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Effects of an Abrupt Diet Change from Hay to Concentrate on Microbial Numbers and Physical Environment in the Cecum of the Pony†

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Microbial numbers, pH, fluid volume, and turnover rate in the pony cecum were measured during an abrupt change from an all-forage to an all-concentrate diet, both fed at maintenance energy levels. Concentrate feeding resulted in increased ($P < 0.01$) numbers of total viable anaerobic bacteria. The numbers of organisms growing on selective starch medium increased ($P < 0.01$) when concentrate was fed, while numbers on xylan and pectin media decreased ($P < 0.025$). Seven days after the diet change to concentrate, the number of bacteria growing on lactate medium increased ($P < 0.01$), followed by a gradual decline. Cellulolytic bacteria occurred in low numbers, ranging from 1.1×10^4 to 4.4×10^4 per g of cecal contents. Feeding all concentrate decreased both the number of genera ($P < 0.01$) and total protozoan numbers ($P < 0.01$) in the cecum. Minimum cecal pH values of 6.4 and 5.8 were obtained when forage and concentrate, respectively, were fed, with the minimum pH occurring 6 h postfeeding. Dry-matter percentage of cecal contents followed a diurnal pattern which was the inverse of the pH curve. During forage feeding, the cecum contained an average of 2.2 liters (1.6 to 3.4 liters), which turned over 3.9 times per day. When concentrate was fed, cecal volume averaged 3.9 liters (0.6 to 8.6 liters), with a mean liquid turnover of 4.2 times per day. Microbial numbers and pH changes in the pony cecum associated with an abrupt change in diet from hay to concentrate resembled those which occur in the rumen under similar feeding conditions.

Overloading with grain or abruptly changing the diet from forage to concentrate may lead to laminitis or colic in the equine. Gardner et al. (7) reported that corn starch administration to horses resulted in large increases in blood lactate within 24 h following the treatment. Willard et al. (21) observed that cecal pH was significantly lower at 4, 5, and 6 h postfeeding for horses fed the concentrate diet compared with forage-fed animals. In addition, these workers noted a decrease in the acetate/propionate ratio from about 5 while feeding hay to 2.6 during concentrate feeding. These data suggest that substantial changes may be occurring in the microbial populations of the horse large intestine when abrupt dietary changes are made, similar to those changes which occur in the rumen following a carbohydrate overload (18). The present study was undertaken to determine total viable anaerobic bacteria and amylolytic, pectinolytic, xylanolytic, lactate-utilizing, and cellulolytic bacteria in the pony cecum before, during, and following an abrupt diet change from hay to concentrate. In addition, the effects of the diet change on cecal protozoa, pH, fluid volume, and liquid turnover are reported.

MATERIALS AND METHODS

A mature Shetland pony weighing about 160 kg was fistulated in the cecum and equipped with a 30-mm-inside diameter polyvinyl chloride cannula. All diets were fed at levels calculated to provide 5,176 kcal (21,670.877 kJ) of digestible energy per day (16). Diet 1 was chopped alfalfa hay (5 cm) containing 13.5% crude protein. It was fed at a level of 2,400 g/day for 8 weeks prior to the start of the experiment. Diet 2, composed of 86.7% ground corn and 13.3% soybean meal, was fed at 1,350 g/day. Diet 3 was chopped alfalfa hay (5 cm) containing 16.75% crude protein, fed at 2,130 g/day. Water, trace mineralized salt, dicalcium phosphate, and ground limestone were provided free choice.

Sampling times have been abbreviated throughout in relation to feeding at 0800 (T_0). Thus, 0700 would be $T - 1$ and 1300 would be $T + 5$, etc. Samples of cecal contents (approximately 64 g) were taken at T_0, just prior to feeding, and again at $T + 5$, with a cutoff 15-mm-outside diameter, plastic centrifuge tube attached to a length of 2-mm-diameter steel wire. The sampling device held about 8 g of material. Samples were obtained from eight regions within the organ and pooled into a container purged of air with oxygen-free carbon dioxide.

The anaerobic culture methods and medium for total and specific carbohydrate-fermenting groups of bacteria have been described previously (4, 5). All media contained 0.2% total added carbohydrate, except the cellulose medium, which contained 0.75% 24-h ball-milled purified cellulose (Sigmacell-20; Sigma Chemical Co., St. Louis, Mo.). Twenty-gram samples of cecal contents, diluted as required, were used as inoculum.

