

REPORT ON

XV CONFERENCE ON RUMEN FUNCTION

held at

Pick Congress Hotel, Chicago Illinois  
November 28-29, 1979

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

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

- |                                    |   |
|------------------------------------|---|
| (a) Microbiology                   | R. B. Hespell for M. P. Bryant (IL)     |
| (b) Physiology and Physiopathology | W. M. Wass (IA)<br>A. D. McGillard (IA) |
| (c) Nutrition                      | J. T. Huber (MI)                        |
| (d) Agronomic                      | J. C. Burns (NC)                        |

MICROBIOLOGY

Comparison of Bermudagrass and Orchardgrass for Differences in The Mode of Microbial Attack of Tissue Types and Variations in Microbial Types Associated with Cell Wall Digestion - Danny E. Akin, USDA, SEA, AR, SR, Richard B. Russell Agricultural Research Center, P. O. Box 5677, Athens, Georgia 30604

Electron microscopy of leaf blades degraded by rumen microorganisms have shown that digestible tissues in orchardgrass (Dactylis glomerata) are more rapidly degraded than similar tissues in bermudagrass (Cynodon dactylon). Research was undertaken to determine microbial associations related to differential fiber digestion of these two forages. Transmission electron microscopy (TEM) revealed differences in the mode of attack by microbes. In orchardgrass, degradation without prior bacterial adherence (i.e., non-localized removal of fiber and generalized loss of electron denseness) occurred in the mesophyll, phloem, parenchyma bundle sheath, and the inner part of the epidermis, i.e., about 65-70% of the area of the leaf cross-section. Conversely, in bermudagrass, bacterial adherence before degradation (i.e., distinct, localized zones of degradation surrounding and in the shape of adhering bacteria) were present in all but the mesophyll and phloem, i.e., only about 30% of the leaf area. The percent of the major bacterial morpho-types involved with the initial attack on cell walls was similar for these forages, but the hemicellulolytic protozoan Epidinium ecaudatum form caudatum preferentially attacked and ingested orchardgrass cells and rarely associated with bermudagrass. Growth studies comparing the numbers of bacteria utilizing xylan, pectin, or cellobiose from populations adapted in vitro to orchardgrass fiber were 80% higher than those from bermudagrass-

adapted populations; xylanolytics were significantly greater ( $P < .05$ ). These studies indicated a greater availability of cell wall components (especially hemicellulosic types) in orchardgrass for rumen microbial digestion. The presence of low levels of syringal-type lignin in bermudagrass cell walls may contribute to its decreased fiber availability.

The isolation of cellulases and other carbohydrates from sheep ruminal contents -- J. M. Gawthorne, School of Veterinary Studies, Murdoch University, Murdoch, Western Australia.

Isolation from rumen contents. A crude mixture of cellulases and other hydrolytic enzymes was isolated from the ruminal contents of sheep fed a mixture of 75% chopped wheat hay and 25% chopped lucerne hay.

Ninety percent of the cellulase activity was associated with the partially digested plant solids. Seven percent with bacteria in the fluid and 3% with cell-free rumen fluid. The enzymes were released from the solids by vigorous shaking with cold, dilute, phosphate buffer pH 6.9. Approximately ten sequential extractions were required for complete extraction.

Cellulase activity extracted in this way fluctuated in rumen contents taken at various times after feeding with a tendency towards two peaks of activity at 5 hr and 16 hr post feeding, at times when the animals were ruminating.

The cellulases could be concentrated in phosphate buffer by filtration on an Amicon XM300 membrane. Between 70% and 95% of the cellulase activity was retrieved in the solution above the filter, indicating that it was associated with material of high molecular weight.

Electrophoresis in polyacrylamide gels revealed a major band of protein at 400,000 MW with several smaller bands in the region 50,000-350,000 MW. Cellulase activity was present in the major band, but was also detected in other bands of smaller molecular weight. Dissociating agents such as urea or sodium dodecylsulfate dispersed all bands, demonstrating that they were complexes of proteins of  $< 50,000$  MW.

In addition to cellulase activity, the material retained by the XM 300 membrane had  $\beta$ -glucosidase,  $\alpha$ -glucosidase, xylanase,  $\beta$ -galactosidase,  $\beta$ -glucuronidase, N-acetyl- $\beta$  glucamidase, sucrase, alkaline phosphatase, lipase and esterase activity. Tests for  $\alpha$ -mannosidase,  $\beta$ -mannanase, protease and acid phosphatase activities were negative.

Attempts to purify the cellulases by sephadex chromatography, ion exchange chromatography or electrophoresis were relatively unsuccessful because of the high adsorption of the enzymes on support materials, low recovery of activity and variable dissociation molecular weight complex. Affinity chromatography on cellulose yielded an enzyme that attacks insoluble cellulose and produces cellobiose. Chromotography on conconavalin A sepharose gave cellulases and B glucosidases in relatively high-purity, indicating that these enzymes are glycoproteins, or are at least firmly associated with carbohydrate polymers containing glucose and/or mannose.

Electron microscopy of suspended cellulose. Pure cellulose powder was enclosed in nylon bags and suspended in the rumen of sheep. Samples were withdrawn regularly over a period of 10 days and studied under an electron microscope. Portions of the same samples extracted with phosphate buffer and assayed for cellulase, glucosidase and xylanase activity.

Electron micrographs showed irregularly shaped bacteria with intracellular granules, attached to the cellulase fibres. The bacteria were enclosed in capsular material, and digestion of the fibres was obvious at the sites of attachment. Cellulase activity and very high levels of xylanase activity was detected in buffer extracts of this material, but there was negligible B glucosidase activity.

Cellulases of Ruminococcus albus -- Ida Yu and R. E. Hungate,  
Department of Bacteriology, University of California,  
Davis, California 95616

Ruminococcus albus, strain 6, isolated from the rumen, produces at least four cellulases, designated I to IV, separable on gel filtration columns, and active on wet pebble-milled Whatman No. 1 filter paper. The molecular weight of the largest enzyme, I, is estimate at  $2 \times 10^6$ d from electronmicrograph, sedimentation rate and gel filtration studies. The smaller enzymes, II, III and IV have molecular weights of approximately  $6 \times 10^5$ ,  $5 \times 10^4$ , and  $4 \times 10^4$ ,d, respectively. The largest of these latter appears to be a component of the  $2 \times 10^6$  complex. Products formed from cellulose are glucose, cellobiose, cellotriase, cellotetraose and possible small quantities of higher polymers.

The strain has at various times shown changes in the vertically elliptical shape of the clearing of the cellulose around colonies of strain 6 in the thin agar on the wall of rumen fluid cellulose agar roll tubes. Strain 6L does not produce any enzyme II, and the clearings are relatively longer in a vertical direction. Strain 6C produces only enzyme II, and shows small circular clearings around colonies. These observations suggest that the largest enzyme sediments at Earth's gravity. Incubation of roll tubes under a

gravitational force of 4 X g results in a greater vertical elongation of the clearing as compared to control tubes at Earth's gravity.

Establishment of the Adherent Microbial Population of the Sheep Rumen -- \*R. E. Mueller, J. M. Asplund and E. L. Iannotti.  
Department of Animal Husbandry, University of Missouri, Columbia, Missouri 65211.

Tissue samples were obtained from 12 well-distributed sites in the ruminoreticulum of a 43 Kg wether on a corn-cottonseedhull based diet. Samples were washed once in 0.85% NaCl, fixed in glutaraldehyde and prepared for scanning electron microscopy (SEM). Each site was examined for approximately 4 hours in an effort to characterize morphologically the adherent population in the normal adult. This examination revealed a complex microbial population consisting of numerous morphotypes, including rods, cocci, spiral, and filamentous organisms. Variation in morphotypes was observed both between sites and within a site between the base and the tip of the papillae. The population was predominately mixed, however cocci were very predominant on the tips of the papillae in 8 of the 12 sites examined. Two rod-shaped microbes were repeatedly seen in close association, with the smaller rod attached to the surface of the larger.

Succession of the adherent population was subsequently studied by obtaining duplicate pieces of wall tissue from 4 sites in the rumen of lambs at 1, 2, 4, 6, 8, and 10 weeks of age, respectively. Tissue samples were washed 3 times in anaerobic mineral solution; one piece was blended in a waring blender with 100 ml of the mineral solution after which dilutions were made for both anaerobic and aerobic culture counts, with the second piece being prepared for examination by SEM. Numbers of aerobes decreased significantly after week 1 and as a percent of anaerobes the aerobes constituted less than 0.7% of the population. There were no significant differences in bacterial numbers between the 4 sites. SEM examination of the tissues is currently underway.

The use of ribonucleic acid, <sup>35</sup>S and diaminopimelic acid as rumen bacterial markers with in vitro studies -- L. N. Rode, B. A. Jones and L. D. Satter, Department of Dairy Science, University of Wisconsin, Madison, Wisconsin 53705.

Two studies were conducted to determine the relative value of ribonucleic acid (RNA), sulfur-35, (<sup>35</sup>S) and diaminopimelic acid (DAPA) as markers for bacterial protein synthesis and the effect of structural carbohydrates on the efficiency of bacterial protein synthesis in continuous culture. Five hundred millilitre, single flow fermenters were run at a dilution rate of 0.07/hr. In trial 1, a purified diet, with all nitrogen (N) supplied as urea, was used.

Bacterial growth measured directly as non ammonia nitrogen flow was 4.90g bacterial N synthesized/100g dry matter (DM) apparently fermented (mean of 3 observations). Growth measured using  $^{35}\text{S}$  RNA and DAPA was, as a percent of that measured directly, 100.2 $\pm$ 2.95\*, 100.8 $\pm$ 3.65 and 97.9 $\pm$ 8.13 respectively. In trial II, three isonitrogenous treatment mixtures of alfalfa-grass hay, corn and casein were used. Each treatment was replicated four times. High (H), medium (M) and low (L) fermentable diets contained 11.9, 25.6 and 38.9% ADF respectively. Daily dry matter addition to the fermenters was 19.2, 24.9 and 33.1g/day for diets H, M and L respectively. Apparent dry matter disappearance was 10.3, 11.7 and 12.5g/day for diets H, M and L respectively. Efficiency of bacterial growth (g bacterial N synthesized/100g apparent DM disappearance) as measured by  $^{35}\text{S}$ , RNA and DAPA was 3.21 $\pm$ .12, 3.18 $\pm$ .14 and 3.25 $\pm$ .26 respectively for diet H; 3.03 $\pm$ .08, 3.08 $\pm$ .08 and 3.05 $\pm$ .17 for diet M and 2.97 $\pm$ .10, 2.99 $\pm$ .09 and 2.96 $\pm$ .15 respectively for diet L. No significant differences were observed between markers, however DAPA showed more variation than either  $^{35}\text{S}$  or RNA. Diet H resulted in significantly more bacterial N synthesized/100g apparent DM disappearance ( $P < 0.05$ ) but when corrected for bacterial dry matter no significant differences were observed.

\*  $\pm$  standard deviation

Use of differential carbohydrate media and anaerobic plating techniques for determining metabolic groups in rumen bacterial populations -- Jane A. Zeigler Leedle and Robert B. Hespell.  
<sup>1</sup>Departments of Dairy Science and Microbiology, <sup>1</sup>University of Illinois, Urbana 61801.

A complete (CC) medium containing nine carbohydrates and a variety of differential (DC) media containing either one or two carbohydrates were prepared by the addition of the carbohydrates to a basal (BC) medium that included incubated clarified rumen fluid, trypticase, yeast extract, hemin, volatile fatty acids and minerals. These media were used for the enumeration and the differentiation of mixed rumina bacteria into metabolic subgroups. Using bacteriological techniques involving an anaerobic glovebox petri plates, the CC medium was found to support 20-50% higher colony counts than did the CC medium prepared using the conventional roll tube method. The lower counts on roll tubes were shown to result primarily from the loss of viability due to heat stress. The methods used in sampling a fistulated dairy cow and preparing the sample for bacterial studies were examined. It was found that blending (1 min) increased direct and viable counts by 26-38%. The DC media were found by plating techniques to be suitable for differentiating mixed rumen bacterial populations into metabolic

groups based upon carbohydrate utilization as shown by: a) correct colony formations by pure cultures of rumen bacteria upon DC and two selective media; b) metabolic group profile differences within solid and liquid fractions of ruminal contents; and c) metabolic group profiles in contents fed animals fed high forage or high grain diets. Supported by these results, it appears that the array of DC media developed is an effective tool with which to assess the metabolic group composition of mixed rumen bacterial populations.

