

REPORT ON

CONFERENCE ON RUMEN FUNCTION

held at

Pick Congress Hotel, Chicago, Illinois

November 30 - December 1, 1977

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TABLE OF CONTENTS

<u>PANEL</u>	<u>Page</u>
<u>PHYSIOPATHOLOGY:</u>	
<u>The Effect of an Experimental Antibiotic and Sodium Bicarbonate on Acidosis Produced by Feeding Wheat to Lambs - W. W. Kezar, et al...</u>	5
<u>The Physical-Chemical Basis of Frothy Bloat - R. E. Howarth, et al.....</u>	6
<u>An Antibiotic Sensitivity Evaluation of Isolated Rumen Organisms from Overloaded Sheep - R. K. Chaplin, et al.....</u>	6-7
<u>Reduction of Ruminal 3-Methylindole Concentration as a Possible Means of Prevention of Acute Bovine Pulmonary Edema and Emphysema - J. R. Carlson, et al.....</u>	7
<u>Physiological and Pathological Responses of Cattle to Rumen Bacterial Endotoxins - T. G. Nagaraja, et al.....</u>	8
<u>Pneumonia: Blood Gas, Radiological and Pathological Studies - J. G. Vestweber, et al.....</u>	8
<u>A Technique for Repeated Prolonged Rumen Infusion without Establishing an Open Fistula - J. D. Olsen</u>	9
<u>NUTRITION:</u>	
<u>Effects of Protein Source and Concentrate in the Ration on Microbial Protein Production In Vitro - J. E. Edwards, et al.....</u>	9-10
<u>Amines in Ruminal Fluid and Blood of Acidotic Ruminants - G. E. Mitchell, Jr, et al.....</u>	10
<u>Influence of Ration on Health and Reproduction of the Periparturient Dairy Cow - Claudie N. Zamet, et al.....</u>	10-11
<u>The Imbalance of Acid and Base Forming Constituents in the Rumen of the Mature Bovine as a Possible Cause of Hypomagnesemia - H. Fenner, et al.....</u>	11-12

<u>Prospective Usages of Soluble Silicates in Ruminant Nutrition: Interactions with Dietary Heavy Metals and Trace Elements -</u> G. Stanley Smith, et al.....	12
<u>Influence of Dietary Nickel on Urease Activity -</u> J. W. Spears, et al....	13
<u>Forage Sulfur and its Influence on Ruminant Nutrient Utilization -</u> D. G. Ely, et al.....	14
<u>The Fraction of Dietary Lactose that Becomes Available for Fermentation in the Large Intestine in Non-Ruminants -</u> N. J. Benevenga, et al.....	14-15
<u>The "Difference" Approach for Estimating Ruminant Protein Degradation -</u> R. A. Zinn, et al.....	15
<u>Effects of Varying Solids Retention Times on Fermentation in Continuous Culture -</u> W. H. Hoover	15-16
<u>Effects of Pentachlorophenol on Rumen Fermentation -</u> L. R. Shull, et al.....	16-17
<u>Long Chain Fatty Acids for Milk Fat Synthesis -</u> D. L. Palmquist	17-18
<u>The Use of Anhydrous Ammonia for Improving the Nutritional Value of Straw -</u> R. K. Chaplin, et al.....	18
<u>A Kinetic Model of Fiber Digestion by Ruminants. I. Development, Justification and Evaluation of the Model -</u> D. R. Mertens, et al.....	18-19
<u>Monensin and Digestibility of Feedlot Rations -</u> J. H. Thornton, et al....	19
<u>Effect of a Selected Buffer Combination on Ruminant Acid Production and Animal Performance -</u> E. L. Herod, et al.....	19-20
<u>Metabolic Effects of Deleting Thiamin from Infused Diets in Sheep -</u> R. E. Mueller, et al.....	20-21
<u>Use of Rare-Earth Elements as Multiple Nutrient Markers in Rate of Passage Studies -</u> G. F. Hartnell, et al.....	21-22
<u>Rumen-Bypass by Reactivation of the Esophageal Groove Reflex in Lactating Cows -</u> F. E. Standaert, et al.....	22-23
<u>Protein Requirements and Ruminant Protein Production in Feedlot Cattle -</u> W. G. Bergen, et al.....	23

AGRONOMIC:

<u>Plant and Animal Aspects of Grass Tetany and Methods of Prevention -</u> J. A. Studemann, et al.....	23-24
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	<u>Page</u>
<u>Plant Characteristics Affecting Animal Intake and Digestion -</u> W. W. Hanna	24
<u>Refeeding Value of Plant Fiber Recovered From Bovine Feces -</u> W. L. Johnson	25
<u>The Use of Organic Acids in Preserving High-Moisture Hay -</u> J. P. Mueller, et al.....	26-27
<u>The Utility of the Meadow Vole as a Bioassay of Forage Quality -</u> W. A. Kendall	27
<u>Differences in Rate of In Vitro Dry Matter Disappearance (Cellulose and Hemicellulose) of Cool- and Warm-Season Forages Harvested at Two Growth Stages - J. C. Burns</u>	28
<u>PHYSIOLOGY:</u>	
<u>Affect of Rumen Insufflation on Rumen Motility -</u> H. W. Colvin, Jr., et al.....	28-29
<u>The Site of Pentagastrin-Induced Inhibition of Reticulo-Ruminal Motility - H. W. Chapman, et al.....</u>	29-30
<u>Distension and Tactile Input from Stomach Compartments as Satiety Signals in Sheep - W. L. Grovum</u>	30
<u>Ruminal Function of Wheat Pasture Stockers - Gerald W. Horn, et al.....</u>	30-31
<u>MICROBIOLOGY:</u>	
<u>The Physiological Importance of Fumarate Reduction to Succinate in Certain Rumen Anaerobes - C. A. Reddy</u> NQ ABSTRACT RECEIVED.....	
<u>Ruminal Oxalate Degradation: Studies with Continuous and Enrichment Cultures - M. J. Allison, et al.....</u>	31-32
<u>Application of a Semi-Continuous Technique to Study the Effect of Monensin on the Rumen Fermentation - D. E. Short, et al.....</u>	32
<u>Isolation and Characteristics of a Skatole Producing Lactobacillus sp. from the Bovine Rumen - M. T. Yokoyama, et al.....</u>	32-33
<u>Urease and Enzymes of Ammonia Assimilation in Two Species of Rumen Bacteria - C. J. Smith, et al.....</u>	33
<u>Digestion of Urea and Epithelial Cells by the Microbial Flora Attached to the Bovine Rumen - K. J. Cheng, et al.....</u>	33-34

	<u>Page</u>
<u>Nutrition of Anaerobic Mycoplasmas from the Rumen -</u> I. M. Robinson, et al.....	34
<u>Effect of Short Term Chilling of Rumen Contents Upon Viable</u> <u>Bacterial Numbers -</u> Burk A. Dehority, et al.....	35
<u>The Effects of Total Nutrient Starvation on the Viability and</u> <u>Physiology of Megasphaera elsdenii -</u> R. W. Mink, et al.....	35
<u>Variations in Morphological Types of Rumen Bacteria Attached to</u> <u>Forage Fiber -</u> Danny E. Akin, et al.....	35-36
<u>Morphological and Chemical Characteristics of the Cell Envelope of</u> <u>Bacteriodes succinogenes -</u> C. W. Forsberg, et al.....	36-37
<u>Isolation and Identification of Adherent Bacteria ("Epimural" Bacteria)</u> <u>from the Ovine Rumen Wall -</u> Lorna J. Mead, et al.....	37

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For the purpose of discussion, the program was divided into five panels. The identity of the panels and the chairman of each follows:

General Chairman --- C. R. Richards ----- CSRS, USDA

(a) Physiopathology	W. M. Wass (IA)
(b) Nutrition	R. M. Cook (MI)
(c) Agronomic	J. C. Burns (NC)
(d) Physiology	A. D. McGilliard (IA)
(e) Microbiology	M. P. Bryant (IL)

PHYSIOPATHOLOGY

The Effect of an Experimental Antibiotic and Sodium Bicarbonate on Acidosis Produced by Feeding Wheat to Lambs - W. W. Kezar and D. C. Church, Department of Animal Science, Oregon State University, Corvallis, Oregon 97331.

Four crossbred wethers with rumen fistulas were used to study the effect of 2% or 4% NaHCO_3 , an experimental antibiotic at 0.25% (A) and the experimental antibiotic plus 2% NaHCO_3 (A + B) in preventing the occurrence of lactic acidosis when the wethers were switched from a grass hay-alfalfa diet to ground white wheat plus chopped alfalfa. Lactic acidosis was provided in all four animals initially by feeding wheat at 30 g/kg $\text{BW}^{.75}$ /day (divided into two feedings 8 hr apart) for two days and then on the 3rd and, if necessary, the 4th days giving 50 g/kg $\text{BW}^{.75}$ divided into three feedings 4 hr apart. Wheat was not given after pH dropped below 4.8. The lowest pH attained and the highest lactic acid levels (mM/l) for the four controls were 4.52, 160.52; 4.34, 148.73; 4.25, 109.11 and 4.36, 132.2. After each animal was readapted to the grasshay and alfalfa, they were started again with the same feeding program plus one of the treatments and continued until each animal received each treatment. All treatments were effective in maintaining the pH above 5.00 and in preventing the sheep from going off feed. The buffer treatments maintained higher pH levels than A or A & B. The lowest pH averages for the treatments were 2% - 5.54, 4% - 5.61, A - 5.17 and A & B 5.33. While total volatile fatty acid values looked very similar for the treatments (2%, 4%, A and A & B), analyses showed the A and A & B treatments resulted in less acetic and more propionic acid. Average C_2/C_3 ratios for the treatments were 2% - 2.19, 4% - 1.86, A - 1.12 and A & B - 0.98. While trying to induce acidosis in two of these sheep, at a later date, pH values of 4.66 and 4.64 were obtained. Analysis of rumen fluid showed that no lactic acid was present but that levels of butyric and valeric acids were abnormally high making up 53.47 and 47.60% of the total volatile fatty acids respectively. At this time, these sheep had been induced with lactic acidosis on four previous occasions.

The Physical-Chemical Basis of Frothy Bloat - R. E. Howarth, B. P. Goplen and R. K. Chaplin, Agriculture Canada Research Station, Saskatoon, and Department of Veterinary Physiological Sciences, University of Saskatchewan.

In 1972 a research project on legume pasture bloat was initiated at the Agriculture Canada Research Station, Saskatoon. The primary objective of this project is the development of a bloat-safe alfalfa cultivar by plant breeding. While pursuing this objective, we have made concurrent studies on the physical-chemical basis of frothy bloat.

We re-examined the proposal that Fraction 1 or 18S protein is the principle foaming agent responsible for pasture bloat using both laboratory studies and animal feeding trials. The results implicated alfalfa fraction II proteins, as well as fraction 1 protein, as causative agents in bloat. Cicer milkvetch is a bloat-safe forage legume and the observation that it does not contain tannins prompted development of the cell rupture hypothesis. This hypothesis provides an alternative to the tannin theory in accounting for the bloat-safe nature of some legumes. The foaming agents of herbage origin are intracellular constituents and they must be released from the plant cells in order to cause frothiness in rumen ingesta. Compared to bloat-causing legumes (alfalfa, red clover, white clover) leaf cells from bloat-safe legumes (sainfoin, birdsfoot trefoil and cicer milkvetch) have greater resistance to mechanical rupture and they are digested more slowly by rumen microorganisms. Consequently there are lower concentrations of herbage proteins in rumen fluid of animals fed the bloat-safe legumes, compared to animals fed bloat-causing legumes. Other observations have led us to believe that soluble proteins, by themselves, do not account for the occurrence of the frothy condition. The formation of bubbles requires a nucleation site which may be provided by fragments of chloroplast lamellae suspended in rumen fluid. This concept in turn raises questions about the validity of "foam strength" in explaining the persistence of frothiness. We do not envisage frothy rumen ingesta as a foam with significant mechanical strength, but rather we regard it as a pliable, gas-in-liquid emulsion in which ruminal contractions do not effectively promote coalescence of gas bubbles.

An Antibiotic Sensitivity Evaluation of Isolated Rumen Organisms from Overloaded Sheep - R. K. Chaplin and G. A. Jones, Departments of Veterinary Physiological Sciences and Dairy and Food Science, University of Saskatchewan.

