REPORT ON

CONFERENCE ON RUMEN FUNCTION

held at

La Salle Hotel, Chicago, Illinois

November 28-29, 1973

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

General Chairman	C. R. Richards	CSRS, USDA
(a) Nutrition		J. T. Huber (MI)
(b) Microbiology		M. P. Bryant (IL)
(c) Physiopathology		R. W. Dougherty (NADC)
(d) Agronomic		J. C. Burns (N.C.)

NUTRITION

Methionine Requirement of Growing Holstein Steers - C. L. Fenderson and W. G. Bergen, Department of Animal Husbandry, Michigan State University, East Lansing, Michigan.

The methionine and lysine requirements of growing steers were studied in 7 Holstein steers by infusing incremental levels of 3.5, 7.0, 11.0, and 15.0 g of methionine or lysine into the abomasum and measuring changes in methionine and lysine levels in the plasma. Nitrogen balances were carried out as confirmatory measures, and lignin to nitrogen ratios were done on both feed and abomasal content to calculate (by the ratio method) the nitrogen passage to the abomasum. The steers had an average body weight of 274 kg and were fitted with soft plastic abomasal cannulas and were fed a 9.5% crude protein ration at the rate of 3% body weight.

There was no difference between the lignin/nitrogen ratio (1.75) of the feed and that of the abomasal ingesta (1.76), thus, indicating that there was no net loss of nitrogen from the rumen.

The quantity of nitrogen, lysine, cystine, and methionine reaching the abomasum per kg of feed consumed was 14.89, 4.5, 1.51, and 1.59 g respectively. The average daily passage of nitrogen, lysine, cystine, and methionine to the abomasum was 105.26, 32.08, 10.68, and 11.25 g respectively.

Plasma methionine level did not increase until at least 7 g of methionine were infused. Thereafter, plasma methionine increased markedly with each successive higher level of methionine infusion. The point of inflection on the curve was regarded as the infused requirement level and was approximately 7 g of methionine. Nitrogen retention increased with each incremental level of infused methionine until after 7 g were infused and then plateaued.

Plasma lysine level increased immediately after the infusion of 3.5 g of lysine and then continued to increase with each successive level infused, thus, indicating that the animal's requirement was satisfied by the lysine reaching the abomasum in the digesta. Nitrogen retention on the other hand decreased concommittantly with each incremental level of infused lysine.

By using 70% as the digestion coefficient for lysine and methionine, as published by Hogan and other workers, the amount of absorbable lysine and methionine reaching the abomasum was determined to be 22.46 and 7.88 g respectively. By also using 7 g of methionine as the infused requirement, the methionine requirement of a growing 274 kg steer was calculated to be 14.88 g per day. Since the lysine requirement was apparently met by the passage of digesta to the abomasum, the lysine requirement of a growing 274 kg steer was estimated to be $\leq$ 22.46 g per day.

Nitrogen Transformations in Corn Silage During Fermentation - W. G. Bergen, Department of Animal Husbandry, Michigan State University, East Lansing, Michigan.

Experiments were conducted to study changes in nitrogenous constituents of corn plant material during ensiling, the biological availability of corn silage nitrogen fractions and the role of the extent of fermentation on voluntary feed consumption (VFC) by mature non-growing wethers. During ensiling, the water soluble nitrogen (WSN) (from proteolysis) content increases approximately 2.4 fold in the first 12 hr. The final product generally contains 40-45% WSN. Amino Acid N (as a % of WSN) increased during the ensiling period, NH_3-N and Urea-N did not change while

the undetermined N fraction (as of % of WSN) declined. The amino acid N fraction of WSN of freshly chopped plant material was characterized by a high glutamic acid and aspartic acid (NH_2) content, but by the end of the ensiling period the essential amino acids (EAA) were predominant. The proteolytic activity of the ensiling corn plant material declined rapidly from day 0 to day 5 with no further measurable activity during the rest of the ensiling period. Non-protein nitrogen (NPN) treatment of the silage appeared to reduce proteolytic activity. The data indicate that proteolysis was primarily a function of endogenous plant proteases. WSN from NPN treated or untreated corn silage supported a significantly ($P<.05$) lower rate of in vitro cellulose digestion than urea. The in vitro protein digestibility of the water insoluble N (RN) of ensiled (treated or untreated) corn was markedly lower than that found for RN from freshly chopped material. The amino acid composition of silage RN was similar to the composition of US no. 2 corn. Four experimental silages with different dry matter content, organic acid content, and WSN were produced. When these silages were fed to non-growing sheep there were no differences in VFC. Simple correlation analysis revealed no significant correlation between silage dry matter, acid content, and WSN on VFC. However, the animals used were most likely not well suited for an intake study of this nature.

In Vitro and In Vivo Ammonia Release Studies on Various Slow-Release Urea Products - J. R. Males and R. R. Johnson, Department of Animal Sciences and Industry, Oklahoma State University, Stillwater, Oklahoma.

The slow ammonia release potential of Golden-Pro and Starea-70, two urea gelatinized starch products, have been examined in vitro and in vivo. In vitro, when Golden-Pro and Starea-70 were incubated at 39°C in a buffered urease solution, the release of ammonia from both products was at the same rate as from an isonitrogenous water-urea mixture. One-hundred percent of the urea-N had been released as ammonia-N by 30 minutes after initiation of the incubation. Golden-Pro, Soybean meal and a corn-urea mix were incubated in rumen liquor from steers fed a roughage ration and a concentrate ration. Ammonia was released from Golden-Pro and from the corn-urea mix at the same rate in both fermentations. Peak ammonia release was reached at 10 to 12 hours in the high roughage rumen liquor and by 2 hours in the high concentrate ration. Twelve fistulated sheep in a 3 x 3 latin square were fed a cottonseed hull ration supplemented isonitrogenously with either corn-urea mix, Starea-70, or Golden-Pro. Rumen samples were collected on days 5, 10, and 16 after rations were switched and ammonia was determined by Magnesium Oxide distillation on samples taken at 0, 1, 2, 4, and 8 hours after feeding. Rumen ammonia-N levels were significantly lower when sheep were fed Starea-70 compared to either Golden-Pro ($P<.01$) or the corn-urea mix ($P<.05$). There were no differences in rumen pH observed.

High-Fiber, Urea-Supplemented Diets in Lactating Dairy Cows - H. R. Conrad and R. Bouchard, Department of Dairy Science, Ohio Agricultural Research and Development Center, Wooster, Ohio.

A diet containing 42% crude fiber and 3.0% nitrogen of which 60% was obtained from urea was compared to a diet where all the nitrogen was supplied by vegetable protein and most of the digestible nutrients were from starch. Dry matter intake was 17.5 and 15.7 kg per day and milk production 23.6 and 21.0 kg per day, respectively, for the protein-starch diet compared with the urea-fiber diet. The plasma amino acid level was generally lower for the cows fed the protein-starch diet and was related to higher milk production and higher plane of energy nutrition in cows fed the control diet. The levels of all the plasma amino acids except for aspartic acid, glutamic acid and cystine increased at a regular rate during the first month of lactation. This elevation was coincidental with an increasing daily rate of feed consumption and a more rapidly increasing milk production. Overall average milk yield of 6100 kg in Holstein cows annually was considered a successful use of the urea-fiber ration.

Influence of Ration Composition on NPN Utilization by Cattle - R. E. Roffler and L. D. Satter, Department of Dairy Science, University of Wisconsin, Madison.

Using continuous culture fermentors, Satter and Slyter (J. Animal Sci., 35:273, 1972) demonstrated that an ammonia concentration of 5 mg NH_3-N/100 ml rumen fluid was sufficient to support maximal rates of microbial growth. Subsequent in vivo studies (Roffler and Satter, J. Dairy Sci., 56:663, 1973) demonstrated that the concentration of ruminal ammonia exceeded 5 mg NH_3-N/100 ml when dietary crude protein (CP) was greater than 13%. Results of these studies were used to develop a system which quantitatively estimates nonprotein nitrogen (NPN) utilization and predicts when NPN supplementation would be beneficial.

The validity of the system has been tested using data from published lactation trials involving 38 comparisons of NPN-supplemented rations to unsupplemented negative control rations (406 cows). The negative control rations contained from 6.8 to 13.8% CP and from 66 to 80% TDN. Simple regression equations relating the percent improvement in milk production from NPN supplementation to (1) % CP in the unsupplemented ration dry matter and (2) predicted ruminal ammonia concentration were computed. Ruminal ammonia concentration was predicted from the % CP and % TDN in the unsupplemented ration using the regression equation:
mg NH_3-N/100 ml = 38.73 - 3.04% CP + 0.171% CP^2 - 0.49% TDN + 0.0024% TDN^2.