Cecal contents for protozoan counts were preserved by diluting with an equal volume of 50% Formalin solution (18.5% formaldehyde). Total numbers and generic distribution were determined according to the procedures described by Purser and Moir (17). Identification of protozoa to the generic level was based on the descriptions of Hsiung (8).

Polyethylene glycol (PEG) (molecular weight, 4,000) was used as an inert fluid marker and infused directly into the cecum at $T - 1$ or $T + 7$ h. At various times after infusion,

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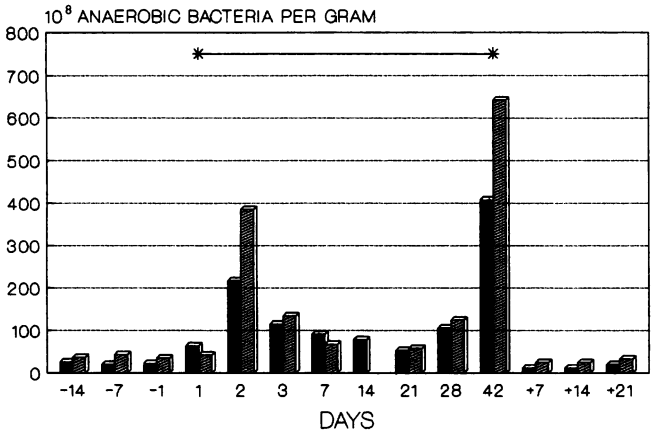

FIG. 1. Total anaerobic bacteria in samples of cecal contents taken just before, at 0 h (■), and at 5 h (▨) after feeding. Diet was alfalfa hay for samples taken on days −14, −7, −1, +7, +14 and +21. Concentrate diet (100% corn-soy, –*–) was fed when samples were taken on days 1 to 42.

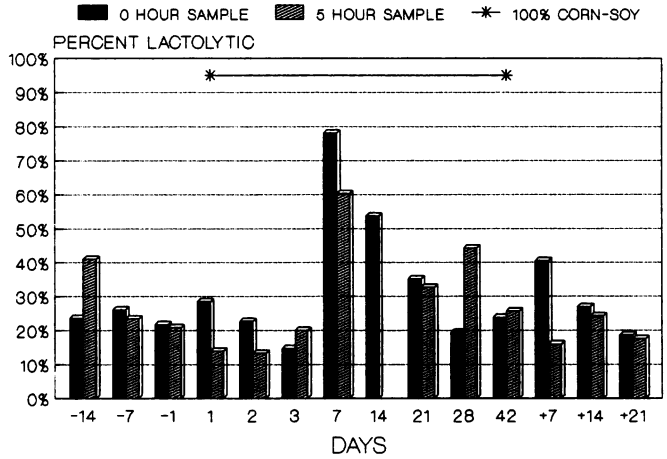

FIG. 2. Lactate-utilizing anaerobic cecal bacteria expressed as a percentage of total anaerobic bacteria. Samples were obtained just before feeding (■) and 5 h (▨) after feeding. See legend to Fig. 1 for diets. Symbol: –*–, 100% corn-soy.

PEG concentration was measured by the method of Hyden (10) as modified by Smith (19). The pH of each sample taken for PEG analysis was measured immediately after removal from the cecum, using a Corning model 125 pH meter with a combination electrode.

Cecal dry matter was determined by drying weighed duplicate samples to a constant weight at 96°C.

For 8 weeks prior to beginning the experiment, 2,400 g of diet 1 was fed at T_0. Cecal fluid volume, liquid turnover, dry matter, and pH were then measured intermittently over a 5-week period, with each measurement being replicated three times. Total viable anaerobic bacteria, along with specific functional groups, were estimated every 7 days during the last 3 weeks. Bacterial and protozoan counts were made at T_0 and $T + 5$ on each sampling day. The diet was then abruptly changed from all forage to all concentrate on an equal caloric basis. Bacterial and protozoan counts were made as described previously at T_0 and $T + 5$ on the following days after the diet change: 1, 2, 3, 7, 14, 21, 28, and 42. After a 6-week period, three replicates of the volume, turnover, and pH studies were conducted as before and the diet was changed abruptly back to all forage. Bacterial and protozoan counts were conducted at weekly intervals for 3 weeks, starting 1 week after the diet was changed.

