Dietary Fermented Ammoniated Condensed Whey and Postprandial Effects on Blood and Urine Acid-Base Metabolites in Lactating Cows

R.A. ERDMAN, L.S. BULL, and R.W. HEMKEN
Department of Animal Sciences
University of Kentucky
Lexington 40546

ABSTRACT
Effects of a fermented whey product containing 38% lactic acid, time postfeeding, and temperature on acid-base metabolism were studied with four lactating Jersey cows in a 4 × 4 Latin square. Treatments included 0, 33, 66, and 100% replacement of soybean meal with fermented whey in a 60% corn silage, 40% concentrate diet (dry basis). Increasing fermented whey to 100% reduced intake and milk production, 27 and 32% compared to animals fed 0 and 33% fermented whey diets. Fermented whey had no effect on blood pH, carbon dioxide tension, bicarbonate, and potassium whereas increasing fermented whey above 33% replacement reduced glucose and sodium in plasma 15 and 40 mg/100 ml from controls. Increasing consumption of fermented whey to 100% replacement increased urinary ammonium excretion 1365/meq per day and slightly decreased urine pH, which suggested changes in acid excretion. Effects of time postfeeding on blood traits were measured from samples taken at 0, 1, 2, 3, 4, 6, and 8 h. Blood pH was higher at 1 to 3 h as compared with 4 to 8 h postfeeding, whereas carbon dioxide tension and bicarbonate increased 10.6 mm mercury and 6.3 meq/liter at 1 h and declined afterward. Period effects for blood acid-based characteristics showed decreasing blood carbon dioxide tension with increasing ambient temperature.

INTRODUCTION
Incidence of anorexia when ruminants are fed high concentrate diets has been attributed to acidic conditions in the rumen (10, 12, 13). Gross symptoms of clinical acidosis have been described (13) and include diarrhea, anorexia, hyperventilation, and dehydration. Ruminant animals can undergo significant changes in acid-base metabolism and not show clinical symptoms (23). Some workers have attributed small intake and production on high concentrate diets to a subclinical acidosis (12).

The importance of blood and urinary metabolites in measurements of acid-base status in ruminant animals only recently has been realized. This has led to sensitive blood gas measurements, which can monitor subtle respiratory responses to changes in acid-base metabolism (2, 12, 27). Some studies dealing with environmental effects on ruminant animal physiology have shown a pronounced effect of temperature on blood and urinary acid-base metabolism (1, 4, 8, 9, 16). Other workers have studied effects of changes of acid load and diet on renal responses in sheep and calves (22, 23, 24).

Only limited study has been given to the effect of time postfeeding on acid-base metabolism. Billitzer and Jarrett (2) found that there was a sharp decline in blood pH and base excess immediately following meals in sheep, which they associated with loss of bicarbonate ion through salivation. More recent work (12) has failed to show this response in beef steers. To the authors knowledge, no experiments have used lactating cows to study the effect of time postfeeding on acid-base metabolism. It was the objective of this study to determine the effect of time postfeeding on changes in blood and urinary acid-base metabolites as part of another larger study dealing with utilization of ammonium lactate in lactating cows (5).

EXPERIMENTAL PROCEDURES
Four lactating Jersey cows in the study were
part of another project of utilization of energy and nitrogen in fermented ammoniated condensed whey (FACW). This product contains 38% lactic acid (approximately 20% D and 80% L isomer) and 7% nitrogen (85% nonprotein nitrogen). Its relatively high lactic acid concentration suggested that it may have an effect on acid-base metabolism (13). Experimental rations consisted of 60% corn silage and 40% concentrate on a dry basis. Treatments consisted of FACW replacement for 0, 33, 66, and 100% of the soybean meal in the concentrate portion of the ration in Table 1. Estimated chemical composition of the complete diet also is in Table 1.

Treatments were assigned in a 4 x 4 Latin square arrangement. Experimental periods were 42 days in length with blood and urine samples taken on day 42 in periods 1, 2, and 4 and day 28 in period 3 because of scheduling conflicts. Days 1 through 21 were for ration adjustment, followed by a 7-day digestion trial. Days 29 through 41 were for indirect calorimetry measurements in (5). The experiment began in late May, 1978, and was completed in November, 1978. To monitor factors related to effects of period, high and low thermometer temperature measurements were on days animals were sampled.

On the afternoon preceding sampling days, jugular catheters were inserted. Catheter extenders fitted with 3-way stopcocks were attached to the catheters and terminated on top of the withers. Catheters and extenders were held in place with surgical tape smeared with bonding cement. Catheters and extenders were filled with heparanized saline (10 IU/ml) to prevent clotting. Urinary catheters (18 French, 75 cm³ ovoid bulb) were inserted at the same time as blood catheters and were connected with polyethylene tubing to carboys for collection of urine.

