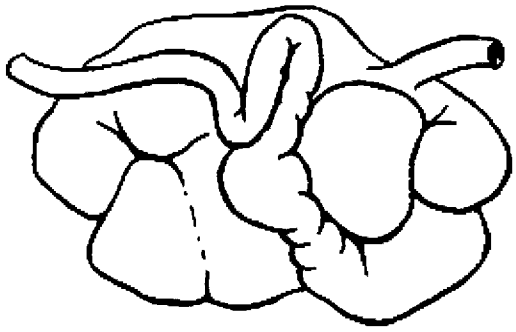
Abstracts

Conference on Rumen Function

Volume 19, 1987



19th Biennial Conference on Rumen Function
Chicago, Illinois
November 17-19, 1987

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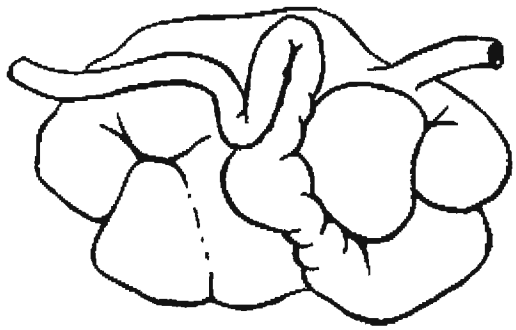

M. J. Allison, Chairman
Panel Chairmen:

Agronomy	J. C. Burns
Microbiology	K. A. Dawson
Nutrition	J. T. Huber
Physio-Pathology	W. M. Wass

Sustaining Members supporting the Mixer and providing coffee for the meeting were:
Eastman Chemicals, Division of Eastman Kodak Company
Hoffmann-La Roche, Inc., Roche Vitamins and Fine Chemicals
Lilly Research Laboratories, Division of Eli Lilly & Company
Purina Mills, Inc.
The Upjohn Company

THE CONFERENCE ON RUMEN FUNCTION

The first CONFERENCE ON RUMEN FUNCTION (CRF) was held November 27-28, 1951. Conferences have been held on odd-numbered years since then, and the conference in 1987 was the 19th such meeting. The first Conference was called by the Adminstrator of the Agricultural Research Administration of the U. S. Department of Agriculture to plan a research program that would assist livestock producers by reducing the incidence of bloat in animals grazing improved pastures.

H. W. Marston, Research Coordinator, U.S.D.A., prepared the report of the first conference which was attended by 71 persons. Panels and chairmen were:
Rumen Physiology - C.F. Huffman;
Physio-Pathology - R.W. Dougherty;
Agronomic - W.K. Kennedy;
Animal Management - H.H. Cole;
Microbiology - W.D. Pounden.

The continued growth of the CRF, which is no longer centered on the problem of bloat, indicates that it meets a need not met elsewhere. The Conference has no formal organization, membership lists or dues. Arrangements for the CRF are made by a Chairman, and H. W. Marston served in that capacity for conferences 1 - 8; C.R. Richards for conferences 9 - 17 and M.J. Allison for conferences 18 and 19.

At the 19th CRF in 1987, R.W. Dougherty was recognized as the only person attending both the first and the 19th Conference. He served as chairman of the Physiopathology Panel of the CRF from 1951 through 1973. Attendees at the 19th CRF were pleased to congratulate Dr. Dougherty for his contributions to the Conference and to ruminant physiology.

A Steering Committee has been appointed to consider questions and suggestions about structure and time and place for the Conference. The Steering Committee agreed that strengths of the CRF that should be maintained and encouraged include: the participation by scientists from several disciplines, the active participation by scientists who are experienced investigators, and the informal character of The Conference. The use of poster sessions, initiated at the 1987 Conference, was also approved.

The 20th CRF is planned for 1989. Unless unforseen problems (or opportunities) develop, it is likely that the next meeting will be held November 15-16, 1989, at the same place as the 19th Conference. Your suggestions are, however, solicited and may be sent to any of the members of this Steering Committee:

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TABLE OF CONTENTS

PAGE

PAGE

PAGE

PAGE

NUTRITION PANEL

Sequence of Corn and Barley Grain Digestion by Rumen Bacteria. L. M. Rode, K.-J. Cheng, Agriculture Canada Research Station, Lethbridge, Alberta T1J 4B1 and J. W. Costerton, Department of Biology, University of Calgary, Calgary, Alberta T2N 1N4

Individual grains of dent corn and barley were cut in half (split) or left undamaged (whole). Samples of split and whole grain were incubated *in sacco* in the rumen of two steers consuming 50% alfalfa hay, 25% steam-rolled barley and 25% steam-rolled corn. Length of incubation ranged from 0.5 to 48 hr. Triplicate bags were incubated for each of 3 days to determine dry matter disappearance (ISDMD). A fourth replicate for each incubation time and treatment was fixed and examined by electron microscopy.

After 48 hr incubation, percent ISDMD was 68.9 and 34.2 for split and 5.9 and 5.4 for whole barley and corn, respectively. The cuticular outer surface of the barley hull did not develop extensive bacterial colonization even after 48 hr of exposure to rumen bacterial populations in nylon bag experiments. The inner surface of this structure was colonized via damage sites and eventually developed very large mixed adherent bacterial populations that effectively digested cellulosic material from within. The endosperm was invaded by bacteria that formed an extensive mixed population in the spaces between starch granules, but granules themselves were specifically colonized by gram-negative rods whose morphological characteristics closely resembled those of the genus *Bacteroides*. These organisms formed extensive adherent microcolonies on the surfaces of the starch granules, showed evidence of extensive amylolytic activity, and stored large amounts of intracellular polysaccharide materials. Comparative examinations of the digestion of corn showed that its starch granules were largely colonized by large gram-negative cells resembling those of the genus *Megasphera* and other smaller cocci which also accumulated very large amounts of intracellular polysaccharide.

These data suggest a sequential bacterial colonization of feed grains in the rumen and that the development of specific bacteria communities digesting starch granules are different for barley and corn. However, for both barley and corn, digestion of starch requires attachment of adherent bacterial populations and proceeds from the inside of the granule, outwards.

EFFECT OF *Aspergillus oryzae* ON RUMEN DIGESTION AND MILK PRODUCTION IN DAIRY COWS

R. GOMEZ-ALARCON, C. DUDAS and J.T. HUBER, UNIVERSITY OF ARIZONA

A series of trials conducted to evaluate the use of *Aspergillus oryzae* (AO) culture-extract, a fungal feed additive, are described here. In a production trial, 46 Holstein cows in early lactation, paired according to pre-treatment milk production and by parity, were assigned to either a control group or an AO supplemented group. The cows were fed a 60% concentrate total mixed ration. The treated group received 3 g of the fungal extract mixed with 87 g of ground milo a day. Cows remained on the trial for at least 90 days. Four digestion trials were conducted with 44 lactating cows. Half of the cows received the AO extract as described above. Chromium oxide, used as marker, was administered for 12 days. Fecal spot samples were collected the last 5 days of the period. Two trials involving ruminally and duodenally cannulated cows were conducted to measure the ration digestibility in the rumen. One trial compared the effects of the fungal additive when the cows had a high and low concentrate ration. The other trial compared AO and yeast culture extracts as feed additives at high concentrate. Cows were managed as in the digestion trial, except that samples were collected every 4 h from duodenal cannulae.

Cows receiving the AO had higher milk yields ($P<.05$) than the control group (40.4 vs. 37.1 kg/d) while feed intake and feed efficiency were similar. In the digestibility trials, feed intakes and dry matter digestibility were higher for the AO than control groups. Even though feed intake increased with AO, total tract digestibility of the low forage ration in the rumen was not depressed. No effects of total tract digestibility were observed with the high forage ration. Both yeast and AO increased the total and rumen digestibility of the ration dry matter and fiber fractions. No changes were observed in the ratio of acetate to propionate in the rumen nor in VFAs produced. As for the digestion of the organic matter in the rumen, in both the low and high forage rations, fermentability increased with AO. Also, microbial yield per unit of organic matter fermented increased, resulting in larger amounts of microbial protein delivered to the duodenum. In the trial with yeast and AO, these effects were not observed. Apparently the higher milk yields with the fungal additive resulted from increased digestion in the rumen with greater synthesis of microbial protein available to the animal.

In Situ Protein and Fiber Digestion of Tropical Forages. J.R. Carpenter* and R.Y. Niino-DuPonte, Dept. of Animal Sci., Univ. of Hawaii, Honolulu, HI 96822

It has long been known that there is a seasonal abundance of forage in tropical and subtropical regions. Even though biomass production is much greater than in the temperate areas, the efficiency of animal conversion of these forages to marketable products is still more efficient in the temperate regions. It is also known that optimum yield per hectare does not correlate well with maximum digestible dry matter production per hectare. The ruminant digestive process is a dynamic system involving the inflow of feed to the rumen and the outflow of liquid, bacteria and undigested feed residues to the lower gastrointestinal tract. Rumen turnover is influenced by both rates of digestion and passage. Physical events relating to particle size reduction of digestible fiber in the rumen are important in regulating both feed intake and rate of digestion, thus animal performance. The addition of energy (corn and/or barley) or protein (fishmeal, cottonseed meal or soybean oil meal) at various levels failed to increase the in vitro dry matter digestion of both kikuyu (Pennisetum clandestinum) and pangola (Digitaria decumbens) grasses (P>.05). These data, along with other data which indicates that nutrient composition of various tropical forages [california grass (Brachiaria mutica), guinea grass (Panicum maximum) and alfalfa (Medicago sativa)] is reasonable, indicate that the limiting factors in the utilization of wet tropical pasture grasses appears to be related to the quantity animals can eat and the rate at which the animals digestive tract can process the fiber component, not the lack of protein or energy concentration. Trials with sheep and steers have demonstrated that wet forages significantly lowers water consumption, rumen pH, raises the specific gravity of the rumen fluid, alters fecal particle size and increases time animals spend ruminanting each unit of cell wall material (P<.01). Kikuyu, california and guinea grasses, and alfalfa, were each harvested at 4 to 6 weeks regrowth and ensiled at 3 different moisture levels in replicated 18.9 liter experimental silos. Forage materials were ensiled fresh, as wilted silage and as haylage and samples were also made into hay. Samples were dried (50 C) and analyzed for total protein, fiber and protein fractions, and in vitro and in situ digestibilities. Moisture level and type of forage affected ensiling characteristics and rate of fermentation (P<.01). Levels of soluble protein increased and insoluble available protein decreased when silage moisture level increased (P<.05). Rates of in situ dry matter, protein and cell wall digestion for various tropical forages are shown below. Values are the slope of the regression of the log(n) of potentially digestible material remaining vs time.

Sample Type	Dry Matter	Protein	Cell Wall
Alfalfa hay	-.07 hr-1	-.08 hr-1	-.05 hr-1
Alfalfa wilted silage	-.05 hr-1	-.09 hr-1	-.05 hr-1
Alfalfa haylage	-.06 hr-1	-.07 hr-1	-.05 hr-1
Kikuyu hay	-.03 hr-1	-.04 hr-1	-.01 hr-1
Kikuyu silage	-.03 hr-1	-.04 hr-1	-.03 hr-1
Kikuyu wilted silage	-.04 hr-1	-.04 hr-1	-.02 hr-1
Kikuyu haylage	-.04 hr-1	-.05 hr-1	-.02 hr-1
Sugarcane silage	-.06 hr-1	-.06 hr-1	-.06 hr-1
Sorghum X sudan silage	-.05 hr-1	not analyzed	-.05 hr-1

The degree to which microbial action may enhance rumination effectiveness through increased fragility of digesta particles and the efficiency with which a bolus is masticated during rumination are still unclear.

Rate and extent of digestion of cell wall fractions in brown midrib and normal genotypes of sorghum x sudangrass hybrids as determined in situ using Holstein steers.

C. L. Wedig and E. H. Jaster, Department of Animal Sciences, University of Illinois, Urbana, IL.

Objectives were to determine compositional differences between normal and brown midrib genotypes of sorghum x sudangrass silages, and to determine rate and extent of digestion of cell wall components of hybrids. Forages were normal and brown midrib genotypes of Redlan x Piper and Redlan x Greenleaf. Four ruminally cannulated steers were used in a 4 x 4 Latin square design. The in situ digestion kinetics were determined in conjunction with a digestibility trial in the steers. Dacron bags were 8 x 16 cm with 20-70 um pore size. Five grams substrate was weighed into each bag. To determine rates of digestion, dacron bags were removed after 0, 6, 12, 18, 24, 36, 48 and 72 h in the rumen.

Brown midrib silages were lower in ADF (40.6% versus 38.0%) and lignin (4.8% versus 3.4%) than were normal genotypes. Extent of 72 h digestion of dry matter, NDF, ADF, cellulose, hemicellulose, and acid detergent lignin were greater for brown midrib genotypes than for normal genotypes. There were no differences between genotypes for rate of digestion of any of the cell wall components. Brown midrib genotypes were higher in the hemicellulosic monosaccharides, arabinose, galactose, and uronic acids, than were normal genotypes.

Extent of Digestion (%)

	Redlan x Greenleaf		Redlan x Piper	
Item	normal	brown midrib	normal	brown midrib
Xylose	51.8	57.8	55.1	60.8
Uronic acids	63.1	70.5	66.4	72.8

Xylose and uronic acids had a higher extent of digestion for brown midrib genotypes than for normal genotypes.

Alkaline hydrogen peroxide treated straw as a source of energy for rumen bacteria in continuous culture. F. J. Bas, M. D. Stern and G. C. Fahey, Jr. Dept. of Animal Sci., University of Minnesota, St. Paul, MN 55108 and Dept. of Animal Sci., University of Illinois, Urbana, IL 61801.

Eight dual flow continuous culture fermenters were used to compare the effect of alkaline hydrogen peroxide treated wheat straw (TS) to other carbohydrate sources on ruminal microbial growth and fermentation. Diets contained either 70% untreated wheat straw (US), solka floc (SF), corn starch (CS) or TS. The remainder of the diet consisted of soybean meal (27.5%) as the only protein source, mineral mix (2%) and urea (.5%). Treatments were arranged in a randomized complete block design with three experimental periods of eight days each and two replicates per period. Some results are presented in the following table:

Item	Diets			
	US	TS	SF	CS
Organic matter digestion, %	19.3^{b}	42.2^{a}	27.9^{b}	45.5^{a}
NDF digestion, %	16.3^{c}	52.5^{a}	38.4^{b}	34.0^{b}
Total VFA, mM	52.9^{c}	93.0^{ab}	75.8^{b}	113.4^{a}
Bacterial N flow, g/d	.7^{b}	1.4^{a}	.6^{b}	1.4^{a}
Bacterial efficiency, g N/kg OMTD	53.5^{a}	46.7^{b}	31.7^{c}	33.8^{c}

^{a,b,c}Means in the same row with different superscripts differ ($P<.05$).

True organic matter digestion was highest ($P<.05$) for the CS and TS diets and lowest ($P<.05$) for the US diet. Neutral detergent fiber digestion was higher ($P<.05$) for the TS diet than any other carbohydrate source, indicating a significant improvement in energy availability with the alkaline hydrogen peroxide treatment. This is consistent with total VFA concentration which was highest with CS but not different ($P>.05$) from the TS diet. The lowest ($P<.05$) concentration of VFA was observed with US, which did not differ ($P>.05$) from the SF diet. Low fermentability of the solka floc was probably due to the highly crystalline nature of its cellulose. Bacterial synthesis, measured as total g of bacterial N flowing out of the fermenter system, was not different ($P>.05$) between the CS and TS diets. In contrast, bacterial flow with the SF and US diets suggests that synthesis was limited by substrate fermentability. Efficiency of bacterial synthesis was also influenced by carbohydrate source, with the two straw-based diets showing higher ($P<.05$) efficiencies than the CS and SF diets.

In summary, improved degradation of cell wall in wheat straw by rumen microorganisms was observed with alkaline hydrogen peroxide treatment under conditions used in this experiment. An increase in bacterial synthesis was also observed, resulting in higher nonammonia N flow in the effluent. In conclusion, alkaline hydrogen peroxide treated wheat straw showed higher DM, OM and fiber digestion when compared to untreated straw and solka floc and was comparable to corn starch as an energy source for rumen bacteria.

