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Diurnal Variations in Bacterial Numbers and Fluid Parameters in Ruminal Contents of Animals Fed Low- or High-Forage Diets

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Differential carbohydrate media and anaerobic replica plating techniques were used to assess the degrees of diurnal variations in the direct and viable cell counts as well as the carbohydrate-specific subgroups within the mixed rumen bacterial populations in cattle fed maintenance (metabolizable energy) levels of either a high-forage or a high-concentrate diet once daily. The rumen was sampled at 1 h before feeding and 2, 4, 8, 12, and 16 h after feeding, and selected microbiological parameters of the isolated bacterial populations were assessed. Corresponding samples of ruminal fluid were assayed for fermentation acids, carbohydrate, ammonia, and pH changes. The data showed that regardless of diet, total bacterial numbers remained fairly constant throughout the day. The number of viable bacteria declined 40 to 60% after feeding and then increased to a maximum at 16 h postfeeding. Changes occurred in the carbohydrate-specific subgroups within the bacterial populations, and some of the changes were consistent with a predicted scheme of ruminal feedstuff carbohydrate fermentation. Regardless of diet, however, soluble-carbohydrate-utilizing bacteria predominated at all times. Xylan-xylose and pectin subgroups respectively comprised about one-half and one-third of the population when the high-forage diet was given. These subgroups, along with the cellulolytics, constituted lesser proportions of the population when the high-concentrate diet was given. The cellulolytic subgroup was the least numerous of all subgroups regardless of diet but followed a diurnal pattern similar to that predicted for cellulose fermentation. There were few diurnal variations or differences in bacterial cell compositions and ruminal fluid parameters between diets. The observed similarities and dissimilarities of the rumen bacterial populations obtained when the two diets were given are discussed. The data are consistent with the versatility and constancy of the rumen as a stable, mature microbial system under the specific low-level feeding regimens used.

The microbial population of the rumen is composed of a complex mixture of bacteria, protozoa, fungi, and yeasts (3, 4, 21). Each of these groups comprises a collection of species that are morphologically and biochemically diverse and whose carbohydrate substrate utilization spectra overlap. This versatility complements the intricate physical structures of the microbial community within the ruminal ecosystem and enables ruminant animals to consume a wide variety of feedstuffs without causing large fluctuations in the amounts or types of microbial products produced (35, 45, 49). However, the species composition of the rumen microbial community has been found to change under different feeding regimens, particularly when high-forage diets are modified to contain high amounts of concentrate (grain) materials (16, 28, 30, 45). The microbial species and cellular composition have also been shown to be affected by feed intake levels, frequency of feeding (21, 35), and the postprandial time at which the rumen was sampled (7, 8, 46).

The diurnal variations of the carbohydrate-specific bacterial groups, if existent, are thought to follow the fermentation of the major feedstuff components. A theoretical scheme for such a ruminal fermentation pattern (Fig. 1) has been suggested and is based upon relative solubilities of feed components and the microbial fermentative pathways observed for the major carbohydrate components in most rations after a once-daily feeding (21, 22, 29). According to this scheme, the highly soluble materials (sugars, some proteins) are fermented rapidly shortly after the feed is ingested. This is followed by the fermentation of less-soluble materials such as

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starches and some pectins, with a lower peak in associated microbial activity. A third peak, representing pectin and dextrin fermentation, is followed by the degradation of the rather insoluble materials of the plant cell wall complex, the hemicelluloses and celluloses. These latter materials undergo fermentation after a relatively long ruminal retention time, with low and broadly based peaks of associated microbial activity. A few studies have examined the diurnal variation of the total rumen population (7, 8, 44, 47), but none has followed the diurnal changes occurring within the bacterial fraction with respect to the major carbohydrate-fermenting groups (e.g., cellulolytic, pectinolytic) in animals fed high-forage or high-concentrate diets at a similar intake level.

Using methodology developed and tested in our laboratory for differentiating the rumen bacterial carbohydrate-specific subgroups from one another, we monitored the diurnal variation within the total rumen bacterial population and the major carbohydrate subgroups within this population after feeding cattle either a high-forage or a high-concentrate diet once daily. In addition, measurements of the cellular composition (dry weight, DNA, RNA, protein, carbohydrate) of the bacterial population and measurements of ruminal fluid parameters (pH, soluble carbohydrate, ammonia, short-chain fatty acids) were made to assess their correlation with the changes in the bacterial populations.

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MATERIALS AND METHODS

Bacteria. Samples of whole-rumen contents containing the mixed rumen bacterial populations to be studied were obtained (25) from rumen-fistulated Holstein steers and contained approximately equal proportions of solids and liquid. These contents were then blended and filtered (25). The resultant fluid was decanted into two or more CO_2-filled wide-mouth polycarbonate centrifuge bottles with air-tight sealing cap assemblies (Ivan Sorvall, Inc.). After centrifugation (1 min; 500 × *g*; 4°C), the supernatant liquid was decanted under CO_2 and recentrifuged (3 min; 650 × *g*; 4°C). The final supernatant liquid, which contained the rumen bacterial population, was washed once, resuspended in S buffer (25), and used.

