Effect of Sodium Bicarbonate on Rate of Passage and Degradation of Soybean Meal in Postpartum Dairy Cows

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ABSTRACT
Effects of sodium bicarbonate on rate of passage and disappearance of soybean meal from the rumen were determined in a change-over experiment with eight cows. Experimental diets containing 50 or 60% roughage were fed over five 21-day periods with four cows per diet. Sodium bicarbonate at 1.0 and 2.5% (diet dry matter) were changed over in periods 2 and 4, whereas periods 1, 3, and 5 served as control. Rate of passage of soybean meal was measured with chromium-mordanted soybean meal and rate of disappearance by nylon bag technique. Effects of diet were similar for all responses. Response to the two percents of buffer was similar for dry matter intake, milk yield, milk fat, and protein. Percent buffer fed was associated positively with ruminal pH and with disappearance of nitrogen from nylon bags. The 0, 1, and 2.5% of buffer resulted in turnover rates of mordanted soybean meal of 8.22, 9.80, and 10.52%/h, but degradation of protein remained relatively constant at 36.0, 38.4, and 38.2%. The influence of rate of passage on ruminal degradability is discussed.

INTRODUCTION
Development of new systems for evaluating protein requirements of ruminants (6, 7, 21, 35, 36, 45) has focused attention on the importance of degradability of dietary proteins and their bypass of the rumen. The total protein reaching the small intestine depends on the extent of microbial synthesis of protein in the rumen and the fraction of dietary protein that escapes ruminal degradation. According to Chalupa (7), modulation of the extent of protein degradation in the rumen is one method of influencing the amino acid supply to the small intestine.

Dietary buffers in dairy rations have been recommended primarily where depression of milk fat is a problem (11). But whereas buffers elevate rumen pH (13, 14), they increase rate of liquid dilution and rate of passage of rumen contents (16, 34). By increasing dilution rate, buffers can enhance the efficiency of microbial protein synthesis (17). It also might be expected that by this mechanism they would increase the dietary protein bypassing the rumen or decrease its degradability. However, no research has been conducted into this aspect with lactating cows. In a companion study on steers, we showed that addition of sodium bicarbonate to the diet did increase the rate of passage of soybean meal (SBM), but it also increased digestion of protein in the rumen with no net effect upon protein degradability (30).

Orskov and McDonald (32) developed a method of estimating degradability of protein in the rumen. This is based upon measurement of disappearance of nitrogen (N) from feedstuffs incubated in nylon bags in the rumen and weighting results according to rate of passage. The purpose of this study was to determine the influence of sodium bicarbonate on degradability of soybean meal N in the rumen by the method of Orskov and McDonald (32) and to compare rate of passage and degradability estimates for lactating cows at relatively high intakes with results from steers (30).

MATERIALS AND METHODS
Effects of sodium bicarbonate at 1 and 2.5% dry matter (DM) were investigated in a change-over experiment with eight multiparous Holstein cows. Cows were prepared surgically with...
rumen cannulae 1 wk after calving and were allowed to recover for 1 mo.

Diets (Table 1) formulated to contain 15% crude protein were composed (DM): diet CS, 50% forage of corn silage only and 50% concentrate; diet CS/HCS, 60% forage, both corn and hay crop silages, and 40% concentrate. Sodium bicarbonate replaced high moisture corn in the diets at 1 and 2.5%. All ingredients except NaHCO₃ were machine mixed, and NaHCO₃ was hand mixed thoroughly into the ration of each cow. The trial was over five periods of 21 days each such that periods 1, 3, and 5 were control, whereas treatments (NaHCO₃) were applied in periods 2 and 4. Two cows on each diet were fed buffer at 1% in period 2, whereas the other two sets of cows received buffer at 2.5%. Buffer percents were reversed in period 4. Cows were fed twice daily at 0700 and 1600 h in amounts sufficient to allow 5% refusals. Water was available ad libitum during the trial.