Improvement in milk production due to NPN supplementation was negatively related to both % CP in the unsupplemented ration and predicted ruminal ammonia concentration. Prediction equations including both linear and quadratic terms gave r^2 values of 0.46 for % CP in the negative control ration and 0.71 for predicted ruminal ammonia concentration. The data indicate that NPN supplementation did not improve milk production if the ration contained more than 12.5% CP prior to supplementation or if the ruminal ammonia concentration was greater than 4 mg NH_3-N/100 ml rumen fluid. These results agree closely with our previous observations regarding the point at which excessive ammonia accumulates in the rumen. They also substantiate our contention that the addition of NPN to many typical dairy rations is superfluous.

Rumen Volatile Fatty Acids in Lactating Cows Fed Hay, Haylage, or Urea Treated Corn Silage Rations - D. J. Schingoethe, G. L. Beardsley, and H. H. Voelker, Dairy Science Department, South Dakota State University, Brookings, South Dakota.

Hay, haylage, .5% urea treated corn silage (UCS), or .5% urea plus 1% dried whey treated corn silage (UWCS) were fed as the sole roughage to one of four groups of 9 lactating cows per group for a 10-week lactation trial. Cows fed hay and haylage were paired on the basis of production and stage of lactation, and averaged 15 weeks into lactation at the beginning of the experiment. Cows fed UCS and UWCS were similarly paired, and averaged 9 weeks into lactation at the beginning of the experiment. Concentrate rations formulated to meet NRC protein, energy, Ca and P requirements were fed at 1 kg/2.5 kg milk produced. Roughage furnished 40.0 to 44.5% of total DM intake of all rations. Average milk production on hay, haylage, UCS, and UWCS was: 20.9, 21.0, 25.0, and 26.7, kg 4% FCM/day, respectively. Rumen samples were taken via stomach tube 3-4 hours after A.M. feeding during weeks 5 and 9; pH of rumen samples from cows fed hay were higher ($P < .05$) than those from cows fed haylage, UCS, or UWCS (6.72, 6.35, 6.40, and 6.46, respectively). Acetate, propionate, butyrate, and total VFA levels (μM/ml) on hay, haylage, UCS, and UWCS were, respectively: acetate, 56.3, 73.0, 66.2, and 67.8; propionate, 17.7, 30.5, 30.4, and 39.8; and butyrate, 7.3, 10.9, 7.2, and 6.8; and total VFA, 88.0, 122.8, 11.8, and 124.3. Total VFA were higher ($P < .05$) on fermented forages which primarily reflected higher ($P < .05$) propionate levels on the fermented forages.

Plasma-Free Amino Acid Concentrations in Lactating Cows Abomasally Infused with Casein or Glucose - J. T. Huber, Lars Vik-Mo, and W. G. Bergen, Departments of Dairy Science and Animal Husbandry, Michigan State University, East Lansing.

Lactating Holstein cows fed above standard allowances for energy and protein were abomasally infused with casein or glucose in four trials to study

effects on milk protein production and related blood parameters. Infusion periods were 5 to 7 days length preceded by and followed by a control period. Milk protein yields were increased ($P<.05$) by abomasal infusion of casein in three of four trials. Erratic intakes of feed masked treatment effects in one trial, but casein infusion still raised ($P<.05$) protein percent in milk. Higher milk yields ($P<.05$) and increased protein in milk both contributed to milk protein responses. Isocaloric infusion of glucose raised protein production above controls but not as much as casein alone. Casein infusions increased blood urea nitrogen suggesting greater deamination and less efficient dietary protein conversion to milk protein as nitrogen intakes were raised; whereas glucose infusions lowered blood urea nitrogen compared to controls. Plasma glucose was increased by both casein and glucose infusions in all but one trial where casein infusion depressed feed intakes. Glucose and casein infusions lowered milk fat content. In trial 1, alpha amino nitrogen in plasma was also raised by casein infusion and lowered by glucose, a pattern similar to that shown for plasma free amino acids. Casein infusions consistently increased the essential to non-essential amino acid ratio in plasma which was associated with significant increases in milk protein production. Mammary transfer efficiencies calculated from the data and corrected for published arteriovenous differences suggested that phenylalanine and lysine were the essential amino acids in most critical supply for milk production regardless of treatment. During control periods, methionine appeared one of those least abundant relative to need, but supplementation of 15 to 30 g per day via abomasal infusion made it one of the most plentiful. Threonine, leucine and histidine frequently ranked among the four most limiting amino acids.

Response of Lactating Cows to Supplemental Feeding of Formaldehyde-Treated Casein - G. A. Broderick and G. T. Lane, Department of Animal Science, Texas A&M University, College Station, Texas.

Formaldehyde-protein treatments may allow improved efficiency of utilization of preformed dietary proteins as well as increased feeding of NPN compounds as alternative sources of ammonia for ruminal microflora. The present studies were conducted to determine the effect of feeding supplemental casein treated with 0.8% w/w formaldehyde (FTC) vs. untreated casein (UTC), or vs. no supplement, on lactating cows being fed an already high protein diet. Eight Holstein cows producing 26 to 30 Kg/day were fed an average of 14.4 Kg/day of a Sorghum-based concentrate (19.4% CP, 5.6% CPE from urea) and 10 Kg/day of sorghum hay (8.0% CP). The cows were divided into 2 groups of approximately equal production, and fed the supplements in a switchback experiment with 14-day periods (28-day cycles). For the

first 2 cycles, group 1 received FTC and group 2 received UTC during the first period of each cycle, followed by a switching of the supplements during the second period. For the third cycle, FTC supplementation was compared to no supplement. Milk was sampled at each milking and 2-day composites were prepared and analyzed for fat, total protein (N x 6.38) and "true" protein (TCA-insoluble N x 6.38). The first 4 days of each period were considered a transitional sub-period and excluded from calculation of results. There were no significant effects on production for FTC vs. UTC supplementation during the first 2 switchback cycles. However, for FTC vs. no supplement (third cycle), significant increases of 4.5% ($P<.05$), 7.1% ($P<.01$), and 8.1% ($P<.01$) were observed for production of milk, total protein, and "true" protein, respectively. Milk content of total protein ($P<.01$) and "true" protein ($P<.05$) was also significantly elevated. Although urea concentrations in jugular vein plasma (obtained on the last day of each period) tended to be higher with FTC feeding, this was not statistically significant. Milk urea tended to comprise a higher proportion of total milk NPN, with the effect during cycle 3 (FTC vs. no supplement) being significant ($P<.05$). However, total milk NPN content was not affected by dietary supplement.

Ruminal in vitro incubations and other studies were conducted with the FTC fed in the lactation study, and with small batches of FTC treated with graded levels of formaldehyde. A curvilinear decrease in net NH_3 release from FTC preparations was observed in vitro with increasing levels of treatment, up to 4.0% w/w formaldehyde. In vitro release of amino acids, however, appeared to plateau at a treatment level of slightly over 1.0% w/w formaldehyde. The FTC preparation fed in the lactation study behaved as if treated with about 1.0% w/w formaldehyde. FDNB-available lysine and reversibly-bound formaldehyde determinations accounted for only a small proportion of the applied formaldehyde, suggesting considerable loss of formaldehyde and/or irreversible binding to amino acid residues other than lysine.

Changes in Unsaturated Fats of Milk with Increasing Amounts of Dietary Fatty Acids - G. P. Dimenna and D. L. Palmquist, Department of Dairy Science, Ohio Agricultural Research and Development Center, Wooster, Ohio.

Four mature lactating Holstein cows were fed alfalfa pellets, corn silage and grain with HEF, a hydrolyzed animal and vegetable fat, added to the grain at four levels - 0, 4, 8, and 12% of the concentrate. When HEF was added to the grain, the corn and soybean oil meal proportions were adjusted to give a constant protein to energy ratio. The diets were fed during four 21-day periods in a Latin Square design. Ration digestibility was measured during the last 5 days of each period. Other items measured were milk production, milk fat, milk protein, and composition and quantity of milk fatty acids. The digestibility of proximate fractions were not significantly ($P = 0.05$) affected by the addition of HEF to the diet, except ether extract digestibility was significantly ($P = 0.06$) increased by the three fat supplemented diets.