On day 2 after the diet change from hay to concentrate, the pony consumed less than half its daily ration. The animal appeared dull and depressed, standing with its head down. No therapy was provided, and by 48 h after the change, the pony seemed normal and alert. Changing the diet abruptly from concentrate to hay resulted in no observable disorders.

Statistical analyses were conducted by the methods described by Little and Hills (13), with differences between means determined by using orthogonal contrasts.

RESULTS

Total viable bacterial numbers. Total viable bacterial counts in the cecum (Fig. 1) remained fairly stable over time when the pony was fed alfalfa hay ($P > 0.01$). Within each forage period the total counts were greater ($P < 0.05$) at 5 h postfeeding than at the time of feeding. When concentrate was fed, the counts at 0 and 5 h after feeding did not differ. The abrupt change in diet from hay to concentrate resulted in greater ($P < 0.01$) numbers of viable bacteria on all days of sampling compared with the numbers observed when hay was fed. At 48 h after the diet was changed from hay to concentrate, the mean viable count (0- and 5-h samples) reached 30.07×10^9/g, which was greater ($P < 0.01$) than the count of 5.28×10^9/g observed 24 h earlier. The counts decreased by 72 h and remained at that level for about 3 weeks after the diet change. Numbers began to increase by week 4 and then rose drastically from a mean value of 11.5×10^9/g to 52.5×10^9/g ($P < 0.01$) between weeks 4 and 6. The reason for this marked increase in numbers after 4 weeks is not known. After the 6-week sample was taken, the diet was abruptly changed back to alfalfa hay. Total numbers were similar to those observed during the earlier forage feeding period and were again higher ($P < 0.05$) at 5 h after feeding than at 0 h.

Specific carbohydrate-utilizing bacterial numbers. The average percentage of total viable bacteria that grew on starch medium was greatest during the concentrate feeding phase ($P < 0.01$). The starch utilizers averaged 73.1% of the total when hay was fed and 85.2% when concentrate was fed. Starch utilizers rose to 92.2% of the total bacterial numbers 24 h after the diet was changed, decreasing to 87.5% by 48 h. Percentages ranged from 77.7 to 85.6% for the samples obtained during the remainder of the concentrate feeding phase. When alfalfa hay was refed, starch utilizers constituted 81.9% of the total population but then decreased significantly ($P < 0.01$) at weeks 2 and 3 (44.2 and 45.5%, respectively).

Percentages of the total bacterial populations utilizing xylan and pectin decreased markedly in the first several days following the ration change ($P < 0.025$). Although quite variable, the percentages increased slowly over the 6-week concentrate feeding period, and when the diet was changed back to hay, they returned to levels observed during the initial hay feeding period.

Lactate-utilizing bacteria (Fig. 2) initially remained at about the same percentage after changing to concentrate; however, between 3 and 7 days they increased rapidly to a peak of 69.2% of total bacterial numbers ($P < 0.01$). Percentages then decreased and from 2 to 6 weeks averaged 33.5% of the total. Changing back to hay lowered the average percentage to 23.7%, which was similar to the 26.1% observed during the first forage period.

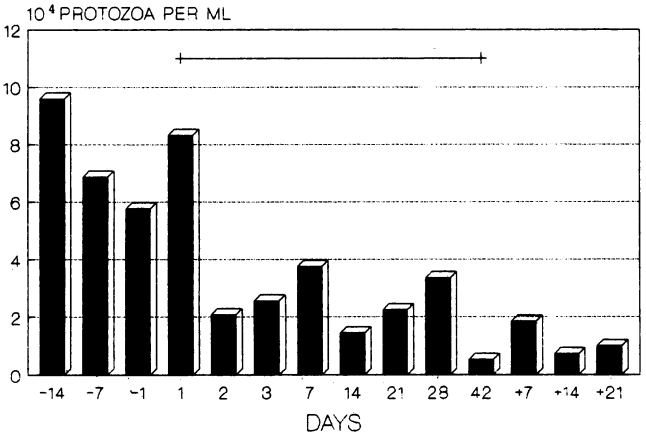

FIG. 3. Average total protozoan numbers for 0- and 5-h samples. See legend to Fig. 1 for diets. Symbols: ■, total protozoa; ┼, 100% corn-soy.