Animals were fed individually their respective rations in equal portions twice daily at 0400 and 1500 h. Feed was offered ad libitum to allow 5 to 10% feed refusals. The bulk of daily feed consumption occurred within 1 to 2 h after fresh feed was offered, although there were instances of smaller meals later during the feeding period. Patterns of feed consumption and milk production comparing means for sampling days and those for 1 wk prior to

| TABLE 1. Ingredient and chemical composition of experimental rations. |
|--------------------------|----------------|----------------|----------------|----------------|
| FACW Replacement         | 0              | 33             | 66             | 100            |
| Concentrate ingredient   | (%) As fed     |
| Corn                     | 50             | 50             | 50             | 50             |
| Soy 44                   | 45             | 30             | 15             | 0              |
| Limestone                | 1.8            | 1.9            | 1.3            | 1.3            |
| Dical                    | 1.8            | 1.7            | 2.2            | 2.2            |
| Dynamate                 | .25            | .60            | .90            | 1.1            |
| TMI salt                 | 1.0            | 1.0            | .90            | .80            |
| ADE source               | .15            | .15            | .15            | .15            |
| FACW                     | 0              | 15             | 30             | 45             |
| Estimated chemical composition (total ration dry matter) |
| NE 1 (meal/kg)⁴         | 1.68           | 1.72           | 1.80           | 1.75           |
| Crude protein %¹         | 16.8           | 17.3           | 17.5           | 16.7           |
| Acid detergent fiber     | 21.1           | 21.0           | 20.3           | 20.7           |
| Calcium %                | .67            | .69            | .64            | .64            |
| Phosphorus %             | .45            | .43            | .45            | .44            |
| Potassium %              | 1.08           | 1.07           | 1.07           | 1.05           |
| Sodium %                 | .21            | .24            | .26            | .29            |
| Magnesium %              | .26            | .27            | .27            | .27            |
| Sulfur %                 | .18            | .18            | .17            | .17            |

⁴ Actual measured components taken from Bull et al. (5).

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Figure 1. Comparison of dry matter intake (●) and milk production (○) between sampling days and means for 1 wk prior to sampling. Line indicates Y = X.

There was no major effect of catheter placement on intake and production on the sampling day that followed.

On sampling days, blood and 12-h overnight urine samples were taken prior to offer of fresh feed at 0400 h. Urine then was pooled over 2-h intervals at 2, 4, 6, and 8 h after feeding for pH measurements. Urine collections taken from 7-day digestion trials were used for ammonia concentration and volume measurements. Blood samples were drawn at 0, 1, 2, 3, 4, 6, and 8 h postfeeding. During blood sampling, heparinized saline first was removed followed by 5 ml of blood into a syringe in which dead space had been filled previously with heparin (1000 IU/ml). Air bubbles were expelled, and syringes were capped and placed tip down into crushed ice for later blood gas analysis. Another 20 ml of blood was drawn and placed immediately in previously heparinized centrifuge tubes. These samples were centrifuged at 7000 x g, and plasma was stored at −20°C for later analysis. Following blood sampling, heparinized saline then was placed back in the catheter to prevent blockage.

Whole blood stored anaerobically was analyzed for carbon dioxide tension (pCO₂, mm Hg) and pH electrometrically. All samples were analyzed within 3 h of the last sampling time during the day. Other workers using similar storage techniques reported that storage time of samples in ice had little effect on pCO₂ and pH concentrations in whole blood. Blood bicarbonate ([HCO₃⁻]) was calculated from blood pH and pCO₂ by a pKₐ of 6.1 fitted into the Henderson-Hasselbalch equation: pH = 6.1 + log ([HCO₃⁻]/0.03·pCO₂).

Potassium (K) and sodium (NA) in plasma were determined by atomic absorption. Glucose of plasma was measured colorimetrically by glucose oxidase enzyme (7) while L-lactate was determined by procedures of Hochella and Weinhous (11). Urinary ammonium ion was measured by an automated modification of procedures described by Bolleter et al. (3).

In the analysis of variance, a 4 x 4 Latin square made up the main plot and time post-feeding made up the sub-plot in a split-plot design (6). Differences between treatment means were tested only after significant F tests by Fisher’s least significant difference method (6).

RESULTS AND DISCUSSION

Milk production and dry matter intake were reduced (P<.05) by increasing FACW (Table 2). Increasing FACW to 100% replacement of soybean meal resulted in 30 and 32% decline in average daily milk production and dry matter intake. This is in contrast to other studies (14) where FACW had no effect on intake and milk production. However, in studies by Huber et al. (14), the highest dietary FACW content would be comparable to 33% in our study where there was relatively little treatment effect. Reasons for this may lie in the relatively high lactic acid content of the rations when lactic acid from silage also was taken into account. Lactate for 0, 33, 66, and 100% treatment groups were 4, 6.5, 8.5, and 11% of total dry matter of the ration (5). Studies with sheep of added lactic acid to raise lactate from 5.4 to 11.3% of the dry matter in perennial ryegrass silage reduced intake 22% (18), similar to our study. However, other workers (14) have not showed an effect on intake from FACW treatments at lower concentrations.
TABLE 2. Effect of treatment on feed intake, milk production, and blood and urine constituents.

