

# EFFECTS OF LEVEL AND SOURCE OF CALCIUM ON DIGESTION OF HIGH CONCENTRATE DIETS BY STEERS

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## Story in Brief

Effects of calcium (Ca) source and level on site of digestion were investigated with four cannulated steers. High concentrate diets containing no supplemental Ca (B), .95 percent calcium chloride (CL), .65 percent limestone ( $\text{CaCO}_3$ ; LL) or 2.50 percent  $\text{CaCO}_3$  (HL) were fed to four steers in a  $4 \times 4$  Latin square. Ruminal pH at <sup>3</sup>2, 6 and 10 hr after feeding and duodenal and fecal pH were not different. However, fecal pH for HL tended to be highest. Ruminal ammonia nitrogen concentrations tended to be higher for LL and HL diets than for B and CL diets. Ruminal organic matter (OM) and starch digestibilities did not greatly differ, but tended to be greatest for B (66.4 and 82.4 percent), lowest for HL (58.1 and 70.7 percent) and intermediate for SR and LR treatments (63.3 and 75.2, 63.9 and 77.9 percent, respectively). Postruminal OM and starch disappearance were slightly enhanced with the HL diet. Soluble Ca levels of ruminal and duodenal fluid tended to be highest for HL (239 and 1128 vs 55 and 497, 165 and 550, 64 and 648 mg/l for HL vs B, CL and LL treatments, respectively). Intestinal Ca disappearance was not greatly different, indicating similar availabilities from all diets.

## Introduction

Limestone addition to high concentrate diets has received considerable research attention in recent years. Results have been extremely variable, which may be partially due to differences in experimental conditions. When dietary Ca levels are low, Ca supplementation increases production, but performance responses generally are not seen when Ca exceeds .45 percent of diet dry matter (Owens et al., 1983). In some trials, buffering actions in the rumen and postruminal digestive tract have been reported, but whether these effects are attributable to buffering or to Ca remains undefined. Generally, the pH of the rumen and ileum are not increased with added limestone, while fecal pH is often increased. The objectives of this study were to investigate effects of source and level of dietary Ca on site and extent of digestion and on Ca levels in ruminal, duodenal and fecal material. Calcium chloride, a more soluble source of calcium in the rumen, was used for comparison to calcium carbonate in these experiments.

## Experimental Procedures

### Experiment 1

Four dairy steers (953 lb) fitted with ruminal and duodenal cannulas were used in this  $4 \times 4$  Latin square design experiment. The

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basal diet (B) contained no supplemental Ca (Table 1). Limestone ( $\text{CaCO}_3$ ) and  $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$  were added to two test diets (LL and CL, respectively) to provide equal Ca levels. For the final diet (HL),  $\text{CaCO}_3$  was included at 2.5 percent of dry matter. All diets contained chromic oxide as an indigestible marker. Animals were fed twice daily at 12 hr intervals at 63 g dry matter (DM) per kg of metabolic body weight.

Periods lasted 14 days with sampling on day 13 and 14. A fluid marker (CoEDTA) was intraruminally dosed prior to the morning feeding on day 13. Samples were taken from the rumen, duodenum and rectum at 2, 6 and 10 hours after feeding on both days. Feed samples were collected throughout the trial. Feed, ruminal fluid, isolated bacterial cells, duodenal (fluid and total digesta) and rectal samples were subjected to all or part of the following analyses: pH, Ca, kjeldahl nitrogen (N), ammonia-N ( $\text{NH}_3\text{-N}$ ), nucleic acid-N (NAN), ash, acid detergent fiber (ADF), starch and chromium.

Table 1. Diet compositions.