Ground barley (40/kg body weight) in water (1:2) was administered through the rumen fistulae of sheep following a 24 h fast. Changes occurring in both rumen metabolite levels and microbial numbers were monitored continuously for the next 72 h. In addition 150 strains of streptococci and 350 strains of lactobacilli were isolated from rumen samples taken up to 8 h and 24 h respectively from the rumen contents. As previously reported (RFC-1973) rumen overloaded induced in this manner typically causes a rapid drop in rumen pH to 4.4 by 12 h after engorgement, a decrease in rumen VFA's to 20 mEq/l by 24 h, an increase in rumen glucose up to 1 g% which then declines rapidly as lactate levels increase 100 fold by 18 h. Rumen streptococci increase from 9×10^5 at 0 h to a peak of 10^9 at 12 h, after which they could no longer be recovered. Lactobacilli, which are low initially ($<10^7$) increase at a slower rate peaking at 10^9 anywhere from

24-30 h post-engorgement. Of the streptococci isolates all were typical Streptococcus bovis and were capable of hydrolyzing starch. When grown with starch or glucose all strains produced L(+) lactate. The strains of lactobacilli could not hydrolyze starch but in a glucose medium produced both L(+) and D(-) lactate. The D(-) lactate accounted for 40-75% of the total lactate produced.

From this sequence of events it appears the streptococci initially metabolize starch and other available carbohydrates producing L(+) lactate. As the pH drops, this is accompanied, and then succeeded, by growth of lactobacilli on carbohydrates other than starch with a further production of L(+) lactate as well as D(-) lactate. In an effort to control the development of grain overload, by reducing the initial changes brought about in the rumen, an antibiotic sensitivity test was conducted on streptococci isolates taken from the first 8 h period. Minimum inhibitory and resistant concentrations of 11 antibiotics were determined on an aerobic trypticase soy broth media and a 20% rumen fluid with 0.2% glucose media. Overall the most inhibitory antibiotics were erythromycin penicillin G and penicillin V at a concentration of 0.1 microgram/ml. It has not been determined at this time whether rapid rumen destruction will make these antibiotics ineffective in vivo.

Reduction of Ruminal 3-Methylindole Concentration as a Possible Means of Prevention of Acute Bovine Pulmonary Edema and Emphysema - James R. Carlson and Andrew C. Hammond, Department of Animal Sciences, Washington State University, Pullman, Washington 99164.

Prior research has shown that ruminal microorganisms can convert tryptophan (TRP) to 3-methylindole (3MI, skatole) and that 3MI causes acute pulmonary edema and emphysema (APE) in cattle, goats and sheep. 3MI has been detected in the ruminal fluid of cattle under conditions associated with naturally-occurring APE. The purpose of this study was to determine whether the conversion of TRP to 3MI can be reduced and thereby prevent the onset of APE. Three groups of 6 cows each were given intraruminal doses of 0.35 g L-TRP/kg BW. Group 1 (control) received no further treatment. Group 2 received 0.7 kg molasses every 12 hr from -24 to +96 hr after dosing and group 3 received 4 g chlortetracycline at -24 and -12 hr and then 2.5 g at 12 hr intervals to 96 hr. Peak ruminal fluid 3MI concentrations were above 15 microgram/ml at 18 and 24 hr in groups 1 and 2 while ruminal fluid concentration remained below 1 microgram/ml in group 3. Three cows in group 1 developed clinical signs of APE and one died at 5 days. Two cows in group 2 developed clinical signs and both died at 5 days. None of the cows in group 3 developed any signs of respiratory distress. The results show that antibiotic treatment reduced ruminal 3MI concentrations and prevented APE while molasses had no effect.

A variety of inhibitors and antimicrobial agents were incubated with TRP and ruminal fluid, in vitro, in an attempt to identify other compounds which inhibit 3MI formation. Monensin and lasalocid were the most potent inhibitors tested in this system resulting in 87-90% inhibition of 3MI production at 5 microgram/ml. Four cows were given rumensin (200 mg/head/day) from -1 to 4 days after a tryptophan dose. Two controls were given tryptophan without additional treatment. One control cow died from APE on day 6 but no clinical signs of pulmonary lesions were observed in any of the rumensin treated cows. In addition, rumensin reduced the mean ruminal fluid concentration of 3MI. These results demonstrate that inhibition of 3MI formation results in lower ruminal 3MI concentrations and prevents tryptophan-induced APE. This rationale will be applied to field conditions in an attempt to prevent naturally-occurring APE. (Supported in part by NIH HL-13645).

Physiological and Pathological Responses of Cattle to Rumen Bacterial Endotoxins -
T. G. Nagaraja, E. E. Bartley, L. R. Fina, H. D. Anthony, S. M. Dennis and R. M.
Bechtle, Kansas State University, Manhattan, Kansas 66506.

The rumen of cattle contain large numbers of gram-negative bacteria that are a potential pool of endotoxin. Rumen bacterial endotoxin, when released and absorbed, may participate in the pathogenesis of certain diet-induced diseases such as lactic acidosis and the sudden death syndrome. Holstein bull calves (3-7 months of age, 75-175 kg body weight) were injected intravenously with endotoxin (1-4 mg/kg weight) extracted from the bacterial fraction of the rumen fluid of cattle fed hay or grain. The injection was repeated on the 15th day with the intention of provoking an anaphylactic response. Blood samples were obtained after each injection and the calves that survived the second injection were euthanized after 24 hours and necropsied with those that did not survive.

The signs observed were hyperventilation gradually leading to dyspnea, profuse salivation, coughing and a profuse diarrhea containing mucus. After injection of endotoxin, calves collapsed usually within 20-30 minutes (lateral recumbency with head, neck and legs stretched). Blood analysis revealed hyperglycemia followed by hypoglycemia, increased blood lactate, and a leukopenia followed by leukocytosis. Serum enzymes SGOT and LDH were elevated. A platelet count associated with hypofibrinogenemia and increased prothrombin time was suggestive of intravascular coagulation (DIC).

The gross lesions observed were the presence of hemorrhages in different organs. The brain was severely congested and showed numerous petechial hemorrhages. The lung exhibited emphysema. Histological preparations showed numerous hemorrhagic areas in the cerebral cortex, cerebellum liver, kidney, and adrenals. The small intestine showed heavy cellular infiltration, particularly with eosinophils in the lamina propria of the mucous membrane. The lung showed bronchiolar constriction, perivascular hemorrhage, scattered emphysema, edema and cellular infiltration in the walls of the alveoli.

The lesions in the lung suggest anaphylactic response of the calves to rumen bacterial endotoxin.

Pneumonia: Blood Gas, Radiological and Pathological Studies - J. G. Vestweber,
M. Guffy and H. Leipold, Kansas State University, Manhattan, Kansas 66506.

The objective of this work was to study the clinical, radiological and pathological changes associated with chronic bronchopneumonia in twenty-five calves. On auscultation of the lungs, normal or harsh vesicular sounds were heard most commonly in the dorsal tripartition, moist or dry rales in the middle tripartition and dry rales in the ventral tripartition. The arterial and venous blood gases were significantly changed when compared to normals. Angiograms revealed decreased blood supply to the areas of the lung affected with chronic bronchopneumonia. Gross pathological changes were atelectosis and consolidation of the anterior ventral aspect of the lungs and emphysema dorsally. Histopathological changes consisted of atelectosis, emphysema, fibrous thickening of alveolar septa, abscess formation, peribronchial lymphocytic hyperplasia, and obstructive bronchiolitis.

A Technique for Repeated Prolonged Rumen Infusion without Establishing an Open Fistula - J. D. Olsen, Poison Plant Research Laboratory, ARS-USDA, 1150 East 14th, North, Logan, Utah 84321.

A method has been developed whereby repeated or prolonged infusion of substances into the rumen can be done with relative ease and with a minimal need for daily care of the preparation. A small part (approximately 6 cm diameter) of the rumen compartment was surgically brought through the body wall and attached subcutaneously in the left paralumbar fossa. When desired, one need only penetrate the skin and rumen wall to gain direct access to the rumen cavity. The skin was shaved and disinfected before insertion of a 10 gauge hypodermic needle into the rumen cavity. A guide wire was passed through the needle and then the needle was removed, leaving the guide wire protruding through the skin and rumen wall. A cardiac catheter (8 French size and approximately 25 cm long) was passed over the guide wire and inserted into the rumen until the luer-lock end was against the skin; then the guide wire was removed. A canvas patch about 16 cm square and having a 2.5 cm diameter hole cut in the center, was placed over the end of the catheter and adhered to the animal's coat with adhesive cement. Suture material was tied around the luer end of the cardiac catheter and then tied through the canvas material to hold the catheter in place. An interconnecting catheter could be attached or detached leaving the rumen catheter in place. When an antibiotic preparation was occasionally placed around the catheter, only a minimal amount of focal cellulitis developed around the rumen catheter. This method has been used in cattle and sheep over the past 3 three years for continuous infusion periods of 1 to 6 weeks and repeated insertion of catheters has been done on the same animal for at least one year. This surgical preparation has also been used to obtain samples of rumen contents and administration of plant material directly into the rumen simply by making a small incision through the skin and rumen wall and ending with a purse-string suture to close the rumen incision.

NUTRITION

Effects of Protein Source and Concentrate in the Ration on Microbial Protein Production In Vitro - J. E. Edwards, E. E. Bartley and R. M. Bechtle, Kansas State University, Manhattan, Kansas 66506.

Rations that contained from 12 to 27% crude protein (CP) were formulated to test the hypothesis that microbial protein cannot be synthesized from Starea if it is added to rations that contain 12% crude protein from natural sources. The control ration (12% CP) contained 25% brome hay, 9.5% soybean meal (SBM), and 65.5% sorghum grain. The quantity of SBM or Starea added to the control ration (12% CP) to increase the protein contents of the experimental rations to 27% CP was at the expense of sorghum grain. The rations were fermented in vitro with rumen fluid for 6 hr and microbial protein was determined by high speed centrifugation and methanol washing. Microbial protein production increased steadily as the CP content of the rations was increased from 15 to 27% using Starea. The rumen ammonia concentration for these rations containing 12, 15, 18, 21, 24 or 27% CP were 5, 15, 29, 44, 60 and 76 mg ammonia N per 100 ml. Microbial protein production declined with incremental additions of SBM to the ration. It was assumed that SBM, when comprising over 19% of the ration, was not extensively degraded in the 6 h fermentation and that it contained less available energy than grain for the conversion of ammonia to microbial protein.

In a second series of experiments, urea, casein and SBM were used as nitrogen supplements. Microbial protein production was not increased as the urea content of the ration was increased. However, incremental additions of casein resulted in incremental increases in microbial protein production. As in the first series of experiments, microbial protein production declined with incremental additions to SBM to the ration. Obviously casein supplied nitrogen in a more available form for microbial protein synthesis than did SBM. It was concluded that microbial protein could be synthesized from urea in the form of Starea but not as unprocessed urea when these supplements were present in rations containing in excess of 12% CP.

Amines in Ruminal Fluid and Blood of Acidotic Ruminants - G. E. Mitchell, Jr., L. N. Irwin, R. E. Tucker, G. T. Schelling and R. D. Long, University of Kentucky, Lexington, Kentucky 40506.

A series of in vivo and in vitro experiments with sheep and cattle have been conducted with the objective of evaluating possible roles of histamine, tryamine and tryptamine in acute acidosis and chronic laminitis. Tissue release of amines appears to be significant but not the major source of amines in the rumen when levels are elevated. Microorganisms in the rumen are capable of converting large quantities of histidine, tryosine and tryptamine to the corresponding amines. Extensive metabolism of amines also occurs in the rumen. We have not been able to establish consistent relationships between ruminal amine levels and either circulating amine levels or the gross symptomatology of either acute acidosis or chronic laminitis.

Influence of Ration on Health and Reproduction of the Periparturient Dairy Cow - Claudie N. Zemet, V. F. Colenbrander, R. E. Erb, N. J. Moeller and C. J. Callahan, Departments of Animal Sciences and Veterinary Sciences, Purdue University, West Lafayette, Indiana 47907.

Much of the nutrition research concerned with increasing milk yield of the dairy cow involves feeding regimes during lactation or continuous feeding through several lactations with little regard to the nutrition of the periparturient cow. The crucial time for disorders related to parturition appears to be during the dry period and shortly after parturition. Of the numerous periparturient disorders the incidences of two, displaced abomasum and fat cow syndrome, have been more apparent and frequently are accompanied by retained placenta, metritis, mastitis, milk fever and ketosis. The objective of our research is to determine the effect of various forage rations on disorders associated with the periparturient cow.

Experimental rations are being fed from 60 days prepartum to 30 days postpartum. Rations designed to test the effect of three basic forages: hay, hay crop silage (HCS) and corn silage (CS), balanced with supplements, are fed prepartum and in complete balanced rations with concentrates during lactation. Numerous parameters of health, reproduction and production are being measured. Since this research is currently underway, our data are still limited and incomplete.

To date about 26 cows per treatment, hay, HCS and CS, have calved. Seven displaced abomasums have occurred, 3 each on CS and HCS and 1 on hay. According to metabolic data, 3 cows, one on each treatment, have died from the "so-called" fat cow syndrome. These cows also had retained placenta, milk fever and fatty

liver. Retained placenta has averaged 44% across groups and nearly all of these cows were treated subsequently for metritis. The incidence of retained placenta was twice as high with HCS and CS fed cows, as with hay-fed cows. The incidence of mastitis was also much higher in silage-fed cows in comparison to those fed hay. Milk fever was the greatest on CS and least on HCS with hay being in between. Dystocia was the greatest among silage fed cows. Cows on CS gained more weight than cows fed HCS or hay during the prepartum period and lost more weight during the postpartum period. Cows fed hay and HCS developed less udder edema than cows fed CS.