Some Physiological Bases of Bacterial Competition in The Rumen --  
J. B. Russell, Department of Animal Science, University of Illinois.

In an effort to better quantitate bacterial competition in the rumen, physiological parameters within a five bacteria (Selenomonas ruminatum, Bacteroides ruminicola, Megasphaera elsdeni, Butyrivibrio fibrisolvens, and Streptococcus bovis), six substrate (glucose, maltose, sucrose, cellobiose, xylose, and lactate) test system were compared. In this model, maximum growth rate differed greatly among the organisms, and relative growth rate was dependent on the particular substrate that was provided. When each microbe was provided with all substrates, some substrates were used to the exclusion of others and injection of "preferred" substrates was found to inhibit the utilization of "repressed" substrates. Comparison of "preferred" and "repressed" substrates indicated that these microbes would preferentially use different substrates. Substrate affinities and maintenance energy expenditures were measured from continuous cultures, and large differences were found among these five microorganisms. Continuous cultures were also used to examine the effect of pH on relative growth yields. At a constant dilution rate of 0.17 hours<sup>-1</sup>, lowering the pH of the medium reservoir caused the yield of each species to decrease, but the magnitude of the growth yield depression and the pH at which culture "washout" occurred varied significantly.

Collectively the data indicate that the physiology of these microbes is quite different, and the niche of organisms in the rumen can be predicted from physiological parameters such as these.

Some Effects of Ammonium-Nitrogen and Glucose Concentration on Growth Yields and Protein Synthesis by Rumen Bacteria --  
W. T. Al-Jumaily, D. R. Caldwell and H. D. Radloff, Divisions of Animal Science and Microbiology and Veterinary Medicine, University of Wyoming, Laramie, 8207.

Four species of ruminal bacteria, Bacterioides ruminicola, Selenomonas ruminantium, Succinivibrio dextrinosolvens and Streptococcus bovis, were used in growth experiments to study the

effects of ammonium-nitrogen concentration, at several levels of fermentable energy, on nutrient utilization and microbial protein synthesis. Three levels each of nitrogen (ammonium sulfate) and carbohydrate (glucose) were used in chemically defined media in factorial design. Ammonium-nitrogen and glucose utilization as well as microbial growth yield and rate were estimated at every stage of growth in an attempt to demonstrate the quantitative capacity of rumen bacteria to utilize ammonium-nitrogen and to establish an optimal level of ammonium-nitrogen and glucose for maximal growth yield. All bacteria studied had a capacity to utilize more than 5.4 mM ammonium-nitrogen with significantly higher protein production in media adequate in glucose concentration (27.1 mM or higher). Growth inhibition and decreased protein production were observed at ammonium-nitrogen concentration of 135.7 mM. Although only slight differences were found in the total quantities of nitrogen and glucose utilized by different organisms, significant variations of growth yields and biosynthetic efficiencies were observed. The utilization of ammonium ion and protein synthesis by rumen bacteria are influenced by energy level, but the effects of energy concentration among organisms varied, a given energy-nitrogen ratio being appropriate for some organisms but suboptimal for others.

Regulation of Assimilation of Nitrogen Compounds by Selenomonas ruminantium -- C. Jeff Smith, J. A. Patterson\*, R. B. Hespell<sup>1</sup> and M. P. Bryant<sup>1</sup> Departments of Dairy Science and Microbiology  
<sup>1</sup>University of Illinois, Urbana Illinois 61801.

The enzymes of ammonia assimilation and various growth parameters of Selenomonas ruminantium strain D were investigated. Urease (UA), glutamate dehydrogenase (GDH), glutamine synthetase (GS) and glutamate synthase (GOGAT) enzymes were found in this organism. UA and GS activities, but not GDH activity, were positively correlated ( $R = 0.96$ ) with one another and seemed to be under coordinate control under a variety of growth conditions. With adequate or high  $\text{NH}_4^+$  concentrations in the growth medium GDH activity was high, but both UA plus GS activities were low. Under  $\text{NH}_4^+$ -limiting growth conditions both UA and GS activities were high, but GDH activity was relatively low. In glucose-limited fed batch cultures, the growth rate was higher with 5mM  $\text{NH}_4^+$  than with 2mM  $\text{NH}_4^+$ . Preliminary data from glucose (energy) limited continuous cultures ( $\text{NH}_2^+$  constant, =10 mM) indicate that at low growth rates GDH predominates whereas at higher growth rates GS predominates. Cell yields, cell composition and fermentation products were also found to change with growth rate. The latter data suggests that changes in cell yields cannot be attributed to changes in a single parameter, such as  $\text{NH}_2^+$  availability, but rather are the result of the interplay of a number of growth parameters.



Adaptation to High Nitrate Diet: Rates of Ruminal Nitrate Metabolism and Predominant Bacteria — C. A. Reddy\*, M. J. Allison, H. M. Cook, and J. A. Bucklin, National Animal Disease Center, USDA, Ames, Iowa 50010.

Nitrate poisoning, an acute toxicity syndrome often encountered in ruminants, is due to accumulation of nitrite ( $\text{NO}_2^-$ ), produced by the reduction of dietary nitrate ( $\text{NO}_3^-$ ) by rumen bacteria. Ruminants can be adapted to high levels of dietary  $\text{NO}_3^-$  by feeding gradually increasing levels of  $\text{NO}_3^-$  in the diet. However, information is not available on the nature of this adaptation or on the rates of  $\text{NO}_3^-$  and  $\text{NO}_2^-$  reduction and the types of and numbers of rumen anaerobes predominant in a  $\text{NO}_3^-$ -adapted animal. The results showed that the rates of  $\text{NO}_3^-$  and  $\text{NO}_2^-$  reduction, followed by determining in vivo rumen  $\text{NO}_3^-$  and  $\text{NO}_2^-$  concentrations at various times post-feeding, increased dramatically on feeding  $\text{NO}_3^-$  diet. The in vitro reduction (nmoles/min/ml of strained rumen fluid) by rumen contents of sheep fed no dietary  $\text{NO}_3^-$  were very low (4.5 and 24.5, respectively), whereas the corresponding rates by rumen contents of sheep fed 3% (w/w)  $\text{NaNO}_3$  in the diet were very high (119 and 65, respectively). There was a  $>20$ -fold increase in the rate of  $\text{NO}_3^-$  reduction within 4 h after the first feeding of  $\text{NaNO}_3$  (1% w/v), suggesting rapid induction of the  $\text{NO}_3^-$  reducing enzymes in the mixed rumen bacterial populations. The rates of  $\text{NO}_3^-$  reduction by in vitro rumen contents (IVRC) of  $\text{NO}_3^-$ -adapted sheep, collected 1, 2, 3, 4 and 12 h post-feeding, were comparable. Exogenous addition of up to 20 mM ammonia N or 0.1-20 ppm Monensin or Lasalocid to IVRC of sheep had no apparent effect on  $\text{NO}_3^-$  or  $\text{NO}_2^-$  reduction rates. Addition of 1, 4 or 20 mM  $\text{NO}_3^-$  to IVRC from the  $\text{NO}_3^-$ -adapted sheep decreased both the rates and total amounts of  $\text{CH}_4$  produced as compared to control without  $\text{NO}_3^-$ -added. The most probable numbers (MPN) of  $\text{NO}_3^-$  reducers/g of rumen contents of  $\text{NO}_3^-$ -adapted and unadapted sheep were  $10.2 \times 10^8$  and  $6 \times 10^7$ , respectively, and accounted for 18.2% and 1.8% respectively, of the MPN of total bacterial numbers. When rumen contents of  $\text{NO}_3^-$ -adapted sheep were cultured anaerobically, nitrate-reducing isolates accounted for 50% and 41% of the total isolates on non-selective roll tube media with and without rumen fluid, respectively. Preliminary studies with the isolates indicate that the predominant  $\text{NO}_3^-$ -reducing organisms can be placed into three main groups.

The Effect of Lasalocid or Monensin on Lactic Acid Producing and Utilizing Rumen Bacteria. S. M. Dennis, T. G. Nagaraja, and E. E. Bartley, Department of Animal Sciences, Kansas State University, Manhattan, Kansas.

The inhibitory effects of lasalocid or monensin were tested on lactic acid producing or utilizing strains of rumen bacteria. Lactobacillus, Streptococcus, and Selenomonas were inhibited by .38 to 3.0 ug per ml of lasalocid. Lactobacillus and Streptococcus were also inhibited by .38 to 3.0 ug per ml of monensin. However, monensin did not inhibit Streptococcus bovis or one strain of Lactobacillus brevis (B<sub>pl</sub>4). Organisms that convert lactic acid to propionic acid (Megasphaera elsdenii, Selenomonas lactilytica, Veillonella aikalescens) were not inhibited by either lasalocid or monensin. When tested in an in vitro system simulating lactic acidosis, lasalocid reduced the production of lactic acid and the concomitant drop in pH. Monensin reduced the pH drop and lactic acid build up, but not to the extent of that shown by lasalocid. When lactic acidosis was induced in vivo, both drugs reduced the drop in rumen and blood pH, and rumen and blood D and L lactic acid concentration. Lasalocid appears to be more effective than monensin in preventing lactic acidosis.

Role of Adherent Bacteria in Urea Digestion in Ruminants -- K. J. Cheng<sup>1</sup>, C. B. Bailey<sup>1</sup>, R. J. Wallace<sup>2</sup>, J. W. Costerton<sup>4</sup>; <sup>1</sup>Research Station, Agriculture Canada, Lethbridge, Alberta; <sup>2</sup>The Rowett Research Institute, Aberdeen, Scotland; <sup>3</sup>The Hannah Research Institute, Ayr, Scotland; and <sup>4</sup>University of Calgary, Calgary, Alberta.

Urea enters the rumen with food and saliva, and by diffusion through the rumen wall, and its conversion to ammonia by urease is an important factor in supporting the growth of bacteria in the rumen contents of animals fed low-quality herbage. While a small proportion of anaerobic rumen bacteria have been reported to produce urease when grown in a urea medium in the absence of ammonia, most of these organisms do not produce the enzyme at the ammonia concentrations typically found in the rumen. We hypothesized that the adherent bacteria of rumen would live in an ecological niche whose urea content would be available by diffusion through the tissue from the blood; therefore, we examined urease production by blocks of rumen tissue with their adherent bacteria, and found high levels of activity of this enzyme. We examined urease production by isolates from the adherent population and found that 10-14% of them produce urease and, more important, that they produce the enzyme under conditions that actually occur in the rumen.

The ureolytic bacteria isolated from the wall were Staphylococcus, Micrococcus, Streptococcus, and Corynebacterium, and were mostly gram-positive facultative cocci and were predominantly catalase-positive Staphylococcus or Micrococcus species. We have removed the rumen contents of hay-fed Hereford steers, washed the rumen, and filled it with buffered fluid. Urease in the buffered fluid increased rapidly to reach levels typical of normal rumen fluid within 24 hr. Of the urease activity in the buffered fluid at 24 hr, 65% was associated with detached epithelial cells. These data are consistent with the suggestion that most of the urease in the rumen originated from the continuous sloughing from the rumen wall of epithelial cells bearing large populations of adherent ureolytic bacteria, thus accounting for the enigmatic but consistent isolation of small numbers of facultative ureolytic bacteria from rumen fluid. Further evidence was obtained from experiments with lambs maintained by direct infusion of bicarbonate-buffer and volatile fatty acids into the rumen. In these lambs, the anaerobic rumen fluid bacterial population was sharply reduced and yet the urease activity of the rumen fluid was within the range found in the normal rumen. Experiments in an artificial rumen (Rusitec) have shown that urease activity in the rumen fluid disappeared within 48 hr. This result agreed with the suggestion that urease activity in the rumen fluid essentially originated from the rumen wall population. However, when urea (0.3 g/L) was added to the artificial saliva and was infused into an artificial rumen, the urease activity in the fluid was returned to a normal level within 48 hr. These results indicated that the induction of urease by urea is required for the constant production of urease in the rumen fluid itself. Since 90% of urea is returned to the rumen via diffusion across the rumen wall and only 10% via the saliva, it is concluded that most of the urease production in the rumen is essentially associated with the rumen wall population. Under certain dietary conditions, production of urease by bacteria in the rumen contents may also be significant.

