REPORT ON THE XVI CONFERENCE ON RUMEN FUNCTION

NOVEMBER 11-13, 1981

AMERICANA CONGRESS HOTEL (PICK CONGRESS)

520 SOUTH MICHIGAN BOULEVARD

CHICAGO, ILLINOIS

REPORT ON THE XVI CONFERENCE ON RUMEN FUNCTION
NOVEMBER 11-13, 1981
AMERICAN CONGRESS HOTEL (PICK CONGRESS)
520 SOUTH MICHIGAN BOULEVARD
CHICAGO, ILLINOIS

TABLE OF CONTENTS

REPORT ON XVI CONFERENCE ON RUMEN FUNCTION
NOVEMBER 11-13, 1981
AMERICANA CONGRESS HOTEL (PICK CONGRESS)
520 SOUTH MICHIGAN BOULEVARD
CHICAGO, ILLINOIS

For the purpose of discussion, the program was divided into four panels. The identity of the panels and the chairman for chairmen of each are as follows:

GENERAL CHAIRMAN -------Clyde R. Richards----------CSRS, USDA

(a)	Agronomic Panel	J. C. Burns, (NC)
(b)	Physiology-Physiopathology	A. D. McGilliard, (IA) and W. M. Wass, (IA)
(c)	Microbiology Panel	M. P. Bryant, (IL) and R. B. Haspell, (IL)
(d)	Nutrition	J. T. Huber, (MI)

AGRONOMIC

Effect of Aluminum on Magnesium and Calcium Utilization in Relation to Grass Tetany - Vivien G. Allen, D. L. Robinson, Louisiana State University, Baton Rouge, Louisiana 70893, F. G. Hembry, and J. P. Fontenot, Virginia Polytechnic Institute and State University, Blacksburg, Virginia 24061

Grass tetany is a metabolic disorder of ruminants characterized as a physiological magnesium deficiency. Recent research has indicated that aluminum may be involved in the etiology of grass tetany. During five years of research, rumen ingesta of cattle which died from grass tetany contained a minimum of 1000 ppm aluminum. Forage samples collected from pastures at the time of grass tetany outbreaks commonly contained 1000 to 14,000 ppm aluminum. The form of aluminum accumulated by forage and the extent of aluminum contributed by soil contamination are unknown. However, ryegrass (Lolium multiflorum, Lam) grown in nutrient solution with aluminum-citrate has accumulated up to 2,000 ppm aluminum in the top growth. When 8,000 ppm aluminum, as sulfate, was added to ryegrass, rumen fluid and buffer solution in a in vitro experiment, the solubilities of magnesium and calcium were depressed by 56 and 74% respectively. Bales of ryegrass hay containing aluminum ranging from 100 to 5000 ppm aluminum contained similar total magnesium and calcium. The water soluble magnesium and calcium, however, varied inversely with the total aluminum content. Although the aluminum was not water soluble, it was 80% solubilized by in vitro digestion and treatment with NDF solution, suggesting the potential for some solubilization to occur within the ruminant digestive tract. Four rumen cannulated steers, maintained on Bermudagrass hay (Cynodon dactylon, L.) were dosed once daily via cannula with 0,4000 ppm aluminum as sulfate, 2000 ppm manganese as sulfate, or the combined aluminum and manganese treatments. Serum magnesium declined in aluminum-treated steers within 24 hours after treatment began and was 32% lower than controls at the end of four days. Serum magnesium returned to normal within three days after treatments were terminated. Serum calcium was not affected. Manganese had no effect. In a second experiment,

eight lactating crossbred cows, maintained on Bermudagrass hay, were randomly allotted to three treatment groups. Animals were dosed once daily for four days by stomach tube with 0, 4000 ppm aluminum or 4,000 ppm aluminum plus 2,000 ppm manganese. Aluminum and manganese were in the sulfate form. Aluminum treatment resulted in serum magnesium values signficiantly lower than controls. Crossbred wethers were dosed twice daily in divided doses via rumen cannula with 0, 1000 or 2000 ppm aluminum as chloride, sulfate or citrate. Six animals per treatment were maintained on fescue hay (Festuca arundinacea, L.). A ten-day adjustment period was followed by a ten-day treatment period with daily collection of blood samples via jugular puncture. Treatment with 1000 ppm aluminum regardless of form had no effect on either serum magnesium or calcium. Treatment with 2000 ppm aluminum resulted in lower serum magnesium values in all aluminum treatment groups. Aluminum citrate resulted in the lowest serum magnesium. Animals receiving aluminum-citrate also excreted detectable levels of aluminum in the urine. Serum calcium levels were not affected by any treatment.

Enhancement of the Value of Low Quality Roughages by Ensiling with Animal Wastes - J. P. Fontenot, W. D. Lamm, W. A. Samuels and J. Moriba, Virginia Polytehnic Institute and State University, Blacksburg, Virginia 24061

Chemical treatment has been used to improve the digestibility of low quality roughages. The four chemicals which have been most commonly used are sodium hydroxide, ammonia, calcium hydroxide and potassium hydroxide. Use of ammonia also increases the crude protein of the roughage. Considerable research has been conducted on ensiling of low quality roughages with animal wastes. This practice may be useful in enhancing the value of low quality roughages, in addition to providing a method for recycling of animal waste. During the last few years experiments were conducted on ensiling swine waste and orchardgrass hay, cattle waste and rye straw, cattle waste and corn stover, caged layer waste and sugarcane bagasse, and caged layer waste and corn stover. Satisfactory ensiling was obtained with optimum proportions of these materials. Ensiling of the wastes with these roughages has increased the crude protein content. The increases have varied, depending on the kind of roughage and kind and level of waste. Generally, apparent digestibility of the ensiled mixtures was higher than that of the roughage alone. Improvements in digestibility were usually greater when waste from cattle fed high concentrate diets was used compared to waste from cattle fed high roughage diets. For example in one experiment organic matter digestibility was 32.9% for straw, compared to 45.4 and 39.3% for ensiled mixtures of straw and high concentrate and high roughage waste, respectively. Usually, higher digestibility values were obtained for ensiled mixtures containing 60% waste, compared to 40%. Improvements in digestibility by ensiling the low quality roughages with animal waste compare favorably with previously reported results with chemical treatment. Combining waste with the roughages also increases the concentrations of most of the required minerals.

Rate and Extent of Nutrient Disappearance from Forages in the Rumen - J. E. van Eys and R. L. Reid, Division of Animal and Veterinary Sciences, West Virginia University, Morgantown, West Virginia 26506

Impaired mineral availability and metabolism have been implicated in the low performance of animals grazing tall fescue. Minerals are known to exert their effects in a number of ways but these are incompletely understood; in particular, the quantitative relationship between concentrations and

availability of minerals in forages and animal digestibility and intake. The reported study was conducted to examine the effects of rate and extent of mineral release on degradation of O.M. in the rumen.

Extent and rate of disappearance of NDF, DM, N, K, P, Ca, Mg, S, Cu and Zn were measured for three forages at six growth stages by means of the nylon bag technique. Forages were Ky.31 tall fescue (TF), Kenhy fescue (KN) and a red clover - Ky.31 tall fescue mixture (RC) in which red clover was the dominant (50-80%) component. Herbage samples were incubated in nylon bags for periods up to 48 hour in fistulated steers grazing replicated pastures. Extent of disappearance was calculated as percent of initial concentration and rate constants as percent/hour of the potentially degradable or available fraction.

NDF degradation occurred at higher rates in RC ($p<.1$) during early tages of digestion while grasses had lower rates but continued digestion of CW after RC had reached maximum extent of NDF degradation. Lag in NDF degradation reflected the importance of rumen microbial adaptation to high fiber diets. Lag phase decreased with increases in CWC content of herbage. Potential degradability of NDF for RC was lower ($p<.01$) than that of grasses, which showed no differences. D. M. disappearance was large during the first 3 hours of digestion, ranging from 26.9% to 55.5%. RC had higher ($p<.05$) rates of D.M. disappearance between 3 and 48 hours, resulting in a higher ($p<.05$) extent of D.M. degradation at 48 hours. Marked effects of plant maturity in decreasing rate and extent of D.M. and NDF degradation ($p<.0001$) were noted.

Differences between forages in rates of N and mineral release were small, but the extent of disappearance at 24 and 48 hours was larger for N, Ca, Mg and S in RC. The various elements differed considerably in their mode of release and in the effects of plant maturity on rates of solubilization. High proportions (>60%) of K, P and Mg were released during the first 3 hours of digestion, with small losses thereafter. N, S, and Cu release during the first 3 hours was lower (40-75%). Initial disappearance rates of Zn and Ca were even lower. Elements reached maximum levels of disappearance between 12 and 48 hours. Highly soluble elements such as K, P and Mg reached maximum disappearance early, at approximately 12 hours, while release of Ca and S continued after 24 hours. Forage maturity caused large decreases ($p<.0001$) in disappearance of N, S, Ca and Mg between 3 and 48 hours; P showed an increase in rate with maturity, while K, Cu and Zn showed no clear effects.

Following 12-24 hours of digestion the ratios of N or mineral concentrations to NDF in the residue increased. Correlation analysis indicated significant partial correlations between residual NDF at 48 hours digestion and the ratios of unavailable S, K, Ca and Zn in undigested cell wall. Linear regression equations indicated particularly close relationships between extent of CW

degradation and the concentrations of S, K and Mg in undigested CW. Significant ($p<.05$) partial correlation coefficients between residual NDF and all elements except P were observed at 12 hours. Residual Mg was also correlated ($p<.05$) with residual NDF at 3 and 6 hours, and Ca at 6 and 24 hours. Residual S was correlated with extent of NDF degradation at all stages of digestion.

Step wise regression analysis relating DM or NDF digestion to rate and extent of nutrient disappearance resulted in the following equations ($p<.001$):

Residual DM at 48 hours to rate of nutrient disappearance,
$Y = 32.9 - 168.6N + 42.8P - 164.3S + 112.9\ Zn$ ($R_2 = .39$)
Residual NDF at 48 hours to rate of nutrient disappearance,
$Y = 53 + 50.2P - 251.3\ Ca - 119.4\ Mg + 138.4\ Zn$ ($R_2 = .39$)
Rate of NDF disappearance was related only to rate of S disappearance, $Y = .02 + .75\ S$ ($R_2 = .38$)

Extent of NDF degradation was related ($p<.001$) to concentrations of lignin, K, Mg and Zn in herbage and rate to NDF disappearance to concentration of N and Ca.

Pro- and Anti-Quality Factors in Tall Fescue Improvement - R. C. Buckner, J. A. Boling, L. P. Bush, R. W. Hemken, P. B. Burrus, II, and J. A. Jackson, USDA-ARS and University of Kentucky, Lexington, Kentucky 40506

Tall fescue (Festuca arundinacea Schreb.) contains several alkaloids. Studies at the University of Kentucky showed that perloline was a principal alkaloid in tall fescue during the summer, and that concentration peaked at a time when cattle grazing tall fescue pastures frequently have severely reduced performance (summer syndrome or summer fescue toxicosis). In vitro fermentation studies showed a concentration-dependent inhibition of cellulose digestion, volatile fatty acid production and growth of ruminal cellulolytic bacteria by perloline. Isolated perloline fed to lambs resulted in decreased cellulose digestion, nitrogen retention, and increased body temperature. Therefore, annual ryegrass (Lolium multiflorum Lam.) x tall fescue hybrid derivatives (2n=6x=42) were used to develop GI-306 and GI-307 experimental synthetic strains. GI-306 had high perloline content; while, GI-307 had low perloline content and improved succulence and digestibility.

A summer grazing study showed that gains of cattle on GI-307 were lower than those on Kentucky 31, Kenhy and GI-306. In a two-year study with lactating dairy cows fed Kentucky 31, GI-306 or GI-307 soilage, cows consuming GI-307 had the lowest intake and milk production, and highest respiration rate. Thus, in selecting for low perloline content, compound(s) more toxic and detrimental to animal performance were increased in GI-307. Further studies were conducted with dairy calves in environmental rooms using GI-307 to determine the influence of temperature on potential toxicity. Calves were fed July-cut GI-306 and GI-307 at 10-13, 21-23 and 34-35 C. Intake and weight change in calves decreased, while body temperature and respiration rate increased on GI-307 at 34-35 C resulting the temperature x forage interactions.

Selection for low perloline in GI-307 resulted in concomitant increases in N-acetyl and N-formyl loline (pyrrolizidine) alkaloids. Epichloe typhina, an

endophytic fungus of cool-season grasses, was associated with the occurence of the loline alkaloids in GI-307.

Parental clones of GI-307 were grown in two separate isolated polycross blocks. Clones in one block were untreated and those in a second block had been previously treated with benomyl, a systemic fungicide, for at least 10 days in a 1:2000 benomyl:soil mixture in a greenhouse to remove E. typhina from the clones before transplanting them to the field. Equal amounts of seed of each clone of treated and untreated blocks were blended to form GI-307 strain and used to establish 2.2 and 3.3 ha swards, respectively. Hay from swards established to seed of benomyl-treated and untreated clones had loline alkaloid levels of 115 and 875 ug/g and infestation estimates by E. typhina of 44 and 95 percent, respectively. Young cattle under high ambient temperature stress (32 C) when fed hay containing 115 ug/g lolines did not have "summer syndrome" symptoms, and had significantly better feed intake and performance than those receiving hay containing 875 ug/g lolines.

Thus, toxic factor(s) in GI-307 are present from May through October, N-acetyl and N-formyl loline concentrations are related to E. tyhphina infestation, and toxic effects to the animal are potentiated by evaluated environmental temperature. Therefore, breeding efforts to improve the pro-quality factors (succulence and digestibility) during summer were negated by the anti-quality components in the grass.

PHYSIOLOGY-PHYSIOPATHOLOGY

A Technique for Biopsy of Rumen Mucosa - W. M. Wass, J. R. Thompson and A. E. Ledet, College of Veterinary Medicine, Iowa State University, Ames, Iowa 50010 and Kurt Walter-Hansen, College of Veterinary Medicine, University of Minnesota, St. Paul, Minnesota 55108

Changes in rumen mucosa associated with the feeding of high energy diets have been previously described. Efforts to study the development of the lesions, however, have been hampered by the necessity for surgical fistulation of the animals or have been limited to observations made at slaughter or at necropsy.

Clinical examination of affected groups or investigations into the pathogenesis of the disease should be greatly enhanced by the development of a rapid, non-invasive technique for collection of samples of mucosa suitable for histopathological evaluation.

Attempts to use a modified gastric suction biopsy instrument similar to those available for human medical use were unsuccessful due to the coarse fibrous nature of rumen ingesta and the generally filled condition of the rumen compartments.

Repeatable successful sampling of the rumen mucosa has been accomplished with a newly designed instrument that approximates the dimensions of a standard large ruminant stomach tube.

The sampler head of the biopsy instrument is metallic and encloses a hinged mechanism with cutting cusps that are activated by mechanical force transmitted by a standard choke wire that is enclosed throughout its length by reinforced tygon tubing.

The metallic edges are carefully rounded so as to be atraumatic and the instrument can be passed orally much like a stomach tube through a standard oral speculum.