The Imbalance of Acid and Base Forming Constituents in the Rumen of the Mature Bovine as a Possible Cause of Hypomagnesemia - H. Fenner and R. A. Damon, Jr., University of Massachusetts, Amherst, Massachusetts 01003.

Six feeding trials, each of 4 x 4 latin square design, with two pairs of rumen fistulated, dry Holstein twins served for determining effects of six different rations on rumen fermentation. Criteria for changes in fermentation pattern were pH, concentrations of ammonia, total VFA's and contents of dissolved Na, K, Ca and Mg in the liquid phase of the cows' rumen contents. Objectives for the different feeding trials were to determine changes created by:

- A. Forage quantity - 1.00%, 1.33%, 1.67% and 2.00% of liveweight as all hay rations lowered significantly pH, concentration of Na and raised that of total VFA's and dissolved Mg in the rumen fluid.
- B. Forage types and consistency - Dry, succulent and two differently proportioned combinations thereof, when offered as dry matter intake constant rations consisting of hay and corn silage, caused in the rumen fluid from the two homogeneous rations, all hay and all corn silage fed cows, high pH values and Na concentrations and low concentrations of total VFA's. The combined but not blended rations created low pH values and Na levels and high concentrations of total VFA's and dissolved Ca and Mg in the rumen fluid.
- C. N-fertilization - Four dry matter intake constant rations of reed canary hay obtained from forage submitted to three levels of ammonium nitrate fertilization, 0, 55, 165 and 220 kg N/ha, raised in the cows' rumen fluid correspondingly the concentrations of ammonia, total VFA's and dissolved K, and reduced the contents of dissolved Ca and Mg.
- D. Urea supplementation - Four intake constant corn silage rations supplemented with three levels of urea 0, 90, 180 and 270 g/day at feeding time raised accordingly in the cows' rumen fluid the pH and the concentration of ammonia and lowered the contents of dissolved Ca and Mg.
- E. Magnesium oxide supplementation - Four intake constant corn silage rations supplemented with three levels of magnesium oxide, Mag-Ox, 0, 60, 120 and 180 g/day at feeding time raised in the cows' rumen fluid the pH, the concentrations of dissolved Ca and Mg and lowered the concentrations of ammonia, dissolved Na and K.

F. Potassium carbonate supplementation - Four intake constant corn silage rations supplemented with three levels of potassium carbonate 0, 120, 240 and 360 g/day at feeding time raised in the cows' rumen fluid the concentration of total VFA's and the contents of dissolved K, Ca and Mg and lowered the concentration of dissolved Na.

The observed negative effect of pH and ammonia concentration on the dissolved portion of Mg in the rumen fluid of cows allows to assume that ammonia and Mg form a complex which is advanced at elevated pH levels. Under these conditions the formation of the ammonium magnesium phosphate complex is the most likely.

Recent studies by European scientists have identified the forestomach region (rumen and omasum) as the location where up to 90% of the total Mg absorption occurs. Also an active transport has been found to be the means of passage through the rumen membrane in the mature animal. With these results and those of the six feeding trials it will be possible to explain more precisely the occurrence of hypomagnesemia at marginal dietary Mg intake. The good response to the MgO supplementation in improving the Mg levels in the rumen will allow the establishment of preventive treatments by better ration formulation.

Prospective Usages of Soluble Silicates in Ruminant Nutrition: Interactions with Dietary Heavy Metals and Trace Elements^{1/} - G. Stanley Smith^{2/}, Leroy Ben Bruce and Joe M. Cadle, New Mexico State University, Las Cruces, New Mexico 88003.

Published research at New Mexico State University (NMSU) has demonstrated biologically and economically important effects of siliceous substances accumulated in rangeland forages and of soluble silicates ingested by mice, rats, chicks, sheep and cattle. Aqueous sodium silicate ingested in drinking water at concentrations of about 400 mg/l (ppm) significantly improved ($P < .05$) body weight gains of intact male rats and castrate male sheep; but exerted negligible effects on females of both species. Opposite effects were noted with male and female chicks. Improved gains and feed efficiency in male sheep occurred despite decreased digestibility of organic matter, notably fibrous components, suggesting primary effects at the host tissue level. Mineral balance studies in rats and sheep demonstrated important effects of ingested soluble silicate on calcium, phosphorus, magnesium and several trace mineral nutrients. Rumen culture studies showed that sodium silicate impaired fiber degradation, apparently by reduced availability of trace mineral nutrients. Known chemical characteristics of the silicates and unpublished data from NMSU experiments suggest that soluble silicates have prospective usages in reducing tissue uptake of certain dietary heavy metals and potentially toxic trace elements by ruminant animals. In view of expected increases in nutrient recycling and in dietary contamination by these elements, prospective means for reducing gastro-intestinal absorption and/or tissue retention by livestock (and humans) deserve increased attention by ruminant nutritionists.

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Influence of Dietary Nickel on Urease Activity - J. W. Spears and E. E. Hatfield.
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Rumen bacterial urease has recently been shown to be a nickel requiring enzyme (J. Dairy Sci. 60:1073, 1977). Experiments were conducted to ascertain if supplemental nickel would affect urease activity and biological responses in ruminants fed practical corn based diets. Nickel was supplemented to the basal diet at a level of 0 or 5 ppm in all experiments.

In the first experiment 12 lambs were fed a 9.5% crude protein diet (77% corn, 1% urea, 10% solka floc, 7% glucose, minerals and vitamins) for a 28-day period. Urease activity was much higher in the lambs receiving supplemental nickel at the end of the experiment. Rumen ammonia concentrations were higher 2 hrs after feeding while serum urea-nitrogen tended to be lower in animals receiving 5 ppm of nickel. Lambs fed the nickel-supplemented diet also tended to consume more feed and gain faster (25% improvement) than the nonsupplemented animals.

In a second experiment 16 lambs were fed ad libitum a 7.5% crude protein diet (66% corn, 0.25% urea, 20% cottonseed hulls, 9% ground wheat straw, minerals and vitamins) for the first 28-day period, then switched to a 12.1% crude protein diet (66% corn, 9% cottonseed meal, 0.75% urea, 19% ground wheat straw, minerals and vitamins) for the second period. Average daily gain (32% improvement) and ruminal urease activity were significantly increased while serum urea-nitrogen and total serum proteins were decreased by nickel in the first period. The increased gain was a result of an increased feed efficiency rather than an increased feed intake.

Serum urea-nitrogen, total serum proteins, urease activity and rumen pH were increased by supplemental nickel in the second period. Gains also tended to be higher (23% improvement) in the nickel supplemental lambs during the second period largely because of an increased feed intake. At the end of the experiment, 6 lambs per treatment were killed and rumen epithelium urease activity was determined. Rumen epithelium urease activity was over 3 times higher in the nickel supplemented lambs than in the nonsupplemented animals.

Twenty steers were fed a 13.1% crude protein diet (50% corn, 15% cottonseed meal, 0.75% urea, 29% ground wheat straw, minerals and vitamins) in another experiment ad libitum for a 42-day period. Average daily gain was not affected by nickel; however, feed efficiency was significantly improved (6%) by nickel supplementation. Urease activity was only slightly higher in the steers receiving 5 ppm nickel while serum urea-nitrogen, total serum proteins and rumen ammonia were not affected by treatment.

These studies suggest that ruminants fed certain practical diets may respond to supplemental nickel, especially when consuming a low-protein diet. The data suggest that nickel may function to enhance the recycling of nitrogen to the rumen by increasing rumen epithelium urease activity. This is in agreement with Houpt's hypothesis (Am. J. Physiol. 214:1296, 1968) that urease present in the rumen epithelium greatly facilitates the transfer of urea-nitrogen from the blood into the rumen.

Forage Sulfur and Its Influence on Ruminal Nutrient Utilization - D. G. Ely, J. W. Spears and L. P. Bush, University of Kentucky, Lexington, Kentucky 40506.

Three experiments were conducted to (1) determine the effect of adding nitrate (NO_3) and molybdenum (Mo) on cellulose digestion when the in vitro media contained different levels of sulfate and sulfide; (2) determine the in vitro cellulose digestion of different forages supplemented with different sources and levels of sulfur (S); and (3) determine the ruminal nutrient utilization of Kentucky (Ky) 31 tall fescue forage when fed to lambs supplemented with S. In the first study, 24-hr in vitro digestion of purified wood cellulose was obtained when .4 and .8% sodium nitrate or 4 and 8 ppm sodium molybdate were added to the media containing either sodium sulfate or sulfide. The S sources were equal in ability to stimulate cellulose digestion. When NO_3 was added to the sulfate treatment, digestion was significantly depressed. Nitrate plus sulfide caused a depression, but not as severe as NO_3 plus sulfate. Additions of Mo to each S source slightly reduced cellulose digestion. In the initial phase of the second study, purified wood cellulose was incubated with different levels of elemental (E) or L-methionine (M) sulfur. In vitro cellulose digestion was greater with M. The second phase of the study utilized the dried forages: Ky 31, Kenhy fescue and orchard grass (OG) harvested in June, August and October. The S content of the forages ranged from .15% for OG harvested in June to .32% for Kenhy harvested in August. Cellulose digestion was greatest for June and August samples of OG and Kenhy fescue, respectively. Additions of .087% S to the August-Kenhy-forage dry matter stimulated cellulose digestion by 50% over the control incubations. Sulfur additions up to .232% of the OG-forage dry matter failed to stimulate digestion above that of the control treatment. The same response was found with both E and M additions. Trial III involved five abomasally cannulated lambs assigned to a 5 x 5 Latin Square design. Ruminal and total gastro-intestinal (GI) tract digestion of neutral-detergent fiber, acid detergent fiber and nitrogen was determined when lambs were fed the following diets: (1) Control (908 g June-cut-Ky 31 fescue per head daily); (2) Control + .05% E; (3) Control + .15% E; (4) Control + .05% M; and (5) Control + .15% M. Sulfur supplementation consistently increased ruminal digestion of fiber components, but had no effect on total GI tract digestion of fiber. The stimulation of fiber digestion was most apparent when .15% was added to the diet. No differences were found between S sources. Availability of forage S may limit ruminal utilization of the forage. Accumulation of NO_3 in the plant may account for some of this limitation. However, dietary supplementation with .15% of either E or M sulfur can alleviate this limitation when tall fescue diets are consumed by ruminants.

The Fraction of Dietary Lactose that Becomes Available for Fermentation in the Large Intestine in Non-Ruminants - N. J. Benevenga, Kyu-Il Kim and R. H. Grummer, University of Wisconsin, Madison, Wisconsin 53706.

A water-soluble marker (Cr-EDTA) was used to estimate the digestion of lactose by the time it reached the terminal ileum. The lactose to marker ratio varied with time after a meal, being high (0.6) when the rate of stomach emptying was high and low (0.3) when the rate of stomach emptying slowed. The fraction of lactose consumed in a single one hour meal that escaped hydrolysis in the small intestine was estimated by measuring the area under a smooth curve of a plot of lactose:marker

ration in the terminal ileum expressed as a fraction of intake, against the fraction of the total marker that passed into the large intestine. Approximately 30 and 31.5% of the lactose consumed by rats previously fed the control or 30% lactose diet respectively, reached the large intestine. This value increased to 43% when rats consumed a 30% lactose diet containing Cr-EDTA ad libitum for one week and than samples were taken at three-hour intervals over 24 hours. When these techniques were applied to Hampshire and Chester White pigs fed a diet containing 40% of whey (30% lactose), with the Cr-EDTA marker, approximately 30-32% of the lactose reached the large intestine. There was no difference between breeds. The lactose that entered the large intestine was apparently fermented to VFA and could account for approximately 18% of the energy used at a metabolic rate of two times basal. In the rat, when the in vitro lactase activity was compared with that which could be calculated from the disappearance of lactose in vivo, it was found that the in vitro rate was substantially greater (50%). This suggests that the full mucosal surface (i.e. enzyme) does not become available to the substrate or that cellular lactase becomes active in vitro which does not contribute in vivo. Although substantial differences (2 1/2 fold) were seen in the lactase activities of the small intestine in the Hampshire and Chester White breeds, this was not reflected in the fraction of dietary lactose available for fermentation in the large intestine, when it was calculated by using the marker technique.

The "Difference" Approach for Estimating Ruminant Protein Degradation - R. A. Zinn, L. S. Bull and R. W. Hemken, University of Kentucky, Lexington, Kentucky 40506.

A method for discriminating protein supplements based on their intrinsic resistance to ruminal degradation is described.