The urease-producing bacteria within the wall-adherent population are ideally situated to intercept the urea that diffuses across the rumen wall from the blood by simple diffusion and, by converting this urea to ammonia, this ureolytic population creates a chemical gradient that favors the passage of more urea towards the rumen. This unique instance of the facilitation of molecular transport in a mammalian organ by a specific population of adherent bacteria is made even more elegant by the regulation of the urease activity of the bacteria by the concentration of ammonia in the rumen.

Methanol-utilizing Anaerobes Isolated from Rumen Fluid and Sewage sludge -- B. R. Sharak Genthner and M. P. Byrant, Departments of Dairy Science and Microbiology, University of Illinois, Urbana, Illinois 61801.

A methanol enrichment medium was used to enumerate methanol-utilizing anaerobes from three natural sources.  $1 \times 10^6$  Methanosarcina-like cocci were present per milliliter of rumen fluid

from steer on a hay/grain (70:30) diet. This organism converted methanol to methane. Two methanol-utilizing anaerobes, previously undescribed, were isolated from either sewage sludge (SI) or ruminal contents (RFI) of sheep fed a diet which included molasses.  $1 \times 10^5$  and  $1 \times 10^9$  bacteria per milliliter were present, respectively. Both isolates were anaerobic, motile, gram-positive rods. SI had rounded ends while RFI tended to become pleomorphic. Using a basal medium containing 5% rumen fluid, vitamins, minerals and  $\text{NH}_4\text{Cl}$ , only a few substrates supported growth. These included methanol, glucose, fructose, glycerol, mannitol, lactate, pyruvate, formate or  $\text{H}_2 + \text{CO}_2$ . Acetate was required for growth on methanol and lactate, but not glucose. Both organisms produced acetate and butyrate as major fermentation products.  $\text{H}_2$  and  $\text{CO}_2$  were produced during growth on glucose, fructose and mannitol.

Cell yields and growth characteristics of an anaerobic oxalate degrader from the rumen. K. A. Dawson\* and M. J. Allison, Department of Animal Sciences, University of Kentucky, Lexington, Kentucky 40546 and National Animal Disease Center, SEA, USDA, Ames, Iowa 50010

Anaerobic, oxalate-degrading populations were selected from mixed populations of rumen bacteria in rumen fluid media containing 45 mM sodium oxalate as a major substrate. Oxalate degradation in these populations was accompanied by the production of 1 mole of methane for each 3.8 moles of oxalate degraded. Methane production was uncoupled from oxalate degradation in oxalate-limiting chemostats at high dilution rates (0.078 to  $0.41 \text{ hr.}^{-1}$ ). The non-methanogenic populations in such chemostats produced formate as a major end product. The relative numbers of oxalate-degrading bacteria in the uncoupled populations were further increased by using a substrate depleted medium.

The final enrichment step allowed for the isolation of obligately-anaerobic, non-motile, gram-negative, curved rods which grew on a medium containing sodium oxalate (45 mM), sodium acetate (1 mM), cysteine and minerals. Growth of the oxalate degraders was directly related to the concentration of oxalate in the medium (0 to 111 mM). The only substrate found to support the growth of these organisms was oxalate which was stoichiometrically degraded to  $\text{CO}_2$  and formate. Cell yields for methanogenic enrichments, uncoupled populations and oxalate-degrading isolated in batch culture were 1.7, 1.0 and 1.1 g dry wt / mole, respectively, and were proportional to the standard free energy changes calculated for degradation to end products in each population.

These organisms are different from any previously described oxalate-degrading bacteria and occupy a unique ecological niche. Their ability to grow in response to increased oxalate concentration in the rumen could explain previously observed adaptations of ruminants to oxalate-containing feeds.

Further studies on the production of skatole and para-cresol by a ruminal Lactobacillus species -- M. T. Yokoyama\* <sup>1</sup> and J. R. Carlson<sup>2</sup>, Department of Animal Husbandry <sup>1</sup> Michigan State University, East Lansing, Michigan 48824 and Department of Animal Sciences<sup>2</sup>, Washington State University, Pullman, Washington 99163.

The isolation of several strains of a rumen bacterium which decarboxylate indoleacetic acid (IAA) to skatole (3-methylindole) has previously been reported. Based on their physiological, biochemical and metabolic characteristics the skatole-producing rumen strains have been assigned to the Lactobacillus. The substrate specificity of the decarboxylation reaction has been examined, and besides skatole, the strains produce para-cresol by the decarboxylation of para-hydroxyphenylacetic acid (p-HPAA). The strains will also decarboxylate 5-hydroxyindoleacetic acid to 5-hydroxyskatole, and 3,4-dihydroxyphenylacetic acid to methylcatechol; but do not produce ortho-cresol, meta-cresol or other phenolic metabolites. The data suggest that a hydroxyl group must be para to the carboxyl group on the ring structure for decarboxylation to occur. When p-HPAA and IAA were combined in a medium at equimolar concentrations, p-cresol and skatole were simultaneously produced by the strains in a final 2:1 ratio. When monensin was added to IAA media at increasing concentrations, a curvilinear decrease in growth was observed, with a monensin concentration of 1.1 ug/ml resulting in a 95% inhibition of skatole production. This is the first demonstration of simultaneous skatole and p-cresol by a bacterium.

#### PHYSIOLOGY AND PHYSIOPATHOLOGY

Butyrate "Overload": A Cause of Feedlot Deaths? -- R. W. Bide, Animal Pathology Directorate, Health of Animals Branch, Agriculture Canada, Animal Diseases Research Institute (W), P. O. Box 640, Lethbridge, Alberta, Canada T1J 3z4.

Single i.v. doses of Na butyrate ( $\geq 1.0$  mmole/kg) administered to 100-200 kg calves produced a toxic effect which mimicked that of succinyl choline. Acidosis enhanced this effect. A serous nasal exudate was the only apparent clinical sign other than loss of muscle tone. No postmortem lesions were observed.

Infusion of Na butyrate (pH 7.4) to similar animals at 0.5 mmole/kg/min produced loss of consciousness in 17 min and death in 50 min. Infusion at 0.25 mmole/kg/min produced loss of consciousness in 60 min, recovery if the infusion was stopped and death in 200 min if continued. The animals became comatose and died without struggling. In the process, eructation ceased and bloat ensued. A serous, occasionally bloody, nasal exudate was observed. No other pathologic sequellae were evident, clinically or postmortem, that could be attributed to the poisoning.

Butyrate can be produced in large quantities by normal rumen fermentation; levels in grain-fed animals can exceed 45 mM in the presence of other acids. An abnormal fermentation producing only butyrate was reported at these meetings two years ago by Kezar et al. Butyrate is rapidly transferred from the rumen to the blood particularly if the rumen is acidic. Blood levels of butyrate reach 11.5 mM within 6 min of the addition of two moles of butyric acid to the rumen. Thus the biologic potential for butyrate poisoning is present. However, definitive diagnosis of the condition is complicated by postmortem degradation of butyrate in the tissues and the production/utilization of volatile acids in the autolytic processes. Attempts to demonstrate butyrate overload will be discussed. The results of these studies suggest that butyrate poisoning from over production of butyrate, i.e., butyrate "overload", may account for a proportion of the unexplained sudden deaths in feedlot cattle.

Tolerance of Cattle to Larkspur (*Delphinium barbeyi*) Extract Given by Rumen Infusion -- John D. Olsen, DVM, PhD, USDA, SEA-AR, Poisonous Plant Research Laboratory, Logan, Utah.

Five female Hereford-type cattle were given repeated and prolonged infusion of larkspur extract into the rumen. Periods of infusion ranged from 2 to 42 days with intermediary periods of no infusion ranging from 2 to 139 days. Effects of a generally sustained high rate and a general slowly increasing rate of infusion were compared. In each case the dose-rate was determined at which the animal was unable to stand. When a sustained dose-rate was initially begun that was equal to or somewhat greater than the dose-rate that would prevent the animal from standing, about 40 to 50 hours were required to reach a steady-state condition where the animal was unable to arise. Later in the experiment one animal was given a lethal dose-rate. Three of the 5 cattle developed an apparent tolerance to the larkspur extract after a period of slowly increasing rate of infusion over 12 or more days. Aging of the cattle may have contributed to the apparent tolerance; however, 2 of the animals did not show an aging effect over a comparable time. The amount of extract tolerated increased by 12 to 25% over the original effective dose-rate.

The Role of Metabolism in the Pulmonary Toxicity of 3MI in Ruminants

-- J. R. Carlson<sup>1</sup>, T. M. Bray<sup>2</sup>, and M. Potchoiba<sup>1</sup> Department of Animal Sciences, Washington State University, Pullman, Washington 99164 and <sup>2</sup> Department of Nutrition, University of Guelph, Ontario, Canada.

3-Methylindole (3MI) is a ruminal fermentation product of tryptophan which causes acute pulmonary lesions in ruminants. 3MI causes selective destruction of alveolar Type I and non-ciliated bronchiolar epithelial cells and damage is initiated within one-half hour after exposure to 3MI. 3MI is rapidly metabolized to 3-methyloxindole (3MOI) and indole-3-carboxylic acid derivatives which are excreted in the urine. Both 3MI and indole can cause direct membrane damage related to their lipophilic properties, but low concentrations of 3MI in the lung suggest that the primary mechanism of lung damage is not related to direct effects. Induction of the mixed function oxidase (MFO) system with phenobarbital (PB) and inhibition with piperonyl butoxide (BT) alters the rate of 3MI metabolism. More severe pulmonary lesions result from 3MI infusion in PB-pretreated goats, and BT-pretreatment significantly reduces the severity of 3MI-induced pulmonary injury. These data indicate that 3MI is metabolized by the MFO system and that metabolism of 3MI is related to pulmonary toxicity. Infusion of 3MOI and indole-3-carbinol (I3C) (primary metabolites in two pathways of 3MI metabolism) into goats did not result in pulmonary lesions which rules out these compounds and their subsequent metabolites in the mechanism of pulmonary injury. Double isotope experiments with <sup>3</sup>H-3MOI or <sup>3</sup>H-I3C and <sup>14</sup>C-3MI indicated that 3MOI and I3C and their metabolites accounted virtually all of the urinary metabolites of 3MI, confirming the existence of two pathways of 3MI metabolism. Evidence for the formation of reactive intermediates was obtained by assessing the extent of covalent binding of <sup>14</sup>C-3MI in in vitro incubations with crude microsomal preparations. Covalent binding was highest in lung microsomes compared to liver and kidney and binding was significantly decreased by addition of BT (MFO inhibitor) or glutathione (conjugating agent). The data indicate that neither 3MI nor the major 3MI metabolites are toxic to the lung. A reactive metabolite is formed by the MFO system in an early step of 3MI metabolism which results in covalent binding of the intermediate and cytotoxicity in specific lung cells. (Supported in part by NIH grant HL-13645).

Factors Affecting the Amplitude or Rumen Contractions --

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Reticulorumen motility is essential for the movement of ingesta back and forth, for the expulsion of the gases evolved during the fermentative process and for the gradual evacuation of ingesta to

more distal regions of the alimentary canal. Extrinsic innervation is essential for this motility. Rumen motility consists of two components, frequency and amplitude. We equate amplitude with strength.

When an air-tight rumen cannula is connected to a pressure transducer so that changes in intrarumen pressure can be recorded, a pattern similar to an exaggerated sine wave is observed. Three possibilities exist to express the amplitude of this wave: (1) the height of the wave from the baseline to the peak of the wave in terms of pressure; (2) the height of the wave as expressed in terms of pressure multiplied by the distance between the beginning and end of the wave in terms of time, in effect, pressure X time which might be called a "contraction index"; and (3) the area under the wave. The contraction index and the area under the wave are probably the most representative of rumen contraction amplitude.

Using a sample of 10 primary contractions from one sheep, we determined the rumen contraction amplitude by all three methods. The coefficients of variation for these methods were as follows: height of the wave, 7.96%, contraction index, 10.64%, and area under the wave, 12.09%.