However, the mean digestion coefficients of all fractions measured were generally increased by the three HEF diets. The intakes of palmitic, stearic, oleic, linoleic, linolenic and total fatty acids were increased with the HEF supplemented diets. Milk production, milk fat and milk protein percentages were not significantly affected ($P = 0.05$) by the dietary treatments. The mean weight percentages of C6:0, C10:0, C14:0, and C16:0 were significantly ($P = 0.05$) decreased by the HEF rations, supporting the theory that increased intake of long-chain fatty acids decreased fatty acid synthesis in the mammary gland. The percentages of C18:0 and C18:2 remained constant, while percentages of C18:1 were 29.71, 33.75, 36.92, and 38.47% in diets of 0, 4, 8, and 12%, respectively. The percentages of C18:3 were also increased by the three HEF diets. In general, milk fatty acid yields were not significantly ($P = 0.05$) affected by the dietary treatments, except C18:1 and C18:3 which were increased by the HEF diets. Milk unsaturated fatty acid percentages and yields regressed on unsaturated fatty acid intakes showed that the unsaturated fatty acid percentages and yields were independent of unsaturated fatty acid intake. The results also show that the 8% treatment was just as effective as soybean flour, which increased the total ether extract percent of the ration to 12%, increasing the percentages of C18:1 and C18:3. However, soyflour, which is high in linoleic acid, was more effective in increasing the percentage of C18:2. The relatively small changes in the milk fatty acid percentages and yields, milk production, and milk fat and protein percentages suggests that the level of production was not sufficient to stress the homeostatic mechanisms regulating food intake. These results suggest that up to 10% fat in the total diet can be fed to dairy cows.

Effects of Grain Processing and Buffers on Rumen pH Changes and Lactic Acid Levels - R. R. Johnson, E. T. Clemens, D. D. Hinman, and N. A. Cole, Oklahoma State University, Stillwater, Oklahoma.

Lactic acidosis resulting from overconsumption of high grain rations is common in beef cattle feedlots and is usually a greater problem when highly processed forms of grain are used. Furthermore, decreased average daily consumption of processed grain rations may be due in part to subclinical acidosis created by the rapid fermentation of processed forms of grain. The effects of processing of sorghum and corn and of buffers on rumen pH and lactic acid (LA) changes were investigated in steers forced to overconsume. PH depressions and LA increases were greater following feeding of micronized sorghum vs. dry rolled sorghum with the LA increases being proportional to degree of micronizing. Depressions in pH were greater when ground, steam flaked or high moisture corn were fed than

when whole shelled corn was fed. Recovery of prefeeding pH levels was slowest with steam flaked corn. Rumen LA levels were much higher when the processed forms of corn were fed. When 2% buffers were added to high moisture corn rations, pH depressions were less with $KHCO_3$ while $NaHCO_3$ and $CaCO_3$ were similar to the control (no buffer).

The Use of Chromium EDTA Markers for Digestion Studies in Lactating Cows - J. F. Bargeloh and H. R. Conrad, Department of Dairy Science, Ohio Agricultural Research and Development Center, Wooster, Ohio.

Chromium (Cr) EDTA is a soluble reference marker which offers analytical advantages in specificity and sensitivity when compared to polyethylene glycol. Rates of excretion and recovery of Cr EDTA following a single dose to lactating cows are presented and compared to rates observed using Cr_2O_3 impregnated paper. Fecal Cr recovery following Cr EDTA administration ranged from 67 to 79% and averaged 73.1 $\pm$ 1.7% after 76 hr. Urinary Cr excretion averaged 3.8 $\pm$ 1.1%. Fecal Cr excretion continued at a low rate after 76 hr. accounting for the low recovery. This observation agrees with the results of Downes and McDonald (Brit. J. Nutr., 18: 153). Analysis of the data by curve peeling revealed a two compartment system with turnover times of 8 and 184 hr. Peak fecal Cr concentration occurred 11 hr. following administration of Cr EDTA and 25 hr. following Cr_2O_3 administration. Rate of fecal Cr excretion was 9.3%/hr. for Cr EDTA and 3.5%/hr. for Cr_2O_3 when cows were fed corn silage (60%) and concentrate (40%). Fecal recovery of Cr_2O_3 averaged 92.2 $\pm$ 6.8% for four observations. Cr EDTA is less desirable than Cr_2O_3 as a digestibility marker due to low recovery and rapid excretion rates. Cr EDTA appears to have merit for use as a marker for estimating liquid phase digesta flow rates.

MICROBIOLOGY

Rumen Microbial Protein Synthesis in Cattle Fed Low Quality Roughage Supplemented with Varying Levels of Soybean Meal and Urea - J. R. Males, J. R. Kropp, and R. R. Johnson, Department of Animal Science and Industry, Oklahoma State University, Stillwater, Oklahoma.

Four 275 Kg Angus steers, fitted with rumen and abomasal fistula, were allotted in a 4 x 4 latin square design. Mature, weathered bluestem hay (crude protein $<$3.0%), supplemented with 100% of the supplemented N as soybean meal, 25% as urea, 50% as urea or 75% as urea, was fed hourly by an automatic feeder to supply 5000 g/head/day. Rumen and abomasal samples were taken on days 10, 11, and 12 after the cattle were started

on a new ration. Lignin was used as a internal marker in order to calculate abomasal passage of various nitrogen fractions. Non-ammonia nitrogen (NAN) concentration as a fraction of total nitrogen in rumen contents was relatively constant on the four rations. The 100% soybean meal ration resulted in no rumen urea-N and a higher fraction of ammonia-N than did the urea rations. Total microbial-N passing through the abomasum was 30.0, 31.8, 30.6, and 31.5 g/day for the four rations, respectively. More nitrogen bypassed the rumen as protein in the 100% soybean meal ration with the smallest bypass of protein nitrogen on the 75% urea ration. Total protein passing through the abomasum was 351.3, 333.8, 320.6, and 295.6 g/day on the four rations, respectively.

Influence of Dilution Rate on Growth Yield and Efficiency of Mixed Rumen Bacteria in Continuous Culture - H. R. Isaacson, F. C. Hinds, F. N. Owens, and M. P. Bryant, Departments of Animal Science, Dairy Science and Microbiology, University of Illinois at Urbana-Champaign, Illinois.

It has been observed that little can be done to affect the nutritive quality of the microbial cell mass leaving the rumen and until recently it was thought that the same was true for the efficiency of production of microbial cells.

In search of good theoretical values for the efficiency of cell or protein production we employed a continuous flow fermenter. In this chemostat, mixed rumen bacterial cultures were grown at three dilution rates (0.02, 0.06, and 0.12 per h) to represent the physiological range of growth rates found in the rumen. At each dilution rate four levels of glucose were employed and these were always limiting. The level of glucose had no effect on any parameter when the parameter was based on per mole of glucose used.

Increasing the growth rate, i.e., dilution rate of the culture, decreased the amount of acetate and butyrate produced per mole of glucose, increased the amount of propionate per mole of glucose, and decreased the amount of methane produced per mole of glucose.

ATP production was based on volatile fatty acid and methane production by assigning two ATP per acetate, three ATP per propionate, and butyrate and one ATP per methane. ATP production was found to be quite constant, 5.0 moles ATP per mole glucose.

Cell concentration, measured by filtration, $Y_{Glucose}$ and Y_{ATP} increased with increasing growth rates. This increase is explained by the effect of a maintenance energy requirement of the bacteria that is satisfied in preference to growth and magnitude of its effect is a function of time or growth rate. This explanation can be likened to an enzyme saturation kinetics model of Michelis-Menton or Lineweaver-Burk. By calculating regression lines based on a double reciprocal plot of cell concentration, $Y_{Glucose}$ or Y_{ATP} vs. dilution rate, one can generate curves to predict these parameters at any dilution rate. From the Y-intercepts maximum cell concentration, $Y_{Glucose}$ or Y_{ATP} can be calculated and from the slopes of the lines the maintenance estimated. Maximum efficiencies and maintenance requirements calculated were 80 g cells per mole of glucose with 0.27 mmoles glucose required per g cells per h and 19.9 g cells per mole ATP with 1.6 mmoles ATP required per g cells per h. At the slow growth rate of 0.02 per h approximately 65 percent of the energy derived from the glucose is being used for maintenance while at the fast growth rate of 0.12 per h 35 percent of the energy is being used for maintenance.

The maintenance energy requirement is described as that energy used for functions not directly resulting in growth. Examples being flagellar motion, lysis, cellular constituent turnover, and osmotic pressure maintenence. This maintenance requirement is not necessarily an energy cost to the host animal--VFA's are produced at the same rate--it is a protein cost.