The procedure is rapid and sample results are readily reproducible.

Effect of Dose and Tissue Glutathione Concentrations on In-vivo Covalent Binding and 3-Methylindole-Induced Lung Injury in Goats - M. R. Nocerini, J. R. Carlson, R. G. Breeze, Z. D. Abraham, Washington State University, Pullman, Washington

Three-methylindole (3MI) causes acute pulmonary edema and emphysema in ruminants as a result of microsomal mixed function oxidase (MFO) metabolism. In-vitro evidence indicates that reactive intermediates are formed which bind covalently to cellular macromolecules. Glutathione (GSH) and cysteine (CYS) as well as MFO inhibitors reduce covalent binding of ^{14}C-3MI in-vitro. To further investigate the mechanisms of 3MI-induced lung injury, we studied the effect of 3MI dose and GSH status on in-vivo covalent binding and lung injury in goats. Groups of 4 goats were given 2 hr jugular infusions of 3MI containing ^{14}C-3MI. In experiment 1, doses of 0.01, 0.02, 0.03, and 0.05 g 3MI/kg BW were used and goats were killed at 4 hr; covalent binding was determined by exhaustive extraction and scintillation counting. In experiment 2, goats were pretreated with CYS to sustain GSH levels, or with diethylmaleate (DEM) to deplete GSH levels prior to infusion of 0.04 g 3MI/kg BW. Covalent binding in lung, liver and kidney, and lung injury were evaluated. Covalent binding was proportional to dose (r^2 = .96) in the lung, but not in liver or blood. CYS pretreatment resulted in longer survival times, less severe lung injury, and lower ($P<.05$) covalent binding than non-pretreated goats infused with 3MI. DEM pretreatment resulted in increased ($P<.05$) covalent binding, shorter survival times, and more severe lung injury. The percent of dose bound in the whole lung was 0.33 $\pm$ 0.05, 0.68 $\pm$ 0.07, and 1.75 $\pm$ 0.15 for the CYS, 3MI control, and DEM groups, respectively ($P<.05$). Covalent binding was higher ($P<.05$) in the lung compared to liver and kidney. Unexpectedly, DEM was toxic to goats at a dose of 0.6 ml/kg BW but did not cause lung injury. Both DEM and 3MI depleted tissue GSH concentrations and the effect of 3MI was greatest in the lung. These experiments demonstrate that covalent binding of ^{14}C-3MI occurs in in-vivo and that there is a relationship between covalent binding and lung injury, organ specificity, and dose. These findings support the hypothesis that 3MI-induced lung injury results from the formation of activated intermediates and that GSH may play a role in detoxification of these metabolites.

Supported in part by NIH grant HL-13645.

Metabolic Measures Associated with Fatty Liver - Jim Liesman, R.S. Emery, Brian Gerloff and T. Herdt

The purpose of this study was to look at the relationship of liver inositol, serum lipids and hepatic triglyceride lipase with liver fat in the lactating Holstein dairy cow. Also comparisons were made between seven animals fasted for 6 days and animals with displaced abomasums (DA) and liver fat greater than 15% (DA > 15%, n=17). For controls, DA animals with less than 15% liver fat (DA < 15%, n=33) and normal lactating cows 2 to 45 days postpartum (n=20) were used. Dextran sulfate perciptable lipoproteins (DSPLP) were determined on serum that had been frozen. Hepatic triglyceride lipase (HTGL) activity was determined by subtracting plasma lipase activity at zero time from activity 5 minutes after injecting 10 units of heparin per kg body weight. Extra hepatic lipoprotein lipase was inhibited with .85M NaCl in the assay.

	DA <15%	DA >15%	Normal	Fasted
Liver fat, %	8.0*	22.1**	7.7*	14.4*
uM inositol/g liver	1.45	1.17**	1.92*	1.65
Serum cholesterol (mg/dl)	88.5	64.9**	109.1*	134.9*
DSPLP cholesterol (mg/dl)	19.2*	10.0**	19.6*	26.7*
Serum triglycerides (mg/dl)	16.6	18.1	14.9	32.7***
DSPLP triglycerides (mg/dl)	3.8*	2.2	3.1	5.5*
HTGL (uM FA/ml plasma-hr)	1.12	0.78**	2.02*	0.69**

* Different from DA > 15% (P<.025).
** Different from normal (P<.025).
*** Different from DA > 15% and normal (P<.025).

No strong correlations were found between the variables measured and liver fat percent. The fasted and normal groups had significantly greater serum and DSPLP cholesterol levels than the DA >15%. Serum and DSPLP phospholipids showed a similar pattern. Serum and DSPLP triglycerides (TG) had a higher concentration in fasted animals than in the control or DA >15% groups. This is probably an indication of increased lipoprotein secretion by the liver or since milk production was greatly suppressed in the fasted animals, slower TG turnover in the serum. Differences found in serum lipids between fasted and DA >15% groups argue against using the 6-day fasted animal as a model for animals with elevated liver fat. Possible considerations that should be made when using this model and which this study did not address are the genetic potential and the degree of conditioning of the animal before fasting.

A Comparison of Rumen and Abomasal Infusion of 3-Nitropropionic Acid in Sheep - John D. Olsen, M. Coburn Williams, and Lynn F. James. USDA Poisonous Plant Research Laboratory, Logan, Utah 84321

The effect of low levels of 3-nitropropionic acid (3-NPA) on 14 sheep was studied by continuous rumen or abomasal infusion for up to 4 weeks. Rumen infusion rates of 3-NPA used were 50 and 75 NO_2 (129 to 194 mg 3-NPA)/kg body weight/24 hours. Abomasal rates used were 5, 15, 30, and 75 mg NO_2/kg/24h. Blood samples were taken at 3 or 4 day intervals before and during infusion. Red and white blood cell counts, packed-cell volume, hemoglobin, methemoglobin, and serum glutamate oxaloacetate transaminase were measured. Abnormal clinical signs due to 3-NPA toxicity were noted for central nervous system and/or

respiratory function in 57% of the sheep in this study. Death of about 20% of sheep receiving a rumen dose of 50 to 75 mg NO_2 per kg body weight per 24 hours, continuously for about 3 weeks, might be expected based on this study. A 100% lethal abomasal dose (15 mg NO_2/kg/24h) was 30% that of a minimal lethal dose given into the rumen suggesting significant protective effect due to processing of 3-NPA in the rumen. Methemoglobinemia increased linearly in sheep given rumen infusions with the maximal average level occurring at about 21 days. Methemoglobinemia also increased in sheep given abomasal infusions, however, the maximal average level plateaued after 7 days of infusion. Mean corpuscular hemoglobin concentration decreased significantly in sheep given a rumen dose of 75 mg NO_2/kg/24h. Remarkable changes in other blood determinations were not observed.

Effect of Lasalocid or Monensin on Feedlot or Grain Bloat - E. E. Bartley, and T. G. Nagaraja, Department of Animal Sciences and Industry, Manhattan, Kansas 66506

Several studies were conducted to test the effects of lasalocid or monensin on feed'ot or grain bloat. Rumen-fistulated adult cattle fed a feedlot bloat producing diet were used. The degree of bloat (0 = no bloat to 5 = severe bloat) was scored 2 to 3 h after feeding. Lasalocid or monensin was fed twice daily with the bloat diet at .33 or .65 mg per kg body weight per feeding.

Lasalocid effectively reduced bloat in 5 to 9 days. The percentage of reduction in bloat score was 90-100 compared to that of control cattle. The effective dose appeared to be .65 mg per kg body weight. Monensin (at .65 mg dose) also reduced the degree of bloat (50-60%) but was not so effective as lasalocid. In two cattle that received treatment for an extended period (64 days) the effectiveness did not diminish as long as lasalocid was fed with the bloat diet. In experiments where lasalocid was fed to nonbloating hay-fed cattle which were then gradually changed to a high-grain bloat producing diet, bloat was prevented with only .33 mg lasalocid per kg body weight per feeding.

Effect of Lasalocid or Monensin with and without Poloxalene on Legume Bloat - M. Katz, T. G. Nagaraja, E. E. Barley, E. Prassman, L. R. Fina and S. M. Dennis, Kansas State University, Manhattan, Kansas 66506

The effect of lasalocid or monensin with and without poloxalene on legume bloat was studied in rumen-fistulated cattle grazing succulent alfalfa pasture.

The degree of bloat was scored after each feeding (0 = no bloat to 5 = severe bloat). Treatments administered intraruminally before the morning grazing were as follows: lasalocid or monensin alone at .65, 1.0 and 1.3 mg/kg body weight or in combination with 11 or 22 mg poloxalene/kg weight. A minimum of six animals were used for each treatment. Treatments were initiated after animals had bloated for two consecutive days (mean bloat score 2.5 or greater). The drugs were tested for a maximum of 7 days.

as follows: lasalocid or monensin alone at .65, 1.0 and 1.3 mg/kg body weight or in combination with 11 or 22 mg poloxalene/kg weight. A minimum of six animals were used for each treatment. Treatments were initiated after animals had bloated for two consecutive days (mean bloat score 2.5 or greater). The drugs were tested for a maximum of 7 days.

Monensin reduced bloat 36, 64 and 72% (compared with controls) at .65, 1.0 and 1.3 mg respectively. Lasalocid reduced bloat by 28% at all three levels. Monensin (.65 mg) plus 11 or 22 mg poloxalene/kg body weight reduced bloat by 46 and 66%, respectively. Lasalocid (1.0 mg) plus 11 or 22 mg poloxalene/kg body weight decreased bloat by 35 and 61% of the control. There were no differences in total volatile fatty acid concentrations and in colony counts of anaerobic bacteria in rumen fluid of control or treated cattle. However, cattle treated with monensin showed significant difference in total protozoal concentration. Similar changes were observed during in vitro rumen fermentation of fresh alfalfa leaves with lasalocid or monensin.

Collection of Pancreatic Secretions from Cattle and Sheep - D. D. Johnson, R. W. Van Hellen, R. E. Tucker, G. T. Schelling and G. E. Mitchell, Jr., University of Kentucky, Lexington

Feeding high concentrate diets to ruminants increases the amount of starch presented to the intestine. This has stimulated interest in secretion rates and activity levels of pancreatic α-amylase. Surgical techniques have been developed for such studies in both the bovine and ovine. The technique involves placement of a re-entrant pancreatic duct cannula in the bovine and re-entrant common bile-pancreatic duct cannula in the ovine. Cannulae are made of tygon tubing with collars placed on one end to aid in securing the cannulae after suturing. One tube is placed in the duct and the other is placed into the duodenum in close proximity to the Sphincter of Oddi. The tubes are then cross-tied and sutured to the duodenum to help secure them in place. Cannula guides are then placed through the skin and muscle layers and the cannulae are externalized through these guides. Cannulae are connected on the outside of the body with a piece of hard plastic tubing to allow normal flow between collection periods. In 10 recent preparations with cattle, cannulae were patent in all of the steers for over 30 days and 4 of 10 were good for over 3 months. In 8 recent preparations in sheep, 2 functioned over 2 months, 5 have functioned for at least 6 months and the eighth is functional after more than 4 months.

Bile-Pancreatic Secretion Rates in Beef Cattle and Sheep - R. B. Muntifering, G. E. Mitchell, Jr., R. E. Tucker and D. D. Johnson, University of Kentucky, Lexington

Quantitative information has been obtained under a variety of experimental conditions with sheep and cattle, each of which was prepared with a re-entrant cannula in the common bile or pancreatic duct enabling total collection and reinfusion of mixed biliary-pancreatic or pure pancreatic secretions. Daily volume of pure pancreatic secretions ranged from 71 to 341 ml, with a mean value of 217 ml in four sheep fed hay. Daily volume of mixed biliary-pancreatic secretions ranged from 557 to 1668 ml in 169 observations with sheep, with a mean value of 1223 ml. Daily volume of pure pancreatic secretions averaged 7,000 ml in 17 observations with steers. No consistent influence of dietary

starch level (0,20 and 40%) of biliary-pancreatic secretion rates was apparent in studies with sheep, but pure pancreatic secretion rates in steers were increased (P<.05) in response to feeding 80 vs 20% concentrate diets; interpretation of an apparent species difference is complicated by the nature of materials collected (pure vs mixed secretions). Duodenal infusions (200 g/day) of starch or maltose had no effect (P>.05) on biliary-pancreatic secretion rates in sheep. Biliary-pancreatic secretion response to either intrajugular or duodenal infusions of glucose was variable, but exogenous insulin administration increased (P<.05) biliary-pancreatic secretion rates in sheep; secretion rate decreased (P<.05) after prolonged administration and subsequent removal of exogenous insulin, or when duodenal infusions of glucose were superimposed onto insulin injections. Pancreatic secretion rates in steers were modulated by plasma insulin and glucose concentrations, and the response patterns differed for 80 vs 20% concentrate diets. Duodenal infusions of coconut or safflower oil depressed (P<.05) biliary-pancreatic secretion rates in sheep, and the magnitude of depression was similar for infusion levels equalling 5 or 10% of feed intake; impairment of gastrointestinal tract fuction may have been implicated. Duodenal infusion of proplonic acid (36 mmole/$W_{kg}^{.75}$/day) caused a transitory reduction (P<.10) of biliary-pancreatic flow rates in sheep, but a similar response was not elicited by feeding monensin (22 ppm). Failure to reinfuse collected biliary-pancreatic secretions in sheep resulted in a 68% depression in flow rate, suggesting that maintenance of the enterohepatic circulation is necessary if meaningful estimates of the volume and composition of secretions are to be obtained. Specific enzyme activity of secretions or of pancreatic tissue homogenates could rarely be equated with total enzyme secretion; such information invariably required quantitation of secretion rates. It is proposed that mechanisms for controlling pancreatic enzyme synthesis and secretion cannot be fully understood unless both specific enzyme activity and secretory rate information is incorporated into a general model of enzyme adaptation to diet.

Renal Magnesium and Calcium Excretion in Cows - L. E. Deetz, R. E. Tucker and G. E. Mitchell, Jr., Department of Animal Sciences, University of Kentucky, Lexington, Kentucky 40546

Twelve nonpregnant, Hereford and Angus cows representing two equally divided age groups, 3 and 8-11 years old, were used to study renal function and the effects of KCl and sodium citrate on plasma concentrations of magnesium (Mg), calcium (Ca) and parathyroid hormone (PTH). The treatments were: control, 1.5 g KCl/kg body weight (BW) and 1.5 sodium citrate/kg BW infused intraruminally. Inulin and p-aminohippuric acid (PAH) were used as measures of glomerular filtration rate (GFR) and renal plasma flow (RPF), respectively, in the control cows. Creatinine clearances were used as a measure of GFR in all cows. Magnesium was infused intravenously (2.4 mg/kg BW) during sample collection to increase the filtered load of Mg and facilitate the measurement of treatment effects.