Daily feed intakes and refusals were recorded during the entire trial. Rations were sampled once weekly and composited every 3 wk for nutrient analysis. Feed dry matter was determined by toluene distillation (10) and crude protein by macro-Kjeldahl (1). Acid detergent fiber (ADF) was measured according to the procedure of Van Soest (44). Gross energy was measured by bomb calorimetry.

Milk yield was recorded twice daily during the entire trial in the morning and afternoon. This milk was sampled twice daily for 5 consecutive days during the last week of control and treatment periods. These samples were analyzed for fat, protein, and lactose by an Infra Red Milk Analyzer (4). Yields of solids-corrected milk (SCM) were calculated by the equation of Tyrrell and Reid (42).

Rates of SBM disappearance were determined by incubating 5-g samples of SBM within nylon bags in the rumen. Nylon bags were made of nitex nylon having a pore size of 10 μm. Bags measured 9 × 16 cm and were designed with double seams and curved corners to facilitate removal of residues. Incubation commenced on day 19 of each period immediately after the morning feeding. Nylon bags containing SBM samples were presoaked for 5 min prior to incubation. Bags were withdrawn at intervals of 2, 4, 6, 9, 15, and 24 h and washed under running tap water until the wash water became colorless. The residue in the bags was recovered into aluminum trays by inverting the bags and washing adhered particles from them with distilled water from a wash bottle. Contents of the trays were transferred carefully into tubes and centrifuged at 1000 × g for 10 min. Finally, residues were transferred into preweighed aluminum dishes, dried at 100°C for 24 h, and weighed.

Rate of passage of SBM through the rumen was measured on days 19 to 21 of each period by estimating the rate of disappearance from the rumen of chromium-mordanted SBM particles (43). A 300-g sample of mordanted-SBM particles (1 to 2 mm particle size) was deposited in equal fractions into six sites in the rumen immediately after morning feeding. Particles then were dispersed as uniformly as possible by hand mixing. Grab samples of

### TABLE 1. Diet ingredients and composition.¹

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>CS</th>
<th>CS/HCS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hay crop silage</td>
<td>13.3</td>
<td></td>
</tr>
<tr>
<td>Corn silage</td>
<td>50</td>
<td>46.7</td>
</tr>
<tr>
<td>High moisture corn</td>
<td>31.5</td>
<td>28</td>
</tr>
<tr>
<td>Soybean meal</td>
<td>16.2</td>
<td>9.7</td>
</tr>
<tr>
<td>Mineral-vitamin mix</td>
<td>2.3</td>
<td>2.3</td>
</tr>
</tbody>
</table>

¹Expressed as a percentage of dry matter.

²Composition (%): dicalcium phosphate 43.5; limestone 23.9; magnesium oxide 6.5; trace mineralized salt 21.7; (guaranteed analysis: salt, 96.5; zinc, .4; iron, .16; manganese, .12; copper, .03; iodine, .007; cobalt, .004); vitamins A, D, and E premix 4.3 (guaranteed analyses: 4,400,000 IU/kg vitamin A; 1,000,000 IU/kg vitamin D; 7,700 IU/kg vitamin E).
rumen digesta were taken at 0, 1, 2, 4, 6, 9, 15, 24, 36, and 48 h following introduction of mordanted-SBM. Composite samples from each cow at each interval were dried at 70°C and ground through a 2-mm mesh screen. The later were analyzed for chromium by atomic absorption spectrophotometry according to the procedure of Arthur (2).

To determine the rate of disappearance of mordanted-SBM particles, linear regression analyses were performed on natural logarithm of chromium concentration in the rumen against time after deposition of mordanted particles. Rate constant (k) was determined as the slope of the regression line. Mean retention time (MRT) was estimated as the reciprocal of k.

