EFFECT OF COLIFORM BACTERIA, FEED DEPRIVATION, AND pH ON RUMINAL D-LACTIC ACID PRODUCTION BY STEER OR CONTINUOUS-CULTURE MICROBIAL POPULATIONS CHANGED FROM FORAGE TO CONCENTRATES

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ABSTRACT

Fecal coliform bacteria were isolated from three herbivores (cattle, horse, and red panda) and shown to produce primarily the D-form of lactate, plus acetate and ethanol when grown anaerobically in 1.0% glucose broth. To evaluate coliform contribution to D-lactate acidosis in cattle, experiments involving a forage-adapted steer (fasted or normally fed) and four 500-ml fermentors were compared during 3 d of grain overload. In both systems, coliforms and D- and L-lactic acid production were greater from fasted than from normally fed steer inoculum. With fasted inoculum, coliform counts peaked (3 x 10^7/ml at 7 h after initial engorgement) and receded to 10^5/ml by the time D-lactate concentration peaked, indicating that bacteria other than coliform were responsible for the delayed peaking of D- (48 h) compared with L-lactate (24 h). Increases in lactobacilli more closely mimicked D-lactate increases than did changes in coliforms. The comparisons between the steer and fermentors showed many similar shifts in end-products and groups of bacteria, more so with the experiment initiated with fasted than with normal inoculum. With normal inoculum, VFA content and moles of butyrate/100 mol of VFA were greater in vitro than in vivo; VFA content presumably was larger because of VFA absorption in vivo. In a separate experiment, cultures initiated with identical inoculum and given the same amount of feed accumulated more lactate when pH was permitted to decrease to 5.0 than when pH was maintained at 5.5 for 6.0 or above, indicating the role buffers can have in controlling acidosis during diet change to concentrates.

Key Words: Ruminal Bacteria, Coliform Bacteria, Lactates, Lactobacillus, Intestines, Feces


Introduction

Lactic acid is produced from glucose by Escherichia coli under anaerobic conditions (Garvie, 1980). In addition to D-lactate, other products produced by E. coli from pyruvate include ethanol, acetate, formate, and succinate (Tammy and Kaplan, 1968). If produced in large amounts, D-lactate causes greater acidosis problems than L-lactate in ruminant tissues after rapid fermentation of high-concentrate diets (Dunlop and Hammond, 1965) because it is metabolized more slowly at high concentrations than L-lactate (Harmon et al., 1984).

The purpose of the present studies was 1) to assess the ability of fecal coliforms from various herbivore species to produce D-lactate and 2) to study the relationship between the predominance of ruminal coliforms and lactobacilli and the accumulation of D- and L-lactate in a steer and in a continuous-culture system whose ruminal microorganisms were from either a normally fed or a fasted steer. Based on known physiological responses by E. coli (Bergeim, 1940; Brownlie and Gran, 1967; Wolin, 1969), we hypothesized that a forage-adapted, fasted steer’s ruminal inoculum, because of its low content of VFA and

1The authors are grateful to M. A. Smith and J. A. Klimper for technical assistance.
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Received August 10, 1990.
Accepted February 12, 1991.

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Experimental Procedures

Experiment 1. The form of lactic acid produced by fecal coliforms from various herbivores was examined. Feces excreted after the 0800 feeding but before 1100 were collected from the following: a beef steer (250 kg BW) fed 2.8 kg (DM basis) of orchardgrass hay each at 0800 and 1600; a lactating dairy cow (545 kg BW) given ad libitum amounts of a 27.5% corn-silage, 22.5% alfalfa haylage and 50% concentrate diet (DM basis); a quarterhorse mare (450 kg BW) given alfalfa hay in ad libitum amounts; a red panda (5.7 kg) fed 154 g (DM basis) of bamboo and gruel per day (Warnell, 1988); and a dairy calf (50 kg) receiving 5 kg of milk and ad libitum amounts of calf starter. The dairy cow concentrates (DM basis) contained the following (% of total): ground corn, 42.0; barley, 31.9; soybean meal (48% CP), 19.4; trace mineral salt, .8; sodium bicarbonate, 1.6; MgO, .4; limestone, 1.6; dicalcium phosphate, 1.1; potassium and magnesium sulfate, .2; Vitamin A (107 IU/kg), .5; Vitamin D3 (1.5 × 107 IU/kg), .125; and Vitamin E as dl-alpha-tocopherol acetate (44,092 IU/kg), .125.

