

GASTROINTESTINAL ACIDITY, PROTEIN AND STARCH DIGESTIBILITY
AND AMINO ACID ABSORPTION IN RUMINANTS FED A HIGH
-CONCENTRATE DIET WITH LIMESTONE, MAGNESIUM OXIDE OR
DEFLUORINATED PHOSPHATE

by

Michael Lee Christiansen

Dissertation submitted to the Faculty of the
Virginia Polytechnic Institute and State University in
partial fulfillment of the requirements for the degree of

DOCTOR OF PHILOSOPHY

in

Animal Science

APPROVED:

Kenneth E. Webb, Jr., Chairman

Forrest W. Thye

Farabee D. McCarthy

Joseph P. Fontenot

Joseph H. Herbein

June, 1988

Blacksburg, Virginia

GASTROINTESTINAL ACIDITY, PROTEIN AND STARCH DIBESTIBILITY
AND AMINO ACID ABSORPTION IN RUMINANTS FED A HIGH-
CONCENTRATE DIET WITH DEFLUORINATED PHOSPHATE, LIMESTONE OR
MAGNESIUM OXIDE

By

Michael L. Christiansen

Committee Chairman: Kenneth E. Webb, Jr.
Animal Science

(ABSTRACT)

Twelve wether lambs (32kg) with abomasal and ileal cannula were fed a 90% concentrate basal diet (800 g/d), basal + 1.5% magnesium oxide (MgO)(812 g/d), basal + 1.5% limestone (812 g/d) or basal + 3.0% limestone (824 g/d) to study the effect of these minerals on intestinal pH, rumen fermentation, N balance, dry matter and crude protein digestibility (DMD, CPD), and small intestinal disappearance of amino acids (AAD). Limestone (3%) increased ($P < .03$) rumen pH. Magnesium oxide increased ($P < .10$) ileal and fecal pH. Limestone significantly increased N absorption and pre-abomasal DMD, but decreased ($P < .03$) AAD. N retention was not improved by the treatments. An 82-d feeding trial was conducted with 72 wether lambs (avg initial wt: 28 kg) to study the effect of 1 or 3% fine (70% $< 53 \mu$) or coarse (85% $> 425 \mu$) limestone on rumen environment, weight gain and feed efficiency of lambs fed an all-concentrate diet. Rumen pH and VFA molar

proportions were not affected by the treatments. Limestone (3.0%) decreased ($P < .10$) total rumen VFA concentrations and increased ($P < .10$) fecal pH. Weight gain was not different ($P > .10$) among the treatments. Coarse limestone increased ($P < .10$) feed efficiency. Five Angus heifers (285 kg) with duodenal and ileal cannulae were fed a 90% concentrate control diet (7.5 kg/d) or the same diet containing 1.60% defluorinated phosphate-regular (5.5%, 19.0% and 33.0% on 1400, 1180 and 850 μ sieves, respectively, DRP-R), 1.60% defluorinated phosphate-coarse (85% evenly among large sieves, DRP-C), 1.28% limestone or .5% MgO to study the effect of limestone or MgO on intestinal pH, DMD, starch digestibility (SD), CPD and AAD in beef cattle fed a high-concentrate diet. Ileal pH was increased by MgO. Fecal pH was increased ($P < .05$) as follows: MgO > DRP > limestone and control. Minerals increased ($P < .05$) duodenal liquid flow. Limestone and DRP-C increased ($P < .05$) acid flow to the duodenum. Total tract DMD, SD and CPD were similar among treatments. Limestone and DRP-R increased ($P < .10$) AAD. DRP-C tended to increase AAD, but differences were not statistically significant.

Dedication

This dissertation is dedicated to

who provided a firm foundation in my early formative years, who taught me the value of work and persistence and who have stood by me throughout my efforts to obtain a university education.

ACKNOWLEDGEMENTS

I would especially like to express my appreciation to Dr. Webb for his encouragement, understanding, patience, professional instruction and advice, surgical skills and editorial expertise. He has been a good friend and very supportive major professor. I am also grateful for the advice and support offered by Drs. Herbein, Fontenot, McCarthy and Thye as committee members.

I express special appreciation to my graduate student colleagues for their friendship and especially for their efforts in helping me accomplish each of my research projects. I especially acknowledge the efforts of _____ and _____ for their dedication and bravery in helping me with halter breaking, replacing cannulae and sampling around the clock during my cattle study.

I thank _____ for his help in doing most of the VFA analysis, and for encouragement and other technical support in the laboratory. I am thankful to _____ for her typing skills and especially for her willingness to be flexible with her time. I am very grateful for the help _____ has offered in typing, but especially for her efforts in finding and copying research articles and helping me organize the literature cited.

TABLE OF CONTENTS

ABSTRACT.....	ii
DEDICATION.....	iv
ACKNOWLEDGEMENTS.....	v
LIST OF TABLES.....	vi
LIST OF FIGURES.....	viii
INTRODUCTION.....	1
CHAPTER 1 REVIEW OF LITERATURE.....	5
Rumen Acid Production and Buffering.....	6
Acid Production.....	6
Rumen Buffers and Buffering Capacity.....	7
Fermentation vs. Buffering Capacity.....	14
A Need For Dietary Buffers.....	21
Effective Dietary Buffers.....	23
Sodium Bicarbonate.....	24
Magnesium Oxide.....	31
Calcium Carbonate.....	34
Acid Production.....	37
Buffering Systems.....	41
A Need For Postruminal Buffers.....	43
Summary.....	49
CHAPTER 2 NUTRIENT DIGESTIBILITY, NITROGEN BALANCE AND AMINO ACID ABSORPTION IN SHEEP FED A HIGH- CONCENTRATE DIET WITH LIMESTONE OR MAGNESIUM OXIDE.....	51
Abstract.....	51
Introduction.....	52
Experimental Procedure.....	54
Animals and Design.....	54
Diet Treatments.....	55
Animal Management and Feeding.....	57
Sample Collection.....	57
Sample Preparation.....	60
Analyses.....	61
Results and Discussion.....	64
Literature Cited.	80
Chapter 3 LIMESTONE PARTICLE SIZE AND DIETARY LEVELS FOR LAMBS FED AN ALL-CONCENTRATE DIET.....	83

Abstract.....	83
Introduction.....	84
Materials and Methods.....	85
Animals.....	85
Experimental Design.....	85
Diet Treatments.....	85
Diet Description.....	86
Animal Feeding and Management.....	87
Sampling and Analyses.....	89
Results and Discussion.....	90
Literature Cited	97
Chapter 4 INTESTINAL ACID FLOW, DRY MATTER, STARCH AND PROTEIN DIGESTIBILITY AND AMINO ACID ABSORPTION IN BEEF CATTLE FED A HIGH-CONCENTRATE DIET WITH DEFLUORINATED PHOSPHATE, LIMESTONE OR MAGNESIUM OXIDE.....	99
Abstract.....	99
Introduction.....	100
Experimental Procedure.....	102
Animals and Experimental Design.....	103
Diet Description.....	102
Animal Feeding and Management.....	103
Sampling.....	105
Sample Preparation.....	107
Analyses.....	111
Results and Discussion.....	113
Literature Cited	140
Chapter 5 CALCIUM AND MAGNESIUM COMPOSITION AND RATE OF REACTIVITY OF LIMESTONE USED IN LIVESTOCK FEEDS WITHIN THE STATE OF VIRGINIA.....	147
Abstract.....	147
Introduction.....	148
Materials and Methods.....	149
Analyses.....	151
Results and Discussion.....	151
Literature Cited.....	155
Chapter 6 GENERAL DISCUSSION.....	156
APPENDIX.....	172
Feed Preparation.....	173
Concentrate Mix For The Lamb Digestion and Metabolism Trial.....	173
Pellet Preparation For The Lamb Feeding Trial....	173
Pellet Preparation For The Beef Cattle Trial.....	175

Lamb Feeding Trial.....	176
Beef Cattle Digestion Trial.....	177
Analysis of Amino Acids in Feedstuffs, Feces And Digesta Using Precolumn Phenylisothiocyanate Derivatization and Liquid Chromotography.....	178
6 N HCl.....	178
Internal Standard Solution (alpha amino-n-butyric acid, AABA, 5.0 μ mol/ml).....	178
Procedure.....	178
Derivatization of Amino Acids with PITC.....	179
Amino Acid Standard Solution.....	179
Analysis of a Casein Standard.....	180
LITERATURE CITED.....	183
VITA.....	201

LIST OF TABLES

Table	Page
1. Composition of Experimental Diets, As-Fed.....	56
2. Particle Size of Limestone and Magnesium Oxide Supplements.....	65
3. The Effect of Limestone or Magnesium Oxide on Rumen pH, Rumen Ammonia and VFA Concentrations, Digesta and Fecal pH, and Blood Urea Nitrogen in Lambs Fed a High-Concentrate Diet	66
4. The Effect of Limestone or Magnesium Oxide on Dry Matter, Organic Matter, and Crude Protein Apparent Digestibility in Lambs fed a High-Concentrate Diet.....	69
5. The Effect of Limestone or Magnesium Oxide on the Partitioning of Dry Matter Digestibility in Lambs Fed a High-Concentrate Diet.....	70
6. The Effect of Limestone or Magnesium Oxide on Nitrogen Balance in Lambs fed a High-Concentrate Diet.....	72
7. The Effect of Limestone or Magnesium Oxide on the Flow of Amino Acids to the Abomasum in Lambs Fed a High-Concentrate Diet.....	74
8. The Effect of Limestone or Magnesium Oxide on Partial Digestibility of Amino Acids in the	

	Small Intestine of Lambs Fed a High-Concentrate Diet.....	76
9.	Supplemental Pellet Composition on an AS-Fed Basis.....	88
10.	Limestone Particle Size and Rate of Reactivity.....	92
11.	Effect of Limestone Source and Dietary Level on Rumen pH, Rumen Volatile Fatty Acid Concentrations and Fecal pH in Feeder Lambs.....	94
12.	Effect of Limestone Source and Dietary Level on the Performance of Feeder Lambs-Cumulative Data by Period.....	96
13.	Diet Composition on an As-Fed Basis.....	104
14.	Mineral Particle Size.....	114
15.	The Effect of Defluorinated Phosphate, Limestone or Magesium Oxide on Digesta and Fecal pH, Duodenal Digesta Acid Concentration and Duodenal Acid Flow in Beef Cattle Fed a High-Concentrate Diet.....	116
16.	Multiple Regression Coefficients for Duodenal Titration Curves: $pH = b_0 + b_1meq + b_2meq^2 + b_3meq^3$	122
17.	The Effect of Defluorinated Phosphate, Limestone or Magnesium Oxide on Dry Matter Apparent Digestibility in Beef Cattle Fed a High-Concentrate Diet.....	124
18.	The Effect of Defluorinated Phosphate, Limestone or Magnesium Oxide on Starch Apparent Digestibility in Beef Cattle Fed a High-Concentrate Diet.....	128
19.	The Effect of Defluorinated Phosphate, Limestone or Magnesium Oxide on Crude Protein Apparent Digestibility in Beef Cattle Fed a High-Concentrate Diet.....	132
20.	The Effect of Defluorinated Phosphate, Limestone or Magnesium Oxide on the Quantity of Amino Acids Flowing into the Duodenum of Beef Cattle Fed a High-Concentrate Diet.....	135

21. The Effect of Defluorinated Phosphate, Limestone or Magnesium Oxide on the Partial Digestibility of Amino Acids in the Small Intestine of Beef Cattle Fed a High-Concentrate Diet.....137
22. Analysis of Feed Grade Limestone Samples Obtained from Various Counties within the State of Virginia.....152

Tables in Appendix:

1. Amino Acid Analysis of a Casein Standard.....181
2. A Comparison Between Actual Values and Expected Values for the Casein Standard Analyzed with the PICO-TAG Amino Acid Analysis System.....182

LIST OF FIGURES

Figures:

1. Duodenal Fluid Titration Curves.....121

INTRODUCTION

The beneficial effects of buffering materials in the diets of cattle and sheep vary widely. There are many factors that dictate what influence these compounds may have on animal metabolism. More information is needed on site and action of buffers on digestive and metabolic processes in ruminants so that these compounds can be more effectively used to benefit the health and performance of the ruminant animal. Some feeding studies have shown improvement in the performance of feedlot cattle when fed a high dietary level of limestone. Results from studies to pinpoint the mode of action of limestone have been quite variable, but some studies have indicated that high levels of limestone can improve postruminal organic matter and starch digestibility. Occasionally, rumen dry matter digestibility has been improved, but total tract digestibility has often not been improved. However, a shift in the digestion of starch from the rumen to the small intestine could provide an energetic advantage to the animal.

Since limestone has a low solubility within the normal pH range of the rumen, some researchers suggest that limestone may aid in neutralizing acid postruminally and provide a more favorable intestinal pH for the activity of digestive enzymes (Wheeler and Noller, 1976).

Research to investigate this hypothesis has spawned some inconsistencies, but results generally have not been very supportive of the postruminal buffering hypothesis.

Duodenal pH has been increased in steers fed a high concentrate diet supplemented with a high dietary level of limestone, but it did not alter postruminal digestion of starch (Zinn and Owens, 1982). Others have reported no change in duodenal pH from feeding a high level of limestone, but reported an improvement in digestion of starch in the small intestine (Laudert and Matsushima, 1982). Teh et al. (1984) reported that buffers such as NaHCO_3 , limestone and MgO may actually increase intestinal acid concentrations. However, the flow of acid into the intestine was not determined in this study. The capability of the intestine to handle an acid load is a combination of the rate and concentration of acid flowing into the duodenum and the rate and concentration of buffer secreted by the pancreas, liver and mucosal cells. The ability of limestone to alter the flow of acid into the duodenum of feedlot cattle has not been investigated.

Major factors that can affect the action of feed buffers include: the concentrate or roughage level of the diet, buffering capacity of the feedstuffs, level of feed intake and reactivity of the buffering compound.

Concentrate or roughage level will affect starch concentration, fermentation rate, saliva production and pH of the gastrointestinal tract. Forages, particularly legume forages, and protein supplements increase the buffering capacity of the diet. As feed intake is increased, the production of acid is also increased in the rumen and intestinal tract and the demand for efficient activity of intestinal enzymes is intensified. Buffer reactivity is not likely a critical factor for consideration for the more soluble compounds such as NaHCO_3 or KHCO_3 , but the reactivity of less soluble compounds such as limestone can vary considerably due to differences in chemical composition, particle size and crystallinity.

The influence of limestone on starch digestion in ruminants has received considerable research attention, but information on the effect of feeding high levels of limestone on protein nutrition in ruminants is lacking in the literature. Mineral buffers can affect protein solubility in the rumen and the quantity of microbial and feed protein reaching the small intestine. Postruminally, buffers may also affect the activity of protein digestive enzymes and the absorptive processes for amino acids.

The research described in this dissertation was designed to: (1) study the effect of feeding a high dietary level of limestone or MgO on gastrointestinal pH, organic matter digestibility, N balance and the partial digestibility of amino acids in the small intestine of lambs limit-fed a 90% concentrate diet; (2) study the effect of dietary level and particle size of feed grade limestone on rate of gain and feed efficiency of lambs fed an all-concentrate diet; (3) study the effect of a high dietary level of deflorinated phosphate, limestone and MgO on flow of acid into the duodenum, partitioning of starch and protein digestion within the gastrointestinal tract, and partial digestion of amino acids in the small intestine of cattle fed a high-concentrate diet near an ad libitum level of intake; and (4) determine the types of feed grade limestone commonly used by livestock producers and(or) feed manufacturers throughout Virginia.

CHAPTER 1

REVIEW OF LITERATURE

The mechanisms involved in maintaining acid-base balance within the body rank very high among homeostatic controls. Keeping the pH of intra- and extracellular fluids within normal limits probably ranks third on the list of metabolic priorities, yielding only to the respiratory drive for oxygen and the need to dissipate heat. Hydrogen ion concentration affects the rate of enzymatic reactions, the conformation of proteins and the transport of ions across cell membranes. Its regulation is crucial to normal body metabolism. Conditions that upset hydrogen ion balance are likely to interfere with the efficiency of metabolic processes and pull energy from functions of lesser metabolic importance (but of significant economic importance) such as growth and lactation.

The ingestion of food by an animal serves to disrupt the balance of protons in the body. Feedstuffs themselves are sources of acids, bases and buffers. The digestion and the metabolism of feed nutrients add acid to the system which must be neutralized or excreted. To a large extent, the amount of bases or buffers in feeds determine how well the body will neutralize or excrete protons.

The ruminant, unlike monogastric animals, must also deal with a major influx of exogenous acids that are products of rumen fermentation. In the ruminant, shifts towards metabolic acidosis are only minor when the diet is composed primarily of slowly fermented forages and contains substantial levels of alkaline materials. However, when the diet is composed primarily of acidic feeds or feedstuffs low in fiber and acid neutralizing materials an added burden is placed on the buffering systems of the body. It is under these conditions that the ruminant is likely to benefit the most from supplemental buffers and(or) alkalizing compounds..

Buffering and alkalizing reagents have commonly been added to beef and dairy cattle rations to control rumen fermentation and counter metabolic problems associated with high energy diets. The site of action of buffers was first thought to be in the rumen only. Though this may be the primary area of action for feed buffers, some research information suggests that certain buffers or acid neutralizing materials may have important action in other areas of the digestive tract.

Rumen Acid Production and Buffering

Acid Production. The pH of rumen digesta does not accurately reflect acid production since under most conditions the rumen is well buffered against acid

addition, but more importantly, because about 50% of the volatile fatty acids (VFA) produced daily are absorbed through the rumen wall and neutralized in the blood (Ash and Dobson, 1963). Davis (1979) pointed out that even though daily bicarbonate and phosphate supplies to the rumen via saliva may be enormous in the lactating dairy cow (estimated at 1-2 kg and .5 kg, respectively) they are capable of neutralizing only about one-half of the fatty acids produced per day. Total daily acid production in the rumen has been estimated to be about 4-5 moles for sheep (Church, 1975), 40-50 moles for beef cattle (Trenkle, 1979) and about 100 moles for the lactating dairy cow (Wiltrout and Satter, 1972). If it is assumed that one-half of the acid produced in the rumen is neutralized in the blood, then 50 moles of VFA require neutralization in the rumen of the dairy cow daily. This production of VFA would correspond to a VFA concentration in rumen fluid of 100 mM which, if not neutralized, would yield a pH of about 3 (Chalupa and Kronfeld, 1983).

Rumen Buffers and Buffering Capacity. The primary buffers in the rumen are phosphate, bicarbonate and volatile fatty acids (Turner and Hodgetts, 1955; Counotte et al, 1979). They regulate rumen pH within the range of 5.5 to 7.3. Bicarbonate and phosphate ions contribute

the most to rumen buffering at the higher pH range 6-7, when fermentation rate is slow to moderate (Emmanuel et al., 1969). When rumen fermentation is rapid and rumen pH drops below 6, bicarbonate and volatile fatty acids become the principle buffers (Turner and Hodgetts, 1955; Emmanuel et al., 1969; Counotte et al., 1979). The bicarbonate system has the greatest buffering potential at a pH of 6.25. VFA buffering capacity is greatest around pH 4.8 (Counotte et al., 1979). The pKa values for VFA vary from 4.7-4.8, so within a normal low rumen pH range they are poorly ionized. As rumen pH approaches 5.0 the bicarbonate system is exhausted and volatile fatty acids become the principle buffers. Even though the VFA buffering system is most effective at a low pH range, studies on rumen buffering in ruminants fed a wide variety of diets indicate VFA still exert some influence on rumen buffering capacity over the pH range of 5 to 7 (Turner and Hodgetts, 1955; Kay and Hobson, 1963; Emmanuel et al., 1969; Counotte et al., 1979).

Phosphate seems to play a very minor role in rumen buffering in cattle fed substantial amounts of grain; though, it still makes an important contribution to the rumen as an acid neutralizer. (Counotte et al., 1979). In saliva (pH = 8.1) 86% of the phosphate is in the deionized form HPO_4^{2-} . Upon entering the rumen at pH =

6.3, it reacts with available protons until 90% is in the ionized form (H_2PO_4^-). This would serve to increase rumen pH, but it also reduces the buffering capacity of the phosphate buffering system.

Ruminants generally produce large amounts of saliva. Saliva contributes more than 70% of the fluid phase in the rumen and is the principle source of bicarbonate and phosphate ions (Baily, 1961; Poutiainen, 1966). Serous secretions from the parotid and inferior molar glands and the highly viscous secretions from the palatine, buccal and pharyngeal glands are strongly buffered with bicarbonate and phosphate. Mixed salivary secretions from other salivary glands (submaxillary, sublingual and labial) are only weakly buffered. Glands producing saliva with the greatest buffering capacity also make the largest contribution to total saliva production. Kay (1960) estimated that the parotids, the paired inferior molar glands and the palatine, buccal and pharyngeal glands produce 47%, 12% and 35% of the saliva, respectively, for a total of 94% of saliva produced in mature sheep. Bartley (1976) estimated that in two mature cattle the parotids contributed 86% of the total resting flow of saliva and 53% of the saliva produced during eating. Saliva secretion occurs at different rates during resting, eating, or ruminating (Bailey,

1961; Bailey and Bach, 1961a; Meyer et al., 1965).

Salivary flow while eating is two to three times greater than during resting and secretion during rumination is about 3.5 times greater than the resting rate.

Since saliva is the major supplier of bicarbonate and phosphate to the rumen, its composition and rate of flow are critical to pH control within the rumen. In addition to contributing buffering and acid neutralizing components, saliva also influences rumen buffering capacity indirectly by altering rumen fluid component concentration, outflow of fermentation substrates and absorption or outflow of fermentation products. When saliva was diverted from the rumen of sheep for approximately 5 h, the pH of the contents decreased and the concentration of VFA increased from 60 to 105 meq/liter; whereas, when saliva was not diverted, rumen pH increased only slightly and VFA concentrations decreased by approximately 30 meq/liter (Kromann, 1976). The volume of saliva secreted by ruminants varies with the type and quantity of feed eaten. Kromann (1976) reviewed important dietary factors that can alter buffering capacity within the reticulorumen. He concluded that the most important measure of buffering potential of a feedstuff or diet is the amount of saliva produced per unit of energy intake, since an increase in

energy intake increases the need to buffer the end products of microbial fermentation within the rumen. The dry matter and chemical composition of a feedstuff and the physical form of the feed or diet influence rumen buffering capacity by altering saliva composition and production, rate and amount of dry matter entering the rumen and the rate of fermentation within the rumen.

Feeds that do not induce the secretion of large quantities of saliva are feeds that are low in dry matter or those which are consumed very rapidly such as finely ground or pelleted hay and feed concentrates. Bailey (1959, 1961) found that the rate of secretion of saliva by any one cow was very similar whether it was eating dairy cubes, hay dried grass or silage, but was lower for fresh grass. Differences in the volume of saliva reaching the rumen seemed to depend primarily on rate of eating or time spent chewing before swallowing. Bailey's (1959, 1961) findings supported results from similar work by Balch (1958), who observed that saliva secretion by cows per 4.5 kg of feed was 19.5-25.9 kg, 5.5-6.8 kg and 2.1-3.4 kg for hay, concentrates and beet fodder, respectively. Even though the rate of secretion of saliva was faster for the concentrates than for hay, the rate of ingestion of the concentrate diet was faster and decreased dry matter in the bolus. This resulted in less

saliva reaching the rumen. Sudweeks et al. (1975) and Sudweeks (1977) observed that chewing time in steers was reduced as the level of concentrate was increased in the diet. Diets of 10, 40 and 70% concentrate (ground corn, citrus pulp or soybean mill feed) resulted in mean chewing times of 713, 490 and 387 min/d, respectively. The type of concentrate had no effect or only a very slight effect on chewing time. Citrus pulp resulted in a slightly greater chewing time than ground corn or soybean mill feed. Long hay (Bermudagrass) and coarsely cut wheat straw acting as the roughage fraction of the diet resulted in a greater chewing time than finely cut sorghum or corn silage.

Yarns et al. (1965) compared daily salivary production in ad libitum fed steers given either a 50:50 ground hay (3.81 cm screen): cracked corn diet or a diet of alfalfa pellets. Salivary production was reduced 26% (38-68 liters/d to 25-51 liters/d, respectively) when the diet was changed from the mixed diet over to the alfalfa pellets. Putman et al. (1966) observed a 30% reduction in resting salivary secretion (measured 1.5-2.0 liters after feeding) when limit fed steers received a pelleted rather than a coarsely ground (3.81 cm screen), 89% hay diet. Feeding a ground, 75% concentrate diet resulted in similar secretion values as the pelleted high-forage

diet. When cattle were fed ad libitum, saliva production increased for all diet types (3.1 vs. 1.9 liters/h); however, the increase in saliva production rate for both high-concentrate diets (ground and pelleted diets) was considerably lower and caused a significant decline in rumen pH. Oltjen et al., (1965) observed a trend for a lower resting saliva production in steers eating pelleted purified and conventional diets. Differences between pelleted and loosely fed diets were not significant, but larger differences may not have occurred due to the fine particle size of the loose diets and the restricted feeding regime used in the experiment.

The physical form and chemical composition of the diet can also affect the chemical composition of saliva. Limit feeding (1.25% of body weight) a pelleted conventional or purified diet reduced the buffering capacity of saliva secreted by steers (Oltjen et al., 1965). Buffering capacity of saliva was lowest for animals fed the purified diet. The mineral additions in the purified diet appeared to be the main reason for the extra low buffering capacities attributed to this diet. The mineral addition provided 3.17% Ca_2HPO_4 , 2.05% K_2CO_3 , .70% MgSO_4 , .5% NaCl and .09% trace minerals. When this mineral mix was combined with conventional feed ingredients, salivary flow was increased by 22% and

saliva buffering capacity declined by 14%. Emery and coworkers (1960) observed a drop in the ash content of saliva as saliva secretion rate and pH increased with increasing increments of hay in the diet (20 to 100%). Significant differences in the Na and K content of parotid secretions have been observed in cattle eating fresh grass versus fresh alfalfa or alfalfa hay (Hawkins and Autrey, 1968).

Fermentation vs. Buffering Capacity. As referred to earlier, VFA become effective buffers as rumen pH approaches 5.0; therefore, volatile fatty acid concentrations within the rumen are of particular importance to rumen buffering at the lower pH range. Rumen VFA concentrations are affected by energy intake, rate of fermentation and absorption, liquid dilution and time post- prandial.

The effect of energy intake on rumen VFA concentrations has been well documented by Rumsey et al. (1970). Total VFA concentrations were increased by increasing intake in steers fed either a pelleted alfalfa-timothy hay or 92% cracked corn diet at .5, 1.0, 1.5 and 2.0% of body weight. Rumen VFA concentrations were increased by feeding either diet at each level of intake with the exception of the 2% level for the high roughage diet. Rumen VFA concentrations were greatest in

steers fed the concentrate diet at each intake level. As feed intake is increased more substrate is available for fermentation increasing the potential for greater VFA production and greater rumen VFA concentrations. VFA concentrations are likely to increase with each increment of intake until the rate of VFA absorption, fluid dilution, and rumen VFA and substrate outflow balances with the rate of VFA production.

Usually there is a greater concentration of VFA in the rumen of cattle or sheep fed finely ground, pelleted diets or diets containing relatively high levels of concentrates (Wright et al., 1963; Kromann and Meyer, 1972). However, the rate of rumen VFA production and VFA concentrations are greatly affected by interactions between the physical form of the diet, the roughage to concentrate ratio, and the level of dry matter intake (Woods and Luther, 1962; Wright et al., 1963; Kromann and Meyer, 1972).

Theoretically, reducing feed particle size increases the total amount of feed surface area that is exposed to the fluid phase of the rumen and the fermentative action of rumen microorganisms. Just like increasing intake, reducing feed particle size serves to increase rumen fermentation rate and VFA concentrations. Fermentation rate of pelleted roughages is often rapid enough to rival

even concentrate supplemented diets in increasing rumen VFA concentrations and lowering rumen pH (Moore, 1964) . An increase in rumen propionate and a decrease in the acetate to propionate ratio have all been observed in ruminants fed ground or pelleted roughage diets (Moody, 1962; Putnam and Davis, 1963).

Reducing feed particle size by grinding or fine chopping also reduces rumen retention time of feed particles (Rodrique and Allen, 1960). Fiber digestibility, particularly in high forage diets, is reduced by fine chopping or grinding. Pelleting ground roughage diets usually increases dry matter intake in animals fed pelleted diets free choice. The increase in intake of pelleted roughage diets is generally enough to compensate for any loss in animal performance that might be suffered due to a faster rate of passage of feed particles through the rumen and the drop in feed digestibility.

Rumen digestibility and fermentation of cereal grains are generally enhanced by steps taken to reduce feed particle size since feed grains are composed largely of readily fermentable components. Starch represents about 70-80% of most feed grains (Rooney and Pflugfelder, 1986). Rumen fermentation of grain starch averages 78.5%, but starch digestibility in the rumen can be quite

variable, ranging from about 65% for limited processed grains to greater than 85% for well processed grains (Owens et al., 1986). Any process that will reduce grain particle size and(or) gelatinize starch granules will increase the rate of ruminal breakdown of that starch.

Rate of fermentation of cereal starches in the rumen varies with grain species and between varieties within species. Thuerer (1986) reported comparative digestibility data for barley, corn and sorghum. Sorghum had the lowest rumen and total tract starch digestibility and lowest total tract protein digestibility. Values for barley were the highest. The digestibility of grain protein and starch are closely related (Hale, 1973; Spicer et al., 1982, 1983).

The composition and physical form of the starch and protein-starch interactions are major factors influencing grain digestibility (Rooney and Pflugfelder, 1986). The ratio of amylopectin to amylose in grain starch can greatly affect its susceptibility to enzymatic activity. Amylose is a linear polymer of starch that is intermediate to only slightly branched and exists primarily in helical form. In addition to being inherently resistant to amyolytic activity, amylose also is thought to orient itself within amylopectin crystallites causing an increase in intermolecular

hydrogen bonding which limits swelling of the starch granule and the enzymatic hydrolysis of the amylopectin. The digestibility of a particular starch is generally inversely proportional to amylose content. Normal cereal starches contain 20 to 30% amylose while waxy starches contain little or no amylose. Waxy cereal starches are among the most digestible of all starches. Protein encapsulation of starch granules within grain can reduce the amount of starch that is actually exposed to moisture. This limits water penetration and swelling of the starch and the access that enzymes have to the starch. Starch granules can be completely embedded in a protein matrix, as in the corneous and peripheral membrane of corn and sorghum. The digestibility of sorghum protein is particularly low in comparison to corn, wheat and barley proteins and is thought to be very important, if not the major reason, for the poorer feeding value of sorghum among the cereal grains (Hale, 1973; Harbers, 1975). In addition to physical restrictions from a protein matrix it is also possible for gelatinized starches to form complexes with proteins that reduce digestion of both starch and protein (Thorne et al., 1983).

Factors that affect rate and extent of rumen digestibility of feed grains have a major impact on rumen

buffering capacity in ruminant animals fed substantial amounts of grain in their diet. Rapid rumen fermentation induced by feeding high concentrate diets to ruminants is characterized by an increase in total rumen VFA concentration, a decrease in rumen pH and a reduction in the rumen acetate to propionate ratio (Topps et al., 1968ab). These characteristics are usually accentuated when grains with more readily fermentable starches and(or) grains that have been more extensively processed are fed.

Orskov et al. (1974) compared rumen fermentation in lambs fed an all-concentrate diet containing either whole or ground pelleted barley, corn, oats or wheat. The decrease in rumen pH was greatest for wheat followed by corn, barley and oats, respectively. Grinding and pelleting the grain decreased rumen pH and increased total VFA and propionate concentrations. Replacing 60% or more of the cracked corn (90% of the diet) with cracked wheat in an all-concentrate diet fed to growing steers decreased rumen pH and increased total rumen VFA concentrations (Oltjen et al., 1966). Average daily gain was greatest for steers fed the corn diets over the 98-d trial. Animal performance was similar for all the diets for the first 70 d; however, during the last 28 d steers fed the wheat diets consumed less feed. Fulton et al.,

(1979) studied intake and rumen fermentation characteristics in steers fed a wheat- or corn-based, high- concentrate diet throughout a 20-d diet adaptation experiment. Cattle were fed each diet free choice. Steers given the wheat-based diet consumed less total feed (6.60 kg/d vs 9.51 kg/d) and experienced wider fluctuations in rumen pH than steers fed the corn based diet (4.60-6.25 vs. 5.27-5.97). Lower pH values were observed for the wheat- fed steers at all levels of feeding over the adaptation period.