Media. The sterile, anaerobic techniques outlined by Hungate (20) and modified by Bryant (5) were used in combination with an anaerobic glovebox (atmosphere, 95% Ar–5% H_2; temperature, 22 to 24°C) and petri plates (60 by 15 mm). The complete carbohydrate (CC) and differential carbohydrate (DC) media and anaerobic replica plating methods used were those developed and described previously (25). These were shown to support the growth of most of the predominant ruminal bacteria and to differentiate the major rumen bacterial carbohydrate-utilizing subgroups from one another. The CC medium contained glucose, soluble starch, pectin, xylan, xylose, and cellobiose (0.05% ([wt/vol] each). Each DC medium contained a single added carbohydrate (0.4% [wt/vol]) of the substrates listed above. The cellulose DC medium, however, was prepared differently. Plates of basal carbohydrate medium (no added substrate) were poured and allowed to dry thoroughly. These were thinly overlaid with a cellulose-slurry medium consisting of the following (percent in medium): minerals one and two (see Table 2 in reference 25), 10% (vol/vol); Trypticase, 0.2% (wt/vol); branched-chain volatile fatty acid solution (isobutyric, *n*-valeric, isovaleric, DL-2-methylbutyric acids; 1 ml of each in distilled water to 100 ml; pH 7.5), 1% (vol/vol); resazurin (0.1% solution), 0.1% (vol/vol); incubated clarified ruminal fluid (25), 30% (vol/vol); cellulose slurry (filter paper ball-milled for 72 h as a 2% [wt/vol] water suspension), 10% (vol/vol); agar (washed [25]), 0.8% (wt/vol); sodium sulfide–L-cysteine hydrochloride solution of 2.5% (wt/vol) each that was added to the medium after autoclaving), 1% (wt/vol); sodium carbonate (8% [wt/vol] solution that was added to the medium after autoclaving), 5% (vol/vol); and CO_2 gas phase, 100%.

Determination of bacterial numbers. Rumen bacterial populations were serially diluted in 10-fold amounts in S buffer, and each of three dilutions was inoculated in triplicate onto plates of CC medium. The plates were incubated under CO_2 for 3 days at 39°C. Afterwards, the colonies were enumerated, and those plates containing 5 to 50 colonies were replica plated onto the various DC media. An average of 95 colonies (range, 50 to 150) were replicated for each bacterial population sampled, and a subsequent 3-day period was allowed for colony development. Each colony showing good growth on a particular plate of DC medium was considered to contain bacteria that were able to use the carbohydrate substrate added. The xylanolytic and cellulolytic subgroups were represented by those colonies surrounded by clearing zones in the respective DC media. We enumerated the pectinolytic portion of

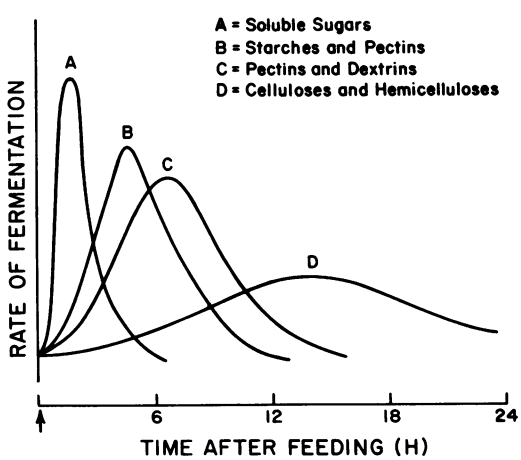

FIG. 1. Ruminal carbohydrate fermentation patterns. The curves are theoretical in nature and are adapted from the work of Johnson (22).

TABLE 1. Dietary components and formulations[a]

Diet	Component	Dry matter consumed		Estimated metabolizable energy intake	
		kg/day	%	Mcal/day	%
High forage	Alfalfa hay[b]	5.45	77	12.59	72
	Concentrate[c]	1.59	23	4.91	28
High concentrate	Alfalfa hay	2.00	32	4.62	27
	Concentrate	4.25	68	13.21	73

[a] Data calculated from nutrient requirements of domestic animals (No. 3. Nutrient requirements of dairy cattle. National Research Council. 1978. Washington, D.C.).

[b] Late vegetative; dry matter, 89%; metabolizable energy, 2.44 Mcal/kg[a].

[c] University of Illinois concentrate mix no. 64 (number in parentheses is percent dry matter as fed): corn, shelled and crushed (84.25); soybean meal, solvent-extracted (13.0); Ca_2PO_4 (1.5); trace mineral mix (1.2); vitamin A plus D (Quadrex 10) (0.05). Calculated dry matter, 89%; metabolizable energy, 3.11 Mcal/kg[a].

the pectin group after visualizing the unhydrolyzed polygalacturonic acid matrix by precipitation effected when each plate was overlaid with 3 ml of a 4% (wt/vol) cetyltrimethylammonium bromide solution (40).

Direct cell counts were made with a Petroff-Hausser counting chamber on samples of bacteria preserved by being diluted at least 1:5 (vol/vol) in a formaldehyde–0.85% (wt/vol) NaCl solution (44). Two separate fillings of the chamber were used to determine numbers. For each sample, the viable cell counts were calculated from CC medium plates containing between 5 and 100 colonies.

Bacterial cell constituents. Nucleic acids were extracted from bacterial cell pellets by a hot 0.5 M perchloric acid procedure (9). Deoxyribose and ribose were assayed by the methods of Burton (9) and Schneider (39), respectively, using these sugars as standards. Correction factors of 2.44 and 4.90 were used to correct deoxyribose and ribose values, respectively, to DNA and RNA equivalents (38). Protein in base-hydrolyzed (0.1 M NaOH; 70°C; 10 min) cell pellets was determined by the procedure of Lowry et al. (26), using bovine serum albumin as the standard. The total carbohydrate content in an acid-hydrolyzed (concentrated HCl; 100°C; 4 h) and neutralized (NaOH) sample was measured. The hydrolysate was assayed by the phenolsulfuric acid method (1), using glucose as the standard. The dry weight of bacterial cell pellets dried to a constant weight (24 h; 90°C) was determined.

Ruminal fluid components. The ammonia concentration in ruminal fluid samples was measured by the method of Chaney and Marbach (11), using NH_4Cl as the standard. The total carbohydrate content was determined as described above. Fermentation acids were determined as their butylated volatile fatty acids by the method of Salanitro and Muirhead (37). Lactic acid was assayed by the method of Barker and Summerson (2), using L-lactate (lithium salt) as the standard.