<table>
<thead>
<tr>
<th>FACW Replacement</th>
<th>0</th>
<th>33</th>
<th>66</th>
<th>100</th>
<th>SE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Average daily dry matter intake (kg/day)</td>
<td>12.4a</td>
<td>11.8ab</td>
<td>10.4b</td>
<td>8.4c</td>
<td>.5</td>
</tr>
<tr>
<td>Average daily milk production (kg/day)</td>
<td>13.5a</td>
<td>13.7a</td>
<td>11.9b</td>
<td>9.4c</td>
<td>.6</td>
</tr>
<tr>
<td>Blood pH</td>
<td>7.387</td>
<td>7.370</td>
<td>7.397</td>
<td>7.379</td>
<td>.042</td>
</tr>
<tr>
<td>pCO₂ (mm Hg)</td>
<td>37.9</td>
<td>37.7</td>
<td>39.6</td>
<td>40.0</td>
<td>2.8</td>
</tr>
<tr>
<td>HCO₃⁻ (meq/L)</td>
<td>32.5</td>
<td>31.9</td>
<td>30.1</td>
<td>30.7</td>
<td>3.1</td>
</tr>
<tr>
<td>Plasma K (mg/100 ml)</td>
<td>16.0</td>
<td>15.7</td>
<td>15.8</td>
<td>16.6</td>
<td>.7</td>
</tr>
<tr>
<td>Na</td>
<td>361.7a</td>
<td>339.4ab</td>
<td>330.8ab</td>
<td>321.2b</td>
<td>19.7</td>
</tr>
<tr>
<td>Glucose</td>
<td>79.2de</td>
<td>83.4d</td>
<td>70.0de</td>
<td>65.2e</td>
<td>6.9</td>
</tr>
<tr>
<td>L-lactate</td>
<td>9.5a</td>
<td>9.3a</td>
<td>9.7a</td>
<td>12.1b</td>
<td>1.8</td>
</tr>
<tr>
<td>Urine pH</td>
<td>8.02</td>
<td>7.94</td>
<td>7.51</td>
<td>7.55</td>
<td>.42</td>
</tr>
<tr>
<td>Weight (kg/day)</td>
<td>10.1a</td>
<td>13.1ab</td>
<td>14.4ab</td>
<td>19.2b</td>
<td>1.6</td>
</tr>
<tr>
<td>Ammonium ion excretion (meq/day)</td>
<td>327a</td>
<td>915b</td>
<td>781b</td>
<td>1692c</td>
<td>284</td>
</tr>
</tbody>
</table>

a,b,c Means in the same row with different superscripts differ (P<.05).
d,e Means in the same row with different superscripts differ (P<.01).

Blood pH, pCO₂, and HCO₃⁻ (Table 2) were not affected significantly by treatment despite increases in lactic acid load which the animals consumed. Other workers did not show significant effects on blood acid-base metabolites when hydrochloric acid was infused into sheep (22, 23) and calves (24) even though net acid excretion was increased. Based on urine pH and ammonium ion excretion data (Table 2), acid excretion was altered. Increasing FACW replacement from 0 to 100% resulted in a nonsignificant .47 pH unit drop in urine pH and a significant (P<.05) 1365 meq/day increase in ammonium ion excretion. Scott (24) indicated from acid loading studies with sheep and calves that urinary ammonium ion excretion was an important homeostatic regulator of acid excretion in ruminant animals. Other workers (16, 17) studying silages preserved with phosphoric acid noted similar increases in urinary ammonia excretion when cows were fed treated silages. Nitrogen balance data from Bull et al. (5) showed that urinary nitrogen excretion increased with increasing FACW. This increase in urinary ammonium ion excretion may have reflected increased ammonium ion intake since 85% of the nitrogen in FACW is in the form of non-protein nitrogen. However, calculations show that ammonia excretion increased even when it was expressed as a percent of total urinary nitrogen.

Urine weight was increased 9.1 kg/day by increasing FACW treatment from 0 to 100% replacement. Reasons for this could be increases in ammonium ion excretion or net acid excretion.

Plasma K (Table 2) was unaffected, but plasma Na was decreased (P<.05) 40 mg/100 ml by increasing FACW treatment from 0 to 100%. Reasons for the decline in plasma Na are not clear, although total Na intake was reduced because of reductions in dry matter intake.

