Effects of Changes in Feed Level, Starvation, and Level of Feed After Starvation Upon the Concentration of Rumen Protozoa in the Ovine

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Four rumen fistulated sheep were used in five experiments to investigate the effect of feed level upon the concentration of rumen ciliate protozoa. The sheep were fed once daily 650 g of a pelleted diet composed of corn cobs, 45%; alfalfa meal, 35%; oats, 12.5%; cane molasses, 5%; urea, 0.4%; and vitamins and minerals, 2%. The concentration of protozoa reached minimum and maximum values at 5 and 22.5 h after feeding, respectively. Thus, to estimate apparent generation rates, concentrations of protozoa were determined at 5 and 20 h postfeeding. Apparent generation rate/h = natural log of ([concentration of protozoa at 20 h divided by concentration at 5 h] divided by the time interval, [T20 to T5]). Alteration of the feed to protozoa ratio by starvation and by changing the level of feed (200 to 900 g/day) showed that as the ratio of feed to protozoa increased, generation rate increased. Measurements of liquid turnover rates in the rumen showed that turnover rate decreased as feed level decreased. Turnover rate was near zero when the sheep were starved. Small quantities of soluble substrates, added directly to the rumen of starved sheep, maintained the protozoal population when rumen turnover was minimal. Furthermore, as rumen turnover rate increased with increased levels of feed, the effect of substrate on maintaining the protozoal population was negated. Thus, at high feed levels, turnover rate may be the dominant factor controlling the establishment and concentration of protozoa in the rumen.

Feed level has been suggested as one of the factors which influences the ciliate protozoal population in the rumen. The proportion of dividing entodinia has been observed to increase as the level of ration increased, whereas the concentration of entodinia showed no consistent relationship with feeding level (10). In comparison to full feeding, feeding at two-thirds of full feed resulted in a decreased rate of passage and increased protozoa numbers (2). Several other studies have also shown that feeding level affects the concentration of protozoa (1, 3, 4).

The present investigation was an attempt to obtain additional information on how feed level affects the concentration of protozoa in the rumen. Particular emphasis has been placed on the short-term effect, in contrast to previous studies where the responses measured have usually been induced at least a week prior to the measurements.

MATERIALS AND METHODS

Four wether lambs weighing approximately 40 kg were prepared with rumen fistulas and housed singly in wooden pens which had a common water trough. They were fed 650 g per day of the following pelleted feed: corn cobs, 45%; alfalfa meal (17% crude protein), 35%; oats, 12.6%; cane molasses, 5%; urea, 0.4%; and minerals and vitamins, 2%.

Samples of rumen contents for ciliate protozoa counts were obtained with a plastic tube through the cannula, immediately fixed in Formalin and then counted (8). Flagellate protozoa were not counted in these studies. Rumen volume and liquid turnover rate estimates were made by using polyethylene glycol (PEG), molecular weight 4,000. PEG in the rumen fluid was determined by the technique of Hyden (11). A preliminary investigation was conducted to determine the diurnal cycle of rumen protozoan concentrations after normal feeding and after 1 day without feed (starvation). The preliminary trial consisted of
collecting rumen samples from the four lambs at 0, 2, 5, 8, 11, 15, 21, 22.5, and 24 h after the regular feeding time for 2 consecutive days on two occasions 10 days apart. On both occasions the lambs were fed their regular level of feed (650 g) the first day and then starved the second day. In subsequent experiments samples of rumen contents for determining protozoan numbers were collected at 0, 5, 20, and 24 h after feeding.

In experiment one, samples were collected on four consecutive days. On day 1 all animals were fed 650 g, and on the subsequent days they were fed either 200, 400, 650, or 900 g of feed. This experiment was replicated three times with each sheep receiving a different feed level in each replicate. Experiment two was similar to experiment one with the exception that the collection period was 5 days in length as a result of adding a day of starvation between the normal intake day and the three days of feeding at the different feed levels. Experiment two was also replicated three times. Experiment three differed from experiment two only in that all animals were fed 200 g of feed on the first day after the day of starvation and then fed at the different levels of feed for the next 3 days.

Experiment four consisted of rumen volume and liquid passage rate measurements on all four sheep. Samples were collected over a 5-day period with a dose of PEG infused into the rumen, 1 h before feeding time on each day. Rumen volume estimates were made on the basis of marker concentration 1 h after dosing. Liquid rumen turnover rate was estimated from the change in marker concentration between the prefeeding and 23 h after feeding samples. On day 1 the sheep were fed 650 g of feed, whereas on day 2 they were left unfed. On days 3, 4, and 5, two sheep received 200 g of feed, whereas the other two sheep were fed 900 g.

