POSTPRANDIAL TRENDS IN ESTIMATED RUMINAL DIGESTA POLYSACCHARIDES AND THEIR RELATION TO CHANGES IN BACTERIAL GROUPS AND RUMINAL FLUID CHARACTERISTICS

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ABSTRACT

In a diurnal study, feedstuff and digesta polysaccharides, ruminal bacterial carbohydrate-fermenting groups, and selected ruminal fluid characteristics (ruminal pH, ammonia and volatile fatty acids) were measured in ruminal-cannulated Holstein steers fed high- or low-forage diets at maintenance level intake once daily. A procedure for the sequential extraction of soluble sugar, starch, pectin, hemicellulose and cellulose from feedstuffs was developed to measure these carbohydrates in dietary and ruminal digesta samples. Recovery of dry matter (determined chemically) using this scheme was 60 to 70%. Data were obtained within the ranges of those in the literature for similar feedstuffs and/or by similar methods. Dietary analysis by the sequential method yielded total recovery across all carbohydrate fractions of 87 and 81% for the high- and low-forage diets, respectively, and similar recoveries were obtained for the digesta samples. Analytical variation was small (< 15% CV), which permitted comparison of the carbohydrate profiles of the digesta over time. From these values, total ruminal digesta polysaccharide content was calculated and, when plotted over time, indicated that the disappearance per fraction corresponded with theoretical curves for ruminal fermentation of major feedstuff components. The postprandial variation within the bacterial population carbohydrate-fermenting groups was small, but changes were consistent with digesta component fermentation. Xylan- and cellulose-fermenting groups followed a pattern compatible with the disappearance of these polysaccharides from the rumen. In contrast, soluble sugar-fermenting groups predominated at all times despite the rapid rise and fall of these components in the digesta. Ruminal fluid pH, ammonia and total carbohydrate supported the digesta and bacterial trends observed. The data are interpreted to suggest that once daily maintenance feeding of high- or low-forage diets permits detection of digesta sugar and polysaccharide changes, supports a relatively stable microbial population while specific groups increase and decrease with the availability of substrate, and results in few differences in ruminal fluid traits.

(Key Words: Polysaccharides, Carbohydrates, Rumen Contents, Bacteria, Ammonia, Volatile Fatty Acids.)

Introduction

Quantification of polysaccharides in feedstuffs fed to ruminant animals and of their disappearance from digesta upon fermentation to volatile fatty acids can be used to describe one level of interaction between the gastrointestinal tract microorganisms and their host. In other words, digestion of soluble sugar, starch, pectin, hemicellulose and cellulose should be related to specific microbes and their activities. This can be measured if the biochemical catalytic pathways for these carbohydrates are known, and if the microorganisms capable of utilizing the various carbohydrates can be enumerated. The anaerobic fermentative pathways for the various sugars are known, although the steps by which complex polysaccharides are enzymatically degraded are still largely unknown (Salyers and Leedle, 1983). Differentiation of mixed populations of ruminal bacteria into carbohydrate-specific groups has been accomplished by use of a minimal
nutrient ruminal fluid-based medium to which only the carbohydrates of interest are added (Dehority and Grubb, 1976; Leedle and Hespell, 1980). Until now, however, the population sizes of these carbohydrate-specific or "functional" groups of bacteria have had to stand without documentation that degradation of a specific type of carbohydrate may have supported the growth of the respective bacterial group. A sequential extraction procedure with solvents and reagents adapted from wood chemistry methods was used to extract and quantify the polysaccharides from digesta (and feed) samples. Although this procedure may not be in complete agreement with other methods (Henneberg and Stohmann, 1864; Tilley and Terry, 1963; Goering and Van Soest, 1970) which attempt to determine differences in bioavailability of carbohydrates in various forages, our intent was to use a chemical method to quantify the sugars representing the soluble sugar, starch, pectin, hemicellulose and cellulose fractions of the feed and digesta. Then, our intent was to use these values to ascertain whether disappearances could be related to predicted fermentation patterns, or postprandial trends in bacterial carbohydrate-fermenting group numbers and other ruminal fluid characteristics.

Materials and Methods

Feed Samples and Preparation. The feed samples used were alfalfa hay (IFN 1--00--054) and University of Illinois concentrate mix number 64. The latter consisted of (percentage of dry matter): shelled corn (IFN 4--02--931), 84.5%, soybean meal (IFN 5--04--604), 12.9%; CaPO₄, 1.4%; trace mineral mix, 1.15%; vitamins A and D, .05%. The alfalfa hay was calculated to contain 2.44 Mcal metabolizable energy (ME)/kg; the concentrate mix 3.11 Mcal ME/kg (NRC, 1978). Samples of the hay and concentrate mix (as well as diet and digesta samples) were dried at 45 C and ground through a 1-mm screen in a Wiley mill.

Diet and Digesta Samples and Preparation. High- and low-forage diets were formulated using the alfalfa hay and concentrate mix, such that 75% of the calculated ME (1 x maintenance) was derived from either the alfalfa (high forage) or concentrate (low forage). On a percent dry matter basis, the dietary proportions were: high forage 77:23 and low forage 32:68, hay to concentrate, respectively. The amounts of dry matter fed to the 500 kg steers were 7.0 and 6.3 kg, high- and low-forage diets, respectively. For analysis, the high- and low-forage diets were prepared by mixing the components in their dry weight ratios.