Influence of chromium concentration of mordanted fiber on digestive kinetics and fecal output estimations. Y.-C. Hsaio, M. D. Hanigan, M. R. Brasche and J. R. Russell, Dept. of Animal Sci., Iowa State University, Ames, IA 50011

Chromium(Cr)-mordanted fiber density is related to its Cr concentration; therefore, it may influence mordanted fiber's passage in the digestive tract. The objective of the present experiments was to evaluate the influence of Cr concentration and fiber type of Cr-mordanted fiber on passage kinetics and in predicting fecal output. Alfalfa hay was ground through 7.62 cm and 1 mm screens, washed with detergent and mordanted with 2, 8 and 14% Cr as sodium dichromate. The actual Cr concentrations and specific gravities of the mordanted fibers treated with 2, 8 and 14% chromium were 2.25%, 1.1936; 3.69%, 1.2509; and 5.52%, 1.4498, respectively. Six steers (mean weight 264 kg) in metabolism crates were fed diets containing ground (7.62-cm) alfalfa and corn stover at ratios of 100:0, 66:34 and 33:67 (dry matter basis) with .45 kg of supplement in two 3x3 Latin Square arrangements. After a 14 day adjustment phase in each period, steers were pulse-dosed with 106, 53, and 30 gm of the mordanted fiber treated with 2, 8 and 14% Cr. Fecal samples were collected from the rectum at intervals up to 144 hours post-dosing, dried, ashed and analyzed for Cr by atomic absorption spectrophotometry. Digesta passage kinetics and fecal outputs were determined by one and two compartment models. During the collection phase, total feces were also collected and dried.

The mean dry matter intakes and digestibilities of the diets containing 100, 66 and 33% hay were 1.66, 1.55 and 1.30 % of bodyweight and 57.4, 48.9 and 44.5%, respectively. Hay concentration in the diet did not affect digesta passage kinetic or fecal output estimations determined either by the one- or two-compartment models. Rate of passage and mean retention time determined by either one- or two-compartment models did not differ between markers. In the one-compartment model, 3.69% Cr-mordanted fiber had a lower ($P<.05$) interval for appearance of marker than did other Cr concentrations. Using either one- or two-compartment models, gut fill and fecal output estimations were lower ($P<.05$) when determined with the 3.69% Cr-mordanted fiber than the 2.25 or 5.53% Cr-mordanted fibers. Mean deviations between true and calculated fecal outputs were 3.05, 2.53; .25, .19; and 1.58, 1.27 kg/d for the 2.25, 3.69 and 5.53% Cr-mordanted fibers using the one- and two-compartment models, respectively. No diet by marker interactions were observed for the digesta passage kinetics or fecal output estimations.

After the last period of the Latin Square, each steer was fed the diet containing 66% alfalfa hay and 34% corn stover (dry matter basis). After adjustment to diet, 2 steers were pulse-dosed with corn stover fiber mordanted with 2, 8 or 14% Cr and fecal samples were collected and analyzed as above. Data were compared with the values obtained from steers receiving the diet containing 66% alfalfa hay in the above experiment. Fiber source did not affect passage kinetics or fecal output estimations.

Influence of Particle Size of Magnesium Oxide and Forage Source on Serum, Urine, and Fecal Magnesium, L.J. Wheeler, C.H. Noller, and J.A. Patterson, Department of Animal Sciences, Purdue University, West Lafayette, IN 47907

A 2x3 factorial experiment was conducted with 25 Holstein heifers, average weight 414 kg, to determine the effect of two forages, corn silage and oatlage, and three magnesium oxide treatments on magnesium in serum, urine and feces. The magnesium oxide treatments were: control, 96g of coarse MgO-A, or 96g of fine MgO-B. A 7-day preliminary period was followed by a 14-day experimental period and a 7-day washout period. The animals were housed in a stanchion barn and fed a complete mixed ration twice daily. Four grams of chromic oxide were mixed with 96g corn grain and fed to all animals during the preliminary and washout phase and to the control animals during the experimental period. During the experimental phase the 4g chromic oxide was combined with the magnesium oxide and then mixed into the ration. Blood serum samples were taken on days 0, 1, 3, 7, 14, 16 and 21 and analyzed for Mg, Ca and P. Urine samples were taken once daily on most days from 0 through 21 and analyzed for Mg and creatinine. Fecal grab samples were taken on days 0 through 21 and analyzed for dry matter, Cr and Mg. Magnesium supplementation increased serum magnesium ($P<.01$) on days 3 through 14 with MgO-B higher ($P<.01$) than MgO-A only on day 14. Serum calcium and phosphorus were not affected. Magnesium excretion in urine increased ($P<.01$) for both magnesium oxides from days 2 through 16. Values for MgO-B were higher ($P<.05$) than for MgO-A. Forage had no effect on urinary magnesium excretion ($P>.05$). Magnesium supplementation increased the amount of magnesium in feces ($P<.01$) for days 2 through 16. Fecal magnesium excretion was higher with MgO-B ($P<.01$) than MgO-A on days 3 and 4. Magnesium in feces decreased more slowly during the washout period when MgO-A was fed as shown by a higher magnesium in feces on day 18 than for either MgO-B, or the control. The more reactive magnesium oxide was more available to the animal as indicated by higher serum magnesium and higher excretions of magnesium in urine. Forage source had little effect on magnesium absorption and excretion.

Influence of Phospholipid Supplements on Ruminal Fermentation. T. C. JENKINS, Dept. of Animal Sci., Clemson University, Clemson, SC 29634

Phospholipids from different sources were added to in vitro substrates or to the diet of lambs to determine the effects on volatile fatty acid (VFA) levels and nutrient digestion. Legume hay (475 mg) was incubated alone or in combination with deoiled soybean lecithin (25 mg) in five in vitro trials. In four of the five trials, lecithin increased ($P<.05$) total VFA concentration an average of 10.9%, but had no effect on neutral detergent fiber (NDF) digestibility. However, NDF digestibility was increased ($P<.05$) in the remaining trial that had no change in VFA concentration. Purified phospholipids as phosphatidylethanolamine (PE) and phosphatidylcholine (PC) were added to the legume hay substrate in another five in vitro trials. The only change was that PE reduced ($P<.05$) total VFA concentration in one of the five trials. Fiber digestibility was not changed by PE or PC in any of the second five trials. The effect of phospholipids on in vivo nutrient digestion was determined with twelve Hampshire wethers randomly assigned to three isocaloric, isonitrogenous diets containing either 2.1% corn oil, 5% deoiled soybean lecithin or 5% crude soybean lecithin. The 21-day study was divided into a 14-day preliminary period to adapt lambs to the diets, and a 7-day collection period for total collection of feces. Dry matter digestiblity was not affected by the source of dietary lipid. Energy digestibilities averaged 67.3, 65.8 and 60.6% for the corn oil, deoiled lecithin and crude lecithin diets, respectively. Significant contrasts for energy digestibility were corn oil vs lecithin ($P<.05$), and deoiled lecithin vs crude lecithin ($P<.05$). Nitrogen digestibilities averaged 73.4, 70.3 and 67.0% for the corn oil, deoiled lecithin and crude lecithin diets, respectively, with only the corn oil vs lecithin ($P<.05$) contrast being significant. The concentration of total VFA and the acetate/propionate ratio in rumen fluid samples taken by esophageal tube on day 21 were not different among the diets. These results suggest that deoiled soybean lecithin contains ingredients other than phospholipids that are fermented to VFA. Also, diets containing commercial soybean lecithin have lower digestibilities of nitrogen and energy than diets containing an equivalent amount of fatty acids from corn oil. (Partial support for this work was provided by Eastman Kodak Co., Kingsport, TN and the commercial lecithin supplements were donated by American Lecithin Co., Atlanta, GA).

EFFECT OF SYNCHRONIZATION OF PROTEIN AND STARCH DEGRADATION IN THE RUMEN ON NUTRIENT UTILIZATION AND MILK YIELDS

R. HERRERA, R. GOMEZ-ALARCON and J.T. HUBER

Four studies were conducted to determine the effect of synchronization of protein and starch degradation on nutrient utilization, microbial protein synthesis and milk production in dairy cows. In Experiment 1, five cereal grains and five protein supplements were compared for extent of solubility and degradability of their starch and nitrogen fractions. Results indicated large differences which permitted their ranking from high to low degradability as follows: grains: oats > wheat > barley > corn > milo; protein supplements: soybean meal > cottonseed meal (CSM) > corn gluten meal > brewers dried grains (BDG) > blood meal. In Experiment 1, the five grains were incubated for varying times in vitro (with added amylase) or in situ to determine rate and extent of degradation of dry matter, crude protein and starch. Results showed that rate of starch degradation followed a slightly different trend than in Experiment 1: wheat > barley > oats > corn > milo. Rates for DM and CP degradation were similar to those for starch.

In Experiment 3, high (barley = HS) and low (milo = LS) degradable starch sources were combined with a high (CSM = HP) and a low (BDG = LP) degradable protein source to formulate four diets: HSHP, HSLP, LSHP and LSLP. Diets were fed to 32 cows, starting two to four weeks postpartum, for a 60-d milk production and digestibility study. Apparent digestibility was calculated using chromium oxide. Organic matter digestibility was higher ($P<.05$) for diets containing barley. Milk production was higher for the HSHP diet, but milk fat percent was depressed on the barley diets. Thus, 3.5% FCM did not differ between the HSHP and milo diets.

A fourth study was conducted to determine the effect of the diets used in Experiment 3 on rumen nutrient utilization and microbial protein synthesis. Four duodenally fistulated cows were used in a 4 x 4 Latin square. Chromium oxide estimated digestibility and rumen output of nutrients, and nucleic acids estimated microbial protein synthesis in duodenal contents. Barley diets had higher ($P<.05$) apparent and corrected rumen digestibility of DM, OM, CP and starch than milo diets. No difference ($P>.05$) was found in nutrient output to the small intestine among diets and microbial CP synthesis was higher ($P<.05$) for barley diets.

Lactational responses of early lactation dairy cows fed diets varying in ruminal degradability of carbohydrate and crude protein. D. P. Casper*, D. J. Schingoethe, and W. A. Eisenbeisz. South Dakota State University, Brookings.

Seventy-six high-producing Holstein cows were randomly assigned in a 2 x 2 factorial design to evaluate two sources of carbohydrates (corn, C; and barley, B) which differed in ruminal degradability with two crude protein sources of different degradability (soybean meal, SBM; and 1% urea, U) in the concentrate mix during wk 4 through 14 postpartum. Total mixed diets, formulated to be isonitrogenous at 16% crude protein, contained (dry matter basis) 40% corn silage, 10% alfalfa hay, and 50% of the respective concentrate mix. Solubility of crude protein (41.0 and 43.0% of CP) was higher for B-SBM than for C-SBM while addition of urea increased crude protein solubility (46.0 and 50.0% of CP) for both C-U and B-U diets. Total nonstructural carbohydrates (40.0 and 39.9%) were similar for C-SBM and B-SBM, similar (42.6 and 42.3%) for C-U and B-U diets but higher when compared to C-SBM and B-SBM diets. Production of milk (32.2 and 31.8 kg/d) was similar, while 4% fat-corrected milk (29.1 and 17.4 kg/d), and solids-corrected milk (29.1 and 27.5 kg/d) were highest for cows fed C and lowest for cows fed B. Percentages of fat (3.39 and 3.22%) and solids-not-fat (8.65 and 8.59%) were lower for cows fed B, while percentages of protein (3.09 and 3.08%) were similar. Solubility of crude protein did not affect production of milk (31.9 and 32.0 kg/d), 4% fat-corrected milk (28.5 and 28.0 kg/d), and solids-corrected milk (28.4 and 28.2 kg/d). Percentages of fat (3.32 and 3.29%), protein (3.07 and 3.10%), and total solids (11.94 and 12.01%) were similar. Milk fat from cows fed B contained lower amounts of short chain fatty acids (C-4:0 to C-12:0) (16.3 and 15.2 g/100 g) and long chain fatty acids (C-18:0 to C-18:3) (29.9 and 28.2 g/100 g) and higher amounts of medium chain fatty acids (C-14:0 to C-16:1) (50.9 and 53.8 g/100 g) than cows fed C. Solubility of protein did not affect fatty acid distribution in milk fat. Dry matter intakes were lower for B than C diets (20.7 and 19.2 kg/d) but similar for SBM and U diets (20.1 and 19.8 kg/d). Ruminal concentrations of acetate (58.3 and 57.3 molar %) were similar for cows fed C and B, while propionate (26.4 and 28.9 molar %) was higher, and butyrate (11.4 and 9.9 molar %) and ruminal ammonia (17.2 and 12.8 mg/dl) were lower for cows fed B. Concentrations of acetate (57.9 and 57.8 molar %), propionate (27.1 and 28.2 molar %) were similar, while butyrate (11.0 and 10.2 molar %) was decreased and ruminal ammonia (12.9 and 17.0 mg/dl) was increased for cows fed U. In this experiment, utilization of a more soluble crude protein source (U) was not improved by providing a more rapidly degradable carbohydrate source (B) in the diet.

Influence of asynchronous energy and nitrogen supply on activity of rumen microorganisms: results of in vitro batch culture studies. J. R. NEWBOLD and S. R. RUST, Department of Animal Sciences, Michigan State University, East Lansing, MI 48824

Short-term, transient asynchrony between N and energy supply to rumen bacteria may result in sub-maximal microbial growth yields. The importance of such asynchrony was assessed in vitro. Treatment substrates were incubated at 37 C with rumen fluid (from corn-fed steers) and a buffer/mineral mixture. To avoid confounding effects of differences in total N and energy supply, microbial growth supported by different treatments was compared when equal quantities of substrate had been degraded. In Experiment 1, glucose and urea solutions were added at 30-min intervals for 12 h. Total gas production (an index of microbial activity) was greater when the amounts of glucose and urea added were synchronized. In Experiment 2, a readily available energy source (corn starch) was incubated with soybean meal or corn gluten meal. The size of the microbial population was calculated from the rate of gas production following addition of an excess of energy (glucose) and N (urea). According to previous in situ degradability measurements, total substrate supply was equal across treatments after 9 h incubation. At this time, microbial growth was greatest for the more readily available N source (soybean meal). In Experiments 3 and 4, optical density was used as an index of microbial growth and substrate availability was estimated from in vitro degradability. At points of equal substrate supply, microbial growth supported by corn starch increased as the rate of protein degradation increased (microbial growth: corn glutenmeal<soybean meal<peptone). With a less rapidly available energy source (corn particles between 1 mm and 2 mm diameter), microbial growth was slightly greater with soybean meal than with peptone. It was concluded that asynchrony is an important determinant of microbial growth efficiency. Rates of degradation of both carbohydrate and protein should be measured and predicted and incorporated into ration formulation system. Further replicated experiments are required to confirm these findings.

Evaluation of diaminopimelic acid and purines as markers for estimating ruminal microbial activity. D. J. Illg, M. D. Stern, P. M. Windschitl, D. M. Waltz, F. J. Bas, and H. M. Metwally, Department of Animal Science, University of Minnesota, St. Paul, 55108.

Concentrations of markers and estimates of ruminal microbial activity determined by diaminopimelic acid (DAPA) and purines from 13 experiments (10 in vitro and 3 in vivo) were evaluated using regression and means analyses. Correlation coefficients (r) between DAPA and purine concentrations (mg/g N) of bacteria and digesta were .87 and .92 ($P<.01$), respectively. Coefficients of variation (CV) for marker concentrations were less for purines than DAPA in bacteria (32.2 vs. 43.6%) and digesta (37.6 vs. 48.4%). The r values for crude protein degradation (%), true organic matter digestion (%) and efficiency of bacterial synthesis (g N/kg organic matter truly digested) determined by the markers were .60, .80 and .84 ($P<.01$), respectively. Lower ($P<.01$) estimates of crude protein degradation (59.0 vs. 69.3), true organic matter digestion (53.9 vs. 58.4) and efficiency of bacterial synthesis (32.4 vs. 34.8) were determined using purines. Purine and DAPA CV values were similar for crude protein degradation (27.4) vs. 25.8%), true organic digestion (18.7 vs. 17.4%) and efficiency of bacterial synthesis 24.5 vs. 24.3%). In general, purines produced lower estimates of ruminal microbial activity than DAPA. However, variability of these estimates was similar for the markers.

Ruminal degradation and intestinal availability in steers of amino acids from soybean meal, corn gluten meal, blood meal and fish meal. Evan C. Titgemeyer, Neal R. Merchen and Larry L. Berger, University of Illinois, Urbana 61801.

The value of soybean meal (SBM), corn gluten meal (CGM), blood meal (BM) and fish meal (FM) in supplying amino acids (AA) to the small intestine (SI) of cattle was studied using 6 steers (336 kg) with ruminal, duodenal and ileal cannulae. Diets (49% corn silage) contained 20% corn starch or starch replaced in part with SBM or CGM at levels supplying 3, 6 or 9% crude protein (CP) or with BM or FM at levels supplying 2, 4 or 6% CP. The trial contained 8 periods (46 observations, 2 missing cells). The basal diet (no supplemental N [SN]) was fed 8 times. Intermediate levels of each source were fed 3 times and highest levels 4 times. Intake was 8.06 kg DM/d. Markers used were Cr_2O_3 (digesta flow) and purines (bacteria). Dependent variables were regressed against SN (within source) such that 4 regression lines with a common intercept were determined.