Inulin and creatinine clearances were not different; therefore, creatinine appears to be a suitable GFR marker. Renal clearances of inulin, creatinine and PAH were not different between the two age groups. However, fractional clearances of creatinine and PAH (GFR/RPF) were lower in the older cows which suggests that the nephrons of older cows filter less efficiently than the nephrons of younger cows. Plasma Mg levels were increased (P<.05) after KCl infusion, and maximal tubular reabsorption of Mg ($T_{M,\ Mg}$) was increased (P.<.002) in the KCl-treated cows. Nc differences in Ca excretion were observed after KCl dosing. Plasma PTH concentrations were increased (P<.03) after KCl

infusion. Potassium has been shown to reduce Mg absorption in the gut; however, it would appear that KCl altered Mg excretion in this study by increasing Mg reabsorption by the kidneys through the action of PTH.

Magnesium and Ca clearances were increased (P<.05) by the citrate treatment. Organic acids such as citric acid may interfere with Mg and Ca reabsorption by chelating the ions. A decrease in renal conservation of Mg (and Ca) due to the antagonistic effects of K and citrate could have a deleterious effect on Mg and Ca balance and may promote the development of grass tetany.

Oxygen Consumption of Beef Cattle During Heat Stress - J. B. Robinson and D. R. Ames, Department of Animal Sciences and Industry, Kansas State University, Manhattan, Kansas 66502

Heat production of cattle exposed to heat stress is unclear. We compared rates of heat production during thermoneutrality (TNZ) and heat exposure of cattle acclimated to cold, TNZ and heat.

Twelve British cross steers were randomly assigned to cold (3C), TNZ (20C), and heat (35C) for an acclimation period of four months. The existance of thermal stress was documented by the fact that both stressed groups gained significantly less weight than the TNZ group. The cattle were exposed to constant lighting, clipped biweekly and were free moving in their environment. They were individually fed once daily and intake was equalized at 4.9 kg/hd/day.

To estimate heat production, oxygen consumption and carbon dioxide production was measured using a Douglas Bag technique. Heat production was calculated using the equation, kcal = (3.87 kcal x liters O_2) + (1.2 kcal x liters CO_2) and expressed in terms of kcal/$W^{.75}$/day. Following acclimation and before measuring heat production the TNZ cattle weighted 350 kg, heat acclimated group 335 kg and the cold stressed cattle 320 kg. Heat production was estimated at 25C, 30C, 32.5C, 35C, 37.5C, and 40C at three and twenty-four hr exposure times. (Except during measurements cattle were maintained in their respective environments).

There was a significant ($P < .01$) linear increase in heat production of the TNZ acclimated cattle exposed to increased temperature at the 24 hr exposure time. In addition, cattle acclimated to heat showed a linear increase ($P < .01$) in heat production when exposed to increased heat stress at the 3 hr exposure. The resting metabolic rate, a point within the TNZ as indicated by minimum heat production, was greater ($P < .05$) for the cold acclimated cattle at 3 and 24 hr exposure times compared with both the TNZ and heat acclimated groups.

Glucagon Mediated Utilization of Propionic Acid and Amino Acids by the Perfused Ovine Liver - W. W. Gill, G. E. Mitchell, Jr., R. E. Tucker, J. A. Boling, G. T. Schelling and R. M. DeGregorio, University of Kentucky, Lexington

An *in situ* perfusion technique was used to study the influence of glucagon on the utilization of propionate and amino acids by the livers of Finnish Landrace ewes. In 2 experiments, physiological levels of propionic acid (.5g/hr) and enzymatically hydrolyzed casein (2g/hr) were continuously infused into the isolated livers of all animals for 2 hours. Treatments in both experiments

consisted of control (0 glucagon) and glucagon (5mg/hr). In experiment 1 [^{14}C]-propionic acid (20uCi/hr) was infused while [^{14}C]-threonine (50uCi/hr) was infused in experiment 2. In experiment 1 glucagon increased ($P<.05$) glucose synthesis from [^{14}C]-propionic acid (total average [^{14}C]-glucose levels =9.3 vs 5.8uCi). Plasma urea nitrogen levels (156 vs 201 mg/dl) were lower and $^{14}CO_2$ levels (1.2 vs 1.0 μCi) were higher ($P<.01$) in the presence of glucagon. In experiment 2, total [^{14}C]-threonine in the perfusate was higher ($P<.05$) with the glucagon treatment (x = 6.7 vs 3.8μCi) while $^{14}CO_2$ was lower ($P<.01$; x= 2.3 vs 6.2 $x10^{-3}$uCi). These results indicate that glucagon mediates the sparing of amino acids by propionic acid.

The Components of Hepatic Portal Flow During Feeding - A. Dobson, R. S. Comline and R. D. Barnes, The Physiological Laboratory, Cambridge, United Kingdom and Department of Physiology, New York State College of Veterinary Medicine, Cornell University, New York 14853, U.S.A.

Nine sheep were trained to consume a ration of lamb pellets and hay within the same 2 hr period each day. Fifteen micron radioactive microspheres were injected into the left ventricle and blood flow was measured by the reference organ technique. Observations were made before feeding, 3-4 min after beginning to eat and postprandially 2 and 4 hr later.

Contribution to Hepatic Portal Flow (%)

	Fore Gut	Abomasum	Small Intest.	Large Intest.	Panc. & Spleen	Gut Fat	Portal Flow (ml/g)
Prefeed	20.9	12.6	19.5	10.2	24.2	12.5	2.83
Feeding	26.2	13.3	17.9	9.9	25.2	7.5	2.76
2 hr	46.0	7.7	14.0	8.5	19.3	4.2	3.30
4 hr	37.3	9.2	17.7	9.0	21.3	5.3	2.81
S.E.	1.2	0.6	0.9	0.4	1.0	1.2	0.16

The major postprandial increase in foregut flow was due solely to increased flow to the epithelium of the ruminoreticulum. Different regions of the foregut respond to feeding in strikingly different ways.

Effect of Elevated Intrarumen Pressure on Rumen Myoelectric Activity in Sheep - H. W. Colvin, Jr., L. Bueno, and Y. Ruckebusch, University of California - Davis and School of Veterinary Medicine, Toulouse, France

To study the physiological events associated with the depression and possible disappearance of rumen contractions during elevated intrarumen pressure, sheep were surgically prepared as follows:

1. Myoelectrodes were implanted in the reticulum, dorsal sac, ventral sac and the posterior ventral blind sac to study myoeletric events.

2. The trachea was transected to study eructation.

3. The rumen was cannulated for the recording of changes in intrarumen pressure resulting from rumen contractions.

Nitrogen was insufflated into the rumen to pressures of 10 and 20 cm HOH and sustained for 5 minutes.

At an intrarumen pressure of 10 cm HOH, the reduction in the amplitude and character of rumen contractions was preceded by a decrease in rumen myoelectric activity. When the intrarumen pressure was sustained at 20 cm HOH, rumen contractions disappeared within 2 minutes; myoelectric spiking was also absent.

Twenty minutes after the end of insufflation in the 20 cm HOH trial, secondary contractions were difficult to differentiate from primaries on the motility record; however, the myoelectrodes enables rapid discrimmination. Forty-seven minutes after insufflation, rumen pressure, rumen contractions, and myoelectric spiking closely resembled pre-insufflation events.

During deflation following the 20 cm HOH trial, gas was expelled via two routes as ascertained by gas traps on the mask and the anterior tracheal cannula. During the eructation reflex, gas escaped via the anterior tracheal cannula on secondary contractions. When the intrarumen pressure exceeded 10-15 cm HOH, gas expultion occurred on some primary contractions at which time intrarumen pressure exceeded the ability of the partially opened cardiac orifice to retain gas. Gas expelled on primary contractions escapes into the atmosphere via the mouth and is not a complex reflex act.

Because of the delay in the myoelectric response to elevated intra-rumen pressure, it is suggested that a humoral mechanism is operating rather than a neural, although both may be involved. Presumably, myoelectric activity is inhibited by the adrenal hormones, epinephrine and norepinephrine, in response to the stress imposed by intrarumen gas insufflation. The possibility of the acting of gastrointestinal hormones of known inhibitory action in other species is not excluded.

As the result of these findings concerning the inhibition of myoelectric activity during elevated intrarumen pressure, there is a distinct possibility that rumen motility ceases when bloat becomes excessive.

Effects of Grain Overload Rumen Stasis Fluid on Ovine Forestomach Acid-Sensitive Epithelial Receptors - E. C. Crichlow and R. K. Chaplin, Department of Veterinary Physiological Sciences, University of Saskatchewan

Although loss of forestomach motility is a salient feature in "grain overload", the mechanism mediating it is still not known. Since rumen fluid collected at rumen stasis, in sheep treated with volatile fatty acids, can activate forestomach acid-sensitive epithelial receptors which reportedly inhibit reticulo-ruminal contractions, the aim of this investigation was to determine whether rumen fluids collected from "grain overload" sheep during loss of forestomach motility were also capable of activating these receptors.

"Grain overload" was induced in 4 sheep with permanent rumen fistulae by introducing ground wheat (40 gm/Kg body weight) in water (1:2) into their rumen. Reticulo-ruminal motility was recorded with a Millar Mikro-tip catheter pressure

transducer and rumen fluids and blood for the jugular vein were collected, at regular intervals, until rumen stasis developed. Blood and rumen fluid pH were measured at collection time as were packed cell volume, plasma protein and plasma osmolarity.

Forestomach acid-sensitive epithelial receptors were isolated in halothane anesthetized sheep using single nerve fiber recordings from the cervical vagus nerve. Rumen fluid samples and solutions of various concentrations of acetic, butyric, and lactic acids were applied to the exposed receptive fields of these receptors. Of 23 acid-sensitive epithelial receptors isolated, 13 were activated by the same minimum concentrations of acetic and butyric acids i.e., 10 mM (3), 50 mM (1), 100 mM (6), 150 mM (2) and 200 mM (1), 8 by minimum concentrations of butyric acid and 1 each by minimum concentrations of acetic acid and lactic acid. Lactic acid elicited responses in 11 receptors, however, the effective concentrations were much greater than that of either acetic or butyric acid.

Although 21 receptors were tested with samples of rumen fluid, responses were only obtained in 7 (33.3%). Of these 7 receptors 4 (57.1%) responded only to rumen fluid collected at rumen stasis. The remaining 3 receptors responded both to rumen fluids obtained at rumen stasis and when forestomach motility was normal i.e. normal rumen fluid. The responses to normal rumen fluids, however, either had longer latencies or smaller number of action potentials than responses elicited by rumen fluid collected at stasis.

These results suggest that rumen fluids obtained at rumen stasis, in "grain overload" sheep are capable of exciting forestomach acid-sensitive epithelial receptors and are more effective than rumen fluid obtained prior to the impairment of forestomach motility. (Supported by Saskatchewan Horned Cattle Trust).

MICROBIOLOGY

The Effect of Carbohydrate Limitation on the Degradation and Utilization of Protein by Mixed Rumen Bacteria - J. B. Russell, USDA and Cornell University, Ithaca, New York 14853

Procedure. Rumen contents from a cow fed a timothy hay diet were sampled one hour after feeding, squeezed through cheese cloth, and centrifuged at 1000 xg, 15 C for 20 minutes to remove feed particles and protozoa. Resultant supernatant was centrifuged at 10,000 xg, 15 C for 20 minutes to harvest bacteria, and the cell-free supernatant was discarded. Isolated bacteria were subsequently inoculated into an artificial medium containing salts, vitamins, volatile fatty acids, sulfide, ammonia, and 40 mg/l mixed carbohydrates (equal parts soluble starch, glucose, sucrose, maltose, and cellobiose) to achieve an optical density of 1.0. Twenty-five milliliters of this medium and bacteria were then anaerobically transferred to incubation bottles containing freeze dried casein.

Experiment 1. When casein was present at 525 mg/l and mixed carbohydrates were added at rates of 0, 40, 80 and 160 mg/l/hr, cell growth ($<.07\ h^{-1}$), and the types of bacteria did not appear to change during 6 hours of incubation. Increases in cell protein were proportional to the rate of carbohydrate addition, and net ammonia production was inversely proportional to carbohydrate availability. Hourly sampling revealed that casein was hydrolyzed rapidly, and that this hydrolysis was accompanied by a marked accumulation of extracellular peptides. Carbohydrate availability had little influence on proteolysis or peptide uptake.

Experiment 2. Incubation of rumen bacteria with 0, 525, 2100, and 8400 mg/l casein under similar carbohydrate feeding strategies showed that proteolysis was subject to saturation kinetics. Increasing casein from 525 to 2100 or 8400 mg/l resulted in 4 times as much extracellular peptide nitrogen, but ammonia production was still minimal if carbohydrate was added at .160 mg/l/hr. When casein was increased from 0 to 525 mg/l and carbohydrate addition was 160 mg/l/hr, there was an 87% increase in the yield of cell protein. Further increases in casein (2100 and 8400 mg/l) caused only a slight increase in cell protein.

Utilization of Proteins from Intact-Forages by Pure Cultures of Rumen Bacteria - H. Hakimzadeh and B. A. Dehority, Ohio Agricultural Research and Development Center, Wooster, Ohio 44691

An *in vitro* procedure was developed to measure forage protein utilization by rumen bacteria. Graded levels of nitrogen, as ammonium sulfate, were added to "N-free" complete media with excess available energy. These media were inoculated with five strains of proteolytic rumen bacteria, and after completion of the fermentation (10 days), the concentrations of major end products were measured and standard curves were established for each organism. Freeze dried samples of 3 legumes and 3 grasses, each at four stages of maturity, were ground to either 40 mesh, 60 mesh or ball-milled, and used as the sole added nitrogen source in the "N-free" medium.

Maturity and particle size did not affect N utilization by the test organisms. Among strains, between species and within species differences for the organisms were significant ($P<.01$). The N utilization from legumes or grasses was not different. Correlation between soluble protein (physiological saline) and total protein digestion was low ($r^2 = 0.16$). The digestibility of the soluble protein fraction was similar among the organisms, but not complete. However, the digestibility of the insoluble protein fraction varied from one strain to another and resembled the total protein digestion from the intact forages.

Adaptation to Dietary Nitrate: Effects on VFA and CH_4 Production Rates - M. J. Allison, C. A. Reedy, and H. M. Cook, National Animal Disease Center, USDA, Ames, Iowa

Ruminal fluid from adult sheep adapted to diets (1 kg/day, 12-hr feeding interval) of alfalfa-corn (3:2) plus $NaNO_3$ (0, 30, and 60 g/day) was collected 4 hours after feeding, strained through cotton gauze and then incubated (2 hr, 38 C) under CO_2 with and without added NO_3^- or NO_2^- (4 and 10 mM). Rates of NO_3^- reduction by ruminal microbes increased by as much as 20-fold during adaptation to dietary NO_3^-, while rates of NO_2^- reduction increased about 3-fold. Quantities of CH_4 produced by microbial populations from animals fed all diets were less when either NO_3^- or NO_2^- was added. Inhibition of CH_4 production by NO_3^- was greater with NO_3^- adapted populations, but inhibition of CH_4 production by NO_2^- was greater with ruminal populations not adapted to NO_3^-. With ruminal populations adapted to NO_3^-, inhibition of CH_4 production by NO_3^- appears to be due to diversion of H to reduce NO_3^- rather than CO_2, while with unadapted populations inhibition of CH_4 production by NO_2^- appears to be due to toxicity of NO_2^-.