Each of the two ruminal-cannulated Holstein steers was fed these diets once daily in a switch-over design with three replicate experiments (one/wk) performed on each steer on each diet. During switch-over, diets were changed in four steps of 5 d each. A 12-d adaptation period was allowed before initiation of sampling on the new diets. For each experiment, composite ruminal digesta samples were collected at -1, 2, 4, 8, 12 and 16 h postprandially (Leedle et al., 1982).

Extraction Scheme and Assays. The extraction scheme used is presented in figure 1. Total sugar content of each extract was determined colorimetrically using D-glucose or D-galacturonic acid as standard. Combinations of standards plus samples yielded additive values. Samples were run in triplicate, averaged and the amount of equivalent sugar calculated by comparison to a standard curve. Results are reported in glucose- or galacturonic acid-equivalents, and were multiplied by the appropriate dilution factors to obtain amounts in milligrams.

Soluble Sugar. The extraction of the soluble sugar component was accomplished by use of a micro-soxhlet extraction apparatus⁴. Samples (125 mg) were continuously refluxed in 20 ml 80% (v/v) ethanol for 3 h. The samples were drained overnight at room temperature. Each sample's solvent phase was brought to a constant volume (25 ml) with distilled water. These and all subsequent extracts were stored in stoppered glass tubes at 4 C until assayed.

Starch. The dried residue from the ethanol step was transferred to a 25-ml screw-cap centrifuge tube and 20 ml of 80% (v/v) dimethylsulfoxide (DMSO) in 8 N HCl added. The capped tubes were vortexed and incubated at 60 C for 30 min. The samples then were centrifuged (10,000 × g, 5 min, 20 C) and the supernatant solvent was transferred to a graduated cylinder. The residue was resuspended in 8 ml distilled water, centrifuged (10,000 × g, 5 min, 20 C), and the supernatant liquid added to

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⁴ Wheaton Scientific, Millville, NJ.
Sample 125 mg (250 mg)

3 hr micro-soxhlet 80% Ethanol (1)

SUGAR extract

25 ml supernatant (2)

residue

80% DMSO:8N HCl, 60°, 30 min (1,3)
Water wash
Formamide, polytron hot water wash

STARCH extract

50 ml (100 ml) supernatant (2)

residue

.5% amm. oxalate - reflux 45 min (1,4,5,6)
.5% amm. oxalate - reflux 90 min
hot water wash

PECTIN extract

25 ml supernatant (5)

residue

24% KOH under N2 (1,6)
60-72 hr at 39° with shaking water wash
neutralize residue .5 N H2SO4

HEMI-CELLULOSE extract

25 ml supernatant (2)

residue

30N H2SO4 - RT4h (2h) (1,6)
dilute 1 to 7, H2O, reflux 3 hr
NaHCO3 washes to neutralize residue
water wash

CELLULOSE extract

50 ml (25 ml) supernatant (2)

RESIDUE residue: dry

Data Analysis. Triplicate subsamples of each diet and digesta sample were extracted. The mean value for each extract of each sample was averaged across the three replicate experiments for each diet type per steer. Differences between the observed and expected carbohydrate

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Figure 1. Sequential scheme to extract and assess sugar, starch, pectin, hemicellulose and cellulose carbohydrate fractions from feedstuffs and digesta samples. Forage and digesta samples were treated identically. Parentheses indicate changes for concentrate only samples. Methods adapted from: (1) Southgate (1976); (2) Ashwell (1966); (3) James and Theander (1980); (4) Dekker and Richards (1972); (5) Blumenkrantz and Asboe-Hansen (1973); (6) Van Soest (1982).

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Note: The DMSO extract. Formamide (10 ml) was added to each residual sample and mixed well. The resultant suspension was disrupted using a Polytron homogenizer. The suspensions were centrifuged as described previously and the supernatant fluids were combined with the DMSO extract. The residual material was resuspended in distilled water (12 ml), centrifuged, and the liquid portion added to the DMSO-formamide extracts until the total combined volume was 50 ml. Using boiling water in these final washes increased the starch recovery.

**Pectin.** To each residue from the previous step, 8 ml of .5% (w/v) ammonium oxalate were added. The samples were refluxed at 130° C for 45 min, cooled, then centrifuged (10,000 x g, 5 min, 20 C). The supernatant fluid was decanted and the extraction was repeated for 90 min. After centrifugation, the residues were washed with hot distilled water (5 ml), centrifuged and the rinse combined with the oxalate extracts. The total extract was brought to 25-ml final volume with distilled water.

**Hemicellulose.** The previous residue was flushed with oxygen-free N2 for 1 min, then 10 ml of N2-equilibrated 24% (w/w) KOH were added and each sample was incubated at 39° C for 60 to 72 h in a shaking incubator. After incubation the samples were centrifuged (10,000 x g, 5 min, 20 C), the supernatant fluid decanted, washed with 8 ml distilled water, centrifuged and the washes were combined with the alkaline extracts. The residues were washed further with .5 N H2SO4, centrifuged and the fluid portion was added to the previous extract. Final volume was 25 ml.

**Cellulose.** Cellulose was extracted from the residue by resuspending it in 2 ml of 30 N H2SO4. The tubes were capped and incubated at room temperature for 4 h, after which 14 ml of distilled water were added and the tubes were refluxed for 3 h. The samples then were centrifuged (10,000 x g, 5 min, 20 C) and the supernatant fluids were transferred to graduated cylinders. To neutralize, the residues were washed twice by resuspending in 1.5 ml of saturated NaHCO3 and 7 ml distilled water. This was followed by distilled water alone to remove excess bicarbonate. The washes were combined with the previous extracts and brought to a final volume of 50 ml.