Primary contraction amplitude is reduced by excessive intrarumen pressure, diets lacking in coarse scabrous material, abomasal distention, and chemical stimulation (VFA) of reticulorumen epithelial receptors. Conversely, rumen contraction amplitude is increased by scabrous diets, eating, slightly elevated intrarumen pressure, and a stimulation of abomasal mucosal receptors by VFA's and coarse material in the diet. These stimuli of gastric origin affect rumen contraction amplitude by reflex action as do impulses arising from receptors in the mouth, pharynx, esophagus and the special senses.

Although it is known that the administration of epinephrine results in a decrease in rumen motility, the effect of a stressor on rumen contraction amplitude has not been investigated. In preliminary studies, sheep were kept in a protected and quiet environment overnight. A person with whom they were familiar recorded their rumen motility the next morning. Immediately after this pre-excitment record, the sheep were briefly exercised in an unprotected area; rumen motility was again recorded. Records from these two periods were compared with records made in a completely different recording environment a year previously. The results are shown in the following table:



Primary Contracting Amplitude (cm HOH)

Sheep	Previous Record	Pre-Excitment	Post-Excitment
1	11.33	31.23	16.83
2	14.82	25.83	12.54
3	10.26	15.68	11.05
4	—	16.29	11.32
<hr/>			
$\bar{X} + S.E.$	12.14 $\pm$ 1.37	22.26 <sup>a</sup> $\pm$ 3.79	12.94 $\pm$ 1.34

<sup>a</sup> Significantly different from other two means ( $P < .01$ ).

Thus, the rumen motility which is observed at any given instant reflects the effect of the summation of afferent neurons on the pool of efferent neurons in the medulla oblongata concerned with motility. Presumably, within this pool are separate centers concerned with frequency and amplitude. It is becoming apparent that impulses from higher centers resulting from psychological factors, one of which being stress, impinge on the medulla and modify rumen motility, especially amplitude.

Visceral Blood Flow During Feeding -- A. Dobson, R. S. Comline, R. D. Barnes and M. Silver, Physiological Laboratory, Cambridge, U.K. and N.Y.S. Coll. Vet. Med., Cornell University, Ithaca, New York 14853, U.S.A.

Blood flow to the ruminant forestomach can be stimulated either neurogenically or by the products of fermentation within the gut lumen. The present study aimed at establishing the normal patterns of blood flow to the viscera as a preliminary to identifying the relative importance of these factors. Three sheep were trained to eat a ration of lamb pellets and hay within the same two hour period each day. Blood flow was measured half-an-hour before feeding, and 3-4 min., 2 hr and 4 hr after feeding each sheep using 15u diameter radioactive microspheres. Changes due to psychic or reflex stimulation during eating should be present at the time of the second sample, when chemical stimulation should be minimal. Chemical stimulation should be maximal 2 to 4 hr after feeding.

A stimulation of flow associated only with eating was observed in the muscle of the dorsal and ventral sac of the rumen, the esophagus + reticular groove and the parotid and mandibular glands. The blood flow to the omasum was inhibited at this time in two of the three sheep. The epithelium of the ruminoreticulum showed maximal stimulation at 2 hr, declining, but not to prefeeding levels, at 4 hr. Most regions of this epithelium showed substantial flow stimulation during feeding. Blood flow through the visceral fat declined markedly throughout the period of observation. A slight overall decline was discerned in the flow to the pancreas, spleen and the combined organs caudal to the ileocecal valve, but no consistent trend between sheep was otherwise apparent in the separate organs caudal to the omasum. An enhanced flow at the 2nd and 4th hour was noted for the flow in the hepatic artery and to the adrenal glands.

The portal flow was low both during eating and by the 4th hour. The fraction of the portal flow originating from the combined flow to the omental and mesenteric fat, the pancreas and the spleen averaged 0.51, 0.41, 0.29 and 0.31 for each of the four times respectively. A large proportion of the portal blood flow thus derived from structures other than the gastro-intestinal tube. The corresponding fractions of portal flow originating from the ruminoreticulum were 0.11, 0.23, 0.37 and 0.29. This confirms the importance of the neurogenic and fermentative effects on flow in this region of the gut.

The Effect of Ration Physical Form and Nitrogen Availability on Propionate and Lactate Metabolism in Rumen Epithelium and Liver --  
J. E. Nocek, T. M. Kenna, J. H. Herbein and C. E. Polan, Virginia Polytechnic Institute and State University.

Fifty-four Holstein bull calves were randomly assigned to one of 9 rations factorially arranged to contain 3 levels of rumen degradable nitrogen [RDN (30, 45 and 60%)] and three physical forms [38% ground hay (GR), 39% chopped hay (CH), and all concentrate (CONC)]. Rumen samples were obtained to characterize fermentation patterns. With respect to physical form, the following significant differences were found: acetate concentration was highest for GR and lowest for CONC rations; propionate concentrations were highest for CONC, intermediate for CH and lowest for GR; both hay rations had higher butyrate concentrations than CONC; lactate concentration and pH were lower for CONC rations. There were no differences in branched chain acids, valerate and  $\text{NH}_3\text{-N}$  concentrations. The Non-Gluconeogenic Ration (NGR) followed the same trend as acetate concentrations with  $\text{GR} > \text{CH} > \text{CONC}$ . With respect to RDN, the following differences were found: NGR was greatest for 30% RDN and lowest for 60% RDN;  $\text{NH}_3\text{N}$  was highest for 60% RDN, intermediate for 45% RDN and lowest for 30% RDN.

Two calves were randomly chosen from each ration for in-vitro rumen epithelium transport studies and enzyme analyses. With respect to physical form, acetate transports were higher for the hay (GR and CH) rations and suggest ration fiber has a primary role in acetate transport. All propionate transport were significantly ( $P < .05$ ) different with  $GR > CH > CONC$ . The Acetate:Propionate ration was significantly ( $P < .001$ ) higher for CH rations. Lactate appearance was not affected by treatment. RDN had no effect on acetate or propionate transport. Rumen epithelium of CONC fed calves was heavier, less uniform and exhibited clumping and embedded hair whereas calves on GR or CH rations had normal appearing rumens.

Lactate and malate dehydrogenase activities were not influenced by treatment. Propionyl-CoA synthetase activity was higher in calves fed CONC rations than in calves fed GR or CH. Glutamate dehydrogenase and aspartate aminotransferase activities were highest in calves fed 60% RDN, regardless of physical form.

Liver incubations were conducted with optimal concentrations of the following substrates: acetate, propionate, butyrate, lactate, pyruvate, glucose and ammonium chloride with  $2-^{14}C$  propionate and  $^{14}C$  lactate used as tracers. Analyses of labeled  $CO_2$  production with respect to physical form indicated that GR fed calves had the lowest  $CO_2$  production from  $2^{14}C$  propionate with 60% RDN fed calves being highest.

The results indicate that: in vitro epithelial propionate transport rates were inversely related to the vivo propionate concentration in the rumen; propionate oxidation in liver decreased as the ratio of acetate to propionate transported across the rumen epithelium decreased. Percent RDN had no effect on acetate and propionate concentration in the rumen or transport across the epithelium; but, percent RDN did influence the activity of rumen epithelium enzymes involved in nitrogen metabolism and the rate of propionate oxidation in liver.

The Effect of Ration Physical Form and Rumen Degradable Nitrogen on Growth, Ration Digestability and Nitrogen Retention -- J. E. Nocek; K. A. Cummins, and C. E. Polan, Virginia Polytechnic Institute and State University.

A growth and nitrogen balance trial was conducted utilizing growing Holstein bull calves between 8 to 20 wk of age. Rations fed were of three physical forms: 1) 38% ground hay, 2) 38% chopped hay, and all-concentrate. The hay containing rations were identical except for particle size of the hay component, being approximately 1.5 cm in the ground hay ration and 10 cm in the chopped hay ration. Within

each physical form were rations of three different levels of rumen degradable nitrogen (RDN): 30%, 45% and 60%. Ration RDN was estimated in previously published studies by suspending feed-stuffs in polyester bags in the rumen of lactating cow and assuming mean ruminal retention times of 12 hr for concentrate and ground ration components and 24 hr for coarse forage components. All rations were isocaloric and isonitrogenous. The hay rations contained approximately 17% ADF while the all-concentrate rations contained approximately 7% ADF. Growth and intake data were collected continuously. Nitrogen balance was measured at 17 wk of age.

Physical form had a significant effect ( $P < .05$ ) on daily gain, body weight and apparent digestible dry matter. Physical form and RDN had a significant effect ( $P < .05$ ) on dry matter intake, apparent digestible nitrogen, corrected digestible nitrogen, percent nitrogen ingested retained, percent nitrogen absorbed retained, and nitrogen retention. Calves fed all-concentrate rations had the higher values for all parameters measured, except dry matter intake. Calves fed chopped hay rations had lower body weights, daily gains, and dry matter intakes than calves fed ground hay or all-concentrate rations. Calves fed ground hay rations had equal body weights and higher dry matter and nitrogen digestibilities along with lower efficiencies of nitrogen utilization. Calves fed chopped hay rations showed better efficiency of nitrogen utilization than calves fed ground hay rations. Nitrogen retention/kg<sup>.75</sup> was highest in the calves fed all-concentrate rations with no difference between the ground and chopped hay rations.

Calves fed 45% RDN rations showed lower performance in all parameters measured compared to calves fed 30% or 60% RDN rations. Calves fed 30% RDN rations had significantly higher body weights, efficiencies of nitrogen utilizations and nitrogen retentions than calves fed 45% RDN rations. Calves fed 60% RDN rations generally had values for nitrogen utilization efficiency and nitrogen retention intermediate to but not significantly different from calves fed 30% or 60% RDN rations. Nitrogen retention and efficiency of nitrogen utilization approached or was equal to that of the calves fed all-concentrate rations only in calves fed 30% RDN rations.

It was concluded that RDN and physical form affect the growth and nitrogen utilization of growing calves. Estimates of RDN by placing feedstuffs in bags suspended in the rumen appear to yield results indicative of more efficient nitrogen utilization and faster weight gain. This may be a useful method for formulating rations for lactating dairy cows.

Hepatic and Exocrine Pancreatic Secretions in Sheep -- J. M. Bergen and G. D. Phillips\*, Department of Animal Science, University of Manitoba, Winnipeg, Canada R3T 2N2

Bile and pancreatic secretions were studied in cholecystectomized sheep in which bile and pancreatic secretions were separately exteriorized through silastic catheters placed in the cystic duct and in an isolated segment of the common bile duct respectively. Between experiments the fluids were returned via a 3rd catheter placed in the common bile duct near the shincter of Oddi. Sheep fed on continuous belt feeders at 600 g alfalfa pellets per day produced bile and pancreatic juice at 0.5 and 0.16 ml/min respectively compared with 1.6 and 0.28 ml on 1700 g/day. While Na and K concentrations tended to increase with increasing rate of secretion Ca, P and Cl decreased. Bile and pancreatic juice secretion rates in starved (48 h) sheep were similar to those in sheep feed 600 g/day. There marked increases in secretion rates of both fluids in the 2 h following feeding on a once-a-day regime, associated with generally decreasing concentrations of electrolytes in bile but increasing concentration in pancreatic fluid. Trypsin, chymotrypsin, lipase, and amylase concentrations were all elevated for 4-5 h following feeding. Secretin injection transiently increased pancreatic secretion rate but had little other effect whereas pancreozymin increased the volume of both bile and pancreatic secretions and also the concentration of pancreatic enzymes. These results indicate a significant role of pancreozymin in sheep but secretin is presumably of lesser importance.

Pancreatic Proteolytic Enzyme Secretion in Sheep -- D. C. Buck and G. D. Phillips, Department of Animal Science, University of Manitoba, Winnipeg, Canada R3T 2N2.