Animals, diets, and diurnal experimental sampling time. Two Holstein steers (no. 3150 and no. 3557 from the University of Illinois, Urbana), each fitted with a rumen fistula and weighing about 500 kg, were used. The bedding consisted of sawdust and woodchips so that possible extraneous feed consumption by the animals would be minimized. The steers were weighed every 6 weeks during the experimental period, and a slight weight gain was observed. Animals were allowed free access to water at all times and were separated by one stanchion to prevent cross-feeding.

Alfalfa hay (late vegetative) and University of Illinois Dairy Herd concentrate mix no. 64 were mixed in proportions that yielded either a high-forage or a high-concentrate diet (Table 1). The diets were fed at 1.0× maintenance level for metabolizable energy content (Table 1). Sufficient quantities of alfalfa-concentrate mix were available to ensure constancy of dietary components. Steers were fed once daily at 7:00 a.m.

Experiments were conducted weekly on three consecutive Mondays. After the third experiment, the alfalfa-concentrate ratio was changed from 3:1 to 3:2, 1:1, 2:3, and finally 1:3 (5-day interval between each change). To allow time for adaptation, we fed the animals the new 1:3 combination for 12 days before the initiation of sampling.

For each diurnal experiment, the sampling times were 6:00 a.m., 9:00 a.m., 11:00 a.m., 3:00 p.m., 7:00 p.m., and 11:00 p.m. These times respectively corresponded with 1 h before feeding and 2, 4, 8, 12, and 16 h after feeding. The sample volume taken each time was 500 ml of fluid plus solid contents.

The data for all parameters were calculated from triplicate determinations for each point of data and then averaged across the three experiments for each animal on each diet. The mean values ± standard deviations are presented. The data for the period between the 16-h sampling and the 23-h (prefeeding) sampling were extrapolated (Fig. 2 through 9, dashed lines) on the basis of the rumen ecosystem relative stability found after analysis of the data (see Results).

RESULTS

Rumen samples. We developed the differential centrifugation procedure used to obtain the rumen bacterial population in conjunction with the results of phase-contrast microscopic examinations of the supernatant fluids and residues as criteria for the successful separation of the bacteria. The bacterial population obtained after differential centrifugation was determined to be nearly devoid of feed particles and protozoa.

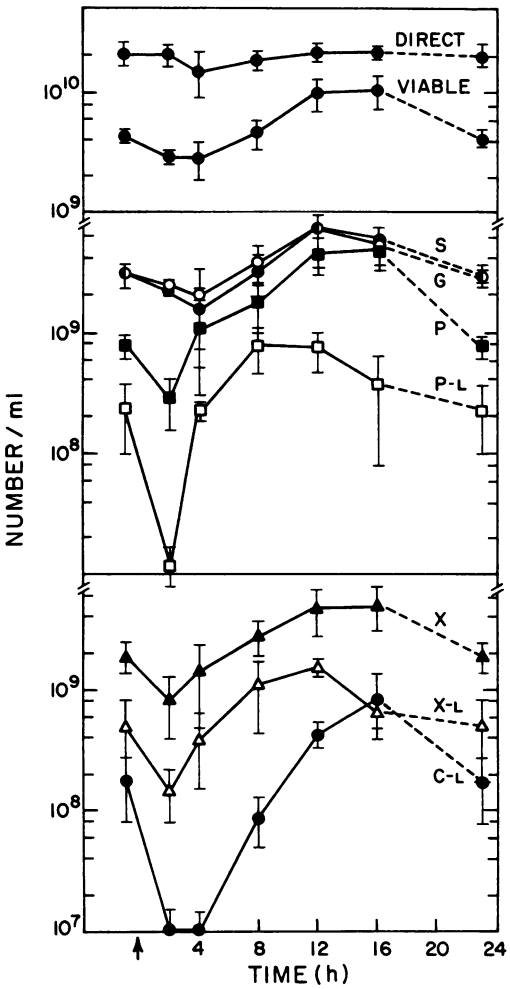

FIG. 2. Diurnal variation in direct and viable counts and carbohydrate-specific bacterial numbers in ruminal digesta from an animal fed the high-forage diet. The numbers of carbohydrate-specific bacteria were determined by replica plating onto various DC media. S, Soluble starch; G, glucose; P, pectin; P-L, pectinolytic; X, xylan; X-L, xylanolytic; C-L, cellulolytic. In this and all subsequent figures, the data are means ± standard deviations from three replicate experiments, and the arrow indicates the time of feeding.

The single washing and resuspension in S buffer (25) reduced residual fermentation acids to less than 1 μmol/ml. Throughout these preparatory procedures, direct and viable counts of the resultant population were unaffected, and the distribution of the carbohydrate-utilizing subgroups remained unchanged. The resultant population was an admixture of the bacterial subgroups present in both the free (fluid) and the associated (particulate) phases of ruminal contents. Samples prepared in this manner were shown to have subgroup distributions between these phases of 50 to 60% and 40 to 50%, respectively (25).

Upon analysis, striking similarities were found in the patterns of diurnal variation in bacterial numbers and ruminal fluid parameters for both animals on the same diet. However, as would be expected, there were differences between individual animals (such as the length of time to consume the daily ration) which caused time to be askewed in the diurnal patterns as well as differences in absolute bacterial numbers between the two animals under the same experimental conditions. Therefore, the two sets of data for the two animals were not combined, and for clarity, only the data (means ± standard deviations of three experiments) for steer no. 3557 are presented.

Diurnal variation in cell numbers (high-forage diet). While the steer was maintained on the high-forage diet, the direct and viable cell counts within the rumen bacterial population ranged from 15.7×10^9 to 22.5×10^9/ml and from 2.8×10^9 to 11.0×10^9/ml, respectively (Fig. 2). Lowest and highest values for both counts occurred at the 4- and 16-h-postfeeding sampling times, respectively. Bryant and Robinson (7) found that the lowest counts are obtained at 2 h and the highest counts are obtained at 12 h when animals are fed a similar diet once daily. The lowest viable proportion of the bacterial population (14.6%) was found at 2 h after feeding, which is generally believed to be the time when maximal rates of fermentation occur in the rumen (10, 21, 43). After the 2-h sampling, the viable portion increased, reaching its highest value (48.6%) at 16 h.