Rumen VFA concentrations were greatest for the corn-fed steers. These data were inconsistent with data obtained by Oltjen et al., (1966), but this discrepancy may have been due more to the large difference in intake between the two groups rather than the type of grain that was fed. Average ruminal lactate concentrations were lowest for the corn diets (122 mg/ml vs 195 mg/ml). Rumen lactate had declined to very low concentrations in all animals by d 10 of the trial even though the animals fed the wheat diet continued to have a lower daily intake to the end of the experiment.

Diurnal fluctuations in rumen VFA concentrations and pH can be of considerable magnitude in ruminants that are meal- fed once or twice daily (Rumsey et al., 1970). The significance of these fluctuations in relation to rumen

buffering capacity and the feeding of high energy diets is evident from studies using multiple feeding management with dairy cattle. Dividing hay and grain feeding into more than five meals per day prevented wide swings in ruminal pH, maintained a higher average pH in the rumen and allowed for a greater intake of grain without causing undesirable changes in the acetate to propionate ratio (Kaufmann, 1976; French and Kennelly, 1985).

A Need For Dietary Buffers

Elam (1983) reviewed the practical need for buffers in livestock rations. His ideas pinpoint some of the critical areas that exist in feeding ruminants high-concentrate diets and how dietary buffers may be of benefit in these situations. His thoughts are summarized as follows:

1. Young calves have responded to the addition of NaHCO_3 with increased feed intake and increased grain. Calves are known to secrete less saliva per unit of feed consumed than mature animals and generally respond to higher levels of NaHCO_3 than more mature animals.
2. In certain instances the addition of highly reactive limestone can improve the performance of cattle fed high concentrate rations. However, dramatic improvements in animal performance from

the addition of limestone or NaHCO_3 should not be expected.

3. NaHCO_3 or limestone is sometimes fed along with potato waste in an effort to prevent acidosis.
4. Nutritionists have found it useful to add Na bicarbonate to the ration whenever acidosis appears to be a particular problem in the feedlot.
5. It is helpful to routinely add NaHCO_3 to starting and transition rations. Symptoms of acidosis are often encountered during these early stages of feeding when cattle are getting started on feed and are being moved to a higher level of concentrate.
6. Buffers can be used to advantage during periods of severe weather change. Feed consumption is often erratic during periods of inclement weather and the incidence of digestive related problems is usually higher than normal. Often it is helpful to increase the roughage and decrease the concentrate in rations.
7. Brahman or Brahman crossbred cattle seem to be more prone to acidosis and founder than the British breeds. Holstein cattle, however, seem to be the most resistant to acidosis and founder.
8. Since the introduction of ionophores in finishing

diets for cattle, there have been fewer problems with acidosis, enterotoxemia, etc. One reason for this may be that ionophores reduce intake and this may reduce the risks associated with the consumption of large amounts of concentrate feeds.

Effective Dietary Buffers

A myriad of mineral compounds have been fed to cattle and sheep to increase the pH within the rumen with varying success. Generally, the term buffer has been very loosely used to describe these compounds. In order for a compound to be useful as a rumen buffer it must be easily and evenly distributed within the rumen medium, it should not create a drastic fluctuation in the pH upon entering the rumen and it should be capable of resisting a pH change as acids are added to the system. Out of 35 compounds evaluated in vitro with rumen fluid extracted from steers fed a high-concentrate diet only NaHCO_3 , KHCO_3 , MgCO_3 , CaCO_3 and bentonite clay exhibited good buffering capabilities after 6 h of incubation (Herod et al., 1978). Compounds such as Ca(OH)_2 , MgO , K_2CO_3 , Na_2CO_3 , K_3PO_4 and NaOH had good acid neutralizing ability, but when added to the system caused a radical change in pH of about 2.0 units. These compounds are better classified as alkalizing agents. Compounds such as Al(OH)_3 , dolomite, MgSO_4 , KH_2PO_4 , NaH_2PO_4 and $\text{Zn(SO}_4)$

were poor acid neutralizers. In vivo evaluations of buffering agents have generally been consistent with results from in vitro determinations of buffering capacity and rate of reactivity (Herod et al., 1978; Keyser et al., 1985; Shaefer et al., 1982).

Sodium Bicarbonate. Sodium bicarbonate has been used extensively as a feed buffer in ruminant diets. It has excellent solubility within the rumen, it is compatible with the ruminant's natural buffering system and when added to a solution containing acid it reacts to neutralize acid without generating a great deal of heat (Weinberg, 1976) or causing a sever increase in rumen pH (Herod et al., 1978). Since NaHCO_3 is so readily dissolved in rumen liquid, its site of action within the GI tract appears to be primarily within the reticulorumen. However, duodenal and fecal pH were increased by NaHCO_3 addition to a 60% concentrate diet (Tagari et al., 1982) and fecal pH was increased in one trial with a 65% concentrate diet (Haaland and Tyrrell, 1982). Cattle were fed ad libitum and some of the bicarbonate may have escaped the rumen. Although, a higher fecal pH could have resulted from a decrease in the availability of substrate for fermentation in the large intestine due to a more favorable fermentation in

the rumen. This idea, however, is not a plausible explanation for the higher duodenal pH.

Sodium bicarbonate supplementation increased ruminal pH in about 70% of the beef cattle trials reviewed by Trenkle (1979). Sodium bicarbonate very consistently increases pH of the rumen under a variety of feeding conditions even though increases may not be statistically significant (Trenkle, 1979).

Buffers can affect rumen pH by neutralizing acid or by affecting the rate of, and time for ruminal fermentation of starch and soluble nutrients. Passage rate of fluid from the rumen increases when NaHCO_3 is added to a high concentrate diet (Hart and Polan, 1982; Rogers et al., 1982). As a soluble mineral salt, NaHCO_3 is capable of altering rumen fluid osmolarity, rumen dilution rate and rate of passage of fluid and particulate matter from the rumen. Kellway et al. (1977) observed that about 43% of the production response in calves was due to rumen dilution effects when fed a pelleted, high-concentrate diet buffered with NaHCO_3 . In theory, when osmolarity of the rumen increases as buffer ions increase in concentration, fluid passes through the rumen wall into the lumen and liquid flow out of the rumen is increased. A faster liquid flow also carries with it constituents associated with the fluid phase such

as ammonia, suspended and undegraded starch, protein and fiber leaving behind coarser, dense food particles. The escape of readily fermentable components will slow fermentation rate and alter VFA concentration. Faster liquid turnover within the rumen can enhance the efficiency of microbial protein synthesis, but may not necessarily increase the total output of microbial protein since available substrates are flushed out (Owens et al., 1983). Faster liquid turnover may also alter metabolic pathways of microbes.

Rogers and Davis (1982) increased rumen pH from 6.07 to 6.35 in steers fed a high-concentrate diet (75:25, concentrate to corn silage, dry matter basis) by adding NaCl (200 g/d) to the rumen and increasing rumen liquid out flow. Infusing NaCl into the rumen also increased the acetate to propionate ratio by reducing propionate production. Total VFA concentrations were also reduced. The infusion of NaHCO₃ (288 g/d) resulted in a rumen pH of 6.55 and similar increases in the acetate to propionate ratio and total VFA concentrations seen with the infusion of NaCl. Okeke et al. (1983b) studied the rate of passage of feed particles out of the rumen in steers fed either a 50:50 or 80:20 concentrate to corn silage diet supplemented with 0, .75, 2.5 and 5.0% NaHCO₃ on a dry matter basis. Diet type had little affect on

experimental results. Values were similar between the low (0, .75%) and the high (2.5, 5.0%) buffer groups. Dilution rate increased 25% when a high level of NaHCO₃ was fed. Rumen pH averaged 6.02 and 6.35 for the low and high buffer levels, respectively. Contrary to results reported by Rogers and Davis (1982), the high buffer level resulted in a greater acetate to propionate ratio (2.58 vs. 2.23).

A buffer will only contribute to the osmolarity of the rumen if it is solublized. This may explain why several researchers have reported no significant change in ruminal dilution rate by feeding limestone, a salt that is poorly solublized within the rumen (Haaland and Tyrell, 1982; Keyser et al., 1985; Rogers et al., 1982; Rust and Owens, 1981).

Fiber digestion is highly correlated with pH of the rumen (Mertens, 1979; Franklin et al, 1981; Wedekind et al., 1986). The optimum pH range for fiber digestion is 6.7 to 7.1 (Mertens, 1979). As rumen pH approaches 6.0, cellulolytic bacteria are inhibited and fiber digestion declines. Franklin et al. (1981) demonstrated the effect of pH on fiber digestibility in situ with sheep and showed the variation that exists between animals in their ability to control pH within the rumen. Sheep were adapted to diets containing different ratios of

concentrate to bromegrass. Sheep were divided into a buffered and nonbuffered group based on their ability to maintain a rumen pH above or below 6.1 when fed a 66% concentrate ration. Within the nonbuffered group, in situ disappearance of bromegrass fiber decreased linearly with increasing levels of concentrate in the diet. Fiber digestion within the buffered group was unrelated to concentrate level, suggesting that rumen pH is the critical component influencing fiber digestibility in mix diets. Goetsch et al., (1983) improved fiber digestion in the rumen of steers fed a high concentrate diet (90%) by increasing ruminal pH with a NaOH-KOH solution. Wedekind et al. (1986) studied the effects of concentrate level and NaHCO₃ on site and extent of forage fiber digestion. A semipurified concentrate was used so forage digestibility information would not be confounded with grain fiber digestibility. Sheep were fed (1.0 kg/d) fescue hay, soybean meal and concentrate in mixtures of 95:5:0, 76:4:20, 57:3:40 and 38:2:60. The feed buffer was added at 0 or .75% of the concentrate. Sodium bicarbonate had no affect on total tract digestion of any fiber fraction, but improved ruminal digestion of NDF, ADF, and hemicellulose digestion was not affected by buffer addition. As the level of concentrate increased in the diet, digestion of dietary fiber was shifted more

postruminally, although the level of concentrate had little affect on total tract fiber digestion. Rumen pH values seemed consistent with information available on rumen pH and fiber digestion (Mertens, 1979); however, they differed from information presented by Franklin et al. (1981) in that the range of mean pH values was 6.70 to 6.30, well above the 6.1 that seemed so critical to the relationship between the concentrate level and the disappearance of bromegrass fiber in situ.

There are very few reports available on the effect of feeding buffers on protein metabolism in ruminants. Okeke et al., (1983ab) studied the effect NaHCO_3 might have on the rate of passage and degradation of soybean meal in steers and early lactating dairy cows. Sodium bicarbonate was fed at 0, .75, 1.0, 2.5 and 5.0% of dry matter intake. Sodium bicarbonate increased liquid dilution and passage rates at 1.0 and 2.5%, but there was no advantage to feeding 5% buffer. Protein degradability was similar among treatments even though there was a slight trend in favor of the buffer treatments. Passage rate for soybean meal was also similar among experimental treatments.

Trenkle (1979) proposed two important ways that feed buffers could enhance protein metabolism in the ruminant. First, buffers that increase ruminal pH above 6.0 and

increase rumen dilution rate have the potential to stimulate efficient microbial protein synthesis. Buffer feeding also increases feed protein degradation in the rumen, but the boost in microbial synthesis due to a more favorable pH is more than enough to prevent excessive urinary nitrogen excretion. Secondly, in feedlot animals that are acidotic glutamic acid is pulled from the muscle and metabolized in the kidney. Ammonia is extracted to act as a carrier of protons in the acid excretion process. A loss of nitrogen in this way could be critical to overall nitrogen balance, particularly in young fast growing animals. Feeding buffers during periods of stress such as during the initial period of changing cattle from a high roughage ration over to a high energy production ration could improve protein efficiency.

Within the last 2 yr a product called Alkaten has been marketed by the Tenneco Minerals Company. Alkaten is the trade name for the natural ore Trona or sodium sesquicarbonate ($\text{Na}_2\text{CO}_3:\text{NaHCO}_3:\text{H}_2\text{O}$) from which NaHCO_3 is manufactured. Trona seems to be more effective than NaHCO_3 in modifying acid-base balance within the rumen (Boerner et al., 1987b). Trona maintains a more stable environment by minimizing very low pH fluctuations that occur postprandial. However, the pH stabilizing

advantage that Trona may have over NaHCO_3 was not reflected in the digestion of feed fiber, protein or starch within the rumen (Boerner et al., 1987a).

Magnesium Oxide. Magnesium oxide (MgO) is often added to production rations for high producing dairy cows to aid in maintaining milk fat percentages. It is a potent alkalizer and more effective per gram of compound than NaHCO_3 for restoring milk fat percentages (Emery, 1976). Magnesium oxide is used in the diet of lactating dairy cows at .75 to 1.0 % of the grain mixture or .30 to .50% of the total diet (Chalupa and Kronfeld, 1983).

Magnesium oxide fed alone or in combination with NaHCO_3 has improved dry matter, organic matter and fiber digestion in lactating dairy cows (DeHaan et al., 1982; Erdman et al., 1980, 1982; Tagari et al., 1982). There is very limited information on the use of MgO as a buffer or alkalizing agent in the diet of beef cattle or sheep. Peirce et al. (1983) reported an improvement in dry matter intake and dry matter digestibility in beef steers that had been abruptly changed from a roughage diet over to a high-concentrate diet supplemented with MgO and NaHCO_3 as buffers. Buffer treatments included a control, .5% MgO , 1.0% NaHCO_3 and .5% MgO + 1.0% NaHCO_3 . Animals were fed for a 2-wk period following the diet change. Sodium bicarbonate alone resulted in the highest intake.

Intake expressed as a percentage of control was: 114% for MgO, 120% for NaHCO₃ and 110% for the combination. Animals tended to eat more the second week even though there was no significant week effect detected. Dry matter and NDF digestibility was greatest for MgO and the buffer combination. Tagari et al. (1982) and Erdman et al (1980) reported increases in fiber digestibility in dairy cows supplemented with MgO and MgO plus NaHCO₃. DeHaan et al. (1982) reported a significant improvement in dry matter and fiber digestibility in steers fed a 50:50 concentrate to corn stalklage diet when supplemented with a NaHCO₃-MgO buffer; however, similar results were obtained with a limestone-MgO buffer combination. In the study by Peirce et al (1983), ruminal pH was similar among all treatments and averaged 6.3. Ruminal pH during the second week of feeding was markedly higher than it was during the first week (6.5 vs 6.0). This was evidence that the rumen had adapted well to the abrupt dietary change. Starch digestibility was greater with MgO in the diet (95.1 vs 93.6%) though the difference was small.

The effects of buffers on animal performance are generally evident only for a short duration. Once the animal has adapted to the new feeding regime, effects due to buffers seem to fade (Emerick, 1976). A 112-d feeding

trial was conducted with Angus x Hereford yearling steers (Grove et al., 1981). Animals were fed an 80:20 concentrate to forage diet. Steers were given the same treatments and switched to the fattening ration abruptly as was reported by Peirce et al. (1983). During the first 28 d of the feeding trial, steers fed the MgO and buffer combination ate 11.5% more feed per d and had a 17.1% greater average daily gain than the control animals. At the end of the experiment, no differences were observed in performance values among treatments.

Particle size, chemical composition, and crystallinity can influence the acid neutralizing capability or mineral bioavailability of a buffer or alkalizing agent. Coarse or slow reacting MgO has been shown to provide less available Mg to the animal and less acid neutralizing capability in the rumen than more finely prilled or fast reacting MgO when supplemented to lactating dairy cows at .4 or .5% of the dry matter (Thomas et al., 1984; Xin et al., 1987). Serum magnesium, milk fat percentages and rumen pH were greater in animals fed the faster reacting minerals. The finely ground MgO depressed intake even though the Mg was readily available for absorption.

Schaefer et al. (1982) evaluated acid neutralizing capacity of two sources of commercial feed grade MgO in

the rumen of fistulated steers fed a corn silage-corn grain diet (60:40, dry matter). Total acid consuming capacity was nearly the same for the two sources (43.68 and 41.92 meq H⁺/g). However, the capacity to change rumen pH was very different between the two sources. Over a 7-d feeding period the rumen pH averaged 5.89, 6.08 and 6.30 for the control, MgO₂-A and MgO₂-B, respectively. The authors did not report any particle size information on the two sources, but they stated that since the two mineral sources were similar in total acid consuming capacity, their ability to change rumen pH was indicative of their reactivity within the rumen. Work by Jesse et al. (1981) demonstrated that commercial grades of MgO may differ in solubility and Mg availability simply due to their chemical or crystalline nature alone.

Calcium Carbonate. In relation to NaHCO₃, limestone is poorly dissolved within the normal pH range of the rumen. Even reagent grade limestone rapidly decreases in reactivity when pH increases to 5.5 or higher (Keyser et al., 1985). Dolomitic limestones are usually harder than calcitic limestones and much less reactive (Ferreira et al., 1979). Only a few researchers have reported a change in ruminal pH by feeding limestone. Haaland et al. (1982) reported a significant increase in ruminal pH in non-lactating cows fed a diet of 60% concentrate and

40% corn silage containing 2.5% of limestone and 11% crude protein. The pH response was lessened by increasing crude protein to 14 and 17% in the diet. In a study involving steers fed 60% concentrate and 40% grass hay, 1.2% limestone in the diet increased rumen pH in similar magnitude as did 1.0% NaHCO₃ or .48% MgO (Teh et al., 1985). Rumen pH has also been increased by mixing the equivalent of 2% limestone or 2% NaHCO₃ in the diet with .75% MgO and placing it in the rumen of steers fed a diet containing 50% corn stalklage (DeHaan et al., 1982). The reason for a limestone pH effect in these few studies is unclear. The poor solubility of limestone within the rumen is a likely explanation for its inability to affect rumen pH, but even within the pH range of the rumen some limestone is dissolved. Tissera et al. (1982) reported that in a pH-stat examination at pH 5.5, 40-50% of a fine fast reacting limestone was solublized within 15-30 min. Factors such as the quality and particle size of the limestone, the amount of limestone in the diet, pH of the rumen, the time and technique of sampling the rumen could influence the pH value determined.

In spite of its low solubility in the rumen, limestone has been shown to affect rumen digestion phenomena. El Tayeb et al., (1984) reported small, but significant increases in rumen fiber digestibility in

sheep fed a high-concentrate diet supplemented with 1.5% limestone. Total tract organic matter digestibility decreased with limestone supplementation. In the same study, 1.5% limestone added to a high-roughage diet (75% grass hay) improved rumen and total tract digestibility of organic matter, starch and fiber. In support of these findings, Zinn and Owens (1980) reported improved rumen digestibility of organic matter, starch and fiber in steers fed an 80% concentrate diet supplemented with either 2.5% CaCO_3 or a mixture of CaCO_3 (2.5%) and NaHCO_3 (1%). Again, it appeared that limestone supplementation simply affected the site of digestion since total tract digestibility was not affected by the limestone treatment. Work by Laudert and Matsushima (1982) also supports this concept, but contrary to results obtained by Zinn and Owens (1980), they saw improved organic matter and starch digestibility in the small intestine rather than in the rumen. Fiber digestibility was not affected in this study. In support of data showing that limestone can affect total tract digestibility, research findings by Rust and Owens (1981) should also be mentioned. Limestone added at the level of 2% of the diet improved organic matter, fiber, starch and nitrogen digestibility in steers fed a 10 or 50% alfalfa diet. Owens et al. (1983) proposed that soluble Ca may have a

stimulatory affect on microbial breakdown of fiber and starch within the gut. A study by Goetsch and Owens (1985) was conducted to examine the effect Ca (from CaCl_2 or CaCO_3) had on the site and extent of digestion within steers fed a high concentrate diet. Calcium concentrations within the rumen actually decreased rumen digestion. Rumen dilution rate was increased by each Ca treatment.

Even though the effects of limestone on rumen digestion are quite variable, several studies have shown that increasing limestone levels in the diet of cattle can improve feedlot performance (Wise et al., 1965; Rouse, 1978; Varner and Woods, 1972). In most cases, though, improvements are small and often insignificant when averaged over an entire feeding period (Owens et al., 1983).

Postruminal Acid Production and Buffering

Acid Production. Gastric acid production and the passage of acidic digesta into the duodenum in ruminants has been studied primarily in sheep, goats and young calves. The ruminant is uniquely different from most monogastrics in that the passage of digesta through the gastrointestinal tract is virtually a continuous process. The rumen and omasum provide the abomasum and the intestines with a nearly uninterrupted supply of digesta

and as such, gastric and enzyme secretions are also continuous. Gastric acid or HCl is actively secreted by the parietal cells within the fundic region of the abomasum (Argenzio, 1984). Acid formation occurs as hydrogen ions are cleaved from bicarbonate molecules within the parietal cell and are transported along with Cl⁻ across the cell membrane against a concentration gradient. Ash and Kay (1963) determined that sheep abomasal gastric juice contained up to 124 meq H⁺/liter, 138-172 meq Cl⁻/liter, 2-19 meq K⁺/liter and 21-167 meq Na⁺/liter. VFA concentrations are usually quite low in abomasal fluid (3 to 14 meq/liter) in comparison to rumen contents (69 to 103 meq/liter) (Masson and Phillipson, 1952).

Anticipation of feeding, rumen reticulum contractions, abomasal distension and the pressure of food within the abomasum are factors that cause an increase in gastric acid production (Ash, 1961; McLeay and Titchen, 1970; McLeay and Titchen, 1974). The presence of VFA also stimulate acid secretion (Ash, 1961). The abomasum is capable of VFA absorption; however, VFA present in high concentrations affect abomasal muscle motility and have been implicated in the etiology of abomasal displacement and abomasal ulcers

(Hull and Wass, 1973; Bolton et al., 1976; Breukink and de Ruyter, 1976).

The heavy amounts of Cl⁻ secreted by the stomach during acid production are reabsorbed primarily in the lower third of the small intestine as well as in the large intestine (Coombe and Kay, 1965). Chloride is thought to be transported via a counter transport mechanism with bicarbonate (Argenzio, 1984). Chloride is transported into the enterocyte in exchange for intracellular bicarbonate. Bicarbonate secretion neutralizes intestinal acids and provides the alkaline environment that is typical of the lower ileum and which often exists in the large bowel of ruminants.

In addition to the abomasum, the large bowel is also a source of acid production in the lower GI tract. Fermentative digestion takes place within the cecum and large intestine in the ruminant and usually leads to the production of significant amounts of organic acids. The large intestine has been estimated to contribute from 4.2% to 26.0% of the total digestible energy in the ruminant animal (Ulyatt et al., 1975). This energy contribution is thought to come primarily from volatile fatty acid production. The cecum and large intestine have present many of the anaerobic bacteria that are common to the rumen (Mann and Orskov, 1973). Cecal

digesta has been shown to have cellulolytic activity greater than or equal to that of rumen digesta (Ulyatt et al., 1975).

The main factors determining the digestible energy contribution from the large bowel seem to be the digestibility of the diet and dietary intake. The more fiber that is digested in the rumen the less there is available for fermentation within the cecum and large intestine (Ulyatt et al., 1975). A larger percentage of a poor quality forage will be fermented in the cecum and the large intestine than will occur with a higher quality forage. The large intestine is pretty effective in picking up the slack in diet digestibility that is passed by the rumen and small intestine. With ground, flaked or high-moisture grains, especially at high levels of intake, grain fiber digestibility in high-concentrate diets fed to cattle is often very low within the rumen because the rumen pH is low and not conducive to fiber digestion (Zinn and Owens, 1983). The dietary fiber is then funnelled on to the lower tract where it is effectively digested in the large intestine (Owens et al., 1986). However, for whole or poorly processed grains, large intestine fiber digestion is quite the opposite. With poorly processed grain more starch reaches the large intestine for fermentation. Starch

fermentation lowers the pH and reduces fiber digestibility within the cecum and large intestine. Generally, the more extensively that grain is processed the greater is its digestibility within the rumen and within the small and large intestines (Owens et al., 1986; Theurer, 1986).

Buffering Systems. Bicarbonate is the principle buffer in the intestines. It is secreted by mucosal cells throughout the small and large intestinal tract and is provided in liberal quantities in pancreatic and biliary secretions. The liver supplies three to five times more bicarbonate than the pancreas (Caple and Heath, 1975). The bicarbonate supplied in pancreatic and biliary secretions is designed to neutralize stomach acid and increase intestinal pH quickly so as to provide a more favorable environment for enzymatic breakdown of feed components and absorption of essential nutrients. However, in the ruminant, pancreatic and biliary secretions appear inadequate to handle the intestinal acid load. Small intestinal pH in the ruminant rises very slowly in comparison to monogastric species. Borgstrom et al. (1957) reported pH values near 6 in the duodenum of human subjects on a digestion and metabolism trial. In sheep the duodenum and upper jejunum are rather acidic ranging from pH 2.5 to 4.5 (Lennox and

Garton, 1968; Kay, 1969; Ben Ghedalia et al., 1974). Intestinal pH does not reach 6 until the lower jejunum. This delayed increase in intestinal pH has not been documented in cattle, though it is assumed it would be comparable to what has been observed in sheep.

The slow neutralization of stomach acid in ruminants may be due to a comparatively low bicarbonate content in pancreatic and biliary secretions. Bicarbonate concentrations in sheep pancreatic juice ranges from 15 to 30 meq/liter (Taylor, 1962) compared to 120-130 meq/liter in human pancreatic juice (Oser, 1965) and 60-148 meq/liter in dog pancreatic secretions (Taylor, 1962). Lower secretion rates may also contribute to the problem. Pancreatic secretory rates expressed per unit of body weight are considerably lower for the sheep (.0067 ml/kg/min) than for the dog (.08 ml/kg/min) (Magee, 1961).

Even though the small intestinal pH is slow in rising in the ruminant, the lower one-third of the small intestine appears adequately buffered. Zinn and Owens (1980) studied the buffering capacity of the small intestine in steers by adjusting the acidity of duodenal chyme up or down one pH unit. The pH adjustment failed to significantly alter the basic pH at the terminal ileum. In fact, numerical differences suggested a trend

opposite from what one would expect from acidifying or neutralizing duodenal contents. A criticism one may offer here is that even though an adjustment of the pH of duodenal contents failed to effect a pH change at the terminal ileum it may still have delayed the pH from rising to 6 or above until further on down the tract. Determining pH changes along the jejunum and ileum would be necessary to access the actual buffering capacity of the small intestine.

Buffering and pH changes in the large intestine have not been given much research attention. Williams (1965) studied the pH in the cecum and colon 6 h and 24 h after feeding in sheep limit-fed (600 g/d) alfalfa chaff, whole wheat grain and alfalfa chaff (20:80, dry basis) and whole corn grain and alfalfa chaff (20:80). He found that pH remained within the basic range in the cecum and colon in animals fed alfalfa chaff, ranging from 7.0 to 7.3 in the cecum and 7.1 to 8.5 in the colon. Cecal pH in animals fed grain ranged from 5.7 to 6.9 at 6 h and 6.8 to 7.2 at 24 h. Colon pH ranged from 5.9 to 7.0 at 6 h and 6.3 to 7.4 at 24 h.

A Need For Postruminal Buffers

Wheeler and Noller (1976) have suggested that when cattle are fed high-concentrate diets above maintenance

the decline in starch digestibility that accompanies an increase in intake may be due to an unfavorable intestinal pH for optimum amylase activity. They supported their hypothesis with intestinal pH data from feeder cattle obtained at the time of slaughter. Data showed that pH in the lower ileum could be below 6 in cattle full-fed a high concentrate diet. The data also indicated that the fecal pH seemed to be a good indicator of pH in the ileum. Wheeler and Noller (1976) further suggested that a feed buffer such as limestone that has rumen bypass capability may be capable of buffering in the lower tract. Limestone added at high levels in the diet of cattle fed high-energy diets has been shown to increase starch digestibility, reduce fecal starch, increase fecal pH and in some cases, improved animal performance (Wise et al., 1965; Varner and Woods, 1972; Owens et al., 1983).

Intestinal pH data obtained from cannulated steers in studies by other researchers have cast doubt on the validity of intestinal pH information obtained at slaughter. In over 200 measurements in steers with intestinal cannulae, the pH of duodenal contents has averaged $2.8 \pm .8$ and the ileal pH has averaged $6.7 \pm .4$ (Zinn and Owens, 1980). The consistently basic pH of the

ileum is not supportive of the low pH values presented by Wheeler and Noller (1977).

A number of applied and more fundamental animal studies have been conducted to further test Wheeler and Noller's hypothesis. Results from these studies have been highly variable, but some important information about intestinal starch digestibility and the action of limestone as an "intestinal buffer" has been gleaned.

Earlier work by Varner and Woods (1972) showed that animal performance could be improved by feeding limestone at fairly high dietary levels. They studied the effects of addition of limestone to diets containing 85% concentrate and 15% roughage for fattening cattle. Increasing Ca from .2 to .31 and .41% increased gains from .97 to 1.10 and 1.18 kg/d. Feed intake was increased slightly, but feed to gain ratios were 9.07, 8.33 and 7.85 for the increasing levels of Ca. Krause and Britton (1979) supplemented cattle eating a diet of high-moisture corn and corn silage with 2% limestone or 2% defluorinated tricalcium phosphate. Average daily gain was about the same for the control and treated diets (1.16, 1.15 and 1.14, respectively). However, the animals fed limestone tended to have the lowest feed to gain ratio (7.26, 7.08, 7.63 for the control, limestone and tricalcium phosphate diets, respectively). Owens et

al. (1983) reviewed results of 40 feeding trials in which limestone was fed as a buffer at various levels. He concluded that the greatest benefit that limestone feeding seemed to provide was an increase in feed efficiency as a result of a lower feed intake. He further stated that most of the benefit came early in the trial, but when averaged over the entire trial it amounted to only a small improvement (2.2% increase). Work by Brink et al. (1984) lend further credence to the conclusion that limestone in some cases seems to give feedlot cattle a slight edge in efficiency. Several cattle feeding experiments were conducted to study the effect of feeding various levels of limestone to cattle eating diets containing 80 to 85% dry (whole and(or) rolled) or high-moisture corn on a dry matter basis. In one experiment with steers fed high-moisture corn, steers fed 1.7% limestone were more efficient than those given .8% limestone. Feeding 2.8% limestone reduced feed intake and rate of gain. When all experiments were combined the probability for a greater F statistic for .8 vs 1.7% limestone were .70, .32 and .17 for gain, dry matter intake and feed/gain, respectively.

Feeding limestone could improve efficiency in cattle in several ways:

1. It could improve feed nutrient digestibility ie.

improve starch and fiber digestibility by providing a more favorable environment within the rumen and within the small and large intestines.

2. It could improve nutrient absorption in the small intestine.
3. It could repartition starch and protein digestibility in favor of the small intestine. Subjecting starch and protein to digestion in the small intestine rather than fermentation in the rumen or large intestine would make more efficient use of feed energy and protein.
4. It could cause two or more of the above.

Results from studies on the effect of limestone on the site of digestion have been variable, but have not closed the door on the idea of a limestone mode of action in the lower tract. Laudert and Matsushima (1982) saw an improvement in small intestine digestion of organic matter and starch by the addition of 1 or 2% limestone in an 80% cracked corn diet fed to steers. However, rumen and total tract digestibility were not altered. Zinn and Owens (1980) saw an improvement in rumen and total tract starch digestibility by feeding limestone, but observed little improvement in starch digestibility in the small and large intestines. Owens et al., (1983) summarized data from 6 Oklahoma trials and reported that limestone

increased ruminal digestion in four of the six trials. While in three of the six comparisons, small intestinal digestion of starch entering the small intestine was numerically increased.

One criticism of the research that has been cited is that they have not considered the impact that ad libitum feeding might have on postruminal phenomena. Animals have been fed 60-70% of maximum intake. Theoretically, higher intake levels funnel more nutrients past the rumen and increase the work load on postruminal digestive and absorptive processes. Under these conditions differences due to postruminal stress or buffering may be more apparent. Work by Brink and Steele (1985) investigated the site and extent of starch and fiber digestion as affected by source of Ca and the level of corn grain in cattle fed ad libitum. Limestone did not affect ruminal digestion, but postruminal starch and fiber digestion were improved with 2% limestone in the diet. In this study, steers with abomasal cannulas were used so partitioning of digestion between the large and small intestines could not be assessed.

Acid flow into the lower tract of feedlot cattle has not been investigated nor has the possibility as to whether or that limestone feeding could actually

influence the quantity of acid reaching the duodenum been thoroughly investigated yet.

Past experiments involving the feeding of high levels of limestone to feedlot cattle have been focused primarily on the effects of postruminal buffering on starch digestion. No one has investigated the possible implications of feeding high levels of limestone or MgO (another mineral compound with potential postruminal effects) on protein digestion and the absorption of individual amino acids.