Glucose of plasma was decreased (P<.01) 10 and 15 mg/100 ml by the 66 and 100% treatments. Changes in glucose may reflect a possible physiological response to, or a mechanism responsible for, lowered intake and production. This response in blood glucose is unusual since other studies using dietary lactate in ketotic cows, in general, have resulted in slight increases in blood glucose (21, 25). Plasma L-lactate (Table 2) was increased (P<.05) by the 100% FACW treatment, which was expected because of its relatively high lactic acid content.
Of particular interest was the effect of time postfeeding on blood acid-base constituents. There was no significant effect of time or of interaction time by treatment for urine pH. However, means for blood pH of samples taken at 4, 6, and 8 h postfeeding (7.367) were significantly (P<.01) lower than of samples taken at 0, 1, 2, and 3 h (7.395) (Figure 2). Other studies have reported declines in blood pH, pCO₂, and HCO₃⁻ immediately following feeding (2, 26). However, average pCO₂ and HCO₃⁻ increased (P<.01) from an average of 33.5 mm Hg and 19.9 meq/liter at 0 h to 44.1 mm Hg and 26.2 meq/liter at 1 h postfeeding (Figure 3). Then there was a decline in average pCO₂ and HCO₃⁻ to a low point at 6 h postfeeding. Reasons for discrepancies between our study and others (2) are unknown. Recent work by Horn et al. (12) in meal fed beef steers failed to show significant effects of time postfeeding on blood acid-base components in beef steers except when means were adjusted for prefeeding measurements for HCO₃⁻. However, they did report a drop in pCO₂ immediately after feeding although changes were non-significant.

Differences between our study and others (2, 12) may reflect methods of feeding. In both sheep (2) and beef cattle studies (12) animals were fed once daily as compared with our twice daily feeding, and relative intakes were much lower. Time spent eating was likely longer than 2 h in the sheep work (2) and 30 min in the steer study (12).

Changes in blood pH, pCO₂, and HCO₃⁻ with time postfeeding are likely to reflect several factors rather than one, as suggested by Billitzer and Jarrett (2). Loss of bicarbonate ion through saliva would lower blood pH and HCO₃⁻ during feeding. However, increased flow of digesta through the abomasum and increased abomasal acid secretion also at feeding would have a converse effect. It is likely that ammonium lactate would increase rumen pH initially since lactate would act as a base because of its low pK' of 3.86, whereas ammonium ion would have little effect on rumen pH because of its relatively high pK' of 9.1. This
likely would raise pH and HCO$_3^-$ concentrations in the blood. However, response in blood to time postfeeding was similar irrespective of FACW treatment. Depressions in rumen pH at 4 to 6 h postfeeding, although not measured in this study, likely are reflected in lower blood pH, pCO$_2$, and HCO$_3^-$ as in (2, 12).

Other blood traits were unaffected by time postfeeding with the exception of plasma Na, which increased from 333 at 0 h to 346 at 4 h and then declined to 325 mg/100 ml at 8 h postfeeding (P<.05). Reasons for this are unknown, but they likely reflect changes in body fluid dynamics.

Of most interest were changes in blood acid-base associated with periods in the analysis (Table 3). Changes in blood pCO$_2$ were inversely related to changes in ambient temperature. This corresponds with (1, 4, 8, 9) where measurements of either pCO$_2$ or total blood carbon dioxide content are decreased by increasing temperature. Since ruminants rely on respiration for temperature regulation, especially at ambient temperatures above 20 to 25°C (15), decreases in blood pCO$_2$ and increases in blood pH correspond to respiratory alkalosis. Blood pCO$_2$ and HCO$_3^-$ data roughly follow this expected pattern, while blood pH was lowest at high ambient temperature, which is unexplained. Data from Dennis and Harbaugh (9) showed that there was a 1.5 and 4.7% linear decline in blood carbon dioxide volume percent for each 10°C increase in ambient temperature with Ayrshire calves. Temperature does affect acid-base metabolism in the ruminant animal. Experiments designed to examine acid-base balance should account for changes in ambient temperature either by controlling temperature or recording it at the time of sampling.

Since ambient temperature affects blood pCO$_2$ and probably HCO$_3^-$ content, the relationship between HCO$_3^-$ in saliva, which is derived from blood HCO$_3^-$, and carbon dioxide needs to be examined. It may be that changes in temperature have a profound effect on composition and buffering properties of bovine saliva because of its effect on blood carbon dioxide content. This suggests possible new areas of work.

We conclude that both time postfeeding and temperature are factors which need consideration in design of experiments measuring blood acid-base criteria. However, these factors probably can be controlled by sampling all animals across treatments on the same day at the same time postfeeding.

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**REFERENCES**