Acetate production rates by both NO_3^- adapted and nonadapted populations were greater when NO_3^- was added. Acetate production rates were increased 1.5 $\pm$ .14 (SD) and 2.0 $\pm$ .67 (SD) fold by addition of NO_3^- at 4 and 10 mM, respectively. Addition to NO_2^- markedly inhibited acetate and butyrate production by microbial populations from sheep not adapted to dietary populations from NO_3^- adapted animals. With microbial populations from sheep not adapted to NO_3^-, added NO_2^- led to accumulation of lactate, but this was not observed with NO_3^- adapted populations. Thus, rates of VFA and CH_4 production are changed by NO_3^- and NO_2^- and these changes are influenced by prior adaptation of ruminal populations to NO_3^-. KEY WORDS: Ruminal microbes, Nitrate, Nitrite, VFA, Methane.

Metabolism of Nitrate and Nitrite in Cell Extracts of Mixed Rumen Bacteria from Nitrate-Adapted Sheep - C. A. Reddy and M. J. Allison, NADC, Ames, Iowa

"Nitrate poisoning" often encountered in ruminants is due to accumulation of toxic nitrite (NO_2^-), produced by the reduction of dietary nitrate (NO_3^-) by rumen bacteria. However, little is known about the metabolism of nitrate and nitrite by cell extracts of mixed rumen bacteria. In this investigation, we studied the biochemistry of nitrate and nitrite reduction by cell extracts of mixed rumen bacteria (extracts) obtained from sheep adapted 30 g/d of dietary NO_3^-. High levels of dissimilatory type NO_3^- and NO_2^- reductase activities were observed in the extracts with H_2 as the electron donor. The specific activities (n mol H_2 utilized/min/mg protein), with benzyl viologen (BV) as the e^- donor, for NO_3^- and NO_2^- reductase activities were 140-160 and 147-221, respectively, for extracts from NO_3^- adapted sheep. The corresponding specific activities for extracts from sheep fed no dietary nitrate were 15-20 fold lower. NO_3^- and NO_2^- were stoichiometrically reduced to ammonia; H_2:NO_3^- and H_2:NO_2^- stoichiometries were 4:1 and 3:1, respectively. The pH optimum for NO_3^- reductase activity was 7.0 whereas that for NO_2^- reductase activity was 7.5.

The specific activity for NO_3^- reduction with methyl viologen (MV) as the e^- mediator was less than 50% of that observed with BV, whereas that with FAD was about 80% of that observed with BV. Little activity was observed with ferredoxin (Fd) from Clostridium pasteurianum as the e^- mediator. NO_3^- reducing activity was primarily membrane-bound and was strongly inhibited by azide and hydroxyquinoline-n-oxide. Chlorate-reducing activity also observed in the extracts was strongly inhibited by azide.

A low potential electron mediator such as BV appeared to be an obligate requirement for NO_2^- reductase activity; MV was a poor substitute for BV and no detectable activity was seen with either FAD or Fd from C. pasteurianum. The NO_2^- reductase activity appeared to be equally distributed between the membrane and cytosol fractions of mixed rumen bacteria but the specific activity with each fraction was quite low. Specific activity increased four-fold when the membrane and cytosol fractions were mixed together. The results on the respiratory nature of NO_2^- reductase activity were inconclusive.

These results indicate the predominance of respiratory type NO_3^- reductase activity in extracts from NO_3^- -adapted sheep. It is attractive to speculate that the predominant rumen anaerobes in NO_3^- -adapted sheep may obtain additional ATP for growth from electron transport phosphorylation coupled to NO_3^- reduction (to ammonia) by e^- donors such as H_2, formate and reduced pyridine nucleotides. This in turn may mean greater microbial cell yields in the rumen of nitrate-adapted sheep.

Nitrate and Formate Metabolism by Whole Cells and Cell Extracts of Selenomonas Ruminantium - S. O. Feighner and C. A. Reddy, Michigan State University, East Lansing, Michigan 48824

Methemoglobinemia is a toxicity syndrome in ruminants which results from the absorption of toxic levels of nitrite, produced by the reduction of dietary nitrate (NO_3) by rumen bacteria. Nitrate metabolism in rumen contents has been investigated in vivo and in vitro but little information is available from pure cultures of rumen bacteria. Selenomonas ruminantium, a predominant member of the rumen microflora in animals fed a variety of diets and known to reduce NO_3, was chosen to investigate the physiology of NO_3 metabolism. Using whole cells and cell extracts we provide evidence which suggests NO_3 metabolism by S. ruminantium occurs by a dissimilatory pathway and can be coupled to formate oxidation to supply energy for growth.

Nitrate metabolism in S. ruminantium strains HD_1 and HD_4 was induced by NO_3. Medium containing ammonia levels (20 mM) four to five times the saturating concentration for growth did not affect NO_3 reducing activity in whole cells. Both nitrite and ammonia were produced from NO_3 in washed whole cell assays using dithionite as the electron (e^-) donor. The production of ammonia from NO_3 and nitrite was, however, only observed in whole cell assays, the nitrite reductase activity was lost during the preparation of cell extracts.

Nitrate reductase activity in S. ruminantium cell extracts was predominatly membrane associated. The apparent pH optimum was 7.0 with Hepes buffer and 7.5 with Hepes-Tris buffer. The specific activity of NO_3 reductase was 1.5-2.0 fold greater with Hepes-Tris buffer than with Hepes buffer. The apparent K_m for NO_3 reductase activity in cell extracts was 0.2 mM. Methyl viologen (MV) and benzyl viologen (BV) were equally effective e^- mediators with dithionite. However, BV was significantly better using H_2 and formate as e^- donors. Clostridium pasteurianum ferredoxin was inactive as an e^- carrier.

Formate dehydrogenase activity in cell extracts was detected spectrophotometrically. Methylene blue and BV were effective e^- acceptors (39.5 and 29.0 nmol formate oxidized/min/mg protein, respectively). MV was only 20% as effective as BV in the spectrophotometric assay.

Nitrate reduction by growing cultures was accelerated when formate was included in glucose-limited synthetic medium. Molar growth yields for S. ruminantium increased in the presence of NO_3. Moreover, NO_3 plus formate significantly increased Yglu compared to glucose alone (80.4 vs 60.4). Also, in the absence of glucose, NO_3 plus formate could function as a source of energy for growth of S. ruminantium.

The results demonstrate the S. ruminantium possesses both a respiratory type NO_3 reductase and a formate dehydrogenase. The NO_3 reductase can be coupled to formate oxidation to generate ATP (oxidative phosphorylation) for growth.

In vitro ^{13}C-Measurements of the Contribution of Megasphaera Elsdenii to Rumen Lactate Fermentation - R. A. Prins and G. H. M. Counotte, Laboratory of Animal Nutrition, Veterinary Faculty, Yalelaan 1, 3584 CL Utrecht, The Netherlands.

Since Megasphaera elsdenii ferments a variable part of DL-lactate to butyrate, measurement of the percentage of DL-lactate fermented to propionate via the acrylate pathway in rumen contents will underestimate the participation of this organism in the fermentation of DL-lactate. Therefore, a technique was described to measure simultaneously the percentage of propionate formed through the acrylate pathway and the part of DL-lactate fermented to butyrate. This technique (described in Appl. Env. Microbiol. 42:649-655, 1981) involves ^{13}C-FT nuclear magnetic resonance measurements of label distribution in propionic and butyric acids formed from DL-[2-^{13}C] lactate after incubation of this compound with rumen contents. From a number of measurements with rumen contents of dairy cattle an average contribution of M. elsdenii to lactate fermentation of 74% (standard deviation, 13%, range 61-97%) was found. Immediately after feeding, the contribution of M. elsdenii to lactate fermentation increased sharply possibly as a consequence of catabolite repression of lactate fermentation by sugars in other rumen bacteria.

Diurnal Changes in Carbohydrate Fermenting Bacterial Groups in the Rumen of Steers Fed Two Different Diets - Jane A. Z. Leedle, Marvin P. Bryant, and Robert B. Hespell, Department of Dairy Science, University of Illinois, Urbana, Illinois 61801

Diurnal variation in bacterial numbers and carbohydrate fermenting groups in the rumen of steers fed two different diets was followed. Mature, rumen-fistulated Holstein steers were fed maintenance level, high forage or high concentrate diets once daily. Maintenance was determined on a metabolizable energy rather than a dry weight basis and diets were formulated in 75/25% proportions of alfalfa hay and concentrate materials. Samples of ruminal contents were manually collected at -1, +2, 4, 8, 12 and 16 h post-feeding. The bacterial populations obtained after blending the samples were assessed for direct and viable counts and the size and distribution of carbohydrate fermenting groups. Carbohydrate groups were determined using anaerobic replica plating techniques with a series of differential carbohydrate media. Corresponding ruminal fluid samples collected were analyzed for fermentation acids, carbohydrate, ammonia and pH.

Results showed that little diurnal change occurred in total bacterial numbers on either diet. Both diets had lowest viable cell counts at 2 h, followed by a gradual increase to highest counts at 16 h post-feeding. Changes in the carbohydrate groups did not correspond entirely with the predicted pattern for fermentation of the feedstuff components. Soluble carbohydrate fermenters predominated at all times in both diets. Bacterial groups fermenting pectin, xylan and cellulose reached maximum population densities at 8, 12 and 16 h post-feeding, respectively. A similar pattern was observed on the high concentrate diet except these groups were lower in proportion. The similarities in the bacterial profiles between the diets were unexpected but probably due to maintenance feeding. The stability of the ruminal ecosystem observed was also supported by the ruminal fluid measurements which exhibited little diurnal change. The nearly identical pH profiles showing no severe depression even on the high concentrate diet suggested that the composition of the diet has little to do with the resultant microbial population. Rather, the data suggest that the overall energy content of the diet will ultimately affect the carbohydrate group composition of the rumen bacterial population.

Maintenance and Selection of Monensin-Resistant Bacteria in the Rumen - K. A. Dawson and J. A. Boling, Department of Animal Sciences, University of Kentucky, Lexington, Kentucky 40546

Habitat-simulating and substrate-specific media were prepared with energy-depleted rumen fluid and were used to compare monensin-resistance and the composition of the anaerobic bacterial population in the rumen of steers on monensin-containing and monensin-free rations. Total anaerobic counts on the habitat-simulating medium ranged from 9.3×10^9 to 8.5×10^{10} CFU/g (dry wt) of rumen ingesta and were not significantly different in animals receiving monensin (33 mg/kg) and animals on monensin-free rations. The mean percentage of the total population resistant to 10 mg monensin/ml of medium was significantly greater ($P<.05$) in animals receiving the monensin ration for 33 days when compared to animals on a similar monensin-free ration (63.6% and 32.8%, respectively). Increased monensin-resistance tended to develop later than the characteristic decreases in acetate: propionate ratios and remain significantly greater in monensin-fed animals for at least 20 days after monensin was deleted from the ration.

Monensin supplementation did not significantly alter the proportions of the total population capable for using glucose, starch, cellobiose or trypticase as energy sources. Percentages of monensin resistant anaerobes with glucose-, starch- and cellobiose-using groups from monensin-fed animals were not significantly different from those on monensin-free rations. However, resistance in trypticase-using populations was higher ($P<.10$) in monensin-fed animals than in animals fed monensin-free rations (64.0% and 18.1% respectively). The contributions of glucose - and starch-using populations to resistance in the total population was significantly lower in monensin-fed animals when compared to animals not fed monensin. However, the contributing of these populations to resistance tended to increase when monensin was removed from the diet.

This study suggests that monensin can select monensin-resistant bacterial strains in the rumen without influencing the total concentration of bacteria. This adaptive change could not be associated with the major saccharolytic groups but was reflected in organisms which use peptones as energy sources.

Phenylpropionic Acid, a Growth Factor for Ruminococcus Albus Strain 8 - R. E. Hungate and Robert J. Stack, University of California-Davis, Davis, California 95616

Phenylpropionic acid can account for part of the stimulatory effect of rumen fluid on the rate of growth and cellulose digestion by cultures of Ruminococcus albus strain 8. The chemically defined medium has supported consistent growth in daily transfers of a one percent inoculum from a 24-hour broth culture containing 0.3% pebble-milled cellulose. Disappearance of cellulose has proven to be a more consistent and reliable criterion of growth than has the increase in optical density of cellobiose broth cultures. As little as 3 μM phenylpropionate gives maximum response.

Fermentation of Cellulose by a Rumen Anaerobic Fungus in the Absence and Presence of Rumen Methanogens - D. O. Mountfort, Cawthron Institute, Box 175, Nelson, New Zealand and T. Bauchop, Applied Biochemistry Division, Department of Scientific and Industrial Research, Palmerson North, New Zealand

From the fermentation of cellulose by an ovine rumen anaerobic fungus, moles of product as a percentage of the moles of hexose fermented were: acetate, 72.7; carbon dioxide, 37.6, formate, 83.1; ethanol, 37.4; lactate, 67.0; and hydrogen, 35.3. When the fungus was cocultured with rumen methanogens acetate was the major product (134.7%) and carbon dioxide increased (88.7%). Lactate and ethanol production decreased to 2.9% and 19% respectively, little formate was detected (1%), and hydrogen did not accumulate. Substantial amounts of methane were produced in the coculture (58.7%). Studies with 2-^{14}C] acetate indicated that acetate was not a precursor of methane. The demonstration of cellulose fermentation by a fungus extends the range of known rumen organisms capable of participating in cellulose digestion and provides further support for a role of anaerobic fungi in rumen fiber digestion. The effect of the methanogens on the pattern of fermentation is interpreted as a shift in flow of electrons away from electron-sink products to methane via hydrogen. The study provides a new example of intermicrobial hydrogen transfer and the first demonstration of hydrogen formation by a fungus.

The Microbial Fermentation in the Termite Hindgut; A Comparison to the Rumen - D. A. Odelson and J. A. Breznak, Michigan State University, East Lansing, Michigan 48824

The abundance of information on the rumen fermentation affords a sound theoretical basis from which to evaluate gut fermentations in other important herbivores. Accordingly, we have sought to understand the fermentation of wood by the hindgut microbiota of termites, Reticulitermes flavipes (Kollar), and to evaluate the relevance of microbial metabolites to the carbon and energy requirements of the insect. Focus has been on volatile fatty acid (VFA) production by the hindgut microbiota, and parameters measured included: the identity and extracellular pool size of VFA's in situ; the in vivo rate of production of VFA's; the contribution of cellulose and hemicellulose carbon to VFA formation; the relative importance of protozoa and bacteria to VFA production; and the importance of microbial VFA's to the termite's respiratory requirements. Although termites resemble ruminants in having a cellulose-dominate nutritive regime, as well as a prominent gut fermentation mediated by both protozoa and bacteria, significant differences were apparent. Among these was the striking dominance of acetate in the VFA pool of the termite hindgut (90 mol %), compared to that of the rumen (60 mol %), and the lack of significant cellulolysis by termite hindgut bacteria. A further difference was the more limited importance of methane as a product of the termite hindgut fermentation. Nevertheless, both animals employ the same strategy to satisfy their energy requirements, i.e., aerobic oxidation of VFA's derived from microbial fermentation of feed polysaccharides. The differences in fermentation patterns in the two animals may reflect a greater influence of protozoa in wood polysaccharide fermentation in the termite hindgut.