This method, the "difference" approach, is to compare the total protein leaving the abomasum of animals fed a test diet consisting of a basal ration plus a test protein with animals fed the basal alone. Adequate NPN is added to all diets tested to insure that nitrogen is not limiting microbial growth. The assumptions underlying this approach as well as the delimitations of the study used to test it are discussed.

Data to be presented represent measurements obtained from 3 Holstein calves ranging in wt. from 160-190 Kgs, equipped with re-entry cannulas at the proximal duodenum. Treatments consisted of a basal diet and 4 test diets containing either casein, soybean oil meal, cottonseed oil meal, or corn gluten meal as supplemental proteins. Calves were fed twice daily, at equal intervals, 1500 gr of 40:60 of chopped alfalfa hay:concentrate diets. A collection period consisted of 36 hours during which calves were sampled continuously using a completely automated sampling device. The estimated ruminal degradability of casein, soybean oil meal, cottonseed oil meal, and corn gluten meal protein was 90, 82, 74 and 70% (+ 2.3 S.E.) respectively.

Effects of Varying Solids Retention Times on Fermentation in Continuous Culture - W. H. Hoover, Department of Animal Science, University of Maine, Orono, Maine 04473; C. J. Sniffen, Department of Animal Science, Cornell University, Ithaca, New York 14853.

A continuous culture device which has a dual effluent removal system was used in these studies. In this system, a known portion of the effluent is withdrawn through a filter by a pump. This controls, assuming a constant buffer input, the

amount of effluent flowing out through the overflow port. Since the flow through the overflow port carries the undigested feed residues, the solids flow rates may be varied, permitting simulation of the differential solid and liquid flow from the rumen.

All studies were conducted using a total turnover rate of 1.5 volumes/day. The effects of solids retention (r) times of 16, 23, 28 and 40 hr were determined on nutrient digestibilities, VFA production, nitrogen utilization and protozoa numbers. Two isonitrogenous diets were compared, differing only in protein source, which was either peanut meal (PM) or zein. Increasing solids r times from 16 to 40 hr significantly increased dry matter (DM) and protein digestibility, VFA production and protozoa numbers. The response of DM digestibility to increased r time was quadratic, leveling off between 30 and 40 hr. Compared to zein, PM supported higher DM digestibility for r times up to 40 hr and higher protein digestion, VFA production and protozoa numbers at all r times. DM digestibilities at solids r times of 16 and 40 hr for the PM diet were 39.7 and 51.0%, respectively and for the zein diet were 31.5 and 51.1%. Zein digestion increased from 36.2 to 77.5% as solids r time increased from 16 to 40 hr, respectively, while the corresponding protein digestibilities for the PM diets ranged from 72 to 97% as r time increased. Regardless of protein source, as protein digestion increased, NH_3 levels increased. Longer solids r times did not increase the efficiency of N utilization for microbial protein synthesis. Nitrogen output as by pass feed protein plus NH_3 remained at approximately 42% of N intake at all r times except 16 hr, where it increased to 49%. Concomitantly, microbial N output averaged 58% at r times of 40, 28 and 23 hr, and 51% at 16 hr.

Effects of Pentachlorophenol on Rumen Fermentation - L. R. Shull, S. K. McCarthy and D. J. Crosswhite, Department of Dairy Science, Michigan State University, East Lansing, Michigan 48824.

Pentachlorophenol (penta) is the active ingredient in a commonly used wood preservative. Recently on several midwest dairy farms adverse effects on herd health and milk production in cattle exposed to penta treated wood have been implied. On these farms treated wood was being used in various capacities such as in bunker silos, feed bunks, free stalls and head boards, fences and gates. There is substantial evidence that metriculation of penta into the cattle occurred by one or more of the three routes, orally, dermally and through the lungs. Various clinical signs of abnormal health have been observed in these cattle but as yet no definitive diagnosis of penta toxicity has been made. Commercial preparations of penta are known to contain various contaminants, among them higher chlorinated isomers of polychlorobidendo-p-dioxins. Both penta and several of the dioxin isomers have been identified quantitatively in body tissues of some Michigan cattle but the significance of this xenobiotic body burden is unresolved. In light of its well-known antimicrobial efficacy, it was of interest to investigate effects of penta on rumen fermentation.

In vitro experiments were conducted to establish the dose-response relationship between penta and fermentation measures. The basic procedure was incubation for periods ranging from 12 to 48 hours at 39°C of buffered and enriched rumen fluid with various concentrations of penta. Measures of fermentation were cellulose

digestion and VFA production. Rumen fluid was collected from a mature cow fed a diet of all alfalfa hay. Technical grade penta (85-90% pentachlorophenol) was an industry composite donated by the American Wood Preservative Institute (AWPI). Desired concentrations of penta were dissolved in 50-100 microgram/l of absolute ethanol and added to the incubates.

Penta was found to exert a significant ($P < .01$) effect on cellulose digestion and propionic acid production at 10 ppm but not a 5 ppm. Acetic acid and butyric acid production on the other hand was significantly ($P < .01$) impaired at 20 ppm but not at 10 ppm. Propionic acid production appeared to be the most sensitive indicator of a toxic effect, and was more than 90% inhibited at 35 ppm. Time studies indicated that the toxic effect of penta occurred after 12 hours suggesting an impairment of bacterial growth. Negligible differences were observed when technical grade penta was compared with pure penta.

These results indicate that penta could interfere with the fermentation process at rumen concentrations of ≤ 10 ppm. The dose-response relationship between penta and rumen function in vivo needs investigating, however, the most critical question needing an answer is whether chronic exposure to penta such as that typically encountered on farms is of significant magnitude to affect milk production and health.

Long Chain Fatty Acids for Milk Fat Synthesis - D. L. Palmquist, Department of Dairy Science, Ohio Agricultural Research and Development Center, Wooster, Ohio 44691.

Long chain fatty acids (LCFA, 16 and 18 carbons) constitute 50-70% of milk fat by weight. Efficiency of milk fat production might be increased if these were provided to the animal performed.

Currently little information is available as to 1) the significance of dietary fat for milk fat production; and 2) the contribution of rumen microorganisms to the fat economy of ruminants.

All 18-carbon and about 1/2 of 16-carbon FA are taken up from the blood triglyceride FA. Non-esterified FA and the sn-1 fatty acids of phospholipid may also contribute.

Evidence from our laboratory suggested that three-fourths of absorbed lipid was utilized directly by the mammary gland. Up to 90% of the milk fat LCFA from plasma were obtained directly from the gut ('chylomicra') and only 10-20% of the LCFA was from endogenous sources (liver synthesis of VLDL triglyceride). Recent experiments by us to test directly incorporation by liver of circulating NEFA into VLDL triglyceride support this conclusion.

Consideration of the low true fat nature of typical dairy rations (about 2.5% FA) shows that the suggested exogenous LCFA requirement for milk fat production is greater than that provided by the diet. This suggests an important role for de novo synthesis of LCFA by rumen microorganisms. Little information is available on this topic, although Sutton et al (J. Dairy Res. 37:97, 1970) found 40-100% more FA reaching the small intestine than was in the diet of sheep. Storry et al

(J. Dairy Sci. 57:1046, 1974) have implicated importance of rumen synthesis of LCFA. However, Viviani (Adv. Lipid Res. 8:267, 1970) reported rates of LCFA synthesis in sheep rumen content that would be too low to support the suggested requirement for lactation and some have questioned the significance of rumen LCFA synthesis because of limiting ATP availability. Synthesis of LCFA from acetate requires 3.5 times as much ATP per unit weight as synthesis of protein from amino acids. Rumen microorganisms will incorporate LCFA into cells if they are available (Viviani, Adv. Lipid Res. 8:267, 1970). The question, then is, does the low fat nature of lactation rations limit synthesis of microbial protein in the rumen, if not milk fat synthesis directly?

The Use of Anhydrous Ammonia for Improving the Nutritional Value of Straw - R. K. Chaplin and J. A. Kernan, University of Saskatchewan and Saskatchewan Research Council.

During the past three-quarters of a century, numerous experiments have been conducted in various part of the world in an effort to improve the nutritional value of low quality roughage for ruminant animals. Except for possibly Norway, little reference can be found indicating any practical use of these processes. Recently, however, with the changes in the supply and price of feed grains, alternative sources of forage have been in greater demand by the livestock industry.

One of the more promising chemical methods for upgrading low quality forage is treatment with anhydrous ammonia. Treated forages are not only higher in digestibility but also retain a portion of the ammonia as a NPN source when fed to ruminants. The process is straightforward and can be conducted at the farm with a minimal capital investment. Basically the material to be treated (straw, corn stover, bagasse, etc.) is tightly enclosed in a covering of 6 mil plastic. A fertilizer dealer injects into the enclosure an amount of anhydrous ammonia equal to 3-4% of the material dry weight. After reacting for about one month the plastic is removed for a few days allowing the excess ammonia to evaporate before the material can be fed. Experimentally the dry matter digestibility of treated straw is improved about 10 units whereas the crude protein content increases from approximately 3% to 9%. Animal performance trials indicate ammoniated straw can provide an alternative source of good quality forage allowing greater flexibility to the livestock operation.

Presently 16 demonstration farms throughout Saskatchewan are evaluating the process under practical conditions. The cost per ton of straw being treated in the prairies is approximately \$9 for the ammonia, \$2-3 for the reusable plastic tarp plus labor and raw material. With average quality hay costing a minimum of \$60/ton the ammoniation of straw becomes a reasonable alternative.

A Kinetic Model of Fiber Digestion by Ruminants. I. Development, Justification and Evaluation of the Model - D. R. Mertens and L. O. Ely, University of Georgia, Athens, Georgia 30602.

Concepts of passage, particle size reduction and digestion were integrated into a model of fiber disappearance from the gastro-intestinal tract of ruminants.

Passage was described as sequential movement from a exponential rumen pool to an exponential intestinal pool to feces as developed by Blaxter et al. (Brit J. Nutr 10:69) and Brandt and Thacker (J. Anim. Sci. 17:218). Particle size reduction was characterized as three sequential exponential pools in the rumen as suggested by Matis (Biometrics 28:597). Fiber digestion was based on the three pool model of Mertens (Fed. Proc. 36:187). Parameter estimates for the complete model were obtained from the literature, except for particle size reduction rates and intestinal and large rumen particle digestion rates which were estimated based on theoretical relationships. Alfalfa, Coastal bermudagrass and fescue composition and digestibility data were used to evaluate the model and make comparisons among forages. Using initial parameter estimates the model predicted fiber digestibilities lower than actual. Rumen pool sizes, pool composition and turnover times were within literature ranges; however, fecal excretion curves suggest that intestinal passage rate constants were underestimated. The model was used to predict ab libitum intake for each forage assuming that fill limited intake. Predicted intake ranked the forages correctly. Model structure appears adequate to predict digestibility and ruminal fill; however, accurate parameter coefficients are needed to refine the model.

Monensin and Digestibility of Feedlot Rations - J. H. Thornton, F. N. Owens and R. W. Fent, Oklahoma State University, Stillwater, Oklahoma 74074.

The influence of monensin and protein concentration on digestibility of a 95% concentrate, whole shelled corn ration was examined using 12 ad libitum fed 285 kg steers in a rotational experiment. Rations contained 0 to 33 ppm monensin with or without supplementation of soybean meal to provide 9.4 and 12.2% protein. Overall, monensin addition reduced intake by 4% and increased dry matter digestibility by 3.6%, so that intake of digestible dry matter was not changed. Protein digestibility was also increased, but nitrogen retention was not. At the low protein level, monensin exhibited its greatest effect, enhancing digestibility of both dry matter and starch by 3.4 percentage points and reducing starch content in feces from 19 to 12% of dry matter. Results suggest that from 25 to 75% of the general monensin effect may be a result of increased digestion of feed dry matter. This in turn may be attributable to an increase in residence time in the rumen.

Effect of a Selected Buffer Combination on Ruminal Acid Production and Animal Performance - E. L. Herod, R. M. Bechtel, E. E. Bartley and A. D. Dayton, Kansas State University, Manhattan, Kansas 66506.

A computer program was used to select combinations of buffers based on their performance when used singly in vitro. Several buffer combinations were selected by computer for both all-concentrate and concentrate-roughage rations. One such combination for the concentrate ration consisted of monobasic potassium phosphate, magnesium carbonate, magnesium oxide, and soda ash combined in a 22:22:35:16 ratio. This combination was fed as a supplement (.5 lb/head/day) to dairy steers consuming an all-concentrate ration ad libitum. Twelve Holstein steers in two groups of six were used in a cross-over design. Ruminal acid production and animal performance were observed. The general appearance of the animals consuming the buffer supplement was inferior to that of the controls.

Severe diarrhea occurred in the buffered animals. This was probably due to a disturbance in the water and electrolyte balance resulting from the high magnesium content of the supplement. A significant ($P < .05$) decrease in feed intake was observed in the buffered animals. There was also a tendency for the supplemented animals to have less efficient weight gain than the controls but this was not significant. Buffered animals had a slightly higher rumen fluid pH, significantly ($P < .01$) higher rumen acetate, significantly ($P < .05$) lower propionate and significantly ($P < .05$) higher lactate concentration than the controls.