Values for SBM, CGM, BM and FM in supplying AA to the duodenum (g AA-N/g SN) were .145, .716, .797 and .500. The amounts of AA absorbed from the SI from SBM, CGM, BM and FM (g AA-N/g SN) were .082, .569, .572 and .359.

Amino acids from each of the four supplemental protein sources which reached the SI were compared to amounts fed such that escape values were calculated for individual AA. Escape values for total AA-N for SBM, CGM, BM and FM were .19, .79, .84, and .60. For all four protein sources, valine, isoleucine and threonine were degraded less extensively than the average of the total AA pool. Leucine, methionine, cysteine, histidine and arginine were ruminally degraded to a greater extent than average for all four protein sources. Aromatic AA (phenylalanine and tyrosine) were more resistant to degradation than average for SBM and FM, but were less resistant to ruminal degradation than average for CGM and BM. Lysine in CGM and SBM was less degradable than average, while BM- and FM-lysine were degraded to an extent near the average for AA-N in those protein sources.

Small intestinal digestibility of AA-N that escaped ruminal degradation was greatest for CGM (79%), intermediate for BM and FM (72%) and lowest for SBM (57%). The SI digestibilities of escaped methionine, valine, isoleucine, leucine and phenylalanine were similar to the average for total AA-N. Escaped cysteine, histidine and threonine were less extensively absorbed than average and escaped arginine was absorbed more extensively than average within each protein source. Escaped lysine followed the pattern for total AA-N for SBM, BM and FM, but was less available from CGM.

Effect of intragastric infusion of methionine, cysteine and/or choline on nitrogen excretion and blood parameters in enterally fed sheep.

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This study was conducted to examine the effects of methionine, cysteine and/or choline on nitrogen excretion and blood metabolites in sheep maintained on total enteral nutrition. Four sheep (35 kg) were arranged in a 4x4 Latin square design in which a purified amino acid mixture devoid of methionine and cysteine, and a vitamin solution devoid of choline were abomasally infused to meet maintenance requirements. Diets included the purified amino acid and vitamin mixture plus: 1) methionine and cysteine (M+C), 2) methionine (M), 3) cysteine and choline (C+Ch), 4) cysteine (C) or 5) a negative control devoid of methionine, cysteine and choline. Methionine, cysteine and choline were all added at levels to meet their respective maintenance requirements. All diets were made isonitrogenous by the addition of glycine. VFA and glucose were infused to provide 104 kcal/MBW. Diets were administered for 3 d periods. Daily urine samples were analyzed for total nitrogen (UN), urea (UUN) and creatinine (CR). Blood samples taken on the last day of each period were analyzed for glucose (PG), free amino-N (AA) and urea (PUN). Inclusion of methionine to diets (M and M+C) resulted in lower ($P < .10$) UN, UUN and AA, and higher CR levels. Methionine-containing diets tended to have lower ($P < .10$) PG values (84.15 vs 105.9 mg/dl). Consequently, methionine addition alone (M) significantly reduced ($P < .10$) UN and UUN. No significant differences ($P > .10$), with the exception of UUN, were observed between M and M+C diets. Comparison of C and C+Ch diets indicated no significant differences ($P > .10$). In summary, three possibilities exist with respect to cysteine requirements at maintenance: 1) that methionine spares the cysteine requirement thru the transsulfuration pathway and/or 2) the metabolic requirement for cysteine is negligible or 3) that there exists a de novo pathway of cysteine synthesis independent of the transsulfuration pathway. Methionine inclusion increased nitrogen balance and apparently increases glucose uptake. Choline addition does not appear to be essential for nitrogen homeostasis at maintenance.

Key Words: methionine, cysteine, choline, sheep, intragastric infusion, nitrogen balance.

RAFTING THROUGH THE RUMINANT INTESTINE. Hector Anzola, Fred Owens and Juan Garza. Oklahoma State University, Stillwater 74078.

Disappearance of feedstuff components in the intestinal tract of cattle was examined in three experiments using 2.5 by 3.5 cm mobile dacron bags (MDB) with 50-70 micron pores. In trial 1, diets containing either 50 or 80% concentrate were fed to four cannulated heifers in a crossover trial and MDB disappearance of these two diets, alfalfa meal and cottonseed meal was measured. Effects of diet on ruminal (15 h) and postruminal (pepsin-HCl for 3 h followed by insertion into duodenal cannula and recovery on defecation) disappearance were estimated. To check postruminal disappearance independent of ruminal effects, bags were transposed at the duodenum among animals fed the two different diets. In the rumen, disappearance of dry matter was greater (33.0 vs 30.2; $P<.05$) with the 50% concentrate diet. In the intestines, disappearance of dry matter was less (55.0 vs 59.2; $P<.05$) while disappearance of protein protein was greater (33.8 vs 26.2; $P<.05$) with the 50% concentrate diet. Results indicate that diet can alter both ruminal and post-ruminal disappearance from MDB. MDB disappearance underestimated in vivo digestibility of DM and starch by 5 to 15%. No diet by feed interactions were detected. Presumably, differences in postruminal disappearance are attributable to varying fermentation activities in the large intestine. In trial 2, the impacts of grain source (oats, wheat, barley, corn, milo) and grain processing (whole, rolled, flaked) on disappearance of DM, protein and starch from MDB from the rumen and the small intestine were measured using calves equipped with ileo-rectal anastomoses to bypass fermentation in the large intestine. Grain processing effects were poorly evaluated with MDB, especially for unprocessed grains for which total tract disappearance values were under 8%. Particle size differences presumably were responsible. Hence, in trial 3, calves equipped with ileo-rectal anastomoses were used to determine the influence of particle size of dry rolled corn on the extent of disappearance of DM, crude protein and starch from MDB in the rumen and the small intestine either with or without previous incubation in the rumen. Extent of DM, protein and starch digestion both in the rumen and in the small intestine increased as particle size decreased. This depression occurred first in the rumen (i.e. ruminal escape was depressed prior to depression of postruminal digestion). To maximize ruminal escape of intestinally disappearing starch and protein, the optimal corn particle sizes were 125 to 250 microns and 250 to 1000 microns, respectively. To increase supply of digestible starch and protein to the small intestine by altering particle size, some sacrifice in total tract disappearance was necessary. Extent of postruminal disappearance of DM quadratically increased with residence time of bags in the postruminal tract (10 to 60 h). In summary, disappearance from MDB is easily, rapidly and repeatably measured provided that the animal's diet and particle size and residence time of the feedstuff are controlled. The MDB can be useful to rank order feeds or protein sources with similar particle sizes and gastro-intestinal retention times. It should prove useful as a tool to determine ruminal protein escape from low protein feedstuffs and small intestinal digestion of protein which escapes digestion in the rumen. Yet, precision in predicting site and extent of digestion with the MDB is limited by ones inability to simulate 1) the particle sizes which the animal and microbes derive from ingested feedstuffs by mastication and dentrition and 2) the specific ruminal and intestinal residence times of these derived particles.

INFLUENCE OF LEVEL AND SOURCE OF DIETARY FAT ON PERFORMANCE AND DIGESTIVE FUNCTION IN FEEDLOT STEERS

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Two trials were conducted to evaluate the influence of level and source of supplemental fat on feeding value of fat for feedlot cattle. Six dietary treatments were compared: 1) steam rolled barley based finishing diet containing to supplemental fat; 2) basal diet plus 4% yellow grease; 3) basal diet plus 4% vegetable fat blend; 4) basal diet plus 8% yellow grease; 5) basal diet plus 8% vegetable fat blend and 6) basal diet plus 6% vegetable fat blend and 2% soybean lecithin. Treatment effects on animal performance and estimated net energy value of the diet were evaluated in a 125-d comparative slaughter trial involving 228 crossbred steers (304 kg). Increasing level of supplemental fat in the diet resulted in linear improvements (P<.01) in weight gain, feed conversion and net energy value of the diet. Fat supplementation resulted in increased (P<.05) carcass fat and marbling score. However, differences in carcass merit may be largely attributable to differences in rate of weight gain. Estimated net energy values of supplemental fat did not appear to be influenced by level of supplementation. The estimated net energy value of yellow grease was numerically greater then that of blended vegetable fat, although these differences were not statistically significant. Inclusion of 25% soybean lecithin did not increase the net energy value of a blended vegetable fat. The estimated net energy value for supplemented fats used in this study averaged 5.781 and 4.61 Mcal/kg, for maintenance and gain, respectively. These estimates are in good agreement with values obtained in 2 previous trials which averaged 6.40 and 4.61 Mcal/kg, respectively, for maintenance and gain, and add further support to the contention that current tabular values (NRC, 1984) underestimate, considerably, the feeding value of supplemental fats. The influence of fat level and source on characteristics of digestion were evaluated in a 6 X 6 Latin square design experiment involving 6 crossbred steers (202 kg) with cannulas in the proximal rumen, proximal duodenum and distal ileam. Increasing level of fat supplementation resulted in linear decreases (P<.01) in ruminal and total tract digestion of organic matter and acid detergent fiber. Intestinal digestion of fat also decreased linearly with increasing level of supplementation (P<.05). Principle differences between yellow grease and blended vegetable fat revolved around their affects on ruminal fiber digestion (P<.01). The negative affects of vegetable fat on ruminal fiber digestion compared to that of yellow grease was largely overcome by the addition of lecithin. The practical significance of this finding could be very important, particularly in higher forage diets where slower rates of fiber digestion might hinder ruminal emptying and thus restrict feed intake.

Effect of Ruminal Protein Degradation of Blood Meal and Feather Meal on Protein Supply to the Small Intestine. D. M. Waltz, M. D. Stern and D. J. Illg, Dept. of Animal Sci., University of Minnesota, St. Paul.

Four lactating Holstein cows fitted with ruminal, duodenal and ileal cannulae were utilized in a 4 x 4 Latin square to examine the effect of blood meal and feather meal on ruminal protein degradation and amino acid supply to the small intestine. Cows were fed four diets consisting of 50% grain mix, 40% corn silage and 10% alfalfa pellets four times daily. Fifty percent of the crude protein in the diets came from one of four test protein sources: Soybean meal (SBM), Blood meal (BM), Feather meal (FM), and a 50-50 combination of blood meal and feather meal (B&F). Experimental periods consisted of 10 days adaptation followed by 4 days of digesta sampling. Digesta samples were collected three times a day and the twelve subsamples were composited for each cow in each period. Chromic oxide was used as a digesta marker and was fed at a rate of 10 grams/day for at least 5 days prior to and during each sampling period. Purines were used as the microbial marker to estimate bacterial nitrogen flow. Some of the results of this experiment are shown in the following table:

Item	Protein source			
	SBM	BM	FM	B&F
Rumen ammonia N, mg/100 ml	32.2^{b}	16.1^{a}	18.1^{a}	17.8^{a}
Crude protein degradation, %	53.2^{b}	43.1^{ab}	35.0^{a}	36.7^{a}
Bacterial synthesis, g N/kg OMTD	33.2^{b}	23.7^{a}	23.4^{a}	23.3^{a}
Apparent CP digestion in GIT, %	78.5^{b}	73.9^{b}	63.4^{a}	66.3^{a}
Nonammonia N absorption in SI, g/d	286	264	285	304
Total AA absorption in SI, g/d	1588^{a}	1647^{ab}	1727^{ab}	1874^{b}

^{a,b}Means in the same row with different superscripts differ (P<.05).

There were no differences (P<.05) among treatments in ruminal organic matter and carbohydrate digestion. Total ruminal volatile fatty acid concentration was highest (P<.05) for cows fed the SBM diet compared to those fed the BM, FM and B&F (118.7 vs 95.2, 90.8 and 100.1 mM, respectively). Molar percent of acetate was lower (P<.05) and propionate higher (P<.05) for the SBM diet.

Ruminal crude protein degradation was lower (P<.05) for the FM and B&F diets compared to the SBM diet. Cows fed these two diets along with those fed the BM diet had lower (P<.05) rumen ammonia concentrations and efficiency of bacterial synthesis than cows fed the SBM diet. Apparent total tract protein digestion was also decreased (P<.05) when cows were fed the FM and B&F diets. Although there were no differences (P<.05) among treatments in nonammonia N absorption in the small intestine, total amino acid (AA) absorption was higher (P<.05) for the B&F diet compared to the SBM diet. Absorption of lysine and histidine was higher (P<.05) for cows consuming the BM diet than the FM diet (134 vs 97 g/d and 63 vs 33 g/d), while isoleucine was lower (P<.05) for the BM diet (56 vs 106 g/d).

In summary, FM and B&F diets had lower estimates of ruminal crude protein degradation than the SBM diet and total amino acid absorption in the small intestine was higher for cows fed the B&F diet than the SBM diet.

Effect of Dietary Protein Level on Diffusion of Blood Urea Nitrogen into the Rumen. L. D. Bunting, J. A. Boling, C. T. MacKown and G. M. Davenport, Dept. of Animal Sciences, University of Kentucky, Lexington, KY 40546

Eight ruminally cannulated heifer calves (234 kg) were assigned to either high (HP; 126.1 g N/d) or low (LP; 66.5 g N/d) protein intake and used to determine the effects of level of dietary protein intake on movement of blood urea nitrogen (BUN)-derived ammonia from the rumen wall into the rumen digesta mass. Each heifer received 4800 g DM of diets containing 70% corn-soybean meal and 30% cottonseed hulls in equal portions 6 times daily. A 7-d training and dietary adaptation period preceded the 10-d experiment. On d 1 of the experiment, heifers on LP and HP received 1.03 and 2.06 g N, respectively, as ^{15}N-urea (68% enriched) as a single injection through a jugular catheter. Blood was collected serially through 72 h post-injection, while ruminal fluid was serially collected through 4 h post-injection from digesta located near the rumen wall (wall-proximate digesta; WPD) and from the center of the rumen digesta mass after manual agitation (center of mixed digesta; CMD). On d 6, heifers on LP and HP also received 1.49 and 2.89 g N, respectively, as $(^{15}NH_4)_2SO_4$ (71.6% enriched) as a single intraruminal injection. Ruminal fluid (CMD) was collected serially through 72 h post-injection. Pool size and production rate for the RAN pool and rate of ruminal BUN influx were determined from the N enrichment data of ruminal fluid obtained from CMD.

Kinetic calculations based on RAN collected from CMD after intraruminal $(^{15}NH_4)_2SO_4$ injection indicated that heifers fed HP had greater (P<.05) RAN pool size (4.7 vs 1.1 g N) and production rate (46.5 vs 17.4 g N/d) than LP. In addition, calculations based on RAN collected from CMD after intravenous ^{15}N-urea injection indicated that 13.9 and 9.7 g BUN/d entered the RAN pools of LP and HP, respectively. Mean RAN concentrations for the 4-h period after ^{15}N-urea injection were higher (P<.05) for HP than LP (180 vs 42 mg N/liter). Site of ruminal fluid collection did not affect (P>.05) RAN concentration. Ruminal urease activities tended (P>.05) to be higher in ruminal fluid collected from WPD than CMD (.260 vs .194 umol urea degraded/min/ml). In addition, mean ruminal urease activities for the 4-h period were lower (P<.05) for HP than LP (.215 vs .305 umol urea degraded/min/ml). Areas under the N enrichment curve (AUC) through 4 h for RAN and bacterial N were similar (P>.05) for WPD and CMD within each protein level. However, the AUC for WPD-derived RAN as a percentage of the AUC for CMD-derived RAN (WPD/CMD x 100) was greater (P<.05) for LP than HP (125.4 vs 107.4%). Similarly, the AUC for WPD-derived bacterial N as a percentage of the AUC for CMD-derived bacterial N tended (P>.05) to be greater for LP than HP (113.7 vs 90.4%). These data suggested that in heifers fed HP, BUN-derived ^{15}N-ammonia entering through the rumen wall was essentially able to equilibrate with the primary RAN pool (CMD). In contrast, in heifers fed LP there appeared to be an enrichment gradient, declining from the rumen wall (WPD) toward the center of the ruminal digesta mass (CMD). Additionally, AUC for bacterial N was 14% greater for WPD than CMD in LP heifers suggesting that a proportion of the ^{15}N-ammonia entering through the rumen wall may have been preferentially utilized by bacteria proximal to the wall.