In experiment five, feed was replaced by a 250-ml intraruminal infusion of soluble substrates. Two levels of soluble substrates were used. The low level included 20 g of starch, 10 g of glucose, and 10 g of casein, whereas the high level was 40, 20, and 10, respectively. Rumen samples were collected at 0, 2, 5, 8, 13, 22, and 24 h after the infusion. Two animals were given each level of infusion.

RESULTS AND DISCUSSION

Figure 1 shows the change in the concentration of rumen protozoa in the 24-h period after either normal feeding or starvation. The mean concentration of protozoa at 24 h after feeding in this preliminary study was 7.4 × 10⁵ per cm². All lambs contained Entodinium and Diplodinium species of protozoa, whereas Dasytricha was found in only two lambs and Isotricha in one. A typical generic distribution as calculated from 1 day during the preliminary study was 91.7% Entodinium, 7.7% Diplodinium, 1.0% Dasytricha, and 0.6% Isotricha. A definite cyclic pattern was observed in the fed animals which had its minimum and maximum at 5 and 22.5 h postfeeding, respectively. Feeding caused a rapid decrease in the concentration of protozoa during the first 5 h, probably due to dilution by feed, saliva, drinking, and passage of ingesta from the rumen. During the 5- to 22-h postfeeding period concentration of protozoa increased in the fed lambs and represents the primary period during which protozoal cell division occurred. During the last 2-h period protozoan concentrations decreased slightly. This may have resulted from substrate shortage, increased rate of passage, or some other factor. In comparison, the concentration of rumen protozoa in unfed animals showed a steady decline which by the end of one day had decreased to 1.3 × 10⁴ protozoa per cm², which was equivalent to a five-fold decrease.

From these data, the time period between 5 and 20 h postfeeding was selected for use in calculating an apparent generation rate. The term-apparent generation rate was used since it was impossible to correct for dilution associated with feeding and rate of passage from the rumen. This was calculated as follows: natural log of ([concentration of protozoa at 20 h divided by concentration at 5 h]) divided by the time interval (20 to 5 h)). The apparent genera-
tion rate for the cycle of fed animals in the preliminary trial shown in Fig. 1 was 0.0395 generations per h.

The effect of changing feed level from 650 g per day to either 200, 400, 650, or 900 g per day upon the concentration of protozoa is depicted in Fig. 2. Decreasing the feed level to 200 g per day resulted in a decreased concentration of protozoa during the first 2 days and a partial recovery of the population on the third day. The apparent generation rates calculated from the 5- and 20-h samples reflect this decrease in concentration, being −0.0117, 0.0113, and 0.0282 generations per h for the 3 days, respectively. In comparison, the change from 650 to 400 g of feed showed only a minor decrease in protozoan concentrations and apparent generation rates of 0.0128, 0.0210, and 0.0193 were obtained for the 3 days, respectively.

The generation rates for the animals fed both 200 and 400 g per day were both less than the rates observed in the animals fed 650 g per day. This suggests that generation rate is influenced by feed level, and agrees with previous observations of Warner (10) which indicated that the proportion of dividing entodinia increased with feed level. Increasing the feed level to 900 g did not increase the concentration of protozoa, which also agrees with the results reported by Warner (10). He concluded that there did not appear to by any consistent effect on protozoan concentrations with increased feed intake, especially at the higher levels of intake. Furthermore, the calculated generation rates were lower than those observed in the lambs fed 650 g. It is believed that generation rates are of more value than simple concentrations, since they reflect the rate of increase in the protozoal population at the various intake levels. On the other hand, apparent generation rates could be misleading; for example, a negative generation rate or decrease occurred on the third day of 900-g feeding. Failure of the protozoal population to increase in concentration under the influence of more feed may have been masked by an increased rate of turnover from the rumen at this higher feed level (5). In other words, the actual generation rate may have increased but is not reflected by concentrations alone, since turnover rate increased with increased feed level (Table 1). The relationship between apparent generation rate and rate of passage will be discussed later.