Summary

1. The rumen is well buffered against acid additions, however, factors which reduce saliva flow to rumen and increase the rate of fermentation reduce the buffering capacity of the rumen.
2. Feed buffers can be most helpful to the ruminant during times of peak acid stress such as moving cattle from high-roughage diets over on to feedlot rations, moving the animal to a higher plane of energy, feeding liquid feeds containing readily fermentable components, feeding highly processed grains or grain with very fast fermentable starch such as wheat, or when getting animals back on feed.
3. Sodium bicarbonate is principally a rumen buffer and can alter rumen pH by neutralizing acid or by

changing rumen dilution rate and liquid flow from the rumen.

4. Magnesium oxide is an alkalizer rather than a buffer. It can affect rumen pH, rumen digestion, and total tract digestion, but its solubility in the rumen is highly dependent upon particle size, chemical composition and crystallinity.
5. Limestone generally is poorly solublized in the rumen and does not usually affect rumen pH, but can affect rumen and total tract digestion of fiber and starch.
6. Results from postruminal buffering studies have been highly variable, but limestone has been shown to improve feed efficiency and postruminal digestion of starch and fiber.

CHAPTER 2

NUTRIENT DIGESTIBILITY, NITROGEN BALANCE AND AMINO ACID ABSORPTION IN SHEEP FED A HIGH-CONCENTRATE DIET WITH LIMESTONE OR MAGNESIUM OXIDE

M. L. Christiansen and K. E. Webb, Jr.

Virginia Polytechnic Institute and State University

Blacksburg 24061

ABSTRACT

Twelve wether lambs (32 kg) equipped with abomasal and ileal T-cannulae were used to study the effect of a high dietary level of MgO or limestone on dry matter, organic matter and crude protein digestibility, N balance, and the intestinal disappearance of amino acids in ruminants fed a high-concentrate diet (90% concentrate and 10% orchardgrass hay). Lambs were allotted to one of four experimental treatments in a randomized complete block design (3 blocks). Two trials were conducted. Diets and levels of feeding (g/d) were basal (800) and basal plus 1.5% MgO (812), 1.5% limestone (812) or 3.0% limestone (824) on an as-fed basis. Rumen pH was increased ($P < .03$) by feeding 3.0% limestone. Abomasal pH was not affected by the mineral treatments, but MgO increased ($P < .05$) pH in the ileum. Magnesium oxide was more ($P < .01$) effective than limestone in increasing

fecal pH. Total rumen VFA concentrations were not appreciably affected by the mineral treatments, but the pre-abomasal apparent dry matter digestibility was improved ($P < .10$) by feeding limestone. Limestone appeared to reduce amino acid flow to the abomasum, and limestone and MgO decreased ($P < .03$) amino acid partial digestibility within the small intestine. It may be concluded that high dietary levels of MgO or limestones can increase rumen and intestinal pH, that a high dietary level of very finely ground limestone can improve rumen dry matter digestion in lambs fed a high-concentrate diet, and that feeding a high level of limestone may also be counter productive since it can reduce amino acid flow to and apparent digestibility in the small intestine.

(Key Words: Lambs, Limestone, Magnesium Oxide, N Balance, Amino Acid Partial Digestibility, High-Concentrate Diet)

Introduction

Limestone is composed mainly of CaCO_3 which is capable of donating mineral ions to the gastrointestinal tract as well as contribute some acid-neutralizing capacity. Its main site of action as a buffer is thought to be in the intestine since studies with cattle and sheep have shown it to be extremely limited in altering the rumen environment (Haaland and Tyrrell, 1982; Haaland

et al., 1982; Schaefer et al., 1982; Prigge and Svonavec, 1984; Round, 1984), but capable of increasing fecal pH (Ferreira et al., 1980; Tissera et al., 1982; James and Wohlt, 1985), total tract starch digestibility (Rogers et al., 1982; Rust and Owens, 1982; Tissera et al., 1982) and postruminal starch digestibility (Owens et al., 1983; Brink and Steele, 1985). Results from studies with intestinally cannulated animals have not been very supportive of the idea that limestone can improve intestinal digestion of starch by neutralizing intestinal acid. Zinn and Owens (1980a) increased the duodenal pH of steers fed an 80% corn diet by feeding a high dietary level of limestone, but intestinal starch digestion was not improved. In other studies with cattle and sheep, feeding high levels of limestone in a high-concentrate diet has failed to increase duodenal pH or reduce duodenal acid concentration (Laudert and Matsushima, 1982; Prigge and Svonavec, 1984; Teh et al., 1985). Some investigators have proposed a possible stimulatory effect by the Ca ion from limestone on pancreatic secretions (Teh et al., 1985) and on the activity of pancreatic amylase (Owens et al., 1983). Mechanisms for nutrient absorption in the intestinal tract are highly dependent on electrical gradients and ionic concentrations inside and outside mucosal cells. Control of gastrointestinal

pH and the presence of large amounts of mineral ions in the lower gastrointestinal tract may not only influence the activity of digestive enzymes, but could affect nutrient uptake as well. The effect of limestone feeding on starch digestion has received considerable attention, but the effect of a high dietary level of limestone on protein digestion and amino acid absorption has not been investigated.

The main objectives of this study were to determine the effect of feeding a high dietary level of limestone or magnesium oxide on the extent of feed nutrient digestion and N balance in sheep fed a high-concentrate diet and to study the impact of these mineral compounds on gastrointestinal pH and amino acid partial digestibility in the small intestine of sheep.

Experimental Procedure

Animals and Design. Twelve cannulated wether lambs, averaging 32 kg, were used for this study. Lambs were equipped with abomasal and ileal cannulae (plexiglas and stainless steel, respectively). Abomasal cannulae were placed in the pylorus and ileal cannulae were placed about 30 cm from the ileocecal junction.

The 12 lambs were grouped according to weight and breed and randomly assigned to treatments in a randomized complete block design. The design included three blocks

and four treatments. Two trials were conducted and treatments were randomly assigned to the animals within each block at the beginning of each trial with the constraint that an animal would not receive the same treatment for both trials.

Diet Treatments. The four diet treatments included a basal diet alone or with addition of 1.5% magnesium oxide (MgO), 1.5% limestone or 3.0% limestone. Particle size distribution of limestone and MgO was determine by sieving duplicate 100 g samples for 20 min through screens with mesh diameters of 425, 250, 106 and 53 μ . Particle size information was expressed as the percent of the total sample retained on each screen.

The ingredient and chemical composition of each diet is presented in Table 1. The basal diet was composed of 90% concentrate and 10% chopped grass hay on an as-fed basis and was formulated to provide 12% crude protein and a 1:1 Ca to P ratio. Hay was prepared for the experiment by chopping it through a 2.5 cm screen. Sufficient hay was chopped and prepared to provide for the entire experiment. Chromic oxide powder served as a digestion marker. It was added to the concentrate mix to provide .5% chromic oxide in the total diet on an as-fed basis. The complete concentrate portions for each diet (basal or basal + treatment) for each trial were prepared in 120 kg

TABLE 1. COMPOSITION OF EXPERIMENTAL DIETS, AS-FED

Item	Basal	Basal +		
		1.5% MgO	1.5% Limestone	3.0% Limestone
Ingredient composition, g/d				
Orchardgrass hay	80	80	80	80
Ground corn	581	581	581	581
Cane molasses, liquid	40	40	40	40
Soybean meal	86	86	86	86
Limestone	5	5	17	29
Magnesium oxide		12		
TM salt	4	4	4	4
Chromic oxide	4	4	4	4
Vitamin A, 1222 IU/kg	+	+	+	+
Vitamin D ₃ , 222 IU/kg	+	+	+	+
Total, g/d	800	812	812	824
Chemical composition, g/d				
Dry matter	688	704	699	714
Organic matter	656	661	658	662
Crude protein	102	99	101	100
Calcium	3.3	3.9	7.0	10.3
Magnesium	1.4	7.0	1.3	1.4

batches in a small horizontal feed mixer. Chromic oxide was used as an external marker and was added to each diet at a level of .5% as-fed.

Animal Management and Feeding. The 12 lambs on study were confined in metabolism crates and housed at the Virginia Tech Ruminant Metabolism Laboratory. Prior to beginning the experiment, lambs were adjusted to the metabolism crates and transferred from a high-roughage maintenance diet (28.8% corn, 61.4% orchardgrass hay and 9.6% soybean meal) to the high-concentrate experimental diets gradually over a 10-d period. After lambs had been transferred to the high-concentrate diet, they were fed for an additional 2 d before beginning Trial 1 of the experiment. Subsequently, lambs were fed 400, 406 and 412 g of the basal, 1.5% MgO, 1.5% limestone, and 3.0% limestone experimental diets, respectively, twice daily at 0600 and 1800 h. Intake of all ingredients were equal across treatments except for MgO or limestone.

Sample Collection. Each trial consisted of a 7-d preliminary period followed by a 7-d collection period and a 6-d sampling period. The preliminary period for Trial 2 began on d 2 following the end of Trial 1.

Feed sampling and collection of feed refusals began 2 d prior to the beginning of and ended 2 d before the end of each collection and sampling period. Hay and each

of the concentrate mixtures were sampled as diets were prepared at each feeding. Feed samples were stored in tightly sealed glass jars. After collection, feed refusals were weighed and then placed in paper bags and dried at 55° C overnight. After drying, refusals were allowed to equilibrate with air moisture for 2 d and then the refusals were reweighed. Refusals were stored in paper bags in a sealed metal container until the end of the experiment.

During the collection period, feces were collected twice daily (a.m. and p.m. feeding) and urine once daily (a.m. feeding). Daily fecal collections were dried at 55° C for 24 h and then stored in preweighed metal cans. Daily urine output was collected in a 3.8 liter plastic jug containing 500 ml of water and 15 ml of 1:1 (w/w) H₂SO₄. Daily urine collections were diluted to a constant weight with water, checked for acidity with indicator paper and reacidified if necessary with 1:1 (w/w) H₂SO₄. A 2% aliquot of the diluted urine was composited daily and stored in a 1 liter plastic container under refrigeration until the final day of the collection period. Urine samples were then frozen and stored at -20° C in a freezer until analyzed. During the sampling period abomasal and ileal digesta were sampled twice daily (a.m. and p.m.) at randomized, even

hours so that at the end of 6 d, a sample collected at each even hour of a 24-h period was represented. Approximately 120 ml of digesta were collected from each cannulation site at each sampling time. Abomasal fluid was collected in polyethylene screw-cap specimen containers and ileal digesta was collected in polyethylene sample bags. The ileal sample was collected by inserting a modified plastic test tube into the cannula to direct digesta flow outwardly into a sampling bag that had been placed on the end of the cannula. Animals were sampled 10 min apart and a sampling time of 1 h per animal was used to collect both digesta samples. At each sampling time, samples were kept on ice until all animals had been sampled and then transferred to a freezer for storage at -20°C until analyzed.

About 300 ml of rumen fluid were taken via a stomach tube 1 hr post-feeding the day following the end of the sampling period. Rumen fluid was strained through four layers of cheese cloth and two 10 ml portions taken for $\text{NH}_3\text{-N}$ analysis and two 5 ml portions taken for volatile fatty acid (VFA) analysis. Samples for VFA analysis were mixed with 1.0 ml of 25% metaphosphoric acid and for $\text{NH}_3\text{-N}$ with two drops of concentrated H_2SO_4 . Rumen fluid (strained) pH was determined at the time of sampling at 25°C with a combination electrode. Samples for $\text{NH}_3\text{-N}$

and VFA analysis were kept on ice until all samples were collected then stored at -20° C until analyzed.

Jugular blood was sampled 6 h postfeeding the day following the end of the sampling period. Blood serum was prepared for serum urea N (SUN) analysis following sample collection by clotting blood in a water bath at 37° C for 30 min and centrifuging for 20 min at $653 \times g$. Serum was deproteinized with tungstic acid (Coulombe and Favreau, 1963) and then stored at -20° C until analyzed.

Fresh feces were sampled for pH determination the day following the end of the second sampling period. Fecal pH was determined for Trial 2 only. Prior to the p.m. feeding, fecal pans were cleaned of daily feces so freshly dropped feces (approximately 30 g) could be sampled. Fecal pH was determined with a combination electrode on a 1:10 suspension of feces in water (20 g feces plus 200 ml deionized water) at 25° C.

Sample Preparation. Feed and feed refusals were prepared for chemical analysis by grinding in a Wiley mill through a 1 mm screen. Refusals were composited for each animal within a collection or sampling period.

Fecal collection cans were weighed to determine total fecal output for each collection period. Dried feces for each animal within a collection period were crushed and mixed thoroughly in a mixer and enough feces

sampled to fill a 3.6 liter plastic bag. Fecal samples were then ground in a Wiley mill through a 1 mm screen.

Samples of abomasal and ileal digesta were composited prior to laboratory analysis. Abomasal composite samples were prepared by pipetting a 25 ml aliquot of each time sample into a 500 ml beaker with a 25 ml wide bore graduated pipette. Ileal digesta was composited by adding a 15 g aliquot of each time sample to a 500 ml beaker. Abomasal and ileal time samples were mixed continuously with a magnetic stir bar while aliquots were taken in order to assure uniform compositing. Digesta pH was determined on composite samples (25° C) with a combination electrode at the time of compositing. Composite samples were transferred to 500 ml glass jars. Jars were covered with three layers of cheese cloth and then placed in the freezer (-20° C) overnight. The frozen composite samples were freeze-dried and then prepared for chemical analysis by grinding in a micro Wiley mill through a 1 mm screen.

Analyses. Sample dry matter was determined by drying in a forced-air oven at 105° C for 24 h. Samples obtained during the collection period were analyzed for ash content by ashing in a muffle furnace at 600° C for at least 2 h (AOAC, 1984). Organic matter in collection samples was calculated as sample dry matter minus the ash

content. Feed, feed refusals, feces and urine were analyzed for N content by the Kjeldahl procedure (AOAC, 1984). The density of each urine solution was determined with a urinometer at the time of N analysis so urinary N could be expressed on a weight basis. A 10 ml sample of each urine solution was used for the N analysis. Chromic oxide concentration in feed, feed refusals and digesta composite samples obtained during the sampling period was determined with a modified colorimetry procedure by Hill and Anderson (1958). Calcium and Mg concentrations in feed were determined using a Perkin-Elmer 403 atomic absorption spectrophotometer. Samples were prepared for analysis by wet ashing in concentrated HNO₃ followed by refluxing for 30 min in 70% HClO₄. After refluxing, samples were evaporated to dryness and then carefully redissolved in 5 ml concentrated HCl while heating. Samples were quantitatively transferred to 100 ml volumetric flasks and diluted to volume with deionized H₂O. Standard and sample dilutions were prepared with 1% (w/v) LaCl₃ for analysis.

Rumen fluid VFA concentrations were determined with a Perkin-Elmer gas chromatograph equipped with a 2 m x 2 mm glass column containing 10% SP-1200/1% H₃PO₄ on 80/100 chromosorb WAW. Column, inlet and detector temperatures were 120, 175 and 180° C, and the flow rate of N₂ was 30

ml/min. Four-methyl-valeric acid was used as an internal standard. In preparation for analysis, samples were centrifuged for 10 min and the clear supernatant filtered through a 2.0 μ Metricel filter. Rumen fluid $\text{NH}_3\text{-N}$ concentration was determined by the Conway procedure (Conway, 1958). Serum urea N (SUN) was determined following the procedure of Coulombe and Favreau (1963). Ten milliliters of 6 N HCl were added to duplicate .1 g samples of digesta in 20 ml ampoules. Ampoules were flushed with N_2 , sealed and heated in an oven at 100° C for 24 h. Hydrolyzed samples were filtered through glass wool. Aliquots (1 ml) of the filtered hydrolysate were dried under vacuum in a dessicator containing NaOH flakes. Dried residues were dissolved in .1 N HCl then analyzed for amino acid composition with the ion-exchange system of the Technicon TSM.

Statistical analyses were done using the General Linear Model Procedure (SAS, 1988). Sources of variation included trial, treatment, block, and the trial x treatment and block x treatment interactions. Treatment comparisons were made using orthogonal contrasts and included comparisons among the control and minerals, MgO and limestone, and 1.5% and 3.0% limestone.

Results and Discussion

The limestone used for this study was a very finely ground, commercial feed grade mineral. Only 4% of the particles were retained on the 106 μ screen (Table 2). The remaining portion was distributed between the 53 μ screen and the pan with the major part (70.9%) retained in the pan. The feed grade MgO was well distributed among all seive sizes. About 11% of the particles were retained on the 425 μ screen. Fifteen and 31% of the sample were retained on the 250 and 106 μ screens, respectively. The remaining 43% was comparable to the limestone in fineness.

Feeding MgO resulted in a substantial increase in fecal moisture. Feces became almost fluid-like in consistency within 6 to 7 d after starting lambs on the MgO treatment. However, adaptation to fecal water losses was visually evident within 4 to 11 d after sheep began to produce the extremely wet feces. Among sheep fed MgO, the extent of adaptation to fecal water losses ranged from the production of feces with a consistency similar to moist cattle feces to that of the production of moist fecal pellets.

Rumen pH was quite low across all treatments (Table 3). Increasing limestone from 1.5% to 3.0% increased ($P < .03$) rumen pH. Magnesium oxide tended to increase

TABLE 2. PARTICLE SIZE OF LIMESTONE AND
MAGNESIUM OXIDE SUPPLEMENTS

Item	Limestone	MgO
Screen size, μ	----- % of total -----	
425	0.0	11.4
250	0.0	14.5
106	4.1	31.4
53	25.0	17.3
Pan	70.9	25.4
Total	100.0	100.0

TABLE 3. THE EFFECT OF LIMESTONE OR MAGNESIUM OXIDE ON RUMEN PH, RUMEN AMMONIA AND VFA CONCENTRATIONS, DIGESTA AND FECAL PH, AND BLOOD UREA NITROGEN IN LAMBS FED A HIGH-CONCENTRATE DIET

Item	Control	Treatment			SKa	Significance of Contrast		
		1.5% MgO	1.5% Limestone	3.0% Limestone		1 vs 2,3,4	2 vs 3,4	3 vs 4
Rumen pH	5.68	5.73	5.65	5.65	.05	NS	NS	.03
Abomasal pH	2.61	2.72	2.75	2.66	.08	NS	NS	NS
Ilcal pH	7.42	6.23	7.72	7.75	.06	NS	.05	NS
Fecal pH ^b	7.61	9.53	8.35	8.37	.12	.01	.01	NS
Rumen NH ₃ -N, mg/dl	25.0	11.8	26.7	35.7	3.2	NS	.01	NS
SUN, mg/dl	1.11	.75	1.03	1.22	.20	NS	NS	NS
Total volatile fatty acids, μmol/ml	100	140	103	100	6.9	NS	NS	NS
Volatiles fatty acid proportions, mol/100 mol								
Acetate	54.7	56.9	53.8	57.0	2.9	NS	NS	NS
Propionate	25.0	25.3	25.9	20.5	1.8	NS	NS	NS
Butyrate	.93	.71	.99	.84	.08	.01	.02	.05
Isobutyrate	13.7	14.1	16.7	18.9	1.14	NS	NS	NS
Valerate	3.57	1.62	3.41	2.48	.63	NS	.10	NS
Isovalerate	1.96	1.00	1.27	1.46	.41	NS	NS	NS
Acetate:Propionate	2.27	2.13	2.35	2.49	.28	NS	NS	NS

^aStandard error of means.

^bMean fecal pH for trial 2 only.

rumen pH, but differences were not significant. Magnesium oxide showed the greatest capacity to affect pH post-ruminally. Magnesium oxide increased the ileal pH above 8.0 ($P < .05$) and fecal pH above 9.0 ($P < .01$). Fecal pH was higher ($P < .01$) for all the mineral treatments. Abomasal pH differences were not significant among treatments, but tended to be higher when mineral acid-neutralizers were fed.

Rumen $\text{NH}_3\text{-N}$ concentration was decreased ($P < .01$) by feeding MgO (Table 3). Consistent with the lower rumen $\text{NH}_3\text{-N}$ values, SUN tended to be lowest for animals fed MgO . Teh et al. (1985) reported a slight decline in rumen $\text{NH}_3\text{-N}$ concentrations in dairy cows when MgO was increased from .4% to .8% of the diet. This decline was observed even when 1% NaHCO_3 was fed in combination with the MgO treatments. In both cases, however, differences were not statistically significant.

In the present study, the rumen VFA profile was affected very little by the mineral treatments. With MgO , total VFA were higher, but the difference was not statistically significant. Generally, the proportion of VFA were not influenced except in the case of butyrate. The average proportion of butyrate was lower ($P < .01$) for the mineral acid-neutralizers. Among these, MgO resulted in the lowest ($P < .02$) proportion. Proportions

Attention Patron:

Page 68 omitted from
numbering

TABLE 4. THE EFFECT OF LIMESTONE OR MAGNESIUM OXIDE ON
 DRY MATTER, ORGANIC MATTER, AND CRUDE PROTEIN
 APPARENT DIGESTIBILITY IN LAMBS FED A
 HIGH-CONCENTRATE DIET

Item	Treatment			SE ^a	
	Control	1.5% MgO	1.5% Limestone		3.0% Limestone
Dry matter					
Intake, g/d	691	704	695	699	8
Digestion, %	84.0	86.4	83.5	84.2	1.4
Organic Matter					
Intake, g/d	659	661	652	649	8
Digestion, %	84.6	87.0	85.5	87.3	1.4
Crude protein					
Intake, g/d	103	99	100	98	1
Digestion, %	77.6	81.2	78.8	80.7	2.2

^aStandard error of means.

TABLE 5. THE EFFECT OF LIMESTONE OR MAGNESIUM OXIDE ON THE PARTITIONING OF DRY MATTER DIGESTIBILITY IN LAMBS FED A HIGH-CONCENTRATE DIET

Item	Control	Treatment			SE ^a	Significance of Contrast		
		1.5% MgO	1.5% Limestone	3.0% Limestone		1 vs 2, 3, 4	2 vs 3, 4	3 vs 4
Dry matter intake	686	708	693	692	14	NS	NS	NS
Dry matter flow, g/d								
Abomasum	444	488	316	384	29	NS	.01	.10
Ileum	187	186	132	158	6	NS	NS	NS
Dry matter digestion, % ^b								
Pre-abomasum	35.4	31.2	54.6	44.4	3.7	.10	.01	.08
Small intestine	40.3	42.7	26.3	32.7	3.3	.07	.01	.10
Dry matter partial digestion, % ^c								
Small intestine	62.3	61.8	57.7	56.1	1.8	NS	NS	NS

^aStandard error of means.

^bPercentage of dry matter intake.

^cPercentage of amount available.

concentrate diet (Zinn et al., 1982). In the present study, with limestone there was actually less ($P < .01$) dry matter digested in the small intestine. Small intestine partial digestion coefficients were lowest for both limestone treatments. The dry matter digestibility data obtained in this study are consistent with observations by Zinn and Owens (1980b) who reported an increase in rumen digestion of starch and organic matter, but a decrease in the intestinal digestion of these nutrients in steers fed a 72% rolled corn basal diet when 2.5% CaCO_3 or 2.5% CaCO_3 + 1% NaHCO_3 was added to the diet.

None of the differences observed for N intake, urinary N or fecal N were significant (Table 6). There was a tendency for MgO to result in a higher urinary N excretion and a lower N retention. This may be associated with the lower ruminal $\text{NH}_3\text{-N}$ observed with MgO. Most occurrences which might normally be observed to produce such responses are not readily apparent here. The tendency for an increased ruminal pH might offer as plausible explanation for both since a higher ruminal pH is usually associated with a more rapid $\text{NH}_3\text{-N}$ absorption. However, one would expect to see a higher level of SUN which was not observed. The high pH of the large intestine might likewise result in increased $\text{NH}_3\text{-N}$

TABLE 6. THE EFFECT OF LIMESTONE OR MAGNESIUM OXIDE ON NITROGEN BALANCE IN LAMBS FED A HIGH-CONCENTRATE DIET

Item	Control	Treatment		3.0% Limestone	SE ^a	Significance of Contrast		
		1.5% MgO	1.5% Limestone			1 vs 2,3,4	2 vs 3,4	3 vs 4
N intake, g/d	16.5	15.8	16.0	15.6	.2 (.2)	NS	NS	NS
Urinary N, g/d	8.1	11.2	9.2	7.3	1.4 (1.6)	NS	NS	NS
Fecal N, g/d	3.7	3.0	3.4	3.0	.3 (.3)	NS	NS	NS
N absorbed g/d	12.7	12.8	12.6	12.7	.3 (.4)	NS	NS	NS
Percent of intake	76.9	76.6	76.8	81.5	1.3 (1.6)	.08	.04	.08
N retained g/d	4.3	1.2	2.1	5.0	1.3 (1.6)	NS	NS	.09
Percent of intake	26.5	8.0	13.1	31.1	8.6 (10.5)	NS	NS	.08
Percent of N absorbed	34.3	10.2	16.3	39.2	11.3 (13.9)	NS	NS	.09

^aStandard error of means (standard error for 1.5% magnesium oxide).

absorption, but again this is not supported by urea data. The 3% limestone treatment resulted in the greatest N retention which was significant when compared to the 1.5% limestone treatment. Interestingly, this 3.0% limestone treatment resulted in the numerically highest ruminal $\text{NH}_3\text{-N}$ and SUN levels.

Amino acid flow data are presented in Table 7. In every case, the quantity of amino acid reaching the abomasum was numerically greatest when MgO was fed and with few exceptions these levels were significantly greater than when limestone was fed. This could account for the lower level of $\text{NH}_3\text{-N}$ with MgO, since a greater passage of amino acids would suggest a lesser degradation of them in the rumen or a more efficient conversion of these to microbial protein. The N excretion and retention data, still do not seem to fit well with these observations.

The lowest levels of amino acids reaching the abomasum were associated with the feeding of the two levels of limestone. Only 85.2 and 78.4% as many grams of essential amino acids reached the abomasum for the 1.5% and 3.0% limestone treatments, respectively, compared to the control. Comparable figures for the nonessential amino acids were 78.3 and 72.4%, respectively. It seemed that the higher the level of

TABLE 7. THE EFFECT OF LIMESTONE OR MAGNESIUM OXIDE ON THE FLOW OF AMINO ACIDS TO THE ABOMASUM
IN LAMBS FED A HIGH-CONCENTRATE DIET

Amino acids	Treatment				SE ^a	Significance of Contrast			
	Control	1.5% MgO	1.5% Limestone	3.0% Limestone		1 vs 2	3 vs 4	2 vs 3	4 vs 3 vs 4
Essential									
Arginine	2.81	4.09	2.50	2.50	.51	NS	.07	NS	NS
Histidine	1.19	1.47	1.08	.98	.11	NS	.06	NS	NS
Isoleucine	2.80	3.21	2.67	2.35	.31	NS	NS	NS	NS
Leucine	6.64	6.92	5.13	5.05	.60	NS	.06	NS	NS
Ivaline	4.51	5.46	4.01	3.48	.33	NS	.04	NS	NS
Methionine	1.87	2.08	1.45	1.25	.18	.07	.01	NS	NS
Phenylalanine	3.63	4.44	2.89	2.76	.28	NS	.003	NS	NS
Threonine	3.92	4.51	3.32	2.92	.31	NS	.03	NS	NS
Valine	3.10	3.54	2.94	2.55	.36	NS	NS	NS	NS
Nonessential									
Alanine	6.42	7.01	4.88	4.60	.48	NS	.04	NS	NS
Aspartic acid	7.37	8.82	6.12	5.40	.56	NS	.02	NS	NS
Cysteine	.76	.79	.64	.58	.07	NS	NS	NS	NS
Glutamic acid	10.52	11.41	7.90	7.25	.80	NS	.03	NS	NS
Glycine	4.54	5.32	3.85	3.35	.36	NS	.02	NS	NS
Proline	5.12	5.23	3.62	3.99	.43	NS	.07	NS	NS
Serine	4.24	4.75	3.32	3.07	.29	NS	.03	NS	NS
Tyrosine	3.42	3.94	2.85	2.42	.29	NS	.02	NS	NS
Residual	30.5	35.3	26.0	23.9	2.9	NS	.05	NS	NS
Nonessential	42.4	47.3	33.2	30.7	3.0	NS	.03	NS	NS

^aStandard error of means.

limestone, the lower the abomasal appearance of amino acids.

Goetsch and Owens (1985) reported a decline in the flow of N to the duodenum with the addition of Ca to the diet in steers fed an 88% concentrate diet. Calcium was supplemented at .40 or .48% of the diet by feeding .95% $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$ or .65% CaCO_3 or at 1.11% by adding 2.5% CaCO_3 to the diet. They determined that the reduced N flow to the duodenum was primarily due to a decrease ($P < .05$) in the flow of microbial N to the small intestine. The authors also reported a decline in organic matter and starch digestibility in the rumen from the addition of Ca to the diet. To suggest that a reduced flow of amino acids to the lower tract observed in the present study may have been due to a decline in microbial protein flow to the lower tract seems almost contradictory to the improvement seen in rumen dry matter digestibility for the limestone treatments (Table 5).

Total amino acid digestibility in the small intestine was decreased ($P < .03$) with the feeding of mineral acid-neutralizers (Table 8). Essential and nonessential amino acid digestibility in the small intestine was reduced ($P < .01$ and $P < .02$, respectively) by the mineral treatments. Limestone feeding resulted in a greater decrease ($P < .03$) in partial digestibility of

TABLE 8. THE EFFECT OF LIMESTONE OR MAGNESIUM OXIDE ON PARTIAL DIGESTIBILITY OF AMINO ACIDS IN THE SMALL INTESTINE OF LAMBS FED A HIGH-CONCENTRATE DIET^a

Amino acids	Control	Treatment			SE ^b	Significance of Contrast				
		1.5% MgO	1.5% Limestone	3.0% Limestone		1 vs 2	1 vs 3	2 vs 3	1, 2 vs 3	
Essential										
Arginine	75.5	72.7	71.2	69.4	2.3	.03	NS	NS	NS	NS
Histidine	69.8	66.5	62.4	64.2	3.8	NS	NS	NS	NS	NS
Isoleucine	74.1	69.1	68.1	69.5	2.9	.05	NS	NS	NS	NS
Leucine	75.5	70.5	68.2	71.4	2.5	.004	NS	NS	NS	NS
Lysine	70.6	69.1	64.4	65.6	2.1	.002	.003	.003	.003	.003
Methionine	78.6	76.2	74.6	75.9	2.3	NS	NS	NS	NS	NS
Phenylalanine	73.7	78.8	66.4	69.9	2.5	NS	.07	.07	.07	.07
Threonine	65.7	63.9	58.1	56.9	2.5	.01	.01	.01	.01	.01
Valine	68.6	61.2	61.6	59.8	4.3	.07	NS	NS	NS	NS
Nonessential										
Alanine	67.5	62.3	59.0	65.8	2.6	NS	NS	NS	NS	NS
Aspartic acid	69.4	67.0	63.2	62.5	1.7	.003	.006	.006	.006	.006
Cysteine	72.0	62.6	59.5	59.1	3.5	.06	NS	NS	NS	NS
Glutamic acid	69.8	67.3	64.2	66.4	1.7	.03	NS	NS	NS	NS
Glycine	51.7	60.2	51.2	48.3	1.5	NS	.05	.05	.05	.05
Proline	77.0	71.5	70.9	71.2	2.7	.09	NS	NS	NS	NS
Serine	65.5	67.3	59.1	59.8	1.6	.09	.01	.01	.01	.01
Tyrosine	74.1	72.0	68.3	67.5	1.7	.01	.02	.02	.02	.02
Essential										
Essential	72.5	65.0	66.0	67.0	3.8	.01	NS	NS	NS	NS
Nonessential	68.2	66.5	62.1	63.6	1.6	.02	.03	.03	.03	.03
Total	70.0	66.1	63.8	65.0	2.3	.03	NS	NS	NS	NS

^aThe apparent absorption of amino acids as a percent of amino acids reaching the small intestine.

^bStandard error of means.

nonessential amino acids than was observed for MgO. From the data obtained from this study, it appears that a high dietary level of MgO or limestone may have influenced intestinal amino acid apparent digestibility either by influencing pre-intestinal digestive processes and the flow of amino acids to the intestines or by affecting digestive and absorptive processes within the small intestine. Van Bruchem et al. (1985) observed an increase in the apparent digestibility of amino acids with an increased flow of protein into the small intestine.