Secretion rates of trypsin and chymotrypsin were studied in sheep fitted with re-entrant cannulae at the proximal and distal ends of the duodenum. Secretions were stimulated by perfusing isosmotic solutions through the isolated duodenal loop for 100 minute experiments, at 5 or 15 ml/min. Solutions of Mannitol or an electrolyte mixture infused a  $\text{pH} > 5$  resulted in the higher enzyme secretion rates at the higher flow rate. Two other infusates with  $\text{pH}$  2.5 and 3.5 gave the opposite effect, and of these solutions the one with 40 mEq/l titrable acid stimulated high enzyme secretion rates than the one with 3 mEq/l titrable acid. Thus the highest secretion rate of pancreatic proteolytic enzymes was found with the solution of high titrable acid, low  $\text{pH}$  and infused at 5 ml/min. These results are consistent with a mechanism for regulating enzyme secretion rates depending on free enzymes in the duodenum acting on receptors in the mucosa to inhibit further secretion, presumably by inhibiting CCK-PZ release. Infusion of solutions containing 3 levels of protein resulted in higher enzyme secretions with the higher protein levels, but the results were not statistically

significant. Trypsin to chymotrypsin ratios varied considerably with the different treatments suggesting that there is not parallel secretion.

## NUTRITION

### The Adaptation of Gas-Liquid Chromatography to Routine Nutritional Evaluation of Feeds and Forages -- S. Bittner<sup>1</sup> and L. E. Harris<sup>2</sup>.

Most research concerned with determination of plant carbohydrate digestability by animals relies on the use of analytical procedures which describe plant carbohydrates with operationally defined categories rather than in terms of their specific chemical composition. Gas-liquid chromatography can be employed for the rapid assessment of plant carbohydrate composition resulting in more accurate evaluation of carbohydrate utilization by animals.

Immature reed canarygrass (Phalaris arundinacea) was harvested, freeze-dried, and ground to pass through a 0.5mm screen. The sample was extracted with boiling water followed by extraction with acetone and delignified with hot acid chlorite solution for 15 minutes. A 30-40mg portion of the residue was hydrolyzed with 72% sulfuric acid at 22°C for 1 h followed by dilution to 2N, degassed and hydrolyzed at 95°C for 3 h. A 100  $\mu$ l aliquot of the hydrolysate was neutralized with ammonia followed by reduction with borohydride for 1 h. Five methanol additions were needed to remove the volatile methanol-borate esters which would interfere with subsequent acetylation. Samples were then acetylated with 250  $\mu$ l N-methylimidazole and 200  $\mu$ l acetic anhydride followed by addition of 1ml water and 200  $\mu$ l chloroform. A 1.0-2.0  $\mu$ l portion of the chloroform layer was injected into a 120 X 0.3cm glass column containing 0.2% DEGS, 0.2% PEGS and 0.4% Silicon XF-1150 coated on a gas Chrom Q (100-120 mesh) solid support. The column temperature was programmed from 140-180°C at 1°C/minute with a carrier gas flow rate of 30 ml/minute.

The chromatogram for the reed canarygrass residue revealed the presence of arabinose, xylose, galactose and glucose in the hydrolysate. This pattern of sugar composition appears to be characteristic for grasses and varies with maturity and species. Monosaccharide composition of plant preparations may accurately reflect polymer composition and with knowledge of polymer linkages can be used for the quantitation of individual plant polymers.

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A Method for Determining In Vivo Rates of Particle Size Degradation, Genesis and Passage In and From the Rumen -- W. C. Ellis<sup>a</sup>, Carlos Lascano<sup>a</sup> and J. H. Matis<sup>b</sup>, Department of Animal Sciences and <sup>b</sup>Institute of Statistics, Texas A&M University, College Station, Texas 77843.

Rate of particle size reduction is an important factor influencing intake, digestability, level of rumen microbial synthesis and composition of digested nutrients. In vivo methods for measuring such rates have been limited by lack of appropriate particulate markers. The rare earths appear appropriate based upon their quantitative recovery on the fiber of a wide array of forage particles considerably smaller and more extensively digested than the dietary forage particle to which they were originally adsorbed. The first model arbitrarily defines 7 particle sizes in the esophageal ingesta (D), rumen digesta (R) and feces (O) based on sieving into sizes ( $\mu\text{m}$ )  $> 1600$  (1), 1000/1600 (2), 800/1000 (3), 500/800 (4), 300/500 (5), 160/300 (6), and  $< 160$  (7).

<sup>169</sup>YbNO<sub>3</sub> (Yb) is adsorbed onto D1, introduced into R and samples of R and O collected at 0, 4, 8, 16, 20, 24, 28, 40, 48, 64, 72, 88, 96, 112, 120, 136 and 144 hr post dose, freeze dried, sieved and assayed for Yb. Since nil of particle size 1 was in feces (O1) then disappearance of R1 was totally by degradation to R2 at a fractional rate (R2, R1) estimated by fitting a time dependent, time independent model without time delay (Ellis et al., Fed. Proc., Dec., 1979) to (Yb/R1) vs. (hr post dose). The slower, time independent rate represents fractional rate (R2, R1) and (Yb dose/Co) equals pool size of R1. Assuming steady state, the mass (g) of R1 exiting to R2 per hr equals ((R1)(R2, R1)). Since g particles entering R2 must equal g leaving R2 by passage (O2, R2) plus degradation to R3 (R3, R2) then (O2,R2 + R3, R2) = (R2, R1) (R1/R2) where (R1/R2) corrects for pool size differences. Pool sizes R2...R7 are determined from total rumen DM pool size multiplied by weight proportions R1...R7. Mean total rumen DM pool size is estimated from (yb dose/Co), where Co is (Yb/g total DM) and is determined from fitting (Yb/g total rumen DM) vs (hr post dose). The proportion (O2,R2/R3,R2) is computed from total fecal output and distribution of Yb therein by particle size; the proportion by passage/degradation equalling ( $\sum$  Yb excreted in O2)/( $\sum$  Yb excreted in O2 and all in fractions smaller than O2). This proportion multiplied by (O2,R2 + R3,R2) equals fractional passage rate O2,R2. R3, R2 is determined by (O2,R2 + R3,R2) - (O2,R2) and, knowing pool size R3, can be used to calculate fractional entry rate into R3 which equals fractional exit rate by passage (O3,R3) plus degradation to R4(R4,R3). These and other rates applicable to

R4...R7 are calculated as described for R3 with the proportion passed vs. degraded from a fraction  $x = (\text{fecal recovery of Yb in } x) / (\sum \text{ fecal recovery of Yb in } x \text{ and all fractions smaller than } x)$ . An important and yet to be tested assumption is sequential genesis of progressively smaller particles. Undoubtedly serial genesis of individual particles occurs however this may not materially affect the deterministic model. A sieving effect by excessive amounts of 1000/1600 particles on that sieve produced erroneously large values for distribution of these size particles (2) and consequently erroneously diluted the respective fractional rates. Rates calculated by this method were statistically responsive to forage factors associated with maturity and grazing selectivity. For 16 grazing animals, mean rates ( $\text{hr}^{-1}$ ) respectively for R1...R6 for degradation were .16, .07, .16, .06, .05, .025 and passage for R2...R7 were .007, .016, .015, .019, .026 and .037. These rates are expressed as fractions of DM in R1...R7. It should be noted that individual pools R2...R7 are the sum of DM derived from further degradation of larger size ruminal particles plus ingested particles of specified size. Thus rates solely applicable to degradation and passage of particles derived from initially marked D1 particles would be larger than those reported here due to the diluting effect of ingested particles on pool size. Methods for correcting this diluting effect and a serial genesis model are being developed.

Factors Associated with Digestive Function as Measured in Calves Equipped with Duodenal and Ileal Re-entrant Cannulas — R. A. Zinn and F. N. Owens, Oklahoma State University, Stillwater, Oklahoma.

Three angus steers with dual re-entrant intestinal cannulas were used in a 3 x 3 latin square experiment to evaluate the influence of level of intake and roughage level on aspects of digestive function. Three treatments consisted of 1) low roughage diet (20% prairie hay, 80% concentrate) fed at 1.5% of body weight, 2) low roughage diet fed at 2.0% of body weight, 3) high roughage (40% prairie hay, 60% concentrate) fed at 2% of body weight. Steers were fed at equal intervals twice daily. All estimates were based on composites of spot samples obtained simultaneously at various sites using chromic oxide as a digesta marker. Mean percent changes as a result of increasing level of intake from 1.5% to 2% of body weight are shown below:

	Rumen	Small Intestine	Cecum Large Intestine	Total Tract
Nitrogen	0	+3	-200	-4
Starch	-16	+14	+16	+1
ADF	-6	---	-100	-23



Mean percent change as result of increasing roughage level from 20 to 40% are shown below:

	<u>Rumen</u>	<u>Small Intestine</u>	<u>Cecum Large Intestine</u>	<u>Total Tract</u>
Nitrogen	-38	+1	+105	-2
Starch	+18	-24	-42	-8
ADF	+5	---	+100	+34

Data obtained from various experiments (44 observations) using dual re-entrant cannulated steers were combined in order to make general observations regarding the fate of nitrogen and starch. Duodenal NAN was found to be a linear function of nitrogen intake ( $DNAN = .19 + .44 N \text{ intake (g/kg BWT)}$ ). Intestinal nitrogen digestion was found to be relatively constant at 66% ( $Ileal N = .001 + .34 DNAN \text{ (g/kg BWT)}$ ), the zero intercept indicating no appreciable endogenous intestinal N contribution to cecal N. Cecal digestion of N was about 20% ( $Fecal N = .024 + .80 Ileal N \text{ (g/kg BWT)}$ ). The intercept indicates that metabolic fecal nitrogen amounted to 2.4 g/kg BWT. Starch appeared to enhance intestinal starch digestion as starch digestion was observed to increase at an increasing rate with increased duodenal starch.

Rumen Turnover: Methodology as Applied to Liquid and Solids Markers  
 -- R. G. Teeter, T. L. Mader and G. W. Horn, Oklahoma State University, Stillwater, Oklahoma.

Ruminal liquid turnover rate estimates using conventional water soluble markers (WSM, CrEDTA, CoEDTA, YbEDTA and PEG) assume single pool kinetics. However, fluid space available to WSM is not greatly altered when solids (quantum sufficient to absorb 75% of the solutions  $H_2O$ ) such as whole corn, ground corn, milo and alfalfa are added to marker solutions. Data suggest that two fluid pools (free liquid and solids liquid) are available to WSM. To examine pool affinity, WSM were ruminally administered to 6 steers receiving either a high concentrate or high roughage diet in a 2 period switchback design. Mean dilution rates for Cr, Co, Yb, Fe and PEG (20,000 MW) on the concentrate and roughage diets were 3.6, 6.0; 4.1, 7.5; 4.5, 10.2; 4.0, 8.1; 4.4, 5.7, respectively. Data indicate that some WSM differ in their liquid pool affinity, however this may be confounded with binding by the solids and/or absorption by the animal.

Animals orally dosed with Yb and Dy labelled prairie hay sampled duodenally and fecally exhibited similar marker kinetics suggesting that they may be suitable for concurrent administration. Duodenal dilution rate, fecal dilution rate as well as duodenal and fecal time delay (hr) for Yb and Dy respectively were: 1.97, 2.42, 10.0, 27.3; 2.08, 2.54, 9.5, 28.1.

Effects of Varying Liquid and Solids Dilution Rates on Fermentation in Continuous Culture -- \* R. J. Crawford, Jr. and W. H. Hoover, Division of Animal and Veterinary Sciences, West Virginia University.

The effects of varying solids retention times (SRT) and liquid dilution rates (D) were investigated using a dual effluent continuous culture system. SRT of 14.3, 22.0 and 29.7 hr were studied, with D of .07, .11 and .15 volumes/hr as subtreatments within a split-plot randomized complete block design. A pelleted ration consisting of 60% grain mixture and 40% hay crop silage was fed at a rate which increased with decreasing SRT.

The pH within the fermenters ranged from 4.57 to 6.78 and increased with increasing D. Within a given D the increased feed input, which was 97, 130 and 201 g/day for SRT of 29.7, 22.0 and 14.3 hr, respectively, resulted in a decrease in pH.

Digestibility coefficients for DM, cellulose, ADF and NDF ranged from 33.32 to 78.03, 8.17 to 69.05, 0 to 61.54 and 0 to 64.24%, respectively. All digestibilities increased with increasing SRT and D. However, in most instances, digestibility reached a plateau at 22.0 hr for the .11 and .15/hr D; increasing the SRT to 29.7 hr resulted in little or no additional increase at these D's.

Maximum protein digestion occurred at the .11 and .15/hr D and 22 hr SRT. At these conditions, 45-48% of the protein by-passed the rumen. Ammonia levels were low at most conditions with a maximum concentration of 6.12 mg/100 ml.