Of the carbohydrate-utilizing subgroups assessed, the soluble-starch and glucose subgroups were the most numerous, ranging from 1.6×10^9 to 7.1×10^9 bacteria per ml and from 2.0×10^9 to 7.0×10^9 bacteria per ml, respectively (Fig. 2). The pectin and pectinolytic carbohydrate subgroups ranged from 0.3×10^9 to 4.6×10^9 bacteria per ml and from 0.01×10^9 to 0.8×10^9 bacteria per ml, respectively. Both subgroups were least numerous at 2 h and increased rapidly to their highest levels at 12 h for the pectin subgroup and at 8 h for the pectinolytic subgroup. The xylan-xylose subgroup ranged from a low of 0.8×10^9 bacteria per ml at 2 h to a high of 5.2×10^9 bacteria per ml at 16 h. Only a portion (12 to 40%) of the xylan-xylose subgroup was xylanolytic, and the lowest number was 0.2×10^9 bacteria per ml at 2 h; however, by 12 h, these organisms had increased to 2.7×10^9 bacteria per ml. The cellulolytic subgroup ranged from 0.01×10^9 to 0.9×10^9 bacteria per ml; the former value was observed at 2 h, and the latter value was observed at 16 h (Fig. 2).

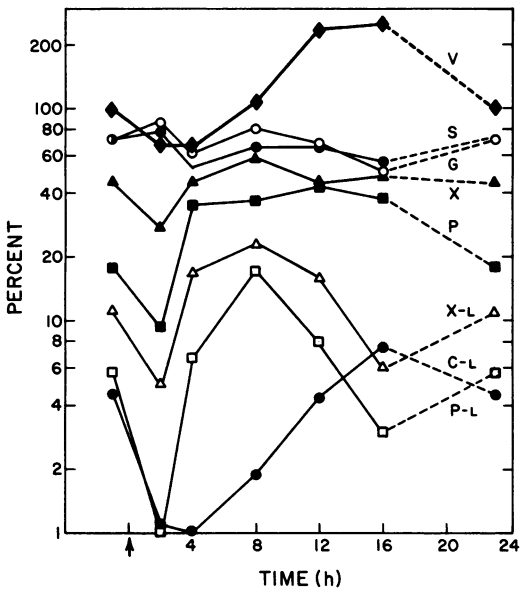

FIG. 3. Diurnal variation in viable and carbohydrate-specific bacterial numbers in ruminal digesta from an animal fed the high-forage diet. The data are expressed as the percentages of viable cell counts at 1 h before feeding. See the legend to Fig. 2 for abbreviation explanations.

When the data were analyzed as percentages of total viable bacteria, similar trends were observed within the rumen bacterial population of the animal fed the high-forage diet (Fig. 3). The soluble-starch and glucose subgroups comprised the largest percentage (50 to 86%) of the population, regardless of time after feeding. The pectin subgroup ranged from 9.6% at 2 h to 44.1% at 12 h (Fig. 3). The xylan-xylose group within the rumen bacterial population ranged from 27.6% at 2 h to 58.5% at 8 h. The cellulolytic subgroup constituted 0.3% of the total at 2 h but increased to 7.7% at 16 h.

Cellular constituents of rumen bacterial populations (high-forage diet). On a dry weight basis, cellular constituents within the bacterial population showed little diurnal variation (Fig. 4). DNA levels varied between 1 and 2% of the dry weight, whereas RNA levels were 10-fold higher than DNA levels. The highest RNA values were found in the sample taken 2 h postfeeding, when rapid bacterial growth would be expected. The protein and carbohydrate contents accounted for approximately 40 and 10%, respectively, of the dry weight.

Ruminal fluid parameters (high-forage diet). Total fermentation acid concentrations ranged from 72 to 110 mM at 1 h before feeding and 8 h after feeding, respectively (Fig. 5). Acetate constituted between 60 and 70 molar % of the acids. Propionate ranged from 9.5 mM (13 molar %) at 1 h before feeding to 20.8 mM (20 molar %) at 4 h postfeeding. Butyrate concentrations ranged from 9 to 12 mM throughout the day. Acetate/propionate ratios calculated from these data remained near 4:1 over the diurnal period.

Minor and branched-chain volatile fatty acids were considerably lower in concentration (Fig. 5). Valerate ranged from 0.51 to 1.0 mM, whereas isovalerate and isobutyrate ranged from 0.35 to 0.65 mM and from 0.37 to 0.51 mM, respectively. Lactic acid concentrations were about 10-fold lower than branched-chain volatile fatty acid concentrations (data not shown). The lactate level did increase, however, after feeding (to 0.2 mM), and then it decreased; the lowest lactic acid level was observed just before feeding.

The total carbohydrate content of the ruminal fluid increased only slightly after feeding (at 2 h [Fig. 6]). Ammonia concentrations increased after feeding from 16.0 to 23.2 mM and then leveled off near 14 mM. The pH of the ruminal fluid ranged from an initial prefeeding value of 7.2 to a low of 6.2 at 8 h, which gradually returned to the prefeeding value (Fig. 6).