In the present study, total amino acid partial digestibility in the small intestine averaged 70.0% in the control animals. Tas et al. (1981) studied the apparent digestibility of amino acids in the intestine of sheep while varying the quantities of microbial and feed proteins in the small intestine by the infusion of a microbial isolate or by dietary manipulation. Amino acid flow to the duodenum averaged 82.1 g/d. Amino acid apparent digestibility in the small intestine averaged 69.0%. Harrison et al. (1973) reported a mean apparent digestibility of 62.0 for total amino acids in sheep fed various forage diets. The diets supplied a similar intake of crude protein and organic matter. Apparent digestibility was highest for fresh red clover (71.0%)

and lowest for alfalfa hay (57.0%) and a ground pelleted red clover hay (55.0%). Across forage types lysine and methionine apparent digestibility averaged 66.0% and 78.0%, respectively. Lysine and methionine digestibility values observed in the present study were similar to those observed for the forage diets (70.6% and 78.6%, respectively). It seems that methionine digestibility is usually highest among the amino acids. The present study is no exception where, numerically, methionine digestibility was the highest and glycine the lowest (51.0%). Similarly, methionine digestibility was greatest (80.0%) in sheep fed alfalfa hay (Coelho da Silva et al., 1972) and in cattle (64.7%) fed a 65:35 concentrate to roughage diet (Koeln and Patterson, 1986).

Amino acid apparent digestibility seems to be quite consistent in spite of an extreme in the concentrate level of the diet. The data observed in the present study appear in good agreement with previously published information even though many of these studies have involved the study of amino acid absorption in sheep fed forage diets.

Results from the present study indicate that a high dietary level of limestone can increase rumen pH and improve rumen dry matter digestibility even though a change in the rumen VFA proportions or total tract

disappearance of dry matter may not occur. In this study, limestone was more effective than MgO in altering rumen pH and improving dry matter digestion in the rumen. This may at first appear in conflict with the general accepted idea that in diets of high-producing dairy cows, MgO is a better agent than limestone in altering rumen pH and fermentation. There are some important factors associated with this study that may explain this difference. The limestone used in this study was extremely fine in particle size compared to the MgO. Both limestone and MgO have low solubility at the normal pH range of the rumen. The fine particle size of the limestone was probably an important factor in determining how each mineral responded in the rumen of the sheep in this study. The rumen pH of the lambs in the present study were much lower than would normally be found in high-producing dairy cows. The extra acid in the rumen complimented the fine particle size of the limestone resulting in a response in the rumen from feeding limestone. Regardless of the improved response in rumen dry matter digestibility, feeding limestone at a high dietary levels actually appears to be counter productive since it also may reduce protein flow and flow of many of the essential amino acids into the small intestine that are required for an adequate growth response.

Furthermore, the digestibility of the amino acid may also be decreased.

Literature Cited

- AOAC, 1984. Official Methods of Analysis (14th Ed.). Association of Official Analytical Chemists, Washington, D.C..
- Brink, D. R. and R. T. Steele. 1985. Site and extent of starch and neutral detergent fiber digestion as affected by source of calcium and level of corn. J. Anim. Sci. 60:1330.
- Coechlo da Silva, J. F., R. C. Seeley, D. J. Thomson D. E. Beever and D. G. Armstrong. 1972. The effect in sheep of physical form and stage of growth on the sites of digestion of a dried grass. Br. J. Nutr. 28:357.
- Conway, E. J. 1958. Microdiffusion Analysis and Volumetric Error (4th Ed.). The McMillan Co., New York.
- Coulombe, J. J. and L. Favreau. 1963. A new simple semi-micro method for colorimetric determination of urea. Clin. Chem. 9:102.
- Ferreira, J. J., C. H. Noller and T. S. Stewart. 1980. Influence of dietary calcium and protein on fecal pH, consistency and rate of passage in dairy cattle. J. Dairy Sci. 63:1091.
- Goetsch, G. L. and F. N. Owens. 1985. Effects of calcium source and level on site of digestion and calcium levels in the digestive tract of cattle fed high-concentrate diets. J. Anim. Sci. 61:995.
- Haaland, G. L. and H. F. Tyrrell. 1982. Effects of limestone and sodium bicarbonate buffers on rumen measurements and rate of passage in cattle. J. Anim. Sci. 55:935.

- Haaland, G. L., H. F. Tyrrell, P. W. Moe and W. E. Wheeler. 1982. Effect of crude protein level and limestone buffer in diets fed at two levels of intake on rumen pH, ammonia-nitrogen, buffering capacity and volatile fatty acid concentrations of cattle. *J. Anim. Sci.* 55:943.
- Harrison, D. G., D. E. Beever, D. J. Thomson and D. F. Osbourn. 1973. The influence of diet upon the quantity and types of amino acids entering and leaving the small intestine of sheep. *J. Agric. Sci.* 81:391.
- Hill, F. N. and D. L. Anderson. 1958. Comparison of metabolizable energy and productive energy determinations with growing chicks. *J. Nutr.* 64:587.
- James, L. G. and J. E. Wohlt. 1985. Effect of supplementing equi-valent cation amounts from NaCl, MgO, NaHCO₃ and CaCO₃ on nutrient utilization and acid-base status of growing Dorset lambs fed high concentrate diets. *J. Anim. Sci.* 60:307.
- Koeln, L. L. and J. A. Paterson. 1986. Nitrogen balance and amino acid disappearance from the small intestine in calves fed soybean meal; toasted soybean meal or corn gluten meal supplemented diets. *J. Anim. Sci.* 63:1258.
- Laudert, S. B. and J. K. Matsushima. 1982. Limestone affects on digestion and gastrointestinal tract pH of finishing steers. *J. Anim. Sci.* 55(Supp. 1):437(Abstr.).
- Owens, F. N., A. L. Goetsch, D. C. Weakley and R. A. Zinn. 1983. Buffers and neutralizers in beef cattle. In: Buffers, neutralizers and electrolyte. pp. 11-24. Symposium Handbook. Natl. Feed Ingrid. Assoc., Des Moines, IA.
- Prigge, E. C. and J. J. Svonavec. 1984. Flow and acidity of digesta entering the duodenum as influenced by buffers and time after feeding in wethers. *Can. J. Anim. Sci.* 64:1055.

- Rogers, J. A., C. L. Davis and J. H. Clark. 1982. Alteration of rumen fermentation, milk fat synthesis and nutrient utilization with mineral salts in dairy cows. *J. Dairy Sci.* 65:577.
- Round, M. H. 1984. Buffers in pelleted diets for exported line wethers. *Proc. Aust. Soc. Anim. Prod.* 15:746.
- Rust, S. R. and F. N. Owens. 1982. Effect of limestone on digestibility of feedlot diets. *Okl. Agric. Exp. Sta. Misc. Publ.* 112:154.
- Schaefer, D. M., C. H. Noller, R. B. Keyser and J. L. White. 1982. Neutralization of acid in the rumen by managesium oxide and magnesium carbonate. *J. Dairy Sci.* 65:732.
- Tas, M. V., R. A. Evans and R. F. E. Axford. 1981. The digestibility of Amino acids in the small intestine of the sheep. *Br. J. Nutr.* 45:167.
- Teh, T. H., R. W. Hemken and R. J. Harmon. 1985. Comparison of buffers and their modes in the rumen and abomasum. *Nutr. Rep. Int.* 32:1339.
- Tissera, G. H., J. H. Vandersall and R. A. Erdman. 1982. Gastrointestinal effects of limestone in the diet of growing steers. *J. Anim. Sci.* 55 (Suppl.1):470(Abstr.).
- Van Bruchem, J., L. J. G. M. Bongers, J. D. van Walsem, W. Onck and P. W. M. von Adrichem. 1985. Digestion of proteins of varying degradability in sheep. 3. Apparent and true digestibility in the small intestine and ileal endogenous flow of N and amino acids. *Neth. J. Agric. Sci.* 33:285.
- Zinn, R. A. and F. N. Owens. 1980a. Expanding our concept of starch digestion in the ruminant. *Minn. Nutrition Conference.* p. 69.
- Zinn, R. A. and F. N. Owens. 1980b. Sodium, calcium and potassium salts for cattle fed high concentration rations. *Okl. Agric. Exp. Sta. Misc. Publ.* 107:131.
- Zinn, R. A., F. N. Owens, D. R. Gill and D. E. Williams. 1982. Limestone and potassium for feedlot steers. *Okl. Agric. Exp. Sta. Misc. Publ.* 112:158.

Chapter 3

LIMESTONE PARTICLE SIZE AND DIETARY LEVELS FOR LAMBS FED AN ALL-CONCENTRATE DIET

M. L. Christiansen, K. E. Webb, Jr. and D. R. Notter
Virginia Polytechnic Institute and State University,
Blacksburg 24061

ABSTRACT

Seventy-two wether lambs of mixed breeding and an average initial weight of 28 kg were used in an 82-d feeding trial to study the effect of limestone particle size and dietary level on feedlot performance. Lambs were fed an all-concentrate diet (70% whole shelled corn and 30% of a protein, mineral and vitamin pellet). Treatments included a fine and coarse limestone fed at 1.0% and 3.0% of the diet as-fed. Rumen pH was not affected by the experimental treatments. Total rumen VFA concentrations were decreased by feeding 3.0% limestone, but VFA molar proportions were not altered by dietary treatments. Fecal pH was increased by feeding 3.0% limestone in the diet. Overall, lamb gains were not significantly altered by the dietary treatments, but lambs fed the coarse limestone had a better ($P < .10$) feed efficiency.

(Key Words: Lambs, Limestone, Particle Size, Dietary Level, Weight Gain, Feed Efficiency)

Introduction

Traditionally, feed grade limestones have been very finely ground for use in livestock diets, but in recent years many commercial feed companies have turned to more coarsely ground limestone. Mineral supplements with a larger particle size are more easily handled by automated feed mixing systems. They are more accurately metered out and more evenly blended during the mixing process. Particle size, however, is an important factor that affects the rate of reactivity of mineral compounds and nutritionists have wondered what effect a larger particle size might have on Ca bioavailability from limestone and limestone buffering capability.

Keyser et al. (1985) observed greater total ruminal volatile fatty acid (VFA) concentrations in rumen fistulated dairy heifers fed a 75:25 concentrate to corn silage diet when supplemented with a fine, fast reacting limestone at 2.88% of the diet dry matter. Rumen pH, volume, dry matter disappearance and dilution rate did not differ between fine and coarse limestones. Nocek et al. (1983) reported a greater milk fat percent and greater milk fat yield by early lactating cows when supplemented with a more finely ground limestone at 2.33% of the diet dry matter. In another dairy cattle study, results indicated that a coarsely ground inorganic Ca

source should be fed at a higher level to optimize feed intake and milk production (Wohlt et al., 1986). Limited information is available on limestone particle size verses animal performance. Particle size could be an important consideration when adding limestone to a diet either as a Ca supplement or as a buffer. Therefore, the purpose of this experiment was to study the effect of limestone source and particle size and dietary level on the performance of lambs fed an all-concentrate diet.

Materials and Methods

Animals. An 82-d feeding trial (June-September) was conducted with 72 wether lambs of mixed breed with an average initial weight of 28 kg. The lambs came from the Virginia Tech Sheep Center and had been raised on expanded metal floors from birth to when they were acquired for the feeding trial. For the feeding trial, animals were housed in 12 partially covered pens. A feeding trough was placed in the covered area of each pen. A nipple waterer was placed near the feeding trough in each pen to give lambs easy access to fresh water at all times.

Experimental Design. The experimental design consisted of a randomized block design with a 2 x 2 factorial arrangement of treatments. Lambs were blocked

according to weight and breed and randomly assigned to 12 pens, six animals per pen and three pens per treatment.

Diet Treatments. Experimental treatments included two types of limestone that differed substantially in particle size and two dietary levels of limestone, 1.0 and 3.0% of the diet on an as-fed basis. Limestone particle size information was obtained by sieving, in duplicate, 100 g samples for 20 min through stacked screens with mesh diameters of 425, 250, 150, 106 and 53 μ using an automatic shaker. Sieving data were recorded as the percent of the total weight appearing on each screen. The rate of reactivity of limestone to neutralize acid at pH 2.7 was determined by pH-stat titrations according to modification of the technique by Jensen et al. (1977). An amount of limestone capable of neutralizing 40 ml of .2 N HCl was used. Rate of acid neutralization was expressed as T₅₀, the time required to neutralize 50% of the carbonate equivalents in the sample. The mineral sample was added to an insulated reaction vessel containing 30 ml of deionized water. The vessel solution was maintained at 37 C throughout the reaction time by circulating heated water through an insulating jacket surrounding the reaction chamber. The fine, feed grade limestone used for this study was manufactured by Gold Bond Building Products, a Division

of the National Gypsum Company in Kimballton, Virginia. The coarse feed grade limestone was provided by Landmark, Inc. in Columbus, Ohio. It was being used extensively in Landmark milled feeds and premixes as a Ca source.

Diet Description. All lambs were fed an all-concentrate diet composed of 70% whole shelled corn and approximately 30% of a protein, vitamin and mineral pelleted supplement (Table 9). Lambs receiving 3.0% limestone were given 14% more pellets to maintain a constant energy and protein intake across treatments. All diets were formulated to contain 14% crude protein and at least .51% Ca (basal + 1.0% limestone) on an as fed basis. Monosodium phosphate was added to the 3.0% limestone pelleted supplement to maintain a 1.4:1.0 Ca to P ratio.

Animal Feeding and Management. Lambs were switched from a 75:25 hay to concentrate creep diet to the all-concentrate test diet over a period of 10 d. During the diet transition, feed intake was maintained at approximately 3.0% of body weight and each day 10% of the creep ration was replaced with an equal weight of the all-concentrate diet until lambs were completely on the experimental diet. Lambs were fed for an additional 2 d before increasing feed intake.

TABLE 9. SUPPLEMENTAL PELLET COMPOSITION
ON AN AS-FED BASIS

Item	Pellet ^a	
	1.0% Limestone	3.0% Limestone
Ingredient composition, %		
Ground corn	21.67	18.90
Soybean meal	55.67	48.55
Cane molasses, dehy.	16.67	14.53
Limestone	3.33	8.72
Sodium monophosphate		6.98
TM salt	1.66	1.45
Ammonium chloride	1.00	.87
Zinc oxide, 100 mg/kg	+	+
Sodium selenite, .26 mg/kg	+	+
Vitamin A, 1,100 IU/kg	+	+
Vitamin D, 200 IU/kg	+	+
Vitamin E, 39 IU/kg	+	+
Total	100.00	100.00

^aLambs fed the 3% limestone treatments (3% fine or coarse limestone) were fed 14% more pellets than lambs fed the 1% limestone treatments to maintain the same proportion of dietary energy, protein, vitamins, salt, ammonium chloride, zinc oxide and sodium selenite across treatments.

The entire feeding trial included the 12-d transition period followed by five, 14-d feeding periods. During the first feeding period, feed intake was increased $76 \text{ g}\cdot\text{hd}^{-1}\cdot\text{d}^{-1}$ until lambs reached a maximum level of intake. Lambs were fed ad libitum the remainder of the trial. Refusals were maintained at approximately 5% of intake. Refusal weights were determined each morning prior to feeding.

Lambs were weighed at the end of each feeding period. Feed intake and weight gains were calculated for each feeding period and for the entire trial. Lambs were fed to an average final weight of 52.2 kg.

Sampling and Analyses. Samples of corn grain and pellets were taken as feed was being weighed for each pen at each feeding so that at the end of the feeding period samples of corn and of each pelleted supplement weighing about 1,400 g had been accumulated. These feed samples were accumulated in tightly sealed 1.9 liter glass jars. Feed samples were prepared for analysis by grinding in a Wiley mill through a 1 mm screen. Feed dry matter was determined by drying in a forced-air oven at 105 C for 24 h. Crude protein in the feed was determined with the Kjeldahl procedure (AOAC, 1984). Feed and limestone samples were analyzed for Ca content by atomic absorption spectrophotometry. Samples were prepared for analysis by

wet ashing duplicate 1 g feed samples or .2 g limestone samples in 5 ml of concentrate HNO₃ followed by refluxing for 30 min in 10 ml of 70% HClO₄. After samples were dry, they were carefully redissolved in 5 ml of concentrated HCl while heating. Samples were quantitatively transferred to volumetric flasks with distilled water and diluted to volume with 1.0% (w/v) LaCl₃. Analyses were performed on a Perkin-Elmer Model 403 Atomic Absorption Spectrophotometer.

Rumen fluid was sampled from each lamb via a stomach tube 1 hr post-feeding at the end of the trial for pH determination and VFA analysis. Fecal grab samples were also taken from each lamb for pH determination. Rumen fluid and fecal pH were determined with a combination electrode at 25 C. Fecal pH was determined on a 10% (w/w) suspension of fresh feces and deionized water.

Statistical analyses were accomplished using the General Linear Model Procedure (SAS, 1988). Sources of variation included replication and treatment. Orthogonal contrasts were made to compare differences due to limestone source and level, and the source x level interaction.

Results and Discussion

The particle size and rate of reactivity information on the limestones used in this study are presented in

Table 10. The two limestones were extremes in particle size distribution. After 20 min of sieving, 25% of the particles in the fine limestone were retained on the 53 μ screen and 70% of the particles passed the 53 μ screen into the pan. Eighty-five percent of the particles in the coarse limestone were retained on the 425 μ screen. The large contrast in particle size between the limestones was reflected in a large difference in the rates of reactivity. The fine limestone had a reaction rate comparable to reagent grade CaCO_3 and 60 times faster than the coarse limestone. Particle size, however, is one of several factors that may influence the reactivity of limestone. Limestone from different sources may vary in rate of reactivity because of differences in particle size, chemical composition and crystallinity (Boynton, 1966; Ferriera et al., 1979; Noller and White, 1979). The implication of these factors may have on the capacity of limestone to neutralize acid in the lower tract in ruminants and the ability of limestone to influence post-ruminal digestive and absorptive phenomena has not been thoroughly studied. Information on the effect that particle size has on limestone bioavailability of Ca in growing cattle and sheep is also lacking in the literature. Bioavailability of Ca for growing chicks has been shown to be dependent

TABLE 10. LIMESTONE PARTICLE SIZE AND
RATE OF REACTIVITY

Item	Fine	Coarse
	----- % of total -----	
Screen size, μ		
425	0.0	84.9
250	0.0	4.0
106	4.1	4.3
53	24.8	1.8
pan	70.4	5.0
Total	100.0	100.0
	----- sec -----	
T ₅₀ , pH 2.7	40	2502

on limestone particle size and feed limestone source (Hillman et al., 1976; Reid and Weber, 1976). However, limestone particle size did not seem to affect Ca bioavailability in a study with growing swine (Pond et al., 1982).

Data showing the effect of limestone particle size and dietary level on rumen pH and VFA concentrations and on fecal pH are presented in Table 11. The rumen was very acidic for lambs across all treatments and limestone treatments did not significantly alter pH. However, even with the low rumen pH, lambs appeared to eat and perform normally throughout the 82-day trial. Total rumen VFA concentrations were lower ($P < .10$) in lambs supplemented with 3.0% limestone, but limestone particle size had little effect on total VFA. Keyser et al. (1985) observed an increase in total rumen VFA concentrations in dairy heifers fed a 75:25 concentrate to corn silage diet when supplemented with fine limestone at 2.88% of the dry matter, and rumen pH was not affected by feeding limestone. In the present study, molar proportions of rumen VFA were not affected by the dietary treatments. Fecal pH was increased ($P < .10$) by an increase in dietary limestone. This is consistent with the idea that limestone has rumen bypass capabilities and can affect large intestinal pH. Course limestone fed at the 1.0%

TABLE 11. EFFECT OF LIMESTONE SOURCE AND DIETARY LEVEL ON RUMEN pH, RUMEN VOLATILE FATTY ACID CONCENTRATIONS AND FECAL pH IN FEEDER LAMBS

Item	Fine		Coarse		SE ^b
	1.0% ^a	3.0%	1.0%	3.0%	
Rumen pH	5.32	5.46	5.41	5.40	.06
Total volatile fatty acids, $\mu\text{mol/ml}^c$	163.7	125.9	162.8	128.2	14.1
Volatile fatty acid proportions, mol/100mol					
Acetate	43.4	46.1	45.7	44.1	1.1
Propionate	42.9	41.9	42.6	43.2	1.4
Butyrate	9.3	8.5	8.5	8.4	.6
Isobutyrate	.17	.11	.15	.17	.05
Valerate	3.7	2.5	3.1	3.6	.50
Isovalerate	.66	.41	.33	.58	.15
A:P ratio	1.03	1.13	1.11	1.03	.06
Fecal pH ^c	6.37	6.43	6.19	6.44	.06

^aPercentage of the diet as-fed.

^bStandard error of means.

^cLevel effect ($P < .10$).

level resulted in the lowest fecal pH, but the interaction was not significant.

Lamb performance data are presented in Table 12. Cumulative data through Period 6 indicate that there was no significant effect of limestone particle size or level on feed intake. However, a significant source by level interaction ($P < .10$) was observed. Lambs fed 3.0% fine limestone ate less than lambs fed the fine limestone at the 1.0% level while lambs fed the coarse limestone at 3.0% of the diet consumed more than their 1.0% counterparts. The fine limestone supplements were difficult to press through the pelleting die when the supplements were pelleted. This resulted in an extra hard supplement pellet. The 3.0% fine limestone pellets were particularly hard. Lambs in two of the pens fed fine limestone at 3.0% of the diet tended to select against the feed pellet. This may have contributed to the lower intake for this treatment. However, feeding high levels of limestone can reduce feed intake (Owens, 1984). In contrast to this, however, lambs fed limestone at 3.0% of the diet tended to eat more than those given coarse limestone at only 1.0% of the diet.

Over the entire trial, weight gains were not significantly different among treatments (Table 12). Weight gain for lambs fed 3.0% fine limestone averaged

TABLE 12. EFFECT OF LIMESTONE SOURCE AND DIETARY LEVEL ON THE PERFORMANCE OF FEEDER LAMBS—CUMULATIVE DATA BY PERIOD

Item	Fine		Coarse		SE ^b
	1.0% ^a	3.0%	1.0%	3.0%	
Feed intake, kg/d					
Period 1 ^c	.91	.91	.91	.91	.00
Period 2	.97	1.00	1.05	1.09	.06
Period 3	1.20	1.15	1.08	1.20	.07
Period 4	1.32	1.17	1.23	1.32	.07
Period 5 ^e	1.38	1.23	1.32	1.41	.06
Period 6 ^f	1.44	1.26	1.36	1.45	.07
Weight gain, kg/d					
Period 1 ^c	.04	.15	.16	.15	.04
Period 2	.19	.20	.20	.23	.02
Period 3	.24	.24	.25	.27	.02
Period 4 ^d	.25	.23	.27	.29	.02
Period 5 ^d	.30	.26	.31	.32	.02
Period 6	.30	.26	.30	.31	.02
Feed/gain					
Period 1 ^c	7.73	6.63	16.60	6.43	6.27
Period 2	5.13	5.07	5.10	4.67	.48
Period 3	5.43	5.13	4.63	4.63	.42
Period 4 ^d	5.26	5.20	4.60	4.67	.22
Period 5 ^e	4.97	4.70	4.40	4.37	.24
Period 6 ^e	4.87	4.80	4.53	4.73	.20

^aPercentage of the diet as-fed.

^bStandard error of means.

^cDiet transition.

^dSource effect (P<.05).

^eSource effect (P<.10).

^fSource x level interaction (P<.10).

the lowest for all the treatments. This, of course, was consistent with the lowest feed intake observed among the lambs in this study. It is interesting to note that the coarse limestone resulted in greater ($P < .05$) weight gains and improved ($P < .05$ and $< .01$) feed efficiencies during periods 4 and 5 of the trial (Table 12). Lambs fed the coarse limestone were 4% more efficient ($P < .10$) than lambs fed the fine limestone for the entire trial. Data obtained from this study indicate that coarsely ground limestones are as effective as finely ground limestones in meeting the needs of the lambs.

Literature Cited

- AOAC 1984. Official Methods of Analysis (14th Ed.).
Association of Official Analytical Chemists,
Washington, D.C..
- Boynton, R. S. 1966. Chemistry and Technology of Lime and
Limestone. (1st Ed.). John Wiley and Sons, Inc. New
York.
- Ferriera, J. J., C. H. Noller, R. B. Keyser and J. L.
White. 1979. Particle size and reactivity of field
grade limestone. J. Anim. Sci. 49(Supp. 1):
367(Abstr.).
- Hillman, R. I., M. C. Pritzland E. W. Kienholz. 1976.
Effect of particle size upon calcium bioavailability
to poults. Poult. Sci. 55:2485.
- Jensen, A., E. Jensen, V. Ravn and A. Unmack. 1977.
Reactivity of inorganic feed additives. I.
Dissolution in vitro at pH 2.7 and 37°C of some
oxides, carbonates and phosphates. Z. Tierphysiol.
Tier. Futter. 38:185.

- Keyser, R. B., C. H. Noller, L. J. Wheeler and S. M. Schaefer. 1985. Characterization of limestones and their effects in vitro and in vivo in dairy cattle. *J. Dairy Sci.* 68:1376.
- Nocek, J. E., D. G. Braund and J. E. English. 1983. Effect of limestone reactivity and percent on production by dairy cows in early lactation. *J. Dairy Sci.* 66:2533.
- Noller, C. H. and J. L. White. 1979. Analytical techniques for evaluating reactivity of limestone. Purdue Univ., Dept. of Anim. Sci.
- Owens, F. N. 1984. Ionophores, buffers and protein for feedlot cattle. Proceedings of the American Association of Bovine Practitioners 16:18-29, Anim. Sci. Dept., Ok. State Univ., Stillwater, Ok. 74078.
- Pond, W. G., J. T. Yen, W. E. Wheeler and D. A. Hill. 1982. Calcium bioavailability from limestones of differing particle size and rate of reactivity for growing nonruminants. *Nutr. Rep. Int.* 26:1027.
- Reid, B. L. and C. W. Weber, 1976. Calcium availability and trace mineral composition of feed grade calcium supplements. *Poult. Sci.* 55:600.
- Wohlt, J. E., D. E. Ritter and J. L. 1986. Calcium sources for milk production in holstein cows via changes in dry matter intake, mineral utilization and mineral source buffering potential. *J. Dairy Sci.* 69:2815.

Chapter 4

INTESTINAL ACID FLOW, DRY MATTER, STARCH AND PROTEIN DIGESTIBILITY AND AMINO ACID ABSORPTION IN BEEF CATTLE FED A HIGH-CONCENTRATE DIET WITH DEFLUORINATED PHOSPHATE, LIMESTONE OR MAGNESIUM OXIDE

Michael L. Christiansen and K. E. Webb, Jr.

Virginia Polytechnic Institute and State University,
Blacksburg 24061

ABSTRACT

Five Angus heifers (285 kg) with duodenal and ileal cannulae were used to study the effect of feeding mineral acid-neutralizers on digesta and fecal pH; intestinal acid flow; DM, starch, CP digestion and amino acid absorption in cattle fed a high-concentrate diet (7.5 kg/d). The experimental design was a 5 x 5 Latin-Square. Treatments included: control, 1.60% deflorinated phosphate of a medium particle size (DRP-R), 1.60% deflorinated phosphate of a coarse particle size (DRP-C), 1.28% limestone or .50% MgO, as-fed. Liquid flow into the duodenum was increased ($P < .05$) by the mineral treatments. Numerically, duodenal acid concentrations were greatest for DRP-R and DPR-C, and least for limestone and MgO. Duodenal pH tended to be highest when DRP and limestone were fed, but differences were not

significant. Ileal pH was increased ($P < .05$) by MgO. Fecal pH was increased ($P < .05$) in the order of MgO > DRP > limestone and control. Total tract DM, starch and CP digestibility were similar among treatments. Crude protein flow into the duodenum was numerically greater for DRP-C and limestone while the control, DRP-R and MgO resulted in similar values. Limestone and DRP-R increase ($P < .10$) the partial digestibility of most essential and nonessential amino acids. Data from this study indicate that high dietary levels of limestone, DRP and MgO increase liquid and acid flow into the duodenum of feedlot cattle and increase the partial digestibility of most amino acids.

(Key Words: Defluorinated Phosphate, Limestone, Magnesium Oxide, Intestinal Acidity, Starch, Amino Acid, Digestibility.)

Introduction

The flow of acid into the small intestine has been studied rather extensively in sheep (Masson and Phillipson, 1952; Phillipson, 1952; Harrison and Hill, 1962; Van Bruchen and van't Klooster, 1980) and young preruminant calves (Bell, 1980; Hill et al., 1970); however, there has been little work published on postprandial acid flow in feedlot cattle. In recent years there has been considerable interest generated in the

postruminal environment in cattle and the possibility that under certain conditions diets containing highly fermentable constituents could increase the acid load on the lower gastrointestinal tract enough to affect nutrient digestion (Wheeler and Noller, 1979; Russel et al., 1981). High dietary levels of limestone have commonly been fed to feedlot cattle to provide some postruminal buffering since limestone is very slowly dissolved within the rumen. Any benefit that has come from feeding limestone as a buffer has been thought to be due to its ability to neutralize gastrointestinal acid. Feeding a high dietary level of limestone to cattle and sheep has either not changed duodenal pH or resulted in only a slight change (Laudert and Matsushima, 1982; Owens and Zinn, 1982; Prigge and Svonavec, 1984; Goetsch and Owens, 1985). There have been no studies to show that a high dietary level of limestone could actually reduce the quantity of acid flowing into the small intestine in feedlot cattle. Work by Teh et al. (1985a) indicated that a high level of limestone in the diet of cattle may actually increase the acid concentration imposed on the small intestine. However, intestinal acid flow was not determined in their study. The purpose of the present study was to estimate acid flow into the small intestine of feedlot cattle receiving a high-concentrate diet at

near ad libitum intake and to determine the impact of feeding several types of potential post-ruminal buffering compounds on intestinal acid flow, starch digestion and amino acid absorption in these cattle.

Experimental Procedure

Animals and Experimental Design. Five Angus heifers averaging 285 kg in weight were spayed and surgically equipped with pliable, plastisol cannulae in the duodenum (15 cm from the pylorus) and ileum (30 cm from the ileocecal junction). Cattle were randomly assigned to treatments in a 5 x 5 Latin-Square design which included five feeding periods and five dietary treatments. Each feeding period included a 10-d preliminary period followed by a 6-d sampling period. The experimental treatments included a control, 1.60% deflorinated rock phosphate of a medium particle size (DRP-R), 1.60% deflorinated rock phosphate of a coarse particle size (DRP-C), 1.28% limestone of a medium particle size and .50% magnesium oxide (MgO_2) of a coarse particle size on an as-fed basis. The limestone treatment was added to the diet on an equal Ca basis with the DRP treatments.

Diet Description. The experimental diet was composed of 80% whole shelled corn and 20% of a protein, mineral and vitamin pellet. The ingredient composition for each of the treatment diets is shown in Table 13. Each diet

was formulated to supply 12.5% crude protein (CP). A blend of 5.7% meat meal and 1.9% corn gluten meal was fed a source of high bypass protein. Urea was supplemented to provide adequate rumen soluble N and elemental S was added to provide a 10:1 nonprotein N:S ratio. The diet also provided at least a 1:1 Ca:P ratio. The ratio of inorganic to organic components in the diet was maintained across treatments by adding a fine, washed sand to the supplemental pellet. The pellet ingredient composition for each of the mineral treatments was the same as for the control pellet except that the sand component was replaced by the appropriate amount of mineral treatment. The DRP, limestone and MgO diets contained .00%, .25% and 1.10% sand, respectively.

External markers to determine the flow rate of the solid and liquid phases of intestinal digesta were incorporated in the pelleted supplements. Cobalt EDTA served as the liquid phase marker and was fed at a rate of 3.4 g·hd⁻¹·d⁻¹. Cobalt EDTA powder was prepared with the LiOH procedure by Uden et al. (1980). Chromic oxide (Cr₂O₃) powder was used to mark the digesta solid phase and was fed at a rate of 34 g·hd⁻¹·d⁻¹.