**Residue.** Neutralized residual materials were transferred to tared aluminum weigh pans. The samples were air-dried to remove excess water and then dried to a constant weight.

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*Brinkman Instruments, Westbury, NY.
*New Brunswick Scientific, Edison, NJ.
values for the dietary samples were tested for significance at the .05 level using the chi-square statistic (Snedecor and Cochran, 1980). The Means procedure (SAS, 1982) was used to generate descriptive statistics for all digesta sample observations. The grand mean values and their pooled standard deviations are presented and used for comparison. The figures were generated using TELLAGRAF™ (figures 2 and 3). Calculated postprandial ruminal digesta carbohydrate curves (figure 3 a and b) were generated using polynomial equations of various orders which were fit to the high- and low-forage data by minimizing the residual mean squares (TELLAGRAF, 1983). This procedure is analogous to fitting data to cubic splines to generate smooth curves, with the exception that the order of the polynomial is selected to minimize deviations about the fitted polynomial.

Calculations. Theoretical values for the blended diets were calculated by first determining the amount of carbohydrate present in each fraction of the concentrate and hay components. These amounts were corrected to 100% total carbohydrate recovered and then multiplied by the appropriate percentages of concentrate and alfalfa for the blended diets.

TELLAGRAF is a trademark of Integrated Software Systems, Corp., San Diego, CA 92121-1698.
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Figure 3. Calculated postprandial ruminal digesta carbohydrate pool sizes: A. High-forage diet, B. Low forage diet. Time_0 indicates feeding. See figure 2 for abbreviations.

Added together, these values yielded the calculated theoretical amounts. In order to address the amount of carbohydrate change in the ruminal digesta samples over time after feeding, it was necessary to have a time zero (T_0) ruminal contents composition. As the rumen was not sampled at T_0 in the experimental regimen used, an estimate was obtained using the preprandial (-1 h) samples and the diet composition. This value assumed that the diet was consumed at once. The proportion of digesta to feed consumed at T_0 was assumed to be 50:50 (Balch and Line, 1957). Thus, total ruminal dry matter at T_0 was 14.0 or 12.6 kg for the high- or low-forage diets, respectively. Carbohydrate values for the six fractions were related to the total dry matter at T_0. Note that the data for the disappearance of carbohydrate from the rumen use the -1-h contents composition as representing 23 h post-feeding.

Bacteria and Ruminal Fluid Components. Samples of whole ruminal contents were obtained as follows: The contents in the rumen first were manually mixed, then handfuls from at least four locations were placed in a CO_2-filled flask to one-half its volume. Additional handfuls were removed and squeezed through two layers of cheesecloth; the resultant liquid portion was added to completely fill the collection flask, which then was sealed with a one-way, check valve stopper and immediately transported to the laboratory. In the laboratory, the ruminal contents sample was blended under O_2-free CO_2, for 1 min at maximum speed in a Waring blender. The blended suspension was squeezed through two layers of cheesecloth into a second CO_2-filled flask and used. The total viable population was cultured on a complete carbohydrate (CC) medium, then anaerobically replica plated onto differential carbohydrate media (Leedle and Hespell, 1980). The carbohydrate fermenting groups assessed were: glucose, starch, pectin, xylan-xylose and cellulose-hydrolyzing. The variables
pH, ammonia, soluble carbohydrate and fermentation acids were measured as described (Leedle et al., 1982).

Results and Discussion

Polysaccharide Extraction Procedure

During development and refinement of the extraction procedure, several methods or modifications of each step were attempted to maximize recovery or to streamline the process. Since these are important for others to consider in evaluating these results relative to other techniques, a few points on each step are presented.

Soluble Sugar. Manual extractions were attempted (Southgate, 1976; James and Theander, 1980; Van Soest, 1982). Each involved centrifugation and decantation steps that proved to be cumbersome. The micro-soxhlet extraction apparatus suitable for 125- to 250-mg samples, eliminated the manual steps, prevented sample loss and was more time-efficient. The recommended extraction time of 6 h (Southgate, 1976) was reduced to 3 h without loss of sugar recovery. Further reduction of extraction time to 2 h reduced sugar recovery by 10%. Glucose was the primary sugar present in the soluble sugar extract as determined by thin layer chromatography on K5 plates developed in n-propanol:methanol:water (70:15:15; Sherma, 1981). Pre-extraction of the feed samples with chloroform or extraction of the ethanol extracts with hexane to remove fats and pigments to reduce assay interference (Southgate, 1976) was determined not to enhance sugar recovery, which was 97%.

Starch. The extraction of starches with boiling water (Southgate, 1976; Van Soest, 1982) proved to be ineffective. Only a 9% recovery was observed. Enzyme-mediated hydrolysis of the alpha-linked glucose chains has been given favorable mention in the literature (Southgate, 1976; Van Soest, 1982) but requires a pre-treatment step to disrupt the still-intact starch granules to disperse the substrate for enzymatic attack. We encountered difficulty in solubilizing the starch granules for quantitative recovery and to provide substrate for the enzymes. Autoclaving the sample in water (James and Theander, 1980) followed by hydrolysis with amyloglucosidase (1.2 IU) and β-glucosidase (.54 IU) for 3 h at 60 C (Southgate, 1976) recovered 22 to 55% of the soluble starch used as an added standard.

When multiple boiling water extracts were replaced by single extractions with DMSO-HCl and formamide, a fivefold increase in yield was observed. To improve further the two-step chemical extraction, mechanical methods for dispersing the granules were tried. The Polytron, with its combination of mechanical shearing and sonic energy disruption, yielded the highest recovery (66%). In combination with the two-step chemical extraction, recoveries to 80% were achieved (data not shown).