Samples of rumen fluid were taken at 0, 2, 4, 6, and 8 h after morning feeding on the day that nylon bags were incubated in the rumen. Their pH's were measured immediately. They then were strained, centrifuged, and the supernatants frozen for later determination of ammonia-nitrogen (NH$_3$-N), volatile fatty acids (VFA), and osmolality. The NH$_3$-N was determined by the Berthelot color reaction with sodium phenolate and sodium nitroprusside (29). Volatile fatty acids were extracted by mixing with methanol followed by centrifugation. They then were separated and quantitated by gas-liquid chromatography. The chromatography utilized a 180 cm column (2mm i.d.) packed with 80/100 mesh chromosorb 101 and a flame ionization detector. Osmolality was measured by freezing point depression with an osmometer.

Data on lactation response were subjected to analysis of variance for cross-over design as described by Cochran and Cox (8). Analysis of variance was on differences between treatments and controls. The average of periods 1 and 3 served as control for period 2 treatments, whereas for period 4 treatments the control was the average of periods 3 and 5. Student's t test was used to assess differences between control and treatments. Data on rate of passage were analyzed according to the procedure for a split-plot design (38) with diets as main plots and percents buffer as subplots. Data on the three control periods were pooled after a t test showed there were no differences between averages of period 1 and 3 and averages of period 3 and 5. Percentages of SBM protein disappearance (p) in nylon bags were analyzed by non-linear least squares regression to predict disappearance at time t (h) by the equation $p = a + b(1 - e^{-ct})$. Constants a, b, c, were fitted by iterative least squares procedures. The percentage of protein disappearing at time t is represented by p, whereas the constant a represents the fraction of protein disappearing in the period before the first withdrawal. The b fraction represents the protein which disappeared subsequent to fraction a and at rate c. The equation $P_e = a + (bc/(c + k)(1 - e^{-(c+k)t})$ (32) was used to estimate degradation of SBM-N in the rumen, where k corresponds to fractional turnover rate and t is the time after feeding. In our case t was set equivalent to mean retention time to estimate degradability.

RESULTS AND DISCUSSION

The two diets were not different (P>.05), and, thus, data for them were pooled.

Lactation Response

Effect of NaHCO$_3$ on dry matter intake, milk yield, and milk composition are in Table 2. There was a tendency for NaHCO$_3$ to depress feed intake, but the effect was not significant (P>.05). Treatment periods had a significant effect on feed intake; DM intake was higher in period 2 relative to controls than in period 4. Addition of sodium bicarbonate to diets stimulated intake in early lactation (14, 23), particularly in the first few weeks postpartum (23). The buffer treatments (Table 2) were imposed by the 8th wk of lactation, which may account for the failure to get response of intake to the feeding of buffer.

There were no differences (P>.05) in total milk yield, solids-corrected milk, and milk protein from NaHCO$_3$ or treatment period. In previous research, NaHCO$_3$ improved fat test (12, 19, 27), and a similar trend existed in the present trial also. Milk lactose was higher (P<.05) with 2.5% buffer compared to 1% in the diet.
TABLE 2. Effects of NaHCO₃ and period on dry matter intake, milk yield, and composition.

<table>
<thead>
<tr>
<th>Source of Variation</th>
<th>Treatment Means</th>
<th>Differences between Control and Treatment Means</th>
<th>Sources of Variation</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>NaHCO₃</td>
<td>Periods</td>
<td>NaHCO₃</td>
</tr>
<tr>
<td></td>
<td>1%</td>
<td>2.5%</td>
<td>2</td>
</tr>
<tr>
<td>Daily dry matter intake (kg/day)</td>
<td>19.27</td>
<td>16.83</td>
<td>18.52</td>
</tr>
<tr>
<td>Milk yield (kg/day)</td>
<td>31.50</td>
<td>29.10</td>
<td>31.40</td>
</tr>
<tr>
<td>Milk, solids corrected (kg/day)</td>
<td>28.20</td>
<td>25.80</td>
<td>27.76</td>
</tr>
<tr>
<td>Milk fat (%)</td>
<td>3.18</td>
<td>3.49</td>
<td>3.16</td>
</tr>
<tr>
<td>Milk protein (%)</td>
<td>3.15</td>
<td>3.06</td>
<td>3.15</td>
</tr>
<tr>
<td>Milk lactose (%)</td>
<td>5.12</td>
<td>5.10</td>
<td>5.14</td>
</tr>
</tbody>
</table>

*a* Means of eight observations.