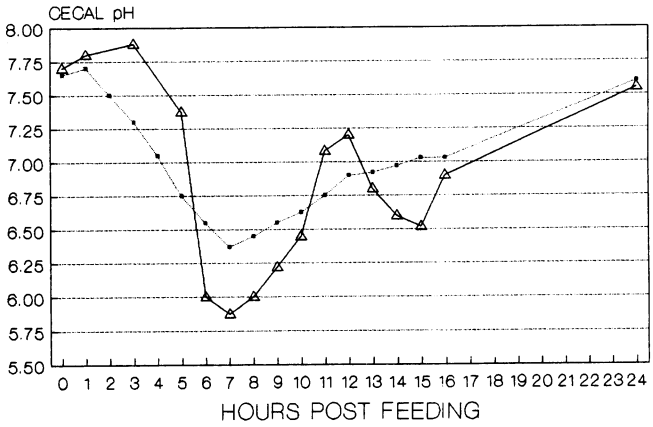

FIG. 4. Effect of feeding on cecal pH. Symbols: ■, forage diet; △, grain diet.

Cellulolytic bacteria were not detected during the initial forage feeding phase and only on day 2 of the concentrate period. Lower dilutions were counted during the final forage period and for the three weekly samples: numbers were 1.8×10^4, 1.1×10^4, and 4.4×10^4/g. This represented 0.00075, 0.00098, and 0.02% of the total bacterial population.

Protozoan numbers and generic composition. Within 48 h after the diet change from hay to concentrate, a marked decrease ($P < 0.01$) in protozoan numbers was observed (Fig. 3). Because time of sampling (0 or 5 h) had no significant effect on total numbers of protozoa in cecal contents, the average is shown in the graph. Values fluctuated over the remainder of the concentrate period, falling to their lowest level at 6 weeks. Changing back to the hay diet had little effect on protozoan numbers, which remained quite low for the entire 3-week period.

A total of 10 genera of protozoa were identified in cecal contents obtained during the initial forage period: *Paraisotricha*, *Cycloposthium*, *Blepharoconus*, *Blepharosphaera*, *Blepharoprosthium*, *Bundleia*, *Blepharocorys*, *Endamoeba*, *Didesmis*, and *Ampullacula*. However, only three genera persisted throughout the concentrate feeding period, i.e., *Paraisotricha*, *Cycloposthium*, and *Didesmis*. These were the only genera observed after changing back to the hay diet, except for a low number of *Blepharosphaera* spp. observed on day 14.

Cecal pH. Means of the three replicate studies comparing cecal pH and time after feeding on both the forage and concentrate diets are shown in Fig. 4. Forage feeding resulted in a linear decrease in pH ($r = -0.99$) from 1 to 7 h postfeeding, followed by a linear increase in pH ($r = 0.98$) over the next 17 h back to the prefeeding level. Values ranged from a low of about pH 6.4 to pH 7.7. With the concentrate diet, cecal pH remained fairly constant for 3 h postfeeding and then fell sharply to a low of about 5.9 at 7 h postfeeding. The pH increased to 7.15 by 12 h, decreased slightly through 15 h, and then rose back to approximately the prefeeding value.

Cecal fluid volume and fluid turnover rate. Preliminary studies during the forage feeding period on fluid turnover rate suggested that turnover was very rapid during the first 7 h after feeding, decreased between 8 and 16 h, and then slowed down even more between 8 and 24 h. Infusing PEG 1 h prior to feeding or 7 h postfeeding produced the curves shown in Fig. 5. The mean slope of three replicate least-squares regression lines for cecal PEG concentration versus time for the first 6 h after feeding was −0.416, the slope for the period from $T + 7$ through $T + 16$ was −0.197, and that from $T + 16$ through $T + 24$ was −0.026, each of which is less ($P < 0.01$) than the slope observed in the previous period. The lowest correlation coefficient observed for any of the three regression lines for any of the three periods was 0.95.

Similar experiments were carried out when concentrate was being fed; however, the results were quite variable. In general, a gradual dilution of the marker occurred until $T + 5$, followed by a very rapid dilution of the marker between $T + 5$ and $T + 6$. Infusion of PEG at $T + 7$ revealed that rapid marker dilution occurred until 9 to 10 h after feeding. Although values were somewhat erratic, turnover rate appeared to decrease after 10 h. Correlation coefficients between marker concentration and time after feeding were very low.