Pectin. Hydrolysis of pectin (5 mg) to uronic acid with 1 mg polygalacturonase10 in 10 ml .1 M acetate buffer was tried (Dekker and Richards, 1972), but due to the high background color, was not adopted. The typical hot water plus chelating agent (ammonium oxalate or diethylene dinitrioltriacetic acid) extraction procedures for pectic materials from plant cell walls (Dekker and Richards, 1972; Blumenkrantz and Asboe-Hansen, 1973; Southgate, 1976; Van Soest, 1982) were tested. In our hands, two reflux extractions (45 and 90 min) with .5% ammonium oxalate followed by a hot water wash was best (recoveries were 70%).

Hemicellulose. A sequential extraction with increasing concentrations of base (5 to 24% KOH) was described in the literature as an effective means for extracting specific hemicellulose fractions (A, B and C; Southgate, 1976; Van Soest, 1982). Since only the total amount was of interest, a single incubation with 24% KOH (under N2) at 39 C for 16 h was tried. Fifty-six percent of a xylan standard was recovered. Increasing the incubation time to 60 h improved recovery to 88%.

Cellulose. Various acid strengths were tried, including 15, 26, 30 or 36 N (concentrated) H2SO4. The most effective (82% recovery) was 30 N H2SO4 (data not shown).

Diet, Digesta and Ruminal Characteristics

The extraction scheme yielded data that were similar to those previously published for comparable feedstuffs or assayed by similar methods (table 1; see figure 1 for references). The differences between the observed and expected dietary carbohydrate values were not significant. Recovery of starch was lowest (62

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10 Sigma Chemical Co., St. Louis, MO.
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For the low-forage diet, some starch-related glucose equivalents may have been recovered in the hemicellulose fraction, which was elevated (136 and 110% for the low- and high-forage diets, respectively). The overall recovery for the total diets of greater than 80% was very good considering the complexity of the dried plant materials assayed.

The high- and low-forage diets contained similar amounts of soluble sugar, pectin, and hemicellulose, but the high-forage diet had 3.5 times as much cellulose, while the low-forage diet had nearly twice as much starch (table 1). At maintenance level feeding, the difference in starch and cellulose should have exaggerated the resultant fermentations, especially with a once-daily feeding regimen. Our expectation was that a single feeding of a low-forage diet would elicit a relatively narrow peak in rate of substrate-associated fermentation (bacterial activity and numbers) after a short postprandial interval. In contrast, no such peak would occur after a single feeding of a high-forage diet.

Literature values (Hungate, 1966; Goering and Van Soest, 1970; James and Theander, 1980; Van Soest, 1982), as well as our data, confirm that dry matter consumed is approximately equivalent to the dry matter degraded or passed on to the lower tract during a 24-h period. Thus, the diet plus the preprandial digesta contributed 50% each to the T₀ composition of the ruminal digesta (table 2 and 3). Values at T₀ were the highest for sugar, starch, and pectin, whose postprandial values then declined, reflecting solubilization in the rumen and utilization by the microflora and microfauna. The same trend from T₀ was not apparent for hemicellulose and cellulose. Although some decrease in these carbohydrates was observed at T₂, the amount thereafter increased. This may be attributed to the relatively slow degradation rate of these polysaccharides; as the more-soluble materials were removed from the digesta, a greater proportion of the less-soluble materials was represented. This phenomenon was most apparent in the low-forage diet (table 3) in which the dietary amount of hemicellulose and cellulose were low, although the amounts in the digesta at T₁₆ were similar to those in the high-forage diet (table 2).
### TABLE 2. SUGAR AND POLYSACCHARIDE COMPOSITION OF DIETARY AND POSTPRANDIAL RUMINAL DIGESTA SAMPLES: HIGH-FORAGE DIET

<table>
<thead>
<tr>
<th>Carbohydrate fraction&lt;sup&gt;b&lt;/sup&gt;</th>
<th>Diet</th>
<th>−1</th>
<th>0&lt;sup&gt;c&lt;/sup&gt;</th>
<th>2</th>
<th>4</th>
<th>8</th>
<th>12</th>
<th>16</th>
</tr>
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<tbody>
<tr>
<td>Soluble sugars</td>
<td>4.18</td>
<td>.38</td>
<td>2.28</td>
<td>1.14</td>
<td>.99</td>
<td>.71</td>
<td>.65</td>
<td>.38</td>
</tr>
<tr>
<td>Starch</td>
<td>19.3</td>
<td>2.42</td>
<td>10.87</td>
<td>7.62</td>
<td>4.72</td>
<td>3.64</td>
<td>2.0</td>
<td>2.83</td>
</tr>
<tr>
<td>Pectin</td>
<td>1.12</td>
<td>.37</td>
<td>.75</td>
<td>.42</td>
<td>.38</td>
<td>.38</td>
<td>.33</td>
<td>.39</td>
</tr>
<tr>
<td>Hemicellulose</td>
<td>12.6</td>
<td>18.55</td>
<td>15.58</td>
<td>15.51</td>
<td>16.77</td>
<td>16.95</td>
<td>16.34</td>
<td>16.95</td>
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<tr>
<td>Cellulose</td>
<td>31.65</td>
<td>29.23</td>
<td>30.44</td>
<td>24.6</td>
<td>27.31</td>
<td>28.69</td>
<td>31.17</td>
<td>36.31</td>
</tr>
<tr>
<td>Total recovered</td>
<td>76.32</td>
<td>65.69</td>
<td>70.88</td>
<td>61.66</td>
<td>64.41</td>
<td>65.87</td>
<td>63.95</td>
<td>73.64</td>
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</table>

<sup>a</sup>Data are mean mg/125-mg sample. Diet samples, n = 6; digesta samples, n = 18.