Ureagenesis and site and rate of urea removal from blood of beef steers fed alfalfa hay or a high energy diet. Gerald B. Huntington, U. S. Department of Agriculture, Agricultural Research Service, Beltsville, MD 20705

Five Hereford X Angus steers 240±6 kg live weight with chronic indwelling catheters in various sites in the splanchnic bed were used to evaluate effects of simultaneous change in dietary energy source and nitrogen (N) level on ureagenesis and site and rate of urea removal from blood. Alfalfa hay (181 g N/d) or a high energy (77% corn) diet (106 g N/d) was fed at similar metabolizable energy intake (13.1 Mcal/d). Daily rations were fed in 12 equal meals every 2 h. Blood was sampled 9 times on each of 2 d for each steer and diet. Urine was collected daily. Urea flux (venoarterial concentration difference multiplied by blood flow) was measured across total splanchnic (TS), liver (L), portal-drained visceral (PDV) and post-stomach sections of PDV tissues. Stomach urea transfer (across the gut wall) was calculated as PDV minus post-stomach flux. Salivary urea transfer was calculated as the difference between TS release and urinary excretion. Rumen urea transfer was the sum of stomach and salivary transfer. Total gut urea transfer was the sum of PDV and salivary transfer. Three steers were measured on both diets and 2 steers on one diet each. Post-stomach data are not available for one steer. Blood flow (dilution of para-aminohippurate) through L (747 L/h) and PDV (599 L/h) was not affected (P>.10) by diet. Similarly, diet did not affect (P>.10) percentage of L blood flow through PDV (81%) or percentage of PDV blood flow though post-stomach tissues (38%). Compared with alfalfa, the high energy diet decreased (P<.05) blood concentrations of urea N from 10.5 to 4.6 mM, L ureagenesis, urinary urea N excretion, transfer of urea N to post-stomach tissues, and salivary transfer of urea N (table). A greater (P<.05) proportion of liver ureagenesis was transferred to the gut when the high energy diet was fed. Diet did not affect (P>.10) rate of urea N transfer to PDV or transfer to the rumen (table); however, percentages of L ureagenesis transferred into the gut shifted among sites in response to diet. With alfalfa, 33% of gut transfer was into post-stomach tissues and 47% was into saliva; with the high energy diet 81% of gut transfer was into the stomach fraction (across the gut wall). Increased intake of readily fermentable carbohydrate at the expense of N and fiber decreased ureagenesis, increased the portion of L ureagenesis recycled to the gut and focused that recycling into the stomach (rumen) in these steers.

Urea N, mmol/h	alfalfa	high energy	SE	P<
TS release	288	83	20	.01
L ureagenesis	366	159	25	.01
PDV transfer	79	76	13	NS
Post-stomach transfer	55	5	16	.05
Stomach transfer	26	72	25	NS
Salivary transfer	70	19	17	.05
Rumen transfer	102	82	21	NS
Total gut transfer	149	95	22	.05
Urinary excretion	218	64	17	.01

Effect of the rate of salivary secretion on the nitrogen content of parotid saliva in sheep. C.A.GOMEZ[1],L.W. GROVUM[2] and L.P. MILLIGAN[1],Dept. of Animal and Poultry Sci.[1] and Biomedical Sci.[2] University of Guelph,Guelph,ON Canada N1G2W1

In ruminants the secretion of parotid saliva is continuous , but there are large variations in the rate of production. Little information is available, however, regarding the extent to which variation in secretion rate of parotid saliva affects its nitrogen composition and content.

Four wethers were anesthetized and a parotid duct cannulated.In the first period of study (PI) saliva samples were taken at different secretion rates which were induced with an inflatable balloon in the esophagus. Plasma urea nitrogen (PUN) was then (PII) immediately raised using a single intravenous injection of urea and the sampling procedure repeated as before. Saliva N content adjusted to an average plasma urea nitrogen level (PI,18.6 ; PII,25.6 mg N/100ml) was inversely correlated (PI: - 0.87 ; PII:-0.71) with salivary flow rate which ranged from PI:0.2-6.2 and PII:0.2-8.0 ml/minute. The regression equations PI:(Y=16.3 - 4.7log(10)X) and PII(Y=22.5 - 5.1log(10)X) express the relationship between flow rate (X;ml/minute) and concentration of nitrogen(Y;mg N/100ml). Urea Nitrogen was 82.6 (PI) and 83.1 (PII) % of the total saliva nitrogen without significant changes in its proportion with different secretion rates.

In a second experiment, the same four wethers were maintained on a hay diet(1.18 % N on a DM basis) given once daily (1200 g DM). The sheep were cannulated cronically in a parotid duct and saliva samples and measurements of secretion rate were taken before (BE) and during the first 30 minutes of eating (E). PUN was 12.7+ 1.3 mg N /100ml during the time of collections and the observed salivary flow rates were BE:0.6 $\pm$ 0.3 and E:2.6 $\pm$ 0.7 ml/minute. There was a decrease in salivary nitrogen content with increased secretion rates, the values being 11.7 $\pm$ 1.3 and 10.6 $\pm$ 1.6 mgN/100 ml before and during eating respectively. Urea accounted for 81.9 (BE) and 71.5(E) % whereas protein N increased from 7.4 (BE) to 24.6 (E) % of the total salivary N.

It is concluded that the rate of salivary secretion did affect the nitrogen content of parotid saliva and as a consequence the provision of N to the rumen is not a direct function of volume of saliva secreted.

AGRONOMIC PANEL

Inoculation of cattle grazing *Leucaena leucocephala* in central Florida with 3,4-DHP degrading ruminal bacteria. A. C. Hammond, M. J. Allison, M. J. Williams, D. B. Bates, G. M. Prine and E. L. Adams, USDA, ARS, Brooksville, FL 34605 and Ames, IA 50010, and University of Florida, Inst. of Food and Agric. Sci., Gainesville, FL 32611

Leucaena leucocephala (leucaena) is a tropically adapted arboreal legume with potential as a forage for the seasonally droughty areas of subtropical Florida but its usefulness may be limited due to its potential toxicity. Leucaena contains a nonprotein amino acid, mimosine, that is converted in the rumen to 3-hydroxy-4(1H)-pyridone (3,4-DHP), a potent goitrogen, although clinical signs of toxicity in ruminants are not observed in all areas of the world. Australian workers have been successful in transferring mixed cultures of DHP degrading ruminal bacteria from Hawaii to Australia and eliminating toxicity associated with grazing leucaena (1). In experiments at Brooksville we found some cattle with the ability to degrade 2,3-DHP, but none with the ability to degrade 3,4-DHP (2). The objectives of the present experiment were to inoculate cattle at Brooksville with 3,4-DHP degrading bacteria and to determine the tolerance of these cattle to grazing leucaena. Four ruminally cannulated Hereford heifers were fed .45 kg fresh chopped leucaena (2.2-3.5% mimosine, DM basis) and 4.5 kg perennial peanut hay daily for 2 wk prior to inoculation with 25 ml of a suspended culture of bacterial strain 78-1 (3) then allowed to graze a 1 ha plot of leucaena (K-8). The inoculum was derived from a 12 liter batch culture harvested after 44 hr of incubation and resuspended in 100 ml of culture media. Two of the inoculated heifers were subsequently deleted from the experiment for reasons unrelated to treatment. Two ruminally cannulated heifers not fed leucaena initially and not inoculated grazed an adjacent 1 ha plot of leucaena and served as controls. At 0, 2, 4 and 6 wk after inoculation, ruminal fluid samples were obtained to test for in vitro 3,4-DHP degradation and blood samples were obtained for analysis of plasma thyroxine (T_4) and triiodothyronine (T_3). One control heifer developed clinical signs of leucaena toxicity (weight loss, alopecia) by wk 5 and was removed from the grazing trial. T_3 and T_4 values were within normal ranges for all heifers but were lowest in the heifer showing signs of toxicity, .23 and 40.3 ng/ml, respectively, averaged over the grazing period. In vitro results indicate that 3,4-DHP degrading ability was not present in any of the heifers at wk 0, was present in at least one of the inoculated heifers at wk 2, and was present in all heifers by wk 6. These data indicate that 3,4-DHP degrading ruminal bacteria were successfully transferred to inoculated heifers and over time were passively transferred from inoculated heifers to control heifers.

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Variation in steer preference for Panicum accessions. J. C. BURNS, D. S. FISHER, and W. H. MORRISON, III, USDA-ARS and Dept. of Crop Science, North Carolina State University, Raleigh, NC, 27695, and USDA-ARS, R. B. Russell Agricultural Research Center, Athens, GA, 30613

In previous preference trials, steers were allowed to graze Panicum accessions in two experiments (1). Twenty-four accessions were evaluated and represented three Panicum taxa. Eight accessions were designated as grazing types and a second eight as intermediate types. These 16 accessions were grouped by type, but all 16 evaluated in each block. In a second experiment, an additional 8 hay types were evaluated. Evaluation occurred three to four times in each of two years and were conducted when forage reached a height of about 60 cm. From a combined analyses of all evaluations (both years) it was evident that certain accessions were either consistently preferred or rejected. One preferred and one nonpreferred accession from within each of the three taxa was selected for further evaluation in stalls. Stall feeding would remove plant canopy structure as a factor that could alter preference.

Special mangers were fitted to a PVC intake manifold to bring in fresh air from outside. Four mangers, each with variable speed fans were attached to the manifold. About 2.5 kg of fresh cut (75 to 150 mm lengths) forage was placed into galvanized tubs with perforated bottoms. The fans were set to force air up through the forage mass. Plywood panels required steers (340 kg) to withdraw their heads before changing mangers (treatments). Trials were video taped for later scoring and terminated before any treatment was entirely consumed (20 to 30 min.). Fresh forage intake, time eating/treatment, and rate of eating were determined. Accession #3 and #8, from the Panicum amarulum taxa, showed wide preference differences in the grazing trial having preference scores of 3.9 and 6.2, respectively (1 = not defoliated and 10 = grazed to the stubble). Stall trials showed a similar preference rank in two October evaluations ($p \leq 0.05$). One year a much stronger preference for #8 was exhibited than in the other. Evaluation from an August harvest showed no difference. Accessions #24 and #23, representing extreme preferences within the Panicum virgatum taxa were also evaluated. Accession #24 gave the highest preference score (8.1) in the grazing trial while accession #23 was intermediate (5.2). No difference ($p > 0.05$) was found between these in fresh forage intake. Animals spent more time ($p \leq 0.01$) examining accession #23 than #24. It appears that volatile constituents can alter steer feeding behavior. Work is continuing to better understand the role of volatile constituents in animal intake.

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Efficacy of Treating Alfalfa Hay with Chemical Drying Agents. S. O. Oellermann, M. J. Arambel, and J. L. Walters, Department of Animal, Dairy and Veterinary Sciences, Utah State University, Logan, UT 84322-4815.

Third cutting alfalfa hay cut at bud stage was treated with a drying agent (potassium carbonate, sodium carbonate, and citric acid) applied at the rate of 280.6 l/ha (1 kg/37.471 water) to 4.27 m wide swaths alternating in the field with 4.27 m wide swaths of untreated hay. Hay samples were taken from the swath at time of cutting and at 4 h intervals during daylight until baling was initiated. Samples were analyzed for dry matter, crude protein, and acid detergent fiber. Hay samples were analyzed for in vitro digestibility. Twenty lactating Holstein cows were allocated at random to untreated and treated alfalfa hay treatments in a switchback design. Individual feed intakes and milk yields were recorded daily with milk compositions being analyzed once weekly. Feed samples were collected weekly, composited every 4 wk and analyzed. Results obtained were drying rates of: 0.40 and 0.48% moisture/hr for control and hay dry treatments, respectively. There was no significant difference between control and hay dry treatments with respect to IVDMD parameters analyzed. Cows fed the hay dry treatment had a significantly higher mean daily milk yield, adjusted for feed intake when compared to control fed animals (33.0 vs. 32.5 kg/day for treatment and control cows, respectively). Milk composition parameters did not differ statistically.

Solid-state carbon-13 NMR, FTIR, and NIRR spectroscopic studies of ruminant silage digestion. F. E. Barton, II, D. S. Himmelsbach, H. Boer, and D. E. Akin, USDA-ARS-Richard B. Russell Agricultural Research Center, Athens, GA, U.S.A. (F.E.B., D.S.H. and D.E.A.) and Department of Animal Nutrition-Agricultural University, Wageningen, The Netherlands (H.B.).

Samples of two silages (21% and 28% crude protein) were digested in sacco in cattle for 0, 12, 24, 48 and 336 hours. The resulting residues were analyzed gravimetrically for: acid detergent fiber (ADF), neutral detergent fiber (NDF); nitrogen, acid detergent fiber (ADIN), lignin and ash. The spectra of intact residues and the cell wall fractions of ADF and NDF were obtained by solid-state carbon-13 nuclear magnetic resonance (NMR), Fourier transform infrared (FTIR), and near-infrared reflectance (NIRR) spectroscopies for compositional analysis.

All three regions of the spectra gave indications of increased amounts of phenolics and lipids along with decreased amounts of carbohydrates and proteins as digestion progressed. The spectroscpic results along with light microscopy confirmed the anti-quality role of both lignified and cuticular material in the ruminant digestion of forage based silages. These studies are an example of the synergism that can result by the use of multiple spectral regions for studying complex analytical problems.

PHYSIO-PATHOLOGY PANEL

Induction and Inhibition of 3-Methylindole Formation in Ruminal Lactobacillus.
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A ruminal Lactobacillus sp produces 3-methylindole (3MI) from indoleacetic acid (IAA) resulting in naturally-occurring acute pulmonary edema and emphysema in ruminants after abrupt pasture change. The purpose of this study was to report the effect of IAA on the growth of Lactobacillus 11201, determine the effect of adding 3MI to the media and evaluate whether the enzyme is constitutive or inducible. The bacteria were anaerobically cultured in glucose, protose peptone and yeast extract. Harvested cells were treated with a variety of detergents, enzymes and physical treatments in an attempt to develop a cell-free assay. Radioactive 3MI formed from [2-^{14}C]-IAA was quantitated. For the induction studies, bacteria were grown in basal media, transferred to fresh media plus IAA (0-.1%) and samples taken over time (1-24 h). In addition to IAA, 16 other indolic and phenolic compounds were evaluated as possible induction agents of the 3MI forming enzyme. Growing the bacteria without IAA resulted in a normal growth curve. Lactobacillus growth was reduced with .02% IAA but at .1% IAA, little microbial protein was formed. Furthermore, addition of 3MI to the cultures reduced protein accretion and inhibited 3MI formation from IAA. Enzyme activity remained with the particulate fraction following all chemical or physical treatments. The presence of most detergents reduced or eliminated enzyme activity. Sonication was also an ineffective method to disrupt this bacteria. Repeated passes through the French Press resulted in supernate DNA (50% of total DNA) plateauing after 3 passes. However, enzyme activity was lost with each pass. No activity was detected when cells were grown in basal media. Peak activity (60 nmoles/mg/min) was detected when cells were grown in .01-.05% IAA for 3 h. In addition, 5-hydroxyindoleacetic acid, indole pyruvate and indole propionate induced synthesis of the 3MI forming enzyme(s) while p-hydroxyphenylacetic acid and 12 other agents did not. In conclusion, 3MI is toxic to this Lactobacillus sp. Attempts to produce a cell-free system thus far have been unsuccessful, and the enzymes that produce 3MI are present only after exposure to an appropriate substrate.

Effects of Dopamine and Alpha-2 Adrenoceptor antagonists on forestomach hypomotility induced in conscious sheep by duodenal acidification.

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Infusions of exogenous acid into the proximal duodenum of conscious sheep induce inhibition of forestomach motility by either a neural and/or hormonal mechanism. Since inhibition of forestomach motility can be induced by the activation of dopamine receptors and/or alpha-2 adrenoceptors, this study utilized metoclopramide, a dopamine receptor antagonist, and tolazoline, an alpha-2 adrenoceptor antagonist, to ascertain whether inhibition of reticular contractions by duodenal acidification involved dopamine receptors and/or alpha-2 adrenoceptors.

Three Suffolk ewes (35-40 kg) were anesthetized and silicon tubing cathethers were implanted in the duodenal bulb for infusions of exogenous HCl. Nichrome wire recording electrodes were imbedded in the muscle layers of the reticulum to record the myo-electrical discharges generated by reticular contractions and force displacement strain gauges were sutured to the serosal surface of the reticulum to record the force of these contractions. The free ends of the nichrome wire recording electrodes and the lead wires from the strain gauges exited through the right flank and were connected to minature electrical connectors which were connected to the input amplifiers of a 4 channel Grass polygraph to obtain tracings of the myoelectrical and strain gauge recordings. After a post-surgical recovery period of 3 weeks, the sheep were individually housed in holding cages with access to hay, water and a salt block at all times. During an experiment the myo-electrical discharges from the reticulum were converted into transistor-transistor logic pulses and fed into an Apple IIe microcomputer system programmed to measure and store the intervals between reticular contractions.