Diurnal variation in cell numbers (high-concentrate diet). Direct cell counts of the bacterial

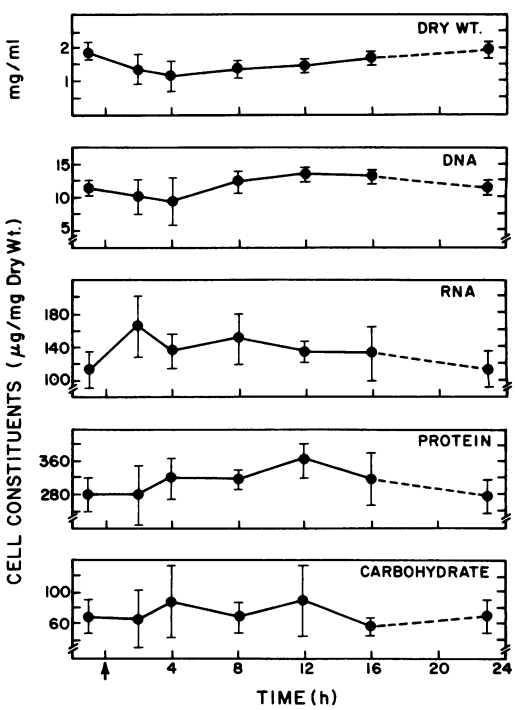

FIG. 4. Diurnal variations in cellular dry weight and composition of bacteria in ruminal digesta from an animal fed the high-forage diet.

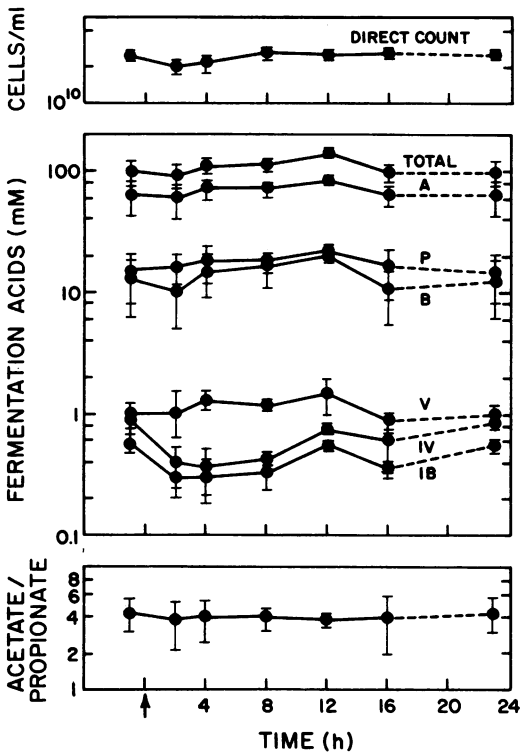

FIG. 5. Diurnal variations in direct-count cell numbers, fermentation acids, and acetate/propionate molar ratios in ruminal digesta from an animal fed the high-forage diet. A, acetate; P, propionate; B, butyrate; V, valerate; IV, isovalerate plus 2-methylbutyrate; IB, isobutyrate.

populations obtained after the steer was fed the high-concentrate diet were lowest at 2 h (20.6×10^9/ml) and highest at 16 h (25.3×10^9/ml) postfeeding (Fig. 7). Viable cell counts ranged from 2.9×10^9 to 18.6×10^9/ml at 2 and 16 h, respectively. The viable proportion of the total cell count went from a low of 14.1% at 2 h to a high of 73.5% at 16 h.

When the animal was on the high-concentrate diet, the soluble-starch and glucose subgroups were the most numerous, as was expected (Fig. 7). The soluble-starch and glucose subgroups peaked at 12.8×10^9 and 7.8×10^9 bacteria per ml, respectively, at 16 h postfeeding. The pectin subgroup varied from 0.6×10^9 bacteria per ml at 4 h to a high of 4.2×10^9 bacteria per ml at 16 h. However, the lowest number (0.1×10^9 bacteria per ml) of pectinolytics was observed at 1 h before feeding, and the highest number (0.9×10^9 bacteria per ml) was at 12 h. The xylan-xylose subgroup ranged from a low of 1.3×10^9 bacteria per ml at 4 h to a high of 8.1×10^9 bacteria per ml at 16 h. The xylanolytic subgroup ranged from 0.2×10^9 bacteria per ml at 2 h to 1.2×10^9 bacteria per ml at 16 h. The cellulose-degrading subgroup increased from a low of 0.03×10^9 bacteria per ml at 2 h to a high of 0.4×10^9 bacteria per ml at 16 h (Fig. 7). The percentages of the total viable rumen bacterial population reprsented by these carbohydrate-specific subgroups when the steer was fed the high-concentrate diet were similar to the percentages observed when the steer was fed the high-forage diet (data not shown). The peak values and postprandial times for each subgroup were as follows: soluble starch, 83% and 8 h; glucose, 71% and 2 h; pectin, 32% and 8 h; pectinolytic, 9% and 8 h; xylan-xylose, 60% and 8 h; xylanolytic, 10% and 1 h before feeding; and cellulolytic, 2% and 8 h.

Cellular constituents of rumen bacterial populations (high-concentrate diet). Calculated on a dry weight basis, cellular DNA constituted between 1 and 2% of the rumen bacterial population. RNA values were 10-fold higher than DNA values. This was similar to the relationship found for the rumen bacterial populations when the animal was fed the high-forage diet (Fig. 4). The amount of RNA within the population increased rapidly after feeding and gradually leveled off thereafter to near 130 μg/mg (dry

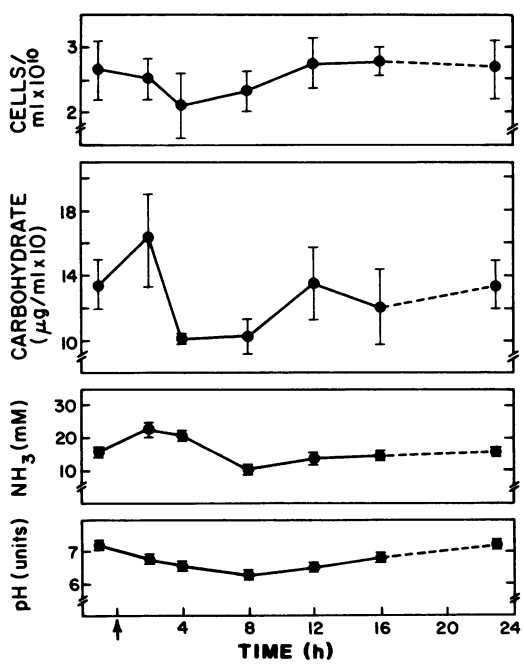

FIG. 6. Diurnal variation in direct-count cell numbers, pH, and carbohydrate and ammonia levels in ruminal digesta from an animal fed the high-forage diet.