<sup>b</sup>Pooled standard deviation of the mean: soluble sugars, .28; starch, 7.3; pectin, .15; hemicellulose, 1.7; cellulose, 2.3; residue, 1.6.

<sup>c</sup>Estimate, see text.

### TABLE 3. SUGAR AND POLYSACCHARIDE COMPOSITION OF DIETARY AND POSTPRANDIAL RUMINAL DIGESTA SAMPLES: LOW-FORAGE DIET

<table>
<thead>
<tr>
<th>Carbohydrate fraction&lt;sup&gt;b&lt;/sup&gt;</th>
<th>Diet</th>
<th>−1</th>
<th>0&lt;sup&gt;c&lt;/sup&gt;</th>
<th>2</th>
<th>4</th>
<th>8</th>
<th>12</th>
<th>16</th>
</tr>
</thead>
<tbody>
<tr>
<td>Soluble sugars</td>
<td>4.06</td>
<td>.68</td>
<td>2.37</td>
<td>4.23</td>
<td>5.24</td>
<td>2.69</td>
<td>1.33</td>
<td>.88</td>
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<tr>
<td>Starch</td>
<td>32.4</td>
<td>3.18</td>
<td>17.78</td>
<td>16.64</td>
<td>9.94</td>
<td>3.62</td>
<td>2.12</td>
<td>3.0</td>
</tr>
<tr>
<td>Pectin</td>
<td>1.07</td>
<td>.32</td>
<td>.69</td>
<td>.40</td>
<td>.37</td>
<td>.40</td>
<td>.41</td>
<td>.38</td>
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<tr>
<td>Hemicellulose</td>
<td>12.0</td>
<td>19.98</td>
<td>16.00</td>
<td>14.88</td>
<td>17.04</td>
<td>17.9</td>
<td>19.46</td>
<td>19.78</td>
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<tr>
<td>Cellulose</td>
<td>9.05</td>
<td>30.19</td>
<td>19.62</td>
<td>21.51</td>
<td>24.64</td>
<td>29.41</td>
<td>27.15</td>
<td>26.24</td>
</tr>
<tr>
<td>Residue</td>
<td>3.93</td>
<td>12.93</td>
<td>8.43</td>
<td>8.27</td>
<td>10.6</td>
<td>11.0</td>
<td>13.23</td>
<td>15.33</td>
</tr>
<tr>
<td>Total recovered</td>
<td>62.47</td>
<td>67.29</td>
<td>64.89</td>
<td>65.92</td>
<td>67.84</td>
<td>65.02</td>
<td>63.69</td>
<td>65.61</td>
</tr>
</tbody>
</table>

<sup>a</sup>Data are mean mg/125 mg sample. Diet samples, n = 6; digesta samples, n = 18.

<sup>b</sup>Pooled standard deviations of the mean: soluble sugars, .74; starch, 1.1; pectin, .14; hemicellulose, 1.8; cellulose, 1.6; residue, 2.4.

<sup>c</sup>Estimate, see text.
Based on the total amount recovered from all fractions, the proportion that each fraction represented at $T_X$ was traced over the postprandial period (figure 2). For the high-forage diet, the soluble sugar content remained at 1 to 2% throughout the postprandial period (figure 2a). Starch was lowest at -1 h (3%), highest at 2 h (12.5%, due to feed intake), then declined. Little change in pectin occurred. Hemicellulose and cellulose were lowest after feeding (25 and 41%, respectively) and reached their highest levels at -1 h (hemicellulose, 28%) and 16 h (cellulose, 50%) post-feeding. The relative increases for these two fractions may be the result of degradation of the other carbohydrates.

When steers were fed low forage, the increase in sugar and starch due to feed intake was obvious (figure 2b). In fact, the soluble carbohydrate showed an impact on the distribution of the insoluble components in the digesta over time. Their disappearance was reflected in larger percentages of the hemicellulose and cellulose fractions. After feeding, the latter components were at their lowest proportions (23% for hemicellulose, 33.5% for cellulose). As the concentrate was digested, these represented greater proportions of the ruminal contents at any time. Cellulose levels peaked at 8 h at 45% of the digesta.

Of interest was the amount of sugar and polysaccharide disappearing from the ruminal contents, regardless of whether it was by solubilization, degradation or passage to the lower tract. In order to calculate the amount disappearing, some assumptions were made. These included values for total ruminal dry matter, turnover rate and percentage digestion, none of which was measured directly in this study. Estimates for these values were taken from Balch and Line (1957) and Hungate (1966) for animals of similar size that were fed similar diets. For our calculations, an average of 7.0 or 6.3 kg ruminal dry matter at the pre-feeding sample time was used along with a 2-d turnover rate. An amount of dry matter equal to that of the diet fed then would disappear (be degraded or passed from the rumen) on a daily basis. For this estimation, 7.0 or 6.3 kg were regressed over time at 4.2% per hour for both diets. Total ruminal dry matter amounts of 14.0, 13.4, 12.8, 11.7, 10.5, 9.3 and 7.3 kg or 12.6, 12.1, 11.6, 10.5, 9.5, 8.4 and 6.6 kg high or low forage, respectively, were used to multiply the carbohydrate values extracted from the digesta samples at 2, 4, 8, 12, 16 and 23 (-1) h after feeding (data from tables 2 and 3).