Intervals between reticular contractions varied from 43.3 $\pm$ 7.8 (mean $\pm$ SEM) during rumination to 72.3 $\pm$ 9.9 seconds during normal contractions. A bolus infusion of 4 mmoles of HCl (pH 2.0) into the duodenal bulb induced inhibition of reticular contraction for 312.0 $\pm$ 46.6 seconds. When the alpha-2 adrenoceptor antagonist, tolazoline at 1 and 2 mg/kg were given 30 minutes before duodenal acid infusions, reticular contraction were inhibited for 117.0 $\pm$ 14.9 and 132.7 $\pm$ 39.3 seconds respectively. When animals were pretreatment with the dopamine antagonist, metoclopramide at dosages of 1.0 and 2.0 mg/kg reticular contraction inhibition lasted for 116.2 $\pm$ 5.8 with the 2.0 mg/kg dosage. No protection against reticular inhibition by duodenal acidification was obtained with metoclopramide at 1.0 mg/kg. Since metaclopramide and tolazoline causes a reduction in the inhibition of reticular contraction it is concluded that both dopamine and alpha-2 adrenoceptors may be involved in the inhibition of forestomach motility which accompanies duodenal acidification with exogenous HCl. However, since metaclopramide can act as an alpha-2 adrenoceptor antagonist, this inhibition may be mediated solely by alpha-2 adrenoceptors.

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MICROBIOLOGY PANEL

Diversity and Changes with Diet Among Rumen Fungi. D. E. Akin, W. S. Borneman, and W. R. Windham, ARS-USDA, P.O. Box 5677, Russell Research Center, Athens, GA 30613.

The diversity of anaerobic fungal types existing within the rumens of cattle was examined by microscopic and cultural methods. Fungal colonies developing in anaerobic media from zoospores in rumen fluid from cows eating various forages included types having monocentric growth similar to those described previously and types showing polycentric growth. High energy supplements such as corn or soybean hulls added to diets of sorghum silage increased fungal numbers in the rumen, but increases were also affected by the history and predisposition of the animal. Mixed fungal types in rumen fluid and pure cultures of isolates showing monocentric and polycentric growth degraded and weakened lignocellulosic tissues; the polycentric type penetrated the cuticle of leaf blades. By weakening or degrading recalcitrant structures in forages, rumen fungi may mitigate structural barriers and alter physical parameters of plants that influence utilization of fiber by ruminants.

Isolation and Characterization of Cellulolytic and Hemicellulolytic Bacteria from Human Feces. K. J. Wedekind*, H. R. Mansfield, and L. Montgomery, University of Illinois, Urbana, IL 61820.

The fibrolytic microbiota of the human colon was examined to determine numbers and types of cellulolytic and hemicellulolytic bacteria present. Fecal samples from each of five individuals studied contained bacteria capable of degrading the hydrated cellulose in spinach and in wheat straw pretreated with alkaline hydrogen peroxide (AHP-WS), whereas only one sample degraded the relatively crystalline cellulose in Whatman #1 filter paper. The mean concentration of cellulolytic bacteria, estimated with AHP-WS as substrate, was 1.2×10^8 per ml feces. Pure cultures of bacteria once isolated from AHP-WS were able to degrade crystalline cellulose, suggesting that interactions with other microbes were primarily responsible for previous low success rates in detecting fecal cellulolytic bacteria. The cellulolytic bacteria included Ruminococcus spp., Clostridium sp., and two unidentified strains. The mean concentration of hemicellulolytic bacteria, estimated using larchwood xylan as substrate, was 1.8×10^{10} per ml feces. The hemicellulose-degrading bacteria included Butyrivibrio sp., Clostridium sp., Bacteroides sp., and two unidentified strains. This work demonstrates that most or all humans harbor intestinal cellulolytic bacteria and that a hydrated cellulose source such as AHP-WS is necessary for their consistent detection and isolation.

Fermentation Products and Cellulolytic Enzymes Produced by Pure Cultures of Monocentric and Polycentric Rumen Fungi. W. S. Borneman, D. E. Akin, Russell Research Center, ARS-USDA, Athens, GA, and Lars G. Ljungdahl, Center for Biological Resource Recovery and Department of Biochemistry, University of Georgia, Athens.

Five anaerobic rumen fungal isolates were grown on Coastal bermudagrass (CBG) and monitored over a 9-day period for substrate utilization, fermentation products, cellulase and xylanase activities. Two of the fungal isolates showed monocentric growth patterns; one had multiflagellated zoospores (isolate MC2) and morphologically resembled the genus Neocallimastix, the other had monoflagellated zoospores (isolate MC1) and resembled Piromonas. Three other isolates (PC1, PC2, PC3) exhibited polycentric growth and have not yet been described in the literature. These isolates were characterized by differences in morphology. All of the isolates degraded CBG to approximately the same extent (70%) in 9 days, but rates of digestion were notably different. Isolate MC2 reached maximum digestion in 4 days, compared to 7 days with isolate MC1. Isolates PC2 and PC3 showed maximum degradation in 5 days and isolate PC1 in 6 days. Fermentation product accumulation was concurrent with substrate utilization, with the exception of lactate production by isolate MC2 and PC3, which had a longer lag time. Analyses of products indicated a mixed acid fermentation. The major fermentation products for all isolates were formate, acetate, lactate, ethanol, carbon dioxide, and hydrogen. In all cultures formate and acetate were the principle end products.

Culture supernatants from growth on 1, 3, 5, 7, and 9 days were assayed for extracellular enzymatic activity towards avicel (exo-glucanase), carboxymethyl cellulose (endo-glucanase) and xylan. Increasing enzyme activity correlated with accumulation of fermentation products and substrate utilization. Enzymatic activities of isolates PC2 and PC3 towards each substrate reached a maximum in 4 days compared with 3 days for isolate MC2, 5 days for isolate PC1, and 7 days for isolate MC1. The maximum activities are listed in the following table.

Isolate	Xylan max. activity (U/ml)*	CMC max. activity (U/ml)	Avicel max. activity (U/ml)
PC1	2.16	.16	.0072
PC2	2.41	.18	.0079
PC3	2.14	.17	.0069
MC1	2.06	.13	.0059
MC2	2.08	.175	.0114

*1 unit is defined as μmoles product formed/minute/ml supernatant.

All isolates produced significant amounts of xylanase (approximately 13-fold that of CMCase), and isolate MC2 had a much higher activity towards avicel. Although obvious differences in morphology and growth pattern exist with these isolates, the ability of all 5 isolates to degrade CBG to the same extent while producing similar fermentation profiles and enzymes indicates a metabolic similarity between the fungi.

Cellulolytic rumen bacteria from buffalo (Bison bison). V. H. VAREL, U.S. Meat Animal Research Center, ARS, USDA, Clay Center, NE 68933

Various studies suggest that the North American buffalo or bison has a superior ability, when compared to cattle, to digest low-quality forages. The objectives of the study were to compare rumen fluid parameters and cellulolytic populations between bison, bison-hybrids, and crossbred cattle (5 steers each), which were fed a ground alfalfa diet (13.8% protein). Approximately 500 to 750 ml rumen fluid was withdrawn from each animal by stomach tube and vacuum pump. No differences were observed in rumen fluid pH, ammonia-nitrogen or individual volatile fatty acid concentrations between the three groups of animals. Neither the total number of viable bacteria, 3.02, 4.22, and 4.62 x 10^9 per ml rumen fluid, or the number of cellulolytic bacteria, 10.9, 7.5, and 7.0 x 10^7 per ml, respectively, for the bison, bison-hybrids, and cattle were different. Significant differences in the proportion of cellulolytic species were observed, although these species differences did not affect 48 h in vitro digestibility of alfalfa cell walls between the animal groups. Bacteroides succinogenes represented 58, 36, and 42% of the total cellulolytic bacteria in the bison, bison-hybrids, and cattle, respectively; and Butyrivibrio spp. represented 7, 29, and 13%, respectively. Ruminococcus albus populations were similar for each group of animals, 29 to 34%; while R. flavefaciens made up less than 3% of the total cellulolytics in each group. Approximately 5% of the cellulolytic populations were not identified. Although differences were observed in the ratio of cellulolytic species between bison, bison-hybrids, and cattle, these differences apparently did not modify the in vitro rumen fermentations enough to suggest that bison have a superior ability to degrade the alfalfa forage used in this study.

The inhibitor in vitro system revisited--further studies on protein degradation by mixed rumen organisms. G.A. Broderick, USDA-ARS, U.S. Dairy Forage Research Center, Madison, WI.

There is a continuing need for methods to rapidly assess ruminal degradation of feed proteins. In vivo methods are costly and tedious, and in situ techniques are subject to several criticisms. In vitro methods which base estimates of degradation on appearance of protein degradation products such as NH_3, usually underestimate degradation because of microbial utilization. Earlier, we reported on a ruminal inhibitor in vitro (IIV) system which incorporated hydrazine (HS) to obtain quantitative recovery of NH_3 and amino acids. However, there appeared to be some direct utilization of amino acids in the presence of HS alone. Hence, chloramphenicol (CAP) also was added to the inoculum. Recoveries of NH_3 and both low and high levels of N (added as amino acids plus NH_3) were very low without inhibitors, but were 94 to 97% with 1 mM HS plus 30 μg CAP/ml. Apparent degradation rates (k_d) for casein and expeller soybean meal (rapidly and slowly degraded proteins, respectively) were increased from .16 and .004 h^{-1}, without added inhibitors, to .40 and .07 h^{-1} with 1 mM HS plus 30 μg CAP/ml. The updated system also contains .33% w/v maltose and 2 mM mercaptoethanol; protein substrates are added at .125 mg N/ml. These concentrations did not reduce hydrolysis of artificial protease substrates or disappearance of azo-dye labelled proteins. There was a tendency for casein to have slightly reduced degradation rates in the presence of inhibitors, possibly due to end-product inhibition. The system was active for at least 8 h, but most degradation studies have been conducted for 4 to 5 h. The relationship between NH_3 release (using conventional in vitro inoculum) and in situ and IIV values was poor (r^2 = .23 and .35, respectively). Although in situ and IIV degradation rates were highly correlated (r^2 = .83), in situ degradation rates averaged only 36% of IIV rates. However, fraction 'A' ("rapidly degraded") from in situ studies averaged more twice that by IIV. As a result, the mean extent of degradation estimated from in situ data was 83% of IIV values. Extent of rumen escape for seven proteins estimated from in situ and IIV data was of similar magnitude to values reported in Ruminant Nitrogen Usage (NRC, 1985). Two very slowly degraded proteins (feather meal and blood meal) had degradation rates too slow to be accurately determined by either technique. The IIV method is described in a paper which is in press (Brit. J. Nutr. 58:Nov., 1987). The in situ-IIV comparisons are the subject of another manuscript submitted for publication.

Attachment of Bacteriophages to Rumen Bacteria. M. T. Yokoyama and T. Tadese, Department of Animal Science, Michigan State University, East Lansing, MI 48824.

Rumen microorganisms have been the object of intensive studies over the years, which has led to a better understanding of how to improve the efficiency of the rumen fermentation. A major gap in our knowledge is the role of rumen bacteriophages. The occurrence of high numbers of bacteriophages (10^7/ml) have been reported in the rumen, but virtually nothing is known about their host specificity, virulence, induction and effect on fermentation efficiency. In preliminary investigations, rumen fluid from a hay-fed cow was centrifuged and the supernatant passed through a millipore filter (0.45 μ). The filtrate was inoculated into a culture of Treponema saccharophilum, a large pectinolytic spirochete previously isolated by the use of rifampin as a selective antibiotic. The association of a bacteriophage with T. saccharophilum was detected by TEM. Other TEM photographs of Butyrivibrio fibrisolvens show the presence of numerous bacteriophages within cells. It is not known if these bacteriophages are either temperate or virulent. We are in the process of screening rumen bacterial strains for their association with bacteriophages.

Evaluation of Saccharomyces cerevisiae Growth in the Rumen Ecosystem. M. J. Arambel and Rung-Syin Tung, Department of Animal, Dairy and Veterinary Sciences, Utah State University, Logan, UT 84322-4815.

Rumen fluid (RF) was collected 2 hrs post-feeding, centrifuged, autoclaved and used as a media source for Saccharomyces cerevisiae growth at 25°C and 39°C in an in vitro trial. Yeast media (YM) was used as a control media source. Aliquot samples were taken every 6 hrs after initiation of the incubation trial and analyzed for yeast cell count and percent viability. Regardless of temperature, mean yeast cell count for YM (3.18×10^7 cells/ml) was significantly different ($P<.01$) from those grown on the RF media (1.87×10^7 cells/ml). Percent viability of yeast grown on YM media was higher than those grown on RF media ($P<.05$). Compared to YM, RF media could not provide optimum nutrients for growth of Saccharomyces cerevisiae. Results indicated that 25°C was more favorable for Saccharomyces cerefisiae growth than 39°C (yeast cell count 3.01×10^7, 2.04×10^7 cells/ml, and viability 75.3, 59.6% for 25°C and 39°C, respectively). Budding of yeast cells was observed in YM at 25°C, but was not present in yeasts incubated at 39°C or in RF media.

Three different size membrane filters (5.0, 1.2, and .22 μ) were used for vivar chambers. Each chamber was inoculated with the same number yeast cells. Buffer solution was used to bring chambers to volume. Yeast cell count was unaffected by membrane filter pore size ($P>.05$). Yeast cell count decreased through time and no budding of yeast cells was observed for all three membrane filter sizes. Based on these results, Saccharomyces cerevisiae cannot maintain a productive population within the rumen ecosystem.

Influence of Sodium and Potassium Concentrations on in vitro Lactic Acid Inhibition and Propionic Acid Enhancement by Lasalocid or Monensin. D. E. Nuzback and T. G. Nagaraja, Department of Animal Sciences and Industry, Kansas State University, Manhattan, KS.

In vitro batch culture fermentation systems were used to study the influence of sodium (Na) and potassium (K) concentrations on lactic acid inhibition and alterations in volatile fatty acid VFA production by lasalocid or monensin. In a 2x4x5 factorial randomized block experimental design, lasalocid or monensin was tested at 5 concentrations in each of four McDougall's buffers. Sodium and potassium concentrations in McDougall's buffer (Na:K ratio, 15:1) were altered by isosmolar substitution to produce Low, Medium and High K buffers with 44:1, 4:1 and 1.4:1 Na:K ratios, respectively. Ruminal fluid inoculum was from a steer fed 80% alfalfa hay and 20% cracked corn diet. The strained ruminal fluid was centrifuged at 8000 x g, the supernatant discarded and the microbial pellet was resuspended in buffer. Substrates for the in vitro fermentation consisted of glucose for lactic acid inhibition and glucose, cellobiose, maltose, xylose, casein hydrolysate, urea and B-vitamins for VFA alterations. Lasalocid caused greater reduction in lactic acid concentration at low K than medium or high K. Based on concentrations of ionophores necessary to inhibit lactic acid concentrations by 50% of the control, lasalocid tended to be more potent at lower K than higher K concentrations. However, K concentrations had no influence on lactic acid inhibition by monensin. The molar proportion of acetate increased with increasing K concentration. Propionate molar proportion decreased and acetate:propionate ratio increased with increasing K concentration. As K concentration increased, molar proportion of butyrate decreased for lasalocid but not for monensin. These studies indicated that as K concentrations increased, the antimicrobial effects of ionophores decreased. However, the effects appeared to be dependent on ionophore concentration.

Subcellular Localization of Starch-degrading Enzymes in Bacteroides ruminicola. K. K. Thurn and S. F. Kotarski, Microbiology and Nutrition Research, The Upjohn Company, Kalamazoo, MI.

In view of the high amounts of starch in diets given to feedlot cattle, we have begun to study the degradation of this carbohydrate by the rumen bacterium, Bacteroides ruminicola 118B. In initial experiments, we examined the distribution of starch-degrading enzymatic activities in the cell. Cell bound pullulanase and amylase activities accounted for at least 80 and 90%, respectively, of the total activity detected in batch cultures containing amylopectin as a carbohydrate source. Seventeen to 20% of the cell-bound amylase, pullulanase and α-glucosidase activities were associated with the membrane fraction. Inner and outer membrane enriched fractions (IM and OM) were obtained by isopycnic sucrose density gradient centrifugation. Membrane associated α-glucosidase activity was detectable only in the IM fraction. Amylose- and pullulan-degrading activities were associated with both membrane types. Using SDS-PAGE, we compared the protein composition of the OM, IM and soluble protein fractions of cells grown on either amylopectin maltose or glucose. Two polypeptides (M_r 57,000 and 105,000) observed in the OM fraction of cells grown on either maltose or amylopectin were not detected in the OM fractions of glucose grown cells. A 165,000-M_r polypeptide was detected only in the IM fraction of cells grown on amylopectin. No differences in protein profiles were detected among the soluble protein fractions.

