UNITED STATES DEPARTMENT OF AGRICULTURE
Agricultural Research Service
Washington, D. C. 20250

February 24, 1964

TO Participants in Conference on Rumen Function

FROM H. W. Marston, Chairman of Conference, Agricultural Research Service, U. S. Department of Agriculture, Washington, D. C.

SUBJECT Report on Seventh Confernce on Rumen Function

Attached is a list of participants in and a copy of abstracts of papers presented at the Conference on Rumen Function held at the Hamilton Hotel, Chicago, Illinois, on December 4 and 5, 1963.

The Conference convened at 9:15 AM on December 4 and was concluded at approximately 2:30 PM on December 5.

The Chairmen of the several panels organized the program for their respective panels. They, together with the persons who presented papers, are commended for the excellent material made available to the group. Those attending the Conference are also commended for the interesting discussions that followed the presentation of the various papers. The Conference was pleased to again have a number of representatives from various institutions participate in our activity.

The participants agreed that a similar conference should be held in 1965 at approximately the same time of the year and also to be held in Chicago. The Chairman requested the group to select his successor but the only satisfaction he obtained was that if he found it impossible to attend the next meeting he select his own successor.

H. W. Marston

Enclosures - 2

CONFERENCE ON RUMEN FUNCTION
held at
Hamilton Hotel, Chicago, Illinois
December 4-5, 1963

The following persons were in attendance during the two-day meeting:

NAME	ORGANIZATION
Adams, John C.	Dept. Bacteriology, Iowa State Univ., Ames
Allen, R. S.	Dept. Biochemistry & Biophysics, Iowa State Univ., Ames
Allison, M. J.	National Animal Disease Laboratory, Ames, Iowa
Barnes, R. F.	Dept. Agronomy, Purdue Univ., Lafayette, Indiana
Bartley, E. E.	Dept. Dairy Sci., Kansas State Univ., Manhattan
Boyd, J. D.	230 W. Washington Sq., Philadelphia 4, Pa.
Brown, L. D.	Dairy Dept., Michigan State Univ., E. Lansing
Brown, Paul B.	Animal Sci. Dept., Louisiana State Univ., Baton Rouge
Buck, W. B.	National Animal Disease Laboratory, Ames, Iowa
Conrad, H. R.	Dairy Sci. Dept., Ohio Agri. Expt. Station, Wooster
Deese, D. C.	Dept. Veterinary Sci., Univ. of Wisconsin, Madison
Dehority, Burk A.	Dept. Animal Sci., Ohio Agri., Expt. Station, Wooster
Diven, R. H.	Abbott Laboratories, North Chicago, Illinois
Donefer, E.	Dept. Animal Sci., Macdonald College, Quebec, Canada
Dougherty, R. W.	National Animal Disease Laboratory, Ames, Iowa
Downee, H. G.	Physiological Sciences, Ontario Veterinary College, Guelph, Canada
Dyer, I. A.	Dept. Animal Sci., Washington State Univ., Pullman
Dziuk, H. E.	Dept. Vet. Medicine, Univ. Minnesota, St. Paul
Elam, C. J.	Consulting Nutritionist, Santa Ynez, California
Ellis, N. R.	Animal Husbandry Res. Div., ARS, USDA, Beltsville, Md.
Emery, Roy	Dairy Dept., Michigan State Univ., E. Lansing
Essig, H. W.	Dept. Animal Husbandry, Mississippi State Univ., State College
Espe, Dwight	Cooperative State Research Service, USDA, Washington, D.C.
Evans, J. V.	Cornell Univ., Ithaca, New York
Everett, J. P.	Ralston Purina Co., 8th & Chouteau Sts., St. Louis 2, Mo.
Fina, Louis R.	Bacteriology Dept., Kansas State Univ., Manhattan
Foote, L. E.	Dept. Veterinary Sci., Louisiana State Univ., Baton Rouge
Gessert, R. A.	The Upjohn Co., Kalamazoo, Michigan
Goetsch, D. D.	Dept. Physiology & Pharmacology, Oklahoma State Univ., Stillwater
Good, A. L.	College Veterinary Medicine, Univ. Minnesota, St. Paul
Graham, A. R.	Physiological Sciences, Ontario Veterinary College, Guelph, Canada
Grainger, R. B.	Monsanto Chemical Co., 800 N. Lindbergh, St. Louis 66, Mo.
Guidry, A. J.	Dept. Dairy Sci., Louisiana State Univ., Baton Rouge
Hartman, P. A.	Dept. Bacteriology, Iowa State Univ., Ames
Hammond, P. B.	Dept. Veterinary Physiology, Univ. Minnesota, St. Paul
Heaney, D. P.	Animal Research Institute, Canada Dept. of Agriculture, Ottawa
Hinds, F. A.	Dept. Animal Sci., Univ. of Illinois, Urbana
Hironaka, R.	Research Station, Lethbridge, Alberta, Canada
Hotchkiss, Don	Statistical Laboratory, Iowa State Univ., Ames
Ingalls, Ray	Dairy Dept., Michigan State Univ., E. Lansing