Increasing D did not result in increased microbial efficiency measured as microbial N/kg DDM or as  $Y_{ATP}$ . Microbial efficiency did increase with increased solids flow (decreased SRT).

Efficiency of VFA production was not affected by D or SRT. The molar percents of acetate, propionate and butyrate were related to both D and SRT. Increasing D resulted in an increase in the proportion of acetate and a decrease in propionate and butyrate. Increasing solids flow (decreasing SRT) decreased the proportion of acetate and increased propionate.

\*Research conducted at the University of Maine, Orono.

Calcium-Lipid Interactions in the Rumen. The Effects of 10% Tallow With and Without 2% Added Calcium on Formation of Insoluble Fatty Acid Salts in Vitro -- T. C. Jenkins and D. L. Palmquist, Department of Dairy Science, Ohio Agricultural Research and Development Center, Wooster, Ohio 44691.

A series of in vitro experiments were conducted to determine the effects of time (0, 3, 6, 9, 12, 24 and 48 hours) and diet (basal, + 10% tallow, basal +10% tallow + 2% calcium supplement) on formation of insoluble fatty acid salts. Dicalcium phosphate and calcium chloride were compared as calcium supplements. Following incubation of the diets, the total fatty acids were extracted and separated into esterified fatty acids (EFA), non-esterified fatty acids (NEFA) and insoluble fatty acid salts (IFAS). The quantities and composition of fatty acids in each fraction were determined by gas chromatography. The IFAS content of the timothy-corn (75:25) basal diet reached a maximum of 19.9% of the total fatty acids after 48 hours of incubation. When 10% of the basal diet was replaced with tallow the IFAS content increased to 48.1% of the total fatty acids after 48 hours. The diet with 2% added dicalcium phosphate had 10 and 25% more IFAS at 24 and 48 hours, respectively, than the high fat diet without added calcium (actual IFAS of 49.9 and 60.4% of the total fatty acids, respectively). However, the diet with 2% added calcium chloride had 63% more IFAS than the 10% tallow diet after only 24 hours of incubation. Neither 2% dicalcium phosphate nor 2% calcium chloride increased the IFAS content prior to 24 hours of incubation. Cell wall digestibilities of the basal and 10% tallow diets were 42.3 and 26.5%, respectively, when averaged over all incubation times. Adding a calcium supplement at the 2% level improved cell wall digestibility, but more for calcium chloride than dicalcium phosphate (cell wall digestibilities of 35.9 and 27.4%, respectively). When the IFAS of individual fatty acids were expressed as a percent of the total pool of each fatty acid not esterified, for example for stearic acid (C<sub>18:0</sub>)

$$\frac{\text{IFAS C}_{18:0}}{\text{NEFA C}_{18:0} + \text{IFAS C}_{18:0}} \times 100$$
, the ranking was C<sub>18:0</sub> > C<sub>16:0</sub> > C<sub>18:1</sub> for all

diets containing 10% tallow. These experiments demonstrate that the amount of increase in both the formation of insoluble fatty acid salts and cell wall digestibility of high fat diets due to adding a calcium supplement depends on the source of calcium used.

Dietary Mineral Oil Enhances Fecal Excretion of Tissue Hexachlorobenzene by Sheep<sup>1</sup> -- G. Stanley Smith and Ina Neiman, New Mexico State University, Las Cruces, New Mexico 88003, and K. Rozman, T. Rozman, and H. Greim, Institute for Toxikologie der Gesellschaft fuer Strahlen- und Umweltforschung, mbH, MUNICH D-8042, West Deuschland.

Contamination of livestock with lipophilic chemicals such as pesticides is common occurrence. Some chlorinated hydrocarbons with prolonged tissue retention pose long-term hazard and great economic costs to the livestock industries. Using hexachlorobenzene (HCB) as a model of chlorinated hydrocarbons, it was shown previously that 5% dietary mineral oil enhanced fecal excretion of tissue HCB by three-fold in rats and ten-fold in Rhesus monkeys. In the present study six crossbred lambs were administered <sup>14</sup>C-labelled HCB in dosage of 90 mg HCB/(kg) <sup>0.75</sup>, a dosage sufficient to provide HCB in adipose tissue at levels in the range 50 to 120 mg/kg. Two weeks after HCB dosage, three lambs were given mineral (paraffin) oil as 5% of diets; whereas three received the untreated, basal diet and served as "controls". Mineral oil increased (P < .01) fecal excretion of HCB about three-fold over levels for untreated controls, and reduced the concentration of HCB in adipose tissue commensurately. Data suggest that fecal excretion did not occur via bile (non-hepatic pathway has been conclusively demonstrated in monkeys). Mineral oil at 5% of diet did not affect feed intake or the digestibility of dry matter and protein. Results suggest dietary mineral oil could be used to great economic advantage in practical circumstances requiring decontamination of contaminated livestock.

<sup>1</sup>Research reported was conducted, in part, with support funds from NMS Project H-159, under contract #EY76-S-04-3626, U. S. Department of Energy, Albuquerque Operations Office, Albuquerque, New Mexico 87115.

Estimation of Ruminal Protein Degradation by Kinetic Analysis -- G. A. Broderick and W. M. Craig, Department of Animal Science, Texas A&M University, College Station, Texas 77843.

The proportion of ruminal protein escape is a major determinant of the value of protein sources fed to ruminants. We compared three in vitro methods in preliminary studies assessing ruminal protein degradation: 1) incubations using high ratios of protein to ruminal

fluid (25-27 mg crude protein equivalent/ml ruminal fluid) at one time point; 2) incubations using more physiological levels of protein (about 2.4 mg/ml) at various times over 4 hours, mathematically analyzed with a biexponential 2-compartment model; and 3) Michaelis-Menton type incubations using small to high levels of substrate (2 to 40 mg/ml) at one time point. The latter two techniques were as described earlier (J. Nutr. 108:181, 1978). Hydrazine (1 mM) was added to all incubations to prevent microbial metabolism of amino acids and ammonia, so these degradation products could be used as a quantitative measure of protein degradation. Using high ratios of protein to ruminal fluid appeared to overestimate ruminal degradation of cottonseed meal (CSM) protein relative to casein. In use of the biexponential kinetic procedure (method 2), data were analyzed assuming CSM contained two protein fractions degraded at two different rates. Estimates of degradation rates for the first CSM fraction (.68 to 1.06/hr) were 2 to 3 times greater than casein (.34/hr). Degradation rates of the second fraction were much slower (.011 to .093/hr). The effect of heat-treating CSM, either by autoclaving or by comparing screwpress and solvent processed meals, was to decrease the proportion of the rapidly degraded fraction, and to both increase the proportion and decrease the degradation rate of the more slowly degraded fraction. Estimated ruminal escape increased with heat-treatment. Intestinally digestible protein (ruminal escape x true digestibility in the rat) averaged 31% for solvent CSM and 50% for screwpress CSM. The Michaelis-Menton procedure appeared to yield estimates of ruminal escape similar to the biexponential technique. The magnitude of the rapidly degraded fraction estimated using the biexponential procedure was similar to the proportion of N soluble in 10% Burrough's buffer. We conclude from these results that methods using excess substrate and N-solubility techniques tend to reflect properties of the more rapidly degraded fractions, and not the degradation properties of the protein source as a whole.

Release of Specific Amino Acids and Nitrogen From SBM In Situ  
Digested in the Rumen -- W. V. Rumpler, L. S. Bull, R. W. Hemken,  
University of Kentucky.

The changes in the amino acid profile of soybean meal during digestion in the rumen were examined. Solvent extracted soybean meal was digested for 2, 4, 6, 8, 10 and 12 hours in nylon bags placed in the rumen of a fistulated steer maintained of an alfalfa hay diet. Four samples were obtained for each time period. All bags containing samples were rinsed, after removal from the rumen, in tap water (39°C) until the rinse water was clear. Four additional samples were also rinsed (designated "0" time) incubation in the rumen. The nitrogen and amino acid content was determined. The amino acid content was express as a % of total amino acids (AAPT) and as a % of essential amino acids (AAPE) remaining in the bag after digestion and/or rinse. Approximately 20% of the nitrogen

washed out of the bags ("0" time) and a linear disappearance of 1.5% per hour was observed from 0 to 12 hours. No significant differences in the amino acid content were seen between the original sample and the "0" time sample. Regression analysis revealed changes in AAPT and AAPE. Leucine and Serine significantly increased (AAPT) with time of digestion. Methionine and Threonine exhibited significant quadratic (decrease then increase) changes and Valine had a significant linear increase and quadratic (increase then decrease) with time. The above four essential amino acids showed the same response in respect to AAPE.

Evaluation of the dacron bag technique for estimating protein degradability in the rumen -- Marshall D. Stern\* and Larry D. Satter, University of Wisconsin, Madison, Wisconsin 53706.

Dacron polyester bags with a pore size of 20-75 $\mu$  were used to examine dry matter (DM) and nitrogen (N) disappearance in the rumen of 26 total mixed rations. All rations were previously studied in vivo. Therefore, a direct comparison between the bag technique and in vivo results was possible. The major N sources in the rations were: soybean meal, brewers' dried grains, distillers dried grains with and without solubles, heated and unheated peanut meal, oats, urea, corn, corn gluten meal, corn silage and alfalfa. Crude protein and soluble N of the rations ranged from 9 to 23 and 4 to 53 percent, respectively. In vivo estimates of ruminal DM digestibility and protein degradability ranged from 28 to 76 and 13 to 98 percent, respectively. Half gram samples of each of the rations were weighed into dacron bags. The bags were placed in the rumen of a fistulated cow 2.5 hr after feeding and were removed at intervals of 1, 4, 8, 12, 17 and 24 hr. The correlation coefficient (r) of 1 hr N disappearance from the bags and 1 hr extraction of soluble N in Burrough's mineral buffer was .80, while the r value between N solubility and in vivo estimates of protein degradability in the rumen was .28. By regressing in vivo estimates protein degradability in the rumen (y) on N disappearance at 24 hr rumen exposure (x), the following prediction equation was derived:  $y = -73.21 + 1.76x$  ( $r^2 = .61; P < .01$ ). Using this equation, the average difference  $\pm$ SD between predicted and observed protein degradability in the rumen was .004  $\pm$ 14.1%.

Some Problems Regarding the Calculation of Microbial Yield In Vivo  
-- P. J. Van Soest, P. H. Robinson and C. J. Sniffen, Department of  
Animal Science, Cornell University, Ithaca, New York.

Equations for estimation of metabolic protein from a dynamic model must be founded on sound physico-chemical theory and calibrated from measured in vitro data. They cannot be empirical. The object is to provide such estimates from feed analyses and predicted feed intake derived in turn from diet cell wall content and productive level. Cell wall intake sets passage rate which is in competition with digestion rates. Rumen escape is a function of competition between passage and digestion and fixes rumen escape of energy and protein. It also determines microbial efficiency. This last factor is the largest single factor affecting rumen output as a function of intake. The modeling of microbial efficiency required literature survey and recalculation of existing data to a common basis. Corrected microbial yield ( $Y_c$ ) expressed as g nitrogen per 100 g true digested organic matter:

$$Y_c = \frac{Y_o}{1 + 10Y_o}$$

Where  $Y_o$  is observed yield in g of nitrogen per 100 g of apparently digested OM. Literature values given as crude protein were converted by the factor .16. The equation is algebraically arrived assuming 10% nitrogen in whole bacteria. Statistical analysis of corrected literature data show that in vivo microbial yield fits Michaelis-Menten kinetics and agrees well with published chemostat measurements. Regression of  $1/Y_o$  with reciprocal of liquid dilution rates ( $K_p$ ) yielded an  $R^2$  of .755, and  $1/Y_c = .0149 (1/K_p) + .1406$ . Maximum yield at infinite dilution rate was 7.11 g nitrogen per 100 g true digested organic matter.

Evaluation of a Protease Procedure for Determining Rates of Protein Degradability in Various Feedstuffs -- C. J. Sniffen,  
U. Krishnamoorthy, P. J. Van Soest, T. V. Muscato and P. H. Robinson,  
Department of Animal Science, Cornell University, Ithaca, New York.