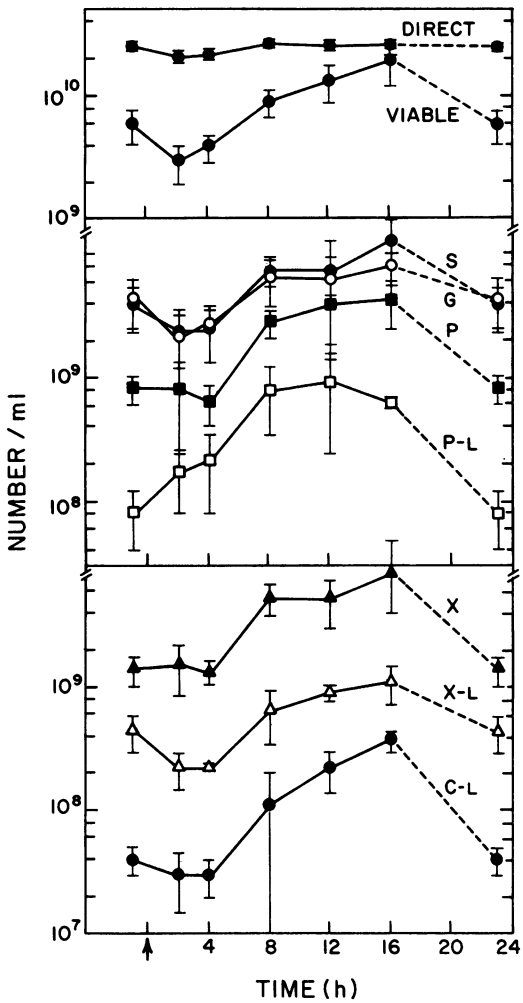

FIG. 7. Diurnal variation in direct and viable counts and carbohydrate-specific bacterial numbers in ruminal digesta from an animal fed the high-concentrate diet. See the legend to Fig. 2 for abbreviation explanations.

weight). Regardless of the time of sampling, protein constituted 30 to 40% of the dry weight. The carbohydrate content of the cells increased rapidly from 127 μg/mg after feeding to 191 μg/mg at 4 h postfeeding and then rapidly declined.

Ruminal fluid parameters (high-concentrate diet). Total fermentation acid concentrations ranged from 94 mM at 2 h to 140 mM at 12 h postfeeding (Fig. 8). Of the total acids, acetate constituted around 65 molar %, regardless of the time of sampling. Propionate and butyrate constituted 16 and 11 molar %, respectively. The acetate/propionate ratios when the high-concentrate diet was given were slightly greater than 4:1.

Of the minor and branched-chain volatile fatty acids, valerate was highest in concentration (0.6 to 1.5 mM) throughout the diurnal period. Isovalerate and isobutyrate varied between 0.5 and 0.9 mM. The lactic acid level averaged less than branched-chain volatile fatty acid levels throughout the day, increasing slightly after feeding (to 0.3 mM) over the prefeeding level of 0.1 mM (data not shown).

The carbohydrate content of ruminal fluid samples obtained after the steer was fed the high-concentrate diet showed a sharp increase from the prefeeding sample to the 2-h-postfeeding sample (Fig. 9). Ammonia concentrations increased slightly after feeding but remained around 14 mM. The pH profile showed an initial value of 6.4 before feeding, a low of 5.7 at 4 h, and values that steadily increased up to the 1-h-prefeeding level (Fig. 9).

DISCUSSION

The direct cell counts of the prepared bacterial suspensions indicated little variation in the

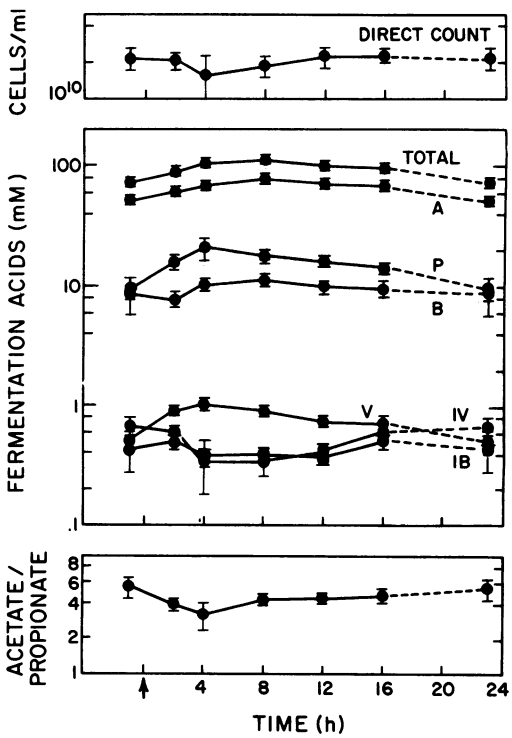

FIG. 8. Diurnal variation in direct-count cell numbers, fermentation acids, and acetate/propionate molar ratios in ruminal digesta from an animal fed the high-concentrate diet. See the legend to Fig. 5 for abbreviation explanations.