*b* Differences between treatment means and the averages of pre- and posttreatment control periods.

*c* NS, not significant (P>.05).

*d* Analysis of variance on b above.

*Significant (P<.05).
**Rumen Measures**

Effects of buffer on ruminal pH and NH$_3$-N concentration are in Figure 1. Without NaHCO$_3$ in the diet, pH decreased with time after feeding, dropping to a low of 5.6 at 4 h. When NaHCO$_3$ was included in the diet, ruminal pH increased with time after feeding, the increase being moderate with 1% buffer, whereas with 2.5% buffer in the diet there was a rapid increase to 6.4% at 2 h after feeding. These results agree with Emery et al. (13), Miller et al. (27), and Nicholson et al. (28). Correlation analysis showed a significant relationship between rate c, at which the b fraction disappeared from the nylon bags, and ruminal pH at 4 h after feeding (r = .89, P<.05). Rumin- nal NH$_3$-N increased with time after feeding, the highest with all diets between 2 and 4 h.

The highest peak concentration of NH$_3$-N was with the 2.5% buffer treatment.

Changes in ruminal osmolality and total VFA with time after feeding are in Figure 2. Ruminal osmolality increased as expected with time after feeding. Peaks were attained at about 2 h after feeding, and rumen osmolality declined thereafter toward prefeeding average. Between diets the highest mean observed at 2 h after feeding was 360 mOsm/kg obtained with 2.5% NaHCO$_3$ in the diet. Harrison et al. (16) reported maximal rumen osmolality of 336 mOsm/kg in response to inclusion of 2.5% NaHCO$_3$ in the diet of sheep. It is unlikely that the high rumen osmolality impeded rumen fermentation, although in vitro data (Okeke and Buchanan-Smith, unpublished) showed that rumen osmolality maintained above 350 mOsm/kg can impede fermentation. There were
moderate increases of concentration of total volatile fatty acids following feeding, but differences between treatments were not significant ($P > .05$). The ratio of acetic to propionic acid, as expected, was higher ($P < .05$) with 2.5% of buffer compared to 1% of buffer. The respective mean ratios of acetic to propionic acid for 0, 1, and 2.5% buffer were 3.29, 3.15, and 3.79 (SE ± .036).

Rate of Passage and Nylon Bag Trial

Table 3 shows effects of buffer on ruminal rate of passage of mordanted-SBM particles. There was a linear increase ($P < .05$) of outflow rate and decrease of mean retention time with increasing sodium bicarbonate in the diet. There was no effect of diet (CS/HCS vs CS) on rate of elimination of mordanted-SBM particles from the rumen.

For interpreting data on rate of passage, determinations were not for the solid phase of rumen digesta, but for SBM particles, which are likely to associate more with the liquid phase in the rumen. Reports (16, 24, 33, 41) showed that dilution rate of ruminal liquid phase increased with infusion of artificial saliva or saline water or with inclusion of mineral salts of artificial saliva in the diet. Few studies have been reported in which specific dietary proteins have been marked to determine their rate of disappearance from the rumen. Percents 4.6 and 6/h were reported by Ørskov and McDonald (32) for SBM under restricted and ad libitum feeding in sheep. Stern et al. (39) reported 4.95, 5.27, and 4.83%/h for SBM, corn gluten meal, and dried brewer’s grains when marked proteins were fed to lactating Holstein cows. The rate of passage of SBM in our trial with steers fed 2.5% NaHCO$_3$ in the diet (30) was lower than the rate with equivalent NaHCO$_3$ in this trial. This difference reinforces (3, 20) that rumen retention time is influenced by feed intake.