Experiment 2: Steer and Fermentor Feeding and Sampling. A ruminally fistulated steer (256 kg BW) was fed twice daily 2.8 kg (DM) of a 90% forage diet (Slyter and Putnam, 1967) for 2 wk (phase 1). The steer was accustomed to an individual pen with automatic water cup and had daily access to outdoors. Diet intake was sufficient to provide 756 g of CP and 11.9 Mcal of ME (calculated), which was at least 1.9 and 2.4 times the protein and ME requirements for maintenance, respectively (NRC, 1984). The surgical procedure to establish the ruminal fistula was performed 2 yr previously. Feed was last offered 48 h and water 24 h before surgery. The steer was cannulated while being restrained, standing in a chute designed with head gate, squeeze, and removable side panels. The surgical procedure was similar to that for the open ruminal fistula (Dougherty, 1955) except that the incision to remove ruminal wall was made during the initial operation rather than 4 to 5 d later. Initially, a ruminal cannula4 7.62 cm in diameter with regular internal and external flanges was inserted. Ten days after surgery, the sutures were removed and a 10.16-cm ruminal cannula4 was inserted. Cannulas were placed in a hot solution of septosol, rinsed with saline, and powdered with furacin before they were inserted.

Before surgery, analgesics (Torbugesic5 and Rompun6, 15 and 8 mg, respectively) were administered into the jugular vein using a temporary catheter. Anesthesia was provided by 2% Lidocaine7 injected subcutaneously and then intramuscularly in a circular pattern 8 cm in diameter. Penicillin (6,600 units per kilogram intramuscularly in rear quarter daily for 3 d) was administered to counter any possible postsurgical infection. The fistula was cleansed with mild soapy water and peroxide as needed. Aseptic technique and sterile instruments were used to conduct surgery.

During the first phase of the coliform study, the steer was fasted for 3 d and all ruminal contents were manually removed and mixed. Before returning the digesta to the rumen, sufficient inocula was removed to fill four 500-ml fermentors to their overflow capacity and to provide samples for microbial enumeration and VFA, glucose, and lactic acid analysis. Remaining digesta (50 kg) was returned to the rumen. After the ruminal contents were returned to the rumen of the steer, the steer was fed 4 kg and the fermentors were given 40 g of the all-concentrate (corn, soybean meal, cane molasses, mineral, vitamin mix) diet described by Bond et al. (1984). The steer consumed all the diet within 90 min. At the second feeding, (8 h, d 1) the steer received 3 kg and the fermentors 30 g of the all-concentrate diet. For the steer, diet that was not consumed within 60 min was placed into the rumen through the fistula. After both the 24- and 48-h samplings the steer and fermentor cultures directly received 1.5 kg and 15 g of concentrates, respectively. Ruminal digesta samples were removed at 0, 2.5, 7, 23.9, 47.9,
and 72 h for pH, VFA, glucose, and lactic acid assays. The pH was immediately determined. The fermentation was immediately inhibited in other samples with 50% H₂SO₄ (1 ml/10g) and the samples were cooled to 3°C. Samples were then centrifuged and the supernatant retained at 3°C until it was assayed (within 3 wk). Except for the 2.5-h sampling, ruminal contents were also removed for microbial enumeration. At 24, 48, and 72 h, the most recently voided steer feces were collected for determining numbers of coliforms. After 3 d of concentrate feeding, the steer was provided 2 liters of ruminal fluid from a steer fed orchardgrass hay. The steer was then fed the same 90% forage diet during a second 2-wk phase that it received in Phase 1. The steer was then not fasted as in the first phase, but the entire ruminal contents were manually removed, mixed, and sampled, and 50 kg was placed back into the steer and 500 g was placed into each of four fermentors as in the first phase; concentrates were fed as in the first period. In both periods, in vitro culture maintenance involved incubation at 38°C in the continuous-culture artificial rumen system described by Slyter (1975). Four fermentors were filled with whole ruminal digesta to their effluent overflow capacity of 500 ml and infused with a modified McDougall’s artificial saliva:tap water in a 60:40 ratio to give a liquid turnover of 1.5 volumes per day. The buffer was slowly sparged continuously with CO₂. McDougall’s saliva (1948) was modified to contain 7.8 mg of urea per 100 ml, and half the sodium bicarbonate and dibasic phosphate, on a molar basis, was substituted with the potassium form. At the start of the experiment a stopper in each fermentor dome was removed and the system was flushed with CO₂ to remove air. Other management and equipment procedures were similar to those described by Slyter and Putnam (1967).