With just the 0- and 24-h values, cecal volume and daily turnover rate were calculated. Mean values for three replicates were 2.2 ± 1.0 liters (1.6 to 3.4 liters) and 3.9 ± 0.37 turnovers per day on forage and 3.9 ± 4.2 liters (0.6 to 8.6 liters) and 4.2 turnovers per day on the concentrate diet.

During two of the forage experiments, samples were obtained from the large colon via a colon cannula. PEG was

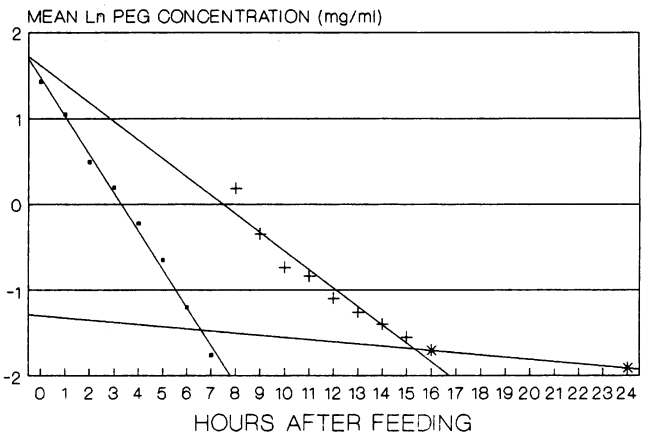

FIG. 5. Changes in ln PEG concentration after feeding (forage diet). Symbols: ■, 0 to 7 h; +, 7 to 16 h; *, 16 to 24 h.

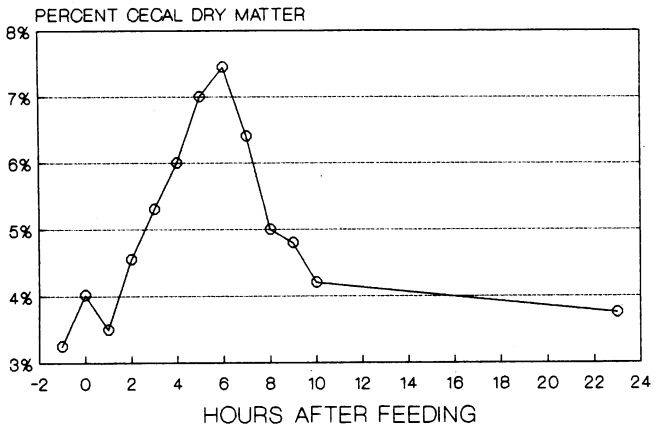

FIG. 6. Effect of feeding on percentage of dry matter in the cecum (forage diet).

infused through the cecal cannulae 1 h prior to feeding and had reached the colon 4 to 5 h after feeding. PEG concentrations remained fairly constant in the colon for the next 4 to 5 h and then began to decline.

Cecal dry matter. Diurnal changes in dry matter of the cecal contents were measured during the forage feeding phase. The percentage of dry matter doubled between 1 and 6 h and then decreased almost back to its original value by 10 h (Fig. 6).

DISCUSSION

Total numbers of cecal bacteria during the forage feeding phase of this study appear to be similar to the values reported by Kern et al. (12) for ponies fed timothy or clover hay. These authors found a significant increase in numbers when the diet was changed to 75% hay–25% grain; however, the numbers were considerably lower than the peak values obtained in the present study when all concentrate was fed. Although the two diets in this study were isocaloric, these data strongly suggest that more readily available energy reaches the cecum when concentrates are fed.

The conditions in the equine cecum following an abrupt diet change from hay to concentrate may be similar to that described by Hungate (9), when a hay-fed ruminant is suddenly fed grain. In the latter case, there is a rapid proliferation of *Streptococcus bovis* which results in a diminution of the ruminal pH, due to lactic acid buildup. The numbers of *S. bovis* then begin to fall, with a concomitant increase in the numbers of lactobacilli. The increase in these organisms results in further production of lactic acid, which peaks at 7 to 24 h after the grain overload (6). The increased concentration of lactic acid then encourages the growth of lactate utilizers. The data from the pony cecum appear to fit this pattern. A general increase in total bacterial numbers (Fig. 1) was observed along with a pH depression (Fig. 4). This was followed by a decrease in total numbers and an increase ($P < 0.01$) in the percentage of the total count that grew on lactate medium (Fig. 2) to peak levels at 7 days. The percentage of bacteria growing on lactate medium decreased 1 to 3 or 4 weeks after the change and then remained fairly stable.