Kinetics of H_2 Consumption in the Bovine Rumen - Joseph A. Robinson and James M. Tiedje, Michigan State University, East Lansing, Michigan 48824

Hydrogen is a key intermediate in the anaerobic degradation of organic matter, since its concentration influences both rates of organic matter processing and the qualitative nature of the products generated in methanogenic habitats. For these reasons, an understanding of the kinetics of hydrogen production and consumption is important. The kinetics of ruminal H_2 consumption and turnover were investigated using a gas-recirculation system that allowed for semi-continuous monitoring of H_2 and CH_4 in gas phases equilibrated with either diluted or undiluted rumen fluid.

The kinetic nature of ruminal H_2 consumption was determined by performing progress curve experiments, which each involved following the consumption of added H_2 in the gas phase of the gas-recirculation system. H_2 consumption by rumen fluid approximates Michaelis-Menten kinetics when rumen fluid is diluted to overcome phase-transfer limitations. H_2 K_m's ranged from 4.2 to 9.2 micromolar. The V_{max} estimates, after being corrected for the dilution factor required to circumvent transfer limitations, ranged from 1.2 to 2.6 micromolar/min.

The turnover of H_2 in undiluted rumen fluid, incubated in the gas-recirculation apparatus, was determined two ways. The first involved measuring endogenous CH_4 production by rumen fluid and multiplying by four to obtain instantaneous rates of H_2 production. Turnover times for H_2 were calculated by dividing the H_2 production rates into H_2 pool sizes that were concomitantly measured with CH_4. The second procedure by which H_2 turnover times were computed employed mean V_{max} and K_m estimates and the H_2 pool concentrations. Thus, we computed H_2 *in vitro* turnover times using H_2 production rates in the first case and H_2 consumption rates in the second. When the H_2 concentration had attained a nearly steady-state concentration the turnover times computed, using rates of H_2 production and consumption, agreed and were approximately 1 second. Turnover of the H_2 pool *in situ* was estimated in a fistulated cow using pool size information and mean V_{max} and K_m estimates. Turnover times for the H_2 pool *in situ* were about the same as those computed for undiluted rumen fluid incubated in the gas-recirculation system (viz., approximately 1 second).

The Influence of Niacin Supplementation and Nitrogen Source on In Vitro Rumen-Like Fermentations - D. R. Shields, D. M. Schaefer and T. W. Perry, Purdue University, West Lafayette, Indiana 47907

A semicontinous culture technique was utilized to investigate the influence of niacin supplementation (100 ppm based on the total weight of flask contents) on rumen-like fermentations. Either NH_4Cl or a soybean meal-like mixture of crystalline amino acids was used during a series of 6 hours or 24 hours incubations having a fluid retention time of 1.33 days. Glucose, maltose and cellobiose were used as the carbon and energy sources. Microbial protein was measured by centrifugation (24,000 x g) of the spent culture followed by washing the pellet with methanol and sonicating to disrupt intact cells. Protein was determined using the Bio-Rad protein assay. Niacin was determined using Lloyd's reagent as the vitamin carrier and CNBr for the color development. Establishment of rumen-like fermentations was confirmed by low H_2 concentrations and relatively normal proportions of acetate, propionate, and butyrate (59:23:13) after 3 days of incubation. Regardless of nitrogen source and length of incubation niacin supplementation increased ($P<.05$) microbial protein synthesis (control 7.70 vs niacin 13.69 mg/g substrate added) and niacin concentration (control 9.2 vs 16.3 ug niacin/g spent culture.) The increased niacin concentration indicates that the catabolism of supplemental niacin was incomplete. This result may be confounded by the probable lack of specificity of the niacin assay for the vitamin. Metabolites of niacin may have been produced during the incubations but detected as the vitamin. Total pyridine nucleotide pool concentration was increased ($P<.05$) by niacin supplementation regardless of nitrogen source during the 6 hours incubations. Ammonia levels were lower during the 6 hours versus the 24 hours incubations which indicated rapid microbial growth during 6 hours incubations. Niacin had no influence on ammonia level. Tryptophan content of microbial protein tended to be higher at 6 hours due to niacin supplementation, however this could be a result of an increased intracellular free trypotophan concentration and not to a higher incorporation into microbial protein. Total gas production or methane production and pH were not influenced by nitrogen source or niacin supplementation. In conclusion, the semicontinous culture technique was useful in establishing rumen-like fermentations in which niacin supplementation increased efficiency of microbial protein synthesis.

A Possible Role for the Rumen Microflora in the Etiology of Polioencephalomalacia - Thomas R. Haven and Daniel R. Caldwell, Division of Microbiology and Veterinary Medicine, University of Wyoming, Laramie, Wyoming 82071

The rumen contents of range cattle suffering from acute polioencephalomalacia as judged by clinical symptoms, brain histopathology, and blood serum thiamine deficiency were examined to determine the quantitative and qualitative nature of the microflora associated with these animals in comparison to that associated with the rumen of a healthy cow on a roughage diet. The colony counts in nonselective medium 98-5 per gram of rumen contents ranged between 1.5 and 2.0 x 10^8 and were statistically indistinguishable. However the morphological character of the associated microbes isolated randomly and nonselectively from the rumens of polioencephalomalacia-affected animals was markedly different from that of a healthy animal on a roughage diet, and contained a high percentage (an average of 77%) of cocci or coccobacillary gram negative rods. Curved rods, of any gram reaction, comprised less than 1% of the isolates. As judged by the relative peak heights at 265 nm of the 590 uM thiamine added to uninoculated broth medium and of thiamine remaining in cultures after growth, between 78 and 100% of the bacteria associated with polioencephalomalacia-affected animals degraded thiamine. Some isolates degraded as much as 60% of the thiamine supplied. Under identical conditions, only 14% of isolates from the healthy animal degraded thiamine and none of the isolates degraded more than 30% of that supplied. Polioencephalomalacia may result from an increased percentage of rumen bacteria capable of thiamine destruction, leading to a ruminant thiamine deficiency.

The Role of Rumen Microbes in Pasture Bloat - K.-J. Cheng[1], R. E. Howarth[2], W. Majak[3], and J. W. Costerton[4]; Agriculture Canada Research Stations, [1]Lethbridge, Alberta; [2]Saskatoon, Saskatchewan; [3]Kamloops, British Columbia and [4]University of Calgary, Calgary, Alberta

Our studies on the initial rate of release of nutrients from forage legumes by leaching (dry matter loss in rumen fluid medium, in the absence of bacteria) and the sequence of events in the digestion of legumes by rumen organisms showed that leaves of bloat-causing legumes (alfalfa, red clover and white clover) are initially leached, invaded, and digested faster than those of bloat-safe legumes (sainfoin, birdsfoot trefoil and cicer milkvetch). These results are consistent with our results obtained with a modified *in vivo* nylon bag technique to measure the extent of leaf tissue disruption of bloat-causing and bloat-safe legumes by rumen microbes.

Daily bloat incidence, rumen fluid composition, and microbial activity were recorded in rumen-fistulated cattle fed fresh alfalfa. Daily measurements of initial rate of digestion by the nylon bag technique were moderately well-correlated with daily bloat incidence. The concentration of chlorophyll in rumen fluid, microbial activity in the rumen fluid, and the flocculation of rumen materials before feeding were higher for bloated cattle than for non-bloated cattle.

These experiments provide a new explanation for legume pasture bloat in that the rapid initial digestion of leaf tissue releases fine particles that act as nuclei for gas formation causing flotation of the digesta. Further, the rapid rupture of mesophyll cells and their chloroplasts accelerates the release of the proteins and fatty acids that promote foam formation.

In short, rumen pasture bloat resulted through higher microbial activity in the rumen due to the inability to clear the flocculated fine feed particles that are colonized with microbial colonies. This, in turn, provided the active inoculants for rapid initial digestion of fresh injested legume to produce foams, and rapid fermentation and microbial colonization of fine particles provided the force for floating of the whole digesta resulting in the condition of pasture bloat.

These studies have provided the basis for breeding a bloat-safe alfalfa cultivar by selecting for low initial rates of digestion. We have suggested that a 25-30% reduction in dry matter loss after 6 hours of digestion would be required to reach the bloat-safe thresnold.

The Establishment of Bacterial Populations in the Digestive Tracts of Newborn Lambs Through the Inoculation of Selected Bacteria - [1]J.-J. Cheng and [2]J. W. Costerton, [1]Agriculture Canada Research Station, Lethbridge, Alberta, and [2]Department of Biology, University of Calgary, Calgary, Alberta.

Inoculating newborn lambs with a mixture of microorganisms isolated from the ruminant digestive tract had a very beneficial effect on rate of gain as compared to uninoculating newborn lambs reared under vivarium conditions. All of the lambs (12) in this study maintained good general health and very little diarrhea was detected at any point throughout the experiment. All the lambs were weighed at slaughter (120 days) and the inoculated group had each gained an average of 23% (0.5 kg) more than those of the control group. The much higher urease activity of the digestive tract tissues of some animals in the inoculated group indicated that the organisms in the inoculum could establish themselves as tissue-adherent populations throughout the tract, and this elevated tissue urease activity was especially notable in the reticulo-rumen and omasum. The inoculated group also showed higher microbial activity in the rumen fluid in that both total bacterial count and fermentation rate were elevated. No obvious differences between the rumen fluid of the inoculated and control groups were seen with respect to pH, alkaline phosphatase, protease, lipase and the number of pectindigesting bacteria. The tissue-associated alkaline phosphatase activites in the duodenum and small intestine were seen to be elevated in most of the lambs in the inoculated group. The higher tissue levels of alkaline phosphatase of the duodenum and ileum in inoculated lambs may be associated with higher rates of absorption by these tissues.

Effect of Feeding Strategy on Rumen Nitrogen and Carbohydrate Escape in a Dairy Cow - P. H. Robinson, C. J. Sniffen, P. J. Van Soest, Cornell University, Ithaca, New York

Dairy cows are increasingly being fed pre-mixed rations. Research on feeding frequency of mixed rations has led to recommendations to increase the number of daily offerings of feed to stimulate productivity. One 540 kg Holestein cow was fitted with a rumen cannual and a cannula (allowing complete digesta diversion) in the proximal duodenum. Ration (70% forage, 22% cracked corn, 7% soybean meal) was fed at 6.56 kg (low or 16.40 kg (High) per day (DM) either in one meal at 15:00 hrs. (1X) or eight equal meals (8X) at three hour intervals at and post 2:00 hours. Collection of 18 rumen and duodenal spot samples were made every three hours over 72 hours for each combination of feeding level and frequency in a way to allow a sample to be taken every 40 minutes on a 24 hour clock. Rumen organic matter digestion was higher with 8X over 1X but showed little effect of feeding level. This difference was primarily composed of organic matter potentially digestable post-ruminally, with acid detergent fibre components showing little effect of feeding frequency. Duodenal non-ammonia nitrogen as a percentage of nitrogen intake was lower for 8X and 1X, again showing little effect of feeding level. This drop reflected decreases in both bacterial and escape feed nitrogen for 8X. The data suggest that if intake is limited increasing the feeding frequency has a negative impact on rumen nitrogen and carbohydrate economy.

Rare Earth Elements: Affinity for and Binding By Cell Wall Fractions - M. Allen, P. J. Van Soest, M. McBurney and P. Horvath, Cornell University, Ithaca, New York

Rare earth elements can be used to measure the cation exchange of plant cell wall surfaces. The binding of metal ions by plant fibers is of interest relative to the buffering capacity of fiber in the rumen, factors influencing the attachment of bacteria, and as passage markers. Reduced lag time and rate of fermentation are correlated with cation exchange capacity of NDF. Eighteen elements of period IIIa (Sc, Y, La) rare earths (Ce, Pr, Nd, Sm, Eu, Gd, Tb, Dy, Ho, Er, Tm, Yb, Lu) and period IVa (Zr, Hf) were examined for suitability as particulate labels. Purchased oxides were converted to acetates (rare earths) or sulfates (Zr and Hf). Reactivities of La, Ce, Sm, Yb, Zr and Hf were tested toward acetic acid, polyphenols, pectic acid, etc. Attachment to isolated NDF of alfalfa and grass was obtained by incubating fibers in neutral buffered solutions. Attachment forces were assayed by soaking in ammonium acetate sodium lauryl sulfate (SLS), EDTA, pepsin-HCl solutions, and 48 hours in vitro digestion with rumen fluid (IVD). La, Ce, Sm and Yb were removed by EDTA, pepsin HCl and partially by acetate but not by IVD or SLS. La, Sm, Ce, Yb, Zr and Hf variably reduced organic IVD. Zr and Hf are very slow to react requiring days to prepare stable mordants that resist digestion and solution in a wide variety of solvents. Yb reduced wettability of fibers and produced uniquely hydrophobic pectate and tannate percipitates. Yb offers new analytical methodologies for pectin and tannin. Sc and Lu have similar interesting physical properties worth investigating.

NUTRITION

The Influence of Feed and Water Withdrawal on the Site and Extent of Weight Loss in Slaughter Steers Under Various Handling Regimens - J. M. Asplund, Department of Animal Science, University of Missouri, Columbia, Missouri 65211

Rate of passage, retention time, turnover rates and other important rumen flow parameters are obviously influenced by the dynamics of liquid flow in the rumen. Previous work with sheep force-fed fixed water:feed ratios indicated that water restriction decreased digesta flow but did not greatly influence fecal or urinary water output over a short term. It was also seen that the physical form of the feed influenced the rate of water intake. In a series of experiments in which slaughter steers had feed and water withdrawn for various lengths of time under a variety of holding and hauling conditions, several observations were made:

1. Excreta loss accounted for one-third or less of the total weight losses observed.
2. Rumen content loss could account for a maximum of 4 kg of total weight losses, which were as high as 30 kg.
3. The moisture level of rumen contents was not altered by withholding of feed and water up to 48 hours.
4. Water loss, apparently from the carcass by insensible routes, appeared to be the largest single factor in weight loss.

While these data are largely inferential, it appears that it would be logical to test the hypothesis that digesta passage from the rumen is significantly influenced by the moisture level in the rumen and that water influx through the rumen wall is not a major factor.

The Effect of Age or Rumen Development on the Drug-Metabolizing Enzymes in Lung and Liver of Goats - T. M. Bray and F. E. Burley, Department of Nutrition, University of Guelph, Guelph, Ontario N1G 2W1

Drug-metabolizing enzyme system appears to be the predominant catalyst for the metabolism of not only drugs, but also of environmental toxins. The principal of the reaction is to convert lipophilic compounds to water soluble products for excretion. The rate of metabolism is an important factor in determining the pharmacological and toxicological properties of various drugs and compounds. Various factors can affect the activity of this enzyme system, such as age, species, sex, nutritional status of the animals, or exposure to other drugs. Information concerning the activity of the drug-metabolizing enzymes in ruminant is limited. The microbial metabolism in the rumen may influence the basal enzyme activity of the animals. The objectives of this study were 1) to measure the effect of age or rumen development on the activity of the drug-metabolizing enzyme system of the lung and liver of goats in 3 age groups: at birth (5 days old), weanling (8-10 weeks old) and mature (3-6 years old). 2) to determine the induction of the enzyme system by sodium phenobarbital (PB) and the inhibition of piperonyl butoxide (PT) in each age group. The cytochrome P-450 contents of the liver and lung were less in the newborn that that of the weanling or mature goats. There was no significant change in these parameters between weanling

and mature goats. The enzyme system in the lung was consistently lower than that of the liver in all age groups. In the lungs of all age groups, there was no detectable benzo [a] pyrene hydroxylase or N-demethylase activity.