We conclude from this study that energy level, while limiting growth, has little effect on efficiency of cell production but with increasing growth rates there will be a greater efficiency of cell production. Within the physiological range of growth rates encountered in the rumen, the maintenance energy requirement has a dramatic effect on cell yield.

Oxidative Phosphorylation in Rumen Anaerobes - C. A. Reddy, Department of Microbiology and Public Health, Michigan State University, East Lansing, Michigan.

The existence of anaerobic oxidative phosphorylation in certain rumen anaerobes is inferred from the fact that they give growth yields much higher than are expected from known substrate phosphorylations involved in the fermentation. Also, a consideration of the reactants and products in mixed fermentations carried out by certain rumen bacteria also suggests the existence of anaerobic oxidative phosphorylation in these microbes. Vibrio succinogenes, a gram-negative, motile, anaerobic vibrio isolated from the rumen by Wolin and coworkers, obtains energy

for growth by oxidizing H_2 or formate with the stoichiometric reduction of malate or fumarate to succinate or of nitrate to ammonia. Particulate preparations have been obtained from Vibrio succinogenes which catalyze the phosphorylation of ADP to ATP in H_2 oxidation coupled to the reduction of fumarate to succinate. This phosphorylation system requires H_2, fumarate, ADP and Pi and is destroyed by boiling the particles. This system routinely gives P/H_2 values up to 0.3 and soluble protein is not required for phosphorylation. Marked inhibition of phosphorylation, but not H_2 oxidation, by pentachlorophenol and gramicidin is observed. Dinitrophenol and methyl viologen are less effective as uncouplers. Oligomycin and antimycin A have no effect either on H_2 oxidation or on phosphorylation. Inhibition of H_2 oxidation as well as phosphorylation by HQNO suggests the involvement of a quinone in the system. H_2 utilized for fumarate reduction is stoichiometric with the succinate formed. Fumarate added was quantitatively recovered as fumarate and succinate at the end of the reaction. Further studies showed that particulate preparations of Bacteroides ruminicola, which is one of the most important species of ruminal bacteria, catalyze phosphorylation coupled to the oxidation of NADH concommitant with the reduction of fumarate to succinate. NADH and fumarate are required. H_2 cannot replace the requirement for NADH. The results suggest that phosphorylation coupled to the reduction of fumarate to succinate with molecular H_2 or reduced pyridine nucleotides may have an important physiological role in the growth of many rumen bacteria.

Evidence for Control of Alternate Amino Acid Biosynthetic Pathways in Rumen Bacteria - M. J. Allison, NADC, Ames, Iowa.

Bacteroides ruminicola and Megasphera elsdenii are ruminal organisms that do not require the branched-chain volatile acids (isobutyrate, 2-methyl butyrate, and 3-methyl butyrate) for growth but when these acids are present, they are reductively carboxylated to synthesize the carbon skeletons of corresponding branched-chain amino acids.

The isopropylmalate pathway for leucine biosynthesis appears to function in B. ruminicola when isovalerate is not present in the growth medium. Evidence for this includes the detection of isopropylmalate dehydrogenase in extracts from B. ruminicola cells (Stieglitz and Calvo, unpublished) and the relatively high specific activity of leucine synthesized when the organism was grown in medium containing 1^4C-acetate and no unlabeled isovalerate.

When 10^{-3} M isovalerate was added to this medium, the flow of ^{14}C from acetate-2-^{14}C into cellular leucine was essentially stopped. When the organism was grown with UL-^{14}C-glucose, the ratio of the specific activity of leucine was 18:11:1 when the initial isovalerate concentration in the

growth medium was 0, 10^{-4}, and 10^{-3} M respectively. When the organism was grown in medium containing ^{14}C-glucose and 20 or 40% ruminal fluid, the incorporation of ^{14}C into leucine, isoleucine, phenylalanine, and valine was very low compared to other amino acids or compared with the same amino acids from cells grown with 5% or no ruminal fluid in the medium.

The results of similar competition experiments with M. elsdenii indicated that the flow of ^{14}C from glucose into branched-chain amino acids was inhibited when branched-chain volatile fatty acids were present in the growth medium.

It is proposed that these organisms are able to use alternate pathways for biosynthesis of the branched-chain amino acids. Mechanisms incorporating intermediates from carbohydrate metabolism are used when branched-chain volatile fatty acids are not present in the growth medium. When these acids are present at concentrations usually found in the rumen, however, reductive carboxylation is the major pathway of biosynthesis and the incorporation of carbon from carbohydrate into these amino acids is greatly reduced.

The existence of alternate pathways for bioshythesis of amino acid carbon skeletons in a single microbe appears to be rare. The nature of the control system that functions to favor utilization of the reductive carboxylation pathway when branched-chain acids are present is not yet known.

Glucose Fermentation by Lactate Fermenting and Non-Fermenting Strains of Selenomonas ruminantium - C. C. Scheifinger, M. J. Latham, and M. J. Wolin, Departments of Dairy Science and Microbiology, University of Illinois, Urbana, Illinois.

A lactate-fermenting strain (HD4) and non-fermenting strain (GA192) of Selenomonas ruminantium were grown in continuous culture in a glucose-limited medium. Below dilution rates of 0.4 hr^{-1}, both strains fermented glucose to acetate and propionate. With increasing dilution rates, a marked shift towards lactate production occurred with both strains but was significantly greater with GA192. At approximately 1.1 hr^{-1}, GA 192 was essentially homolactic whereas HD4 converted 63% of the glucose C to lactate with the remainder converted to propionate, acetate, and CO_2. All further experiments were with batch cultures. GA192 forms only L(+) lactate and HD4 forms mainly L(+) with a small amount of D(-) lactate. HD4 uses L(+) but not D(-) lactate as an energy source. Both strains possess NAD-linked, L(+) lactic dehydrogenases, NADH-oxaloacetate oxidoreductases, and only very low, NAD-independent L(+) lactate: dichlorophenolindophenol oxidoreductase activity. No major differences were found in specific activities of these enzymes and NADH: fumarate oxidoreductase activity when growth was with limiting glucose and assays were at mid-log and after glucose disappeared or when HD4 was grown with lactate. We

confirmed that glucose prevents lactate utilization by HD4 and that rapid growth on lactate requires a factor in yeast extract which is not required for rapid growth on glucose. In addition, we found that CO_2 is required for growth of HD4 on lactate but not for growth on glucose. It is clear from the continuous culture studies that a control mechanism connected with growth rate determines whether carbon from glucose flows to propionate, acetate and CO_2 or lactate in both strains. There was no indication from our limited enzyme studies of the reason for the inability of GA192 to grow on lactate. It is possible that the inability of HD4 to use lactate when glucose is present may be a reflection of the more fastidious nutritional requirements and slower growth rates on lactate rather than a catabolite repression phenomenon.

Fermentation of Cellulose by Combined Cultures of Ruminococcus flavefaciens and Methanobacterium ruminantium - M. J. Latham and M. J. Wolin, Departments of Dairy Science and Microbiology, University of Illinois, Urbana, Illinois.

Ruminococcus flavefaciens C94 was grown with cellulose as an energy source in the presence and absence of Methanobacterium ruminantium PS. Fermentation products were measured after 6-7 days incubation at 37 C in a CO_2 atmosphere. The following values were obtained and are the means of 5-6 separate single and combined culture fermentations, respectively (in mole/mole initial hexose). R. flavefaciens alone: acetate, 74; formate, 35; succinate, 94; H_2, 33; and CH_4, 0. R. flavefaciens plus M. ruminantium: acetate, 145; formate, 3; succinate, 25; H_2, 0; and CH_4, 63. Growth of M. ruminantium depended on growth of R. flavefaciens. A significant increase in acetate formation in the combined culture accompanied the decrease in succinate formation by R. flavefaciens. The results are consistent with a shift of approximately 70 moles pyruvate and 140 moles NADH per mole hexose from the formation of succinate by R. flavefaciens alone to acetate and H_2 formation in the presence of the H_2- and formate-using M. ruminantium. Lowering of the partial pressure of H_2 by methane formation presumably permits an NADH to H_2 reaction of R. flavefaciens to proceed favorably in the combined culture. We suggest that the combined culture fermentation more realistically represents the natural ecosystem fermentation of R. flavefaciens than the single culture fermentation and raises the question of the quantitative significance of succinate formation by R. flavefaciens in the rumen.

Propionate Formation from Cellulose by Combined Cultures of Bacteroides succinogenes and Selenomonas - C. C. Scheifinger and M. J. Wolin. Departments of Dairy Science and Microbiology, University of Illinois.