More studies will be needed to determine rates of ruminal outflow of supplemental proteins in dairy diets. Until these are done, Ørskov et al. (31) have suggested an outflow rate of 9.0%/h as appropriate for high yielding cows where roughages, such as silage or hay, form a substantial part of the diet. Our estimates of SBM passage rates out of the rumen are in close agreement with rates suggested by these authors.

Percentages of N disappearance from nylon bags are in Table 4 for incubation intervals of 4, 15, and 24 h. Buffer had no effect ($P > .05$) on protein disappearance from nylon bags during the first 4 h of incubation. However, from 6 through 24 h, there were linear and quadratic increases ($P < .05$) of nitrogen disappearance as buffer in the diet increased. Across diets, NaHCO$_3$ fed at 1 and 2.5% caused relative increases of 19.2% and 22.2% of nitrogen disappearance over the control at 24 h of incubation.

The increase in disappearance of nitrogen

<table>
<thead>
<tr>
<th>Buffer (%) DM</th>
<th>CS Diet</th>
<th></th>
<th>CS/HCS</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>k (%)/h</td>
<td>MRT (h)</td>
<td>k (%)/h</td>
</tr>
<tr>
<td>0</td>
<td>8.20</td>
<td>12.17</td>
<td>8.24</td>
</tr>
<tr>
<td>1</td>
<td>9.75</td>
<td>10.27</td>
<td>9.84</td>
</tr>
<tr>
<td>2.5</td>
<td>10.42</td>
<td>9.59</td>
<td>10.62</td>
</tr>
<tr>
<td>SE</td>
<td>.13</td>
<td>.14</td>
<td>.11</td>
</tr>
<tr>
<td>Orthogonal contrast$^a$</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>L</td>
<td>*</td>
<td>*</td>
<td>*</td>
</tr>
<tr>
<td>Q</td>
<td>*</td>
<td>*</td>
<td>*</td>
</tr>
</tbody>
</table>

$^a$L and Q are linear and quadratic contrasts.

$^*P < .05$.  

BICARBONATE AND PROTEIN DEGRADATION

from the nylon bag could be primarily from effects of NaHCO₃ on rumen environment. Because an increase in ruminal dilution rate increased protein disappearance may have been from increased microbial activity. Support for this argument is in the steer trial (30), in which increasing buffer in the diet resulted in concomitant increases in dilution rate of rumen fluid and increased N disappearance from the nylon bags. Furthermore, increased ruminal pH associated with feeding NaHCO₃ may have provided an optimum ruminal environment for protein degradation. This was the case in the steer trial in which the positive correlation between rumen pH and protein degradation was highly significant. Further support is provided by the work of Ganev et al. (15), who fed either a barley or all grass diet to sheep and reported higher N disappearance for proteins of plant origin incubated in sheep receiving the all grass diet. Because the barley diet would depress rumen pH relative to the grass diet, their work is interpreted as indicating pH may be important in determining degradation of proteins in the rumen.

Ruminal pH above 6.0 for the 1 and 2.5% buffer in this trial are within the range of 6 to 7 reported by Blackburn and Hobson (5) and by Lewis and Emery (25) to be optimum for proteolysis in the rumen. Correlation analysis relating rumen pH at 4 h after feeding to protein disappearance in nylon bags at 24 h gave a correlation coefficient of .92 (P<.05), further emphasizing the role of rumen pH in modulating disappearance of ruminal protein. Relationship was similar for cows (26). It is likely, therefore, that the higher pH and probable increase of microbial activity accounted for most increases of N disappearance through addition of buffer to the diet.