Diet Ingredient, % of DM	Experiment 1				Experiment 2			
	B	CL	LL	HL	B	CL	LL	HL
Corn grain, rolled	79.8	79.2	78.9	77.3	55.9	55.2	55.4	54.1
Cottonseed hulls	7.0	7.0	7.0	7.0	14.9	14.9	14.9	14.9
Prairie hay, chopped	0	0	0	0	20.0	20.0	20.0	20.0
Alfalfa, dehydrated	5.0	5.0	5.0	5.0	3.5	3.5	3.5	3.5
Soybean meal	5.0	5.0	5.0	5.0	3.5	3.5	3.5	3.5
Molasses, cane	2.5	2.5	2.5	2.5	1.75	1.75	1.75	1.75
Salt, trace mineralized	.50	.50	.50	.50	.35	.35	.35	.35
Chromic oxide	.20	.20	.20	.20	.14	.14	.14	.14
Calcium carbonate	0	0	.65	2.50	0	0	.46	1.75
Calcium chloride dihydrate	0	.95	0	0	0	.67	0	0

### Experiment 2

Four animals (666 lb), fitted with large ruminal cannulas, were used in a 4 x 4 latin square study with 14 day periods. Animals were fed at 12 hr intervals at 1.25 percent of body weight. Dacron bags containing 1.5 g (air dry) finely rolled corn were ruminally incubated for 4, 8, 12, 24 and 72 hr, washed and dried. Rate of DM disappearance was calculated from disappearance of potentially digestible residual DM (with subtraction of 72 hr DM residue) and expressed as a percent of the preceding residue.

## Results and Discussion

### Experiment 1

Diets were formulated to contain .11, .34, .34 and .96 percent Ca (for B, CL, LL, and HL diets, respectively) but, laboratory analysis revealed higher Ca concentrations in all diets (.25, .40, .48 and 1.11



percent). Isolated bacterial cell nucleic acid and N content for steers receiving all treatments was similar. Ruminal pH at 2, 6 and 10 hr after feeding (Table 2) did not differ. Limestone may not increase ruminal pH when basal diets produce a relatively high pH (Owens et al., 1983). In this trial, ruminal pH with all treatments was higher than expected for a high concentrate diet. Limiting feed intake may have helped prevent lower pH values. No differences in duodenal pH were observed, in agreement with most other reports. Fecal pH did not significantly differ but tended to be greatest for the HL diet.

Table 2. Digestive tract measurements, experiment 1.

Item	Time after feeding	Diet			
		B	CL	LL	HL
Ruminal pH	2 h	5.99	5.93	6.31	6.05
	6 h	6.37	6.14	6.32	6.19
	10 h	6.61	6.38	6.59	6.47
Duodenal pH	Mean	2.29	2.24	2.35	2.37
Fecal pH	Mean	5.64	5.79	5.73	5.94 <sup>b</sup>
Ruminal ammonia-N, mg/dl	2 h	3.0 <sup>a</sup>	3.4 <sup>a</sup>	5.8 <sup>b</sup>	5.4 <sup>b</sup>
Ruminal fluid					
dilution rate, %/h		3.72	4.50	5.32	5.05
volume, liter		88.1	63.4	56.4	63.3

a,<sup>b</sup> Means in a row with different superscripts differ (P<.05).

Ruminal NH<sub>3</sub>-N (Table 2) was higher (P<.05) for LL and HL steers than for B and CL animals. This is probably due to reduced ruminal digestion of OM and reduced use of ammonia for synthesis of protein by microbes. Microbial N (MN) entering the duodenum tended to be greater for B and CL diets (44 and 49 vs 36 and 36 g/day for B and CL vs LL and HL diets, respectively). Also, ruminal fluid dilution rates tended to be lower with the B and CL diets (Table 2) than the LL and HL diets. Ruminal fluid volumes were similar and trends were generally opposite those of fluid dilution rate. Fluid volume and dilution rate were inversely related (r=-.72; P<.01). Ruminal fluid dilution rate and pH at 2 hr after feeding were correlated (r=.64; P<.01) but relationships at 6 and 10 hr were less apparent. Greater quantities of readily digestible substrates would be present in the early hours after feeding, but faster fluid dilution rates may carry feed particles out of the rumen which would reduce the amount of fermentation acids produced which would otherwise reduce ruminal pH.

True ruminal OM digestion coefficients (Table 3) tended to be highest for the B diet and lowest for the HL diet. Ruminal Ca tended to be increased more with the CL than the LL diet. This may reflect greater solubility and hydrolysis of the chloride salt than the carbonate form of Ca.