Animal Feeding and Management. Diet transition was begun 20 d following surgery on the last animal. Cattle were transferred from a maintenance diet composed of

TABLE 13. DIET COMPOSITION ON AN AS-FED BASIS

Item	Treatment				
	Control	DRP-R	DRP-C	Limestone	MgO
Ingredient composition, %					
Whole shelled corn	80.00	80.00	80.00	80.00	80.00
Pellet					
Ground corn	3.44	3.44	3.44	3.44	3.44
Wheat flour	3.71	3.71	3.71	3.71	3.71
Corn gluten meal	1.98	1.98	1.98	1.98	1.98
Meat meal, rend.	5.65	5.65	5.65	5.65	5.65
Beet pulp	1.84	1.84	1.84	1.84	1.84
Urea	.50	.50	.50	.50	.50
DRP-R	-	1.60	-	-	-
DRP-C	-	-	1.60	-	-
Limestone	-	-	-	1.29	-
Magnesium oxide	-	-	-	-	.50
Sand	1.60	-	-	.31	1.10
Dynamate	.10	.10	.10	.10	.10
KCl	.38	.38	.38	.38	.38
TM salt	.25	.25	.25	.25	.25
Markers	.55	.55	.55	.55	.55
Total	100.00	100.00	100.00	100.00	100.00
Chemical composition, %					
Dry matter	92.4	92.4	92.3	92.4	92.3
Crude Protein	12.4	12.4	12.4	12.4	12.4
Starch	57.7	57.7	57.8	58.0	58.1

56.3% mixed grass hay, 40.0% ground corn, 3.5% soybean meal and 1.1% minerals to the high-concentrate diet within a 5-d period. During the diet transition, feed intake was maintained at 4.6 kg/d and animals were fed in two equal portions at 0800 and 1700 h. The maintenance diet was replaced with the high-concentrate diet by 10% increments at each feeding. After the cattle were consuming only the high-concentrate diet, intake was increased gradually to 6.8 kg/d. Intake was maintained at this level for the remainder of the study. In addition to the whole corn and pellets, cattle were fed .68 kg chopped grass hay (4 cm). The hay was mixed with the concentrate portion of the diet at each feeding.

Sampling. Cattle were maintained on the same daily feeding schedule during each preliminary and sampling period of the experiment as previously mentioned for the diet transition period. Feed sample collection and the handling of feed refusals were accomplished with methods described in Chapter 2.

During the sampling period, duodenal and ileal digesta and feces were sampled twice daily (a.m. and p.m.) at randomized, even hours so that at the end of 6 d a sample collected at each even hour of a 24-h period was represented. Approximately 300 ml of duodenal digesta,

200 ml of ileal digesta and 250 g of feces were collected from each animal at each sampling. A time restraint of 1 h/animal was used for sampling. If the animal failed to defecate within the sampling time, a rectal sample was taken by hand.

Digesta and feces samples were placed in plastic sample containers and frozen within 20 min following collection from the animal in an ethanol-dry ice bath. The sample containers were wrapped in two plastic bags and then submerged into the ethanol and dry ice. After samples were frozen, they were transferred to an ice chest containing dry ice until the end of the sampling time. After each sample collection time, digesta and feces were stored at -20° C.

Immediately upon collection from the animal and before freezing, approximately 160 ml of the duodenal sample was taken for pH determination and acid titration. Upon collection, the duodenal sample was first placed in a Waring blender and blended for 30 sec at moderate speed to break up large feed particles so duodenal digest could be sampled uniformly with a wide bore, 10 ml plastic pipette tip and syringe. The entire duodenal sample was transferred to a 1 liter beaker and 160 ml of the sample was pipetted into four, 40 ml centrifuge tubes and then centrifuged at $3000 \times g$ for 5 min. After centrifugation,

exactly 50 ml of the supernatant was pipetted into a reaction vessel containing 20 ml of deionized water. The sample solution was heated to 37° C by heated water circulating through the insulating sleeve surrounding the reaction vessel and then titrated to neutrality with .2 N NaOH. All sample titrations were performed at this constant temperature with an autotitrimer¹. Volume and pH changes were recorded throughout the titration with a strip recorder. Only single titrations were performed on each of the duodenal time-samples since minimizing time following sampling was important and repeatability was extremely high.

Before freezing, 8 g of feces were taken for fecal pH determination. Fecal pH was determined on a 10% (wt/wt) suspension of feces and deionized water at 25° C.

Sample Preparation. Feed samples and feed refusals were prepared for analysis by grinding in a Wiley mill through a 1 mm screen. After grinding, corn grain samples and samples for each pelleted supplement were composited across feeding periods to form one sample each for the experiment. Feed refusals were composited by animal within each feeding period.

¹Fisher Titrimeter II Titration Systems, Models 380, 381, 385 and 395.

Digesta time-samples were composited and the sample composite separated into solid and liquid phases before being analyzed for chemical constituents. A 480 ml duodenal composite sample was prepared by combining eight 5 ml portions from each of the 12 time samples. Each time sample was placed in a 600 ml Berzelius beaker and stirred rapidly enough with a magnetic stir bar to form a deep vortex. Each of the eight sample fractions was removed by lowering a 5 ml plastic ladle into the center of the vortex to the bottom then rapidly raising it up through the stirring solution. After compositing, the sample composite was divided equally between two, 250 ml plastic centrifuge bottles. After tightly capping, heavy digesta solids were allowed to settle to the bottom. They were then displaced to one side of the bottle by lightly tapping the bottle on the counter top. Allowing the solids to settle, especially the large corn particles, was important to good, solid pellet formation during centrifugation. The bottles were carefully placed in the centrifuge head so that the solid residue remained at the lowest point when the bottle was slid into position. Duodenal digesta was separated into solid and liquid phases by centrifuging at 7000 x g for 10 min.

After centrifuging, about one-half of the supernatant was poured out into a liter beaker. The cap

was replaced and the bottle tipped into a horizontal position with the pellet at the bottom. The bottle was then gently rocked back and forth so the sample liquid loosened the lightly colored layer (bacteria and fine feed particles) on the surface of the pellet. The bottle was rocked far enough so the liquid flowed from one end of the pellet to the other. About 20 oscillations were required to bring the light layer into suspension. After the light layer had been removed and mixed into suspension, this liquid portion was added to the rest of the supernatant in the beaker. This was done because it was felt that these bacteria and light particles were more likely to pass with the liquid rather than solid phase of the digesta. After thorough mixing with a magnetic stir bar, two 10 ml aliquots of the duodenal supernatant were pipetted into plastic culture tubes each for dry matter (DM), Co, Cr, N and starch determinations. Two 7 ml aliquots of liquid phase were placed in glass scintillation vials for subsequent amino acid analysis. Liquid samples were then stored at -20° C until analysis. The solid phase pellet was placed in a preweighed plastic sample container, the container plus sample was weighed, covered with three layers of cheese cloth and frozen at -20° C. Solid samples were then freeze-dried. After the samples had dried, they were then allowed to equilibrate

with atmospheric moisture for 2 d and then ground in a Cyclotec mill through a 1 mm screen for lab analyses.

A 420 g composite sample of ileal digesta was prepared by adding one 35 g portion from each of the 12 time samples to a 500 ml beaker. Each ileal time sample was mixed thoroughly with a stainless steel spoon immediately before removing the aliquot with the spoon. After compositing, the composite sample was mixed thoroughly then transferred in equal portions to two 250 ml plastic centrifuge bottles. Ileal solid and liquid phases were separated with the same method described above for separating the duodenal solid and liquid phases. The distinct light colored layer observed on the surface of duodenal pellet, was not observed on the ileal pellet. Also, the thick, viscous supernatant of the ileal samples did not seem to disturb the pellet surface as easily as was observed in the duodenal samples. The ileal supernatant was sampled for analysis as previously described for the duodenal samples. The ileal solid phase also was prepared for analysis as previously described for the duodenal samples.

A 252 g fecal composite sample was prepared by adding 21 g of each of the 12 feces time samples to a 500 ml plastic beaker. The composite sample was blended thoroughly by hand with a stainless steel spoon then

transferred to a plastic sample container. Fecal samples were then prepared for analysis following the same procedures used for the duodenal solid-phase samples.

Analyses. Dry matter composition of feed, feces and the digesta solid phase was determined by drying for 24 h at 105 C. Dry matter composition of the digesta liquid phase was determined by pipetting, in triplicate, 1.5 ml of the liquid phase samples into preweighed dry plastic scintillation vials (20 ml). A wet sample weight was determined, the samples were frozen at -20° C and then freeze-dried. Before samples were removed from the freeze-drier, they were warmed to room temperature. Samples were then transferred to a dessicator and a dry weight determined. Feed, feces and digesta crude protein (CP) was determined by the microKjeldahl procedure.

Cobalt concentration in solid and liquid samples was determined by extracting Co from the sample with the procedure of Hart and Polan (1983) and then determining the Co content in the extraction filtrate by atomic absorption spectrophotometry. The filtrate was analyzed for Co without dilution. Samples were analyzed in triplicate.

All solid samples were analyzed for Cr₂O₃ content with the modified procedure of Hill and Anderson (1958).

Duodenal liquid phase samples were not analyzed for Cr. The ileal liquid phase was prepared for Cr determination with the procedure by Hill and Anderson (1958), but analyzed for Cr concentration with inductively-coupled atomic plasma spectrophotometry using a Jarrell-Ash AtomScan 2400 System².

Starch content of solid and liquid samples was determined by the starch hydrolysis procedure of Leonard and Croom (1985) and C. J. Croom, Jr. (personal communication) in combination with the glucose oxidase assay³.

Digesta flow rates for both the solid and liquid phases along the small and large intestinal tract were calculated with procedures published by Armentano and Russell (1985). Nutrient intake and nutrient content of digesta (solid and liquid phases) and feces were expressed on a DM basis for all digestibility calculations.

Statistical analysis was accomplished with the SAS (1982) General Linear Model procedure and the Fischer's protected LSD test for comparisons among treatment means.

²Samples were analyzed for Cr by the Soil Testing and Plant

Analysis Laboratory, 145 Smyth, Virginia Polytechnic Institute and State University, Blacksburg 24061.

³Diagnostic Kit No. 510, SIGMA Chemical Co.

Results and Discussion

The mineral supplements used as dietary treatments in the present study were all coarse commercial feed grade supplements. DRP-C represented the coarsest of the mineral additives (Table 14). Eighty-five percent of the particles were about evenly distributed among the 1400, 1,180 and 850 μ screens. Limestone was the finest ground feed grade mineral, though 82% of the particles were about equally distributed between the 425 and 250 μ screens and 11% of the sample was retained on the 150 μ screen. Magnesium oxide was slightly more coarse than DRP-R in particle size, but both were intermediate in particle size relative to DRP-C and limestone. Nineteen and 47% of the DRP-R and MgO particles were retained on the 850 μ screen, respectively, while 46 and 34% of the particles were distributed between the 425 and 250 μ screens. The shape of the MgO particles was considerably different compared to the other mineral additives. Magnesium oxide particles were very flat compared to the more spherical shaped DRP and limestone particles. Particle size and shape are important criteria affecting mineral reactivity. Both factors dictate the amount of surface area of the mineral that is actually exposed to the acidic solvent in the gut. The size and shape of mineral particles in addition to chemical composition and

TABLE 14. MINERAL PARTICLE SIZE

Item	Mineral source			
	DRP-R	DRP-C	Limestone	MgO
	----- % of total -----			
Screen size, μ				
2,000		1.1		
1,700	.2	6.7		
1,400	1.6	31.3		
1,180	5.5	30.3		
850	19.0	25.5		47.4
425	33.5	2.4	37.0	23.1
250	12.3	.4	45.2	10.5
150	12.1	.3	10.5	7.5
106	4.4	.2	3.3	4.1
75	4.1	.2	1.7	2.6
Pan	7.3	1.5	2.3	4.8
Total	100.0	100.0	100.0	100.0

crystallinity are the major factors which determine mineral reactivity (Boynton, 1966; Ferreira et al., 1979; Noller and White, 1979).

Deflorinated phosphate and limestone tended to increase pH at the proximal duodenum, but differences were not statistically significant (Table 15). Magnesium oxide was effective in increasing ($P < .05$) pH at the distal ileum. Generally, limestone feeding has not been very effective in changing duodenal pH in ruminants fed high concentrate diets, though limestone has ranged from .9% to 3.0% of the diet DM (Laudert and Matsushima, 1982; Goetsch and Owens, 1985; Prigge and Svonavec, 1984). Zinn and Owens (1980) increased duodenal pH in cattle fed an 80% corn-based concentrate diet when limestone was fed at 2.5% of the DM, but it did not alter pH at the terminal ileum or in the rectum. In our earlier work, ileal pH in lambs was increased one pH unit by feeding MgO at 1.5% of the diet (Chapter 2).

In the present study, MgO consistently resulted in fecal pH values very near or above pH 7.0 resulting in higher ($P < .05$) fecal pH than the other treatments. Fecal pH averaged 9.5 in lambs limit-fed a 90% concentrate diet containing 1.5% magnesium oxide (Chapter 2). In other studies, MgO has increased fecal pH in beef and dairy cattle when added to high-energy rations at a level

TABLE 15. THE EFFECT OF DEFLUORINATED PHOSPHATE, LIMESTONE OR MAGNESIUM OXIDE ON DIGESTA AND FECAL pH, DUODENAL DIGESTA ACID CONCENTRATION AND DUODENAL ACID FLOW IN BEEF CATTLE FED A HIGH-CONCENTRATE DIET

Item	Treatment					SE ^a
	Control	DRP-R	DRP-C	Limestone	MgO	
Duodenal pH	2.56	2.66	2.71	2.66	2.55	.06
Ileal pH	7.49 ^b	7.52 ^b	7.49 ^b	7.50 ^b	7.85 ^c	.09
Fecal pH	6.54 ^b	6.86 ^c	6.75 ^c	6.86 ^c	7.18 ^d	.08
Duodenal liquid flow, liter/d	60.5 ^b	70.0 ^c	75.7 ^c	72.7 ^c	71.3 ^c	3.0
Duodenal acid concentration, meq/liter	58.3 ^{b,c}	65.4 ^d	64.0 ^{c,d}	55.5 ^b	54.4 ^b	2.0
Duodenal acid flow, Eq/d	3.51 ^b	4.53 ^c	4.82 ^c	4.02 ^d	3.88 ^{b,d}	.16

^aStandard error of the mean.

^{b,c,d}Means in the same row with no common superscript differ (P < .05).

between .4 and .8% of the DM (Erdman et al., 1980; Peirce et al., 1983; Teh et al., 1985ab). All of the mineral supplements increased fecal pH, but differences among the control and DRP-R, DRP-C and limestone were not significant. The feces is the main route for the excretion of excess Ca and P and this, along with unabsorbed residues, is likely the major reason for the elevated pH in the treated animals. Fecal pH has been shown to increase linearly as the level of Ca has been increased in the diet (Krause and Britton, 1979; Haaland and Tyrrell, 1982; Haaland et al., 1982; Laudert and Matsushima, 1982; Goetsch and Owens, 1985). The large particle size in combination with the high dietary level could have increased passage of unreacted mineral into the large bowel.

The mineral treatments resulted in similar liquid flow rates and all resulted in an increase ($P < .05$) in liquid flow into the duodenum compared to the control (Table 15). Liquid flow into the duodenum may have increased due to an increased flow of liquid from the rumen and(or) increased fluid production by the abomasum. A heavy intake of dietary minerals can increase rumen dilution rate and the flow of liquid and associated components from the rumen. This is a result of fluid passage through the rumen wall in response to osmotic

pressure changes from dissolving minerals. Increased water consumption usually also accompanies the heavy intake of minerals since urinary output of fluid is often increased. Rumen soluble mineral salts such as NaHCO_3 and NaCl have been shown to increase rumen dilution rate in cattle fed a high-concentrate diet (Hart and Polan, 1984b; Rogers and Davis, 1982ab; Rogers et al., 1982). In several studies, limestone has not altered rumen dilution rate (Haaland and Tyrrell, 1982; Rogers et al., 1982; Rust and Owens, 1982; Keyser et al., 1985). Goetsch and Owens (1985), however, reported an increase in rumen dilution rate when steers were limit-fed (1.6% of body wt.) an 88% concentrate diet supplemented with .95% CaCl_2 or .65 and 1.11% reagent grade CaCO_3 . Mineral level of the basal diet, intake of the animals, reactivity of the mineral additive and roughage or concentrate level are important factors that can influence the effect of mineral additive on rumen liquid turnover (Owens et al., 1983).

In the present study, rumen pH was not measured, but it may have been low enough to allow for enough mineral to react to effect a change in rumen osmotic pressure. Water intake of the animals was not monitored in the present study, but was likely increased by the high dietary level of minerals. There have been few data

reported on the effect of an increased fluid passage rate on the flow rate of liquid into the duodenum. Data reported by Kellaway et al. (1978) indicated a positive relationship between the two factors though differences in dilution rate and duodenal flow between the control and animals fed mineral salts were not significant.

Acid secretion by the abomasum is stimulated by the infusion of phosphate and bicarbonate buffers (Ash, 1961). The increased liquid flow into the duodenum seen in the present study may have been a result of an increase in acid secretion by the abomasum to counter the neutralization of gastric acid and a heavy increase in ion concentrations resulting from the minerals reacting in the acidic environment of the abomasum.

Duodenal acid concentration was observed to be highest ($P < .05$) in animals fed DRP-R and DRP-C while animals fed limestone and MgO tended to have the lowest duodenal acid concentrations. Duodenal acid concentrations observed in the present study for limestone and MgO were in slight contrast to observations by Teh et al (1985a). They observed a trend for MgO (.48%) and limestone (1.2%) to increase duodenal titratable acidity (44 vs 46.5 meq/liter) in steers full-fed a 60:40 concentrate to orchard grass hay diet.

Sodium bicarbonate added at 1% of the diet DM actually increased duodenal acid concentrations (49 meq/liter).

In the present study, when liquid flow and acid concentration data were combined, duodenal acid flow was stimulated and found to be increased ($P < .05$) with the feeding of mineral acid-neutralizing compounds. Highest ($P < .05$) flows were recorded when DRP-R and DRP-C were fed. Limestone resulted in higher ($P < .05$) levels than the control and MgO was intermediate.

Phosphate forms a weak acid when in solution and is capable of donating about one and a half H^+ per mole when neutralized with a strong base. The divalent form of the acid has a pK_a of 6.68. The higher duodenal acid concentration for both DRP treatments (Table 15) was likely due to the elevated level of phosphoric acid in the duodenal fluid and the contribution of the extra proton as pH of the duodenal fluid was increased by titration to 7.0.

The duodenal fluid titration curves for each of the experimental treatments are presented in Figure 1. These curves represent neutralization of the acidic intestinal fluid as it might occur in the animal. The titration data was best represented by a cubic model and differences among curves were found with both linear ($P < .0001$) and cubic ($P < .01$) comparisons. Differences in

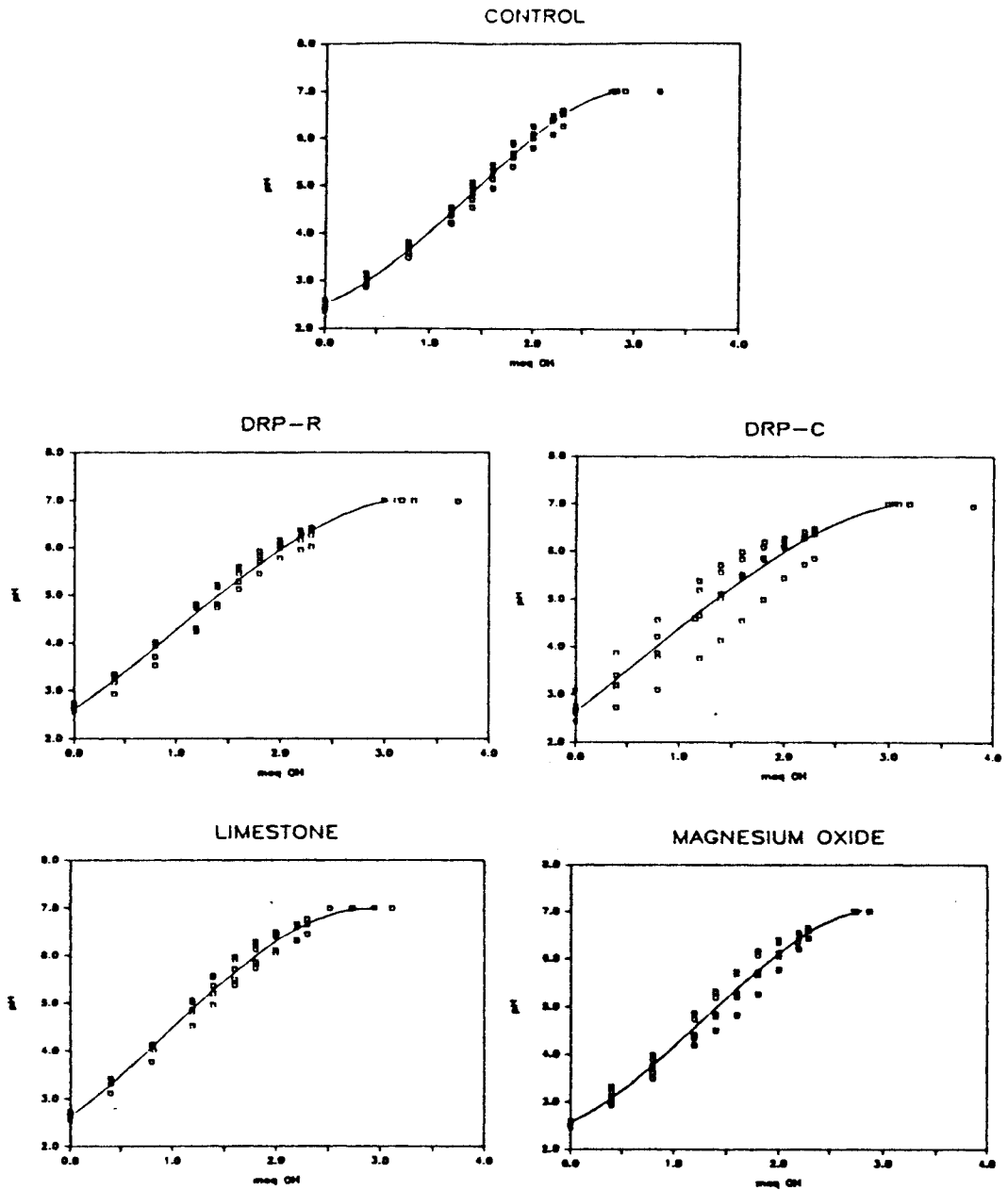


Figure 1. Duodenal fluid titration curves for each dietary treatment were determined by titrating 50 ml duodenal fluid with .2 N NaOH. Curves were determined for the control, DRP-R, DRP-C, limestone, and MgO_2 . Each square symbol represents the mean from 12 titration curves (one curve for each of the 12 sampling times) for each of five feeding periods. Multiple regression coefficients for each curve are shown in Table 16.

TABLE 16. MULTIPLE REGRESSION COEFFICIENTS
 FOR DUODENAL FLUID TITRATION CURVES:
 $\text{pH} = b_0 + b_1\text{meq} + b_2\text{meq}^2 + b_3\text{meq}^3$

Treatment	Coefficients				R ²
	b ₀	b ₁	b ₂	b ₃	
Control	2.52	.780	.966	-.240	.95
DRP-R	2.61	1.431	.346	-.112	.95
DRP-C	2.64	1.682	.148	-.075	.95
Limestone	2.64	1.477	.552	-.187	.95
MgO	2.57	.982	.838	-.221	.95

the linear coefficients among the curves appeared to be due mainly to the much slower rise of the DRP curves as pH increased from about pH 6.0 to 7.0. This is consistent with the idea that the duodenal fluid from animals fed DRP contained substantial amounts of phosphoric acid and that additional protons were removed from the multivalent acid as pH approached 7.0. At pH 7.0, the monovalent form of phosphoric acid is about 50% dissociated. Differences among the curves with the cubic comparison appeared to be due to the accentuated s-curvature in the control, MgO, and limestone curves in comparison to the curves for DRP-C and DRP-R. The chemical reason for these differences in shape of the curves is not known, but is indeed envincive of the complex chemical nature of the duodenal fluid and indicates that the mineral treatments used in this study were capable of affecting how the acid was neutralized in the intestinal tract.

Dry matter intake was similar among treatments (Table 17). Dietary treatments did influence the partitioning of DM digestibility within the various segments of the digestive tract. The mineral treatments apparently provided a slight advantage to fermentation of DM within the rumen. The DRP-R resulted in a greater ($P < .10$) DM disappearance within the stomach compared to the control.

TABLE 17. THE EFFECT OF DEFLOURINATED PHOSPHATE, LIMESTONE OR MAGNESIUM OXIDE
ON DRY MATTER APPARENT DIGESTIBILITY IN BEEF CATTLE FED
A HIGH-CONCENTRATE DIET

Item	Treatment				MgO	SE ^a
	Control	DRP-R	DRP-C	Limestone		
Dry matter intake, %/d	6857	6704	6906	6796	6881	122
Dry matter digestion, % ^b						
Stomach	18.4 ^d	32.9 ^a	25.7 ^d	29.6 ^{ac}	28.8 ^d	5.2
Small intestine	49.6	37.6	39.4	39.2	37.5	5.0
Large intestine	6.6 ^d	5.1 ^d	8.9 ^e	8.1 ^d	9.0 ^e	1.3
Total tract	74.8	75.5	74.1	76.9	75.4	1.4
Dry matter partial digestion coefficient, % ^c						
Small intestine	59.9 ^d	55.0 ^d	52.6 ^e	55.3 ^d	52.4 ^e	2.9
Large intestine	20.0	17.5	25.6	25.4	26.3	3.7

^aStandard error of means.

^bPercentage of dry matter intake.

^cPercentage of amount available.

^dMeans in the same row without a common letter differ (P < .10).

The other mineral treatments resulted in an intermediate DM digestibility within the stomach, though values were not significantly different from those of DRP-R and the control. Dry matter digestion in the stomach of animals fed the mineral acid-neutralizers averaged 11 percentage units higher than the control.

The lower DM digestion within the stomach of the control animals tended to increase DM digestion within the small intestine. Small intestinal digestion of DM in the control animals was numerically greater than in animals fed the mineral treatments, but differences were not significant. Magnesium oxide and DRP-C resulted in a greater ($P < .10$) DM digestibility within the large intestine than DRP-R. Dry matter disappearance within the large intestine of animals fed DRP-C, limestone and MgO averaged about 3.0 percentage units greater than in the control or animals fed DRP-R. The greater digestion of DM in the stomach for DRP-R and greater DM disappearance in the small intestine appeared to be the major reason behind these differences. The coarser or less reactive mineral acid-neutralizers or minerals capable of exerting a greater change in intestinal pH could have provided a slight advantage in hind-gut fermentation of DM. The greater fecal pH for the mineral treatments indicated a passage of unabsorbed mineral

residues or the excretion of excess Ca and P into the large bowel, but increases in fecal pH were not completely consistent with the pattern in DM digestion within the large intestine. DRP-R resulted in an increase in fecal pH, but did not provide any apparent advantage to DM digestion within the large intestine.

Total tract DM digestibility was not different ($P > .10$) among the experimental diets. Total tract DM digestion averaged 75.3. Slightly larger values were reported by Turgeon et al. (1983) with steers fed ad libitum an 85% whole, 50:50 whole to cracked, or cracked corn diet. This may be partly explained by the lower pre-intestinal disappearance of DM recorded in the present study. Dry matter digestion within the stomach region averaged 18.4 and 29.3% for the control and mineral treatments, respectively, compared with 41.8% reported by Turgeon et al. (1983). The lower rumen DM digestibility values observed in the present study may be partially due to an extra low pH in the rumen. Difficulties with intake were experienced periodically throughout the study and seemed to be due to problems with acidosis. Dry matter disappearance expressed as a percent of the amount reaching the small or large intestine (partial digestibility) followed the same trend as apparent digestibility within these areas of the

tract. The partial digestibility of DM in the small intestine for the control was significantly greater than for DRP-C and MgO, but numerically greater than for all the mineral treatments. Feeding DRP-C, limestone and MgO resulted in a numerically greater DM partial digestibility within the large intestine than was observed for the control or DRP-R.

Brink and Steele (1985) have compared the effect of limestone and dicalcium phosphate (.70% dietary Ca) on the apparent digestibility of organic matter in steers fed diets containing 50, 70 or 90% corn plus corn silage. Ruminal organic matter digestibility was lower for limestone compared to dicalcium phosphate; however, postruminal organic matter digestibility and partial digestibility were higher for limestone.

Differences observed among the experimental treatments for the apparent digestibility of starch were not statistically significant, but some consistent trends were noted (Table 18). Numerically, starch digestion within the stomach was similar for the control and DRP-C and averaged about 8.5 percentage units below starch digestion in the stomach of animals when DRP-R, limestone and MgO were fed. The lower starch digestion coefficients in the stomach region for the control and DRP-C were consistent with the lower rumen DM digestion

TABLE 18. THE EFFECT OF DEFLUORINATED PHOSPHATE, LIMESTONE OR MAGNESIUM OXIDE ON STARCH APPARENT DIGESTIBILITY IN BEEF CATTLE FED A HIGH-CONCENTRATE DIET

Item	Treatment			MgO	SE ^a
	Control	DRP-R	DRP-C		
Starch intake	3900	3813	3926	3937	65
Starch digestion, % ^b					
Stomach	57.1	66.6	58.8	63.4	6.4
Small intestine	30.0	21.6	27.5	24.4	5.7
Large intestine	3.0	2.7	5.5	4.9	1.3
Total tract	90.1	90.9	89.9	92.0	1.3
Starch partial digestion coefficient, % ^c					
Small intestine	64.9	64.4	62.9	66.5	4.7
Large intestine	19.2 ^d	13.2 ^d	35.1 ^d	38.0 ^e	23.1 ^d

^aStandard error of means.

^bPercentage of starch intake.

^cPercentage of amount available.

^dMeans in the same row without a common letter differ (P < .10).

coefficients also observed for these two treatments. Small and large intestinal digestion of starch appeared to compensate for differences in the disappearance of starch within the stomach region. Small intestinal apparent digestibility of starch tended to be slightly greater for the control and DRP-C since these treatments resulted in lower digestibility of starch pre-intestinally.

Similar to DM digestibility in the large intestine, the apparent digestion of starch within the large intestine tended to be greatest in animals fed DRP-C, limestone and MgO which seemed related to the particle size or the ability of the mineral acid neutralizer to effect a pH change in the lower gastrointestinal tract.

The capacity of the lower tract to compensate for lower starch digestibility within the stomach region resulted in similar total tract apparent digestion coefficients among the treatments. Total tract apparent digestion of starch compared well with information presented in the literature. For this study, starch digestibility of the whole shelled corn diet averaged 90.7. Owens et al. (1986) reported a mean starch digestibility of 91.7 for whole shelled corn in a review of literature on factors that affect starch digestion in the ruminant small intestine. Turgeon et al. (1981)

observed a mean total tract starch digestibility of 91.5 in steers full-fed a diet containing 85% whole shelled corn. In research reviewed by Theurer (1986), total tract starch digestibility in cattle fed whole shelled corn diets ranged from 77 to 94 and averaged 89.

Partial digestibility of starch within the small intestine was similar among all treatments except for MgO. Starch partial digestibility within the small intestine averaged about 9 percentage units lower for MgO, though the difference was not statistically significant. This difference may be partly related to the capacity of MgO to increase pH within the lower tract. Optimum pH range for starch hydrolysis in the small intestine is about 6.4 to 7.2 (Zinn and Owens, 1980a). Even though ileal pH for all the treatments was above the optimal range, the extra capacity of MgO to increase intestinal pH may have served to increase small intestinal pH a lot sooner and may have reduced amylolytic activity within the small intestine.

The limestone resulted in greater ($P < .10$) partial digestion of starch within the large intestine than DRP-R. Similar to the apparent digestion of starch within the large intestine, large intestinal partial digestibility of starch for DRP-C, limestone and MgO was

numerically greater than observed for the control and DRP-R.

Crude protein intake was similar and negative CP digestibility in the stomach was observed for each of the diets (Table 19). In the stomach region, negative values are expected because of the potential contribution of protein and N from saliva and abomasal secretions. A larger ($P < .10$) negative CP digestion coefficient in the stomach was recorded for animals fed limestone than those fed MgO which was indicative of a greater amount of CP reaching the duodenum in relation to CP intake in the animals given the limestone treatment. Limestone and MgO resulted in 176 and 46 g/d more CP reaching the duodenum than was eaten, respectively. These data represented the maximum and minimum flows of CP among the experimental treatments. Numerically, the CP digestion coefficient in the stomach for DRP-C was similar to that for limestone while the control and DRP-R resulted in intermediate values.