**Estimated Degradation (Pe)**

Adjustment of the nylon bag N-disappearances for passage of SBM particles out of the rumen resulted in a substantial reduction of Pe of SBM relative to the percentage disappearance from the nylon bag at 24 h (Table 4). No differences (P>.05) were observed for Pe between control and buffer, although values for the 1 and 2.5% buffer tended to be higher than control. The low Pe's—35.95, 38.40, 38.20, for 0, 1, and 2.5%—indicate that under the condi-

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**TABLE 4. Effect of NaHCO₃ on mean retention time (MRT), N-disappearance from nylon bags, degradation (Pe) at MRT.**

<table>
<thead>
<tr>
<th>Buffer (%)</th>
<th>N Disappearance</th>
<th>Pe (%)</th>
<th>MRT</th>
<th>CS Diet</th>
<th>Pe (%)</th>
<th>MRT</th>
<th>CS/HG Diet</th>
<th>Pe (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>15</td>
<td>24</td>
<td></td>
<td>0</td>
<td>15</td>
<td>24</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1%</td>
<td>15</td>
<td>24</td>
<td></td>
<td>1</td>
<td>15</td>
<td>24</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2%</td>
<td>15</td>
<td>24</td>
<td></td>
<td>2</td>
<td>15</td>
<td>24</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2.5%</td>
<td>15</td>
<td>24</td>
<td></td>
<td>2.5</td>
<td>15</td>
<td>24</td>
<td></td>
<td></td>
</tr>
<tr>
<td>SE</td>
<td>1.0</td>
<td>.94</td>
<td></td>
<td>.4</td>
<td>NS</td>
<td>.35</td>
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<td>.05</td>
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<td>Orthogonal contrast</td>
<td>L</td>
<td>Q</td>
<td>10</td>
<td></td>
<td></td>
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<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td></td>
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</tr>
</tbody>
</table>

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*Incorporated hours. b L and Q are linear and quadratic contrasts.

c NS, P>.05

dP<.05
tions of this trial, over 60% of SBM protein escaped ruminal degradation. These estimates of rumen degradability of about 40% for SBM in the rumen were considerably lower than 68% reported by Stern et al. (39) for lactating Holstein cows fed ad libitum a diet containing 60% alfalfa hay and 40% of a basal grain mix. The large variation of estimates of ruminal degradability between different trials may be partly from differences of ruminal passage rates of proteins under conditions of the various studies. Turnover rates of Stern et al. (39) were much lower than those now reported.

The significance of ruminal passage rates on degradation of proteins has been emphasized by Ørskov et al. (31) and Tamminga (40). The former authors estimated degradability mathematically by weighting N disappearance from nylon bags with a range of ruminal turnover rates and observed that degradability decreased with increasing flow rates. They concluded that there cannot be a single constant for degradability of a protein and that effects of turnover rates may vary between proteins. The estimate of protein degradation was lower in cows (37.5%) than in steers (48.7%) (30). Feed intakes were higher in the trial with cows as were rates of passage of mordanted-SBM particles. The SBM samples incubated in the two trials were compared for differences by incubating both simultaneously in the rumens of two cows at intervals up to 30 h. Disappearance of N from the two samples was not different (P>.05) at all incubation intervals. It seems, therefore, that ruminal turnover is an important determinant of ruminal protein degradation and that the low estimates of degradation in our study must be interpreted in the light of the relatively high turnover rates.

Although feeding NaHCO₃ in this trial contributed little to ruminal bypass of SBM, its use in dairy rations, nevertheless, has been beneficial. Buffers have been used to alleviate acidosis of rumen and low milk fat test associated with increased energy intake by postpartum cows (13, 22, 27). Reports (14, 23) showed that feeding sodium bicarbonate to postpartum dairy cows moved the intake peak earlier, thus helping to reach positive energy balance earlier in lactation. Also the elevated ruminal pH and the dilution rate associated with buffer feeding would be expected to increase microbial protein synthesis (18, 37) and yield (9, 24). Thus, total protein reaching the small intestine might be expected to increase.

ACKNOWLEDGMENTS

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REFERENCES


