.4 x 10⁷ (sheep 1) and 4.2 x 10⁷ (sheep 2) H₂

TABLE 2. Fermentation products (corrected for controls) in methanol and H₂-CO₂ cultures used for MPN estimates and inoculated after sheep had been on the molasses diet for 73 days

<table>
<thead>
<tr>
<th>Product</th>
<th>Methanol</th>
<th>H₂-CO₂</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acetate (mM)</td>
<td>7.1 ± 1.3</td>
<td>-2.6 ± 0.9</td>
</tr>
<tr>
<td>Propionate (mM)</td>
<td>0.0 ± 0.6</td>
<td>-0.4 ± 0.1</td>
</tr>
<tr>
<td>n-Butyrate (mM)</td>
<td>8.7 ± 1.6</td>
<td>-0.4 ± 0.1</td>
</tr>
<tr>
<td>Isovalerate (mM)</td>
<td>0.1 ± 0.4</td>
<td>-0.4 ± 0.2</td>
</tr>
<tr>
<td>Valerate (mM)</td>
<td>0.5 ± 0.1</td>
<td>-0.1 ± 0.0</td>
</tr>
<tr>
<td>Methane (mM liter⁻¹)</td>
<td>5.5 ± 4.0</td>
<td>39.1 ± 2.3</td>
</tr>
</tbody>
</table>

* Cultures for MPN estimates were incubated for 2 weeks, and one tube of each dilution that was considered positive based on growth and corresponding controls and was minus methanol or with 4:1 N₂-CO₂ replacing 4:1 H₂-CO₂ was analyzed. The MPNs were 10⁻³ through 10⁻⁴ (sheep 1, methanol medium), 10⁻³ through 10⁻⁴ (sheep 2, methanol medium), and 10⁻¹ through 10⁻⁴ (both sheep, H₂-CO₂ medium). Numbers are means ± standard deviation.
FIG. 1. Phase-contrast photomicrographs of the microbiota in the rumen of two sheep. The samples were equally diluted twofold. A, Sheep 1; B, sheep 2. Bars, 10 μm.

FIG. 2. Total VFA, pH, molasses consumption, and molar percent of individual VFA in the rumen of two sheep 67 days after the molasses diet was begun. ——, Sheep 1; - - - - , sheep 2; ●, acetate; ○, propionate; □, butyrate; △, 2-methyl butyrate plus isovalerate; ▲, valerate. Each point except those for pH and molasses consumption is the mean of two subsamples of rumen contents. Molasses (■) is that consumed between h 0 and 1, h 1 and 2, h 3 and 4, etc.

The H₂ users seen in positive tubes were all of the *Methanobrevibacter* morphotype (5). The dominant morphotype in the methanol MPN estimates was that of *E. limosum*, except that most of the tubes inoculated with the 10⁻³ dilution contained clumps of the *Methanosarcina* morphotype. Normal numbers of methanol- and methylamine-using *Methanosarcina* spp. in the rumen are 10³ to 10⁴ ml⁻¹ (10).

Table 2 shows fermentation products found in some culture tubes used for MPN estimates. The standard deviations, which were large relative to the means for acetate, butyrate, and methane, found with the methanol tubes were likely due to the use of some methanol by *Methanosarcina* spp. in tubes inoculated from the 10⁻³ dilutions. Less than 0.1 nmol of methane liter⁻¹, and usually none, was found in tubes inoculated with higher dilutions. The major morphotypes correlated with fermentation products observed for both sheep for both methanol and H₂-CO₂ MPN estimates.

**Rumen pH, VFA, and molasses consumption.** The samples taken just before feeding the sheep (0 or 24 h) had the highest pH (7.2), lowest total VFA (20 mM), highest acetate/propionate molar ratio, and highest moles percent of isovalerate plus 2-methyl butyrate of any of the samples from...
either sheep. The rumen contents of both sheep showed peaks in total VFA at 1 and 5 h after feeding and minor peaks at 9 h. The higher moles percent of butyrate in sheep 1 compared with that in sheep 2, especially 6 to 10 h after feeding, seemed to be related to the 10-fold-higher counts of presumptive *E. limosum* in sheep 1.

Measurement of the number of grams of liquid molasses consumed at hourly intervals (Fig. 2) indicated that between 0 and 1 h, moderate amounts were consumed; between 1 and 2 h, none was consumed; and within 10 h, all was consumed. Peak consumption was between 3 and 7 h. Total VFA and pH seemed to be related to some extent to hourly consumption.

The reason(s) for the large numbers of Quin’s ovals found in this study, compared with the large number of methanol-using bacteria, *Methanosarcina* (13) and *E. limosum* (2), found in other studies, is not known. Possible reasons include that (i) different sheep were used, (ii) different diets could have been used during the periods before the sheep were placed on the molasses diet, (iii) different weather and different geographic areas were involved, and (iv) different lots of sugarcane molasses were used. Molasses is known to vary greatly in pectin and ester-linked methoxyl content (4). We did not save any of the molasses from this study for chemical analyses, and no analyses were done in the previous studies.

This research was completed in 1983. In a study in progress to obtain a large amount of cells to determine the phylogenetic position of Quin’s oval via 16S rRNA nucleotide sequencing, we have obtained huge numbers of Quin’s oval in a wether fed alfalfa pellets plus a large amount of molasses.

The finding in this study of Quin’s oval as the dominant microbe agrees with findings in several previous studies of the sheep rumen in which diets containing relatively large amounts of molasses, glucose, sucrose, or high-quality alfalfa were fed (1, 12, 15). These sugars plus fructose are the main sugars in molasses (4).

ACKNOWLEDGMENTS

We thank Lee Krumholz for taking the photomicrographs. This research was supported by grant 35-331 from the U.S. Department of Agriculture and by the Agricultural Experiment Station of the University of Illinois.

LITERATURE CITED