Administration of PB induced hepatic cytochrome P-450, however, PB was not a suitable inducer in the lung of goats. Piperonyl butoxide inhibited this enzyme system in weanling and mature but not in newborn goats.

Influence of Captan and Difolatan on Ruminal Metabolism in the Ovine - S. I. Smith and J. A. Boling, Department of Animal Sciences, University of Kentucky, Lexington, Kentucky 40546

Two experiments were conducted to determine the effect of administration of captan and difolatan on ruminal metabolism in lambs. In the first experiments, 0,80,260, or 440 ppm of difolatan were added to a diet containing 11% crude protein. Twenty-four lambs were allotted to the four diets. Rumen ammonia values were lower (P<.08) in animals fed the drug indicating a possible supression of proteolysis and/or deamination. Shifts in volatile fatty acid percentages were also observed. The percentages of acetate and butyrate were lowered (P<.05); that of propionate was increased (P<.05) indicating potentially improved energetic efficiency. The second experiment was designed to investigate the effects of captan on ruminal metabolism, as well as the mediation of these effects according to frequency of administration. Twenty wether lambs were randomly allotted to four diets providing the equivalent of 160 ppm captan in the total dietary dry matter, which was administered 1, 2, or 4 times daily. Again, there were shifts in ruminal volatile fatty acids. The percentage of acetate decreased (P<.08) and that of butyrate increased (P<.08). There were also effects due to frequency of administration of captan. The percentage of propionate was highest (P<.10) in animals receiving captan only once daily. Rumen ammonia and pH were not effected (P>.10). Both of these drugs showed potential for improving nutrient utilization by the ruminant, but further work is warranted to determine optimum dosage levels.

Effects of Monensin Upon Intake and Utilization of Grazed Forages and Interaction with Forage Digestibility - W. C. Ellis, K. R. Pond and D. S. Delaney, Animal Science Department, Texas A&M University, College Station , Texas 77843

Effects of supplemental monensin (0 or 13-89 ppm) upon digestibility (DOM), rate of digesta passage (KP), fecal output (FO), undigested dry matter fill (UDMF), forage intake (FI) and intake of digestible DOM (DOMI) was measured in 14 grazing trials. DOM of control forage (DOMC, without supplemental monensin) ranged from 37.4 to 65.4. Monensin increased DOM via increasing digestibility of fiber (DNDF) of the magnitude which was inversely related to the reduction in KP due to monensin. This suggests the increased DNDF was caused by increased residence time in the rumen (1/KP) due to monensin. Monensin influenced FO, FI, UDMF and DOMI in a cubic interactive fashion with DOMC. Maximum positive response to monensin (monensin - control/control) occured with forages of 55

DOMC with no response with forages of 44 and 64 DOMC as estimated from a cubic regression model. These same measurements were negatively affected by monensin at DOMC <44 and >64. These results suggest that, when supplemented to medium quality forages (DOMC of 45-64), monensin increases grazed forage intake via increasing UDMF and DNDF. When supplemented to poorer quality forages (DOMC <44) monensin reduces FO and FI more than is compensated for by DNDF and thereby reduces DOMI. Apparently this occurs by reducing KP in a digestive system which already has maximum fill and cannot further compensate by increasing UDMF. The progressively reduced response to monensin when supplemented to forages between 55 and 64 DOMC is suggested the result of a progressively reduced involvement of fill in determining intake with metabolic regulation becoming dominant at 64 DOMC. These results suggest a major effect of monensin in foraging animals is upon KP and consequently upon DNDF and UDMF. The ability of the animal to accomodate increased UDMF and the importance of physical vs metabolic regulation of intake then determines the consequential responses in FO, FI and DOMI.

Digestible Energy and Estimated Net Energy in Soyhulls for Dairy Cows - W. O. Odwongo and H. R. Conrad, Ohio Agricultural Research and Development Center, Wooster, Ohio 44691

Evidence accumulated over the past several years indicates that soyhulls contain polysaccharides that are readily digestible in the gut of ruminants. Consequently, it has been observed that soyhulls have a high potential as ingredients that could be routinely or regularly included in dairy rations to provide readily fermentable carbohydrates. An experiment was conducted, therefore, to study the energy intake and efficiency of utilization by dairy cows fed diets containing soyhulls in which soyhulls, Solka Floc, and urea replaced the corn and soybean meal ingredients of a conventional dairy ration. The second objective was to evaluate milk yield and composition in animals fed diets with soyhulls. The third objective was to assess the body weight changes of the animals fed such diets. The diet contained 37.5% soyhulls, 21% beet pulp, 20% Solka Floc, and 4.2% urea in the concentrate portion. In addition 2.5-3.5 kilograms of hay were fed. The conventional ration contained 40% concentrate, 40% hay, and 20% corn silage on a dry weight basis. A switch-back trial over two lactations was used to evaluate the diets. The results showed that the difference in digestibility was the main effect. The average digestibility of the soyhull diet was approximately 60% of the digestible energy; conventional diet - 69% of the digestible energy. The energy of the soyhulls was utilized to meet the requirements of the dairy cows for milk production with the same efficiency as the conventional diet.

Digestibility and Utilization of Wheat Straw Diets Supplemented with Bypass Protein - J. R. Males and R. H. Pritchard, Washington State University

Soybean meal-urea, formaldehyde treated soybean meal-urea, high urea (50% of supplemental nitrogen) and soybean meal (SBM) protein supplements were compared to determine their effect on nitrogen utilization (NU) and dry matter digestibility (DMD) of wheat straw (WS) diets. The composition of the 4 pelleted diets was: (1. 91.6% WS, 0.4% barley, 0.2% fat, 0.4% urea, 7.4% SBM

(9.4% crude protein); (2) 91.6% WS, 0.4% barley, 0.2% fat, 0.4% urea, 7.4% F-SBM (8.6% crude protein); (3) 84.5% WS, 13.6% barley, 0.6% fat, 1.3% urea (8.4% crude protein); and (4) 86.1% WS, 3.2% barley, 0.3% fat, 10.4% SBM, (9.5% crude protein). Diets were arranged in a Latin square design, and fed to 16 yearling wethers confined to metabolism crates. DMD was higher ($P<.05$) with diet 1 than 2. Coefficients of DMD were 44.0 $\pm$ 2.9, 27.3 $\pm$2.1, 40.0 $\pm$ 1.9 and 42.3 $\pm$ 1.8 for diets 1, 2, 3, and 4, respectively. Digestibility of acid detergent fiber was similarly affected by diet. Apparent nitrogen (N) digestion was lower with formaldehyde treated SBM than all other treatments ($P<.05$). Type of protein supplement also affected the utilization of N apparently absorbed. The percentage of N absorbed that was retained was highest with diets 1 and 4 and differed from 2 ($P<.05$). The mean percentage of absorbed N retained for each diet was 14.4 $\pm$ 10.2, -29.1 $\pm$ 10.2, -4.4 $\pm$ 4.1 and 14.1 $\pm$ 5.5%, respectively. Three ruminally fistulated lambs were randomized across the experiment and fed these same diets to help characterize ruminal fermentation. Two hours after feeding, the mean levels of ruminal ammonia (mg/dl) and volatile fatty acids (umoles/ml) (acetate, propionate and butyrate) were 10.5 $\pm$ 2.8, 7.7 $\pm$ 2.7, 12.2 $\pm$ 6.1, 6.6 $\pm$ 3.6 and 66 $\pm$ 3, 57 $\pm$ 5, 70 $\pm$ 3, 73 $\pm$ 13 for the 4 diets, respectively. These data reflect that urea can successfully replace as much as 15% of the supplemental N in a WS diet (1), but may be of limited value when included at higher levels (3). This occurred in spite of the fact that urea was fed at or below the urea fermentation potential of the diets involved. Formaldehyde treatment of SBM limited crude protein availability resulting in lowered dry matter digestibility and nitrogen utilization. Whether this is the result of overprotection of SBM protein or decreased ruminal fermentation of WS cannot be derived from these data. However, depressed acid detergent fiber degradation indicates that fermentation was depressed. This points out the need for further studies to evaluate the relative usefulness of supplementing low quality forages with bypass protein.

Amino Acid and Fatty Acid Digestion in Intestinally Cannulated Cows Fed Heat Treated or Raw Soybeans - Marshall D. Stern, Department of Animal Science, University of Minnesota, St. Paul, Minnesota 55108; and Larry D. Satter, Department of Dairy Science, University of Wisconsin, Madison, Wisconsin 53706.

Four lactating Holstein cows, surgically prepared with rumen fistulas and simple t-type cannulae in the duodenum and ileum were used in a 4 x 4 Latin square experiment. Whole soybeans (RAW), Soybean meal (SBM) and whole soybeans extruded at 132C (132) and 149C (149) each provided 50% of the protein in diets comprised of grain, corn silage and hay. Each experimental period was 14 days in duration including a 10-day adjustment period. Samples of ruminal, duodenal, ileal and fecal contents were taken on days 11-14. Lanthanum was used as a marker to measure flow and digestibility of nutrients. Long chain fatty acid composition of the grain mixture containing extruded soybeans at 149C decreased by 38% when compared to the grain mixture containing raw soybeans. This was probably due to oxidation of polyunsaturated fatty acids when soybeans were heated. C18:1 fatty acid absorption in the small intestine (SI) was two times greater for cows fed the extruded soybean diets than the SBM and raw soybean diets. Ruminal degradation of dietary amino acids (determined by difference using DAP as a microbial marker) and amino acid (AA) digestion are presented

	RAW	SBM	132	149
Degradability of AA in rumen, %	74	72	59	58
AA intake, g/d	2064	2081	2085	2097
AA flow to duodenum, g/d	2090	2265	2314	2361
AA absorption in SI, g/d	1459	1617	1749	1777
AA absorption in SI, %	70	71	76	75

Results from this experiment indicate that protein degradation in the rumen was lower when soybeans were extruded at 132 and 149C. Total amino acid flow to the duodenum and subsequent absorption in the small intestine (g/d) was lowest for raw soybeans. Essential amino acid absorption from the small intestine was also lowest for raw soybeans, while cows fed whole soybeans extruded at 149C had greater absorption of histidine, arginine and phenylalanine when compared to the SBM diet.

Rationale for Calcium Soaps in Ruminant Nutrition - D. L. Palmquist and T. C Jenkins, Department of Dairy Science, Ohio Agricultural Research and Development Center, Wooster, Ohio 44691

Milk production response to added fat in dairy rations is well-documented. Conventional U.S. dairy rations contain 300-400 g fatty acid/cow/day. Additional of 500 g fatty acid is readily accepted in Europe; studies with "protected" fats show responses in milk and fat production up to at least 1 kg added fat/cow/day. At the latter intakes free fat can have severe inhibitory effects on rumen cellulolytic activity, with decreased acetate/propionate ratio and milk fat content. In earlier experiments we showed dietary calcium to have important influence on total dry matter and acid detergent fiber (ADF) digestibility. We theorized that calcium interacted with fat in the rumen to form insoluble soaps, removing their inhibitory action. Subsequently, studies in vitro and in vivo showed Ca soap formation was only 40-50% complete, and saturated fatty acids were preferentially precipitated. Next we found that preformed calcium soaps had no inhibitory effects on fiber digestion in vitro or in the rumen, as measured by duodenal sampling. Five percent tallow fatty acids added to the ration dry matter reduced digestibility of ADF (from 51.5 to 44.9%) in lactating cows, whereas the same amount as Ca soaps had no effect (49.6%). Digestibility of soap fatty acid was equal to conventionally-fed fat. Fat corrected milk production of cows fed 5% calcium soap was 8% greater than no fat controls, and 9.6% greater than 5% fat conventionally fed (preliminary data). Ca soaps of soy fatty acids fed (1250 g/day) to a cow in mid-lactation increased milk linoleic acid content from 3.2% to 10.10% in 48 hours. Oleic acid increased from 26.3% to 36.1%; linolenic acid increased from 0.7% to 1.6%; stearic and butyric acids were unchanged, whereas other major milk fatty acids decreased.

We suggest that calcium soaps may prove to be a viable alternative to formaldehyde-treated proteins as a means of protecting rumen microbiota from the toxic effects of feeding high levels of fat.

Gnotobiotic Lamb Responses to Linoleic Acid- G. G. Bruckner, Department of Food Science, Cornell University, R. E. Tucker, Department of Animal Science, University of Kentucky, H. A. Gordon, Department of Pharmacology, University of Kentucky, G. T. Schelling, Department of Animal Science, Texas A&M University, and G. E. Mitchell, Jr., Department of Animal Science, University of Kentucky.

A factorial experiment involving gnotobiotic (GN) and conventional (CV) colostrum deprived lambs and diets formulated to be adequate (Cunningham and Loosli, J. Anim. Sci. 13:453, 1954) or deficient in linoleic acid was conducted to determine the effect(s) of the microflora on the availability and utilization of an essential fatty acid (EFA) and the physiological consequences of an EFA deficiency. Futhermore, it was anticipated that parameters associated with GN and CV lamb lower bowel function, i.e., Cl^-, Na^+, K^+, etc., might be characterized. Germfree isolators and equipment were constructed for housing and maintaining the animals. Lambs were obtained by sterile surgical procedures and housed in the sterile isolators or in conventional metabolism stalls for 60 days. Skimmed cows' milk with 6% hydrogenated coconut oil and vitamins A, D, and E added with (L) or without (D) 0.32% of the total calories as linoleic acid was homogenized, bottled and autoclaved. It was fed to appetite 3-4 times daily. GNL lambs gained significantly faster and more efficiently than the other treatment groups. The absence of dietary linoleic acid decreased liver and spleen weights, and in general, suppressed organ development with exception of the brain. Red blood cell hemolysis was not affected by treatment. Plasma prostaglandins indicated a slightly decreased plasma PGB level for the linoleic acid deficient groups. The ratios between 5, 8, 11 eicosatrienoic (tri)/5, 8, 11, 14 eicosatetranoic (tetra) acids suggest an EFA deficiency condition if values are greater than 0.4. The liver and plasma tri/tetra ratios of the GN and CV lambs indicate the following: (1) tri/tetra values for all treatment groups were above 0.4 even with linoleic supplementation at 0.32% of the total calories (2) absence of dietary linoleic acid resulted in a 5-10 fold tri/tetra ratio increase in the plasma and liver samples. Furthermore, kidney and brain tri/tetra ratios were greater than 0.4 in GND and CVD lambs, but not in the L-supplemented counterparts. Brain fatty acid ratios did not reflect the EFA status of the animal as well as the other samples tested. The influence of the presence of the microflora on the tissue fatty acid profile was primarily reflected in the medium chain fatty acids, with significant reductions observed in the percent of C-14 fatty acids in the CV versus GN liver samples. The 18:1 to 18:2 ratio (with exception of brain tissue) appears to correlate well with the EFA status of the neonatal lamb. The lower bowel parameters which were assessed indicate that the GN neonatal ruminant, although showing signs of "chronic milk diarrhea", differs in Cl^- and dry matter percent of its lower bowel contents from the "classic rodent model." In conclusion, the data show that neonatal colostrum deprived lambs have an EFA requirement anc suggests that this level is in excess of 0.32% of the total caloric intake as linoleic acid.