Experiment 3. Ruminal inoculum was obtained from a ruminally fistulated steer (245 kg BW) different from the steer used in Exp. 2. The steer was fed 2.8 kg (DM basis) of the 90% forage diet and was fistulated and managed in the same way as the steer in Exp. 2. Fluid, squeezed from the digesta through cheesecloth, was placed into each of six fermentors to their 500-ml effluent overflow volume. The management of fermentors and infusion of buffers was as in Exp. 2, with these exceptions: A .48-cm pelleted diet rather than the ground diet described by Bond et al. (1984) was fed, and, in addition to the 40 g fed initially, 16 g of diet was fed daily for 3 d. The latter involved feeding an average of .67 g of pelleted diet at hourly intervals using a timer and a gravity-fed pellet dispenser (Slyter, 1975). Culture pH was not permitted to go below 5.0, 5.5, or 6.0 in each of two fermentors by infusing a 10% sodium hydroxide solution to supplement the buffer that was infused as in Exp. 2. The sodium hydroxide solution infusion was controlled by individual pumps as directed by a pH controller (Slyter, 1975). After the first day, the infusion of only the artificial saliva:tap water buffer was required to maintain the culture pH above the minimum desired. Based on the results of Slyter et al. (1974), samples were collected at 0, 1, 3, 8, 16, 24, 48, and 72 h for glucose, lactic acid, and VFA analysis.

Microbial Assays. In Exp. 1, feces were placed in a dilution fluid as described by Bryant and Burkely (1953) and aerobically diluted and inoculated into MacConkey’s Agar. Each of two plates was inoculated with .1 ml of 10⁻¹ to 10⁻² diluted sample and 15 to 20 ml of MacConkey’s Agar Medium was added; upon solidification the plates were inverted and incubated for 24 h at 38°C. Bright red colonies were picked into MacConkey’s Agar slants and incubated overnight at 38°C. Freshly grown cells from the slant’s water of syneresis were transferred using a Pasteur pipette into 3 ml of pH glucose medium of Bryant and Small (1956) modified to contain clarified ruminal fluid No. 1 of Bryant and Robinson (1961) instead of whole ruminal fluid. This transfer was done using the anaerobic technique of Hungate (1950) and a 20% CO₂-80% N gas phase. After a 7-d incubation, 1 ml of broth was removed into 2 ml of 12% perchloric acid and assayed for D- and L-lactic acid. The remainder was acidified with .02 ml 50% (vol/vol) H₂SO₄ and assayed for VFA and ethanol.