Estimated total carbohydrate disappearing was 62 and 55% including residue for the high- and low-forage diets, respectively (table 4). These values are slightly higher than the 50 to 55% disappearance values in the literature (Hungate, 1966). Overall, 4,108 and 3,010 g carbohydrate were solubilized, degraded or passed from the rumen for the high- and low-forage diets, respectively. When steers were fed the high-forage diet, an estimated 1,704 g cellulose were degraded compared with 394 g when fed low forage, while 1,626 g starch were degraded when fed low forage compared with 1,076 g when fed high forage (table 4). The estimated amounts of cellulose and starch degraded relative to the amounts fed were: 96 and 100% (high forage) and 86 and 100% (low forage), respectively. Similar amounts of soluble sugar, pectin and hemicellulose disappeared postprandially regardless of diet.

Rates at which the polysaccharides disappeared were represented by the computer-generated lines (figure 3). For the high-forage digesta samples (figure 3a), most of the soluble sugar and starch disappeared by 8 h; thereafter their levels were < 30 and 200g, respectively. Little postprandial change in observed pectin concentrations may be due to the dynamics of this pool to which the contribution of pectin degradation is balanced by microbial utilization of galacturonic acid moieties. Hemicellulose and cellulose were expected to follow similar postprandial trends, but hemicellulose followed a gradual trend downward, while cellulose exhibited a biphasic curve. The cellulose curve showed an initial decrease between $T_0$ and $T_2$, which may have been due to physical displacement out of the rumen, followed by a plateau during which this carbohydrate may have been beginning to undergo extensive degradation. Finally, there was a decrease after $T_{16}$ wherein cellulolytic bacteria may have succeeded in removing a sizeable portion of this material, or else the feed particles may have been reduced to a size sufficiently small to readily pass through the reticulo-omasal orifice. Although this trend may be explained, it may be that our estimated data and calculation method, in common with most current methods, is not sensitive enough to distinguish the behaviour of cellulose in these samples.

The low-forage diet (figure 3b) starch disappearance curve suggested that sampling at 2 h post-feeding may have missed the greatest
### TABLE 4. POSTPRANDIAL LEVELS OF DIGESTA POLYSACCHARIDES IN THE RUMENa

<table>
<thead>
<tr>
<th>Diet</th>
<th>Carbohydrate fractionb</th>
<th>Ruminal digesta sample postprandial time, h</th>
<th>% disappearance (T₀ − T₂₃)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>0</td>
<td>2</td>
</tr>
<tr>
<td>High forage</td>
<td>Soluble sugar</td>
<td>255</td>
<td>122</td>
</tr>
<tr>
<td></td>
<td>Starch</td>
<td>1,217</td>
<td>818</td>
</tr>
<tr>
<td></td>
<td>Pectin</td>
<td>84</td>
<td>45</td>
</tr>
<tr>
<td></td>
<td>Hemicellulose</td>
<td>1,745</td>
<td>1,665</td>
</tr>
<tr>
<td></td>
<td>Cellulose</td>
<td>3,409</td>
<td>2,641</td>
</tr>
<tr>
<td></td>
<td>Residue</td>
<td>1,228</td>
<td>1,328</td>
</tr>
<tr>
<td></td>
<td>Overall disappearance</td>
<td>62</td>
<td></td>
</tr>
<tr>
<td>Low forage</td>
<td>Soluble sugar</td>
<td>239</td>
<td>409</td>
</tr>
<tr>
<td></td>
<td>Starch</td>
<td>1,793</td>
<td>1,608</td>
</tr>
<tr>
<td></td>
<td>Pectin</td>
<td>70</td>
<td>39</td>
</tr>
<tr>
<td></td>
<td>Hemicellulose</td>
<td>1,612</td>
<td>1,438</td>
</tr>
<tr>
<td></td>
<td>Cellulose</td>
<td>1,978</td>
<td>2,079</td>
</tr>
<tr>
<td></td>
<td>Residue</td>
<td>850</td>
<td>799</td>
</tr>
<tr>
<td></td>
<td>Overall disappearance</td>
<td>55</td>
<td></td>
</tr>
</tbody>
</table>

aData are g carbohydrate/total ruminal contents estimated as described in text.

bPooled standard deviation of the mean high-forage diet: soluble sugars, 28; starch, 658; pectin, 13; hemicellulose, 153; cellulose, 183; residue, 144; low-forage diet: soluble sugars, 60; starch, 89; pectin, 11; hemicellulose, 129; cellulose, 130; residue, 195.
starch concentration in the rumen because this substrate can be degraded (solubilized) within a short time after feeding (Takahashi and Nakamura, 1969). Degradation and release of glucose units from the less-soluble starch concentrate materials may have contributed to the soluble sugar profile, which peaked at T₄ and thereafter declined. Similar in total amount as their high-forage counterparts, hemicellulose and residue also followed similar decreasing postprandial trends. The shape of the cellulose curve may indicate a different fermentation pattern than observed with the high-forage diet. From T₂ to T₈, the amount of cellulose increased, perhaps due to disproportionate disappearance of the more-readily digestible components; from T₈ to T₁₆, total cellulose content in the rumen decreased perhaps due to fermentation and passage. On the other hand, the technique and data manipulations may not be refined enough to get a clear picture for all the polysaccharide fractions, particularly cellulose between T₀ and T₁₂.