Inhibition of *Selenomonas ruminantium* Growth by Ammonium Salts - S. C. Ricke and D. M. Schaefer, Dept. of Meat and Animal Science, University of Wisconsin, Madison, WI.

The present understanding of rumen bacterial ammonia requirements is incomplete as evidenced by the lack of agreement among literature estimates for a minimum ammonia concentration which provides for maximum bacterial growth and by the absence of substantive explanations for the discrepant estimates. The concept of a lower limit is not new and has often been used exclusively as an indicator of optimum. Based on preliminary batch culture work with a variety of rumen bacteria, an upper ammonia concentration limit appears to exist for growth. It was decided to focus on the rumen bacterium *Selenomonas ruminantium* for further work with nonlimiting ammonia concentrations since it is prevalent under a variety of *in vivo* dietary conditions and is the best characterized rumen genus for ammonia assimilation pathways and regulation. The objective of this study was to determine the maximum ammonia concentration tolerated by *S. ruminantium* by comparing growth rates over a broad range of concentrations in order to achieve complete inhibition. Strain HD_4 when grown in batch with cysteine and ammonia as nonlimiting nitrogen sources (15 mM glucose as the energy source) had an observed reduction in growth rates at ammonia concentrations above 200 mM. Initially the inhibition constant (K_i) was estimated from a single reciprocal plot but this was an overestimate of the actual value where substrate concentration reduced maximum growth rate (μ_{max}) by half. However, when the data were transformed to $\log_{10}$ and graphed as a Hill plot all the data points were retained as a linear fit and the calculated K_i (.12 - .22 mM) accurately estimated the substrate concentration where 1/2 μ_{max} occurred. Of the factors examined that might influence the ammonia inhibition estimate: 1) Reduction in continuous culture μ_{max} was similar to reduction of batch culture growth rate for respective ammonia concentrations. 2) Basal medium with or without yeast extract had no influence on the estimated K_i value. 3) Anion species associated with ammonia may effect the degree of inhibition observed as the K_i was lower for ammonium sulfate (.12 mM) than ammonium chloride (.21 mM). Therefore, optimum concentration of ammonia should be defined as a range with an upper as well as lower limit rather than just a single minimum concentration.

Nutritional requirements of the rumen bacterium *Syntrophococcus sucromutans* . J. Doré and M. P. Bryant, Dept. of Animal Sci., University of Illinois, Urbana, IL 61801.

In an attempt to develop more defined culture conditions for the rumen bacterium *Syntrophococcus sucromutans* strain S195 (S195) requiring rumen fluid (RF) for growth, we have been brought to describe a specific lipid requirement. This investigation was undertaken prior to further study of the unique physiology of S195, which was isolated as the most numerous species degrading methoxylated lignin monoaromatic derivatives in the rumen. It has an absolute requirement for an electron acceptor system when grown on sugars and producing acetate. Formate or a hydrogen utilizer can substitute for the methoxyaromatic as electron acceptor system.

The RF requirement of S195 was such that it gave a linear growth response (yield measured as maximum OD 600 of a culture) to the RF content of the medium up to the 30% (v/v) tested. None of the most commonly used growth factors could replace RF for growth of S195.

Attemps to characterize the biological nature of the growth factor lead us to observe that a total lipid extract of RF retained almost quantitatively the effect of RF for growth of S195. Chromatography of lipid extracts showed that the phospholipid fraction concistantly retained most of the activity, and we could propose a more defined medium based on the replacement of RF by crude phospholipids. A 60% pure phosphatidylcholine preparation fron egg yolk showed optimum levels of 200µg/ml medium.

Enzymatic treatments and chemical deacylation indicated that the fatty acids (FA) might be the fraction of the phospholipid molecule stimulating growth, and when free acids were used as lipid suplement, S195 appeared to be a natural auxotroph for18 carbons monoenoic isomers, oleic, elaidic and t-vaccenic acids. FA were suspended at or above melting temperature by sonic dispertion in a bile salt, and added to the medium at a concentration of 150µM.

S195 was further observed to closely adapt its cellular simple lipids composition to that of the medium. It was also shown to synthesize aldehydes, witch we suspect to be in its plasmalogens. The observation that S195 made more aldehydes of the longer chain lengths and conversely more FA of the shorter chain lengths suggests a strategy to maintain membrane fluidity.

When t-vaccenic acid was the sole lipid suplement, 99% of the cellular FA, and 80% of the aldehydes were of the C 18:1 type suggesting that the FA may not be metabolized at all.

We made use of (1-^{14}C) oleic acid to address this question. Cells grown with 150µM oleic acid (10µCi/l) incorporated 20% of the label, none being released as CO_2.

We could observe that about 55 % of the label incorporated was recovered in the cellular simple lipids, with a ratio of FA to aldehydes of 2 to 1. The label recovered in the non lipidic fraction was further shown to be 99% TCA precipitable, and differencial fractionation of the cell components showed that 75% of the label from the disrupted cells was in the membrane preparation, versus 25% in the cytosolic fraction.

Description of *Acetitomaculum ruminis*, a new hydrogen and carbon dioxide utilizing acetogen that was isolated from a forage fed steer. Jane A. Z. Leedle* and Richard C. Greening, Microbiology and Nutrition Research, The Upjohn Company, Kalamazoo, MI

A new genus and species of fastidiously anaerobic, hydrogen-oxidizing, carbon dioxide-reducing bacteria which produce a homoacetic fermentation has been isolated by selective enrichment from the rumen of a mature steer fed a forage diet. Suspensions of rumen bacteria, prepared from digesta collected 7 h after feeding, were incubated in a minimal medium containing rumen fluid under 3 atmospheres of either H_2:CO_2 (80:20) or N_2:$C0_2$ (80:20) headspace gas. The selection criterion was an increment of acetate in the H_2:CO_2 enrichments over the N_2:CO_2 enrichments. Periodically, the enrichment broths were plated onto agar media and presumptive acetogenic bacteria were screened for acetate production. Selected acetogenic bacteria utilized the pressurized atmosphere of H_2:CO_2 to form acetate in quantities up to 8 fold higher than when grown under N_2:CO_2. All isolates were obtained from either the 10^{-7} or 10^{-8} dilutions of the original rumen contents. Five (5) strains were characterized. All were gram positive rods and had a limited substrate spectrum. In addition to hydrogen and carbon dioxide, other growth substrates were formate, glucose and CO. Acetate was produced with a stoichiometry of $4H_2 + 2HCO_3^- + H^+ \rightarrow CH_3COO^- + 4H_2O$. One strain required and all were stimulated by rumen fluid. No spores were observed with phase contrast microscopy and two strains were motile. Temperature and pH optima were 38° and 6.8, respectively. Cell wall amino acid analysis revealed a ratio for serine : glutamic acid : alanine : diaminopimelic acid : ornithine : lysine of 1 : 2.6 : 2.3 : 0.4 : 0 : 1.8. Together these data suggest that the microorganisms represent a new genus and species of rumen bacteria. The name *Acetitomaculum ruminis* is proposed. The isolation and characterization of these bacteria indicate that they are indigenous inhabitants of the rumen. Their role may be to participate in the hydrogen economy of the rumen to conserve feed energy as acetate, a useful product for the host.

Characterization and Partial Purification of an Extracellular Xylanase from Butyrivibrio fibrisolvens Strain 49. S. Evans, R. J. Stack, and R. B. Hespell, USDA-ARS, Northern Regional Research Center, 1815 N. University St., Peoria, IL

Butyrivibrio fibrisolvens strain 49 produces extracellular xylanolytic activity when grown in defined medium with glucose, maltose, arabinose, xylose or cellobiose as the carbon source. Highest activity was obtained on xylose (17 μmoles pentose released/ml supernatant/hr at 37°C with larchwood xylan as the substrate). Activities on arabinose, maltose, cellobiose and glucose were 13-, 11-, 5- and 5 μmoles pentose released/ml supernatant/hr, respectively. A xylanase was partially purified by a relatively simple procedure involving concentration of the culture supernatant, dialysis, and isoelectric focusing. Culture supernatant was lyophilized to dryness, redissolved in one-tenth volume of water, and then dialyzed against water. Approximately 90% of the total protein precipitated during dialysis; however, 40-60% of the xylanase activity remained soluble and was recovered in the supernatant fluid after centrifugation to remove the precipitated protein. A single peak of xylanase activity was obtained from gel exclusion chromatography of the concentrated supernatant fraction, but attempts to estimate the molecular weight of the xylanase were unsuccessful due apparently to interaction of the enzyme with column matrices. A single xylanase activity (pI = 5.3) also was obtained after isoelectric focusing (IEF) in polyacrylamide gels over the pH range 3 to 11 or 4 to 6.5 SDS-PAGE of the protein recovered from the active fraction of the IEF gel indicated the presence of only two proteins (M_r = 66,000 and 72,000). The partially purified xylanase yielded a mixture of xylooligosaccharides (principally xylotriose through xylohexaose) as hydrolytic products with larchwood xylan as the substrate, though a trace amount of xylobiose was detected.

Transfer of Bacteroides succinogenes (Hungate) to Fibrobacter gen. nov. as Fibrobacter succinogenes comb. nov. and Fibrobacter intestinalis sp. nov. L. Montgomery[1], B. Flesher[2] and D. Stahl[2], Departments of Animal Sciences[1] and Veterinary Pathobiology[2], University of Illinois, Urbana, IL 61801.

Comparison of 16S rRNA sequences showed that strains classified as Bacteroides succinogenes are not closely related to other species of Bacteroides, including the type species Bacteroides fragilis. Comparison of sequences indicate that Flavobacterium heparinum, for example, is more closely related to B. fragilis than is B. succinogenes. Therefore, we propose that B. succinogenes strains be renamed as members of a new genus, Fibrobacter. Based on the 16S rRNA sequence divergence between two subgroups within that genus, two species have been formed. Isolates from the rumen are placed in F. succinogenes; the neotype strain is S85 (ATCC 19169). Isolates from the ceca of nonruminant animals are placed in F. intestinalis; the type strain is NR9. Members of F. succinogenes can be differentiated from F. intestinalis by their requirement for biotin; the site of isolation may not be diagnostic. Fibrobacter succinogenes consists of two subspecies; cells of subsp. succinogenes strains are pleiomorphic and coccoid, whereas cells of subsp. elongata are slender rods.

4-O-[1-Carboxyethyl]-D-galactose: A Biochemical Marker for Differentiating Isolates in the Genus Butyrivibrio? Robert J. Stack and Thomas M. Stein, USDA-ARS, Northern Regional Research Center, 1815 N. University Street, Peoria, IL.

Isolates tentatively classified as Butyrivibrio fibrisolvens represent a phenotypically diverse group of ruminal bacteria. Nearly all examined strains produced extracellular polysaccharides, or slimes, when grown in pure culture on a variety of carbohydrate substrates. These slimes were purified and analyzed in order to ascertain whether the degree of relatedness between strains might be inferred from compositional data. During these studies several peaks not corresponding to any known carbohydrate standards were detected by GLC of prepared alditol acetate derivatives. The identity of the compound giving rise to one of these unknown peaks was established as 4-O-[1-carboxyethyl]-D-galactose on the basis of ^{13}C-n.m.r. and ^{1}H-n.m.r. spectroscopy, mass spectrometry, and chemical degradation studies. This novel acidic sugar was found in the slimes from about half of nearly 40 strains examined, and may serve as a useful biochemical/taxonomic marker for B. fibrisolvens.

Differentiation of Butyrivibrio fibrisolvens Strains into Several Species by G+C Content and DNA-DNA Hybridization Data. B. M. Mannarelli and D. Lee, Northern Regional Research Center, USDA-ARS, 1815 N. University Street, Peoria, IL 61604.

Thirty-nine strains assigned to the strict anaerobic species Butyrivibrio fibrisolvens were examined for DNA relatedness. Guanine-plus cytosine base contents (buoyant densities) of chromosomal DNAs of 27 strains was 39-42 mol %. Another nine strains had G+C contents of 42-45 mol %, and three other strains had higher contents of 46.4-49.2 mol %. Genetic relationships among the strains were determined by DNA hybridizations while using both spectrophotometric and membrane filter techniques. The relationships derived by both methods are compared. Results indicate that the strains comprise a genetically heterogeneous group and represent a number of distinct species, and, possibly, several genera. Strain D1, the type strain, was not closely related to any of the other 38 strains. Five groups of related bacteria, containing 20 Butyrivibrio strains, were identified as forming 5 separate species. The other 19 strains were not closely related to any other strain. The largest group identified by DNA relatedness as belonging to the same species contained only six strains.

Genetic Analysis of Starch Utilization by Bacteroides. K. L. Anderson and A. A. Salyers, University of Illinois, Urbana.

Previous studies of starch utilization by Bacteroides have not detected extracellular starch-degrading enzymes. Only cell-associated enzymes have been found, and these are regulated by maltose and starch. Thus, the bacteria may have to transport polysaccharides through the outer membrane, and starch breakdown probably involves not only a number of degradative enzymes but also transport and regulatory proteins.

Mutants were used for further study of the proteins involved in starch utilization. Mutants of B. thetaiotaomicron were obtained by transpositional mutagenesis, using the Bacteroides' transposon Tn4351, which has been inserted into the self-mobilizing E. coli plasmid R751. Ten mutants were obtained that were unable to grow on either amylose, amylopectin, or pullulan. Physiological analysis of these mutants suggested that the presence of polysaccharidases alone are not sufficient for utilization of polysaccharides, and that α-glucosidase may not be co-regulated with amylase and other polysaccharides. Also, transport of amylose may differ from transport of oligomers up to maltoheptose. Finally, there may be more than one pathway for maltose utilization.

Endo-β-1,4-D-Glucanase Components of Ruminococcus flavefaciens FD-1. K. C. Doerner and B. A. White, Dept. of Animal Sci., University of Illinois, Urbana, IL 61801.

Three endo-β-1,4-glucanase components from Ruminococcus flavefaciens FD-1 have been identified and partially purified. Endocellulase activity was monitored during identification and purification by measuring reducing sugars released from carboxymethyl cellulose (CMC) using the ferricyanide reducing sugar assay. Over 85% of the CMCase activity was found to be extracellular, once the filter paper was degraded (7 days). Culture supernatant was harvested and protein concentrated by ultrafiltration. The retentate, (≥300,000 MW) containing most of the activity against CMC, was then fractionated using a TSK DEAE-5PW HPLC column. This yielded three peaks of CMCase activity. A sharp peak of CMCase activity (Endo A) eluted just prior to exo-β-1,4-glucanase A (Exo A). A major peak of CMCase activity (Endo B) followed Exo A with a broad shoulder of less active enzymatic activity designated Region C. Further purification has not yet been achieved due to the unstable nature of the enzyme preparations and the low concentrations of enzyme recovered from the DEAE column. Endo A has a M_r of 38,000 as determined by gel filtration chromatography. These three enzyme peaks show differential inhibition by methyl cellulose. Endo A is not sensitive to methyl cellulose inhibition suggesting that it may be a cellulodextrinase. Endo B and Endo C are sensitive to methyl cellulose inhibition suggesting that these peaks are composed of true cellulase enzymes.

Molecular Cloning and Expression of Cellulase Genes from Ruminococcus albus 8 in Escherichia coli bacteriophage λ. G. T. Howard and B. A. White, Dept. of Animal Sci., University of Illinois, Urbana, IL 61801.

A genomic library of Ruminococcus albus 8 DNA was constructed using the Escherichia coli bacteriophage λ vector λDASH. R. albus 8 chromosomal DNA was partially digested with the restriction endonuclease SauIIIA, to obtain DNA fragments with a size range of 9 kilobases (kb) to 23 kb. The size fractionated DNA was then ligated with BamHI digested E. coli phage vector λDASH, in vitro head packaged, and plaqued on the appropriate E. coli host. Recombinant phage were screened for cellulolytic activity by plating in soft agar (0.7%) overlays containing either 1% carboxymethyl cellulose (CMC), 1 mg/ml 4-methylumbelliferyl-β-D-cellobioside (MUC) or 1% (w/v) Ostazin brilliant red-hydroxyethyl cellulose (OBR-HEC). CMC hydrolysis was detected by the Congo Red staining procedure. MUC hydrolysis was detected by scoring for fluorescence under UV light. OBR-HEC hydrolysis was detected by scoring clear zones in the red background around plaques. One hundred and three recombinant phage exhibiting activity against OBR-HEC were found and these fell into 4 classes based on the size of the zone of hydrolysis. Twenty-one recombinant phage exhibiting activity against CMC and 19 recombinant phage exhibiting activity against MUC were isolated. Six OBR-HEC+, 5 CMC+, and 8 MUC+ clones were further analyzed by restriction endonuclease mapping and cellulase substrate specificity to identify unique clones, and determine their cellulase type. Three different clone types were identified representing endocellulase activity. Three clones that appear to encode exocellulase type activity, and 4 clones that have a mixed specificity, including β-glucosidase activity, were also identified.

