Some Factors Affecting Fermentation Capacity and Net Growth of Rumen Microorganisms

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The fermentation rate of rumen microorganisms obtained from a sheep fed on hay was determined at different hours after feeding. The highest rate of fermentation was obtained immediately after feeding whereas the lowest rate was always before feeding. The effect of adding glucose, sucrose, and starch alone or in combination with urea, sodium citrate, or succinic acid on the maximal fermentation rate was studied. In no case was the maximal fermentation rate greater than that obtained in the presence of concentrate and straw. An investigation on the effect of C/N ratios, dilution, and substrate concentration on the net growth of rumen microorganisms was undertaken. The highest net growth value was obtained with a C/N ratio of 16.5 to 17.5, which is the ratio found for the ration fed to the animal. It was possible to increase net growth approximately threefold by using the proper dilution and fivefold by using the proper feed concentration.

A method for measuring the microbial growth in the rumen was proposed by el-Shazly and Hungate (9) based on the changes in fermentation capacity taking place within a short period of incubation of rumen contents. The diaminopimelic acid in the same rumen samples was estimated. The growth measurements by the two methods were in good agreement (10).

Zaki el-Din and el-Shazly (14) produced evidence of the reliability of the method in measuring the bacterial and protozoal growth.

The present paper describes factors affecting the net growth rates of rumen microorganisms, e.g., C/N ratio, dilution, and substrate concentration.

MATERIALS AND METHODS

Animals and sampling. The experiments were carried out by using two fistulated sheep fed on berseem hay (2 kg once in the morning) or concentrate plus wheat straw in the ratio 3:1 (1 kg once in the morning). The concentrate mixture consisted of undecorticated cottonseed cake and rice bran in the ratio 1:1 plus calcium hydroxide and 1% common salt.

Collection and preparation of samples were done as described previously (14). The rate of fermentation was measured at different times after feeding on samples obtained from the sheep fed hay.

Measurements of maximal fermentation rate and net growth. Maximal fermentation rates and net growth rates of rumen microorganisms were determined by the method of el-Shazly and Hungate (9) as modified by Zaki el-Din and el-Shazly (14).

The effects of substrate concentration, sodium bicarbonate, and type of carbohydrate (glucose, sucrose, and starch, 5 to 10 g alone or together with urea, sodium citrate, and succinic acid) on maximal fermentation rate were tested.

Effect of carbon-nitrogen ratio on net growth. Rumen samples (about 200 g) obtained through the rumen fistula from a sheep fed concentrate plus straw were collected at different hours after feeding. Urea or starch were added to samples (200 g) to alter the C/N ratio, and the net growth rate was compared with that in samples without these additions.

The effect of C/N ratio on net growth was also studied on one large sample divided into several small samples (50 g) to each sample, urea or starch was added to study the whole range of C/N ratios in one experiment.

Carbon was determined by the method of Walkley (12) utilizing o-phenanthroline as indicator. Nitrogen was determined by the Kjeldahl method according to the official methods of the Association of Official Agricultural Chemists (7). In the above experiments the range of C/N ratios used was obtained by using samples collected at different times.

Effect of dilution on net growth. The effect of diluting the rumen sample with mineral solutions A and B (1) on the net growth was studied in the following experiments. The maximal fermentation rate was measured in the usual manner on a 50-g rumen sample. Similar samples were incubated for 1 hr with different volumes of the mineral solution. After 1 hr of incubation the maximal rate of fermentation was determined after making the final volume 200 ml with mineral solution.

Effect of feed concentration on net growth. The effect of adding different amounts of ground concentrate plus straw from the same ration consumed by the animal on the net growth values was tested on rumen
samples obtained before and after feeding. The optimum dilution level found from the previous experiments was employed throughout these trials. The initial maximal fermentation rate was first measured, then several subsamples (about 50 g each of rumen contents) were incubated for 1 hr with the following concentrations of the feed: 0, 5, 10, 15, 20, and 25 g. At the end of the incubation period, the feed was increased to 20 g, and the mineral solution to 200 ml. The final maximal rate of fermentation was then determined, and net growth was estimated.

RESULTS

Normal rates of fermentation. The fermentation rates vary with time after feeding. The highest rate was obtained immediately after feeding (15 to 30 min), whereas the lowest rate was found before feeding (Fig. 1).

Factors affecting maximal rates of fermentation. The effect of rumen sample concentration, feed concentration, dilution, and sodium bicarbonate concentration on the maximal rates of fermentation were studied and found similar to those found by el-Shazly and Hungate (9).

The effect of adding glucose, sucrose, and starch alone or in combination with urea, sodium citrate, or succinic acid was compared with that of adding the feed. In no case was the maximal rate of fermentation greater than that obtained in the presence of concentrate plus straw (Table 1).

Net growth rates of rumen microorganisms. Figure 2 shows typical curves of maximal fermentation rates before and after 1 hr of incubation at 39 C. From the difference of the slopes of

![Graph](https://example.com/graph1.png)  
**Fig. 1.** Fermentation rates of rumen samples collected from a fistulated sheep fed berseem hay. Different curves represent different days.