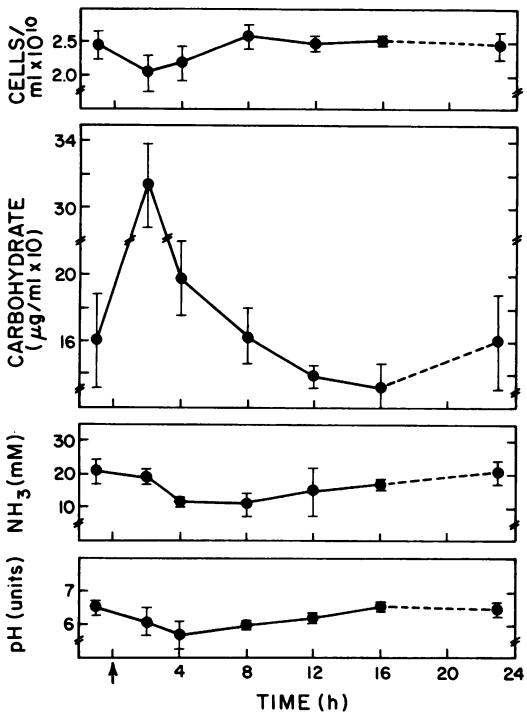

FIG. 9. Diurnal variation in direct-count cell numbers, pH, and carbohydrate and ammonia levels in ruminal digesta from an animal fed the high-concentrate diet.

total numbers of bacteria over the diurnal period (Fig. 2 and 7). These data suggest that rumen bacterial numbers remain fairly constant in animals fed maintenance (metabolizable energy)-level, high-forage diets once a day. Maintenance of such a constant bacterial density indicates that production of cells (multiplication), detachment from particulate materials, or both are balanced by (i) the passage of cells to the lower tract, (ii) the engulfment by protozoa, (iii) cell death and lysis, and (iv) attachment of bacteria to particulate materials. The diurnal trends in direct and viable cell counts, although comparable, did not always match (Fig. 2 and 7). The decreases in direct cell counts observed after feeding (20 to 30%) were much smaller than the decreases in viable cell counts (40 to 60%). Thus, the loss of viable bacteria was not simply due to increased passage of digested materials (hence, passage of total cells) from the rumen at feeding, nor was it completely due to the dilution of the ruminal contents with feed and water. Rather, the fact that the losses in viability were greater than the losses in total bacterial numbers may be more related to rapidly changing ruminal conditions at feeding.

After feeding, some ruminal bacteria might uncouple their metabolism to adapt to the changed ruminal conditions, resulting in a phenomenon known as substrate-accelerated death (13, 31). Water intake at feeding could lead to osmotic shock effects (33, 34), but this phenomenon has not been studied with ruminal bacteria. Water and feed influxes usually cause the temperature of the ruminal contents to decrease. However, chilling the ruminal contents (14) or the roll tubes during preparation (25) has been shown to have little effect on culturable bacterial numbers. Ruminal bacteria are sensitive to pH values below 6.0 (41), but in the present study, the pH values were all within the tolerable range. Viability loss might have been due to oxygen entering via the water and feed; oxygen might have restricted those fermentative bacteria innately more susceptible to it (e.g., cellulolytics [21]).

The decline in viable bacterial numbers (as well as total cell numbers) after feeding might have been due to the attachment of ruminal bacteria to incoming feed particles. Initial colonization has been found to be rapid (within 15 min) as well as tenacious (C. G. Orpin, Soc. Gen. Microbiol. Q. 7:174, 1980). This attachment probably was greater when the animal was fed the high-forage diet, as the numbers of cellulolytic bacteria decreased more substantially than they did when the high-concentrate diet was given (cf. Fig. 2 and 7). The blending procedure used in our studies probably was not sufficient to dislodge all of these newly attached bacteria from the particulate materials.

After the decreased viability seen postfeeding, the numbers of viable bacteria increased steadily, reaching their highest values at the 16-h sampling time (Fig. 2 and 7). Since total cell numbers did not increase as rapidly, an increasing proportion of the population was viable as a result of growth. As feed particles undergo degradation, the associated bacteria might become more easy to dislodge by blending. Thus, the increased proportion of viable cells might have been due to growth, dislodgement, or both.

Diurnal variation in soluble-carbohydrate-utilizing subgroups within the rumen bacterial population generally followed the pattern of viable bacterial numbers (Fig. 2 and 7). Over time, the soluble-starch and glucose subgroups were consistently the most numerous of all tested subgroups. The reason may be simply that soluble starch and glucose, readily utilizable by most ruminal bacteria, permitted the growth of these subgroups on the recovery medium. The pectin-fermenting subgroup declined initially, particularly when the steer was fed the high-forage diet (Fig. 2, 3, and 7), but later increased, reaching maximal levels by 12 h. The increase reflected

the use of pectin substances, as these growth substrates can constitute 10 to 20% of the cell wall carbohydrate complex in alfalfa hay (23). The pectinolytic subgroup was composed of bacterial colonies surrounded by clearing zones after precipitation of unhydrolyzed polygalacturonic acid in the pectin DC medium with cetyltrimethylammonium bromide and constituted about one-third of the pectin group for each diet. For the population obtained when the high-forage diet was given, the contribution of *Bacteroides succinogenes* colonies to the pectinolytic subgroup might help explain the precipitant drop in this group after feeding. This drop was not observed in the pectin subgroup as a whole but was observed in the cellulolytic subgroup (Fig. 3).

The xylan-xylose and xylanolytic subgroups represented the hemicellulose-utilizing portion of the rumen bacterial population. Thus, the xylan-xylose DC medium supported the growth of bacteria which hydrolyzed the xylan backbone and thereby cleared the medium (the xylanolytic subgroup) and bacteria which utilized the xylose portion. The number of xylan-xylose-fermenting bacteria obtained when the high-concentrate diet was given was found to be nearly equal to the number obtained when the high-forage diet was given (cf. Fig. 2 and 7). However, the xylanolytic portion was much less numerous when the high-concentrate diet was given, probably owing to the lesser amounts of hemicellulosic materials in the high-concentrate feed.

Although the high-forage diet contained a great deal of cellulosic materials, the cellulose-degrading bacteria constituted the least numerous group of those tested (Fig. 2). When the high-forage diet was given, we observed that immediately after the animal was fed, the numbers decreased 85 to 95%, the most severe decline in viability observed. Rapid recovery ensued, however, with numbers increasing to the maximum at the 16-h sampling time. These numerical increases were probably due both to multiplication and to higher degrees of dislodgement from disintegrating feed particles.