Impact of Dietary Phosphorus on Calcium Homeostasis in the Periparturient Dairy Cow - B. A. Barton, N. A. Jorgensen, and H. F. DeLuca, Department of Dairy Science and Biochemistry, University of Wisconsin, Madison, Wisconsin 53706

A study was conducted to determine the influence of phosphorus concentration in the dry cow's diet on vitamin D metabolism and the incidence of parturient paresis. Thirty aged cows (10/group) were fed one of three experimental diets for 28 days prior to calving. Daily calcium intake was 3X the maintenance requirement for each cow while daily phosphorus intake was .7X, 1X, and 3X the maintenance requirement. Maintenance requirements for calcium and phosphorus were based on the 1978 NRC-Dairy. The incidence of parturient paresis was 20% (2/10) in each group. Precalving and calving plasma phosphorus concentration reflected dietary phosphorus intake. Plasma magnesium concentration was not influenced by treatement. Hypocalcemia accompanied calving in all groups. Cows fed the low phosphorus diet (.7X requirement) had higher plasma calcium levels at 3 and 5 days postcalving than did cows fed phosphorus at 1X and 3X maintenance requirements. Dietary phosphorus had no effect on plasma hydroxyproline, 1,25-dihydroxyvitamin D, or 24,25-dihydroxyvitamin D levels. In conclusion, it appears that dietary phosphorus concentration (range .7X, 1X, 3X requirement) did not have a significant impact on calcium homeostasis during parturition. However, cows fed the low phosphorus diet did have higher plasma calcium levels at 3 and 5 days postcalving, which may reflect a preconditioning effect of low dietary phosphorus on vitamin D target tissues.

Relationships between Rumen Fermentation Parameters and B Vitamin Nutrition of Steers - R. A. Zinn, F. N. Owens and R. L. Stuart, Animal Science, Department, Oklahoma State University, Stillwater, Oklahoma 74078 and Hoffman LaRoche, Inc., Nutley, New Jersey 07110.

Four Angus steers (258 kg) with cannulas in the proximal duodenum and distal ileum were used in a 4 x 4 Latin square experiment to study quantitative aspects of B vitamin (thiamin, riboflavin, B_6, niacin, B_{12}) nutrition. The 80% concentrate, corn based diet was fed at 1.2, 1.5, 1.8 and 2.1% of body weight of steers. Rations were meal fed at equal intervals twice daily. Estimates of passage of digesta were based on composites of spot samples obtained from the duodenum, ileum and rectum using chromic oxide as a marker. Steers fed at 2.1% of body weight had net microbial dry matter flow to the duodenum of 619 g/day, (based on nucleic acids) and fermented 2234 g of organic matter in the rumen. Daily intake, duodenal flow, ileal flow and fecal output of vitamins were: Thiamin - 7.2, 33.2, 7.6, 5.0 mg; Riboflavin - 26.6, 49.8, 38.7, 21.9 mg; B_6 - 14.3, 58.1, 8.3, 10.0 mg; niacin - 130, 616, 106, 76 mg, B_{12} - 0, 10.9, 4.9, 5.6 mg. Regression (R^2) of duodenal flow of B vitamins and net microbial flow to the duodenum were: Thiamin, .66; Riboflavin, .29; B_6, .77; Niacin, .74; and B_{12}, .53.

Effect of Heated or Unheated Soybean Meal with or without Niacin on Rumen Protozoa - S. M. Dennis, M. J. Arambel, E. E. Bartley, D. O. Riddell, and A. D. Dayton, Kansas State University

Four rumen-fistulated Holstein cattle with reentrant duodenal cannulas were assigned to each of four treatments in a 2 X 2 Latin square design. The diets were unheated soybean meal (SBM), unheated SMB + niacin, and the concentrate was a pelleted mixture of corn starch, dextrose, SBM, fat, minerals, and vitamins. The cattle were kept in individual stalls and fed three times daily. Niacin (2g) was placed directly into the rumen at each feeding. Cattle fed the heated SBM (table 4) had fewer Entodinium, Diplodinium, total protozoa ($P<.01$), Ophryoscolex, and Isotricha ($P<.05$) than those fed unheated SBM. Addition of niacin to heated SMB increased Entodinium, Diplodinium, total protoza ($P<.01$), Dasytricha and Isotricha ($P<.05$) in rumen fluid above that of the untreated controls. Only Diplodinium ($P<.01$) was increased when niacin was added to unheated SBM. Niacin addition to heated SBM increased protozoal numbers so that they equalled or were greater than what was found with unheated SBM with or without niacin. Increases in protozoa, especially Entodinium and Diplodinium. may be responsible for increased propionate and ammonia production observed when niacin is added to the diet.

Evaluation of Several Methods Used for Estimating Rumen Microbial Protein Synthesis - M. J. Arambel, E. E. Bartley, S. M. Dennis, G. S. Dufva, T. G. Nagaraja, D. E. Nuzback, D. O. Riddell, A. D. Dayton and S. J. Galitzer, Kansas State University

Two rumen-fistulated cattle were assigned randomly to each of three virtually protein-free semipurified diets that varied in ratio of concentrate to roughage: 30:70, 50:50 and 70:30. Following a 14 day adaptation rumen samples were collected on three consecutive days to isolate mixed rumen bacteria and protoza. On days 21, 23 and 25 rumen contents were hand mixed and sampled prior to and at 1, 2, 3, 4 and 5h after feeding (0800h). Total VFA production increased from 67.2 umoles/ml for cattle fed the low concentrate diet to 81.1 umoles/ml in cattle fed the medium concentrate diet. Total VFA production declined in cattle fed the high concentrate diet (73.2 umoles/ml). Butyric and valeric acids increased as concentrate fed increased. Rumen ammonia and urea were higher in cattle fed the medium concentrate diet (16.6 and 17.4 mg/dl, respectively) compared to high concentrate (11.5 and 11.2 mg/dl) and low concentrate diets (9.0 and 9.7 mg/dl, respectively). Total nitrogen content of rumen fluid increased as concentrate fed increased (2.3, 2.7 and 3.1%, respectively). Total rumen bacterial numbers were lowest in the low concentrate diet ($.03X10^{10}$/ml), but did not differ in the medium ($6.14X10^{10}$/ml) or high concentrate ($5.69X10^{10}$/ml) diets. Total rumen protozoa did not differ in cattle fed the low ($1.84X10^{4}$/ml) or medium concentrate ($2.44X10^{4}$/ml) diets, but were higher in cattle fed the high concentrate diet ($4.07X10^{4}$/ml). Increasing the amount of concentrate in the diet increased ATP production (10.4, 19.2 and 34.4 ug/ml rumen fluid for low, medium and high concentrate diets). Aminoethylphosphonic acid (AEP) was not found in isolated rumen bacteria, while diaminopimelic acid (DAP) was not found in isolated rumen protozoa. Rumen microbial protein production (Barr method) decreased as level of concentrate fed increased (224.0, 192.1 and 67.4 mg microbial N/g total N, respectively). Using RNA as a microbial marker resulted in increased microbial synthesis (361.6, 586.8 and 839.1 mg N/g total N, respectively) as level of concentrate fed increased. Microbial protein production using DNA as a marker

was unaffected by diet. Using total amino acid nitrogen as the reference microbial marker resulted in lower estimations of microbial protein synthesis (regardless of diet) with the Barr, RNA, DNA, DAP and AEP methods.

Effect of Diet on Amino and Nucleic Acids of Mixed Rumen Bacteria and Protozoa - M. J. Arambel, E. E. Bartley, G. S. Dufva, T. G. Nagaraja and A. D. Dayton, Kansas State University

Seventeen species of gram-positive and -negative rumen bacteria were grown anaerobically in pure culture. The amino acid composition between gram-positive and -negative organisms was not different. The total nitrogen content of gram-negative bacteria (10.80%) was higher than gram-positive organisms (9.93%). The DNA-N:total N ratio differed between gram-positive (8.76 mg/g) and gram-negative (18.90 mg/g) bacteria, whereas there was no difference between bacteria in the RNA-N:total N ratio. Total carbohydrate content between organisms did not differ. In a second experiment six rumen-fistulated cattle were fed either a high roughage (85% alfalfa hay plus 15% concentrate) or high concentrate ration (15% alfalfa hay plus 85% concentrate). Cattle were adapted 14 days and rument contents sampled on three consecutive days. Rumen microbial yields increased in cattle fed the high concentrate diet (2.78 mg dry cells/ml for bacteria and 1.81 mg/ml for protozoa) compared with the low concentrate diet (1.56 and .40 mg dry cells/ml). Nitrogen content of bacteria was similar for both diets. The DNA-N:total N ratio in bacteria decreased from 27.19 mg/g for cattle fed the low concentrate diet of 20.89 mg/g in cattle fed high concentrate diet. Nitrogen content of protozoa from cattle fed low concentrate was higher (8.41%) than that in protozoa from cattle fed high concentrate diet (7.85%). There were differences among sampling days in the ratio of DNA-N to total N in both bacteria and protozoa. There was a difference among sampling days in the ratio of RNA-N to total N for protozoa but not for bacteria. RNA and DNA content of mixed rumen bacteria are affected by bacterial species present, diet and day of sampling. These nucleic acids may serve as markers for estimating microbial production in the rumen if sources of variation are recognized and adequate corrections made.

Relationship Between the In Situ Bag Technique and an In Vitro Protease Method for Determining the Rate of Insoluble and Soluble Protein Disappearance of Feedstuffs - J. E. Nocek, C. E. Polan, and J. H. Herbein, Virginia Polytechnic Institute and State University, Blacksburg, Viginia.

Eleven protein and five energy feed sources were evaluated for protein degradation either by the *in situ* polyester bag technique or an *in vitro* protease procedure. Enough feed material was solubilized in .1M phosphate-bicarbonate buffer (50 mg feed nitrogen (N)/200 ml buffer) to render 50 g of insoluble feed material. Bags containing 4 g of ground (2mm) insoluble feed material were suspended in the rumen of a fistulated cow and percent N disappearance was measured at 2, 6 and 12 hour. An aliquot of insoluble feed material was hydrolyzed with 6N HCL to determine total amino acid (AA) concentration. Feed material to contain 6.25 mg of protein was placed in a centrifuge tube with 5 mg protease (Streptomyces griseus) and 2.0 ml of

phosphate-bicarbonate buffer (.05% sodium azide) and incubated in a water bath at 39°C. Reactions were terminated (2, 6 and 12 hour) by addition of concentrated perchloric acid, centrifuged at 20,000xg for 15 min and analyzed for percent released AA. Degradation constants (Kd=fractional disappearance/hr of N or AA) were determined for the bag technique (Kd_N) and protease method Kd_{AA}), respectively.

Calculated quantities of feed material were solubilized in .1M phosphate-bicarbonate to render 4.2 mg of soluble protein per ml in the filtrate. The appropriate amount of filtrate, protease and buffer were added to each incubation tube to obtain 6.25 mg soluble protein with 5 mg of S. griseus in a final volume of 2 ml. Duplicate determinations were incubated for 2 hour at 39°C. Blanks without protease were conducted to correct for soluble amino acids. Rates of AA liberated per mg of soluble protein per hour were compared to AA liberated per mg of insoluble protein per hour for the insoluble fraction from 0 to 12 hour.

Analysis for heterogeneity of regressions showed significant ($P<.01$) differences for most protein sources when comparing the two methods. The following sources showed no significant ($P>.10$) differences between methods from 0 to 12 hour: corn gluten feed, dehydrated alfalfa, poultry blend, meat meal, corn grain, wheat middlings, wheat bran, whole wheat, barley and oats. Correlation coefficients (r) of Kd_N and Kd_{AA} for the bag vs protease technique at 0 to 2, 2 to 12 and 0 to 12 hour were -.21, -.35 and -.58, respectively for all feeds.

Degradation of the soluble protein fraction for several protein sources ranged from 100 to 697 uM AA/mg soluble protein/hour for dehydrated alfalfa and soybean meal (48%), respectively. Overall AA release for the insoluble protein fraction remained relatively constant for protein sources (182 to 255 uM AA/mg insoluble protein/hour for meat and bone meal and corn gluten meal, respectively). Corn gluten feed and dehydrated alfalfa had soluble fractions which degraded at a slower rate than the insoluble portion.

Wheat middlings had the fastest rate of soluble protein degradation while corn grain had the slowest of the energy sources tested (2005 and 351 UM AA/mg soluble protein/hour, respectively). Energy sources ranged from 194 to 258 UM AA/mg insoluble protein/hour for cracked and ground corn, respectively.

These results suggest amino acid release *in vitro* and nitrogen disappearance *in situ* are not synonymous for all feedstuffs and there are considerable differences in the degradation potentials of soluble proteins.

Rate and Extent of Fiber Degradation of Various Feedstuffs In Situ - G. A. Varga and W. H. Hoover, Division of Animal and Veterinary Sciences, West Virginia University, Morgantown, West Virginia.

The rate and extent of neutral detergent fiber (NDF) disappearance were determined for 22 grains and six forages *in situ*. Polyester bags (13 x 21 cm) containing 5 g of forage or 8 g of grain were suspended in the rumen of fistulated steers .5 hr after feeding. Two bags were removed at 2, 4, 6, 8, 12, 16, 24, 30, 36 and 48 hour after exposure to rumen contents. A control feed was also removed at 4, 8, 12 and 36 hours to monitor animal variability in rumen

fermentation. Forages and whole grains were ground through a 4 mm screen, while processed grain products were used as provided.

Disappeaance rates of NDF were determine using non-linear regression analyses computed as follows:

$$R = D_o E^{-K(T-L)} + U \quad (t > L)$$ (Mertens, unpublished)

where:
R = residual fiber; D_o = potential digestibility; K = rate of degradation; T = time; L = lag ; U = indigestible fraction. The rate of NDF disappearance for oats and peanut hulls were .27 and .21 hr^{-1} with 26.4 and 13.5% of the NDF degraded at 24 hours, respectively. Barley and wheat midds each had rate constants of .14 hr^{-1} and on the average 55% of the NDF was degraded at 24 hours. Values for wheat bran, distilled grains w/solubles and brewers grains ranged from .071 to .077 hr^{-1}. Beet pulp, soybean meal, corn gluten meal and peanut meal had rate constants ranging from .055 to .048 hr^{-1} with the amount of NDF degraded ranging from a low of 42.3% for corn to a high of 78% for soybean meal. Rate constants for hominy, corn cobs and soy hulls were .041, .034 and .011 hr^{-1} and the extent of degradation: 45.1, 27.5 and 38.4, respectively.