Bacteroides succinogenes S-85, a cellulolytic rumen species, produces succinate and acetate as major products of carbohydrate fermentation. Selenomonas ruminantium HD4 another rumen species, produces propionate and acetate

from carbohydrate via the succinate pathway. These organisms were grown together to determine if Selenomonas decarboxylated succinate formed by Bacteroides. Analysis of products and total counts of independent and mixed cultures showed that an interdependent propionate-acetate fermentation took place in mixed cultures. With competitive energy sources. i.e., glucose or cellobiose, no succinate was found in mixed cultures although a significant amount was expected from the number of Bacteroides present. Propionate production per Selenomonas was significantly greater in mixed versus independent cultures indicating that Selenomonas decarboxylated exogenous succinate produced by the Bacteroides. Propionate and acetate were produced and no succinate accumulated. These results provide a model for in vivo interactions involved in propionate formation in the rumen microbial ecosystem.

Characterization of Rumen Bacteria Isolated from Alaskan Reindeer (Rangifer Tarandus L.) - B. A. Dehority, Department of Animal Science, Ohio Agricultural Research and Development Center, Wooster, Ohio.

A total of 141 strains of rumen bacteria were isolated on a non-selective medium from semi-domestic Alaskan reindeer (Rangifer tarandus L.). Seventy-one strains were obtained from two animals being fed alfalfa pellets, 49 strains from an animal feeding on lichen, browse, and native pasture, and 21 strains from this same animal after two weeks on a ration of dried lichens. Based on general morphology, the 21 strains from this latter isolation series were divided into seven groups. Thirteen strains, at least one from each morphological group, were then reisolated to insure culture purity, and studied in detail. Three morphological types, varying considerably in physiological characteristics and fermentation end products, all appear to belong to the genus Butyrivivrio. The four remaining morphological types have been classified as belonging to the genera Selenomonas, Treponema, Streptococcus and Lactobacillus. Microscopic examination of the 49 cultures isolated from this same animal just prior to changing to the dried lichen feed revealed that the majority of organisms were morphologically similar to those described above. However, a considerably higher percentage of coccus types was observed. Using a selective cellulose medium, 21 strains of bacteria were isolated from the alfalfa pellet fed semi-domestic reindeer. Eight strains morphologically resembled organisms in the genus Butyrivibrio and 9 the genus Ruminococcus. All of the Ruminococcus type strains were extremely active in their ability to digest cellulose.

Anaerobic Mycoplasmas from the Rumen of Sheep and Cattle - I. M. Robinson, M. J. Allison, and P. N. Hartman, NADC, Ames, Iowa, and Iowa State University, Ames, Iowa.

Obligately anaerobic mycoplasmas were detected in ruminal contents of cattle and sheep. Mycoplasmas capable of hydrolyzing cells of gram-negative

bacteria and/or casein (skim milk) were usually present at between 10^5 and 10^7 viable units per gram of ruminal contents. Mycoplasmas not possessing these proteolytic or bactoclastic capabilities were consistently present at higher concentrations in both sheep and cattle (10^7-10^8 per gram). Neither lytic nor nonlytic mycoplasma-like organisms were detected in cultures from cecal material from rabbits, hamsters, horses, pigs, turkeys, or deer.

On the basis of typical colonial appearance, lack of cell wall, filterability through 450 nm membrane filter, absence of reversion to a bacterium under appropriate conditions and growth inhibition by homologous antiserum, these organisms fit the proposed minimal standard for classification of an organism in the order Mycoplasmatales. The growth of these organisms was inhibited by oxygen. They were sensitive to thallous acetate, bacitracin, and streptomycin but insensitive to penicillin.

In the rumen fluid-free medium, all strains of anaerobic mycoplasmas studied required bacterial lipopolysaccharide for growth. In addition, the lytic strains and some nonlytic strains required cholesterol, while some nonlytic strains did not require cholesterol but were stimulated by it.

Serological studies showed that nonlytic and lytic strains were heterogenous species. Also, a comparison of these strains with known bovine mycoplasma strains in reciprocal tube agglutination tests demonstrated no heterologus agglutination.

We propose that a new genus be established for these obligately anaerobic mycoplasmas. The name Anaeroplasma is proposed for the new genus. The specific epithet would denote these organisms lytic (proteolytic and bactoclastic) and nonlytic capabilities.

Cholesterol is required for growth of some mycoplasmas, but a nutritional requirement for lipopolysaccharide is unusual and the role it plays in the growth of these organisms is not understood.

The physiology of these organisms and their role in the rumen ecosystem is not known.

Coenzyme M, 2-Mercaptoethanesulfonic Acid, Essential for Growth of a Rumen Strain of Methanobacterium ruminantium - C. D. Taylor, B. C. McBride, R. S. Wolfe, and M. P. Bryant, Departments of Microbiology and Dairy Science, University of Illinois, Urbana, Illinois.

In earlier studies (Bryant, Tzeng, Robinson, and Joyner, pp. 23-40. In F. G. Pohland (Ed.), Anaerobic Biological Treatment Processes. Advances

In Chemistry Series No. 105, Amer. Chem. Soc., Wash., D. C. 1971), strain M1 was shown to require H_2-CO_2 or formate as energy source, NH_4^+ as nitrogen source, and acetate as a major carbon source for growth. Several amino acids and 2-methylbutyrate and an unidentified growth factor present in rumen fluid were also essential to growth. Further studies showed that the latter factor as present in rumen fluid was a highly polar, relatively strong acid of low molecular weight and that at least two forms of somewhat differing polarity were present. The factor was found to be produced by diverse species of methanogenic bacteria including a sewage sludge strain of M. ruminantium, Methanospirillum hungatii, Methanosarcina barkeri, and Methanobacterium formicicum (strain MOH) but not by nonmethanogens so far studied. Coenzyme M, a compound involved in methyl transfer reactions in methane formation by the latter organism (McBride and Wolfe, Biochem. 10: 2317, 1971) has recently been identified, chemically synthesized, and shown to be 2-mercaptoethanesulfonic acid (Taylor and Wolfe, Fed. Proc. 32: 589, 1973). We now find that coenzyme M is the previously unknown growth factor. Either coenzyme M or its oxidized form, 2,2' dithiodiethanesulfonic acid, allows growth of strain M1 in a complex medium containing formate, H_2-CO_2, volatile acids, Trypticase, yeast extract, sulfide, cysteine and minerals. Three to four ng of either chemically synthesized compound allow half-maximal growth 3,3' Dithiodipropionic acid is inactive.

A New Method for Studying Microbial Decomposition of Cellulose in Rumen Fluid with a Cotton Fiber Substrate - Paul B. Marsh and Marion E. Simpson, Nutritional Microbiology Laboratory, Nutrition Institute, ARS, USDA, Beltsville, Maryland.

Measurement of the degree of swelling of cotton fibers in 18% sodium hydroxide has been found to be useful as a sensitive technique to investigate low levels of microbial cellulose decomposition in rumen fluid. After incubation in rumen fluid, a weighed sample of the fiber is immersed in the alkali, centrifuged to remove excess alkali remaining between the fibers, and re-weighed to determine percent increase in weight during the swelling, which is termed the "alkali-centrifuge value" or "AC value."[1/] Major changes in this value occurred during incubation in rumen fluid when weight losses of the unswollen fiber were 2% or less.

Data to illustrate the use of the AC method in detecting low levels of degradation were provided from incubations at 21 C and 51 C, also from incubations in the presence of added glucose or penicillin. Cotton fiber incubated in rumen fluid which had been centrifuged to remove bacterial cells exhibited no change in AC value, indicating a lack of free cellulase in the fluid. A similar inactivity was obtained with rumen fluid autolyzed for 72 hours at 37 C before incubation with the fiber. Data also showed that rumen fluid inactivated an added fungal cellulase.

No constant ratio was observed between the increase in the AC value and the loss in weight of the unswollen fiber. The latter change represents essentially the loss of cellulose throughout the entire fiber wall, while the AC change is known from prior data to relate specifically to damage to the cellulose in the outer wall of the fiber. The two properties may be used conveniently with fiber from a single incubation to measure cellulose decomposition in both the earlier and later phases of the microbial action.

1/ For details of the basic alkali-centrifuge method, see Textile Research Journal 23:831-841, 23:878-888, and 40:859-860.

Studies on the Sodium Requirement of Rumen Anaerobes - D. R. Caldwell, R. F. Hudson, and S. J. Hufsmith, Division of Microbiology and Veterinary Medicine, University of Wyoming, Laramie, Wyoming.