The Effects of Cellulose Ethers on Ruminal Cellulose Degradation. M. A. Rasmussen,[1,2] R. M. Gardner,[1,2] B. A. White[1] and R. B. Hespell[3] Department of Animal Sciences, Univeersity of Illinois, Urbana[1], and Northern Regional Research Center, USDA-ARS, Peoria, IL.[3]

Highly substituted, water soluble ether derivatives of cellulose were shown to inhibit ruminal degradation of cellulose based upon the type of ether-linked cellulose substituent. Methyl cellulose and hydroxypropyl methyl cellulose inhibited ruminal cellulose degradation 100 and 88%, respectively, whereas carboxymethyl cellulose and hydroxypropyl cellulose lacked inhibitory activity. Results obtained with crude cellulase preparations from Ruminococcus flavefaciens strain FD1 suggested that methyl cellulose functioned as a competitive inhibitor of p-nitrophenyl-β-D-cellobioside hydrolysis. This fact was confirmed with purified exo-β-1,4-D-glucanase A isolated from R. flavefaciens FD1. In contrast to crude cellulase activity, the exoglucanase activity also was inhibited by carboxymethyl cellulose. These data support the hypothesis that exoglucanases do not efficiently hydrolyze carboxymethyl cellulose.

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OCCURRENCE OF 2-AMINOETHYLPHOSPHONIC ACID IN FEEDS, RUMEN BACTERIA AND DUODENAL DIGESTA FROM DEFAUNATED SHEEP

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A quantitative method for 2-Aminoethylphosphonic acid (AEP) was developed using reversed-phase high performance liquid chromatography (HPLC). Conditions were similar to those used for analyzing amino acids in protein hydrolysates and physiological fluids. The procedure entailed pre-column derivatization of protein hydrolysate with ortho-pthalaldehyde and measurement of the isoindole derivatives with a fluorescence detector. The coefficient of variation was 2.1% for AEP levels ranging from 100 μmolar to a detection limit of 15 nMolar. The analysis was linear over this range with a correlation coefficient of 0.999. Mean recovery of AEP added to rumen fluid from faunated sheep was 98.2%.For AEP to be of use as a rumen ciliate protozoa marker it is critical that any AEP from lysed protozoa or other sources would be degraded completely in the rumen. The fermentability of pure AEP was determined *in vitro* using inoculum from defaunated sheep, and AEP disappearance rates from 3-12 hrs and 12-24 hrs of incubation were 2.63%/hr and 10.07%/hr, respectively. When AEP was added at a concentration of 22.6 μg/ml, 78% disappeared during the 24 hr incubation. Using the HPLC technique AEP was readily detected in preparations of mixed rumen ciliate protozoa, and quite unexpectedly also found in mixed and pure strains of rumen bacteria, feedstuffs, and rumen fluid and duodenal digesta from defaunated sheep. Its occurrence in feed and bacterial hydrolysates was confirmed by isolating the compound and estimating the quantity of carbon bound phosphorus. This value was then compared to the calculated AEP-phosphorus concentration determined by the HPLC technique. The AEP concentration in mixed rumen protozoa was three times greater than the concentration in mixed rumen bacteria, i.e., 4,304 and 1383 μg/g DM, respectively. AEP values for pure rumen bacterial cultures ranged from 733 to 1,166 μg/g DM in *Bacteriodes succinogenes* B21a and *Butyrivibrio fibrisolvens* H17c respectively. Rumen fluid and duodenal digesta from defaunated sheep contained AEP concentrations of 30 μg/ml and 90 μg/g DM, respectively. The concentration of AEP in feedstuffs varied from a high value of 263 μg/g DM in ground oats to a low value of 25 μg/g DM in wheat straw. Because AEP is not specific to rumen ciliate protozoa, occurring in variable quantities both in rumen bacteria and several feedstuffs, it is of little use as a marker.

Microbial population of the cecum in cattle fed a high grain or high forage diet.
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Existence of a fermentation in the cecum and colon of ruminants is widely accepted. Classically, this fermentation has been considered to be energy limited and, consequently, of little importance to the animal's nutritional status. Thus, extensive descriptions of cecal-colonic bacterial populations in cattle fed practical diets are lacking. Therefore, the objective of this study was to describe the bacterial population and presumptively identify major species in the cecum of steers fed high forage or high grain diets.

Two mature Holstein steers fitted with cecal cannulas were fed either a high grain (20% hay, 80% grain) or a high forage (80% hay, 20% grain) diet. Cecal contents taken from each steer 3 times during a 1 week sampling period were used to inoculate roll tubes from which individual isolates were obtained.

Changing from the high forage to the high grain diet resulted in a five-fold increase in bacterial numbers. Additionally, the cecal bacterial population was altered by diet. Bacteroides species were the predominant organisms on both diets. The second and third most numerous bacterial species on the high forage diet (Butyrivibrio fibrisolvens and Peptostreptococcus productus) were among the least numerous organisms on the high grain diet. Conversely, Eubacterium ruminantium and E. cellulosolvens made up the second most numerous group on the high grain diet. E. ruminantium was present in low numbers when steers received the high forage diet while E. cellulosolvens was not detected. The results of this and previous studies indicate that the bovine cecal-colonic fermentation is readily altered by changes in diet.

FACTORS AFFECTING THE MIGRATION AND NUMBERS OF RUMEN PROTOZOA IN THE FAMILY ISOTRICHIDAE

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Migration of the isotrichids (primarily Isotricha and Dasytricha) into the rumen at feeding time is believed to be a chemotactic response to soluble sugars in the feed. However, increases in isotrichid concentrations have also been observed to occur prior to feeding. A normal cycle generally consists of a rise in conc. just prior to feeding, a further increase immediately after feeding, followed by a fairly rapid decline. The decrease in conc. is believed to be the result of the isotrichids sequestering on the walls of the reticulum and ventral rumen. Migration of the isotrichids prior to feeding was not affected by handling or sampling of the animals. If feeding was delayed 4.5 h, isotrichid conc.increased markedly, falling again when feed was provided. In general, chemotactic migration could be elicited after 12 h, or migration without feeding occurred after 22-24 h. In animals fed once daily, the conc. of isotrichids in rumen contents of an unfed animal about 3 h after the normal feeding time is probably the best estimate of true total numbers. Two migrations of the same isotrichids appeared to occur in animals fed twice a day. It is proposed that feeding level controls the amount of storage polysaccharide in the cell, which in turn controls migration of the isotrichids into the rumen. The conc. of storage polysaccharide appears to decrease enough in 12 h to allow migration of the isotrichids on the basis of a chemotactic response. Approximately 20 to 22 h after feeding a depletion type response seems to occur, i.e., the isotrichids migrate into the rumen contents when the cellular storage polysaccharide falls below a specific level.

In situ Measurements of Ruminal Hydrogen in Cattle Fed Once Versus Eight Times Daily. W. J. Smolenski and J. A. Robinson, The Upjohn Company, Kalamazoo, MI.

Dissolved hydrogen was measured in the bovine rumen using an in situ hydrogen probe coupled to a mercury-reduction detector. The probe can quantitate dissolved hydrogen from low nM concentrations to saturation. In the rumens of steers fed a maintenance diet once a day, the basal hydrogen level increased after feeding and steadily drifted to lower concentrations until the next feeding. Although the magnitude of hydrogen fluctuations varied between steers, the response patterns to feeding were comparable. When the maintenance diet was fed eight times/day hydrogen fluctuations still occurred, but a more stable basal hydrogen level was maintained and the steady downward drift was not observed. The hydrogen fluctuations differed depending on the location of the probe. In the reticulum, where feed first enters the rumen, the increase in hydrogen after feeding before a hydrogen increase was observed after feeding. Basal hydrogen levels in all diets ranged between 0.7 and 1.8 μM.

Associative Effects of Probiotics and Diet on Ruminal Fermentation. K. E. Newman and K. A. Dawson, Department of Animal Science, University of Kentucky, Lexington.

The effects of two probiotic feed supplements on microbial activity in rumen-simulating continuous cultures being fed either a high concentrate or a high roughage ration was evaluated. One supplement contained an active yeast culture (Yeasacc) and the other contained both an active yeast culture and lactic acid bacteria (Lactosacc). In all instances supplementation with probiotics led to greater ($p<0.1$) concentrations of live yeast when measured relative to cultures not receiving the supplements. Fermenters receiving Yeasacc and a high roughage ration had a higher pH (6.55 vs. 6.36) and concentrations of cellulolytic organisms (2.5×10^9 vs. 9.37×10^8 CFU/ml), lower ammonia concentrations (25.6 vs. 28.9 mg/dl) and acetate:propionate (A:P) ratio (2.98 vs. 3.12) than fermenters receiving the same diet and no supplementation. Yeasacc fed with a high concentrate ration yielded none of the above effects except a decrease in A:P ratio in one instance. Lactosacc supplementation effects were also dependent on the diet used and resulted in lower pH (6.02 vs. 6.50) and A:P ratio (2.45 vs. 2.89) together with a greater total VFA concentration (152.6 vs. 132.4 mM) in fermenters receiving the high concentrate ration. pH was greater (6.55 vs. 6.36) and total VFA concentration lower (125.3 vs. 157.8 mM) in fermenters receiving Lactosacc and a high roughage diet than in those receiving only the high roughage diet. Heat treated Yeasacc had no effects ($p>0.1$) on pH, total VFA, or A:P ratio when compared to a control receiving no supplementation and a high roughage ration. Results indicate that the effects of probiotic supplementation are dependent on the type of supplement used and the composition of the ration.

Performance and Ruminal Changes of Early-weaned Calves Fed Lasalocid. K. L. Anderson, T. G. Nagaraja, J. L. Morrill, P. G. Reddy, T. B. Avery and N. V. Anderson, Department of Animal Sciences, Kansas State University, Manhattan, KS 66506.

Twenty-two neonatal bull calves, fed colostrum until 3 d of age, were assigned to a control or lasalocid-fed group. Calves in both groups then received whole milk until weaned at 3 wk of age. They were also fed a prestarter diet from 3 d of age until they consumed 227 g/d and then a mixture of 227 g pre-starter daily and starter diet ad libitum. The lasalocid-fed group received lasalocid in milk at 1 mg/kg body weight daily from 4 to 7 d and at .5 mg/kg body weight daily in milk and medicated pre-starter diet (88 mg lasalocid/kg) during the second week. After 2 wk, lasalocid-fed calves were given medicated pre-starter and starter (44 mg lasalocid/kg) diets. Four calves in each group were ruminally cannulated at 3 to 5 d of age, and ruminal fluid samples were obtained at 1, 2, 3, 4, 5, 6, 8, 10, and 12 wk of age to monitor microbial activity. Feed intake, weight gain, and fecal scores of all calves were recorded. Rectal fecal samples were collected at 4, 8, and 12 wk of age from all calves and examined for coccidial oocysts. Lasalocid-fed calves had a greater weekly feed intake and weight gain than control calves after 6 wk of age. Ruminal pH of lasalocid-fed calves was higher at 1 and 2 wk of age, but thereafter tended to be lower than that of control calves. Total ruminal volatile fatty acid concentrations and molar proportions of isobutyrate and isovalerate were higher, but acetate:propionate ratio and molar proportion of valerate were lower in lasalocid-fed calves than control calves. Total viable anaerobic and amylolytic bacterial counts were higher in lasalocid-fed calves than control calves. No significant treatment effect was found for ruminal ammonia-N concentration or ruminal lasalocid-resistant, lactobacillus, lactate-utilizing, cellulolytic, or methanogenic bacterial numbers. Also, there was no evidence of coccidial infestations in either group and fecal scores were unaffected by lasalocid feeding. In general, lasalocid-fed calves had greater feed intake and weight gain and, therefore, greater ruminal microbial activity than the calves fed no lasalocid in the diet.

Soluble Carbohydrate Transport by Selenomonas ruminantium. S. A. Martin[1] and J. B. Russell[2], Animal and Dairy Sci. Dept., University of Georgia, Athens[1] and USDA-ARS[2] and Dept. of Animal Sci., Cornell University, Ithaca, NY[2].

Toluene-treated cells of Selenomonas ruminantium HD4 used phosphoenolpyruvate (PEP) to phosphorylate glucose and sucrose. Glucose activity was constitutive, while the phosphorylation of sucrose was inducible. Competition experiments indicated that separate phosphotransferase system enzymes II were present for glucose and sucrose, but it appeared that maltose was hydrolyzed by an inducible extracellular maltase and then transported by the glucose PTS. S. ruminantium HD4 grew more slowly on maltose than glucose or sucrose and specific activity of maltase was rate limiting. The maltase was competitively inhibited by glucose and sucrose. Xylose was not phosphorylated by PEP or ATP, and uptake was inhibited by carbonyl cyanide m-chlorophenylhydrazone, dicyclohexylcarbodiimide, dinitrophenol, and chlorhexidine diacetate. The absence of PEP-dependent phosphorylation and the effects of protonophores and an ATPase inhibitor suggested that xylose was transported by an active transport mechanism.

Effect of dietary forage:grain ratio on digestion measurements and digesta passage rate in lactating dairy cows. J. Kleinmans, T.R. Dhiman, H.D. Radloff, L.D. Satter, U.S. Dairy Forage Research Center, USDA-ARS, Madison, WI.

The objective of this study was to obtain information on digestion kinetics, rumen fill, and digesta passage when high quality alfalfa silage was fed in various forage:grain ratios. Four lactating cows were fed total mixed rations containing 38%, 58%, 78% or 98% high quality forage in a 4x4 Latin square design. Ruminal digestion kinetics were determined by the in situ dacron bag method. Rate of passage of alfalfa silage, high moisture corn and liquid were estimated using La, Sm and Cr-EDTA as indigestible markers. Yb was used to determine dry matter digestibility. Rumens were emptied manually for rumen fill data. Results are summarized below:

Item	% Alfalfa			
	38	58	78	98
Diet, CP%	20.5	21.2	21.9	21.1
NDF%	27.4	32.4	37.5	42.7
ADF%	18.4	23.9	29.3	34.7
DM intake, kg/d	23.0^{a}	23.6^{a}	22.1^{ab}	20.2^{b}
% BW	3.73^{a}	3.77^{a}	3.67^{a}	3.22^{b}
DMD %	63.4^{ab}	65.0^{a}	53.4^{c}	55.0^{bc}
Milk yield, kg/d	37.7^{a}	35.8^{a}	32.8^{b}	27.2^{c}
Milk Fat, %	3.57	3.16	3.38	3.47
Milk Protein, %	3.00^{a}	2.79^{cb}	2.83^{b}	2.70^{c}
Rumen Fill:				
Wet Digesta, kg	67.4^{a}	72.7^{b}	74.5^{b}	86.3^{c}
Dry Digesta, kg	10.2^{ab}	10.4^{ab}	10.0^{a}	11.6^{b}
Ruminal Retention Time ($1/k_p$):				
La (forage), h	11.5	10.2	10.0	11.1
Sm (grain), h	12.0	12.2	10.6	--
Cr-EDTA (liquid), h	5.4	4.7	4.2	4.0
In Situ Digestion				
Alfalfa NDF:				
Potentially digested, %	49.8	50.1	49.1	49.0
Rate (k_d), h^{-1}	.054^{a}	.066^{ab}	.089^{bc}	.106^{c}
DM (High Moisture Ear Corn):				
Potentially digested	26.0	28.2	30.2	28.4
Rate (k_d), h^{-1}	.067	.071	.069	.071

Forage:grain ratio had little effect on passage of solids. Rate of digestion of forage, however, decreased with increasing amount of grain. Dry matter intake was lower and rumen fill of DM was higher with all forage diets, however, differences in rumen dry matter contents were small.

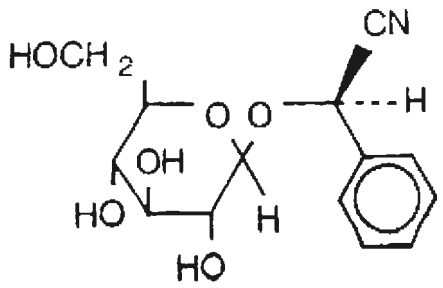
Diurnal Changes in Rates of Degradation of Cyanogenic Glycosides in Bovine Rumen Fluid -- W. Majak, R.E. McDiarmid, Agriculture Canada Research Station, Kamloops, B.C. V2B 8A9 and K.-J. Cheng, Agriculture Canada Research Station, Lethbridge, Alberta T1J 4B1.