Metabolic Effects of Deleting Thiamin From Infused Diets in Sheep - R. E. Mueller and J. M. Asplund, University of Missouri, Columbia, Missouri 65201.

Thiamin was deleted from the diets of sheep in two infusion trials in order to evaluate the effects of thiamin deletion from diets of mature wethers. The relationship between an uncomplicated thiamin deficiency and polioencephalomalacia (PEM) was of particular interest.

In a preliminary trial, six mature wethers, average weight 37 kg, were fitted with rumen cannulae and bilateral jugular catheters in preparation for a dual infusion dietary regime. Glucose, amino acids, B-vitamins and antibiotics were prepared aseptically and infused intravenously via jugular catheter. Volatile fatty acids (acetic, propionic, butyric), McDougal buffer, minerals, sodium bisulfite and antibiotics were infused intraruminally. Vitamins A, D, E were given by intramuscular injection on a weekly basis. This dietary was formulated to provide a maintenance level of energy ($104 \times BW^{.75}$ kg) and 7.0 gm N/day. The rumens of all animals were emptied, rinsed and filled with polyethylene beads. Antibiotics were then used in an effort to suppress bacterial numbers intraruminally. Thiamin was deleted from the diets of animals #1 and #2, while animals #3, #4, #5 and #6 received 100 mg thiamin/day and served as controls. The control animals were in an infusion experiment to determine the metabolic requirement for leucine.

Animal #1 was euthenized on day 28 showing signs of incoordination, hyperirritability and bradycardia. Animal #3 died on day 31 showing arrhythmia. No signs of PEM were seen upon necropsy of either animal. Both animals 1 and 2 had elevated plasma lactic acid and pyruvic acid, while urinary thiamin reached undetectable levels.

In an effort to deplete the animals more rapidly a second trial was run in which several changes were made: (1) The animals were cecectomized; (2) Duodenal infusion replaced intravenous infusion; (3) A solution of glucose + maltose was used to provide an increased proportion of energy from carbohydrate; (4) The VFA solution contained only propionic acid; (5) Nolvasan, a disinfectant, was infused intraruminally along with antibiotics to suppress ruminal bacteria; and (6) The level of sodium bisulfite was raised to 9.0 gm/day. Five wethers, weighing an average of 38 kg, were used in the second trial. Animals #1, #2 and #5 received no thiamin, while animals #3 and #4 received 5 mg of thiamin/day and served as controls. Bacterial counts (MPN)₅ of rumen contents showed a depression of bacterial numbers to levels of 10^5 - 10^6 bacteria/ml. Animal #2 died on day 6 of unknown etiology. Animal #5 died on day 11 showing signs of ataxia and animal #1 died on day 23 with bradycardia. The control animals #3 and #4 were maintained for 32 days at which time they were killed for necropsy. No signs of PEM were

evident in any of the 5 animals. Animals #1 and #5 showed elevated plasma lactate and pyruvate while urinary thiamin reached undetectable levels. Animal #5 had elevated plasma glucose, while animal #1 showed depressed transketolase activity and a maximum thiamin pyrophosphate effect (TPP effect) of 95%.

These experiments support the view that PEM is not a simple or uncomplicated thiamin deficiency, but rather an induced deficiency. The level of 5 mg of thiamin/day was found to be marginal in satisfying the metabolic requirement for thiamin in the control animals of trial II.

Use of Rare-Earth Elements as Multiple Nutrient Markers in Rate of Passage Studies - G. F. Hartnell²⁷ and L. D. Satter, Dairy Science Department, University of Wisconsin, Madison, Wisconsin 53706.

Samarium (Sm), Cerium (Ce), and Lanthanum (La) treated hay (1 g of element applied per .9 kg of hay) were fed to four rumen fistulated Holstein cows for five days. At the beginning of the sixth day, unmarked hay was fed and removed from the rumen every six hours for 24 hours. At 6, 12, 18 and 24 hours, 4.4, 5.8, 5.9, and 10.1% (ppm basis) of the elements were detected on particulate fractions other than the one upon which they were originally placed. Possible reasons for the small amount of marker movement were discussed.

Sm, La, and Cr-EDTA were used as multiple nutrient markers in assessing transit times and turnover rates of hay, grain, and liquid through the gastro-intestinal tract of four lactating Holstein cows. Sm, La, and Cr in grab samples of rumen contents, grab samples of feces and composite samples of feces from a total feces collection were analyzed using neutron activation.

Liquid, hay, and grain ruminal turnover times, total mean retention times, and transit times were calculated by fitting a two compartmental model (Grovm and Williams, 1973)²⁷ to the fecal excretion data (grab samples and total feces collection).

$$\begin{array}{l}
 Y = A e^{-k_1 (T - TT)} - A e^{-K_2 (T - TT)} \quad T \rightarrow TT \\
 Y = 0 \quad T \leftarrow TT
 \end{array}$$

Ruminal turnover times calculated by fitting the model to the data obtained by grab sampling feces (Method 1), by fitting the model to the cumulative percent recovery curve from the total feces collection data (Method 2), and by calculating the rate of disappearance using the rumen grab sampling method (Method 3) were 11.5, 12.5, and 10.3 hours for liquid; 22.2, 22.7, and 22.7 hours for grain; and 28.6, 27.0, and 25.6 hours for hay. Total mean retention times were 42.3, 36.0, and 41.5 hours for grain; 52.6, 45.3, and 53.6 hours for hay, and 27.8 and 22.1 hours for liquid for Method 1, Method 2, and stained particle technique respectively.

It was concluded that rare-earth elements can be used as multiple nutrient markers for dairy cows and if used in conjunction with the method of taking grab samples of feces from the rectum, ruminal turnover rates and overall mean retention times can be calculated.

- 1/ Present address: Allied Mills, Inc., Research and Development Center, Post Office Box 459, Libertyville, Illinois 60048.
- 2/ Grovum, W. L. and V. J. Williams, 1973. Rate of Passage of Digesta in Sheep IV. Passage of Marker Through the Alimentary Tract and the Biological Relevance of the Rate-Constants derived from the changes in Concentrations of Marker in Feces. B.J. Nutr. 30:313.

Rumen-Bypass by Reactivation of the Esophageal Groove Reflex in Lactating Cows - F. E. Standaert, J. T. Huber and R. S. Emery, Michigan State University, East Lansing, Michigan 48824.

Two groups of 22 month old pregnant Holstein heifers (group 1, 6 heifers, Group 2, 13 heifers) were used in 2 different trials, 5 months apart, to determine if heifers this age could be trained to suckle from a nipple pail. Slightly different training procedures were used in each trial. Group 1 was limit fed hay, 2-3 kg/day for the first three days of the training period which was approximately 14 days in length. Whole milk was force fed through nipple pails twice daily during the training period. The procedure was changed after the third day because the animals were not responding. They were given hay ad libitum and limited water, 10-15 liters/day. In the second trial, the heifers were fed this way on the first day of approximately a 10 day training period. Water was force fed once a day in the second trial. Free choice water was gradually re-introduced 2-3 days before the end of each training period. A greater percentage of the animals in trial 1 maintained the suckling habit for a longer period of time than those in trial 2. At the end of the training periods, 83% and 100% of the heifers in trial 1 and 2 respectively, suckled willingly. One month after training 67% and 62% were still suckling and after 5 months, 67% and 15%. After the training period there were 2-day change-over design trials to determine if a glucose solution would by-pass the rumen when suckled. This was done 3 times with group 1 and once with group 2. The first day, half the animals were allowed to suckle 500 grams of glucose dissolved in 4 liters of water. The others were given the same type of solution in such a way that it would enter the rumen. In trial 1, normal drinking and stomach tubing were used; in trial 2, 500 grams of glucose was fed in 1-2 kg grain. Blood samples were taken before and 1/2, 1, 2, 4 and 6 hours after feeding the solution. The second day, animals and treatments were switched. Plasma was analyzed for glucose by the glucose oxidase colorimetric method. The mean plasma glucose level for the suckled animals were significantly higher ($P < .05$) at 2 hours than for the controls at the same time, in all the trials except for the one utilizing normal drinking for the control. The plasma glucose of these controls rose as high or higher than those that were suckled. There were a few animals that did not show a glucose peak and were assumed not to be getting rumen by-pass. Four heifers from each training group that did show glucose peaks within 2 hours after feeding were used in a 2 period changeover design production trial. Periods lasted 3 weeks. Half of the animals were allowed to suckle whole milk to obtain approximately 500 g supplemental protein/day. The other half received 500 g casein in the concentrate/day. They all received the same corn silage-haylage mix ad libitum and concentrate at the rate of 1 kg/3 kg milk produced. Average milk production parameters for the suckled and control groups were as follows:

milk yield, 22.6 and 22.0 kg/day respectively; milk protein %, 3.52 and 3.43; milk fat %, 3.06 and 2.87; milk protein yield, 789 and 745 grams/day; and milk fat yield, 683 and 628 grams/day.

Protein Requirements and Ruminant Protein Production in Feedlot Cattle - W. G. Bergen and J. R. Black, Michigan State University, East Lansing, Michigan 48824.

A system for balancing the protein component of feedlot rations has been developed. This system, called Net Protein System, is based on: (1) Estimates of protein needs for maintenance and growth from carcass and composition data, (2) Estimation of the utilization coefficient (Net Protein) of common feedlot feedstuffs, and (3) An upper limit of ruminal microbial protein synthesis and NPN utilization. Net Protein (Np) for maintenance and growth were derived from the following equations:

$$Np_m = (70.4W^{.734}) (0.0125)$$
 and
$$Np_g = (.235 - 0.00026W) ADG$$
 where W = empty body weight.

Net utilization coefficients of feeds (Np values) were determined from nitrogen balance studies with growing cattle. The upper limit of ruminal microbial protein synthesis was termed Ammonia Utilization Potential (AUP) (or N utilization potential) and is defined as follows:

AUP = (ATP yield in the rumen) x (efficiency of microbial protein synthesis). AUP in this context is not a function of dietary N level. Dietary and physiological factors that influence ruminal and post-ruminal metabolism were discussed in relation to the Np system.

AGRONOMIC

Plant and Animal Aspects of Grass Tetany and Methods of Prevention - J. A. Stuedemann and S. R. Wilkinson, Southern Piedmont Conservation Research Center, Agricultural Research Service, Watkinsville, Georgia.

Grass tetany is a metabolic disorder of cattle and sheep whose daily consumption of available magnesium is too low. Many factors have been associated or thought to be involved in the occurrence of grass tetany. These include temperature, rainfall, cloudiness, type and availability of forage, soil conditions, age and reproductive status of the cow, and the cow's production level.

Kentucky-31 tall fescue pastures were fertilized with three different levels of N. Levels of N fertilization included a high N supplied by topdressing with broiler litter at approximately 22.0 metric tons/ha, a moderate N (224 kg N/ha), and a low N (74 kg N/ha) supplied as ammonium nitrate. All pastures were grazed on a year round basis with Angus brood cows stocked at one cow-calf unit per 0.40 ha.

Increased levels of N fertilization resulted in greater number of cases of hypomagnesemia and tetany. No grass tetany occurred on the low-N pasture. Herbage samples taken from the high-N pasture at the time of grass tetany contained higher concentrations, of N, P, K, Na, Cu, and Zn than herbage from low- and moderate-N pastures. Herbage samples from the moderate-N pasture at the time of tetany were slightly higher in N and K than those from the low-N pasture.

Dusting tall fescue pastures with MgO prevented grass tetany. However, plant tissue analyses and blood serum Mg analyses suggests less than complete protection in severe grass tetany challenge situations. The dust appeared to be easily washed or blown from the grass leaves. Including MgO in a mineral mix did not provide consistent elevation of blood serum magnesium levels. Spraying a MgO-bentonite-water slurry either a 3- or 6-week intervals, or at 50 or 100% cover at 3-week intervals prevented grass tetany. It gave complete protection against the occurrence of milk hypomagnesemia (< 1.61 mg Mg/ml) for an average of approximately 16 days, or against the occurrence of severe hypomagnesemia (< 1.11 mg Mg/100 ml) for an average of approximately 24 days. The MgO-bentonite-water slurry provided complete restoration of normal serum Mg levels in all cows within 48 hours, and is recommended for controlling severe outbreaks of tetany. Bentonite sticks the MgO to the grass and assures adequate consumption of Mg.

Successful hypomagnesemia and/or grass tetany prevention techniques must assure that every cow will consume an adequate quantity of available magnesium on an almost daily basis. The success of various oral supplements is highly dependent upon the palatability of the supplement and the given environmental situation which includes supplement accessibility, species and quality of grazing, weather, and the overall herd management program. Combining cost per unit of magnesium and biological availability, generally results in magnesium oxide serving as the predominant form of magnesium used to supplement ruminant diets. However, if supplementation via water is desired a more soluble form such as magnesium sulfate, magnesium chloride, or magnesium acetate must be used.