Sodium, previously shown an obligate growth requirement for rumen Bacteroides species, has now been shown an obligate growth requirement for most of the currently recognized predominant rumen bacterial species. Non-Bacteroides species thus far shown to require Na^+ include Eubacterium ruminantium, Butyrivibrio fibrisolvens, Succinivibrio dextrinosolvens, anaerobic Lactobacillus sp., Fusobacterium sp., Lachnospira multiparus, Selenomonas ruminantium, organisms similar to strain B 385-1, and Ruminococcus albus. Neither Streptococcus bovis nor Peptostreptococcus (Megaphaera) elsdenii could be shown to require Na^+.

Although quantitative differences were found among species in the quantities of Na^+ required for abundant growth, Na^+ concentration affects the growth yields and rates of all the Na^+-requiring species, and also appeared to affect the lag times from small inocula. The quantities of Na^+ over which growth was affected varied among species, but the critical range was usually between 5 and 50 mM. The Na^+ requirement is independent of K^+, which is also obligately required for growth of most rumen bacteria. The concentration range of the latter ion which affected growth rates and yields was between 0.1 and 2.0 mM. The K^+ requirement of a few organisms could be partially replaced by Rb^+ or Cs^+. No related monovalent cation could replace the Na^+ requirement.

The concentrations of Na^+ which support abundant growth of Na^+- requiring rumen bacteria indicate that these organisms are slight halophiles. The functions of Na^+ in the physiology and metabolism of these organisms remain to be determined. Membrane filtration studies with cells grown in growth-limiting concentrations of $Na^{22}Cl$ indicate that Na^+ is not accumulated against the concentration gradient by Na^+- requiring species, apparently precluding an active Na^+ transport mechanism. It seems probable that the function(s) of Na^+ involve, primarily, its influence on the utilization of other nutrilites. The rumen is the first relatively

low-salt-terrestial environment thus far studied in which the majority of the predominant bacterial species require Na^+. Na^+ appears a more frequent growth requirement for terrestial bacteria than has been previously believed.

Sulfate and Nitrate Reduction by a Rumen Strain of Desulfovibrio Desulfuricans - M. R. Bennink and M. P. Bryant, Michigan State University and University of Illinois.

Sulfate reducing bacteria were found in 10^6 numbers per milliliter of rumen fluid from sheep. A dissimulatory, sulfate-reducing bacterium was isolated and characterized as follows: a gram negative, curved rod (1 to 2 by 0.2) with a single polar flagellum; an obligate anaerobe; contained a C type cytochrome, desulfoviridin and a hydrogenase; no spores and the guanidine plus cytosine content of DNA was 55.1%. The bacterium was identified as a strain of Desulfovibrio desulfuricans which would reduce sulfate to sulfide and nitrate to ammonia when lactate was fermented. Nitrate is not reduced by other known dissimulatory sulfate-reducing bacteria. Two moles of lactate were oxidized to acetate and CO_2 per mole of either sulfate reduced to sulfide or nitrate reduced to ammonia. Nitrate and sulfate were reduced at equal rates and each competed equally with the other for reduction. The ability to reduce nitrate was inherrent (i.e., didn't need to be induced).

PHYSIOPATHOLOGY

Inhibitory Effect of Acid in the Intestine on Rumen Motility in Sheep - L. A. Bruce and T. L. Huber, University of Georgia, Athens, Georgia.

A relationship between duodenal acidification and rumen motility in sheep was investigated. Both the intravenous infusion of secretin (6.88 U/kg/hr.) and the duodenal infusion of lactic acid (pH 2.0, 0.7 M/l) inhibited amplitude and frequency of contractions. A more complete inhibition was obtained by acid infusion. Observations from cross-circulation and blood transfer experiments indicated that one or more hormones in addition to secretin was released by duodenal acidification which had stimulatory effects on intestinal motility and inhibitory effects on rumen motility similar to that described for cholecystokinin. It is concluded that intestinal hormones play a role in regulating rumen and intestinal motility during acid overloads of the rumen.

Microbial Changes in the Rumen of Sheep during the Onset of Induced Grain Overload - R. K. Chaplin and G. A. Jones, Departments of Veterinary Physiological Sciences and Dairy and Food Science, University of Saskatchewan, Saskatoon, Canada.

Although rumen microbial changes accompanying acute grain overload involve an initial increase in numbers of streptococci followed by lactobacilli, the time sequence of these events is poorly documented. Rumen fistulated sheep accustomed to brome-alfalfa hay were starved 24 hours. A slurry of ground barley (45 g/kg BW) in warm water (1:2) was then administered in a single dose. Rumen microbial counts and rumen fluid and blood metabolities were measured at -24, 0, 4, 8, 12, 18, 24, 32, and 48 hours.

Counts of total bacteria, starch hydrolyzing bacteria and lactate utilizing bacteria were made in a 40% rumen fluid medium containing the appropriate energy source. Total counts of each group were made at each time period using the anaerobic roll-tube technique of Hungate.

Lactobacilli were counted using the medium of Schaedler and Dubos, with pH adjusted to 4.5, in plates incubated under 100% CO_2. Streptococci were initially counted using the thallous acetate medium of Barnes in plates incubated under 100% CO_2. After characterizing these organisms, lactobacilli were found to have grown on the thallous acetate medium with the streptococci from 12 hours onward. This greatly distorted the streptococcal counts and gave a false impression (J. Dairy Sci. 56:671, 1973). Streptococci have since been isolated on a bile esculin azide medium. Although the lactobacilli also grew on this medium esculin was hydrolyzed only by the streptococci allowing differential counts to be made.

Counts of total bacteria, starch hydrolyzers and lactate utilizers all appeared to decrease from 24 hours onward. It has not been determined whether this was a reduction in absolute numbers or the result of rumen dilution. With only limited data using the bile esculin azide medium, streptococci seem to peak at about 12 hr. then decrease rapidly. Lactobacilli, although slow to increase initially, reach a peak between 24 and 48 hr. and maintain this level up to 72 hr. Protoza die between 8 and 12 hr. corresponding to the time rumen pH values drop to 5.0 and below.

Further Results with Thiamin and Polioencephalomalacia in Cattle and Sheep - F. M. Loew, Animal Resources Centre and Department of Veterinary Physiological Sciences, University of Saskatchewan, Saskatoon, Canada.

Evidence from England and Canada, supported by clinical observations in numerous countries, clearly implicates an inadequacy of thiamin, probably

thiamin diphosphate (TDP), in the causation of ruminant polioencephalomalacia (PEM). Current research in this laboratory is directed at detecting a possible defect in phosphorylation of thiamin to TDP or even the triphosphate (TTP) in rumen or intestinal wall, liver, or brain; analytical methods for accurately measuring the low concentrations of these phosphates have not been perfected, however. Concurrently, the distribution of thiamin in blood between erythrocytes and plasma is being correlated with the proportion of grain to roughage in bovine rations, since epidemiological patterns as well as chronology of reports of occurrence of PEM suggest a relationship to the development and onset of intensive (i.e., high grain, low forage) beef production systems. Field work by the author in Cuba in 1972 and 1973, where PEM can occur in epic proportions in the molasses/urea/low forage feedlots developed there, supports this contention. A further hypothesis in this context implicates a hypoglycemia in causation of PEM, said to result from shortages of glucose precursors in the rumen fermentation characterized by high instantaneous concentrations of butyric acid and low concentrations of propionic acid. Little supportive evidence for this hypothesis currently exists.

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Metabolic Disturbances in the Dairy Cow Influenced by Modern Managerial Practices - Case Reports and Epidemiologic Studies - J. L. Noordsy, R. A. Frey, D. L. Carnahan, J. Vestweber, M. G. Robl, H. W. Leipold, G. Kennedy, J. R. Dunham, and T. E. Chapman, Department of Surgery and Medicine, Dykstra Veterinary Hospital, Manhattan, Kansas.

Clinical observations on metabolic and/or infectious diseases in the aged dairy cow admitted to Dykstra Veterinary Hospital along with field examinations of associated herds has lead to a summarization of several common demoninators.

Clients are primarily associated with above-average producing cows. Complaints have greatly increased in the last three to five years.

Common complaints have involved: 1) increased reproduction problems, 2) chronic, non-responsive mastitis problems, 3) poor response to clinically diagnosed hypocalcemia cases, 4) abomasal dilation and/or displacements, 5) decreased milk production, and 6) above average death loss-primarily following parturition.