Analysis of the cellular composition of the mixed bacterial populations for DNA, RNA, protein, and total carbohydrates (Fig. 4) indicated that the distribution of these components was as expected and was similar to previously published data, calculations for mixed ruminal bacteria, or both (12, 17–19). The bacterial population obtained when the high-concentrate diet was given increased in carbohydrate content, indicating possible intracellular carbohydrate storage.

Ruminal fluid parameters did not exhibit large diurnal variations. Shortly after feeding is the period of greatest fermentation activity (10, 15, 21, 43), and increased volatile fatty acid levels (Fig. 5 and 8). The number of viable bacteria at this time, however, does not reflect the increased microbial activity. Our observations support the concept that nonviable bacteria in the rumen continue to ferment and degrade feed materials and that these activities are not necessarily translated into replication. The acetate/propionate ratio remained near 4:1 (Fig. 5 and 8) for each diet. Such ratios have been observed in other studies with animals fed high-forage diets (43) but not with high-concentrate diets.

The total carbohydrate content of the clarified ruminal fluid showed a definite increase after feeding (Fig. 6 and 9). This represented release of soluble substrates into the fluid from the ingested feeds, particularly when the high-concentrate diet was given. Ammonia levels were nearly constant over time (ca. 14 mM; Fig. 6 and 9). The rather low fluctuations might have been due to the maintenance-level feed intake, the once-daily feeding regimen, the continual influx of plasma urea via the saliva into the rumen, or any combination of the three factors. Other studies in which animals were fed and sampled more frequently (48) have shown much greater variation over time, although the ranges in ammonia concentrations were similar. The pH range of 6.1 to 7.2 observed when the animal was fed the high-forage diet was well within what is considered normal. The pH profile of ruminal contents observed when the high-concentrate diet was given, however, was not as expected (Fig. 9). Generally, when diets containing large proportions of readily fermentable carbohydrate are fed, considerable depressions in pH values result (27, 28, 35). Except for the fact that the pH values were somewhat lower when the high-concentrate diet was given, as compared with the high-forage diet, the diurnal variation was similar.

In an overall sense, when the data obtained with the high-forage and high-concentrate diets were compared, there were more similarities than dissimilarities. The extent of these similarities was not expected, as the once-daily maintenance feeding regimen used in the studies was chosen to exacerbate the differences between the two diets. Higher intake levels (e.g., 1.5 to 2.5× maintenance levels), more frequent feedings, or both were not used because it was reasoned that these conditions would have caused a semicontinuous simultaneous ruminal fermentation of the different feed polysaccharides. Consequently, the resultant overlapping degradation patterns most likely would have precluded both the extrapolation of any individual-carbohydrate fermentation curves and the observations of diurnal changes in the microbial

population and ruminal environmental parameters. Our data did, however, show some discernible trends. When either diet was fed, the peak population densities of the complex-polysaccharide (cellulose, xylan, pectin)-fermenting subgroups were observed at or near the times (Fig. 2 and 7) during which the major portion of these substrates were predicted (Fig. 1) to undergo substantial degradation and fermentation. In addition, these subgroups generally constituted lesser percentages of the bacterial populations obtained when the high-concentrate diet was fed, as would be expected since this diet probably contained considerably lesser amounts of these complex polysaccharides.

The general rumen stability observed in our studies differs from results of previous studies that have shown substantial ruminal changes with diets, feed intake levels, or both (6, 16, 24, 42, 45, 49). Both types of data, however, are compatible when one considers evolutionary and ecological aspects of the ruminants and rumen microbes. The normal, evolutionarily developed, ruminal ecosystem behaves as a mature ecosystem able to withstand moderate changes in the amount and type of food supply without its structural base(s) collapsing. The microbial structural base is primarily composed of the complex-polysaccharide-degrading bacteria, whose activities can provide a relatively constant supply of nutrients to the other bacterial species. Without such a base, the ruminal ecosystem would resemble a primary type within which the numbers and types of bacteria could fluctuate unpredictably. Development of a primary-type ecosystem for the rumen could cause perturbations of the microorganisms and environmental parameters with ensuing collapse of the ecosystem structural base. Such microbial changes would most likely result upon the ingestion of low roughage-high concentrate diets that contain, on a dry matter basis, more components that are readily digestible, than do their high-forage counterparts. With high-concentrate rations, high-level feed intakes can be attained that elicit intense microbial fermentations in which acid production can no longer be adequately neutralized by the animal. The resultant ruminal pH depression will exert a detrimental effect upon the community structural base, as ruminal cellulolytic bacteria and protozoa are sensitive to a low pH (32, 36, 41). The population could become overwhelmed by acid-tolerant soluble-carbohydrate fermenters. Maintenance of these altered pH values and other environmental parameters would eventually result in selection for a primary ecosystem. This ecosystem is inherently less stable. The existence of unstable, primary ruminal ecosystems is indirectly supported by the limited diversity of microbial species observed in animals fed diets at high-intake levels (27, 28).

The aforementioned perturbations in the ruminal ecosystem were not observed in the present study because neither diet disturbed the ecosystem structural base(s). Had the diets been formulated on a dry matter basis (3:1), more energy would have been included in the high-concentrate diet. Since diets with equal amounts of energy were fed and the resultant ruminal populations were similar, the "form" of the diet (e.g., cellulose versus starch) must have caused relatively minor changes in the rumen. The maintenance-level daily feeding restricted the amount of available soluble nutrients (energy) to levels which permitted ruminal fermentations well within the normal buffering range. Consequently, the effects resulting from low pH and other drastically altered environmental factors did not occur. In this study, the ruminal ecosystem and its microbial community remained a relatively unperturbed, mature ecosystem regardless of the diet fed. From these observations, however, one could speculate that in lactating dairy cows or feedlot steers fed high levels of low-roughage feeds, the ruminal ecosystem would be primary-like and hence unstable and subject to large fluctuations in bacterial numbers and species diversity.

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