Plant Characteristics Affecting Animal Intake and Digestion^{1/} - W. W. Hanna,
Coastal Plain Station, University of Georgia, Tifton, Georgia 31794.

In recent years plant and animal scientists have become more aware of the effects of physical plant characteristics on forage quality. Many of these characteristics are genetically controlled and can be used to improve plants. The cuticle and associated waxes have been shown to be deterrents to rumen microbe penetration into the plant tissue. Plants without trichomes appear to be more palatable and more insect resistant than plants with trichomes. Diversity for cuticle waxes and trichomes is present in many of the forages and small grains. Dwarf genes reduce the amount of stem, one of the most limiting quality components of the plant. Animals make better daily gains on the dwarfs. Genes that reduce lignin and also increase in vitro dry matter digestibility in sorghum have tremendous potential in this species as well as other species. Short day sensitivity is a genetic controlled response in plants whereby the plant remains vegetative until a certain day length is reached. Short day plants have more uniform production of high quality forage over a longer period of time. Cell size and arrangement also affect rate and extent of digestion.

^{1/} Cooperative investigations of the Agricultural Research Service, U.S. Department of Agriculture, and the University of Georgia, Coastal Plain Station, Tifton, Georgia 31794.

Refeeding Value of Plant Fiber Recovered from Bovine Feces - W. L. Johnson, Department of Animal Science, North Carolina State University, Post Office Box 5127, Raleigh, North Carolina 27607.

A vibrating screen (8 mesh/cm) separation process has been used to recover fibrous solids from dairy cattle manure. The product, "Screened Manure Solids" (SMS), has been refeed to dairy heifers, steers, and lactating cows. The cattle from which the SMS were obtained received a ration of 57% corn silage, 29% corn grain, 13% soybean oil meal and 1% minerals. Dry matter composition of SMS has varied from 6 to 9% in CP, 30-42% CF, 4-20% ash, 72-84% NDF, 23-36% cellulose, 7-15% lignin, .21-.29% Ca and .09-.13% P; dry matter content has ranged from 13 to 20%. Palatability of SMS is highest if fed within a few hours of collection. If stored anaerobically without preservatives the pH will drop from 6.5 (fresh) to less than 4.5 within 3 days, stabilizing at about 4.0 within 15 days. The ensiled product is equally palatable to the fresh material.

Results of feeding trials - (1) The ration fed to Jersey heifers contained 50% ensiled SMS, 25% chopped alfalfa hay, 16.9% ground corn, 7.4% soybean oil meal and 0.7% minerals. Rate of gain was 438 ± 88 g/day for 20 heifers, from 12 months of age until diagnosed pregnant (at 17 ± 1.5 months). (2) Sixteen Jersey steers, initial weight 210 ± 22 kg. gained 880 ± 170 g/day when fed 30% SMS, either fresh or ensiled; 20% corn silage; 37% ground corn, 12% soybean oil meal and 1% minerals. (3) Lactating cows (Holsteins) received six different rations (4 cows per ration) with 0-25% of the dry matter supplied from SMS. Mean 305-day milk yield was 5,570 kg with no significant treatment effect. In all three trials, no problems of general health or reproduction were encountered which could be attributed to SMS refeeding.

Results of digestibility trials - (1) In vivo DM digestibility of diets with 20, 30, 40, 50 and 60% SMS averaged 66, 61, 61, 54 and 48% in a trial with 4 or 5 Jersey heifers per treatment. Mean voluntary DM intakes were 107, 96, 90, 74 and 62 g/kg BW^{0.75} for the same treatments. (2) In a trial with Hereford steers, mean DM digestibility was 66, 65 and 70% when 30% of the ration was fresh, ensiled, or NaOH-treated (7%) SMS. When treated SMS was increased to 45 or 60% of ration DM, mean DM digestibility declined to 66 and 64%. (3) In a trial with mature, open, non-lactating Holstein cows, mean DM digestibility was 66.4, 68.5 and 64.6% when 22% of the ration DM was provided by fresh untreated, fresh NaOH-treated, or stored (7 days) treated SMS.

Among various laboratory analyses that were conducted, the in vitro digestibility of the cell wall fraction of SMS rations showed the highest correlation with in vivo digestibility and intake.

Estimates of the energy value of SMS for growing, fattening and lactating cattle place it in a category with high fiber feedstuffs, such as cereal straws and other crop residues.

Among alternative uses for SMS that have been considered, the manufacture of pressed particle board has shown some promise. Fresh SMS can also be used as bedding material, without prior processing of any kind.

The Use of Organic Acids in Preserving High-Moisture Hay - J. P. Mueller, R. E. Welty and J. C. Burns, Department of Crop Science and Plant Pathology, North Carolina State University, and ARS, Post Office Box 5847, Raleigh, North Carolina 27607.

The production of high quality hay requires careful attention to such management factors as plant maturity, harvesting and processing methods, weather forecasts and plant moisture at baling. Plant moisture determination is a critical factor in the hay-making process. Hay is often baled too wet or too dry because the decision to begin baling is usually based on subjective methods of moisture determination. When hay, especially legumes, is baled at less than 20% moisture, considerable loss of dry matter may occur due to leaf shattering. A perhaps more serious problem may result when hay is baled at greater than 25% moisture. Inadequately cured hay allows the proliferation of fungi that cause heating and results in appreciable losses in dry matter and quality. Recent research indicates that certain organic acids or mixtures of acids have the potential for significantly reducing fungi growth and subsequent losses in high-moisture hay.

Knapp, Holt and Lechtenberg (1976) treated 32% moisture alfalfa hay with several rates of propionic acid. They found that propionic acid applied at 1.0% of wet forage weight (WFW) was required to prevent excessive heating and losses of dry matter and other quality parameters.

Sheaffer and Clark (1975) found no significant difference in preserving ability between Ammonium isobutyrate (AIB) and propionic acid when applied to alfalfa-timothy hay. Hay at 40% moisture required rates of 3-5% WFW while 30% hay was adequately preserved with rates of 1.5-2.0% WFW.

Mueller et al. (1976) concluded that alfalfa or timothy hay (25-30% moisture) treated with 1.0-2.25% WFW AIB or ChemStor (80% propionic-20% acetic mixture) at the baler resulted in hay of acceptable quality.

McNemar, Sheaffer and Clark (1977) modified a conventional hay baler for field application of AIB to high-moisture hay. AIB was applied to rates of 1.5, 2.0 and 3.0% WFW to alfalfa-timothy hay containing an average moisture content of 39%. All rates of AIB reduced storage temperatures relative to wet, untreated hay.

In North Carolina we have conducted some preliminary work with small lots of sorghum-sudangrass hay reconstituted to 40% moisture. Three preservatives, propionic acid, ChemStor III and Haysavor were applied at 0, 1.0, 2.0 and 3.0% WFW. Visual observations and mold counts from the 1.0% treatments revealed that propionic acid and ChemStor were more effective than Haysavor in preserving the forage. All three chemicals appeared to be equally effective at the 3.0% rate. Principal fungi identified included various species of Penicillium, Aspergillus, Streptomyces and Paecilomyces. Adequately preserved forages averaged above 60% IVDMD and NDF while wet untreated forage averaged 39% IVDMD and 47% NDF.

Systems for field application of hay preservatives are presently being tested. The practical field application of hay preservatives within favorable economic margins remains a challenge.

REFERENCES

- Knapp, W. R., D. A. Holt and V. L. Lechtenberg. 1976. Propionic Acid as a Hay Preservative. *Agron. J.* 68:120-123.
- McNemar, J. H., C. C. Sheaffer and N. A. Clark. 1977. Modification of a Hay Baler for Applying Organic Preservatives to High-Moisture Hay. *Agron. J.* 69:331-332.
- Mueller, J. P. et al. 1976. Chemical Preservation of High-Moisture Hay. Prog. Rep. 359. The Pennsylvania State University, Agricultural Experiment Station, University Park, Pennsylvania 16802.
- Sheaffer, C. C., and N. A. Clark. 1975. Effects of Organic Preservatives on the Quality of Aerobically Stored High-Moisture Baled Hay. *Agron. J.* 67:660-662.
- The Utility of the Meadow Vole as a Bioassay of Forage Quality - W. A. Kendall, USDA-ARS, U.S. Regional Pasture Research Laboratory, University Park, Pennsylvania 16802; and J. S. Shenk, The Pennsylvania State University.

This report reviews a number of experiments designed to evaluate further the use of meadow vole (Microtus pennsylvanicus) as a bioassay to (a) estimate the quality of forages for large animal production and (b) aid in the identification and evaluation of some antiquality constituents in forages. These experiments were conducted by several scientists and graduate students that were affiliated with the Pennsylvania State University and/or the Agricultural Research Service, USDA. The generous supply of plant materials and sharing of research data by scientists at various agricultural experiment stations is gratefully acknowledged.

Good agreements were obtained between vole weight gains in our laboratory and large animal performances when both were fed samples of the same forages in the following comparisons: cow milk production with alfalfa hay that was grown and fed in Wisconsin, sheep grains with tropical grasses that were grown and fed in Australia, sheep gains with temperate grasses that were grown and fed in West Virginia, sheep gains with alfalfa hay that was grown and fed in Michigan. Good agreement also was obtained between vole short-term (individual meal) intake values and sheep palatability rating of reed canary-grass clones that were grown and fed in Minnesota. High correlations were obtained between vole weight gains and vole short-term intake values with diets that contained predominately either alfalfa or orchardgrass forages.

The weanling vole was used as a bioassay in a study which led to the identification of B-nitropropionic acid as the toxic constituent in crownvetch. Intake and weight gains of weanling voles were used to detect adverse effects of foliar diseases on alfalfa quality. The short-term intake bioassay was used to estimate the relative toxicity and interactions of several alkaloids that occur in reed canary-grass. An alkaloid of tall fescue and fescue forage that was toxic to cattle in Kentucky did not affect vole intake or weight gains.

We conclude that meadow voles provide a useful bioassay of forage quality for several types of investigations. Further knowledge of the vole digestive system and vole responses to numerous other forages is needed. Additional research appears to be warranted.

Differences in Rate of In Vitro Dry Matter Disappearance (Cellulose and Hemicellulose) of Cool- and Warm-Season Forages Harvested at Two Growth Stages - J. C. Burns, Department of Crop Science and ARS, North Carolina State University, Post Office Box 5847, Raleigh, North Carolina 27607.

Four forages, harvested at two growth stages were evaluated for in vitro dry matter disappearance (IVDMD) at ten fermentation times through 96 hours. The residues after fermentation were extracted with acid detergent solution to obtain estimates of acid detergent fiber (ADF), lignin and ash. These values were corrected for rumen fluid contents and cellulose and apparent hemicellulose losses calculated.

The forages and their respective growth stages were alfalfa, vegetative and full bloom; orchardgrass, vegetative and heading; SX-16 (a sorghum-sudangrass hybrid), vegetative and heading; and Coastal bermudagrass, 4 week and 8 week old growth.

Examination of neutral detergent fiber (NDF), ADF, lignin, cellulose and hemicellulose concentrations suggests that immature alfalfa should have highest nutritive value, mature alfalfa and immature orchardgrass similar, and next in nutritive value followed by immature and mature SX-16 and finally the two bermudagrasses. However, after 72 hours of fermentation IVDMD values were greatest for immature orchardgrass with alfalfa occurring intermediate between the two maturity stages of SX-16 and bermudagrass.

Rate of dry matter disappearance (0 through 24 hours of fermentation) for the immature forages was highest for alfalfa, followed by orchardgrass then SX-16 and bermudagrass. The same rank occurred for the mature forages except SX-16 replaced orchardgrass in having the second highest rate. Cellulose disappearance (mg) was similar for immature alfalfa, orchardgrass and SX-16, but lower for bermudagrass. In the mature forages alfalfa showed an appreciably higher rate of cellulose disappearance with maximum losses occurring by 20 hours. Apparent hemicellulose losses after 96 hours of fermentation (based on the 12 hour levels) were 101 and 106% for immature and mature alfalfa, 98 and 56%, respectively for orchardgrass, 84 and 77% for SX-16 and 61 and 44% for bermudagrass. Because cell walls (hemicellulose and cellulose) are a high proportion of the total plant dry matter, especially of warm-season grasses, more consideration should be given to their rate of breakdown and disappearance in programs concerned with improving the nutritive value of forages.

PHYSIOLOGY

Affect of Rumen Insufflation on Rumen Motility - H. W. Colvin, Jr., J. A. Louvier, G. Ishizaki, G. Iwamoto and H. R. Parker, University of California, Davis, California 95616.

Previous insufflation studies are incomplete with respect to the influence of different gases and different pressures on rumen motility, especially as concerns the frequency of primary and secondary contractions.