Due to the derivation of the data in table 4 and figure 3, the values must have the same variation, but in the computer-generated curves (figure 3) the variation was removed. Although calculated and based on the assumptions outlined above, these trends are consistent with predicted theoretical fermentation patterns for the predominant carbohydrates in the rumen. The latter set of theoretical curves is based upon solubilization and fermentation properties of each carbohydrate type found in most feedstuffs (Johnson, 1976; Leedle et al., 1982). Expressed as rates of fermentation, the theoretical curves predict that soluble carbohydrates like starch are solubilized rapidly in the rumen soon after ingestion and likewise are removed rapidly via the microflora and microfauna. This is followed in time by the slightly less-soluble pectic substances, which is followed by the lesser-soluble polysaccharides, hemicellulose and cellulose, which require a longer ruminal residence time before the majority of these materials is extensively degraded. In general, these trends are reflected in those of figure 3a and b.

Bacterial carbohydrate-fermenting group postprandial profiles were consistent with those of the digesta carbohydrates. Total viable bacteria for either diet showed a downward trend after feeding (figures 4 and 5). Accounting for this decrease may be passage of digesta from the rumen, death by oxygen intrusion with the feed and water consumed, a raising of the redox potential due to feeding events, attachment of microbes to freshly ingested feed particles (subsequently not removed by blending), or an uncoupling of cellular metabolism such that viability is not maintained (Dawes and Ribbons, 1962; Postgate and Hunter, 1964). For the high-forage diet the soluble sugar (glucose) and starch fermenters remained at high population levels throughout the period and rapidly fermented these carbohydrates by 4 to 8 h after feeding. The pectin group, although numerous, could not be assessed relative to its digesta pectin profile because the latter was too low in concentration (figure 3). The xylan (hemicellulose)- and cellulose-fermenting bac-

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**Figure 3.** Postprandial variation in the soluble, starch, glucose, pectin and xylan concentrations in ruminal digesta from high-forage diet fed steers. S = soluble starch, G = glucose, P = pectin, X = xylan-xylose, C-L = cellulolytic. Data are means and standard deviations from three replicate experiments; arrow indicates time of feeding.
tential groups, followed their respective digesta polysaccharide profiles. Postprandial variation of the xylan-xylose group mirrored the calculated changes in ruminal hemicellulose. Likewise the decrease and subsequent increase in cellulolytic bacteria corresponded to the plateau and subsequent decrease in cellulose content.

Many of the same trends in bacterial numbers were observed for the low-forage diet (figures 3b and 5). Although the digesta soluble sugar and starch showed distinct disappearance curves on this diet, no corresponding change was observed in these carbohydrate-fermenting groups. This supports the fact that these carbohydrates are used readily by most ruminal bacteria and that the ruminal soluble sugar pool, which arises from the degradation of all plant polysaccharides, is a dynamic one. Additionally, its composition is dependent upon the composition of feedstuff materials being degraded. The types of sugars in the pool then change over the fermentation cycle, for example, between T₀ and T₈, the pool may be predominantly from starches and pectin, and between T₈ and T₂₄ it may be of sugars from xylans and cellulose. The flux through this pool would support a consistently large population of soluble-sugar-utilizing bacteria, as was observed in this study.

For the low-forage diet, the pectin group exhibited postprandial changes, but digesta pectin levels were too low to correlate trends (figure 5). The xylan-xylose group numbers paralleled the starch and glucose groups with time. After the initial decrease between 2 and 4 h, the cellulolytic bacterial group increased in size as the ruminal cellulose content decreased. This relationship suggests that a critical population size may need to be reached before ruminal cellulose disappearance can be detected.

Ruminal fluid fermentation acid concentrations in the samples taken when steers were fed the high- and low-forage diets are given in figures 6 and 7, respectively. Total fermentation acid concentrations were just above 100 mM. Acetate represented 60 to 70 mol/100 mol of the total. The acetate to propionate ratios were 4 to 1 regardless of diet, which may have been due to the once-daily maintenance feeding regimen. The branched-chain fermentation acids and valerate averaged between .5 and 1.5 mM, with no distinct trends apparent.

Corresponding values for pH, ammonia and total carbohydrate are given in table 5. Ruminal pH values dropped from a prefeeding level of 6.5 and 6.7 to lows of 6.1 and 5.6 at 8 h for the high- and low-forage diets, respectively, before returning to the prefeeding values. Neither diet caused extended periods below pH 6.0. Thus, it is probable that little selective pressure was placed on the microbial population of the rumen as a result of low pH. Ruminal ammonia concentrations increased slightly after feeding, 22.8 to 24.2 mM on the high-forage diet and 21.3 to 22.8 mM on the low-forage diet, before leveling off at 18 or 15 mM, respectively. Ammonia was not limiting microbial growth in this study. Total carbohydrate content in ruminal fluid varied only slightly on the high-forage diet, while showing a distinct peak (210
In conclusion, the sequential extraction scheme for the major polysaccharides in feedstuffs and digesta worked well except for the low recovery of pectin. Use of this technique permitted us to calculate a postprandial ruminal digesta fermentation profile. The once-daily maintenance feeding regimen was chosen to exaggerate the disappearance of specific components while minimizing overlapping fermentation curves. Indeed, the observed rapid rise and fall of sugar and starch followed later on by the more gradual disappearance of hemicellulose and cellulose, suggested that maintenance feeding separated the fermentation of these feedstuff components. Although concomitant changes in glucose- and starch-fermenting bacterial groups did not occur, consistent increases in xylan and cellulose groups were observed. Regardless of whether the high- or low-forage diet was fed, similar bacterial populations, fermentation acid profiles, pH, ammonia and carbohydrate concentrations were observed. An interpretation is that dietary composition alone may not be the major determinant in species makeup or fermentation acid or pH change in the rumen. Rather, it may be the overall energy content of the diet fed, as opposed to the form of the caloric components, that affects the fermentation and the distribution of functional groups in the ruminal ecosystem. These conclusions are particularly applicable to ruminants fed at maintenance levels. Whether similar conclusions can be drawn for ruminants fed high feed-intake levels associated with intensive dairy or beef (feedlot) farming practices needs to be examined.