Feed samples containing 50 mg of potentially available true protein nitrogen [PATPN = Total N-(soluble NPN + ADF-N)] was incubated with 25 mg of enzyme (Str. griseus, (.9 unit/mg enzyme) in 40 ml of bicarbonate-phosphate buffer ( $\text{NaHCO}_3$  9.81 g/l,  $\text{KH}_2\text{PO}_4$  1.55 g/l,  $\text{Na}_2\text{HPO}_4$  1.43 g/l) of pH 7 to 7.5 at 38C for different intervals of time. To prevent bacterial contamination of the samples during prolonged incubations, 2.5 ml of 2% sodium azide solution was added to the incubation mixture. Sodium azide at this concentration did not have any effect on nitrogen solubility in soybean meal or protease activity. The effectiveness of t-butyl alcohol to stop the enzyme activity at different intervals of time

was tested and it was found to be ineffective. The enzyme-substrate reaction was stopped at different intervals of time by removing the flasks from the waterbath and filtering immediately on whatman no. 54 filter paper, followed by thorough washing with distilled water. The residual N was estimated by Kjeldahl method. Using residual nitrogen values at different time intervals, different pools of nitrogen in feed samples could be characterized by means of "curve peeling" technique. There could be two or three pools in each sample.

The N disappearance in in-vitro protease technique was compared with that obtained by Dacron-bag technique (M.D. Stern and L. D. Satter). The nitrogen disappearance at one hour obtained by these two techniques were close to each other. But at 24 hours, the percent N disappeared in in-vitro technique was more than that observed in Dacron-bag technique.

N disappearance (% Total N) in in-vitro protease and Dacron-bag technique.

Sample	Time			
	1 hr.		24 hrs.	
	In-vitro protease	Dacron bag	In-vitro protease	Dacron bag
Grain mix 1	40.85	42.20	66.96	62.20
Grain mix 2	32.11	28.40	70.49	54.50
Grain mix 3	25.88	28.50	70.43	51.10
Grain mix 4	19.55	24.30	61.74	42.40
Hay	45.44	34.70	-	-
Silage	59.73	58.60	85.25	78.70

The level of enzyme and the level of substrate was found to have a positive relationship with the percent protein degraded. At protein (mg)/enzyme(mg) ratio of 2.2, potentially available insoluble nitrogen degraded [PAIN = Total N-(soluble N + ADF-N)] in soybean meal was 31.54 as against 45.81 at the protein/enzyme ratio of 0.6. Similarly at a given enzyme level as the substrate level increased, percent protein degraded also increased. At protein/enzyme ratio of 0.6, with the substrate level of 0.2 g, percent PAIN degraded was 45.81 as against 70.65 percent degraded at the substrate level of 1.1 g at the same enzyme level.

Antimicrobials and free amino acid survival in the rumen --

G. T. Schelling, T. O. Lindsey, H. R. Spires, S. E. Koenig, D. K. Beede, W. W. Gill, G. E. Mitchell, Jr., R. E. Tucker, University of Kentucky, Lexington 40546.

Studies with a variety of antimicrobial agents were conducted to assess their potential for allowing the survival of amino acids in the rumen. In vitro rumen fluid studies with 2 ppm of oxytetracycline (OTC) indicated that the rate of free lysine degradation was decreased to 31.6% of the control value. Plasma amino acid studies indicated that feeding 1.0 gram



of OTC per sheep per day with 8.0 grams of supplemental free lysine resulted in plasma lysine levels 4-fold those occurring when lysine was fed alone. In vitro studies revealed that capreomycin, neomycin, cycloserine and penicillin were not effective for protecting free amino acids in the rumen, but streptomycin and zinc bacitracin were somewhat effective, chlortetracycline, erythromycin and bacitracin were moderately effective, and monensin was highly effective. Feeding studies with chlortetracycline and monensin using 300 kg heifers fed supplemental free lysine resulted in elevated ruminal free lysine levels when the antimicrobials were fed, and in elevated plasma lysine when chlortetracycline was fed. A similar animal study with calves that were 7 weeks of age indicated that both of these antimicrobials elevated plasma lysine levels. This series of studies indicates that certain antimicrobial agents can increase the survival of free amino acids in the rumen to some extent.

The Role of the Hindgut in Nitrogen Metabolism in the Ruminant --  
D. O. Jensen and J. M. Asplund, University of Missouri, Columbia,  
Missouri 65211.

A digestion trial was conducted to determine the effect of cecectomy on nitrogen metabolism in the ovine. A total of 32 sheep were used, 16 of which were cecectomized. Two protein levels were fed consisting of 8% and 16% crude protein. Half the animals received formaldehyde-treated SBM (.8% on a protein weight basis) while the other half received untreated protein. The treatments were arranged factorially.

Cecectomization had no significant effect on nitrogen balance (NB), plasma alpha amino nitrogen (AAN), dry matter digestability or urinary urea nitrogen (UUN). There was a slight increase in gain ( $P < .01$ ) within the cecectomized group. Treatment of the SBM resulted in a 21% increase in AAN ( $P < .01$ ). The 16% crude protein resulted in higher nitrogen balance, gain, AAN, UUN and percent of dry matter digested. From these data it would appear that the absence of the cecum had little effect on the parameters examined. The higher protein level and the treatment of the SBM with formaldehyde did improve the performance of these animals.

A separate trial was conducted to determine if ammonia and amino acids are absorbed from the cecum. Four wethers were surgically prepared by the placement of catheters in the cecal vein and artery. With the cecum exteriorized and the animals under anesthesia 1 g of lysine was injected directly into the cecum by using a syringe with a 22 gauge needle. Blood samples were taken at frequent intervals for 4 hours. The cecum was kept warm and moist by covering it with a towel and bathing the towel with warm isotonic saline.

It was found that venous ammonia levels were consistently higher than arterial levels. The difference between venous and arterial ammonia levels reached a peak of 1 hour, with a second increase again at 4 hours. Alpha amino nitrogen values were also higher in venous blood than in arterial blood with peak levels at 1 hour. It would appear from these data that both ammonia and AAN are absorbed from the cecum. It is not clear whether this is by active or passive absorption.

A Computerized Net Protein System for Formulating Rations for Beef and Dairy Cattle, Using Feed Analysis Data -- D. G. Fox, C. J. Sniffen, P. J. Van Soest and P. H. Robinson, Animal Science Department, Cornell University.

A net protein system is being developed to formulate rations for cattle based upon feed analysis data. A discussion of specific steps follows. Development of any protein model will be a continuous process, to improve accuracy as new information becomes available. Thus, estimates given for various functions that are being used initially for purposes of sensitivity analysis are tentative. Preliminary calculations indicate that most are likely within 10%. Those that the authors feel to be reasonably adequate or that are less sensitive so they can be used without committing large errors will be indicated. Those for which the authors are developing more correct estimates or which need further study, however, will also be identified.

Requirements are based upon body composition data and protein content of milk. Maintenance requirements ( $NP_m$ ) reflect tissue turnover. The equation being used initially is that of Smuts;  $(70.4 W_{kg}^{.735} (.0125))$ . Correction for differences in mature weight will be developed, based upon known differences among cattle types in body composition at a specific weight.

Requirements for weight gain ( $NP_g$ ) reflect protein content of weight gain at a given stage of growth. the equation of Fox et al. is being used initially;  $NP_g = (.235 - .00026 WE) (ADG)$  where WE is equivalent weight (weight of average frame size cattle that is similar in chemical composition). Further refinement in this equation will be made to adjust for the influence of rate of gain on composition gain. Requirements for pregnancy (NP) is estimated as  $(1.136 W_{kg}^{.7})$ , which reflects  $NP_p$  needs during the last 60 days of pregnancy. Further work is needed to refine this expression, to include an adjustment for number of days pregnant. Requirements for lactation ( $NP_l$ ) = kg milk (% protein in milk) or alternatively  $1.9 + .4$  (% milk fat).

Dry matter intake is estimated for growing cattle, using the equations of Fox and Black. Base daily DM intake is; To 364 WE,  $W_{kg} \cdot .75$  (100 g), at 432 kg, base intake declines  $W_{kg} \cdot .75$  (95g), then declines to  $W_{kg} \cdot .75$  (90g) at 477 WE. Adjustments for NE concentration, breed, age and feed additives are made as described by Fox and Black. Intake of dry cows is assumed to be  $W_{kg} \cdot .75$  (100); for lactating cows,  $DMI = W_{kg} \cdot .75 (.255 FCM_{kg} + 8.769/100)$ .

The key carbohydrate and protein fractions in individual feeds in the diet are identified through feed analysis. A = soluble or rapidly degraded; B = insoluble available; and C = unavailable or bound. Microbial protein synthesis is estimated with a series of calculations. First, the escape of energy and protein is calculated, based upon individual B fraction degradation rates, lag times and dilution rates. Ruminally digested plant organic matter is estimated by subtracting the "C" and escaped "B" fractions. Then microbial yield (MCP) is calculated (gm/100 gm true organic matter digest ruminally);  $1/\text{bacterial N} = .1406 + .0149 (1/\text{liquid fractional turnover rate/hr})$ .

A "check" calculation is made, to see if proportions of N arising from the A & B protein pools are adequate to meet the needs of the  $MCP_1$  and  $MCP_2$  pools.  $MCP_1$  arises from the fermentation of the "A" carbohydrate fraction and  $MCP_2$  rises from the fermentation of "B" fraction carbohydrate.

Intestinal digestion and absorption of MCP is assumed to be 64%, based upon a content of 80% of N as true protein and 80% digestibility of microbial true protein. Escaped feed protein is estimated to have a digestibility of 90%, with the "C" fraction removed. Efficiency of utilization of absorbed protein is assumed to be 60% for tissue deposition and 70% for milk protein synthesis. Further work is needed to identify factors influencing utilization of absorbed protein, as it is a sensitive calculation and for which limited data are available.

Validation of the system will be conducted by comparing predicted vs. actual performance, using independent data for which adequate description of feed protein and energy fractions, dry matter intake, cattle type and condition and other key factors influencing performance and accuracy of predictions are available. Initial comparisons of predicted vs. actual performance with rapidly growing lightweight cattle fed under carefully controlled conditions resulted in a difference between predicted and actual performance of approximately 3%. Sensitivity analysis indicated that estimation of dilution rate is especially critical. In calculations made with high producing lactating cows, it was revealed that microbial yield must be calculated as a function of dilution rate as well as estimated true digestibility of plant organic matter in the rumen to adequately predict microbial yield. Calculations also indicate that

tabular feed NP values are impractical, because of the dependency on the interaction with intake, protein and energy fractions in other dietary ingredients and with protein requirements of the animal. This is not expected to be a major limitation of the system, however as low cost microcomputers are expected to become widely used in the near future.

### AGRONOMIC

Effect of Minerals and Mineral Availability on Nutritive Quality of Forages -- R. L. Reid, Division of Animal and Veterinary Sciences, West Virginia, Morgantown, West Virginia 26506.

With the exceptions of silicon, little attempt has been made to relate the role of minerals in forages quantitatively to measures of nutritive quality such as digestibility and intake. The effect of minerals may be exerted either indirectly, by modification of plant cell wall or non-structural organic components or directly, possibly by (a) supplying requirements of rumen microorganisms for minerals for specific functions such as cellulose degradation; (b) alteration of osmotic pressure, buffering action, dilution rates in the rumen; (c) depletion of tissue and blood levels, with consequent effects on intake. Earlier studies at W.V.U. showed: (a) Ca fertilization of orchardgrass increased intake by 15% in sheep, with no effect on digestible dry matter (DDM); (b) P fertilization of fescue increased TNC concentration, rate of in vitro cellulose digestion, palatability, with no effect on in vivo DDM; (c) Mg fertilization of orchardgrass on tetanigenic soils increased DDM, with no effect on intake; (d) micro-element fertilization of orchardgrass in 3 years increased DDM of first cutting hays over NKP alone; NKP increased intake over non-fertilized hays. Recent trials with Mg indicate: (a) live weight increases in pregnant beef cows wintered on low-quality forage or corn stover rations in response to MgSO<sub>4</sub> supplements in water or MgO in grain-mineral mix; (b) increased intake by lambs of alfalfa fertilized with high levels of kieserite (448 kg Mg/ha), with no effect on DDM; (c) in 3 years, increased intake of alfalfa by sheep (5%) and beef cows (13%) in response to MgSO<sub>4</sub> but not S fertilization; no effect on DDM; (d) increase in intake (10%) of timothy by lambs with Mg supplement, not with Mg fertilization; no effect of either Mg source on DDM.