The rate constants for ryegrass, alfalfa, timothy, orchardgrass, clover and hay crop silage were: .079, .062, .056, .068 and .023 hr^{-1} and NDF degraded at 24 hours ranged from 32.5% for hay crop silage to 66.3% for ryegrass. Variable lag times were associated with the NDF degradation of 11 of the feedstuffs. The lag times ranged from .24 to 5.41 hours. These results indicate that the rate and extent of NDF degradation varies a great deal among feedstuffs and these values may need to be considered in the formulation of diets for ruminants.

Influence of Dietary Protein and Energy on Disappearance of Various Types of Forage Dry Matter from Dacron Bags Suspended in the Rumen - V. P. de Faria and J. T. Huber, Department of Animal Science, Michigan State University, East Lansing, Michigan 48824

In two experiments, the influence on intakes, in vivo digestibility, disappearance of dry matter of three forage types from bags suspended in the rumen from in vitro fermentations of varying crude protein in corn silage rations and changing energy concentrations in alfalfa haylage rations was studied with six rumen-fistulated steers. Increasing corn silage protein from 8.1 to 13.3% by urea addition increased dry matter intakes, but did not alter in vivo dry matter digestibilities or disappearance of dry matter of three forage types (corn silage, alfalfa hay and orchardgrass hay) from dacron bags suspended in the rumen. A small, but significant decrease as nitrogen increased was shown for in vitro dry matter disappearance. Additions of ground corn to comprise 60% of the dry matter in alfalfa haylage rations increased voluntary intakes and in vivo dry matter digestibilities, but did not change acid detergent fiber digestibility. Disappearance of dry matter from dacron bags was slightly depressed, but disappearance from in vitro fermentations was not significantly affected. Similar rankings for the three test forages in dry matter disappearance from dacron bags were shown for all rations. These data suggest that wide differences in ration type and protein and energy content will give similar results with the suspended bag method. Impregnation of bags with rumen

contents and digesting after removal in a pepsin solution were factors which influenced results.

Influence of Increasing Density on Rate of Particulate Passage in Dairy Cows - Fred R. Ehle, Research Animal Scienctist (Nutrition), USDA-ARS and Assistant Professor, Department of Animal Science, University of Minnesota, St. Paul, Minnesota 55108

Chromium (Cr) solutions were used to mordant the cell wall isolated from chopped alfalfa hay. The mordanted fibers were evaluated for their suitability as particulate rate of passage markers and used to study the effect of particle density on rumen particulate turnover rate. Two pregnant nonlactating Holstein cows were fed ad libitum equal portions of a total mixed ration three times a day. A switchback design was used with five 15-day experimental periods. The animals were fed a pulse dose of the marker and fecal grab samples were collected at scheduled intervals for 10 days. The undigested cell wall was isolated and Cr analysis was performed by atomic absorption spectroscopy. Linear regression analysis was used to determine the slope of the line that fit the declining phase of the marker excretion curve. Rumen turnover rates and densities were: .0155, not determined; .0191, 1.111; .0303, 1.250; .0340, 1.478; and .0415, 1.667 for alfalfa cell wall mordanted with the 2, 4, 8, 16 or 32% Cr solutions, respectively. The regression of rumen turnover rate of alfalfa hay on Cr concentration of the mordanting solution was: Y =.0008X + .0181 (r = .920). These data demonstrate the suitability of the 2 and 4% chromium solutions for preparation and use of Cr-labeled alfalfa as a particulate rate of passage marker. Results indicated that particle density may be an important factor affecting particle turnover rate in the rumen.

A Kinetic Method for Measuring Rates of Genesis and Passage of Varied Size Forage Particles in the Rumen - K. R. Pond, J. H. Matis, J. Guerrero, Carlos Lascano and W. C. Ellis, Texas A&M University, College Station, Texas 77843

Ingested forage particles entering the rumen have two fates: passage to the feces without size reduction or size reduction followed by passage. Large particles (>1600 um) labeled with ^{141}Ce were dosed into the rumen of 18 cattle. ^{141}Ce was measured after separation by particle size in serial samples taken from the rumen and feces. Rumen content and fecal ouput of varied particle sizes was determined from a continuous infusion of Cr DTPA and dry sieving of samples. Rates of breakdown and passage were simultaneously estimated by compartmental analysis based on marker recovered over time within each particle size in the rumen and feces. Initially only serial breakdown to successively smaller particles was assumed. However, addition of concurrent breakdwon to all smaller particles yielded larger model sum of squares, smaller error sum of squares and indicated concurrent breakdown should be included in model ($P<.05$). The rate parameters for degradation (.3 - .7) were much larger than rate parameters for passage from rumen (.03 - .09) and rate parameters for passage increased as size of particle decreased. Rate of serial genesis (.3 - .6) was greater than rates of concurrent genesis (.1 - .2). These results indicate the breakdown of particles to smaller sizes may not be the major limiting step in their passage from the rumen although smaller particles do pass faster from the rumen.

Sodium Diacetate: A Stimulant of Feed Intake in Early Weaned Calves - D. K. Roseler and H. R. Conrad, Ohio Agricultural Research and Development Center, Wooster, Ohio 44691

In previous studies it was found that sodium diacetate was a preferential feed flavor for mature dairy cattle. Also, we observed that young calves would eat a larger amount of dry feed in the post-weaning period under circumstances of early weaning. However, there were only six calves per group in the earlier experiments and there were insufficient data to evaluate the magnitude of the stimulus and the statistical significance. The objective of the present study was to evaluate the specific effect of sodium diacetate in a high roughage calf pellet on the feed intake and gain in body weight of calves during the post weaning period. A high roughage diet was composed of 66% roughages and 33% concentrate and was designed as a complete feed. Twenty-eight percent of the diet was furnished through a milk replacer used in the total diet as the major proportion of the concnetrate. Sodium diacetate was included in the diet at the level of.5%. The experimental design was comparisons of groups. In the first experiment the comparisons at weaning age of 3 weeks and 5 weeks were used. In experiment 2 calves were weaned between 5 and 6 weeks of age with many of them being delayed to 6 weeks. In experiment 3 all calves were weaned at the age of 30 days. Sixty-six calves were used to evaluate sodium diacetate. The results of the experiment showed that sodium diacetate caused an increase in feed intake in calves during the period of reduction in milk just previous to weaning and immediately after weaning of 278 grams of dry matter per day. There was a simultaneous increase in body weight gain of 61 grams daily. We concluded that the growth response in young calves from the presence of sodium diacetate in the dry feed was a simple input-output response from greater feed consumption and consequently more gain in body weight.

High Moisture Ground Ear Corn, High Mositure Barley or NaOH-Treated Barley for Lactating Cows: Milk Production and Ration Utilization - L. Kung, Jr., D. S. Brecht, B. W. Jesse, A. Shanan, J. W. Thomas, J. T. Huber and R. S. Emery, Department of Animal Science, Michigan State University, East Lansing, Michigan 48824

Whole barley was treated with sodium hydroxide (NaOH) in a laboratory trial. Dry matter disappearance of whole barley treated with 0, 2.5, 3.5, 4.9% NaOH or untreated ground barley from nylon bags suspended in the rumen for 30 hr was 0, 59.6, 72.4, 93.0 and 82.2%.

In a lactation trial 24 Holstein cows (6 per treatment) were fed complete rations consisting of high moisture ground ear corn (GEC), high moisture rolled barley (HMR-barley) or whole high moisture barley treated with 3.5% NaOH (NaOH-barley). Rations of 45% forage (78% corn silage, 22% hay) and 55% concentrate were balanced for protein, minerals and vitamins (NRC). Daily milk yields were greatest for cows fed HMR-barley (26.5 kg), next for GEC (25.1 kg) and least for NaOH-barley (23.0 kg). Milk composition did not differ significantly between treatments. Dry matter intake was greatest for cows fed GEC and lower of the barley rations. Per kg of dry matter cows on HMR-barley produced 1.34 kg milk; whereas, conversion efficiencies for cows fed GEC and NaOH-barley were 1.01 and 1.10.

Alpha-linked glucose and pH of feces were similar for GEC and HMR-barley rations, but fecal pH was lower and alpha-linked glucose concentrations three times greater for NaOH-barley suggesting a reduction due to NaOH treatment in rumen and/or intestinal starch utilization.

Ration digestibilities (%) of dry matter, ADF and N were 61.4, 25.3, 64.7 for GEC; 64.4, 38.0, 67.1 for HMR-barley; and 56.8, 43.2, 54.8 for NaOH-barley. Rumen pH and molar % acetate were highest for NaOH-barley. Rumen solids turnover, estimated by excretion of ytterbium in feces was greatest for NaOH barley (9.09% hr), lowest for HMR-barley (4.93% hr), and intermediate for GEC (6.10% hr).

Cows fed HMR-barley produced as much milk, but more efficiently than those fed GEC. Feeding NaOH-barley reduced milk production probably because of decreased starch utilization and lower ration digestibility associated with a faster rumen turnover.

Sodium Bicarbonate for Dairy Cows Fed Hay Crop Silage Diets - M. R. Stokes and L. S. Bull, University of Maine, Orono, 04469

Feeding behavior, milk production and composition, and rumen fluid turnover rate were observed in three cows fed 0 (C), 68, (L) or 113.5 (H) g $NaHCO_3$/d. Diets fed contained 70%, 50% or 30% concentrate DM plus hay crop silage and were fed sequentially, each for 90 days, beginning 2 days postpartum. Buffers were fed in 3 x 3 Latin squares within each diet. Each buffer period lasted 30 days. Behavior was measured on several days after 2 weeks adaptation and rumen turnover was measured with PEG on days 29-30. Eating behavior did not differ between diets. For diets containing 70, 50, 30% concentrate, least squares means were: meals/day, 11.1, 9.4, 8.9; minutes eating, 240, 291, 290; meal length (min), 25.3, 32.3, 37.9; meal size (kg), 3.3, 5.2, 6.5; eating rate (g/min/MBW), 1.13, 1.37, 1.51; eating rate (g DM/min/MBW), .55, .55, .51; meal as % total eaten, 10.4, 11.0, 12.6. Buffer addition increased DM intake with 70% and 50% concentrate diets and increased milk yield with 70% concentrate diet. Buffer increased time spent eating with 70% concentrate diet but reduced it with 30% concentrate diet. Eating rates were increased by buffer addition to 30% concentrate diet, and low buffer was greater than high buffer. Mean milk composition and rumen fluid turnover were not affected by diet or buffer treatments. Least squares means for diets containing 70, 50, 30% concentrate were: milk fat %, 3.25, 3.67, 3.74; milk crude protein %, 3.07, 3.16, 3.21; milk solids-not-fat %, 8.68, 8.57, 8.84; liquid turnover (%/hr), 16.0, 16.4, 15.5. Corresponding means for buffer treatments (C, L, H) were: 3.74, 3.31, 3.61; 3.07, 3.20, 3.16; 8.58, 8.76, 8.74; 15.7, 15.7, 16.5. Additional $NaHCO_3$ appears to be beneficial to early lactation cows receiving high concentrate diets based on hay crop silage.

Effect of Magnesium Oxide and Sodium Bicarbonate on the Environment and Digestion in the Rumen of Lactating Cows - H. Tagari, N.S. Reddy and L. D. Satter, Dairy Science Department, University of Wisconsin, Madison, Wisconsin 53706

Five Holstein cows fitted with rumen cannulae and T-type cannulae at the proximal duodenum and terminal ileum were used in a 5 x 5 Latin square design experiment having 14 day periods. The five experimental diets contained grain mix and corn silage in an approximate ratio of 60:40 (DM basis). Lanthanum was used as a digestibility marker. The grain mix was supplemented with: 1) no buffers (control); 2 .64% MgO (Basic Chemicals, Inc.); 3) 1.28% $NaHCO_3$; 4) .64% MgO (Basic Chemicals, Inc.) plus 1.28% $NaHCO_3$; 5) .64% MgO (Martin Marietta) plus 1.28% $NaHCO_3$. Though the values were not significant, buffers supplemented either alone or in combination reduced feed intake per Kg $W^{0.75}$. Average daily milk yield was not altered, but significant treatment effects were observed for daily milkfat yield: .56, .62, .73, .62, and .60 kg; daily milk protein yield: .67, .67, .74, .65, and .64 kg; duodenal pH: 2.84, 2.83, 3.01, 3.03, and 2.91; pH of feces: 6.01, 6.32, 6.14, 6.33, and 6.52; organic matter flow at duodenum: 17.2, 13.8, 15.2, 14.2, and 13.1 kg; acid detergent fiber flow at duodenum: 2.37, 1.89, 2.09, 1.81, and 1.96 kg and apparent acid detergent fiber digestibility: 34.5, 44.0, 42.7, 44.9, and 40.1%. Addition of buffers at the levels used in this experiment did not exhibit an additive effect on percent apparent digestibilities of organic matter: 61.1, 64.2, 64.5, 65.3, and 61.9; starch: 92.6, 93.3, 93.4, 93.0, and 92.7; crude protein: 72.1, 66.8, 70.3, 62.3, and 68.3; non ammonia nitrogen: 64.7, 63.8, 64.7, and 63.3; total amino acids: 71.8, 70.0, 66.8, 67.4, and 69.2 and essential amino acids: 69.3, 70.3, 68.2, 66.6, and 69.5. The differences observed for volatile fatty acid concentrations mM/l; 109.7, 106.8, 106.2, 109.9, and 105.1 and ammonia nitrogen mg/100 ml, 13.0, 13.5, 13.0, 12.5, and 12.9 were also not influenced by the treatments. Water intake and digesta flow at the intestinal cannulae were not affected by treatment. The most important effect of feeding buffers was on fecal pH, milk fat production and fiber digestibility.

Evaluation of the Acid-Neutralizing Capability of pH-Regulating Materials by pH Stat Tiration - L. J. Wheeler, C. H. Noller and J. L. White, Departments of Animal Science and Agronomy, Purdue University, West Lafayette, Indiana 47907.

Reactivities of pH-regulating compounds $CaCO_3$, $MgCO_3$, and MgO and $NaHCO_3$ were compared. Total acid consuming capacity test (TACC) was used to measure total capacity to neutralize acid. The pH-stat titration measured rates of reaction at specificed pHs, and *in vitro* rumen fluid incubations measured buffering activity in poorly buffered media. Total acid consuming capacity values for reagent grades $CaCO_3$, $MgCO_3$, MgO and $NaHCO_3$ were 19.8, 20.7, 43.7 and 12.2 meq H^+ consumed per gram, respectively. The pH-stat titrations of the respective materials at pHs ranging from 3.0 to 7.5 showed that $CaCO_3$ lost reactivity rapidly at a pH above 5.5 and $MgCO_3$ above pH 6.5. Magnesium oxide lost reactivity between 3.0 and 6.0 and then tended to maintain reactivity above 6.0. Sodium bicarbonate began to lose reactivity above pH 6.0. Addition of the above materials, in amounts of equal TACC, to weakly buffered rumen fluid media produced changes in media pH when measured at hourly intervals from 0 to 6 hours. The order of increases in pH of the medium was: control < $CaCO_3$ <MgO < $MgCO_3$ < $NaHCO_3$.