μg/ml) at 2 h post-feeding on the low-forage diet, corresponding well to the digesta starch profile.

In conclusion, the sequential extraction scheme for the major polysaccharides in feedstuffs and digesta worked well except for the low recovery of pectin. Use of this technique permitted us to calculate a postprandial ruminal digesta fermentation profile. The once-daily maintenance feeding regimen was chosen to exaggerate the disappearance of specific components while minimizing overlapping fermentation curves. Indeed, the observed rapid rise and fall of sugar and starch followed later on by the more gradual disappearance of hemicellulose and cellulose, suggested that maintenance feeding separated the fermentation of these feedstuff components. Although concomitant changes in glucose- and starch-fermenting bacterial groups did not occur, consistent increases in xylan and cellulose groups were observed. Regardless of whether the high- or low-forage diet was fed, similar bacterial populations, fermentation acid profiles, pH, ammonia and carbohydrate concentrations were observed. An interpretation is that dietary composition alone may not be the major determinant in species makeup or fermentation acid or pH change in the rumen. Rather, it may be the overall energy content of the diet fed, as opposed to the form of the caloric components, that affects the fermentation and the distribution of functional groups in the ruminal ecosystem. These conclusions are particularly applicable to ruminants fed at maintenance levels. Whether similar conclusions can be drawn for ruminants fed high feed-intake levels associated with intensive dairy or beef (feedlot) farming practices needs to be examined.

Figure 6. Postprandial variation in direct count bacterial numbers, fermentation acids, and acetate/propionate molar ratios in ruminal digesta from high-forage diet fed steers. A = acetate, P = propionate, B = butyrate, V = valerate, IV = isovalerate plus 2-methylbutyrate, IB = isobutyrate. Data are means and standard deviations from three replicate experiments; arrow indicates time of feeding.

Figure 7. Postprandial variation in direct count bacterial numbers, fermentation acids and acetate/propionate molar ratios in ruminal digesta from low-forage diet fed steers. See figure 6 for abbreviations. Data are means and standard deviations from three replicate experiments; arrow indicates time of feeding.
### Table 5. Variation in Direct Microscopic Count Bacterial Numbers, Soluble Carbohydrate, Ammonia and pH in Rumenal Fluid from Steers Fed a High- or Low-Forage Diet

<table>
<thead>
<tr>
<th>Diet</th>
<th>Variable</th>
<th>Ruminal digesta sample postprandial time, h</th>
<th>-1</th>
<th>2</th>
<th>4</th>
<th>8</th>
<th>12</th>
<th>16</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>High forage</td>
<td>Direct microscopic count, ( \times 10^{-9} )/ml</td>
<td></td>
<td>26.7</td>
<td>23.8</td>
<td>21.7</td>
<td>28.4</td>
<td>24.0</td>
<td>27.4</td>
</tr>
<tr>
<td></td>
<td>Soluble</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>carbohydrate, ug/ml</td>
<td></td>
<td>155.5</td>
<td>157.9</td>
<td>127.4</td>
<td>166.0</td>
<td>148.4</td>
<td>147.4</td>
</tr>
<tr>
<td></td>
<td>Ammonia, mM</td>
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<td>22.8</td>
<td>24.2</td>
<td>20.0</td>
<td>16.6</td>
<td>14.0</td>
<td>16.0</td>
</tr>
<tr>
<td></td>
<td>pH</td>
<td></td>
<td>6.5</td>
<td>6.5</td>
<td>6.4</td>
<td>6.1</td>
<td>6.4</td>
<td>6.6</td>
</tr>
<tr>
<td>Low forage</td>
<td>Direct microscopic count, ( \times 10^{-9} )/ml</td>
<td></td>
<td>23.1</td>
<td>20.4</td>
<td>19.0</td>
<td>21.1</td>
<td>22.2</td>
<td>24.9</td>
</tr>
<tr>
<td></td>
<td>Soluble</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>carbohydrate, ug/ml</td>
<td></td>
<td>125.7</td>
<td>204.5</td>
<td>104.6</td>
<td>117.3</td>
<td>126.4</td>
<td>115.8</td>
</tr>
<tr>
<td></td>
<td>Ammonia, mM</td>
<td></td>
<td>21.4</td>
<td>24.9</td>
<td>21.0</td>
<td>11.8</td>
<td>13.5</td>
<td>16.8</td>
</tr>
<tr>
<td></td>
<td>pH</td>
<td></td>
<td>6.7</td>
<td>6.3</td>
<td>5.8</td>
<td>5.6</td>
<td>5.8</td>
<td>5.9</td>
</tr>
</tbody>
</table>

\(^a\)Data are means \( n = 18 \). Pooled standard deviations high-forage diet: count, 1.2; carbohydrate, 2.0; ammonia, 2.3; pH, 4; low-forage diet: count, 1.4; carbohydrate, 4.9; ammonia, 2.8; pH, .8.
Literature Cited