Rumen cannulas were established in four ewes that had been subjected to the tracheal transection procedure. Rumen motility was measured by connecting the rumen cannula to a Statham pressure transducer by means of rubber tubing. The transducer was interfaced to an electronic recorder. Eructation frequency and volume were measured in order to correlate rumen motility with eructation.

After recording rumen motility and eructation for 30 minutes, the tubing between the mask, the anterior teacheal cannula and the eructated gas collecting equipment were clamped shut and gas insufflated into the rumen to a pressure of 10, 20, or 30 cm HOH. Using this procedure makes the continuous insufflation of gas to maintain the desired intrarumen pressure (IRP) unnecessary. Rumen motility was monitored continuously and eructation measurements resumed one minute after the cessation of insufflation. The gases insufflated included nitrogen, compressed air, carbon dioxide:methane (60:40) and human expired air. There were no fewer than 7 trials/gas/pressure level/sheep.

At high levels of IRP, it is difficult to distinguish secondary from primary contractions; however, this problem was largely circumvented by measuring eructation.

When the rumen was insufflated to 10 cm HOH, there was a linear relationship between IRP and the primary contraction frequency; there were no differences due to type of gas observed. At an initial IRP of 20 or 30 cm HOH, quadratic relationships were observed between IRP and primary contraction frequency. Those gases containing no carbon dioxide, i.e., nitrogen and compressed air, had a significant depressing effect on primary contraction frequency at IRP greater than 16-18 cm HOH.

At all IRP levels tested, the insufflation of nitrogen and compressed air resulted in a greater frequency of secondary contractions than when carbon dioxide:methane and expired air were insufflated. With all gases and all IRP levels tested, the relationship between IRP and secondary contraction frequency was linear.

Our evidence supports the previous neurophysiologic experiments which suggest separate but highly integrated centers in the medulla for primary and secondary contractions. In addition to pressure and tactile influences on these centers, in view of our findings, it now becomes necessary to consider the nature of the gas present in the rumen.

A maximum rumen contraction rate probably exists and is dictated by refractory periods in the motility centers and smooth muscle of the rumen. Because of the influence of IRP and different gases on the frequency of primary and secondary contractions, reciprocal innervation of centers controlling these contractions undoubtedly occurs.

The Site of Pentagastrin-Induced Inhibition of Reticulo-Ruminal Motility - H. W. Chapman, W. L. Grovum and J. C. Newhook, University of Guelph, Canada and Massey University, New Zealand.

Ballons were used to detect reticular contractions in 5 sheep during continuous infusion of pentagastrin into the carotid artery or the jugular vein. The dose (0.6-3.0 microgram/kg⁻¹ h.⁻¹) was adjusted for each sheep so that little or no inhibition was produced during jugular infusion. Injection of the same dose into the carotid in all sheep produced greater inhibition of motility. The amplitude and frequency of contractions were both decreased and in 5 experiments (3 sheep) contractions were completely abolished by carotid infusions. The site of action of the synthetic hormone is apparently cranial since infusion into the cranial vascular supply has more effect than infusion into the drainage. Pentagastrin, cholecystokin, and caerulein had previously been shown to inhibit motility in a dose-related fashion when infused into the jugular vein (Grovm, unpublished). An additional peripheral effect of higher doses is not ruled out by these experiments. To our knowledge this is the first report of a cranial effect of a gastro-intestinal hormone on motility.

Distension and Tactile Input from Stomach Compartments as Satiety Signals in Sheep - W. L. Grovm, Department of Biomedical Sciences, Ontario Veterinary College, University of Guelph, Guelph, Canada N1G 2W2.

The intake of ground and pelleted alfalfa by hungry sheep was depressed by distending the reticulum (0.21 g food in 30 min/ml distension) and the abomasum (0.11 g/ml) but not the rumen. Intake was also suppressed by the presence in the reticulum of a polyethylene probe with collapsed ballons on the end. Since the instrument regularly made the sheep ruminate it must have generated a significant amount of afferent activity from mechanoreceptors in the epithelium of the reticulum or the reticulo-ruminal fold. Intakes were not affected by placing a similar probe made from polyvinyl tubing in the abomasum. The reticulum through its sensitivity to distension and to tactile input may be limiting food intake by sheep.

Ruminal Function of Wheat Pasture Stockers - Gerald W. Horn, Larry A. Bruce and Denzil F. Frost, Animal Science Department (Horn and Frost), Oklahoma State University, Stillwater; and Department of Physiology (Bruce), Southwestern Medical School, Dallas, Texas.

Frothy bloat is a significant health problem of stocker cattle grazed on winter wheat forage of the Southern Great Plains. The objective of these studies was to determine whether wheat pasture bloat of stockers is a primary bloat or a secondary bloat as a result of a reduced ruminal motility and/or reduced functionality of the cardia. Ruminal motility of stocker steers was measured at approximately weekly intervals during the 1975-76 and 1976-77 wheat pasture grazing seasons. Mean amplitudes of ruminal contractions (mm Hg) are listed below. Method of measuring ruminal motility is enclosed in parentheses, mean pre- or post-wheat pasture control values are underscored, and * indicates value is significantly different ($P < .05$) from control value. 1975-76 (pressure transducers surgically implanted in dorsal ruminal sac) 6.7, 23.2*, 24.9*, 23.7*, 16.7*; 1975-76 (water-filled, ballon cannulas attached to an external pressure transducer) 12.9, 18.6, 22.7*, 23.5*, 25.2*, 17.9; 1976-77 (implantable pressure transducers placed in dorsal ruminal sac through small ruminal cannulas) 17.1, 17.3, 16.5, 18.0, 15.9, 15.9, 23.5*, 33.5*, 25.4*, 15.2, 12.4, 26.6*, 11.0*, 15.9, 15.1.

Frequencies of ruminal contractions remained about the same or were increased in steers grazed on wheat pasture as compared with control values. Possible effects of wheat forage on stocker cardiac function was determined by measuring the effect of 1000 x g supernatant rumen fluid on force and frequency of contraction of (1) rat antral tissues and (2) bovine lower esophageal sphincteric (LES) tissues in vitro. (Bruce et al. 1977. IRCS Medical Sci. 5:266). Since spontaneous contractions of these tissues are very irregular, the tissues were chemically stimulated with bethanechol chloride in order to establish baseline contraction data. Mean force (gram-seconds/contraction) and frequency of contractions (contraction/minute) of rat antral tissues in response to 4 ml of control rumen fluid (underscored values) and wheat pasture rumen fluid taken on 4 dates from 4 steers that grazed on wheat pasture were: 6.18 (force), 3.25 (frequency); 2.64*, 5.96*; 6.17*, 6.04*; 2.80*, 6.39*; 4.51, 6.69*. Rumen fluid taken at different dates from wheat pasture stockers had differential effects on the mechanical muscle activity of rat antral tissues. Decreased contraction forces accompanied by increased frequencies of contraction would be indicative of spasmodic, functionally-impaired contractions. Mean contraction forces of bovine LES tissues in response to control and wheat pasture rumen fluid were 13.3 ± 3.8 and 30.1 ± 10.6 respectively. Frequency of contractions was of no consequence since all LES tissues responded with a single tetanic contracture for the entire 10 minutes recording period. These studies indicate that ruminal motility is not decreased in stockers grazed on wheat pasture, but that cardiac and/or esophageal function may be altered to the extent of being an etiologic factor in the bloating of wheat pasture stockers.

MICROBIOLOGY

Ruminal Oxalate Degradation: Studies with Continuous and Enrichment Cultures - M. J. Allison, K. A. Dawson and H. M. Cook, National Animal Disease Center, Post Office Box 70, Ames, Iowa 50010.

The rate of oxalate degradation by mixed ruminal populations from a cow or from sheep fed alfalfa hay was consistently less than 0.2 uMoles/ml/hr. When the mixed population was grown in a fermenter under continuous culture conditions, the rate of oxalate degradation increased 10-40 fold if the fermenter was gradually adapted by feeding it oxalate containing plant material (Halogeton glomeratus) or alfalfa plus gradually increasing quantities of sodium oxalate. Selection of populations with high rates of oxalate degradation also occurred when the fermenter was fed a medium that contained glucose, rumen fluid, trypticase and salts plus an increasing gradient of sodium oxalate. Selection of oxalate degrading microbes in vitro thus appears to resemble the selection observed in cattle or sheep.

Enrichment cultures which contained a less diverse microbial population were obtained by serial transfer in a 10% rumen fluid medium with oxalate as the only added fermentable substrate. In these cultures oxalate was usually not degraded during the first 2-3 days of incubation, but when oxalate degradation occurred it was accompanied by methanogenesis (1uMole CH_4 /3.8 uMoles of oxalate degraded). When formate was added to these cultures, methanogenesis occurred but oxalate was not degraded. Inhibitors of methanogenesis inhibited oxalate degradation in both the enrichment batch cultures and the continuous cultures.

None of the isolates obtained from oxalate adapted animals or from enrichment cultures degraded oxalate in pure culture. Experiments to test these for oxalate degradation in co-culture with hydrogen scavenging methanogens and desulfovibrio proceed but have not yet yielded positive results.

Our results are consistent with the hypothesis that the oxalate degraders live in an intimate association with methanogens. If this is true, interesting questions are posed because of the limited energy available in oxidation of oxalate to CO₂ and H₂. An alternate hypothesis is that the oxalate degrader is a methanogen other than Methanobacter ruminantium strain M1, or the strains of methanogens we have isolated from an oxalate adapted continuous culture.

Application of a Semi-Continuous Technique to Study the Effect of Monensin on the Rumen Fermentation - D. E. Short, M. P. Bryant, F. C. Hinds and G. C. Fahey, Departments of Animal Sciences, Dairy Science and Microbiology, University of Illinois, Urbana, Illinois 61801.

A semicontinuous culture technique for the study of rumen-like fermentation was developed which combined the simplicity of batch culture with many of the capabilities of continuous culture. Fifty ml of buffered medium containing cellulose (0.3%), casein hydrolysate (0.3%), starch (0.1%), glucose (0.1%) and clarified rumen fluid (10.0%) was inoculated with an equal volume of strained rumen fluid. The incubation vessel consisted of a 125ml Erlenmeyer flask fitted with an aluminized mylar gas bag. Cultures were constantly shaken and incubated under a CO₂ gas phase at 39½C. At 24 hour intervals half the contents of each culture was transferred to a similar culture vessel containing 50 ml of fresh medium. "Steady state" or "steady end point" conditions were obtained no later than six days after the start of culture. The addition of monensin (1 ppm) to "steady state" cultures of microorganisms which had never been exposed to monensin resulted in dramatic decreases in cellulose digestibility (P<0.01) and cell yield (P<0.025). No difference was found, due to monensin (1, 2, or 4 ppm), in cellulose digestibility or cell yield when inoculum was obtained from sheep receiving monensin in their diet. Hydrogen production was unchanged whereas methane production was decreased (P<0.005). Ammonia nitrogen levels decreased linearly (P<0.001) whereas the level of peptide (P<0.001) and alpha amino acid nitrogen (P<0.05) increased linearly as the level of monensin increased. The data suggest that monensin decreases proteolytic and deaminase activity.

Isolation and Characteristics of a Skatole Producing Lactobacillus sp. from the Bovine Rumen - M. T. Yokoyama, J. R. Carlson, and L. V. Holdeman, Department of Animal Husbandry, Michigan State University; Department of Animal Sciences, Washington State University; The Anaerobe Laboratory, Virginia Polytechnic Institute and State University.

Skatole (3-methylindole) is a common metabolite found in the feces of all mammalian and avian species. Its occurrence has also been shown in the bovine rumen. In elucidating the etiology of bovine pulmonary edema and emphysema induced by L-tryptophan administration into the rumen, skatole was found to be the metabolite responsible for the syndrome. The conversion of L-tryptophan to skatole in the

rumen occurs as a two-step process involving (1) the degradation of L-tryptophan to indoleacetic acid (IAA) and (2) the decarboxylation of IAA to skatole. A bacterium that is capable of decarboxylating IAA to skatole has been isolated from an L-tryptophan enrichment of bovine rumen fluid. The bacterium is a gram-positive, non-motile, non-sporeforming, pleomorphic rod. Deep colonies are minute, lenticular, cream to white in color, with entire margin and smooth surface. It is an obligate anaerobe, and will grow at 37°C, but not at 15°C or 45°C. Initial growth in liquid cultures appear as a white swirl, turning to a ropy, brown sediment after 3 days. Isolated strains produce predominately D- (-) - lactic acid, with smaller amounts of L- (+) - lactic acid and acetic acid from sugar. Fructose, glucose, maltose, and mannose were fermented by all strains, salicin by two strains and sucrose by one strain. All strains were positive for esculin hydrolysis and arginine hydrolysis. Although skatole is produced from IAA, all of the strains demonstrated a negative indole test, because they cannot convert L-tryptophan directly to skatole. Addition of sugars to media containing IAA reduced skatole production; with sucrose, maltose, and mannose completely inhibiting the reaction of specific strains. Although isolated from the bovine rumen, the habitat of this bacterium may extend to the intestinal tract of mono-gastric species. Based on their physiological, biochemical, and metabolic characteristics the skatole producing strains are being assigned to the genus Lactobacillus.