Common managerial and feeding programs are: 1) dry-lot confinement, 2) feeding of high yielding corn silage and/or haylage as a roughage, 3) emphasis on "lead feeding," and 4) altered calcium-phosphorus mineral supplement with emphasis on increased phosphorus intake as a possible

hypocalcemia prevention. Almost all client complaints have come from herds that have been associated with this type of program for at least 2 to 3 years.

Common clinical signs observed are: 1) chronic, unresponsive mastitis problems, 2) anovulatory estrus, 3) slow parturition, 4) retained placentas, 5) metritis, 6) atonic uteri, 7) abomasal dilatation and/or displacement, 8) negative or slightly positive serum ketones, 9) abnormal serum calciumphosphorus ratios, 10) thrombocytopenia, 11) leukopenia, 12) neutropenia, and 13) marginal anemia.

Prominent necropsy lesions include: 1) mastitis, 2) metritis, 3) enlarged diffuse "fatty livers," 4) bone marrow depression with lipid deposition, and 5) osteoporosis.

A very prominently associated key to the typically affected cow is a lack of favorable response to emergency surgical procedures such as abomasoplexies.

Field studies with subsequent alterations of the nutritional programs have stimulated satisfactory, although delayed, results. The changes recommended have included: 1) careful determination and adjustments of the total calcium-phosphorus intake, 2) increased Vitamin D intake, 3) adequate "dry-cow" period with emphasis on increased roughage intake and restricted "lead feeding" of concentrates. Records indicate that herds have responded satisfactorily to this program after approximately 4 to 6 month periods.

Data accummulated at this point would stimulate a conclusion that the common managerial and/or feeding practices of the affected herds have apparently affected calcium metabolism. Smooth muscle atony appears to be a prominent common denominator. The problem appears to be a complicated interrelated metabolic syndrome influencing disease entities. The role of hormones associated with parturition and environmental stresses, especially hyperthermia, is also questioned. It is conceded that they do influence the incidence of clinical cases.

Gut Motility and Biochemical Studies of Cattle and Sheep before and during Induced Grain Overload - R. W. Dougherty, M. J. Allison, H. M. Cook, J. A. Bucklin, and K. S. Coburn, Physiopathological Laboratory, National Animal Disease Center, Ames, Iowa.

Motility studies of the digestive tract of 4 sheep were made using radiotelemetric equipment. After normal records were made from each sheep, they were overfed with 70 gms. of grain per kilo of animal weight. The

ruminoreticulum did not become static until the ingesta pH was lower than 5. The cecum followed the same motility and pH patterns as the ruminoreticulum, but recovered more quickly in the surviving sheep. The abomasum and small intestine were more erratic. These experiments did show that considerable quantities of grain (substrate for microbial growth) reached the cecum before gut motility was inhibited.

The pH of the abomasum showed a sharp rise a few hours after overfeeding, indicating that significant amounts of rumen ingesta were passing through the abomasum. The pH of the cecum decreased from an alkaline state to about the same pH as the rumen (about pH 4). In the sheep that survived the cecal pH returned to normal levels much more quickly than did the rumen pH. These studies have lead to the initiation of studies of the bacteriological changes occurring in the lower gut which may be as significant as the changes that occur in the ruminoreticulum.

Radio Telemetry of Reticular Motility of Unrestrained Sheep - A. R. Graham, Department of Biomedical Sciences, Ontario Veterinary College, University of Guelph, Guelph, Ontario, Canada.

Previous work has established different patterns of cutaneous electrical potentials are associated with the different patterns of reticular motility during rumination and non-rumination. In the present investigation one phase of the work discussed involved the design of electrodes suitable for chronic subcutaneous implantation and assessment of their ability to detect a subcutaneous electrical analogue of the cutaneous potential. Another aspect of the work involved evaluation of performance of three different small FM transmitters (commercially available) for inclusion in a total telemetry system for monitoring subcutaneous motility in the unrestrained sheep. Two of the transmitters tested were suitable for encapsulation and have been tested as part of a totally implantable system. One system with the transmitter external continues to transmit reliable data four months after electrode implantation. Another electrode-transmitter combination totally implanted subcutaneously still functions reliably a month after total implantation; the maximum transmission life of this system is yet to be determined. Ancillary information covering heart rate, electrocardiogram, and breathing rate can be determined in records of the totally implanted system.

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AGRONOMIC

Studies of the Rate and Extent of Degradation of Plant Tissues by Rumen Microorganisms - Warren G. Monson, Research Agronomist, Agricultural Research Service, USDA, and the University of Georgia, College of Agriculture Experiment Stations, Coastal Plain Station, Tifton, Georgia.

The digestion of fresh forage *in vitro* is a valuable additional tool in our forage breeding and evaluation programs. Leaf disks or segments are incubated in rumen fluid for varying lengths of time and rate and progression of digestion observed. Digestion occurs readily at cut or broken surfaces but the cuticle generally forms a barrier to entry by the micro-organisms. Differences in rate and extent of digestion between species and between genotypes within a species are apparent. This procedure also preserves the fine structure of the leaf which permits detailed study of undigestible fractions.

The digestibilities of leaves of pearl millet, napiergrass, and bermudagrass have been increased by several treatments. The 48 hour *in vitro* dry matter loss of 5 grasses was increased an average 2.5 times by reducing the length of cut from 2.5 to .3 cm. Pressing leaves on a rough surface or abrading them with sand paper to break the cuticle increased dry matter losses from 2.5 to 5 times those from 2.5 cm leaf sections. A harvesting method which would abrade or break the cuticle would appear to enhance the digestion of forage.

The *in vitro* digestion of fresh bermudagrass and pearl millet leaves after 12, 48, and 96 hours in rumen fluid was studied histologically. Our observations indicate that the relative amounts of the leaf made up by vascular bundles, cutinized and lignified walls, mesophyll and other anatomical structures varied between varieties within a species. The rate at which digestion progressed through the leaf tissue also varied between varieties indicating a permeability factor. The variation observed indicates that forages of higher digestibility could be obtained by breeding for cell types and arrangements that rumen micro-organisms readily digest. The micro-organisms consistently digested the larger and less compactly arranged cells of the mesophyll first. Bundle sheath cells and other cutinized or lignified cell walls remained undigested after 96 hours in rumen fluid.

Relationship of Microanatomy to Digestibility in Forage Grasses Revealed by Electron Microscopy - Danny E. Akin and Donald Burdick, ARS, USDA, Russell Laboratory, Athens, Georgia.

Leaf samples of warm-season and cool-season forage grasses were microscopically examined for differences in sites of lignification and microanatomy related to *in vitro* degradation by rumen microorganisms. Lignified cells, as

determined histochemically with acid phloroglucinol, were located in the vascular bundles of the leaves. The inner bundle sheath of the bermudagrasses totally surrounded the xylem and phloem and stained intensively for lignin. Large vascular bundles in Pensacola bahiagrass possessed a lignified, partial inner bundle sheath that delimited only the phloem. The inner bundle sheaths of the cool-season grasses examined were complete, but in some species only the inner portions of the cell wall were lignified. Quantitatively, in the warm-season grasses, vascular tissue made up 15 - 40% of the total leaf area whereas the cool-season leaves consisted of 10 - 20% vascular tissue.

Examination of scanning electron micrographs revealed that tissue types differed in the rate in which they were degraded by rumen microorganisms. In the grasses examined, the mesophyll and phloem appeared to be degraded first with thick-walled cells (the outer bundle sheath cells and epidermal cells) degraded later. Lignified vascular tissue and sclerenchyma were not significantly degraded after 72 hours incubation with rumen microorganisms. Anatomically similar leaf tissues in all grasses examined were degraded faster in cool-season than in warm-season species; however, leaves of Coastcross-1 bermudagrass were degraded faster than those of Coastal bermudagrass.

Results indicated that differences in the amounts of particular tissues among species are important in determining the rate and extent of forage degradation by rumen microorganisms. However, microscopic observations of different grasses also indicated that anatomically similar tissues are degraded at different rates which implies that the chemical composition or organization of cell walls in particular grasses is also important in the utilization of forage grasses by ruminants. Possibilities exist for improving the nutritive value of warm-season forages by breeding plants for a higher ratio of rapidly digestible to slowly digestible tissues or by improving the digestibility of specific forage tissue by processing techniques.

Chemical and Physical Treatment of Crop Residues - Terry Klopfenstein, University of Nebraska, Lincoln, Nebraska.