![Graph](https://example.com/graph2.png)  
**Fig. 2.** Normal and maximal rates of fermentation at two different intervals after feeding sheep on concentrate plus straw, 3:1.

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<thead>
<tr>
<th>Table 1. The effect of adding glucose, sucrose, and starch alone or in combination with urea, sodium citrate, or succinic acid on the maximal fermentation rate</th>
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* Concentration is expressed in grams per 200 ml of buffer.
the two curves the net growth was calculated. The net growth (per cent per hour) for these curves was 62 when the sample was collected 2 hr after feeding, and 17 when the sample was collected 0.5 hr after feeding.

Effect of C/N ratios on the net growth rates of rumen microorganisms. The net growth rates in the first experiment (results not reported) differed widely from one trial to another, but it was obvious that C/N ratios close to those found in the feed consumed by the animal gave highest net growth rates. This was confirmed in three experiments whereby urea or starch or both were added in different concentrations to portions of the same rumen sample, thus altering the C/N ratio. The highest net growth values were found when the C/N ratio was between 16.5 and 17.5 (Fig. 3) or close to the values of the C/N ratio in the feed the animal was consuming.

The mean value of total N of 11 rumen samples obtained from a sheep (60 kg) fed on concentrate plus straw 3:1 was 2.47 ± 0.24 g per 100 g of dry matter.

Effect of dilution on net growth rates. Dilution of the rumen sample about 3 times (150 ml of mineral solution to 50 g of rumen sample) gave the highest net growth value (Fig. 4); a higher dilution caused a slight drop in the net growth rate of rumen microorganisms.

Effect of feed concentration on net growth rates. The results (Fig. 5) indicate that 15 g of feed (concentrate plus straw, 3:1) gave the highest net growth rate (increased approximately 5 times). A higher concentration of the feed (20 or 25 g) caused a drop in the net growth rate of rumen microorganisms. In two other experiments where samples were collected after feeding and the initial maximal fermentation rates were high (of the order of 140 μliters per g per min), the addition of substrate to the fermentation media in vitro resulted in depression rather than improvement of net growth.

DISCUSSION

The fermentation rates vary with time from feeding; the highest rate was obtained immediately after feeding (Fig. 1). This was because of the readily fermentable carbohydrates present in the ration. A second smaller peak was observed about 6 to 8 hr after feeding and probably repre-
Fig. 5. Effect of concentration of feed on the net growth rate of rumen microorganisms obtained from a sheep fed concentrate plus straw in two different experiments.

Microorganisms may have a lower nitrogen content, 6.8% (5); therefore, an average microbial N of 9.32% is indicated (68 × 0.105 + 31 × 0.068 = 9.32), which yields 16.7 to 22.1 g of microbial cells/100 g of dry matter or 117 to 155 g (taking the dried rumen contents of the sheep as 700 g). The average net growth rate equals 12.6% per hr (Table 4 of reference 14); in 24 hr it amounts to 302%. The microbial cells formed in 24 hr would equal 353 to 468 g. Hungate (2) calculated a value of 175 g for sheep (40 kg) fed hay.

The results of studies to determine the effect of dilution with Hungate's mineral mixtures A and B (1) on the net growth rate indicated that a dilution of about three times that of the rumen sample (150 ml of mineral solution to 50 g of rumen sample) gave the highest net growth rate. An increase of from 2.6 to 3.4 times was obtained due to this dilution. A higher dilution caused a slight drop in the net growth rate of rumen microorganisms. The effect of dilution is probably due to reduction of concentration of end products.

When the ideal dilution was used and the substrate (concentrate plus straw, 3:1) concentration was varied, 15 g of the feed for every 50 g of the sample gave the highest growth rate of rumen microorganisms.

It was possible in this work, by adjusting C/N ratio, dilution, and substrate concentration, to increase the net growth rate in vitro about fivefold. It is therefore suggested that it might be economical to propagate rumen microbial cells in vitro under ideal conditions to obtain microbial food rich in protein of high biological value (5) from low quality roughage and concentrates, including urea and some minerals. Purser and Buechler (8) showed that bacterial and protozoal cells contain about 10% lysine, which is one of the limiting amino acids in plant proteins.

Theoretically, based on net growth rate results (Fig. 5), it is possible to produce 0.5 to 0.95 × 10^8 kg (dry weight) of microbial cells per hr from 10^8 kg (dry weight) of rumen microorganisms (which could be contributed from about 10^4 kg or more of dry rumen contents). From the results of volatile fatty acid (VFA) production (Table 3 of ref. 14) of 50 μmoles of VFA per 100 g of wet rumen contents per min, assuming ratios of acetic to propionic to butyric of the order of 70:20:10, it could be calculated that 10^4 kg of dry rumen contents (or 10^4 kg of wet rumen contents) would yield 126, 44.4, and 26.4 kg per hr of the acids, respectively, and roughly 16 kg of methane. Under conditions of maximum fermentation, the rates of acid and gas production would be accelerated (probably fivefold) and a greater yield would be indicated. It is expected that when incubation takes place for more than 1 hr the net growth rate
will decrease unless ideal conditions of dilution, feed concentration, and C/N ratio could be regulated as the process of fermentation proceeds.

LITERATURE CITED