Experiment two was an attempt to obtain additional information relating feed level to increases in the concentrations of protozoa. This was accomplished by using the same four feed levels in conjunction with a protozoal population which was reduced in concentration; the reduced population was obtained by starving the lambs for 1 day. The effect of these same feed levels upon a smaller initial protozoal population are shown in Fig. 3. All feed levels increased the concentration of protozoa. The increase observed in the lambs fed 200 g of feed was less on all 3 days than the increases observed with the other feed levels. In addition, the apparent generation rates were lowest on the 200-g feed level. Equal concentrations of protozoa were observed after 3 days of feeding either 400, 650, or 900 g of feed. However, the apparent generation rates were highest on the 650-g feed level, being 0.0550, 0.0742, and 0.0550 generations per h for the 3 days, respectively. The increase in generation rate obtained with

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**Fig. 2.** Total protozoan numbers on the 3 days after a change in feed level from 650 g to either 200, 400, 650, or 900 g/day. The numerical values in the figure are the apparent generation rates for each feed level on each day, respectively.

**Fig. 3.** Total protozoan numbers as influenced by feed level on the 3 days after starvation. The numerical values in the figure are the apparent generation rates for each feed level on each day, respectively.
the 650-g feed level over that recorded on the 400-g level can be explained on the basis of more substrate. Failure of the 650-g feed level to result in a larger population at the end of 3 days is probably due to a faster rate of passage from the rumen. An additional increase in the rate of passage from the rumen would explain the apparent decrease in generation rate observed when feed level was increased to 900 g per day.

In comparing the generation rates of experiments one and two, several points should be considered. First, the generation rates obtained in experiment two were considerably higher. This can probably be attributed to the larger ratio of feed to protozoa; however, rate of passage of ingesta from the rumen may have been reduced from the day of starvation. Second, in general the apparent generation rates recorded on day 2 of the differential feeding periods in experiment two were higher than those from days 1 and 3. This was not expected; the largest generation rates were expected on day 1 since the population was smallest at that time. However, this unexpected result may indicate that some protozoa were being counted which were not viable and thus unable to contribute to an increase in cell division on day 1.

Experiment three incorporated 1 day of feeding 200 g of feed on the day after starvation and prior to the 3 days at different feed levels. This was an attempt to eliminate any countable nonviable protozoa. The results of experiment three are shown in Fig. 4. In general, there were no major differences between the results obtained in experiments two and three. Thus it appears that either the 1 day of 200 g feed did not eliminate countable nonviable protozoa or that factors other than this are involved. Two minor differences between experiments two and three were noted. First, the apparent generation rates were lower in this experiment and second the generation rates observed on the 900-g treatment were above that from the 650-g treatment on days 1 and 3. We have no explanation for these differences.

The effect of feed level upon apparent generation rates in these three trials are summarized by Fig. 5. The top left portion of Fig. 5 refers to the average response on all 3 days, whereas the other quadrants depict responses on days 1, 2, and 3, respectively. In general, as feed level increased to 650 g, generation rate increased, whereas the increase to 900 g resulted in a slight decrease in generation rate. Furthermore, imposing the starvation treatment prior to feeding different levels (experiments two and three) resulted in higher generation rates. Thus, this figure depicts the change in generation rate as the ratio of feed to protozoa changed. Increasing the feed to protozoa ratio (experiments two and three versus experiment one) by starvation increased the generation rate. This, along with the increase in generation rate with the different feed levels up to 650 g, suggests that feed level is a primary factor controlling protozoa concentration up until the time that feed level caused rumen turnover to increase and override the effect of increased substrate. This conclusion supports in vitro data (9) which showed increased protozoa concentrations with increases in starch levels.

Since apparent generation rates do not take into account the passage of protozoa out of the rumen with the ingesta, attempts were made to obtain rate of passage information for sheep on the different feed levels. Experimental procedures designed to measure rate of passage of
The liquid turnover rate after the normal 650-g feeding was 2.00 turnovers per day. This compared very well with other reported values (5). In comparison, the turnover rate after starvation was a \(-0.17\) per day. It is important to note that the value was calculated from samples collected 23 h apart. In starved sheep, the PEG concentration did decrease slightly for about 14 h and then during the last 9 h its concentration in the rumen fluid actually increased, resulting in a negative turnover rate. Complete interpretation of occurrences in the rumen during starvation are not obvious at this point; however, the data suggest that the liquid turnover rate is minimal or nonexistent. In reference to changes in the concentration of protozoa noted in the previous experiments, it thus appears that the decreases in the concentration of protozoa with starvation were in fact due to nutrient depletion and not rumen washout. Experiment five will deal with this further.