In Exp. 2, three microbial assays were used: MacDonkey’s Agar as described for Exp. 1; lactobacilli medium of Rogosa et al. (1951), as modified by Slyter et al. (1968); and the nonselective 98-5 medium of Bryant and Robinson (1961). The lactobacilli medium was inoculated with .1 ml of 10⁻³ to 10⁻⁷ dilutions, two 18 × 150-mm roll tubes at each dilution. The 98-5 medium was inoculated with .1 ml each from 10⁻⁵, 10⁻⁶, and 10⁻⁷ dilutions, two
18 × 150-mm roll tubes at each dilution. Aseptic technique, the dilution fluid of Bryant and Burkey (1953), a CO₂ gas phase, and the anaerobic technique of Hungate (1950) were used to dilute the contents before incubating the lactobacilli and 98-5 media in roll tubes at 38°C for 2 and 4 d, respectively. Colonies were counted manually using a marking pencil and an AO darkfield Quebec Colony counter.

**Chemical Assays.** Fermentor and steer ruminal contents were assayed for pH using a pH meter and combination electrode. Samples were assayed for VFA (Slyter, 1979), ethanol (Slyter et al. 1986), and methane (Slyter and Putnam, 1967) by gas chromatography. Glucose (Sigma, 1983) and D- and L-lactic acid (Sigma, 1976) were assayed enzymatically. Ethanol from coliforms in Exp. 1 was also assayed enzymatically (Sigma, 1989).

**Statistical Methods.** In Exp. 2, effects of inoculum source on ruminal microbes and fermentation products were evaluated using the GLM procedure of SAS outlined by Barr et al. (1976). Main effects were inoculum source and culture unit. Error term was interaction between inoculum source × culture unit. Comparisons in tables were made across all sampling times. Comparisons in figures were also made for each sampling time. In Exp. 3, effects of pH on fermentation products were evaluated by GLM with pH × culture unit interaction serving as the error term. The multiple comparison procedure of Duncan was used to test for significant differences between means.

**Results and Discussion**

**Herbivore and Coliform Fermentation of Glucose.** Regardless of herbivore species or diet fed, the lactate produced from glucose under anaerobic conditions by the fecal coliforms isolated was primarily of the D form (Table 1). These results are consistent with those for E. coli as reported by Garvie (1980) and Tarmy and Kaplan (1968) and are taken as evidence that if E. coli becomes predominant in a population, D-lactic acid production for E. coli might cause acidosis problems. However, because coliforms produce a number of end-products, they are less likely to lower pH of the rumen as much as homolactic bacteria such as *Streptococcus bovis* and lactobacilli. At lower pH, E. coli converts a greater proportion of glucose to lactate than at pH 6.8, the pH of the broth we used. Up to 50% of glucose is fermented to lactate at pH 6.3, compared with only 20% at pH 7.0 and 3% at pH 7.5 (Tikka, 1935). It is not clear whether coliforms contributed to ethanol accumulation from fasted inoculum in Exp. 2. About 20% of the glucose fermented has been reported to form ethanol (Tikka, 1935) regardless of pH. Because ethanol is slowly metabolized in the rumen (Moomaw and Hungate, 1963), its production by coliforms could result in its accumulation. In this regard perhaps ethanol would serve as an indicator of coliforms should they become predominant in the rumen unless they fail to produce it in a mixed culture, as does *Ruminococcus albus* (Wolin, 1975). The coliforms were shown to produce relatively similar amounts of D-lactate, ethanol, and acetic acid plus traces of L-lactate.