A number of native and cultivated forages contain cyanogenic glycosides which can be degraded to hydrogen cyanide (HCN), an acute toxin that is lethal to ruminants at 2 mg/kg bodyweight. Prunasin is the cyanogenic glycoside that occurs in saskatoon serviceberry (Amelanchier alnifolia), chokecherry (Prunus virginiana) and black cherry (P. serotina). Linamarin is a component of cassava (Manihot esculenta) and triglochinin occurs in arrowgrass (Triglochin maritima).

In rumen fluid, two steps are involved in the degradation of cyanogenic monosaccharides. Initially, microbial β-glucosidases hydrolyze the glycosidic bond linking the sugar to an α-hydroxynitrile (cyanohydrin) and then, the HCN is dissociated from the cyanohydrin non-enzymatically. Rumen bacteria capable of prunasin hydrolysis and linamarin hydrolysis have been identified. Approximately 50% of the strains that were screened showed hydrolytic activity.

The in vitro degradation of prunasin in bovine rumen fluid was re-examined in greater detail to elucidate the factors that control the production of HCN. In these studies cattle were maintained on a diet of fresh alfalfa herbage. The non-enzymatic dissociation of HCN from mandelonitrile (the cyanohydrin aglycone) was pH dependent. Higher rates of dissociation occurred at higher pH. In rumen fluid, pH increased with duration of fasting and the rate of dissociation of mandelonitrile also increased. When prunasin was incubated in rumen fluid, diurnal changes in rates of HCN production also occurred but the rates decreased when cattle were fasted for 48 h. The most rapid rate of HCN production from prunasin occurred after a 24 h fast when the pH of the rumen fluid was elevated but when hydrolytic enzymes were still active. Similar results were obtained with linamarin or when rumen inocula were obtained from cattle on orchardgrass hay (Dactylis glomerata).

These results indicate that the precondition of the rumen may well determine whether HCN poisoning can occur in cattle ingesting cyanogenic forages. A lower pH in the rumen favours a gradual release of HCN and a greater rate of detoxification. At higher pH the risk is greater, especially when hydrolytic enzymes are active.



(R)-Prunasin

Enumeration of Selected Bacterial Groups in the Cecum and Colon of Pigs During the Growing and Finishing Phase. Tala J. Butine and Jane A. Z. Leedle, Microbiology and Nutrition Research, The Upjohn Company, Kalamazoo, MI 49001.

Populations of selected bacterial groups in cecal and colonic contents of clinically healthy pigs fed a corn-soybean meal diet were determined at 3 times during the growing-finishing phase of growth. Eight pens of 4 pigs each were used in this study. Pigs were sacrificed after 4, 8 or 11 weeks on feed, corresponding to phases I, II and III. Cecal and colonic contents samples were taken from each pig at necropsy and pooled by pen. Anaerobic techniques and rumen fluid-based media with agar plate or most probable number methods were used to estimate the total anaerobic population and the cellulolytic, pectin fermenting, pectin hydrolyzing, xylan fermenting, xylan hydrolyzing, sulfate reducing and methanogenic bacterial groups. An analysis of variance was performed on these bacterial group variables to examine the effects of phase (weeks on feed), site (cecum or colon) or their interaction (phase by site). Results indicated that the population of total anaerobes was twice as dense in the colon as in the cecum (2 vs. 1 x 10^{10} per g wet weight; $p = .001$). The proportion of cellulolytics was lower at phase I than at the other 2 phases ($p = .026$), while the proportion of pectin fermenting bacteria depended on the interaction of phase with site ($p = .021$). No significant differences between phases were observed for the proportions of pectin hydrolyzing, xylan fermenting, or xylan hydrolyzing bacterial groups. Numbers of sulfate reducing and methanogenic bacterial groups were significantly higher in the colon than the cecum ($p = .014$ and 002, respectively). These results suggest that the intestinal microbial community is at least 2 fold higher in the colon than in the cecum with respect to the total number of anaerobes and those bacterial groups that utilize hydrogen, namely the sulfate reducing and methanogenic bacteria. These populations, as well as the proportions of bacteria which degrade the more recalcitrant, complex polysaccharides entering the pig cecum and colon appear to be stable over the growing-finishing phase.

Effects of Mineral Cations on Ionophore Catalyzed Proton Flux into Ruminal Bacteria. D. B. BATES, W. R. SCHWINGEL and S. C. DENHAM. Department of Animal Science, University of Florida.

Earlier research in our lab (Schwingel et al., 1987. J. Anim. Sci. (Suppl. 1) 65:456) indicated that increasing potassium or sodium concentrations of in vitro fermentations with mixed ruminal microorganisms resulted in significant, but different, qualitative and quantitative changes in volatile fatty acid production in the presence of lasalocid or monensin relative to control fermentations carried out in the absence of ionophore. Increasing potassium linearly increased ($P = .0013$) the acetate:propionate ratio (A:P) of lasalocid containing fermentations ($y = .0033x + 1.41$), whereas monensin containing fermentations showed a decline ($P < .0001$) in A:P when potassium was increased from 50 to 150 mM. Increasing sodium concentrations had significant ($P < .0001$) quadratic effects on A:P and total VFA production in fermentations with monensin and lasalocid; a downturn in each of these parameters occurred as sodium concentrations were increased.

In the experiments reported in this abstract, 9-amino acridine (9AA) was used to monitor ionophore catalyzed proton flux in *Streptococcus bovis*, *Ruminococcus albus*, *Selenomonas ruminantium* and *Bacteroides rumincola*. Principle objectives included (1) testing the hypothesis that the ruminal response to ionophore (decreased acetate and methane as a proportion of fermentation end-products) can be attributed to ionophore catalyzed proton flux into ruminal bacteria, (2) determining if mechanistic differences exist in the action of lasalocid and monensin on ruminal bacteria when ionophore catalyzed proton flux is modified through alteration of extracellular cation concentration, and (3) determining the relative sensitivity of gram positive and gram negative ruminal bacteria to ionophores. Bacterial cells were harvested anaerobically from the exponential phase of growth and suspended in assay buffer in which the relative amount of extracellular mineral cation and pH were varied. 9-amino acridine (2.5 μM) was added and fluorescence monitored before and after addition of ionophore using a filter fluorometer electronically coupled to a strip chart recorder. Proton influx was measured as fluorescence quenching in relative fluorescence units (%) adjusted to a constant mg dry matter ml^{-1}. Maximum fluorescence quenching following addition of lasalocid was affected by potassium ($P < .0001$) and sodium ($P < .0001$) with means of -93.6, -58.8, and -15.3% (SE = 1.61) when potassium was varied in the assay buffer at 5, 70, and 140 mM; and -87.3 and -18.5% (SE = 1.2) when sodium was increased from 5 to 140 mM. Increasing sodium concentration from 5 to 140 mM changed monensin catalyzed 9AA quench in *R. albus* and *S. bovis* from -77.2 to +3.2%. Therefore, ionophore catalyzed proton flux into ruminal bacteria can only partially explain the functional response of the rumen microbial ecosystem to ionophore perturbation. Furthermore, monensin and lasalocid exhibited mechanistic differences that went beyond established differences in their relative selectivity and affinity for mineral cations. While the effect of both ionophores on proton flux appears to be predicated on antiporter efflux of intracellular mineral cation, monensin antiports K^+/H^+ whereas lasalocid does not; there was no K * pH interaction ($P = .2627$) on lasalocid catalyzed proton flux as measured with the 9AA procedure. Species differences exist in the relative effects of ionophores on proton flux in ruminal bacteria, but a general pattern of ionophore action is indicated. Gram negative bacteria respond more slowly to ionophores but respond in much the same fashion as do gram positive organisms.

Physiology of Starch Degradation by Ruminal Bacteria: Amylolytic Activities and Degradation Products Formed from Starch. M. A. Cotta, USDA-ARS, Northern Regional Research Center, 1815 N. University Street, Peoria, IL.

A variety of ruminal bacteria were grown in a complex medium containing glucose, maltose, or starch as the carbohydrate source, and amylase activity was determined by the dinitrosalicylic acid procedure. Of those species tested, the highest levels of amylase were produced by Streptococcus bovis JB1 and Ruminobacter amylophilus H18. Other species that grew well on starch and produced amylase included Butyrivibrio fibrisolvens strains A38 and 49, and Bacteroides ruminicola strains 23 and B_14. Varying the carbohydrate source resulted in changes in the growth rate and level of amylase produced by these strains. All strains grew rapidly in starch-containing media, and their rates of growth were generally more rapid than those observed for maltose-grown cultures. For S. bovis JB1, B. ruminicola 23 and B_14, B. fibrisolvens A38 and 49, amylase was produced when growth was on maltose or starch, but this activity was greatly reduced in glucose-grown cultures. The distribution of amylolytic activity between cellular and extracellular fractions was sometimes affected by the carbohydrate provided for growth. If S. bovis JB1 and B. fibrisolvens 49 were grown on starch, amylase was largely associated with cell pellets; however, when grown on maltose these strains produced activities that were almost entirely present in the extracellular fluid fractions. While not as dramatic, a similar shift in the location of amylase activity was noted for the two B. ruminicola strains when grown on the same substrates. Growth on maltose or starch had little influence on either the predominantly cell-associated activity of B. fibrisolvens A38 or the activity of R. amylophilus H18, which was equally divided between the cell pellet and extracellular fluid fractions. Digestion of amylose by extracellular amylolytic activities produced by these six strains yielded mixed oligosaccharides (glucose through maltoheptaose). Based on these data, these strains of ruminal bacteria produce amylases with endo-splitting activity similar to alpha-amylase.

The Phylogeny of Quin's Oval Based on Its 16S rRNA Sequence. Lee R. Krumholz, Marvin P. Bryant, William J. Brulla, John L. Vicini, Jimmy H. Clark and David A. Stahl.

A rumen population consisting mainly of "Quin's oval" was obtained by feeding sheep a diet of alfalfa pellets and mollasses. "Quin's oval" organisms were enriched from the rumen contents by differential centrifugation. The total RNA was extracted and the complete sequence of the 16S fragment was determined using the dideoxy sequencing procedure. By comparison of the 16S sequence with that of other organisms, a relative phylogenetic relationship can be obtained. "Quin's oval" was determined to be phylogenetically within the Gram positive group and most closely related to Selenomonas ruminantium as well as a related group of organisms including Clostridium quercicolun, Sporomusa paucivorans and the rumen bacterium, Megasphaera elsdenii. The relative sequence homologies of "Quin's oval" with these organisms ranged from 0.816 to 0.839 indicating that "Quin's oval" forms a distinct group of organisms likely making up a new genus.

Evidence for Acetyl Xylan Esterase and Other Esterase Activities in Butyrivibrio fibrisolvens Strains - Robert B. Hespell, USDA-ARS, Northern Regional Research Center, 1815 N. University Street, Peoria, IL.

Butyrivibrio fibrisolvens is a major ruminal bacterium with many strains showing a wide metabolic diversity including hydrolysis of xylans, starches, pectins, cellulose, and proteins. Lipolytic strains have been isolated on selective media containing tributyrin or other lipid materials, but it is not known whether lipolytic/esterase activity is a common trait for this species. Thirty strains of B. fibrisolvens from diverse geographical isolation were examined for esterase activity by using naphthyl esters of acetate, butyrate, caprylate, laurylate, or palmitate. All strains possessed some esterase activity. Highest levels were observed in strains 49, H17c, S2, AcTF2, and LM8/1B. For all B. fibrisolvens strains tested, naphthyl fatty acid esterase activity paralleled culture growth and was predominantly cell-associated. With strains 49, CF4c, and S2, the activity was retained by protoplasts made from whole cells. While esterase activity was detected with all strains when grown on glucose, higher levels (5- to 12-fold) were sometimes observed with growth on other substrates (larchwood xylan; citrus pectin) for strains D16f, D30g, 787, or X6C61. When nitrophenyl esters of fatty acids were used to measure esterase activity, generally 4- to 7-fold higher activity levels were detected. For a number of strains, substantial esterase levels were found in the culture fluid. With two of these strains, H17c and NOR37, the cultures contained xylanase and acetyl xylan esterase activities, neither of which was associated to any great extent with the cells.

Molecular Cloning and Expression of Endoglucanase Genes from Ruminicoccus flavefaciens FD-1 in Escherichia coli Bacteriophage λ. B. A. White and G. T. Howard, Dept. of Animal Sci., University of Illinois, Urbana, IL 61801.

A genomic library of Ruminococcus flavefaciens FD-1 DNA was constructed using the Escherichia coli bacteriophage λ vector λDASH. R. flavefaciens FD-1 chromosomal DNA was partially digested with the restriction endonuclease SauIIIA. The partially digested chromosomal DNA was size fractionated using sucrose gradient centrifugation (10-40%) to obtain DNA fragments with a size range of 9 kilobases (kb) to 23 kb. The size fractionated DNA was then ligated into E. coli phage vector λDASH which had been digested with BamHI. The resulting ligation mixture was then in vitro head packaged and plaqued on the appropriate E. coli host. Recombinant phage were screened for cellulolytic activity by plating in soft agar (0.7%) overlays containing either 1% carboxymethyl cellulose (CMC) or 1% (w/v) Ostazin brilliant red-hydroxyethyl cellulose (OBR-HEC). Two recombinant phage exhibiting activity against OBR-HEC and 9 recombinant phage exhibiting activity against CMC were isolated. Two OBR-HEC+ and 2 CMC+ clones were further analyzed by restriction endonuclease mapping and cellulase substrate specificity to identify unique clones, and determine their cellulase type. The four clones have in common a 7.0 kb, a 2.2 kb, and a 1.0 kb EcoR1 fragment. The two clones with higher activity also share 6.5 and 5.4 kb EcoR1 fragments. The substrate specificity data suggest that the two larger clones have a mixed activity. This may be due to the cloning of a portion of a cellulase operon containing more than one cellulase gene.

Estimation of rumen Microbial Protein Production by Fecal Nitrogen Fractionation. B. Haryanto and W. L. Johnson, Dept. of Animal Sci., North Carolina State University, Raleigh, NC 27695-7621.

The highly digestible rumen microbial nitrogen (Storm et al., 1983) and the available technique for fecal nitrogen fractionation (Mason, 1969) allow the estimation of rumen microbial protein production (RMPP) as:

$$RMPP_{est.} = [FO*(MEN_f) - (MBS*.037)]*4*6.25 \quad (g/d)$$

where FO is daily fecal dry matter output, MBS is W ($kg^{.75}$), and MEN_f is fecal microbial + endogenous nitrogen.

In an attempt to apply the formula, four treatments, i.e., 1) control, 2) supplemented with urea + L. leucocephala leaves, 3) supplemented with zinc, and 4) supplemented with zinc, urea + L. leucocephala leaves, each has been imposed to 4 goats fed with ad libitum mixed native grass forage from rubber tree plantation area, Bogor, Indonesia. Estimates of RMPP are shown in Table 1.

TABLE 1. Daily intakes of digestible neutral detergent solubles (NDS), cellulose and hemicellulose, estimates of rumen microbial protein production (RMPP), microbial dry matter (RMDM) and efficiency of microbial growth (Y_{ATP})

	Without zinc		With zinc		
	-(N)[a]	+(N)	-(N)	+(N)	SEM
Treatments	Control	2	3	4	
Intakes, g/d					
NDS	142	245	130	242	7.75**
Cellulose	123	134	104	113	11.66
Hemicellulose	106	111	91	102	7.74
ATP prod. (moles)	7.0	9.4	6.1	8.7	.51*
RMPP, g/d	24.9	29.3	21.3	40.0	1.64**
RMDM, g/d	71.0	83.7	60.8	114.3	4.67**
Y_{ATP}	10.8	9.0	9.8	14.1	.80
Gain, g/d	0	10.0	1.3	30.8	18.43**

[a]-(N) - No supplementation.
+(N) - Supplemented with urea + L. leucocephala leaves.
*Significantly different among treatments ($P < .05$).
**Significantly different among treatments ($P < .01$).

The positive endorcing effect of zinc on RMPP was noted ($P < .05$), and it would seem likely that RMPP was positively related to the daily gain.

References:

1. Mason, V.C. 1969. Some observations on the distribution and origin of nitrogen in sheep faeces. J. Agric. Sci. Camb. 73:99-111.
2. Storm, E., E.R. Orskov, and R. Smart. 1983. The nutritive value of rumen microorganisms in ruminants. 2. The apparent digestibility and net utilization of microbial N for growing lambs. Br. J. Nutr. 50:471-478.