The abrupt diet change resulted in a drastic decrease in the percentages of xylanolytic and pectinolytic bacteria. These results might be anticipated since the levels of pectic substances and xylans present in grain (3, 15, 22, 23) are below those observed in forages (20).

The extremely low numbers of cellulolytic bacteria in cecal contents might suggest that the large colon is the major site of cellulose digestion in the pony, the cecum acting only as an organ of inoculation. However, it has been reported (14) that cellulose clearing in roll tube cultures of rat cecal material could best be observed when 0.5% agar is used. Unpublished studies conducted by one of us (B.A.D.) have demonstrated the inability of several ruminal cellulolytic bacteria, particularly *Bacteroides succinogenes*, to produce zones of clearing in 2% agar medium. The present medium contained 2% agar, and thus it remains to be seen whether cellulolytic organisms from the pony cecum could be detected in a low-agar medium or perhaps by using most-probable-number procedures.

A previous report on the cecal pH pattern in horses (21) agrees quite well with our work, in that pH levels above neutrality are observed for a few hours following feeding. Minimum pH values were observed near $T + 6$, and concentrate feeding resulted in minimal pH measurements which were less than the minimum levels observed during forage feeding. The delay in pH depression may be because there is a postprandial delay of about 2 h before any material reaches the cecum (2, 11). During the concentrate feeding phase, following a rise in pH at 7 to 12 h after feeding, an additional pH depression was observed for a few hours. This could be explained by the arrival of feed particles which travel more slowly than the soluble material (2).

If the situation in the cecum is similar to that in the rumen, the decreased pH resulting from concentrate feeding may have caused the depression observed in protozoan numbers (Fig. 3). It has been noted (17) that any material which depresses ruminal pH also depresses protozoan numbers. However, completely different protozoan genera are present. Kern et al. (12) did not observe any decrease in protozoan numbers when 25% concentrate was included in the diet. The reason for protozoan numbers remaining low after switching from concentrate back to the hay diet is unknown. However, the loss of several genera of protozoa during concentrate feeding and isolation of the pony from other animals, preventing possible reinoculation, could be important factors. Adam (1) has reported that two unique protozoan populations occur in the equine hindgut, one in the cecum and anterior portion and the other in the posterior portion of the colon. He observed that five genera (*Didesmis*, *Blepharoprosthium*, *Paraisotricha*, *Blepharocorys*, and *Cycloposthium*) comprised the majority of the protozoa observed anterior to the pelvic flexure, which was also the case in this study. In addition to these five genera, low numbers of *Bundelia*, *Endamoeba*, *Ampullacula*, *Blepharoconus*, and *Blepharosphaera* spp. were also observed in the present study. Hsiung (8) has reported the presence of these genera in the cecum of the equine.

The mean cecal fluid volume of 2.2 liters during forage feeding agrees quite well with published data. Argenzio et al. (2) reported pony cecal volumes ranging from nearly 0.5 liter to approximately 3 liters from feeding through 8 h postfeeding. Kern et al. (11) indicated that pony ceca contained 2.8 kg of ingesta 3 h postprandially. In contrast, extreme variation was noted in our studies during concentrate feeding, with a range in calculated volumes from 0.6 to 8.6 liters.

The PEG data collected during the forage feeding phase suggest the presence of three fluid turnover rates (Fig. 5). It appears that there is a rapid dilution of the fluid marker from T_0 through $T + 6$, at which time the rate decreases and remains the same through $T + 16$, when a further decrease in rate occurs. It is interesting to note that the first change in

dilution rate occurs at $T + 6$, which is also the time when pH was at minimum levels and percent dry matter was at its maximum. These data would support the pattern of fluid movement described by Argenzio et al. (2). Although their animals were fed every 12 h, they observed an early influx of fluid followed by a gradually increasing efflux of fluid up to the next feeding.

The magnitude of bacterial and protozoan numbers appeared to vary somewhat with sampling time, i.e., just prior to feeding or 5 h postfeeding. However, in most cases these differences were not significant and did not affect the overall patterns associated with the dietary changes. Thus, it would seem appropriate to sample at only one time period.

ACKNOWLEDGMENTS

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