**Effect of Grain Overload and Ruminal Inoculum Source on Ruminal Coliform and Lactobacilli Proliferation, and Glucose and D-Lactate Accumulation.** Although inoculum from a fasted steer did result in greater
coliform growth ($P < .05$) in a forage-adapted ruminal population given concentrates than did inoculum from a normally fed steer (Table 2), their numbers only reached 7.4 to 7.7 (log10/ml) from the fasted inoculum (Figure 1). This occurred at 7 h, much sooner than the 30-mM peak of D-lactate that occurred at 48 h (Figure 2). In Exp. 2, the lack of synchronization between the coliform bacteria (Figure 1) and D-lactate accumulation (Figure 2), plus the observation that coliforms made up at most <1% of the lactobacilli or of the total ruminal bacterial count (Table 2 and Figure 1), is taken as evidence that coliforms were not major contributors to acidosis problems in this experiment. When they have composed a large portion of the gut’s microbial population in a sheep with grain overload their increases did not coincide with an increase in lactate (Allison et al., 1975). Though $E. coli$ are not ordinarily predominant in ruminal bacterial populations (Mann et al., 1954; Kern et al., 1974), they are more predominant in the small and large intestines of ruminants (Kern et al., 1974; Allison et al., 1975) and intestines of other herbivores (Kern et al., 1974; Warnell, 1988). These differences in $E. coli$ numbers between the rumen and the lower intestines would be consistent with the inhibitory effect of fatty acids on coliforms, particularly at pH < 6.5 (Bergeim, 1940; Wolin, 1969), which are more common to the rumen (Kern et al., 1974). Reduced feed intake, which has been shown to increase growth of $E. coli$ in the bovine rumen (Brownlie and Grau, 1967), is likely to precede a change from forage to concentrates if cattle are shipped long distances to feedlots. Then, if concentrates were available ad libitum, cattle would have a greater chance for acidosis if more coliforms proliferated in the rumen and lower intestines due to cattle’s fasted condition. This, in turn, could cause more total D-lactate production and acidosis problems. In some cases, D-lactate has been the predominant form of lactate in ruminal fluid of sheep (Ryan, 1964; Huntington and Britton, 1978). The above comments are consistent with the thesis that cattle on full feed should always have concentrates available (Elam, 1976) because this will minimize feed deprivation followed by grain engorgement and acidosis problems.

TABLE 2. EFFECT OF INOCULA FROM A FASTED OR NORMALLY FED STEER ON RUMINAL ACCUMULATION OF END-PRODUCTS OR ON COLIFORM, LACTOBACILLI, AND TOTAL VAILABLE BACTERIA IN RUMINAL CULTURES CHANGED FROM FORAGE TO ALL-CONCENTRATE DIET (EXP. 2)

<table>
<thead>
<tr>
<th>Item</th>
<th>Normally fed</th>
<th>SE*</th>
<th>$P$-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glucose, mM</td>
<td>1.0</td>
<td>.32</td>
<td>&lt;.05</td>
</tr>
<tr>
<td>Lactate, mM</td>
<td>10.2</td>
<td>3.33</td>
<td>&lt;.05</td>
</tr>
<tr>
<td>L-Isomer</td>
<td>56.4</td>
<td>11.45</td>
<td>&lt;.05</td>
</tr>
<tr>
<td>Bacteria, colonies/ml, log10</td>
<td>4.69</td>
<td>1.95</td>
<td>.87</td>
</tr>
<tr>
<td>Lactobacilli</td>
<td>9.14</td>
<td>7.85</td>
<td>.54</td>
</tr>
<tr>
<td>Total</td>
<td>9.40</td>
<td>9.30</td>
<td>.17</td>
</tr>
<tr>
<td>pH</td>
<td>5.8</td>
<td>6.2</td>
<td>.29</td>
</tr>
<tr>
<td>Total VFA, mM</td>
<td>19.0</td>
<td>101.3</td>
<td>12.97</td>
</tr>
<tr>
<td>Molar proportions, mol/100 mol</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Acetate</td>
<td>70.2</td>
<td>58.6</td>
<td>3.34</td>
</tr>
<tr>
<td>Propionate</td>
<td>18.8</td>
<td>21.6</td>
<td>3.32</td>
</tr>
<tr>
<td>Isobutyrate</td>
<td>1.8</td>
<td>1.1</td>
<td>.37</td>
</tr>
<tr>
<td>Butyrate</td>
<td>4.1</td>
<td>14.5</td>
<td>2.53</td>
</tr>
<tr>
<td>Isovalerate</td>
<td>2.5</td>
<td>2.3</td>
<td>.62</td>
</tr>
<tr>
<td>Valerate</td>
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<tr>
<td>Hexanoic</td>
<td>.5</td>
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<td>.23</td>
</tr>
<tr>
<td>Ethanol, mM</td>
<td>2.0</td>
<td>6</td>
<td>1.02</td>
</tr>
</tbody>
</table>