Stepwise regression analysis of DDM and intake values for 221 forages (158 grasses, 63 legumes; as cut herbage or hay) on organic and inorganic composition gave the following regressions:  $Y$  (DDM%) =  $88.19 - .39$  (ADF%) -  $1.13$  (lignin%) +  $.40$  (crude protein%) -  $46.3$  (Mg%).

For intake,  $Y (I, \text{g/kg BW}^{.75}) = 68.59 - .93 (\text{ADF}\%) - 4.57 (\text{silica}\%) + 27.01 (\text{Ca}\%) + 31.34 (\text{P}\%) + 47.44 (\text{Mg}\%)$ . Other grazing studies with cool-season perennial grasses have indicated that mineral availability (apparent absorption, retention) may be independent of mineral concentration; P concentration and availability were major factors affecting herbage intake. It is suggested that a low availability of minerals in tall fescue may account in part for poor animal performance on this grass. Studies on the form in which minerals are present in herbage, and on their rate of ruminal release, are in progress to examine further the observed effect on quality of forages.

Straw Digestibility of Winter Wheat, Spring Wheat, Barley and Oat Cultivars in Eastern Montana -- Larry M. White, Glenn P. Hartman, and Jerald W. Bergman, Range Scientist, North Plains Soil and Water Res. Ctr., and Agronomists, Eastern Agric. Res. Ctr., P. O. Box 1109, Sidney, Montana 59270.

Straw from small cereal grains represents one of the largest potential sources of feed for maintenance of ruminant animals. This study was conducted to determine if differences existed in straw digestibility among and between cultivars of winter wheat (Triticum aestivum L.), spring wheat, [T. aestivum L. and T. turgidum L. (durum)], barley (Hordeum vulgare L. and H. distichon L.), and oats (Avena stiva L.) grown in eastern Montana. In vivo dry matter digestibility, crude protein, and phosphorus content of straw were measured on 8 to 25 cultivars of each crop for 2 years. We also measured heading date, plant height, lodging severity, and grain yield to determine if selecting for higher straw digestibility would adversely affect these important traits for grain production.

The average straw digestibility of winter wheat, spring wheat, barley, and oats was 38, 38, 42, and 45% the first year, respectively, and 42, 43, 47, and 46% respectively, the second year. Greater differences in straw digestibility existed among cultivars within a crop than between crops. Straw digestibility of winter wheat, spring wheat and barley cultivars each differed by 7 to 11 percentage units depending upon crop and production year. Straw digestibility of oat cultivars differed by 6 percentage units the first year but only 3 percentage units the second year. Differences in straw digestibility were consistent between years with few exceptions.

Straw of some cultivars of oats and barley contained enough crude protein and energy to maintain a mature cow during the middle third of pregnancy if supplemented with phosphorus and probably vitamin A. It was estimated that pregnant cows fed straw of cultivars with the lowest digestibility would lose from one-fifth to one-half kg weight/day. Selecting cultivars with higher straw digestibility apparently would not adversely affect important agronomic traits for grain production. Cultivars with higher straw digestibility were not associated with higher lodging or less grain yield. Neither was straw digestibility consistently associated with heading date or plant height. Crude protein and phosphorus content of straw was not consistently associated with higher straw digestibility.

Adaptation and forage potential of U. S. Panicum species as warm-season perennials -- D. H. Timothy, J. C. Burns, R. D. Mochrie, and J. O. Rawlings, Department of Crop Science, Animal Science, and Statistics, North Carolina State University and SEA-AR, Box 5155, Raleigh, North Carolina 27607.

The Virgata section of the genus Panicum offers great potential as warm-season perennial forage. Switchgrass, P. virgatum, is found in all but five states of the continental U.S. Its relatives are all maritime and found along the Atlantic and Gulf Coasts, except for a relict species of geological lake beds in Texas. These species contain great amounts of variation for agronomic, morphological, nutritive feedstuff characteristics, and adaptation. They should, therefore, be amenable to improvement of desirable characteristics through plant breeding.

A collection from along the U. S. Atlantic and Gulf coasts of 12 accessions of P. virgatum ( $2n = 36$ ,  $2n = 72$ ) and its maritime relatives represented by 33 accessions of P. amarum var. amarum ( $2n = 54$ ) and 44 accessions of P. amarum var. amarulum ( $2n = 36$ ) was evaluated for two years. Percent in vitro dry matter disappearance (IVDMD) of the forage was higher than anticipated. The negative association of IVDMD and maturity (plant growth) was relatively minor. For example, on May 10, June 7, and July 8 plant height of P. amarum was 59 cm, 111 cm, and 132 cm, while percent IVDMDs were 76, 69, and 57, respectively. P. amarulum was somewhat taller, but IVDMDs were similar to those of P. amarum. P. virgatum, more upright and tussocked than the other species, but of comparable plant height, had IVDMDs of 71%, 62% and 45% on May 10, June 7, and July 8, respectively.

A recurrent selection program was begun with the non-maritime, low-land form of P. virgatum (switchgrass). A selected population of 161 plants from 11 different germplasm sources was evaluated for yield and IVDMD. From these, 33 plants were selected on the basis

of yield x IVDMD. The 33 plants were intermated and the half-sib family progenies evaluated. Fifteen clones of the original 161 were selected at random for use as a check population, representing the original population, in the half-sib progeny trials. Of these 15, 4 are also parents of half-sib families in the experiment. The material was sampled at 28-day intervals in April, May, and June for two years. IVDMDs appear comparable to those of the maritime collection. The average increase in yield of the Syn I over the Syn 0 cycle was 132%.

Nutritive Attributes of U. S. Panicum Species -- J. C. Burns, D. H. Timothy, R. D. Mochrie, and J. O. Rawlings, Departments of Crop Science, Animal Science and Statistics, North Carolina State University and USDA, SEA-AR, Raleigh, North Carolina 27650.

Perennial, tropical forages provide grazing for ruminants, or a source of stored feed during the midsummer when temperate forages are either unproductive or dormant. The source of nutrients is extremely important for ruminant enterprises that require year-round feeding.

Tropical forages presently used by producers are characteristically high in fiber which increases appreciably as the plant matures. Inherent fiber concentrations, their nature and subsequent changes with maturity are associated with low animal gain.

A number of ecotypes representing three species of the genera, Panicum, i.e., virgatum, amarum and amarulum were characterized for their fiber contents and subsequent changes with maturity. The populations examined ranged widely in maturation (head emergence) date. The widest range in head emergence of initial growth was found in P. amarulum varying from July 11 to October 25. Examples of neutral detergent fiber (NDF) concentrations present in ecotypes in P. virgatum having highest in vitro dry matter disappearance (IVDMD) were 65.6, 64.7 and 72.1% for May, June and July harvests, respectively. The ecotype having lowest IVDMD for the July harvest contained 65.7, 66.3 and 74.0% NDF for the respective harvest dates. Acid detergent fiber (ADF) was 30.4, 33.5 and 37.8% for the former ecotype and 31.7, 34.6 and 41.1% for the latter. Hemicellulose varied little among harvest dates within and between ecotypes (ranged from 31.2 to 35.2%).

In the Panicum amarum and amarulum populations, IVDMD was generally higher and fiber fractions lower than in P. virgatum. Concentrations of the fiber fractions were frequently similar between ecotypes while IVDMD concentrations changed appreciably. For example, an ecotype with high IVDMD from the P. amarum population (averaging 76.9, 72.1 and 58.3% for the three harvest dates) contained NDF percentages of 54.7, 60.0 and 65.0. A selection having appreciably lower IVDMD in harvest three (47.0 vs 58.3%) averaged 56.6, 61.4 and 64.5% NDF. ADF values were similar between the ecotypes for each harvest ranging from 29.2 to 36.5%. Hemicellulose values of the latter ecotype were generally two percentage units higher, being 27.5, 28.3 and 30.5% for the three harvests.

Contrasting these values with 4-, 6- and 8-week-old Coastal bermudagrass is rather revealing. Respective concentrations of NDF, ADF and hemicellulose are 74.9, 77.5 and 78.0; 38.5, 43.1 and 42.7; and 36.4, 34.4 and 35.3. Selections from the Panicum genera offers potential of developing tropical perennials with reduced fiber concentrations and possibly higher nutritive value.

Turfgrass Clippings - An Untapped Agricultural Resource -- A. J. Turgeon, F. C. Hinds, D. H. Baker, and W. A. Torello, University of Illinois.

Currently, several large sod growers in Wisconsin, Illinois, California and Colorado are collecting, processing, and marketing turfgrass clippings for use in poultry feeds. At approximately five percent of total intake, the clippings provide a desirable coloration to the skin and shanks of the broilers and to the egg yolks of layers. Thus, the current market value of the clippings is a function of the pigmentation potency or bioavailable xanthophyll content, and sale price is typically twice that of dehydrated alfalfa meal. In 1975, work was initiated to determine the influence of turfgrass cultivars and cultural practices on the concentrations of bioavailable xanthophyll, crude protein and other nitrogen forms in the clippings removed with mowing. Results showed that the xanthophyll content varied from 72 to 358 mg per kg of fresh weight depending upon the turfgrass cultivar, and that xanthophyll increased significantly, but in small increments, with increasing rates of nitrogen fertilization (2). The crude protein level of dried clippings also varied with turfgrass cultivar; at the same intensity of culture, "Campina" Kentucky bluegrass ranked highest of 52 cultivars with 32.7 percent while "EVB-307", an experimental selection, was lowest at 22 percent (3). In a cultural study with Kenblue-type Kentucky bluegrass comparing three mowing heights (1.9, 3.8 and 6.9 cm), three mowing frequencies (1, 3, and 5 mowings per week), and four fertilization rates (0, 28, 56, and 112 kg N/ha/month), the crude protein and nitrate-nitrogen contents of clippings increased with: decreasing mowing height; increasing



mowing frequency; and increasing fertilization rate. In a similar study with tall fescue, the same trends were observed. The highest concentrations of crude protein and nitrate nitrogen measured in any of several studies was approximately 34 and 0.16 percent, respectively.

In sheep-feeding studies, the dry-matter digestibility of turfgrass clippings was above average, the clippings were an excellent supplemental protein source, and the amino acid composition of the protein was equal or superior to that of soybean meal (1). Both dehydrated-pelleted and ensiled clippings were found to be feasible for use in feeding ruminants. In poultry-feeding studies, shanks of chicks; the material was found to contain approximately four times the bioavailable xanthophyll of corn gluten meal (4). When isonitrogenous, isocaloric diets containing either methionine-supplemented soybean meal or turfgrass clippings as the sole protein source were fed to chicks, the gain/feed and gain/protein-intake ratios were uninfluenced by protein source. Furthermore, no increase in chick performance resulted when sulfur-containing amino acids were added to the turfgrass clippings, indicating that these amino acids were not deficient in the clippings. REFERENCES

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Videothermometry for Assay of Fescue Food in Cattle --  
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The study of tall fescue toxicosis in cattle has led to an independent objective measure that allows early assessment of the

nutrition-related disorder and provides a basis for comparing forages or fractions therefrom.

Chemical fractionation of alcohol extracts from toxic tall fescue produces four major fractions: cations, including alkaloids; anions, particularly organic acid salts and lactones; neutral compounds, lipids and simple sugars; and residue, complex carbohydrates. Components of these fractions are identified and measured by gas chromatography and gas chromatography-mass spectrometry. Reliable assay of their biological activities is accomplished in beef cattle by I. P. infusion during cold weather. Simultaneous observation of foot temperatures on a daily basis via videothermometry (measurement of surface temperatures with an infrared sensitive system) shows that the weighted average coronary band temperatures of animals exhibiting clinical fescue foot differ significantly from those of normal animals, and usually correlate with the onset of visual signs of fescue foot. Several varieties of tall fescue have been compared by this quantitative procedure; each has the potential to produce toxic forage, but the degree of potency can vary from variety to variety. Also under test is the toxicity of synthetic mixtures similar in composition to active fractions from toxic fescue. New evidence gained from the quantitative test suggests that fescue foot is a syndrome caused by more than one constituent of toxic grass.