Crop residues are inefficiently utilized by ruminants because of high content and poor digestibility of the fibrous fraction. This poor digestibility is related to the extent of lignification of the cell wall component of these low quality forages. Some of our more basic studies have indicated that crop residues respond to hydroxide treatment. Animals will consume crop residues with the hydroxide remaining in the crop residue and the moist mixture of crop residues and chemical can be successfully ensiled. From this information, we have conducted several animal growth studies to determine the feasibility of hydroxide treatment of crop residues. Lamb performance indicated that the residues showing most response

to treatment are milo residue and corn husklage. A lamb trial comparing performance of lambs fed corn cobs treated with either 4% NaOH, a combination of NaOH and calcium hydroxide or a combination of sodium, calcium and potassium hydroxide indicated that sodium and calcium hydroxide was as effective as straight sodium hydroxide. This would have the benefit of reducing the sodium content, reducing the cost and supplying some calcium in the ration which would be needed. Calves fed treated cobs gained 1.6 lb. a day compared to calves fed control cobs which gained about .7 lb. a day. Feed required per unit of gain was nearly halved. The performance on the treated cobs was similar to that normally obtained with corn silage rations. Calves fed treated husklage supplemented with soybean meal gained about 90% as rapidly and as efficiently as calves fed corn silage. A urea supplement was definitely not as nutritionally acceptable as the soybean meal supplement. The corn gluten meal-urea supplement gave performance somewhat less than the soybean meal supplement but considerably greater than the urea supplement. Hydroxide treatment of certain crop residues appears to have some potential in increasing digestibility and feeding value of crop residues. The crop residues which seem to have the most potential for treatment are corn husklage and milo residue. These materials are probably also the most easily collected at harvest time. However, the crop residues are low in protein and only a portion of this can be supplied by urea. Therefore, the cost of natural protein may limit the use of treated crop residues. Another type of treatment which has been conducted at Nebraska is a high temperature, pressure treatment where forage is subjected to steam pressure from 100 to 400 PSI (lb/sq in). Treatment with water alone at 250 or 300 PSI increased in vitro dry matter disappearance of corn cobs by approximately 20 percentage units. Addition of hydrochloric acid did not appreciably increase digestibility but did lower the pressure necessary for optimum digestibility. Sodium hydroxide addition had little effect on the response of the cobs to high pressure treatment. On a larger scale, treatments at 250 PSI increased animal digestibility of cobs by more than 16 percentage units. Sodium metabisulfite may be of some advantage in increasing digestibility either by the effect of the metabisulfite on delignification or by decreasing the amount of non-enzymatic browning. In a lamb growth trial, cobs comprised 70% of the ration. Daily gains were nearly doubled by treatment at 200 PSI and tripled by treatment at 250 PSI. Efficiencies of gains were increased accordingly. The use of sodium metabisulfite improved effeciency slightly but not daily gains.

Supplemental Protein Needs of Growing-Finishing Cattle - R. L. Preston, Ohio Agricultural Research and Development Center, Animal Science Department, Wooster, Ohio.

Removal of supplemental protein from the ration of growing-finishing calves after reaching 350 kg. live weight did not alter feedlot performance or carcass composition in two separate experiments. The ration

consisted of approximately 40% limestone-treated corn silage and 60% corn grain (dry matter basis). Soybean meal was the source of supplemental protein. Daily gain and dry matter efficiency were 1.02, 1.15, 1.15, and 1.16 kg/day, and 7.15, 6.53, 6.55, and 6.48, respectively, for calves that did not receive supplemental protein for the entire feeding time (168 days), or supplemental protein for the first 56 or 112 days, or continuously. When supplemental protein was removed from the ration, a similar quantity of supplement made up with corn grain was used; minerals were added to provide equal levels of Ca, P, K, and S to that found in soybean meal. Two additional experiments with yearling cattle have confirmed these results. Thus, once cattle are on feed and weigh 350 kg., their apparent protein requirements do not exceed 8.2 - 8.6% of the dry matter.

Survival and Possible Increase of Aspergillus Terreus in the Rumen of Fistulated Steers - M. C. Futrell, W. E. Poe, Leon Turner, D. R. Farnell, V. H. Watson, and R. E. Coats, Mississippi Agricultural and Forestry Experiment Station and USDA-ARS.

Populations of Aspergillus terreus Thom increased from 0 to over 1000 propagules per gram of rumen fluid in fistulated steers during a 29-day grazing period on fescue that had been fertilized with chicken manure plus litter. A. terreus remained viable through the entire digestive tract of 3 fistulated steers in 1973. In preliminary studies in the artificial rumen, A. terreus showed a slight increase in growth in the liquid portion of centrifuged rumen fluid taken from fistulated steers grazing fescue. These data indicate that A. terreus can survive and possibly increase in the rumen of fistulated steers. Thiabendazole, a drug with known anthelmintic and antifungal properties, prevented fescue toxicosis when administered orally at the rate of 5.0 gm per 45.5 Kg body weight at 7-day intervals. Under high rates of nitrogen fertilization applied as ammonium-nitrate in 1972, the number of propagules of A. terreus increased in fescue, but similar increases did not occur in 1973. Fescue toxicosis has not been observed in fescue pastures during the first year after establishment. Fescue toxicosis has occurred only in older pastures that have been established for a number of years. This seems to lend additional evidence that fungi are increasing in older pastures and may be involved in the fescue toxicosis syndrome.

TWELFTH RUMEN FUNCTION CONFERENCE

ATTENDEES

NAME	ORGANIZATION
Danny E. Akin	USDA--Athens, Georgia
Milton J. Allison	USDA--Ames, Iowa
Maurice Bennink	Michigan State University
W. G. Bergen	Michigan State University
Rus Berzins	University of Saskatchewan
David T. Bochman	Ruminant Nitrogen Products Adrian, Michigan
Ronald L. Boman	Michigan State University
Glen A. Broderick	Texas A&M University
M. P. Bryant	University of Illinois
Richard C. Bull	University of Idaho
Donald Burdick	USDA--Athens, Georgia
J. C. Burns	USDA--Raleigh, North Carolina
Daniel R. Caldwell	University of Wyoming
Jane A. Carstairs	Michigan State University
William Chalupa	Smith, Kline Corporation West Chester, Pennsylvania
R. H. Chaplin	University of Saskatchewan
Herbert L. Chapman, Jr.	University of Florida Ona, Florida
H. R. Conrad	Ohio Agricultural Research and Development Center Wooster, Ohio
Roger Crickenberger	Michigan State University
R. A. Dehority	Ohio Agricultural Research and Development Center Wooster, Ohio
P. G. Dimenna	Ohio Agricultural Research and Development Center Wooster, Ohio
R. W. Dougherty	USDA--Ames, Iowa
Roy S. Emery	Michigan State University
Duane Erickson	North Dakota State University
W. J. Esdale	Miracle Feeds, Montreal
C. L. Fenderson	Michigan State University
Theodore Ferris	Michigan State University
M. C. Futrell	USDA--State College, Mississippi
Roland A. Gessert	Ayerst Labs, New York City, New York
Frank C. Hinds	University of Illinois
Ronald L. Horst	University of Wisconsin

Attendees (Continued):

Ron Isaacson	University of Illinois
John Thomas Johns	Michigan State University
Ronald R. Johnson	Oklahoma State University
Terry Klopfenstein	University of Nebraska
W. M. Knight	Silver Spring, Maryland
Maud Knutsson	University of Illinois
Hector H. Li	University of Wisconsin
David Libby	Tuskegee Institute
F. M. Loew	University of Saskatchewan
James R. Males	Oklahoma State University
Paul B. Marsh	USDA, Beltsville
Warren G. Monson	USDA--Tifton, Georgia
J. L. Noordsy	Kansas State University
Fred N. Owens	University of Illinois
John Peters	Michgian State University
R. L. Preston	Ohio Agricultural Research and Development Center Wooster, Ohio
C. Adina Gayano Reddy	Michigan State University
Clyde R. Richards	USDA--Washington, D.C.
C. R. Richardson	University of Illinois
I. M. Robinson	USDA--Ames, Iowa
W. E. Roe	University of Saskatchewan
Robert E. Roffler	University of Wisconsin
Larry D. Satter	University of Wisconsin
David J. Schingoethe	South Dakota State University
Charles G. Schwab	University of Wisconsin
H. T. Shin	University of Illinois
Marion E. Simpson	USDA--Beltsville, Maryland
William H. Smith	Purdue University
J. E. Sullivan	University of Illinois
Wallace M. Wass	Iowa State University
J. M. Willinison	Michigan State University
Larry Wilson	University of Illinois
W. Mark Wilson	University of Illinois