*Standard error of treatment mean based on samples collected from four fermentors and one steer at 2.5, 7.0, 23.9, 47.9, and 72 h for glucose and lactate and these same times minus the 2.5-h sampling for coliforms, lactobacilli, and total bacteria.
Figure 1. Effect on in vitro and in vivo numbers of total ruminal viable bacteria, lactobacilli, and coliforms over time as influenced by inocula source after changing from a 90% forage to an all-concentrate diet. Inoculum was provided by either a fasted or a normally fed steer adapted to a 90% forage diet. Fasted, in vivo (→→); fasted, in vitro (O--O); normally fed, in vivo (++++); and normally fed, in vitro (O--O). Asterisk at base, along time scale, indicates significant difference due to inoculum source. Asterisk at top indicates that fermentor and steer response differed (P < .05, .01, .001 = *, **, ***). Common standard error of treatment x time means calculated from GLM analysis was .3 for total viable bacteria, .9 for lactobacilli, and 1.4 for coliforms.
Figure 2. Effect on in vitro and in vivo ruminal concentrations of glucose and D- and L-lactate over time as influenced by inoculum source after changing from a 90% forage to an all-concentrate diet. Inoculum was provided by either a fasted or a normally fed steer adapted to a 90% forage diet. Fasted, in vivo (→); fasted, in vitro (○○); normally fed, in vivo (+-+); and normally fed, in vitro (○○○). Asterisk at base, along time scale, indicates significant difference due to inoculum source. Asterisk at top indicates that fermentor and steer response differed (P < .05, .01, .001 = *, **, ***). Common standard error of treatment × time means calculated from GLM analysis was 1.1 for glucose, 2.5 for D-lactate, and 6.1 for L-lactate.
The present results do not show a consistent relationship between glucose accumulation and proliferation of coliforms. Glucose peaked in the cultures from the fasted steer at 2.5 and 24 h, reaching levels of 2 mM each time, in contrast to approximately 0.25 mM at 7 and 48 h (Figure 2). The second peak of glucose did not promote growth of coliforms, as did the first. Numbers were between 1.5 and 5.5 (log_{10}/ml) at 48 h or 2 to 6 log_{10}/ml less than at 7 h (Figure 1). With inocula from the fed steer, lactobacilli count did not increase until the 24-h sampling, and coliform count did not increase with time (Figure 1). Lactobacilli accounted for an appreciable portion (>10%) of viable bacteria when >10 mM lactate accumulated (Figures 1 and 2).

The greater accumulation of glucose ($P < .05$) in the cultures from the fasted steer than in those from the fed steer (Table 2) seems to be more an effect of inoculum than an effect of pH, because reducing pH in Exp. 3 to 5.0, 5.5, or 6.0 with the same inocula (Table 3) gave similar ($P > .10$) glucose concentrations. Ruminal turnover of both lactate and glucose have been shown to be related to the amount of readily fermentable carbohydrate in the diet (Mackie et al., 1984).

Figures 1 and 2 shown that the microbial L- and D-lactate responses from initially identical populations were very similar in fermentors and in ruminal fluid of steers during the first day but were less alike after 72 h of refeeding concentrates. Greatest microbial differences between the fermentors and steer occurred with inoculum from the fed steer; the fermentors in this case contained fewer coliforms (Figure 1).

**TABLE 3. EFFECT OF pH ON RUMINAL GLUCOSE, LACTIC ACID, AND VOLATILE FATTY ACID (VFA) ACCUMULATION OF IN VITRO CULTURES CHANGED FROM FORAGE TO ALL-CONCENTRATE DIET (EXP. 3)**