Crude protein digestion in the small intestine followed an inverse pattern as CP digestion in the stomach. Limestone resulted in a greater ($P < .05$) CP apparent digestibility in the small intestine than MgO. Numerically, the DRP-C value was similar to limestone while the control and DRP-R resulted in intermediate

TABLE 19. THE EFFECT OF DEFLUORINATED PHOSPHATE, LIMESTONE OR MAGNESIUM OXIDE ON CRUDE PROTEIN APPARENT DIGESTIBILITY IN BEEF CATTLE FED A HIGH-CONCENTRATE DIET

Item	Control	Treatment			MgO	SE ^e
		DRP-R	DRP-C	Limestone		
Crude protein intake, g/d	909	889	924	902	919	18
Crude protein digestion, % ^b						
Stomach	-9.6 ^{de}	-9.7 ^{de}	-17.7 ^d	-20.2 ^d	-5.0 ^e	4.7
Small intestine	70.8 ^{de}	72.6 ^{de}	78.1 ^d	80.7 ^d	65.6 ^e	4.7
Large intestine	5.5	4.2	3.5	6.6	6.6	2.3
Total tract	66.7	67.1	63.9	67.1	67.2	1.7
Crude protein partial digestion coefficient, % ^c						
Small intestine	64.1 ^{de}	66.0 ^{de}	66.3 ^{de}	66.9 ^d	61.6 ^e	2.0
Large intestine	13.3	11.4	8.8	16.4	16.8	5.6

^aStandard error of means.

^bPercentage of crude protein intake.

^cPercentage of amount available.

^{d,e}Means in the same row without a common superscript differ (P < .10).

values. These data are in keeping with the concept that CP apparent digestibility in the small intestine is usually positively correlated with the CP supply to this region (Schneider and Flatt, 1975). On the other hand, CP apparent digestibility in the large intestine is not necessarily correlated to the amount of CP reaching the hindgut because of the potential protein contribution from the microbial population that inhabits the cecal and colonic regions (Orskov, 1982). In the present study, the pattern in CP apparent digestibility in the large intestine was not related to CP apparent digestibility in the small intestine. Crude protein tended to be highest for limestone, MgO and the control and lowest for DRP-R and DRP-C.

In spite, of the differences among the treatments observed in the partitioning of CP digestibility between the various compartments of the digestive tract, total tract CP apparent digestibility was quite similar among the dietary treatments. Rogers et al. (1982) observed a decrease in CP apparent digestibility of over 10 percentage units in dairy cows fed a 75:25 concentrate to corn silage diet supplemented with 2.4% limestone on a DM basis. They suggested that limestone may have increased cecal and colonic pH, thereby allowing for increased

microbial activity, greater breakdown of starch, and larger amounts of microbial N excreted in the feces.

In the present study, except for MgO, CP partial digestion within the small intestine was rather constant among the dietary treatments . Partial digestibility of CP in the small intestine tended to be lower for MgO. This was probably the result of the trend for animals fed MgO also to have a greater CP disappearance in the stomach region. Partial digestion of CP within the large intestine followed much the same pattern observed for the apparent digestibility of CP in this region, but differences were not significant.

The quantity of amino acids reaching the duodenum per day tended to be greatest in cattle fed DRP-C and limestone (Table 20). A numerical increase in flow of amino acids for DRP-C and limestone was apparent for most individual amino acids. Compared to the control, DRP-C resulted in a significantly greater flow of histidine, threonine, alanine, proline and serine into the duodenum. When compared to the control, limestone significantly increased the flow of threonine into the duodenum. Adding DRP-C and limestone to the diet increased the duodenal flow of all essential amino acids by 9.4% and 7.3%, respectively, over that observed for the control. The flow of nonessential amino acids was increased 8.9%

TABLE 20. THE EFFECT OF DEFLUORINATED PHOSPHATE, LIMESTONE OR MAGNESIUM OXIDE ON THE QUANTITY OF AMINO ACIDS FLOWING INTO THE DUODENUM OF BEEF CATTLE FED A HIGH-CONCENTRATE DIET

Amino acids	Control	Treatment			MgO	SE ^a
		DRP-R	DRP-C	Limestone		
----- g/d -----						
Essential						
Arginine	46.1	43.6	50.7	48.8	47.6	3.1
Histidine	20.7 ^a	22.3 ^{ab}	24.4 ^b	23.1 ^{ab}	22.6 ^{ab}	1.2
Isoleucine	44.7	46.7	48.9	48.1	45.4	2.0
Leucine	88.4	91.5	97.8	93.8	88.7	4.8
Lysine	49.5	54.1	52.0	53.3	50.2	3.1
Methionine	15.0	15.2	15.8	15.9	15.6	.5
Phenylalanine	47.6	50.3	53.0	51.4	48.5	2.2
Threonine	34.0 ^a	34.8 ^{ab}	37.0 ^b	37.1 ^b	35.1 ^{ab}	1.2
Valine	49.0	48.6	52.4	52.0	49.6	1.6
Nonessential						
Alanine	59.1 ^a	59.8 ^a	65.9 ^b	64.4 ^{ab}	60.8 ^{ab}	2.2
Aspartic acid	65.3	65.3	67.1	65.5	61.9	2.6
Cysteine	2.7	2.4	2.6	2.9	2.8	.3
Glutamic acid	124.3	122.6	135.5	130.8	123.8	5.4
Glycine	44.5 ^a	50.7 ^b	49.6 ^{ab}	48.7 ^{ab}	47.3 ^{ab}	2.1
Proline	56.6 ^a	57.8 ^{ab}	63.6 ^b	60.7 ^{ab}	57.0 ^{ab}	3.0
Serine	34.9 ^a	34.5 ^{ab}	37.2 ^b	35.8 ^{ab}	34.5 ^{ab}	1.6
Tyrosine	47.1	46.8	50.0	49.5	48.3	1.8
Essential	394.9	407.4	431.9	423.7	403.4	17.1
Nonessential	433.1	440.0	471.5	457.9	436.3	17.1
Total	827.9	847.4	903.4	881.6	839.7	33.4

^aStandard error of means.

^bMeans in the same row without a common letter differ ($P < .10$).

and 5.9% over control values when DRP-C and limestone were fed, respectively. This tendency for a greater amino acid flow in cattle fed DRP-C and limestone was consistent with the trend for larger negative rumen CP apparent digestion coefficients calculated for these treatments (Table 19) indicating the propensity for a greater amount of protein reaching the duodenum in these cattle.

Previously published information indicates that partial digestibility of amino acids in the small intestine is influenced by the quantity of protein entering the small intestine (Van Bruchem et al., 1985). Metabolic sources of protein remain relatively constant even though the total quantity of protein entering the small intestine may increase. Partial digestibility of amino acids generally increases as the quantity of amino acids flowing into the small intestine also increases. In the present study, increases in amino acid partial digestibility did not follow the same pattern as the duodenal flow data (Table 21). Though DRP-R did not appear to have much of an impact on duodenal amino acid flow, DRP-R together with limestone resulted in significantly greater partial digestibility values for most of the amino acids when compared to the control. DRP-R significantly increased the partial digestibility

TABLE 21. THE EFFECT OF DEFLUORINATED PHOSPHATE, LIMESTONE OR MAGNESIUM OXIDE ON PARTIAL DIGESTIBILITY OF AMINO ACIDS IN THE SMALL INTESTINE OF BEEF CATTLE FED A HIGH-CONCENTRATE DIET^a

Amino acids	Treatment				MgO	SE ^b
	Control	DRP-R	DRP-C	Limestone		
Essential						
Arginine	75.8 ^e	76.8 ^{ef}	77.5 ^{ef}	77.6 ^{ef}	79.8 ^f	1.4
Histidine	60.0 ^e	63.9 ^{ef}	62.7 ^{ef}	64.9 ^f	61.7 ^{ef}	2.3
Isoleucine	73.5 ^e	76.9 ^{ef}	75.5 ^{ef}	76.9 ^{ef}	73.9 ^e	1.1
Leucine	76.7 ^e	80.0 ^{ef}	78.6 ^{ef}	80.4 ^f	78.8 ^e	1.3
Lysine	67.7 ^c	75.0 ^d	72.0 ^{cd}	73.1 ^d	71.0 ^{cd}	1.5
Methionine	76.2 ^c	81.0 ^d	77.1 ^{cd}	80.6 ^d	78.9 ^{cd}	1.3
Phenylalanine	73.3 ^c	77.5 ^d	76.0 ^{cd}	77.3 ^d	74.2 ^{cd}	1.2
Threonine	61.9 ^c	67.7 ^d	66.0 ^{cd}	66.7 ^{cd}	63.2 ^{cd}	1.7
Valine	69.2 ^e	72.8 ^{ef}	71.3 ^{ef}	72.9 ^f	69.3 ^{ef}	1.4
Nonessential						
Alanine	66.8 ^e	70.6 ^{fg}	69.4 ^{ef}	71.2 ^f	66.9 ^{ef}	1.5
Aspartic acid	71.8 ^e	78.8 ^f	76.6 ^{fg}	80.0 ^f	73.9 ^{ef}	1.8
Cysteine	71.9	70.5	72.8	70.2	68.4	4.1
Glutamic acid	70.6 ^e	76.3 ^f	74.0 ^{ef}	77.5 ^f	70.7 ^e	1.8
Glycine	64.2 ^e	71.6 ^f	67.1 ^{ef}	68.6 ^{fg}	65.7 ^{ef}	1.2
Proline	66.9 ^e	72.4 ^f	70.4 ^{ef}	73.1 ^f	68.2 ^e	1.5
Serine	65.0 ^e	69.5 ^f	67.2 ^{ef}	68.3 ^{ef}	65.1 ^e	1.6
Tyrosine	70.8 ^e	74.4 ^f	73.9 ^{ef}	74.6 ^f	71.8 ^{ef}	1.3
Essential	71.6	75.6	74.1	75.4	72.9	.3
Nonessential	68.6	74.1	71.9	74.4	69.5	2.6
Total	70.15	74.9	72.9	74.9	71.1	2.0

^aThe apparent absorption of amino acids as a percent of amino acids reaching the small intestine.

^bStandard error of means.

^c,^dMeans in the same row without a common letter differ (P < .05).

^e,^f,^gMeans in the same row without a common letter differ (P < .10).

of all amino acids except arginine, histidine, leucine, alanine and cysteine. Limestone improved partial digestibility of all amino acids except arginine, threonine, alanine, cysteine, glycine and serine. Among essential amino acids, the partial digestibility of lysine received the greatest boost from feeding DRP-R and limestone, averaging 6.4 percentage units greater than the control value for both treatments. The average increase in methionine partial digestibility for DRP-R and limestone was increased 4.5 percentage units over that observed for the control. These observations suggest a potential nutritional advantage for cattle fed DRP-R and limestone at the dietary levels used in this study since lysine and methionine are thought to be the most common limiting amino acids in ruminant diets.

Partial digestibility for essential and nonessential amino acids averaged 3.9 and 5.7 percentage units greater, respectively, for DRP-R and limestone compared to the control. In comparison, partial digestibility for essential and nonessential amino acids averaged 2.5 and 3.3 percentage units greater, respectively, for DRP-C compared to the control. However, differences in partial digestibility for all essential or nonessential amino acids observed among treatments were not statistically significant.

In the present study, methionine (78.6%) and leucine (78.5%) appeared to have the greatest relative absorption among all the amino acids, while histidine had the least (62.6%) (Table 21). Koeln and Patterson (1986) also observed methionine to have the highest (64.7%) and histidine the lowest (43.7%) relative absorption in calves fed a cottonseed hull-corn based diet with a soybean meal, toasted soybean meal or corn gluten meal supplement. This same absorption pattern has also been observed in sheep fed alfalfa hay (methionine = 80.0%; histidine = 47.0%) (Coelho de Silva et al., 1972).

Methionine uptake from the small intestine appears to be rather efficient since transport of methionine seems to occur via several of the proposed amino acid transport systems and methionine absorption does not appear to be inhibited by competitive inhibitors (Phillips, 1974 and 1976; Christensen, 1984; Guerino and Baumrucker, 1987).

In conclusion, it appears that the dietary levels of DRP-R, DRP-C, limestone and MgO used in the present study may actually serve to increase rather than decrease the quantity of acid flowing into the small intestine of beef cattle fed a high-concentrate diet by increasing liquid flow into the duodenum. The mineral treatments provided a slight advantage in rumen fermentation of DM and starch. Differences in DM, starch and CP digestibility

in the small intestine were closely related to digestion differences in the rumen rather than to any special action of the mineral treatments on postruminal digestion. A lower DM, starch or CP digestibility in the rumen was associated with a greater digestibility of the nutrient in the small intestine. There was a tendency for the digestion of DM and starch in the large intestine to be influenced by minerals with a larger particle size or having a greater potential to increase pH postruminally, but differences were small. Total tract digestibility of DM and starch were similar among the experimental diets. Limestone and DRP-C tended to increase the flow of CP and amino acids into the duodenum, but DRP-R, rather than DRP-C, and limestone significantly increased the partial digestibility of most individual amino acids. Factors other than the quantity of amino acid flow into the duodenum seemed responsible for the effect of DRP-R on partial digestibility of amino acids.

Literature Cited

- Armentano, L. E. and R. W. Russel. 1985. Method for calculating digesta flow and apparent absorption of nutrients from nonrepresentative samples on digesta. *J. Dairy Sci.* 68:3067.
- Ash, R. W. 1961. Stimuli influencing the secretion of acid by the abomasum of sheep. *J. Physiol. (Lond.)*. 157:185.

- Bell, F. R. 1980. The mechanisms controlling abomasal emptying and secretion. In: Y. Ruckebusch and P. Thivend. (Ed.). Digestive Physiology and Metabolism. pp. 81-100. A. V. I. Publishing Company, Inc., Westport.
- Bidlingmeyer, B. A., S. A. Cohen, T. L. Tarvin and B. Frost. 1987. A new rapid, high sensitivity analysis of amino acids in food type samples. J. Assoc. Anal. Chem. 70:241.
- Boynton, R. S. 1966. Chemistry and technology of limes and limestone. (1st Ed.). John Wiley and Sons, Inc. New York.
- Brink, D. R. and R. T. Steele. 1985. Site and extent of starch and neutral detergent fiber digestion as affected by source of calcium and level of corn. J. Anim. Sci. 60:1330.
- Christensen, H. N. 1984. Organic ion transport during the seven decades: the amino acids. Biochem. Biophys. Acta. 779:255.
- Coehlo Da Silva, J. F., R. C. Seeley, D. J. Thomson, D. E. Beever and D. G. Armstrong. 1972. The effect in sheep of physical form and stage of growth on the sites of digestion of a dried grass. Br. J. Nutr. 28:357.
- Erdman, R. A., R. L. Botts, R. W. Hernken and L. S. Bull. 1980. Effect of dietary sodium bicarbonate and magnesium oxide on production and physiology in early lactation. J. Dairy Sci. 63:923.
- Ferreira, J. J., C. H. Noller, R. B. Keyser and J. L. White. 1979. Particle size and reactivity of feed grade limestones. J. Anim. Sci. 49 (Suppl.1): 367(Abstr.).
- Goetsch, A. L. and F. N. Owens. 1985. Effects of calcium source and level on site of digestion and calcium levels in the digestive tract of cattle fed high concentrate diets. J. Anim. Sci. 61:995.
- Guerino, F. and C. R. Baumrucker. 1987. Identification of methionine and lysine transport systems in cattle small intestine. J. Anim. Sci. 65:630.

- Haaland, G. L. and H. F. Tyrrell. 1982. Effects of limestone and sodium bicarbonate buffers on rumen measurements and rate of passage in cattle. *J. Anim. Sci.* 55:935.
- Haaland, G. L., H. F. Tyrrell, P. W. Moe and W. E. Wheeler. 1982. Effect of crude protein level and limestone buffer in diets fed at two levels of intake on rumen pH, ammonia-nitrogen, buffering capacity and volatile fatty acid concentrations of cattle. *J. Anim. Sci.* 55:943.
- Harrison, F. A. and K. J. Hill. 1962. Digestive secretions and the flow of digesta along the duodenum of the sheep. *J. Physiol. (Lond.)*. 162:225.
- Hart, S. P. and C. E. Polan. 1984a. Simultaneous extraction and determination of ytterbium and cobalt ethylenediamine-tetraacetate complex in feces. *J. Anim. Sci.* 67:888.
- Hart, S. P. and C. E. Polan. 1984b. Effect of sodium bicarbonate and disodium phosphate on animal performance, ruminal metabolism, digestion and rate of passage in ruminating calves. *J. Dairy. Sci.* 67:2356.
- Hill, F. N. and D. L. Anderson. 1958. Comparison of metabolizable energy and productive energy determinations with growing chicks. *J. Nutr.* 614:587.
- Hill, K. J., D. E. Noakes and R. A. Lowe. 1970. Gastric digestive physiology in the calf and piglet. In: A. T. Phillipson (Ed.). *Physiology of Digestion and Metabolism in the Ruminant*. pp. 166-179. Proceeding of the 3rd International Symposium. Oriel Press, Cambridge, England.
- Kellaway, R. C., D. E. Beever, D. J. Thomson, A. R. Austin, S. B. Cammell and M. L. Elderfield. 1978. The effects of NaCl or NaHCO₃ on digestion in the stomach of weaned calves. *J. Agric. Sci. (Camb.)* 91:497.

- Keoln, L. L. J. A. Paterson. 1986. Nitrogen balance and amino acid disappearance from the small intestine in calves fed soybean meal toasted soybean meal-or corn gluten meal-supplemented diets. *J. Anim. Sci.* 63:1258.
- Keyser, R. B., C. H. Noller, L. J. Wheeler and D. M. Schaefer. 1985. Characterization of limestones and their effects in vitro and in vivo in dairy cattle. *J. Dairy Sci.* 68:1376.
- Krause, V. E. and R. E. Britton. 1979. Tri-calcium phosphate or limestone as buffers in finishing rations for steers. *J. Anim. Sci.* 49(Suppl. 1):384. (Abstr.).
- Laudert, S. B. and J. K. Matsushima. 1982. Limestone effects on digestion and gastrointestinal tract pH of finishing steers. *J. Anim. Sci.* 55 (Suppl.1): 437(Abstr.).
- Leonard, E. S. and W. J. Croom, Jr. 1985. A simplified method for the analysis of fecal and digesta starch. *J. of Anim. Sci.* 61(Suppl. 1):64. (Abstr.).
- Lu, C. D., N. A. Jorgensen and L. D. Satter. 1988. Site and extent of nutrient digestion in lactating dairy cows fed alfalfa protein concentrate or soybean meal. *J. Dairy Sci.* 71:697.
- Macrae, J. C. and D. G. Armstrong. 1968. Enzyme method for determination of x-linked glucose polymers in biological materials. *J. Sci. Food Agric.* 19:578.
- Masson, M. J. and A. T. Phillipson. 1952. The Composition of the digesta leaving the abomasum of sheep. *J. Physiol. (Lond.)* 116:98.
- Noller, C. H. and J. L. White. 1979. Analytical techniques for evaluating reactivity of limestone. Purdue Univ., Dept. of Anim. Sci.
- Orskov, E. R. 1982. Protein Nutrition In Ruminants. Academic Press Inc., New York.
- Owens, F. N. and R. A. Zinn. 1982. Calcium nutrition and buffers for feedlot cattle. *Proc. Am. Feed Mfr. Assn.*, p. 1.

- Owens, F. N., A. L. Goetsch, D. C. Weakley and R. A. Zinn. 1983. Buffers and neutralizers and Electrolyte Symposium Handbook. Natl. Feed Ingrid. Assoc., Des Moines, IA.
- Owens, F. N., R. A. Zinn and Y. K. Kim. 1986. Limits to starch digestion in the ruminant small intestine. *J. Anim. Sci.* 63:1634.
- Peirce, S. B., L. D. Muller and H. W. Harpster. 1983. Influence of sodium bicarbonate and magnesium oxide on digestion and metabolism in yearling beef steers abruptly changed from high forage to high energy diets. *J. Anim. Sci.* 57:1561.
- Phillips, L. L. and M. L. Caldwell. 1951. A study of the purification and properties of a glucose-forming amylase from *Rhizopus delemar*, gluc amylase. *J. Am. Chem. Soc.* 73:3559.
- Phillipson, A. T. 1952. The passage of digesta from the abomasum of sheep. *J. Physiol. (Lond.)*. 116:84.
- Price, S. G., L. D. Satter and N. A. Jorgensen. 1988. Dehydrated alfalfa in dairy cow diets. *J. Dairy Sci.* 71:72.
- Prigge, E. C. and J. J. Svonavec. 1984. Flow and acidity of digesta entering the duodenum as influenced by buffers and time after feeding in wethers. *Can. J. Anim. Sci.* 64:1055.
- Raabo E. and T. C. Terkidse. 1960. On the enzymatic determination of blood glucose. *Scand. J. Clin. Lab Invest.* 12:402.
- Rogers, J. A. and C. L. Davis. 1982. Effects of intraruminal infusions of mineral salts on volatile fatty acid production in steers fed high-grain and high roughage diets. *J. Dairy Sci.* 65:953.
- Rogers, J. A., C. L. Davis and J. H. Clarks. 1982. Alteration of rumen fermentation, milk fat synthesis and nutrient utilization with mineral salts in dairy cows. *J. Dairy Sci.* 65:577.

- Russel, J. R., A. W. Young and N. A. Jorgensen. 1981. Effect of dietary corn starch intake on ruminal, small intestinal and large intestinal starch digestion in cattle. *J. Anim. Sci.* 52:1170.
- Rust, S. R. and F. N. Owens. 1982. Effect of limestone on digestibility of feedlot diets. *Ok. Agric. Exp. Sta. Misc. Publ.* 112:154.
- Schneider, B. H. and W. P. Flatt. 1975. *The Evaluation of Feeds Through Digestibility Experiments.* The Univ. of Georgia Press, Athens.
- Teh, T. H., R. W. Hemken and R. J. Garmon. 1985b. Magnesium oxide interactions with sodium bicarbonate in early lactating cows. *J. Dairy Sci.* 68:881.
- Teh, T. H., R. W. Hemken and R. J. Harmon. 1985a. Comparison of buffers and their modes in the rumen and abomasum. *Nutr. Rep. Int.* 32:1339.
- Theurer, C. B. 1986. Grain processing effects on starch utilization by ruminants. *J. Anim. Sci.* 63:1649.
- Turgeon, O. A., Jr. D. R. Brink and R. A. Britton. 1983. Corn Particle size mixtures, roughage level and starch utilization in finishing steer diets. *J. Anim. Sci.* 57:739.
- Turgeon, O. A., Jr., D. R. Brink and R. A. Britton. 1981. Corn particle size and starch digestion in steers fed ad libitum. *J. Anim. Sci.* 52(Suppl.1):440 (Abstr.).
- Uden, P., P. E. Colucci and P. J. Van Soest. 1980. Investigation of Chromium, cerium and cobalt as markers in digesta. Rate of passage studies. *J. Sci. Food Agric.* 31:625.
- Van Bruchem, J. A. T. van't. Klooster. 1980. Effect of protein on abomasal secretions of acid in sheep. *Br. J. Nutr.* 44:307.
- Wheeler, W. E. and C. H. Noller. 1976. Limestone buffers in complete mixed rations for dairy cattle. *J. Dairy Sci.* 59:1788.

- Williams, H. H., Leo V. Curtin, Jean Abraham, J. K. Loosli and L. A. Maynard. 1954. Estimation of growth requirements for amino acids by assay of the carcass. *J. Biol. Chem.* 208:277.
- Zinn, R. A. and F. N. Owens. 1980. Sodium, Calcium and potassium salts for cattle fed high concentration rations. *Ok. Agric. Exp. Sta. Misc. Publ.* 107:131.

Chapter 5

CALCIUM AND MAGNESIUM COMPOSITION AND RATE OF REACTIVITY
OF LIMESTONE USED IN LIVESTOCK FEEDS WITHIN THE STATE OF
VIRGINIA

M.L. Christiansen and K.E. Webb, Jr.

Virginia Polytechnic Institute and State University
Blacksburg 24061

Abstract

A sample survey was conducted in 20 counties of Virginia to assess the types and quality of feed grade limestone used in livestock feed throughout the state. Ten of the counties responded to the survey. Five of the 10 limestone samples received came from the same manufacturer. This limestone was very finely ground and extremely reactive. The T50 rate of reactivity for this limestone averaged 40 seconds. Two limestone samples from a different manufacturer were also evaluated as finely ground and highly reactive. Two of the limestone samples received were very coarsely ground, similar in particle size distribution, but were very different from each other in rate of reactivity. They were about 12 and 23 times slower in reactivity than fine, fast reacting limestone samples. The finely ground, fast reacting calcitic limestone appears to be the most widely used by producers and feed mills in the State of Virginia.

Chemical composition, particle size and rate of reactivity appeared to be important criteria in evaluating the quality of a feed grade limestone.

(Key Words: Calcitic, Limestone, Particle Size, Rate of Reactivity.)

Introduction

In conjunction with the study on the effect of limestone level and particle size on lamb performance as reported in Chapter 3, there was also interest in the quality of feed grade limestones being used by feed manufacturers and livestock producers throughout the State of Virginia. Pulverized and ground limestone manufactured and sold for liming purposes within the state must meet minimum requirements for particle size, Ca and Mg content and CaCO_3 equivalents (Agricultural Lime Law, 1974). Similar guidelines are followed in regulating the manufacture and sale of feed limestones, but there have been no laws established specific for the regulation of feed grade limestone within the state.

Feed limestones require acidic conditions for adequate reactivity. However, limestone from different sources vary in their reactivity because of differences in particle size, chemical composition and crystallinity (Boynton, 1966; Ferreira et al., 1979; Noller and White, 1979). Rate of reactivity in an acid medium at normal

physiological temperature may be more indicative of the actual quality of a feed grade limestone than simply particle size and chemical information alone.

Bioavailability of Ca for growing chicks has been shown to be dependent on limestone particle size and feed limestone source (Hillman et al., 1976; Reid and Weber, 1976). This type of information on Ca bioavailability is lacking for growing cattle or sheep.

The objective of this study was to assess the particle size and reactivity of feed grade limestone commonly used by feed manufacturers and(or) livestock producers within the counties of Virginia.

Materials and Methods

A sample survey was conducted in 1981 in cooperation with Dr. H. John Gerken, Jr., Virginia Tech Animal Science Extension Specialist, and the extension agents in 20 counties in the State of Virginia to assess the type and quality of feed grade limestones that were being fed to livestock within the state. County extension agents were asked to mail to our laboratory a 500 g sample of feed grade limestone that was commonly being used by livestock producers and(or) feed mills within their county. They were asked to identify the source of the limestone (plant or quarry) if this information was available. In our laboratory, limestone samples were

evaluated on the basis of Ca and Mg content, particle size and rate of reactivity at pH 2.7 at 37 C.

The following counties in Virginia were surveyed:

<u>County</u>	<u>County Agent</u>
1. Albemarle	Donald J. Bowman
2. Appomattox	James Smith
3. Augusta	Jerry M. Swisher
4. Botetourt	Bobby E. Leonard
5. Fauquier	Douglas Cooper
6. Frederick	Gary C. Deoms
7. Greensville	Bobby L. Flippen
8. Halifax	Larry L. McPeters
9. Isle of Wright	Robert D. Goeger
10. Lee	Harley Young
11. Montgomery	Billy R. Mckinnon
12. Rockbridge	Allen G. Strecker
13. Rockingham	James R. Mckenna
14. Russell	Roy J. Kiser
15. Shenandoah	Edward M. Conklin
16. Smyth	Walter J. Robinson
17. Southampton	Wesley C. Alexander
18. Tazewell	James L. McDonald
19. Washington	Joe W. Derting
20. Wythe	Benny D. Burkett

Analyses. Calcium and Mg content was determined by using a Perkin Elmer 403 Atomic Absorption Spectrophotometer. Samples were prepared for analysis by wet ashing duplicate .2g samples of the mineral supplement in 5 ml of concentrated HNO₃ followed by refluxing for 30 min in 10 ml of 70% HClO₄. Samples were then evaporated to dryness and redissolved thoroughly in 5 ml concentrate HCl while heating. Samples were quantitatively transferred to volumetric flasks with distilled water and diluted to volume with distilled water. Standard and sample dilutions for analysis were prepared with 1% (w/v) LaCl₃. Particle size information was obtained by using standard sieves and an automatic shaker. Particle size distribution was calculated as the percent of the total sample weight retained on each screen. Screen sizes used were 53, 75, 106, 150, 250 and 425 μ . The rate of reactivity of the limestone was determined by pH-stat titrations as described in Chapter 3.

Results and Discussion

Limestone samples were received from 10 of the 20 counties surveyed. Results of the sample analysis are presented in Table 22. All of the samples analyzed for Ca content were calcitic limestones. Calcium content averaged 35.6%. The sample received from Tazwell county

Table 22. ANALYSIS OF FEED GRADE LIMESTONE SAMPLES OBTAINED FROM VARIOUS COUNTIES WITHIN THE STATE OF VIRGINIA

County	Source	Particle size, μ							Pan	Reactivity ^a T ₅₀	Ca% Mg%	
		425	250	150	106	75	53	53				
		-----% of Total-----							-----sec-----			
Augusta	W.S. Frey, Clearbrook, VA	.4	.8	6.0	12.5	10.3	9.8	8.0	60.0	42	36.6	.42
Frederick	Producer	0	.5	3.4	6.9	6.9	7.8	74.5	84	84	37.4	.24
Frederick	Producer	45.9	25.6	17.3	6.5	1.6	.7	2.5	908			
Guiles	Gold Bond Building Products, Kimballton, VA	0	0	0	3.5	10.8	13.2	72.0	38	38	35.7	.40
Halifax	Gold Bond Building Products, Kimballton, VA	0	0	0	2.6	10.5	15.9	70.0	34	34	37.5	.29
Isle of Wright	Gold Bond Building Products, Kimballton, VA	0	0	0	2.5	11.1	15.2	70.5	60	60	35.7	.41
Montgomery	Gold Bond Building Products, Kimballton, VA	0	0	0	2.9	9.8	15.3	70.7			35.4	.24
Shenandoah	W.S. Frey, Clearbrook, VA	.1	.6	5.0	11.0	9.5	9.0	64.4	38	38	38.1	.33
Southampton	Southern Materials Corp., Ocala, FL	39.5	25.9	18.5	5.4	1.2	.8	8.5	472	472	36.1	.18
Tazewell	Coal Industry Services Co., Founding Mill, VA	1.3	.8	5.9	12.0	11.2	11.5	57.2			30.0	3.00
Washington	Gold Bond Building Products, Kimballton, VA	0	0	0	3.4	11.3	14.6	70.0	28	28	33.9	.52

^aRate of reactivity determined at pH 2.7 and 37° C.

contained 3.0% Mg. Magnesium content ranged only .18 to .52% for the other limestones. One sample was not analyzed for Ca and Mg content because of an insufficient quantity of sample.

Ground limestone must contain at least 33% Ca and it usually ranges between 33-37% Ca. Dolomitic limestone averages about 23% Ca and must contain at least 10% Mg. Chemical impurities affect the quality of the limestone. Silica and Al_2O_3 are the most common impurities and when combined usually equal 85 to 95% of the total impurities (Boynton, 1966).

The source for five of the limestone samples received was the Gold Bond Building Products in Kimballton, VA. This company is a division of the National Gypsum Company and manufactures a very finely ground, highly reactive limestone. Thirty percent of the particles were between 106 and 53 μ in diameter while the remaining 70% were less than 53 μ in diameter. The T50 rate of reactivity for this limestone averaged 40 seconds in comparison to 30 seconds for reagent grade $CaCO_3$. Two limestone samples received were similar in particle size distribution and rate of reactivity to the Gold Bond limestone, but were manufactured by the W. S. Frey Co. in Clearbrook, Va. Two of the limestone samples received were very coarsely ground and very slow in reactivity.

The coarse sample received from Southhampton County was a limestone manufactured in Ocala, Fl. Both coarse limestones had similar particle size distributions, but were extremely different in rate of reactivity. Forty-three percent of the particles were 425 μ in size, 43% of the particles were between 150 and 250 μ in diameter and the remaining 14% were 106 μ or smaller. The rates of reactivity varied about 12 and 23 times slower than values observed for the fast reacting limestones. From the data available it is not known if the slowest reacting, coarse limestone actually had a higher Mg content. Magnesium in limestone causes a tighter crystalline structure which increases hardness and reduces reactivity to stomach acid (Noller and White, 1979). The crystal structure and hardness of the limestone can also vary due to petrological type and contamination from Fe and other heavy metals (Boynton, 1966). These factors affect reactivity and likely affect bioavailability of the Ca and Mg in limestone. However, Mg is the major deterrent to mineral availability in limestone (Axe, 1988).