Ruminal soluble Ca (Table 4) tended to be greatest for the HL treatment. Bacterial cell walls may bind Ca (Durand and Kawashima, 1980). Since added Ca reduced the extent of ruminal digestion with the HL diet, possibly binding of Ca to bacterial cells may have interfered with activity of cell wall associated enzymes or secretion of extracellular enzymes.

**Table 3. Digestibilities, experiment 1.**

Item	Diet			
	B	CL	LL	HL
Organic matter, %				
Ruminal	66.4	63.3	63.9	58.1
Postruminal	.6	9.3	4.9	14.5
Total tract	67.0	72.6	68.8	72.7
Starch, %				
Ruminal	82.4	75.2	77.9	70.7
Postruminal	8.3	16.6	13.2	22.5
Total tract	90.7	91.7	91.1	93.2
Fiber, acid detergent, %				
Ruminal	61.4	56.0	66.8	52.2
Microbial efficiency, g MN/kg organic matter fermented	23.1	25.4	20.1	22.6
Ruminal bypass of protein, % of intake	51.5	56.5	52.5	49.9

**Table 4. Calcium intakes, concentrations and disappearance, experiment 1.**

Item	Diet			
	B	CL	LL	HL
Intake, g/d	14.4	23.0	27.7	63.3
Soluble in ruminal fluid, mg/l	54.6	165.4	63.6	239.3
Soluble in duodenal fluid, mg/l.	497.2	549.6	647.8	1018.4 <sup>b</sup>
Total duodenal passage, g/d	16.9 <sup>a</sup>	31.3 <sup>a</sup>	30.0 <sup>a</sup>	110.2 <sup>b</sup>
Total fecal passage, g/d	3.1 <sup>a</sup>	4.6 <sup>a</sup>	4.1 <sup>a</sup>	8.3 <sup>b</sup>
Postruminal disappearance g/d	13.9 <sup>a</sup>	26.7 <sup>a</sup>	26.8 <sup>a</sup>	102.0 <sup>b</sup>
% of intake	94.0 <sup>a</sup>	112.2 <sup>a</sup>	96.6 <sup>a</sup>	158.6 <sup>b</sup>
% of Ca entering duodenum	81.7	84.4	86.4	92.7

<sup>a,b</sup> Means in a row with different superscripts differ (P<.05).

Effect of Ca on ruminal digestion also may be mediated through ruminal washout of digestible particles. Ruminal OM digestion and fluid dilution rate were negatively related ( $r=-.59$ ;  $P<.03$ ). Fluid dilution rate and duodenal passage of MN were negatively correlated ( $r=-.52$ ;  $p<.04$ ). Elevated liquid dilution rates are commonly believed to be positively related to efficiency of microbial growth and flow of MN from the rumen. Flow of microbes from the rumen might be expected to be more closely related to particulate dilution rate than to fluid dilution rate when microbes extensively colonize feed particles which do not follow the same ruminal exit pattern as fluids. If elevated fluid dilution rates washed out potentially digestible materials, or if time of fermentation is inadequate for maximal microbial colonization, total microbial output may be reduced when dilution rate is elevated. Relative rates of 1) microbial dilution 2) feed removal 3) fermentation rate and capacity and 4) time lag for fermentation are needed to assess the total effect of altered dilution rates on microbial output from the



rumen. A lower dilution rate may enhance the proportion of duodenal chyme which is microbial protein. Postruminal OM digestion (Table 3) tended to compensate for ruminal digestion, being greatest for the HL diet, lowest for the B diet and slightly greater for the CL than for the LL diet. Total tract OM digestibilities tended to be higher for CL and HL diets.

Ruminal starch digestion (Table 3) tended to be greatest for NC, lowest for HL and intermediate for SR and LR treatments. Correlations between ruminal soluble Ca and starch digestion were nonsignificant. Postruminal starch digestion trends (Table 3) followed OM digestion differences. It has been postulated that increased intestinal Ca level might enhance host enzyme activity through stabilization effects (Owens et al., 1983). However, correlations between total and soluble duodenal Ca levels and postruminal starch disappearance (as percents of starch intake or as a percent of the starch entering the duodenum) were not significant. Dietary Ca level and postruminal digestion measures were linearly related ( $P < .05$ ). Postruminal starch digestion coefficients for CL and LL diets were similarly increased compared with the B diet despite the form of Ca ( $\text{CaCl}_2$  vs  $\text{CaCO}_3$ ). Since the effect of limestone did not differ from that of supplying Ca in the chloride form, this suggests that the calcium, not the carbonate was probably responsible for altered digestibility. Total tract starch digestion tended to be greatest with the HL diet, largely due to increased postruminal digestion of starch. But whether this was due to increased digestion in the small intestine or increased fermentation of starch in the large intestine plus colon was not determined in this study.