<table>
<thead>
<tr>
<th>Item</th>
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<tbody>
<tr>
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<tr>
<td>Lactate, mM</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>51.7c</td>
</tr>
<tr>
<td>D-lactate</td>
<td>4.3c</td>
</tr>
<tr>
<td>L-lactate</td>
<td>47.4d</td>
</tr>
<tr>
<td>Total VFA, mM</td>
<td>106.3b</td>
</tr>
<tr>
<td>Molar proportions, mol/100 mol</td>
<td></td>
</tr>
<tr>
<td>Acetate</td>
<td>55.2b</td>
</tr>
<tr>
<td>Propionate</td>
<td>27.7c</td>
</tr>
<tr>
<td>Isobutyrate</td>
<td>7.7</td>
</tr>
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<td>Butyrate</td>
<td>13.5c</td>
</tr>
<tr>
<td>Isovalerate</td>
<td>1.0</td>
</tr>
<tr>
<td>Valerate</td>
<td>1.5</td>
</tr>
<tr>
<td>Glucose, mM</td>
<td>2.2</td>
</tr>
<tr>
<td>Methane, mM/d</td>
<td>22.0d</td>
</tr>
</tbody>
</table>

*Standard error of treatment based on two fermentors and 0, 1, 3, 8, 16, 24, 48, and 72 h samplings except for methane, which involved a composite 0- to 72-h sample.

b,c,dValues on same row that do not have a common superscript letter differ ($P < .05$).
in Exp. 3 by transient reduction in pH than was acid production by forage-fed cultures kept at pH 5.0 on a continuous basis (Slyter et al., 1966). The effects of pH on bacterial population shifts have been stated to be related to the length of time pH remains below a critical value (Mackie et al., 1984). The critical pH is dependent on physiological traits of the bacteria present and the dynamics of the ecosystem, such as flow rates. In this study, the potential for propionate production by the lower pH cultures in both Exp. 2 and 3 was indicated by the much greater amounts of propionate plus lactate accumulating in these cultures (Tables 2 and 3).

A minimum pH of 5.0 resulted in lower \( P < .05 \) production of butyrate and methane (tested only in Exp. 3) (Table 3), results consistent with pH effects noted with forage-fed cultures (Slyter et al., 1966). Less methane was also produced at pH 5.5. The high molar proportion of acetate corresponds to the low proportion of butyrate of cultures from fasted inoculum in Exp. 2 (Table 2).

The accumulation of ethanol likely indicates instability or change in the ruminal microbial population. Ethanol accumulation was greater \( P < .05 \) in cultures from fasted than in cultures from fed inoculum. Ethanol has been shown to accumulate during acidosis in ruminants (Allison et al., 1964), probably partly due to lower pH and its effect on fermentation (Slyter et al., 1966). Competition for hydrogen by methanogenic bacteria keeps organisms that, as pure cultures, are capable of producing ethanol from producing it in mixed cultures (Wolin, 1975). Thus, the lower amounts of methane produced in Exp. 3, and presumably by cultures from fasted inoculum in Exp. 2, are consistent with the increases in ethanol accumulation observed (Table 2).

The addition of concentrates to cultures from fed inoculum in Exp. 2 (Table 2 and Figure 3) increased lactate less than did concentrate addition to cheesecloth-strained, fed inoculum in Exp. 3 (Table 3). Lactate also accumulated less in cultures from fed inoculum than in cultures from fasted inoculum in Exp. 2 (Table 2 and Figure 3). It is not possible to differentiate between effects of animal source or processing (straining) of inoculum on fermentation response to concentrates. Increased forage content in the inoculum that was not strained, via its ability to buffer or stabilize pH, may have reduced lactate accumulation. Whole sheep ruminal contents have been shown to have faster lactate disappearance rates than strained ruminal contents (Kunkle et al., 1976). In this regard, there is need to compare the ratio of lactobacilli (or lactate-producing bacteria) to lactate-utilizing bacteria in whole and strained ruminal contents.