The limestone samples received in this survey study, though limited in number, may be fairly representative of the kind of feed grade limestones used by feed mills and livestock producers throughout the state. Samples were

received from counties well distributed throughout the state. About 80% of the samples received were finely ground and fast reacting limestones.

Literature Cited

- Agricultural Lime Law, 1974. Rules and regulations for the enforcement of Virginia Agricultural Liming Materials law of 1974. Promulgated pursuant to section 3.1-126.12 of the Code of Virginia, 1950.
- Axe, D. 1988. Limestone: The not so generic calcium source. *Feedstuffs* 60:16.
- Boynton, R. S. 1966. Chemistry and technology of lime and limestone. (1st Ed.). John Wiley and Sons, Inc., New York.
- Ferriera, J. J., C. H. Noller, R. B. Keyser and J. L. White. 1979. Particle size and reactivity of feed grade limestone. *J. Anim. Sci.* 49(Suppl.1):367 (Abstr.).
- Hillman, R.I., M.C. Pritzl and E.W. Kienholz. 1976. Effect of limestone particle size upon calcium bioavailability to poults. *Poult. Sci.* 55:2485.
- Noller, C. H. and J. L. White. 1979. Analytical techniques for evaluating reactivity of limestone. Purdue Univ., Dept. of Anim. Sci.
- Reid, B. L. and C. W. Weber. 1976. Calcium availability and trace mineral composition of feed grade calcium supplements. *Poult. Sci.* 55:600.

Chapter 6

General Discussion

As previously mentioned, there are several factors that can affect the action of buffers or acid-neutralizing compounds within the body of the ruminant. These include differences in feed processing, concentrate or roughage level in the diet, buffering capacity of feed ingredients, and dietary level and(or) reactivity of the buffering or alkalizing agent. In the experiments reported here, dietary concentrate was maximized so as to maximize acid production within the gastrointestinal tract and minimize the buffering capacity of the diet exclusive of the mineral compounds added as buffer treatments. Effort was made to create the type of environment that can exist within the digestive tract of feedlot cattle. Typical cattle fattening rations contain 80-100% concentrate. Roughage is considered expensive to feed and is used very sparingly. A cheaper roughage source such as cottonseed hulls is preferred to harvested forages. Level of dietary intake, and the dietary level and(or) particle size of the mineral compounds seemed to be the predominating factors influencing the action of the mineral acid-neutralizers in the animal experiments described in this report.

Rumen pH was increased by 3% limestone in the lamb metabolism trial where animals were limit-fed a 90% concentrate diet, but feeding 3% limestone in the lamb feeding trial where sheep received an all-concentrate diet ad libitum did not affect the pH of the rumen. Rumen pH averaged 5.68 for the control lambs in the metabolism trial and averaged only 5.32 in the feeder lambs fed 1% limestone. In lambs fed the 90% concentrate diet, the hay fraction in the diet may have provided some benefit towards maintaining a higher pH in the rumen by increasing chewing time and saliva production, but the difference in intake between the two studies was probably the most important factor that created the difference in rumen pH between these two studies. In the feeding trial, acid production from fermentation within the rumen was probably great enough to overwhelm any benefit coming from the limestone treatment.

Magnesium oxide is generally more effective than reagent grade CaCO_3 or limestone in raising rumen pH (Schaefer et al., 1982; Chalupa and Kronfeld, 1983). It is often used in production rations for dairy cows to help maintain normal milk fat percentages because of its capacity to increase rumen pH and increase the acetate to propionate ratio within the rumen (Erdman et al., 1980; Erdman et al., 1982). Its effectiveness as an alkalizer

within the rumen seems to be dependent primarily on differences in particle size (Jesse et al., 1981). The larger particle size of MgO used in the lamb metabolism trial reduced its capacity to compete with limestone in altering rumen pH and DM digestibility. In this study, the finely ground limestone increased rumen pH and rumen DM digestibility while MgO had little affect on the rumen environment.

In both the lamb and cattle metabolism studies, a trend was observed for the mineral treatments to increase duodenal pH. Differences, however, were not statistically significant. Also, when MgO was fed at only .5% of the diet, as in the cattle study, duodenal pH averaged nearly the same as in the control animals. The increases in duodenal pH observed in the present research were not accompanied by any improvements in digestion of dry matter, starch or protein in the small intestine. These results were not surprising, considering the variability in results reported by other researchers who have studied the capacity of a high dietary level of limestone to affect the small intestinal environment. Zinn and Owens (1980a) increased duodenal pH in steers fed an 80% concentrate diet by feeding 2.5% limestone, but organic matter and starch digestion in the small intestine were not improved. Laudert and Matsushima

(1982) reported no change in duodenal pH, but saw an increase in starch digestion in the small intestine by increasing limestone from 1 to 2% of the diet DM. Based on available information, it is unclear as to why small intestinal digestion of dry matter or starch is sometimes improved by feeding high levels of limestone, but it does not appear that simple changes in duodenal pH is a valid explanation (Owens et al., 1983).

An area of gastrointestinal physiology that perhaps needs further research attention is the relationship of total acid load on the intestinal tract verses the intestine's ability to neutralize that acid. The ability of the intestinal tract to neutralize the acid that is imposed on it is a combination of the concentration and rate of acid actually imposed on the intestinal tract verses the concentration and the rate of buffer secreted by the intestinal buffering systems. In the small intestine, the primary source of acid neutralizing capacity comes from pancreatic, biliary and mucosal secretions. Ileal digesta becomes quite heavily saturated with carbon dioxide as a result of ample secretion of bicarbonate. The large intestine controls pH by absorbing acids produced by fermentation and by neutralizing them with secretions from the mucosal lining. Acid production by the abomasum and the flow of

acidic digesta into the duodenum of sheep has been studied rather extensively, but these studies were not conducted from the perspective of the sheep's ability to control pH within the intestinal tract under different feeding conditions. Acid flow and intestinal buffering in cattle has received little attention. A few studies with cattle fed a high-concentrate diet, including the one presented in this report, indicate that feeding buffers or alkalizing agents may at times actually serve to increase rather than decrease acid flow into the duodenum. Teh et al.(1985a) observed a trend for MgO and limestone to increase duodenal titratable acidity in steers full-fed a 60:40 concentrate to orchardgrass hay diet. Sodium bicarbonate added a 1% of the diet DM actually increased duodenal acid concentrations. In the cattle study described in this report, limestone and MgO tended to reduce duodenal acid concentrations. However, when liquid flow and acid concentrations were combined to calculate acid flow into the duodenum, feeding limestone and MgO actually resulted in a greater flow of acid into the duodenum when compared to the control.

Monitoring pH changes along the intestinal tract is difficult. There have been no studies conducted that have looked at the ability of limestone or any other "postruminal" buffer or alkalizing compound to effect pH

changes in the midregions of the small intestine or in the large bowel. Cannulation studies have documented the slow rise in pH in the small intestine of the ruminant (Lennox and Garton, 1968; Ben-Ghedalia, 1974), but research has not been conducted to study how well the small intestine in the ruminant can regulate pH under different feeding regimes. What happens to small intestinal pH when cattle are undergoing a diet transition in the feedlot? How much variation exists between animals? What differences exist between breeds of cattle?

Zinn and Owens (1980a) observed that when pH of the digesta was adjusted down one full pH unit in steers, the small intestine was able to increase pH of the digesta well within the normal basic range by the time it reached the lower region of the ileum. In fact, an over correction was made in neutralization so an increase in acidity at the duodenum actually resulted in a higher pH at the ileum. This information seems to suggest that buffering in the small intestine is adequate to handle a substantial acid load, but this study did not indicate how long the rise in pH might have been delayed by the additional acid load or what implications the additional acid load had on digestive or absorptive efficiency within the small bowel.

The optimum pH range for starch hydrolysis is 6.4 to 7.2 (Zinn and Owens, 1980a) and the optimal pH range for most intestinal proteases is 7 to 8 (Bergen, 1978). Duodenal pH is too low for normal activity of intestinal enzymes and pH in the lower portions of the ileum tend to be on the high end for effective starch hydrolysis. Mineral additives that increase pH in the small intestine could improve the activity of digestive enzymes in the upper regions of the small intestine, but feasibly could reduce activity of enzymes in the ileum. The finely ground limestone used in the sheep metabolism trial tended to increase pH at the ileum, but differences were not significant. In cattle fed limestone at 1.28% of the diet, pH at the terminal ileum was not different from the control animals. Magnesium oxide was very effective in increasing ileal pH. Ileal pH was increased over one full pH unit to 8.23 in lambs fed 1.5% MgO. This pH was well beyond the optimum range for starch hydrolysis and marginal for protein hydrolysis. Magnesium oxide fed at .5% also increased pH beyond the optimum range for starch hydrolysis at the terminal ileum in cattle to 7.65.

It seems clear from the data presented in this publication that fecal pH is influenced very heavily by the quantity of fermentable constituents reaching the large bowel as well as the type and level of minerals

provided in the diet. Data do not support previous assumptions that fecal pH is a good indicator of pH in the lower ileum (Wheeler and Noller, 1976). All the mineral treatments increased fecal pH over that observed in the control animals. The feces is the main route of excretion of excess Ca and P. Fecal pH has been shown to increase linearly with the level of Ca in the diet (Krause and Britton, 1979; Haalan and Tyrrell, 1982; Haaland et al., 1982; Goetsch and Owens, 1985). Excretion of excess minerals into the lower tract is probably a very important factor affecting fecal pH when high levels of limestone are fed, but undissolved mineral residues may likely be the main contributor to an increase in fecal pH. Fecal pH in the cattle averaged below pH 7.0, but pH at the terminal ileum averaged 7.57. In the lambs, the pattern was much different. Fecal pH averaged 8.47 compared to an average pH at the ileum of 7.78. A difference in intake was likely the major factor that created the difference in fecal pH observed between the cattle and sheep. When more starch enters the digestive tract more starch is likely to pass into the large intestine for fermentation. However, other factors such as corn processing and rumen fermentation of starch play an important role in determining the quantity of starch reaching the large intestine in the cattle.

Cracking, rolling and flaking corn grain increases starch digestibility in the rumen of cattle (Thuerer, 1986). Large intestinal pH is generally lowest in cattle fed whole corn because of a greater amount of undigested starch reaching the large intestine for fermentation (Owens, 1986).

It seems apparent from the few studies that have been conducted to investigate the effect of limestone particle size on the performance of feedlot cattle that factors such as chemical composition and crystallinity may be more important than particle size when it comes to reactivity or buffering capacity of limestone. Supplementation of high-concentrate diets with limestone separated into groups that did or did not pass through a 600 μ sieve, showed no difference in weight gains of steers fed diets supplemented with those limestones (Matsushima et al., 1955). Similarly, Williams et al. (1981) found that adding limestones of various particle sizes and reactivities to diets containing 40 or 15% cottonseed hulls did not affect weight gain, feed efficiency or N retention of feedlot steers. However, contrary to the results published by these authors, Brink et al. (1984) observed greater gains in steers fed a fine limestone over those fed a limestone with a larger particle size. Feed conversion for the fine and coarse

limestones were 7.57 and 7.79, respectively. Results from the lamb feeding trial described in the present report seem supportive of the idea that particle size may not be that important to limestone reactivity in the feedlot animal, however, the coarser particle size did result in an improvement in feed efficiency. There was little difference between the limestones in their effect on rumen pH or total VFA concentrations in the lambs. This was unexpected considering the extreme difference between the limestones in particle size and rate of reactivity. Intake level and the extra low rumen pH observed in the feeder lambs were probably sufficient to eliminate any differences that may have occurred in the rumen parameters even though these limestones were very different in particle size. The improvement in feed efficiency appears to be somewhat paradoxical to what was seen in the rumen data and is opposite to what Brink et al. (1984) observed in cattle. A plausible explanation for this is not known.

In the present research, the mineral treatments used in the sheep and cattle sampling studies showed the capacity to influence rumen fermentation when added to a high-concentrate diet. A high dietary level of limestone increased rumen pH and the pre-intestinal disappearance of DM in lambs limit-fed a 90% concentrate diet. Cattle

fed DRP-R also showed a greater DM digestion in the stomach region. In the cattle study, all the mineral treatments tended to provide an advantage for DM and starch digestion within the rumen. Differences in nutrient digestibility in the small intestine in both the sheep and cattle studies were closely related to digestion differences in the rumen rather than to any special action of the mineral treatments on postruminal digestion. A greater nutrient digestibility in the rumen was associated with a lower digestibility of the nutrient in the small intestine and visa versa. Post-ruminal digestion seemed to compensate for differences in rumen digestibility so total tract digestibility of DM, starch and crude protein were similar among the experimental diets. Zinn and Owens (1980b) reported an increase in rumen digestion of starch and organic matter, but a decrease in the intestinal digestion of these nutrients and little change in total tract digestibility in steers fed a 72% rolled corn basal diet supplemented with either 2.5% CaCO₃ or 2.5% CaCO₃ + 1% NaHCO₃. The reasons behind specific improvements in postruminal digestion by feeding a high-dietary level of a mineral compound such as limestone have not been determined. However, based on current experimental data, either published or presented in this report, it seems unlikely that a capacity for

these minerals to increase small intestinal pH or reduce acid flow into the duodenum are valid reasons for these changes in small intestinal digestion.

The primary interest in the sampling studies was to determine the effect of feeding a high dietary level of a mineral buffer or alkalizer on protein digestion and apparent absorption of amino acids in the small intestine. Previous studies by other researchers have focused on the effect of buffers or alkalizing agents on starch digestion, but little attention has been given the effect of buffering compounds on protein nutrition in the ruminant. Data presented in this dissertation demonstrated that limestone and MgO can affect protein digestibility and amino acid partial digestibility when fed at a high dietary level. A restricted intake and the use of a highly processed limestone increased rumen dry matter digestibility and reduced the flow of amino acids reaching the small intestine in sheep. This in turn reduced the partial digestibility of amino acids in the small intestine. Action of buffers or alkalizers in the rumen can increase pH and improve microbial fermentation of feed fiber, starch and protein. In lambs limit-fed the 90% concentrate diet, microbial synthesis of protein apparently was not good enough to compensate for the loss of protein to fermentation. Nitrogen absorption was

increased by limestone feeding, but nitrogen retention was not improved. Serum urea levels seem to indicate a loss of protein to fermentation. In the case of the lambs that were limit-fed the high-concentrate diet, the use of a limestone buffer appeared to be counter productive since it reduced the flow of protein to the small intestine and concomitantly reduced apparent absorption of amino acids in the small intestine.

In contrast to results obtained in the sheep metabolism trial, the mineral treatments used in the cattle study tended to enhance amino acid flow to the small intestine and improve the partial digestibility of amino acids. Compared to the control, there was a trend for DRP-R, DRP-C and limestone to increase the flow of most amino acids into the duodenum of the cattle. Adding DRP-C and limestone to the diet seemed to result in the greatest changes. Numerically, the quantity of histidine, lysine, phenylalanine and threonine reaching the small intestine averaged 15, 6, 9 and 10% greater, respectively, in cattle fed DRP-C and limestone than in control animals. Together with methionine, these essential amino acids are thought to be important limiting amino acids in the diets of sheep and cattle (Owens and Bergen, 1983). In the present study, the quantity of methionine reaching the duodenum was not

affected by the mineral treatments. Numerically, greater negative rumen CP digestion coefficients were determined for cattle fed DRP-C and limestone. This indicated a trend for a greater amount of protein reaching the duodenum in these cattle and was also consistent with the trend observed among the amino acid flow data for DRP-C and limestone.

In contrast to the sheep metabolism trial, differences in amino acid partial digestibility among the treatments in the cattle trial did not necessarily follow the trend for the flow of amino acids into the duodenum in this study. This seemed to suggest that factors other than the difference in the quantity of amino acids entering the small intestine were involved in influencing amino acid partial digestibility. Compared to DRP-C and limestone, DRP-R seemed less effective in increasing amino acid flow to the duodenum, but predominated over DRP-C in increasing amino acid partial digestibility in the small intestine. Numerically, DRP-R and limestone increased the partial digestibility of nearly all amino acids. Feeding DRP-R significantly increased the partial digestibility of all amino acids except arginine, histidine, leucine, alanine and cysteine. Limestone improved the partial digestibility of all amino acids except arginine, threonine, alanine, cysteine, glycine

and serine. In keeping with the trend for an increased flow of amino acids into the duodenum observed for DRP-C, DRP-C also tended to increase amino acid partial digestibility, but statistically, coefficients for DRP-C were not different from those of the control.

When considering the effect the mineral treatments had on amino acid flow and partial digestibility in the cattle study, it seems logical that the improvement in performance seen occasionally in feedlot cattle fed a high level of limestone could be contributed, at least in part, to an improvement in the protein nutrition of these animals. However, caution must also be emphasized when making this point. In the cattle study, rumen DM digestibility was quite low. If rumen DM digestibility had been higher, it is a good possibility the effect of the mineral treatments on protein flow would not have been manifest. The treatment effects in this study, particularly on protein flow, seemed rather small. Had rumen fermentation been increased, microbial protein supply to the duodenum would likely have been high enough in all animals to over shadow the small differences observed in protein flow. This would likely have altered partial digestibility as well. Further research is recommended to study dietary factors affecting rumen pH and rumen liquid turnover and their relationship to the

type and quantity of protein reaching the duodenum and their impact on amino acid absorption in feedlot cattle.

APPENDIX

Feed Preparation

Concentrate Mix For The Lamb Digestion and Metabolism

Trial. Chopped hay was prepared by chopping orchardgrass hay through a 2.5 cm screen. Hay was chopped and mixed in the university's feed mill and vertical mixer. Enough hay was prepared for the entire experiment. The complete concentrate portions for each diet (basal or basal + treatment) for each trial were prepared in 120 kg batches in a small horizontal feed mixer. Dry ingredients were first added to the mixer and mixed for 5 min. The wet molasses was then added and all ingredients mixed for 13 min. After 10 min of mixing, half the concentrate mix was emptied from the bottom of the mixer and added again through the top opening and all ingredients mixed for an additional 3 min. The concentrate mix for each diet for the entire study was stored in two plastic lined metal cans with lids. The first half of the concentrate mix to empty from the mixer was fed during the collection and sampling periods. This was done on the assumption that it was the most uniformly mixed since some sifting probably occurred as feed was emptied from the mixer.

Pellet Preparation For The Lamb Feeding Trial. The treatment supplements were pelleted in 909 kg batches at the Big Spring Mill in Elliston, Virginia. A 250 kg mineral and vitamin premix for each supplement was

prepared beforehand in a horizontal mixer at the Virginia Tech Swine Center. Each premix contained the complete ground corn, mineral and vitamin fraction for each of the supplements to be pelleted.

In preparing the premix, the ground corn was added to the mixer first followed by the limestone, sodium monophosphate and ammonium chloride additives. These ingredients were mixed for 3 min. The mixer was turned off and the zinc oxide, sodium selenite and vitamins were added to the mixer by mixing some of the premix from the mixer with the additive and sprinkling it uniformly over the corn mineral mix. All ingredients were then mixed for 10 min.

At the feed mill, the soybean meal and molasses fractions of the supplement were added to the batch mixer first and mixed thoroughly. Then the mineral mix was added and all ingredients thoroughly mixed for 10 min. The pelletizing die was cleaned initially with oats before pelletizing began and again each time a new diet supplement was processed. The first 10-15 kg of pellets produced for each supplement mix was discarded to avoid diet contamination from feed residues remaining in the feed processing equipment. Pellets were placed in new 45.5 kg nylon feed sacks at the mill and stored in the university mill storage shed until needed for the trial.

For storage at the barn, corn grain and each supplement was placed in a metal drum with a lid and plastic liner.

Pellet Preparation For The Beef Cattle Trial. Feed pellets were manufactured by the Big Spring Mill in Elliston, Virginia. The ingredients for each diet pellet were mixed prior to going to the mill in a 227 kg capacity horizontal mixer at the Virginia Tech Swine Center. Three 227 kg batches of a transition supplement (the same as the control supplement except the external markers were replaced with .55% sand) plus 227 kg each of the treatment supplements were prepared at the Swine Center. Prior to mixing the pellet ingredients at the Swine Center, a mineral premix was prepared in a small mortar mixer at the Animal Sciences Building. The mineral premix contained the ground corn fraction in the pellet plus all the mineral and vitamin additives. The premix was prepared by weighing out the corn and dietary minerals into the mixer first. The corn was placed in the mixer first followed by the urea and other mineral supplements. Care was taken to make sure that all the mineral compounds were placed on top of the corn so they would be blended directly into the corn carrier. The mixer was turned on and let mix for 1 min. After 1 min, with the mixer still running, the external markers were added slowly and uniformly onto the ingredients as they

were blended. After the markers were added, all ingredients were mixed for 10 min. The mixer was cleaned thoroughly between each treatment mix with a brush and vacuum.

At the Swine Center, pellet ingredients were prepared by first mixing the wheat flour, corn gluten meal, meat meal and beet pulp for 3 min. With the mixer off, the premix was poured uniformly over the ingredients in the mixer. All ingredients were then mix for 10 min. The horizontal mixer was cleaned thoroughly with a brush and vacuum between each treatment mix.

At the feed mill, pellet premixes were added to the feed processing system very near the pellet mill to minimize feed loss and contamination. The pelletizing die was cleaned initially with oats and again after each diet and the first 10-15 kg of pellets produced for each diet were discarded to prevent diet contamination. Diet pellets were transported from the mill in nylon feed sacks and then placed in metal drums with lids and plastic liners for the animal trial.

Animal Health

Lamb Feeding Trial. On d 16 of the feeding trial, seven lambs exhibited a lack of appetite and lathargy as a result of a bronchial infection and an elevated body temperature. A veterinarian prescribed treating the

infected lambs with 6 cc oxytetracycline per d for four consecutive d and feeding all lambs on the study an expectorant for 3 wk to prevent the development of pneumonia in lambs suffering from the infection.

Beginning on d 17 of the trial, the expectorant Paladide-20 was administered at the rate of 10 g.pen⁻¹.d⁻¹ for 23 d. Ten g of the expectorant were dissolved in 500 ml of tap water and mixed with the a.m. feed each d. For the remainder of the feeding trial, any lambs that were observed coughing excessively and having high fevers were treated with oxytetracycline as previously described. Before the study was completed, 47 out of the 72 lambs on trial were treated with the antibiotic for respiratory infection.

Beef Cattle Digestion Trial. During the trial some difficulty was experienced in keeping the neoprene stopper in the end of the duodenal cannula for some of the cattle. Animal movement caused the loss of the stoppers and if enough duodenal fluid was lost before the stopper was replaced, the animal's shifted into metabolic alkalosis. This resulted in anorexia and reduced water intake. The condition was corrected by drenching with an electrolyte solution that is routinely used by veterinarians at Virginia Tech for the treatment of dairy cows with displaced abomasums.

Analysis of Amino Acids in Feedstuffs, Feces And
Digesta Using Precolumn Phenylisothiocyanate
Derivatization and Liquid Chromotography

6 N HCl. Prepare by adding 500 ml of concentrated HCl to a 1 liter volumetric flask and dilute to volume with deionized water.

Internal Standard Solution (alpha amino-n-butyric acid, AABA, 5.0 μ mol/ml). Prepare by weighing out .2578 g AABA (Mol. wt. 103.1) into a 500 ml volumetric flask and dilute to volume with 0.1 N HCl.

Procedure.

1. Weigh out a sample equivalent to 20 mg protein into 16 X 150 mm Pyrex culture tubes with screw caps. Tubes should be washed with 6 N HCl, rinsed with deionized water and dried prior to being used. Be sure to use screw caps with a Teflon seal.
2. To the contents of each tube add 15 ml 6 N HCl. After adding the acid, the sample should be thoroughly mixed with the acid by vortexing and the tube flushed thoroughly with N₂ and tightly capped. The contents of the tube are more easily mixed if half the acid volume is added to the tube, the tube vortexed to mix the sample with the acid, the remaining portion of the acid added to the tube and the tube capped and inverted to obtain uniform

mixing.

3. Place sample tubes into a metal rack and the tube rack into a glass Pyrex tray and then place sample tray in the autoclave at 132° C for 6 h.
4. Following hydrolysis, cool samples to room temperature and then quantitatively transfer each sample solution into a 25 ml volumetric flask that contains 5 ml of the internal standard solution. Dilute the sample solution to volume with deionized water.
5. Filter 1 ml of the sample solution through a .45 µM filter for analysis. To store the filtered sample hydrolysate, flush the sample vial thoroughly with N₂, cap the vial tightly and place it in the freezer.

Derivatization of Amino Acids with PITC. For information on preparing derivatizing reagents and the procedure for derivatizing sample amino acids, see the Operation section (p. 3-1) in the PICO-TAG Amino Acid Analysis System Operators Manual. A sample volume of 10 µl was derivatized for analysis.

Amino Acid Standard Solution. Prepare an AABA solution containing 2.5 µmol AABA/ml by combining 1 ml of the internal standard solution with 1 ml 0.1 N HCl. Prepare the amino acid standard solution by combining 500 µl of

the AABA solution (2.5 μmol AABA/ml) with 500 μl of a Pierce H Standard.

Analysis of a Casein Standard. Prior to beginning the amino acid analysis on the digesta samples for the beef cattle trial a casein standard was analyzed to test the accuracy of the amino acid analysis procedure. In this study it was of particular interest to see if modifying the redrying procedure would have an effect on the accuracy of quantifying amino acids concentrations in a sample. The treatments included: The use of the recommended 10 μl vol of redrying reagent (1X), the use of 20 μl redrying reagent (2X) or processing the samples through the recommended redrying step twice before derivatizing (1X+1X). Prior to weighing out the casein standard, it was dried overnight at 50° C in a vacuum oven to make sure sample weights were on a dry basis. Standard hydrolyzates were prepared in duplicate for each treatment. Data obtained from the analysis is presented in Appendix Tables 1 and 2.

APPENDIX TABLE 1. AMINO ACID ANALYSIS OF A CASEIN STANDARD

Amino acid	Redrying Method								
	1X			2X			1X+1X		
	mg/g	dif	%dif	mg/g	dif	%dif	mg/g	dif	%dif
Asp	52.1			42.9			39.3		
	27.6	24.5	89	25.6	17.3	69	18.0	21.3	118
Glu	175.6			162.4			152.4		
	155.8	19.8	13	142.6	19.8	14	123.8	28.6	23
Ser	41.0			37.5			34.7		
	40.4	0.5	1	38.4	1.0	2	24.7	10.0	41
Gly	15.9			15.1			14.4		
	16.4	0.5	3	16.2	1.1	7	14.8	0.5	3
His	24.1			21.4			20.8		
	23.8	0.2	1	22.7	1.3	6	21.6	0.8	4
Arg	32.8			27.6			26.6		
	33.5	0.7	2	33.5	5.9	17	22.9	3.8	16
Thr	33.0			30.3			27.6		
	32.1	0.9	3	31.0	0.8	2	23.2	4.3	19
Ala	25.8			24.7			24.0		
	27.9	2.1	8	27.6	2.9	10	30.0	6.0	20
Pro	87.6			87.1			82.0		
	95.1	7.6	8	97.0	10.0	10	95.6	13.6	14
Tyr	47.2			44.6			42.2		
	49.7	2.5	5	48.2	3.6	8	43.2	1.0	2
Val	55.3			52.9			51.6		
	59.8	4.5	7	59.2	6.3	11	59.0	7.4	13
Met	21.8			18.4			17.1		
	22.9	1.1	5	21.5	3.1	14	21.2	4.1	19
Cys	1.9			.8			3.4		
	3.5	1.5	44	1.6	0.8	51	2.8	0.6	20
Ile	45.5			42.9			41.8		
	48.6	3.2	7	47.8	4.9	10	47.9	6.0	13
Leu	77.5			74.1			73.1		
	83.8	6.4	8	82.2	8.0	10	81.3	8.1	10
Phe	44.8			41.6			40.5		
	46.7	1.9	4	46.1	4.5	10	45.1	4.7	10
Lys	64.1			59.5			54.8		
	66.5	2.4	4	61.6	2.1	3	51.8	3.0	6

APPENDIX TABLE 2. A COMPARISON BETWEEN ACTUAL VALUES AND EXPECTED VALUES FOR THE CASEIN STANDARD ANALYZED WITH THE PICO-TAG AMINO ACID ANALYSIS SYSTEM

Amino acid	Actual value	Expected value	dif	%dif
	----- mg/g -----			
Asp	52.1	65.0	12.9	25
Glu	165.7	209.0	43.3	26
Ser	40.7	54.0	13.3	33
Gly	16.2	18.0	1.8	11
His	24.0	26.0	2.0	8
Arg	33.2	36.0	2.8	8
Thr	32.6	38.0	5.4	17
Ala	26.9	26.0	.9	3
Pro	91.4	117.0	25.6	28
Tyr	48.5	53.0	4.5	9
Val	57.6	57.0	.6	1
Met	22.4	26.0	3.6	16
Cys	2.7	4.0	1.3	48
Ile	47.1	48.0	.9	2
Leu	80.7	88.0	7.3	9
Phe	45.8	50.0	4.2	9
Lys	65.3	74.0	8.7	13

Literature Cited

- Agricultural Lime Law. 1974. Rules and regulations for the enforcement of Virginia Agricultural Liming Materials law of 1974. Promulgated pursuant to section 3.1-126.12 of the Code of Virginia, 1950.
- AOAC. 1984. Official Methods of Analysis (14th Ed.). Association of Official Analytical Chemists, Washington, D.C..
- Argenzio, R. A. 1984. Secretory functions of the gastrointestinal tract. In: M. J. Swenson. (Ed.) Dukes' Physiology of Domestic Animals. pp. 290-300. Cornell University Press., London.
- Armentano, L. E. and R. W. Russel. 1985. Method for calculating digesta flow and apparent absorption of nutrients from nonrepresentative samples on digesta. J. Dairy Sci. 68:3067.
- Ash, R. W. 1961. Stimuli influencing the secretion of acid by the abomasum of sheep. J. Physiol. (Lond.) 157:185.
- Ash, R. W. and A. Dobson. 1963. The effect of absorption on the acidity of rumen contents. J. Physiol. (Lond.) 169:39.
- Ash, R. W. and R. N. B. Kay. 1963. Digestive secretions and the flow of food material in the sheep. In: D. P. Cuthbertson (Ed.) Progress in Nutrition and Allied Sciences. pp. 127-140. Oliver and Boyd, Edinburgh.
- Axe, D. 1988. Limestone: The not so generic calcium source. Feedstuffs 60:16.
- Bailey, C. B. 1959. The rate of secretion of mixed saliva in the cow. Proc. Nutr. Soc. 18:13.
- Bailey, C. B. 1961. Saliva secretion and its relation to feeding in cattle. 3. The rate of secretion of mixed saliva in the cow during eating, with an estimate of magnitude of the total daily secretion of mixed saliva. Br. J. Nutr. 15:443.

- Bailey, C. B. and C. C. Balch. 1961a. Saliva secretion and its relation to feeding in cattle. 1. The composition and rate of secretion of parotid saliva in a small steer. *Br. J. Nutr.* 15:371.
- Bailey, C. B. and C. C. Balch. 1961b. Saliva secretion and its relation to feeding in cattle 2. The composition and rate of secretion of mixed saliva in the cow during rest. *Br. J. Nutr.* 15:383.
- Balch, C. C. 1958. Observations on the act of eating in cattle. *Br. J. Nutr.* 12:330.
- Bartley, E. E. 1976. Bovine Saliva: Production and function. In: M. S. Weinberg and A. L. Sheffner (Ed.) *Buffers in Ruminant Physiology and Metabolism* pp. 61-81. Church and Dwight Co., Inc., New York.
- Bell, F. R. 1980. The mechanisms controlling abomasal emptying and secretion. In: Y. Ruckebusch and P. Thivend. (Ed.) *Digestive Physiology and Metabolism*. pp. 81-100. A. V. I. Publishing Company, Inc., Westport.
- Ben-Ghedalia, D., H. Tagari and A. Bondi. 1974. Protein digestion in the intestine of sheep. *Br. J. Nutr.* 31:125.
- Berger, W. G. 1978. Postruminal digestion and absorption of nitrogenous components. *Fed. Proc.* 37:1223.
- Bidlingmeyer, B. A., S. A. Cohen, T. L. Tarvin and B. Frost. 1987. A new rapid, high sensitivity analysis of amino acids in food type samples. *J. Assoc. Official Anal. Chem.* 70:241.
- Boerner, B. J., F. M. Byers, G. T. Schelling, C. E. Coppock and L. W. Greene. 1987a. Trona and sodium bicarbonate in beef cattle diets: Effects on site and extent of digestion. *J. Anim. Sci.* 65:303.
- Boerner, B. J., F. M. Byers, G. T. Schelling, C. E. Coppock and L. W. Greene. 1987b. Trona and sodium bicarbonate in beef cattle diets: Effects on pH and volatile fatty acid concentrations. *J. Anim. Sci.* 65:309.