Treatment means for ruminal ADF digestion (Table 3) were similar. Soluble ruminal Ca level (Table 4) was negatively related to ruminal ADF digestion ( $r = -.50$ ;  $P < .05$ ). Since cellulose digesting enzymes are extracellular or associated with bacterial cell walls, Ca may interfere with digestion. Depressed fiber digestion could effect starch digestion by increasing the lag time for digestion of cell walls to expose starch or by increasing the amount of particulate residues, ruminal motility and rate of exit of particles containing starch from the rumen. ADF in this study was low and came largely from cottonseed hulls. It may be erroneous to extrapolate results to other forms or sources of roughage. Ruminal N digestion and microbial efficiency estimates (Table 3) were not changed by treatment.

Ruminal soluble Ca levels are in the normal range (Table 4; Durand and Kawashima, 1980). Based on ruminal fluid volume estimates (Table 2), the total amount of Ca soluble in ruminal contents was 4.8, 10.5, 3.6 and 15.2 g for B, CL, LL and HL diets, respectively. These amounts correspond to ruminal soluble Ca/daily Ca intake ratios of .33, .46, .13 and .24, respectively. By difference, ruminal solubility of  $\text{CaCO}_3$  in the HL diet was estimated to be 21 percent. In contrast, soluble Ca levels in duodenal fluid were directly proportional to Ca intake indicating solubilization by abomasal acid. Duodenal Ca flows for all diets slightly exceeded dietary Ca intakes. Diet is presumed to be the primary source of Ca in the digestive tract (Durand and Kawashima, 1980). Several explanations may be offered. Analytical problems may have occurred or dietary adaptation periods should have been longer. Gut contributions to digesta Ca could partially explain results with the B, CL and LL diets. With the HL diet, a blood overload may have increased Ca flow into the gut lumen. Recycling of Ca to the abomasum or rumen would aid in activation of pancreatic exoenzymes such as alpha-amylase. Intestinal Ca availability did not greatly vary with source or level.

## Experiment 2

Diets were the same as in experiment 1, except for addition of 30 percent roughage to prevent leakage around the ruminal cannulas. Hence, differences in dietary Ca levels are slightly less than in the first trial.

Dry matter disappearance during the first 4 hr of ruminal incubation was not different ( $P < .05$ ) due to dietary treatment (Table 5), though disappearance values for LL and HL diets were slightly greater than for B and CL diets. Substrate DM disappearance during 72 hr of incubation were similar. Rate constants of DM disappearance from 4 to 24 hr of ruminal exposure did not markedly vary, though trends for B, CL and LL treatments corresponded with treatment rankings in experiment 1. The discrepancy between in situ and in vivo results with the HL diet may be explained by differences in ruminal residence times. In conclusion, Ca source and level exerted no major influence on site of starch or organic matter digestion. However, ruminal starch digestion was slightly depressed while postruminal digestion was slightly enhanced by 2.5 percent  $\text{CaCO}_3$  supplementation.

Table 5. Dry matter disappearance, experiment 2.

Item	Treatment			
	B	CL	LL	HL
4 h	5.3	4.8	6.4	5.7
72 h	57.2	56.7	55.5	52.1
Rate from 4 to 12 h, $h^{-1}$	.0130	.0103	.0103	.0110

## Literature Cited

- Durand, M. and R. Kawashima. 1980. Digestive Physiology and Metabolism in Ruminants. Y. Ruckebusch and P. Thivend (Eds.) p. 375.
- Owens, F. N. et al. 1983. Buffers, Neutralizers and Electrolytes. NFIA, Des Moines, IA.