Overall, the in vitro cultures mimicked in vivo microbial and fermentation changes of the steer well. Similarity of response was better between fermentors and steer in Exp. 2 when concentrate was added to cultures initiated with inoculum from a fasted rather than from a fed steer. The total VFA and moles of butyrate/100 mol was about twofold greater in the fermentors than in the steer within 24 h after concentrate was added to fed inoculum (Figure 3). The moles of acetate/100 mol was about 50 in vitro and about 60 in vivo (data not shown). The reduced acetate and increased butyrate found in vitro from the fed inoculum is consistent with the presumed greater conversion of acetate to butyrate in vitro than in vivo. Similarities in response to inoculum (Table 2) in Exp. 2 between the fermentors and steer include the following: with fed inoculum both in vitro and steer cultures had higher pH \( P < .05 \), less glucose and lactate, and fewer lactobacilli and coliform \( P < .05 \) than with fasted inoculum. In addition, lactate was predominant for cultures initiated with fasted inoculum and VFA production was predominant for cultures initiated with fed inoculum.

The fecal coliform count was approximately 100 times greater than the ruminal coliform count in the steer (Exp. 2, data not shown). Further experiments involving D-lactate measurements will be required to determine whether the coliform populations in the lower tract can contribute sufficiently to cause acidosis to the host animal. A sheep engorged with grain did not have much lactate but had high coliforms (Allison et al., 1975). There is also need to determine what organisms are responsible for increased ruminal production of D-lactate after long-term feeding of concentrates to sheep (Huntington and Britton, 1978). In the latter study, because lactate utilization was not decreased and racemization was judged to have been minimal, the accumulation of D-lactate could be the result of proliferation of a strictly D-lactate producer. To our knowledge, such a group of organisms common to the rumen have not been demonstrated.
Figure 3. Effect on in vitro and in vivo ruminal concentrations of total volatile fatty acids, total lactate, and moles of butyrate/100 mol VFA as influenced by inoculum source after changing from 90% forage to all-concentrate diet. Inoculum was provided by either a fasted or a normally fed steer adapted to a 90% forage diet. Fasted, in vivo (— — —); fasted, in vitro (○—○); normally fed, in vivo (——); and normally fed, in vitro (○——○). Asterisk at base, along time scale, indicates significant difference due to inoculum source. Asterisk at top indicates that fermentor and steer response differed (p < .05, .01, .001 = *, **, ***). Common standard error of treatment × time means calculated from GLM analysis was 2.5 for total VFA, 4.8 for total lactate, and 3.0 for butyrate.
Lactobacilli, however, could contribute to the accumulation of D-lactate. As pH drops, eventually S. bovis growth is inhibited and almost a monoculture of lactobacilli can develop (Dawson and Allison, 1988), and these lactobacilli produce D-lactate. It would seem that the later peak of D-lactate than of \( \text{L-lactate} \) that occurs with a diet change from forage to concentrate as observed here (Figure 2) and in earlier studies (Slyter et al., 1974; Bond et al., 1984) but does not occur in fasted, concentrate-adapted cattle refeeding concentrate (Bond et al., 1984) is a result of S. bovis, which produces L-lactate growing out first followed by lactobacilli and other bacteria that produce D- or both D- and L-lactate then growing out. It does not seem that this later peak of D-lactate is the result of coliforms becoming predominant and producing D-lactate, as we proposed before the present studies were conducted.

**Implications**

The bacterial and fermentation responses due to diet change that occurred with the in vitro artificial rumen and the steer’s rumen were more alike initially than on the third day; this indicates that some differences may exist between the systems. Still, the same major changes in types of bacteria and fermentation were noted, giving credence to the use of the artificial rumen to study the ruminal contribution to acidosis in ruminants. Second, differences during the first day between concentration of end-products in the ruminal digesta of the fermentors and the ruminant might serve to estimate differences in absorption rates of lactate producer in the present experiments, the lactobacilli after grain overload in feeddeprived cattle in contrast with the changes in types of bacteria and fermentation between the systems. Still, the same major differences during the first day between concentration of end-products in the ruminal digesta of the fermentors and the ruminant might serve to estimate differences in absorption rates of lactate producer in the present experiments, the lactobacilli after grain overload in feeddeprived cattle in contrast with the changes in types of bacteria and fermentation between the systems.

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