- Bolton, J. R., A. M. Merritt, G. M. Carlson and W. J. Donawick. 1976. Normal abomasal electromyography and emptying in sheep and the effects of intra-abomasal volatile fatty acid infusion. *Am. J. Vet. Res.* 37:1387.
- Borgstrom, B., A. Dahlgrist, G. Lundh and J. Sjovall. 1957. Intestinal digestion and absorption in the human. *J. Clin. Invest.* 36:1521.
- Boynton, R. S. 1966. *Chemistry and Technology of Lime and Limestone.* (1st Ed.). John Wiley and Sons, Inc. New York.
- Breukink, H. J., and T. de Ruyter. 1976. Abomasal displacement in cattle: Influence of concentrates in the ration on fatty acid concentrations in ruminal, abomasal, and duodenal contents. *Am. J. Vet. Res.* 37:118.
- Brink, D. R., D. A. Turgeon, Jr., D. L. Harmon, R. T. Steele, T. L. Moder and R. A. Britton. 1984. Effects of additional limestone of various types on feedlot performance of beef cattle fed high corn diets differing in processing method and potassium level. *J. Anim. Sci.* 59:791.
- Brink, D. R. and R. T. Steele. 1985. Site and extent of starch and neutral detergent fiber digestion as affected by source of calcium and level of corn. *J. Anim. Sci.* 60:1330.
- Caple, I. W. and T. J. Heath. 1975. Biliary and pancreatic secretions in sheep: Their regulation and roles. In: I. W. McDonald and A. E. I. Warner. (Ed.) *Digestion and Metabolism in the Ruminant* pp. 91-100. Univ. New England Publ. Univ. Armidale, Australia.
- Chalupa, W. and D. S. Kronfeld. 1983. Buffers for Dairy Cattle. *Anim. Nutr. Health* 38:50.
- Christensen, H. N. 1984. Organic ion transport during the seven decades: the amino acids. *Biochem. Biophys. Acta.* 779:255.
- Church, D. C. 1975. *Digestive Physiology and Metabolism of Ruminants Vol. I. Digestive Physiology.* O. & B. Books, Corvallis, OR.

- Coehlo da Silva, J. F., R. C. Seeley, D. J. Thomson, D. E. Beever and D. G. Armstrong. 1972. The effect in sheep of physical form and stage of growth on the sites of digestion of a dried grass. *Br. J. Nutr.* 28:357.
- Conway, E. J. 1958. *Microdiffusion Analysis and Volumetric Error* (4th Ed.). The McMillan Co., New York.
- Coombe, J. B. and R. N. B. Kay. 1965. Passage of digesta through the intestines of the sheep. Retention times in the small and large intestine. *Br. J. Nutr.* 19:325.
- Coulombe, J. J. and L. Favreau. 1963. A new simple semi micro method for colorimetric determination of urea. *Clin. Chem.* 9:102.
- Counotte, G. H. M., A. T. van't Klooster, J. Van der Kuiten and R. A. Prins. 1979. An analysis of the buffer system in the rumen of dairy cattle. *J. Anim. Sci.* 49:1536.
- Davis, C. L. 1979. The use of buffers in the rations of lactating dairy cows. In: W. H. Hale and P. Meinhardt (Ed.) *Regulation of Acid-Base Balance*. pp 51-64. Church and Dwight Co., Inc., Piscataway, NJ.
- DeHaan, K. A., T. J. Klopfenstein, O. A. Turgeon, R. A. Britton and D. R. Brink 1982. Effect of buffers on fiber digestion in vivo. *J. Anim. Sci.* 55(Suppl. 1):416(Abstr.).
- El Tayeb, A. E., M. L. Galyean and H. E. Kiesling: 1984. Influence of limestone level in high-concentrate and high-oughage diets on site and extent of digestion in lambs. *J. Anim. Sci.* 59:217.
- Flam, J. 1983. Physiological basis and the need for buffers in livestock rations. In: *Buffers, Neutralizers and Electrolyte Symposium Handbook*. Natl. Feed Ingrid. Assoc., Des Moines, IA.

- Emerick, R. J. 1976. Buffering acidic and high-concentrate ruminant diets In: M. S. Weinberg and A. L. Sheffner (Ed.) *Buffers in Ruminant Physiology and Metabolism* pp. 127-139. Church & Dwight Company, Inc., New York.
- Emery, R. S. 1976. High energy feeds for milk production. In: M. S. Weinberg and A. S. Sheffner (Ed.) *Buffers in Ruminant Physiology and Metabolism*. pp. 149-159. Church and Dwight Co., Inc., Nutley, NJ.
- Emery, R. S., C. K. Smith, R. M. Grimes, C. 7. Huffman and C. W. Duncan. 1960. Physical and chemical changes in bovine saliva and rumen liquor with different hay-grain rations. *J. Dairy Sci.* 43:76.
- Emery, R. S., L. D. Brown and J. W. Bell. 1965. Correlation of milk fat with dietary and metabolic factors in cows fed restricted-roughage rations supplemented with magnesium oxide or sodium bicarbonate. *J. Dairy Sci.* 48:1647.
- Emmanuel, B., M. J. Lawler and D. M. McAleese. 1969. The rumen buffering system of sheep fed pelleted roughage-concentrate rations. *Br. J. Nutr.* 23:805.
- Erdman, R. A., R. L. Botts and R. W. Hemken. 1980. Effect of dietary-sodium bicarbonate and magnesium-oxide on production and physiology in early lactation. *J. Dairy Sci.* 63:923.
- Erdman, R. A., R. W. Hemken and L. S. Bull. 1982. Dietary sodium bicarbonate and magnesium oxide for early postpartum lactating dairy cows: Effects on production, acid-base metabolism and digestion. *J. Dairy Sci.* 65:712.
- Ferreira, J. J., C. H. Noller and T. S. Stewart. 1980. Influence of dietary calcium and protein on fecal pH, consistency and rate of passage in dairy cattle. *J. Dairy Sci.* 63:1091.
- Ferreira, J. J., C. H. Noller, R. B. Keyser and J. L. White. 1979. Particle size and reactivity of feed grade limestone. *J. Anim. Sci.* 49(Suppl. 1):367(Abstr.).

- Franklin, K. K., J. E. Winch and G. K. MacLeod. 1981. The effect of concentrate on the digestion of bromegrass constituents. *Can. J. Anim. Sci.* 61:935.
- French, N. and J. J. Kennelly. 1985. The effect of frequency of feeding on rumen parameters and on blood insulin concentration in dairy cows. *Can. J. Anim. Sci.* 64:1075.
- Fulton, W. R., T. J. Klopfenstein and R. A. Britton. 1979. Adaptation to high concentrate diets by beef cattle. I. Adaptation to corn and wheat diets. *J. Anim. Sci.* 49:775.
- Goetsch, A. L. and F. N. Owens. 1985. Effects of calcium source and level on site of digestion and calcium levels in the digestive tract of cattle fed high-concentrate diets. *J. Anim. Sci.* 61:995.
- Goetsch, A. L., F. N. Owens, C. E. Kautz and K. W. Milner. 1983. Effect of ruminal pH alteration on digestion in steers fed a high concentrate diet. *Ok. Agric. Exp. Sta. Misc. Publ.* 113:10.
- Grove, R. J., H. W. Harpster, L. D. Muller, and E. H. Cash. 1981. Sodium bicarbonate and magnesium oxide addition to high energy rations for yearling beef steers. 73rd Annual Meeting of the American Society of Animal Science, p. 402 (Abstr.).
- Guerino, F. and C. R. Baumrucker. 1987. Identification of methionine and lysine transport systems in cattle small intestine. *J. Anim. Sci.* 65:630.
- Haaland, G. L. and H. F. Tyrrell. 1982. Effects of limestone and sodium bicarbonate buffers on rumen measurements and rate of passage in cattle. *J. Anim. Sci.* 55:935.
- Haaland, G. L., H. F. Tyrrell, P. W. Moe and W. E. Wheeler. 1982. Effect of crude protein level and limestone buffer in diets fed at two levels of intake on rumen pH, ammonia-nitrogen, buffering capacity and volatile fatty acid concentrations of cattle. *J. Anim. Sci.* 55:943.
- Hale, W. H. 1973. Influence of processing on the utilization of grains (starch) by ruminants. *J. Anim. Sci.* 37:1075.

- Harbers, L. H. 1975. Starch granule structural changes and amyolytic patterns in processed sorghum grain. *J. Anim. Sci.* 41:1496.
- Harrison, D. G., D. E. Beever, D. J. Thomson and D. F. Osbourn. 1973. The influence of diet upon the quantity and types of amino acids entering and leaving the small intestine of sheep. *J. Agric. Sci.* 81:391.
- Harrison, F. A. and K. J. Hill. 1962. Digestive secretions and the flow of digesta along the duodenum of the sheep. *J. Physiol. (Lond.)*. 162:225.
- Hart, S. P. and C. E. Polan. 1984a. Simultaneous extraction and determination of ytterbium and cobalt ethylenediaminetetraacetate complex in feces. *J. Anim. Sci.* 67:888.
- Hart, S. P. and C. E. Polan. 1984b. Effect of sodium bicarbonate and disodium phosphate on animal performance, ruminal metabolism, digestion and rate of passage in ruminating calves. *J. Dairy Sci.* 67:2356.
- Hawkins, G. E. and K. M. Autrey. 1968. Relationship among rate of bovine parotid saliva flow, saliva composition, and ration fed. *J. Dairy Sci.* 51:627(Abstr.).
- Herod, E. L., R. M. Bechtle, E. E. Bartley and A. D. Dayton. 1978. Buffering ability of several compounds in vitro and the effect of a selected buffer combination on ruminal acid production in vivo. *J. Dairy Sci.* 61:1114.
- Hill, F. N. and D. L. Anderson. 1958. Comparison of metabolizable energy and productive energy determinations with growing chicks. *J. Nutr.* 64:587.
- Hill, K. J., D. E. Noakes and R. A. Lowe. 1970. Gastric digestive physiology in the calf and piglet. In: A. T. Phillipson (Ed.). *Physiology of Digestion and Metabolism in the Ruminant*. pp. 166-179. Proceeding of the 3rd International Symposium. Oriel Press. Cambridge, England.

- Hillman, R.I., M.C. Pritzl and E.W. Kienholz. 1976. Effect of limestone particle size upon calcium bioavailability to poult. *Poult. Sci.* 55:2485.
- Hull, B. L. and W. M. Wass. 1973. Causative factors in abomasal displacement. 1: Literature review. *Vet. Med. Sml. Anim. Clin.* 68:283.
- James, L. G. and J. E. Wohlt. 1985. Effect of supplementing equi-valent cation amounts from NaCl, MgO, NaHCO₃ and CaCO₃ on nutrient utilization and acid-base status of growing Dorset lambs fed high concentrate diets. *J. Anim. Sci.* 60:307.
- Jensen, A., E. Jensen, V. Ravn and A. Unmack. 1977. Reactivity of inorganic feed additives. I. Dissolution in vitro at pH 2.7 and 37° C of some oxides, carbonates and phosphates. *Z. Tierphysiol. Tier. Futter.* 38:185.
- Jesse, B. W., J. W. Thomas and R. S. Emergy. 1981. Availability of magnesium from magnesium oxide particles of differing sizes and surfaces. *J. Dairy Sci.* 64:197.
- Kaufmann, W. 1976. Influence of the composition of the ration and the feeding frequency on pH-regulation in the rumen and on feed intake in ruminants. *Livest. Prod. Sci.* 3:103.
- Kay, R. N. B. 1960. The rate of flow and composition of various salivary secretions in sheep and calves. *J. Physiol. (Lond.)* 150:515.
- Kay, R. N. B. 1969. Digestion of protein in the intestines of adult ruminants. *Proc. Nutr. Soc.* 28:140.
- Kay, R. N. B. and P. N. Hobson, 1963. Reviews of progress of dairy science. Part I. The physiology of the rumen. *J. Dairy Res.* 30:261.
- Kellaway, R. C., D. E. Beaver, D. J. Thomson, A. R. Austin, S. B. Cammell and M. L. Elderfield. 1978. The effects of NaCl or NaHCO₃ on digestion in the stomach of weaned calves. *J. Agric. Sci. (Camb.)* 91:497.

- Kellaway, R. C., D. J. Thomson, D. E. Beever and D. F. Osbourn. 1977. Effects of NaCl and NaHCO₃ on food intake, growth rate and acid-base balance in calves. *J. Agric. Sci. (Camb.)* 88:1.
- Keyser, R. B., C. H. Noller, L. J. Wheeler and D. M. Schaefer. 1985. Characterization of limestones and their effects in vitro and in vivo in dairy cattle. *J. Dairy Sci.* 68:1376.
- Koeln, L. L. and J. A. Paterson. 1986. Nitrogen balance and amino acid disappearance from the small intestine in calves fed soybean meal- toasted soybean meal-or corn gluten meal-supplemented diets. *J. Anim. Sci.* 63:1258.
- Krause, V. E. and R. E. Britton. 1979. Tri-calcium phosphate or limestone as buffers in finishing rations for steers. *J. Anim. Sci.* 49(Suppl. 1):384.(Abstr.).
- Kromann, R. P. 1976. Buffering capacity as influenced by dietary physical characteristics. In: M. S. Weinberg (Ed.) *Buffers In Ruminant Physiology and Metabolism.* pp. 107-126. Church and Dwight Company, Inc., New York.
- Kromann, R. P. and J. H. Meyer. 1972. Rumen metabolism in sheep as influenced by interactions among the ration's energy content, physical form and buffers. *J. Anim. Sci.* 34:813.
- Laudert, S. B. and J. K. Matsushima. 1982. Limestone effects on digestion and gastrointestinal tract pH of finishing steers. *J. Anim. Sci.* 55(Suppl. 1):437(Abstr.).
- Lennox, A. M. and G. A. Garton. 1968. The absorption of long-chain fatty acids from the small intestine of the sheep. *Br. J. Nutr.* 22:247.
- Leonard, E. S. and W. J. Croom, Jr. 1985. A simplified method for the analysis of fecal and digesta starch. *J. of Anim. Sci.* 61(Suppl. 1):64.(Abstr.).
- Lu, C. D., N. A. Jorgensen and L. D. Satter. 1988. Site and extent of nutrient digestion in lactating dairy cows fed alfalfa protein concentrate or soybean meal. *J. Dairy Sci.* 71:697.

- Macrae, J. C. and D. G. Armstrong. 1968. Enzyme method for determination of x-linked glucose polymers in biological materials. *J. Sci. Food Agric.* 19:578.
- Magee, D. F. 1961. An investigation into the external secretion of the pancreas in sheep. *J. Physiol. (Lond.)* 158:132.
- Mann, S. O. and E. R. Orskov. 1973. The effect of rumen and post-rumen feeding of carbohydrates on cecal microflora of sheep. *J. Appl. Bact.* 36:475.
- Masson, M. J. and A. T. Phillipson. 1952. The Composition of the digesta leaving the abomasum of sheep. *J. Physiol. (Lond.)* 116:98.
- Matsushima, J., T. W. Dowe, C. L. Comar, S. L. Hansard and W. J. Visek. 1955. The effect of particle size on the utilization of bone meal and limestone by beef cattle. *J. Anim. Sci.* 14:1042.
- McLeay, L. M. and D. A. Titchen. 1970. Abomasal secretory responses to teasing with food and feeding in the sheep. *J. Physiol. (Lond.)* 206:605
- McLeay, L. M. and D. A. Titchen. 1974. Effects of the amount and type of food eaten on secretion from fundic abomasal pouches of sheep. *Br. J. Nutr.* 32:375.
- Mertens, D. R. 1979. Effects of buffers upon fiber digestion. In: W. H. Hale and P. Meinhardt (Ed.) *Regulation of Acid-Base Balance.* pp. 65-75. Church and Dwight Co., Inc., Nutley, NJ.
- Meyer, R. M., J. L. Decker, E. E. Bartley, W. E. Stewart, J. L. Noardsey and D. A. Stiles. 1965. Salivation studies in cattle with bilateral submaxillary and parotid gland cannulas. *J. Dairy Sci.* 48:790.
- Moody, E. G. 1962. Pelleted alfalfa and coastal Bermudagrass as dairy roughages. *J. Dairy Sci.* 45:694(Abstr.).

- Moore, L. A. 1964. Symposium on forage utilization: Nutritive value of forage as affected by physical form. I. General principles involved with ruminants and effect of feeding pelleted or wafered forage to dairy cattle. *J. Anim. Sci.* 23:230.
- Nocek, J. E., D. G. Braund and J. E. English. 1983. Effect of limestone reactivity and percent on production by dairy cows in early lactation. *J. Dairy Sci.* 66:2533.
- Noller, C. H. and J. L. White. 1979. Analytical techniques for evaluating reactivity of limestone. Purdue Univ., Dept. of Anim. Sci.
- Okeke, G. C., J. G. Buchanan-Smith and D. G. Griene. 1983a. Effect of sodium bicarbonate on rate of passage and degradation of soybean meal in postpartum dairy cows. *J. Dairy Sci.* 66:1023.
- Okeke, G. C., J. G. Buchanan-Smith and W. L. Grovum. 1983b. Effects of buffers on ruminal rate of passage and degradation of soybean meal in steers. *J. Anim. Sci.* 56:1391.
- Oltjen, R. R., P. A. Putnam and R. E. Davis. 1965. Salivary and metabolic studies with steers fed pelleted or unpelleted conventional and purified rations. *J. Anim. Sci.* 24:1126.
- Oltjen, R. R., P. A. Putnam, E. E. Williams, Jr. and R. E. Davis. 1966. Wheat verses corn in all-concentrate cattle rations. *J. Anim. Sci.* 25:1000.
- Orskov, E. R. 1982. Protein Nutrition In Ruminants. Academic Press Inc., New York.
- Orskov, E. R., C. Fraser and J. G. Gordon. 1974. Effect of processing of cereals on rumen fermentation, digestibility, rumination time and firmness of subcutaneous fat. *Br. J. Nutr.* 32:59.
- Oser, B. L. 1965. Hawk's Physiological Chemistry. (14th Ed.). McGraw Hill Book Co., New York. p. 511-521.
- Owens, F. N. 1984. Ionophores, buffers and protein for feedlot cattle. Proceedings of the American Association of Bovine Practitioners 16:18-29, Anim. Sci. Dept., Ok. State Univ., Stillwater, Ok.

- Owens, F. N. and W. G. Bergen. 1983. Nitrogen metabolism in ruminants: Historical perspective, current understanding and future implications. *J. Anim. Sci.* 57:498.
- Owens, F. N. and R. A. Zinn. 1982. Calcium nutrition and buffers for feedlot cattle. *Proc. Am. Feed Mfr. Assn.*, p. 1.
- Owens, F. N., A. L. Goetsch, D. C. Weakley and R. A. Zinn. 1983. Buffers and neutralizers in beef cattle. In: *Buffers, neutralizers and electrolyte*. pp. 1-24. *Symposium Handbook*. Natl. Feed Ingrid. Assoc., Des Moines, IA.
- Owens, F. N., R. A. Zinn and Y. K. Kim. 1986. Limits to starch digestion in the ruminant small intestine. *J. Anim. Sci.* 63:1634.
- Peirce, S. B., L. D. Muller and H. W. Harpster. 1983. Influence of sodium bicarbonate and magnesium oxide on digestion and metabolism in yearling beef steers abruptly changed from high forage to high energy diets. *J. Anim. Sci.* 57:1561.
- Phillips, L. L. and M. L. Caldwell. 1951. A study of the purification and properties of a glucose-forming amylase from *Rhizopus delemar*, gluc amylase. *J. Am. Chem. Soc.* 73:3559.
- Phillips, W. A. 1974. In vitro absorption of valine, threonine and methionine by the small intestine of sheep. M. S. Thesis. Virginia Polytechnic Institute and State University, Blacksburg.
- Phillips, W. A. 1976. Characteristics of threonine, valine and methionine absorption in the jejunum and ileum of sheep. Ph. D. Dissertation. Virginia Polytechnic Institute and State University, Blacksburg.
- Phillipson, A. T. 1952. The passage of digesta from the abomasum of sheep. *J. Physiol. (Lond.)*. 116:84.
- Pond, W. G., J. T. Yen, W. E. Wheeler and D. A. Hill. 1982. Calcium bioavailability from limestones of differing particle size and rate of reactivity for growing nonruminants. *Nutr. Rep. Int.* 26:1027.

- Poutiainen, E. 1966. The production of saliva in the fluid flowing through the reticulorumen of the cow. *Ann. Agr. Fenn.* 5:342.
- Price, S. G., L. D. Satter and N. A. Jorgensen. 1988. Dehydrated alfalfa in dairy cow diets. *J. Dairy Sci.* 71:727.
- Prigge, E. C. and J. J. Svonavec. 1984. Flow and acidity of digesta entering the duodenum as influenced by buffers and time after feeding in wethers. *Can. J. Anim. Sci.* 64:1055.
- Putnam, P.A. and R.E. Davis. 1963. Feeding pelleted rations to beef cattle. Production and Research Report No. 69. U.S.D.A.
- Putnam, P. A., R. Lehmann and R. E. Davis. 1966. Feed intake and salivary secretion by steers. *J. Anim. Sci.* 25:817.
- Putnam, P. A., D. A. Yarns and R. E. Davis. 1966. Effect of pelleting rations and hay: grain ratio on salivary secretion and ruminal characteristics of steers. *J. Anim. Sci.* 25:1176.
- Raabo E. and T. C. Terkildse. 1960. On the enzymatic determination of blood glucose. *Scand. J. Clin. Lab Invest.* 12:402.
- Reid, B. L. and C. W. Weber. 1976. Calcium availability and trace mineral composition of feed grade calcium supplements. *Poult. Sci.* 55:600.
- Rodrigues C. B. and N. N. Allen. 1960. The effect of fine grinding of hay on ration digestibility, rate of passage and fat content of milk. *Can. J. Anim. Sci.* 40:23.
- Rogers, J. A. and C. L. Davis. 1982. Effects of intraruminal infusions of mineral salts on volatile fatty acid production in steers fed high-grain and high-roughage diets. *J. Dairy Sci.* 65:953.
- Rogers, J. A., C. L. Davis and J. H. Clark. 1982. Alteration of rumen fermentation, milk fat synthesis and nutrient utilization with mineral salts in dairy cows. *J. Dairy Sci.* 65:577.

- Rooney, L. W. and R. L. Pflugfelder. 1986. Factors affecting starch digestibility with special emphasis on sorghum and corn. *J. Anim.* 63:1607.
- Round, M. H. 1984. Buffers in pelleted diets for exported line wethers. *Proc. Aust. Soc. Anim. Prod.* 15:746.
- Rouse, G. 1978. The effect of calcium source and level on the performance of yearling steers fed high-grain diets. A. S. Leaflet R 271. Iowa State Univ. Agric. and Home Ec. Exp. Sta. Report. AS-482.
- Rumsey, T. S., P. A. Putnam, J. Bond and R. R. Oltjen. 1970. Influence of level and type of diet on ruminal pH and VFA, respiratory rate and EKG patterns of steers. *J. Anim. Sci.* 31:608.
- Russel, J. R., A. W. Young and N. A. Jorgensen. 1981. Effect of dietary corn starch intake on ruminal, small intestinal and large intestinal starch digestion in cattle. *J. Anim. Sci.* 52:1170.
- Rust, S. R. and F. N. Owens. 1982. Effect of limestone on digestibility of feedlot diets. *Ok. Agric. Exp. Sta. Misc. Publ.* 112:154.
- Schaefer, D. M., C. H. Noller, R. B. Keyser and J. L. White. 1982. Neutralization of acid in the rumen by managesium oxide and magnesium carbonate. *J. Dairy Sci.* 65:732.
- Schneider, B. H. and W. P. Flatt. 1975. The Evaluation of Feeds Through Digestibility Experiments. The Univ. of Georgia Press, Athens.
- Spicer, L., B. Theurer and M. C. Young. 1983. Ruminal and post-ruminal utilization of protein from feed grains by beef steers. *J. Anim. Sci.* 57(Suppl. 1):470(Abstr.).
- Spicer, L., J. Sowe and B. Theuer. 1982. Starch digestion of sorghum grain, barley and corn based diets by beef steers. *Proc. West. Sec. Amer. Soc. Anim. Sci.* 33:41.
- Sudweeks, E. M. 1977. Chewing time, rumen fermentation and their relationship in steers as affected by diet composition. *J. Anim. Sci.* 44:694.

- Sudweeks, E. M., M. E. McCullough, L. R. Sisk and S. E. Law. 1975. Effects of concentrate type and level of forage on chewing time of steers. *J. Anim. Sci.* 41:219.
- Tagari, H., N. S. Reddy and L. D. Satter. 1982. Effect of magnesium oxide and sodium bicarbonate on the environment and digestion in the rumen and the gastrointestinal tract of lactating cows. *J. Anim. Sci.* 55(Suppl. 1):468(Abstr.)
- Tas, M. V., R. A. Evans and R. F. E. Axford. 1981. The digestibility of amino acids in the small intestine of the sheep. *Br. J. Nutr.* 45:167.
- Taylor, R. B. 1962. Pancreatic secretion in the sheep. *Res. Vet. Sci.* 3:63.
- Teh, T. H., R. W. Hemken and R. J. Harmon. 1985a. Comparison of buffers and their modes in the rumen and abomasum. *Nutr. Rep. Int.* 32:1339.
- Teh, T. H., R. W. Hemken and R. J. Harmon. 1985b. Magnesium oxide interactions with sodium bicarbonate in early lactating cows. *J. Dairy Sci.* 68:881.
- Theurer, C. B. 1986. Grain processing effect on starch utilization by ruminants. *J. Anim. Sci.* 63:1649.
- Thomas, J. W., R. S. Emery, J. K. Breaux and J. S. Liesman. 1984. Response of milking cows fed a high concentrate, low roughage diet plus sodium bicarbonate, magnesium oxide, or magnesium hydroxide. *J. Dairy Sci.* 67:2532.
- Thorne, M. J., L. U. Thompson and D. J. A. Jenkins. 1983. Factors affecting starch digestibility and the glycemic response with special reference to legumes. *Amer. J. Clin. Nutr.* 38:481.
- Tissera, G. H., J. H. Vandersall and R. A. Erdman. 1982. Gastrointestinal effects of limestone in the diet of growing steers. *J. Anim. Sci.* 55 (Suppl. 1):470(Abstr.).
- Topps, J. H., R. N. B. Kay and E. D. Goodall. 1968a. Digestion of concentrate and of hay diets in the stomach and intestine of ruminants. 1. Sheep. *Br. J. Nutr.* 22:261.

- Topps, J. H., R. N. B. Kay and E. D. Goodall. 1968b.
Digestion of concentrate and hay diets in the stomach
and intestine of ruminants. 2. Young steers. Br. J.
Nutr. 22:281.
- Trenkle, A. H. 1979. Sodium bicarbonate in beef
nutrition. National Feed ingredient Association. Des
Moines, Iowa.
- Turgeon, O. A., Jr. D. R. Brink and R. A. Britton. 1983.
Corn Particle size mixtures, roughage level and
starch utilization in finishing steer diets. J. Anim.
Sci. 57:739.
- Turgeon, O. A., Jr., D. R. Brink and R. A. Britton. 1981.
Corn particle size and starch digestion in steers fed
ad libitum. J. Anim. Sci. 52(Suppl. 1):440(Abstr.).
- Turner, A. W. and V. E. Hodgetts. 1955. Buffer systems in
the rumen of the sheep. II. Buffering properties
inrelation to composition. Aust. J. Agric. Res.
6:125.
- Uden, P., P. E. Colucci and P. J. Van Soest. 1980.
Investigation of Chromium, cerium and cobalt as
markers in digesta. Rate of passage studies. J. Sci.
Food Agric. 31:625.
- Ulyatt, M. J., D. W. Dellow, C. S. W. Reid and
T. Bauchop. 1975. Structure and function of the large
intestine of ruminants. In: I. W. McDonald and A. E.
I. Warner (Ed.) Digestion and metabolism in the
Ruminant. pp. 119-133. Univ. New England Publ. Unit.,
Armidale, Australia.
- Van Bruchem, J. and A. T. van't. Klooster. 1980. Effect
of protein on abomasal secretions of acid in sheep.
Br. J. Nutr. 44:307.
- Van Bruchem, J., L. J. G. M. Bongers, J. D. van Walssem,
W. Onck and P. W. M. von Adrichem. 1985. Digestion of
proteins of varying degradability in sheep. 3.
Apparent and true digestibility in the small
intestine and ileal endogenous flow of N and amino
acids. Neth. J. Agric. Sci. 33:285.
- Varner, L. W. and W. Woods. 1972. Calcium levels in high
grain beef cattle rations. J. Anim. Sci. 35:415.

- Wedekind, K., R. B. Muntifering and K. B. Barker. 1986. Effects of diet concentrate level and sodium bicarbonate on site and extent of forage fiber digestion in the gastrointestinal tract of wethers. *J. Anim. Sci.* 62:1388.
- Weinberg, M. S. 1976. Summary and review of the role of buffers in ruminant digestion and physiology. In: M. S. Weinberg (Ed.) *Buffers in Ruminant Physiology and Metabolism*. pp.160-169. Church and Dwight Company, Inc., New York.
- Wheeler, W. E. and C. H. Noller. 1976. Limestone buffers in complete mixed rations for dairy cattle. *J. Dairy Sci.* 59:1788.
- Wheeler, W. E. and C. H. Noller. 1977. Gastrointestinal tract pH and starch in feces of ruminants. *J. Anim. Sci.* 44:131.
- Williams. H. H., L. V. Curtin, J. Abraham, J. K. Loosli and L. A. Maynard. 1954. Estimation of growth requirements for amino acids by assay of the carcass. *J. Biol. Chem.* 208:277.
- Williams. V. J. 1965. Microbial metabolism in the forestomachs and the large intestine of sheep. *Aust. J. Agric. Res.* 16:77.
- Williams, D. L., D. E. Hodge and W. C. Sadler. 1981. The effect of various limestone sources on ration digestibility and feedlot performance of steers. *J. Anim. Sci.* 53(Suppl. 1):121.
- Wiltout, D. W. and L. D. Satter. 1972. Contribution of proprionate to glucose synthesis in the lactating and nonlactating cow. *J. Dairy Sci.* 55:307.
- Wise, M. B., T. N. Blumer, H. B. Craig and E. R. Barrick. 1965. Influence of rumen buffering agents and hay on performance and carcass characteristics of steers fed all-concentrate rations. *J. Anim. Sci.* 24:83.
- Wohlt, J. E., D. E. Ritter and J. L. Evans. 1986. Calcium sources for milk production in holstein cows via changes in dry matter intake, mineral utilization and mineral source buffering potential. *J. Dairy Sci.* 69:2815.

- Woods, W. and R. Luther. 1962. Further observations on the effect of physical preparation of the ration on volatile fatty acid production. *J. Anim. Sci.* 21:809.
- Wright, P. L., A. L. Pope and P. H. Phillips. 1963. Effect of physical form of ration upon digestion and volatile fatty acid production in vivo and in vitro. *J. Anim. Sci.* 22:586.
- Xin, Z. X., R. W. Hemken and W. B. Tucker. 1987. Influence of dietary magnesium oxide reactivity rate on acid-base balance, calcium, metabolism and magnesium bio availability. *J. Dairy Sci.* 70(Suppl. 1):201(Abstr.)
- Yarns, D. A., P. A. Putnam and E. C. Leffel. 1965. Daily salivary secretion by beef steers. *J. Anim. Sci.* 24:173.
- Zinn, R. A. and F. N. Owens. 1980a. Expanding our concept of starch digestion in the ruminant. *Minn. Nutrition Conference.* p. 69.
- Zinn, R. A. and F. N. Owens. 1980b. Sodium, calcium and potassium salts for cattle fed high concentration rations. *Ok. Agric. Exp. Sta. Misc. Publ.* 107:131.
- Zinn, R. A. and F. N. Owens. 1983. Site of protein digestion in steers: predictability. *J. Anim. Sci.* 56:707.
- Zinn, R. A., F. N. Owens, D. R. Gill and D. E. Williams. 1982. Limestone and potassium for feedlot steers. *Ok. Agric. Exp. Sta. Misc. Publ.* 112:158.

**The vita has been removed from
the scanned document**