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Urease and Enzymes of Ammonia Assimilation in Two Species of Rumen Bacteria - C. J. Smith, D. M. Schaefer, R. B. Hespell, and M. P. Bryant, Department of Dairy Science, University of Illinois, Urbana, Illinois 61801.

The enzymes of ammonia assimilation in Bacteroides amylophilus strain H-18 and Selenomonas ruminantium strain D were investigated using batch culture, continuous culture, and nutrient limited batch culture. Both organisms demonstrated an 8-15 fold increase in the levels of glutamine synthetase as ammonia became the limiting substrate for growth. Asparagine synthetase was also induced by limiting ammonia concentration but was less important for ammonia assimilation. Glutamate dehydrogenase was the major ammonia incorporating enzyme when ammonia was in excess and its activity decreased during ammonia limited growth. Glutamate synthase activity was very low and did not change during and growth condition tested; the significance of this is discussed. Urease activity in S. ruminantium was highest when ammonia was limiting and the regulation of urease by glutamine synthetase is considered.

Digestion of Urea and Epithelial Cells by the Microbial Flora Attached to the Bovine Rumen - K. J. Cheng and J. W. Costerton, Research Station Agriculture Canada, Lethbridge, Alberta, Canada, and Department of Biology, University of Calgary, Calgary, Alberta, Canada.

Urease-producing bacteria are responsible for the conversion of the urea, entering the rumen in feed and saliva, or by diffusion across the rumen wall, into ammonia.

This ammonia is necessary for the formation of bacterial protein which is finally absorbed by the animal as amino acids in the gastro-intestinal tract. This essential conversion of urea to ammonia in the reticulo-rumen is carried out by facultative anaerobic bacteria that are adherent to the epithelium surface. The urease activity found in rumen fluid represents the enzyme activity of formerly attached bacteria that have been released into the rumen by natural sloughing off of the stratified squamous epithelium. Urease activity has been quantitated using epithelial tissue samples. The adherent ureolytic bacteria have also been isolated and identified and their production of urease has been shown to be constitutive. This study finally locates the elusive urease-producing bacterial population of the rumen and it also provides a unique example of an adherent microbial population whose enzyme activity plays an essential role in the physiology of a mammalian system. Further, examination of the bacteria colonizing of keratinized cells being sloughed off from the surface of the stratified squamous epithelium of the rumen revealed that these dead cells were being digested by the adherent bacteria. This utilization of dead epithelial cells by the adherent bacteria represents an important nutrient and energy conservation mechanism in the ruminant.

Nutrition of Anaerobic Mycoplasmas from the Rumen - I. M. Robinson and M. J. Allison, National Animal Disease Center, Post Office Box 70, Ames, Iowa 50010.

Both sterol- and nonsterol-requiring strains of obligately anaerobic mycoplasmas have been isolated from the rumens of sheep and cattle. These organisms were placed in a new genus Anaeroplasma, and have cultural, biochemical and serological properties that distinguish them from previously reported bovine mycoplasmas. Soluble starch stimulates growth of all strains. The metabolic products from starch include acetate, formate, lactate, succinate, ethanol, H₂ and CO₂. In medium containing starch, trypticase, minerals, vitamins, volatile fatty acids and cholesterol, growth of Anaeroplasma abactoclasticum, type strain 6-1, was dependent upon the addition of lipopolysaccharide (LPS). The LPS growth factor was destroyed by alkaline hydrolysis, but after mild acid hydrolysis (0.1 N formic acid, 100 C/60 min), it was extracted into the organic phase by chloroform-methanol (2:1). Growth was obtained when LPS was replaced by phosphatidyl choline (PC) and to a lesser degree by phosphatidyl ethanolamine, phosphatidyl glycerol, diphosphatidyl glycerol, and lysophosphatidyl choline. Glycerolphosphoryl compounds without esterified fatty acids or free fatty acids in various combinations with glycerol and choline would not replace the intact phospholipids. Anaeroplasmas (strain 6-1) grew with either oleic or linoleic esterified to glycerolphosphoryl choline was added to the growth medium but did not grow when the esterified fatty acids were saturated fatty acids (lauric, myristic, palmitic or stearic acids). The failure of Anaeroplasma (strain 6-1) to incorporate C-14 labelled acetate into cellular constituents during growth, suggests that it is unable to synthesize significant quantities of long-chained fatty acids from acetate. Definition of the lipid requirement made it possible to grow the organisms in a completely defined medium containing PC, cholesterol, acetate, amino acids, vitamins, and minerals. All strains of Anaeroplasma appear to require phospholipids and either PC or LPS meets this need.

Effect of Short Term Chilling of Rumen Contents Upon Viable Bacterial Numbers -
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Anaerobic storage of whole rumen contents at 0°C for 8 and 24 hr resulted in viable colony counts which were 113 and 92%, respectively, of the colony count obtained with an unstored sample. Numerous factors were investigated as possible explanations for the increase in bacterial numbers observed after 8 hr. Growth and multiplication of bacteria, sub-sampling of rumen contents, susceptibility to oxygen, lysis of protozoa with subsequent release of viable intercellular bacteria, and rumen sampling time did not appear to be involved. The effect of storage time at 0°C indicated that a significant increase in colony count occurred after 4 hr. Based on these data, 6 hr was used as the standard cold storage period. Circumstantial evidence supported the hypothesis that the increase in viable counts from cold storage resulted from either a breakdown in bacterial attachment to particulate matter, a breakdown in bacterial clumping, or both. Addition of a surfactant to the anaerobic dilution solution (ADS) significantly increased total colony count of rumen contents to a similar extent as chilling on ice for 6 hr. However, use of the surfactant containing ADS with samples stored 6 hr at 0°C also gave a significant increase in total colony count. Thus, storage of rumen contents for 6 hr at 0°C appears to alter or breakdown the material responsible for cell to cell or cell to particulate matter attachment. An additive effect upon this breakdown occurs when a surfactant is added to the dilution solution. In general, increases in colony count ranged from 0 to 300%.

The Effects of Total Nutrient Starvation on the Viability and Physiology of
Megasphaera elsdenii - R. W. Mink, R. B. Hespell and J. A. Zeigler, Department of Dairy Science, University of Illinois, Urbana, Illinois 61801.

Glucose-cultured Megasphaera elsdenii was grown in batch culture, harvested, and the cells were subjected to starvation in a mineral buffer solution after being washed by centrifugation with this buffer. Cells harvested at mid-exponential growth phase and late exponential growth phase exhibited an 85% decrease and 26% decrease in viability, respectively, after 24 hours of starvation. A simultaneous decrease in cellular RNA (51% and 35% respectively) and carbohydrate levels (31% and 39% respectively) occurred along with only modest decreases in protein and dry weight levels. The data suggest that storage material and ribosomes are degraded simultaneously under conditions of total starvation. Mixed rumen populations, when starved, exhibited a drop of 95% of initial viability in 24 hours along with a concomitant drop in DNA (41%) and greater drop in RNA (57%) suggesting some endogenous utilization of RNA by most organisms during starvation. Tentatively, it would appear that rumen bacteria have little ability to withstand long-term starvation as compared to other bacteria which have been studied.

Variations in Morphological Types of Rumen Bacteria Attached to Forage Fiber -
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The interrelationship of various types of rumen bacteria that associate with the fiber of forage grass leaves during digestion has been studied using transmission electron microscopy. For leaves of Coastal bermudagrass (CBG) and Kentucky-31 tall fescue (Ky-31), about 70% of the attached bacteria degrading forage fiber were of two distinct types: (1) an encapsulated coccus ('ec') closely resembling Ruminococcus spp. of the rumen based on the regular, oval to coccoid shape, size of 0.8 to 1.8 microns, abundant electron-dense capsule, appearance in diploids or short chains, gram-positive cell wall anatomy, digestion of rigid fiber, and similarity to micrographs of pure cultures of R. spp. and (2) an irregular-shaped bacillus ('ib') resembling Bacteroides succinogenes based on a pleomorphic shape due to the lack of an apparent rigid cell wall, size of 1.0 to 2.0 x 0.4 microns, lack of a capsule, close attachment to fiber during degradation, and similarity to pure cultures of B. s. The other 30% of the bacteria that associated with fiber was comprised of a heterogeneous group of bacilli and cocci not closely attached to the fiber and unlike 'ec' and 'ib'. For CBG, the percent of 'ec' was significantly higher ($P \leq .02$) than on Ky-31 (43% vs. 10%). Conversely, significantly ($P \leq .02$) more 'ib' attached to the cell walls of Ky-31 than to those of CBG (61% vs. 23%). Chemical analysis of leaf fiber revealed 47 mg/g more xylan in CBG than in Ky-31. This difference in constituents could have elicited a higher proportion of 'ec' since R. spp. degrade xylan while Bacteroides succinogenes does not. For leaves of other forage grasses (i.e., bahiagrass, digitgrass, orchardgrass, and timothy), 'ec' and 'ib' also comprised about 70% or more of the attaching, fiber-digesters. However, a consistent relationship between the proportions of attached bacteria types and the ease of tissue digestion in forage leaves did not occur. For example, both CBG and orchardgrass, which differed widely in the ease of their tissue digestibility, had high percentages of 'ec'. In bermudagrass cultivars, the initial high ratio of 'ec' to 'ib' at 6 h changed so that proportionately more 'ib' were attached to the fiber by 24 h. Inherent characteristics of the leaf tissues appeared to effect differences in the digestion of tissue with time more than did proportions of the types of rumen bacteria that were elicited and attached to the forage fiber.

Morphological and Chemical Characteristics of the Cell Envelope of Bacteroides succinogenes - C. W. Forsberg, Department of Microbiology, College of Biological Science, University of Guelph, Guelph, Ontario, Canada N1G 2W1; and J. W. Costerton, Department of Biology, University of Calgary, Calgary, Alberta, Canada T2N 1N4.

Many hydrolytic enzymes of rumen bacteria including proteases, amylases, and cellulases are reported to be either partially or wholly bound to the bacterial cell. To gain an understanding of how readily the bound hydrolytic enzymes interact with particulate substrates, a knowledge of the cell envelope ultrastructure and chemistry is essential. To attain this objective with the cellulolytic bacterium, B. succinogenes, we have devised a method for fractionating the cell envelope of this bacterium.

When exponentially growing cells of B. succinogenes were washed in a salts solution corresponding to that in the culture medium and then resuspended successively in 0.5 M NaCl, membrane vesicles were released that accounted for 3.5% of the cell dry weight. Subsequent successive resuspensions of the cells

in 0.5 M sucrose resulted in the release of membranous fragments and non-sedimentable materials which accounted for 8.9% and 3.9% of the cell dry weight respectively. Analysis of the sedimentable and non-sedimentable materials revealed that all contained protein, carbohydrate and phosphorus, but little hexosamine. All fractions exhibited similar compositions with the exception of the membranous fraction released during the third suspension of cells in sucrose, which was lower in protein. The results suggest that the membranous fractions were released from the outer cell wall layer of the cell. The non-sedimentable fraction released into sucrose after fragmentation of the outer membrane presumably originated from an underlying location between the outer and cytoplasmic membranes. This fractionation technique should prove to be valuable for studies on the cellulase of B. succinogenes and may also be suitable for fractionation of the cell envelope of B. amylophilus, thus enabling the definitive location of the surface bound amylase and protease activities of that bacterium.

Isolation and Identification of Adherent Bacteria ("Epimural" Bacteria) from the Ovine Rumen Wall - Lorna J. Mead and G. A. Jones, Department of Dairy and Food Science and Microbiology, University of Saskatchewan, Saskatoon, Saskatchewan, Canada.

Duplicate pieces of wall tissue were cut from four sites in the rumen of sheep fed hay or a hay-grain ration, and washed six times in an anaerobic mineral solution; one piece in each case was examined by scanning electron microscopy and the other piece used to obtain isolates of the adherent flora which were subsequently characterized. Quantitation of viable bacteria in the washings showed that only the more firmly adherent bacteria remained attached to the epithelium after the washing process, but visualization of the tissue revealed that these bacteria represented many morphological types. Of the 152 isolates obtained, most were morphologically consistent with the types seen in situ and 92% were assigned to genera and species of common occurrence in the rumen, including Bacteriodes, Borrelia, Butyrivibrio, Lachnospira, Propionibacterium, Ruminococcus, Selenomonas, Streptococcus and Succinivibrio. Characteristics of eleven strains did not correspond with those of previously described rumen bacteria. The results showed that the existence of a taxonomically specific dominant flora in association with the rumen epithelium is unlikely but did not rule out the possibility that the adherent flora may play a specific functional role in the rumen ecosystem.