The liquid turnover rates observed when sheep were fed 200 or 900 g per day show that a reduced feed intake results in a reduced rumen turnover. The liquid turnover rate during the feeding of 200 g per day was approximately 0.95 turnovers per day, whereas the turnover rate in sheep fed 900 g averaged 2.14 turnovers per day. The turnover rates obtained for the sheep fed 900 g increased on each of the 3 days after 900-g feeding, and may be indicative of the fact that feed residues in the rumen were lower during days 1 and 2. These data suggest that at reduced levels of feed, rumen turnover rate is reduced and thus may be of little importance in regulating the protozoal population. Consequently, establishment of increased concentrations of protozoa should occur more readily. This agrees with previous reports (2, 3) which suggested that protozoan concentrations increased when rumen turnover rate was reduced by reduced feed intake.

The effect of nutrients upon the concentration of protozoa was further investigated by infusing small quantities of soluble substrates into the rumen of unfed sheep. It was assumed that the turnover rate in the rumen was essentially zero as suggested in experiment four. These results are shown in Table 2. Data from the preliminary trial for starved and fed animals are included for reference. During starvation without added nutrients, protozoan concentrations fell to 19% of the normal prefedding level in 24 h. In comparison, the low and high infusions of starch, glucose, and casein maintained protozoa numbers at levels equivalent to 68 and 94%, respectively, of the normal prefeding concentrations. Since 70 g of soluble substrate was able to maintain the total rumen protozoal population when turnover was minimal, it is suggested that substrate could be the major factor controlling the protozoal population at low feed levels.

### Table 1. Rumen volume and liquid turnover rate as influenced by feed level, starvation, and level of feed after starvation

| Day of expt. | Level fed | Rumen volume* (liters) | Liquid turnover rate* per day |
|--------------|-----------|------------------------|-----------------------------|
|              | Level of feed (g/day) | Level of feed (g/day) | Level of feed (g/day) |
| 1            | 650       | 0                      | 200 | 650 | 900 | 0 | 200 | 650 | 900 |
| 2            | 0         | 3.82                   | 4.39 | -0.17 | 2.00 |
| 3            | 200 and 900 | 3.26            | 4.22 | 1.03 | 1.79 |
| 4            | 200 and 900 | 2.85            | 3.91 | 0.88 | 1.97 |
| 5            | 200 and 900 | 0.86            | 2.66 |

* Rumen volume estimates were made immediately before feeding and thus are presented on the basis of the previous day's feed level.

* Liquid turnover rate estimates are based on samples collected immediately before feeding and 23 h after feeding.
The effect of feed level upon protozoan concentrations and liquid passage rates have both been determined, thus it should be possible to calculate more reliable estimate of protozoal generation rate than those based solely on protozoan concentrations at two different time intervals. The sum of the turnover rate constants for the 200 and 900 g per day feed levels obtained in experiment four (Table 1) and apparent generation rates from experiment two (Fig. 3) should give an adjusted generation rate closer to the true value for the three-day period following a day of starvation. Hungate et al. (6) have shown that species of Entodinium pass out of the rumen at a rate similar to PEG, and since 90% or more of the protozoa in the animals studied were in this genus, the use of fluid turnover rate as an estimate of protozoan passage appears warranted. The turnover rate constant is negative in sign, but this has been ignored in the present calculations since we are estimating an increase in protozoal generation rate. These adjusted generation rates are presented in Table 3. The marked effect of turnover rate on apparent generation rate at the 900-g feed level clearly emphasizes the importance of this parameter in estimating overall protozoal growth at high feed levels. In contrast, turnover rate at the low feed level was fairly constant over the 3 days, suggesting that feed level was of more importance than passage rate in controlling protozoan concentrations. The results of experiment five on the infusion of soluble substrates would appear to support this conclusion. The liquid turnover rate when all four animals were fed 650 g per day was fairly close to that obtained at the 900 g per day level (Table 1). Although low, the relative relationship between apparent generation rates would be valid when turnover rates are similar.

Using the adjusted generation rate on day 3 of the 900-g feed level, a division rate for the protozoa of once each 6.8 h is obtained. This compares quite well with a division time of 6.55 h calculated from the total turnover of rumen contents on that day (2.66 times), which assumes all of the protozoa passed out of the rumen at the same rate as PEG. These values are similar to previously reported division times (5, 6).

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