NAME	ORGANIZATION
Jacobson, D. R.	Dept. Dairy Sci., Univ. of Kentucky, Lexington
Jacobson, N. L.	Dept. Animal Sci., Iowa State Univ., Ames
Jones, B. L.	Abbott Laboratories, Waukegan, Illinois
Klavano, Paul A.	Washington State Univ., Pullman
Lee, A. M.	Animal Disease & Parasite Res. Div., ARS, USDA, Beltsville, Md.
Loomis, W. E.	Iowa State Univ., Ames
Marston, H. W.	Agricultural Research Service, USDA, Washington, D. C.
McArthur, J. M.	Research Station, Summerland, British Columbia, Canada
McCloud, D. E.	Crops Research Division, ARS, USDA, Beltsville, Md.
Miltimore, J. E.	Research Station, Summerland, British Columbia, Canada
Mitchell, G. E. Jr.	Dept. Animal Sci., Univ. of Kentucky, Lexington
Mullenax, C. H.	National Animal Disease Laboratory, Ames
Nichols, R. E.	Dept. Vet. Sci., Univ. of Wisconsin, Madison
Pedersen, M. W.	Crops Research Division, ARS, USDA, Logan, Utah
Phillips, G. D.	Dept. Animal Sci., Univ. of Manitoba, Winnipeg, Canada
Raine, A. P.	Eli Lilly & Co., Greenfield, Indiana
Richards, Clyde,	Cooperative State Research Service, USDA, Washington, D.C.
Roberts, W. K.	Univ. of Manitoba, Winnipeg, Canada
Roe, W. E.	Dept. Physiology, N. Y. State Veterinary College, Cornell Univ., Ithaca, N. Y.
Scheidy, S. F.	School of Veterinary Medicine, Univ. of Pennsylvania, Philadelphia
Sellers, A. F.	Department of Physiology, N. Y. State Veterinary College, Cornell Univ., Ithaca
Sibbald, I. R.	Research Dept., John Labatt Ltd., London, Ontario, Canada
Shazly, Khaled I.,	Bacteriology Dept., Univ. of California, Davis
Stevens, C. E.	Physiology Dept., N. Y. State Veterinary College, Cornell Univ., Ithaca
Stone, Edward J.	Dept. Dairy Sci., Louisiana State Univ., Baton Rouge
Swenson, Melvin J.	Dept. Physiology & Pharmacology, College Veterinary Medicine, Iowa State Univ., Ames
Synhorst, S. H.	Dept. Bacteriology & Biophysics, Iowa State Univ., Ames
Thomas, J. W.	Dairy Dept., Michigan Sate Univ., E. Lansing
Troelsen, J. G.	Experiment Farm, Swift Current, Saskatchewan, Canada
Underbjerg, G. K. L.	Dept. Physiology. College Veterinary Medicine, Kansas State Univ., Manhattan
Van Horn, H. H.	Animal Sci. Dept., Iowa State Univ.,Ames
Whiting, Frank	Research Branch, Canada Dept. of Agriculture, Ottawa
Williams, Grady F.	Mound Valley Branch, Kansas State Univ., Mound Valley
Wilsie, C. P.	Dept. Agronomy, Iowa State Univ., Ames
Worthington, Earl	Dept. Animal Sci., Iowa State Univ., Ames

REPORT ON
CONFERENCE ON RUMEN FUNCTION
held at
Hamilton Hotel, Chicago, Illinois
December 4-5, 1963

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

(a)	Agronomic	-	D. E. McCloud
(b)	Physio-Pathology	-	R. W. Dougherty
(c)	Animal Management	-	J. M. Boda
(d)	Rumen Physiology	-	N. L. Jacobson
(e)	Microbiology	-	C. K. Smith

(Drs. Boda and Smith were unable to attend the Conference)

AGRONOMIC PANEL

Varietal Differences in the Saponin Content of Alfalfa - M. W. Pedersen, U. S. Department of Agriculture, Logan, Utah, and G. Allan Taylor, Utah Agricultural Experiment Station, Logan

Toxic components are not uncommon in forage crops. For example, coumarin occurs in sweet clover, alkaloids in lupines, prussic acid in sudangrass, and saponin in alfalfa. The importance of toxic compounds in the crops mentioned except in alfalfa has been well established because of their extreme toxicity, which frequently results in sudden death to the grazing animal. Saponin is known to inhibit the growth of chicks, but a question remains in regards to its importance in hay and pasture for ruminants.

Samples of forage of the two varieties of alfalfa grown in replicate were harvested at weekly intervals throughout the 1962 growing season. The dry samples were separated into leaf and stem portions and analyzed for saponin by the carbon pyridine procedure. The relation of saponin percentage to leaf percentage was checked by covariance.

"F" values for saponin content in the foliage of these varieties were 113 for dates, 548 for varieties, and 15 for the variety by date interaction; by adjusting for leaf percentage, the values were changed to 38, 374, and 13 respectively. The appreciable reduction in the "F" value for dates indicates that at least part of the change in saponin content was accounted for by a change in leaf percentage. Although there was a reduction in the "F" value for varieties, the residual value was high enough to confirm a true varietal difference in saponin content. The reduction in the "F" value for the interaction of varieties by dates was very small.

The average saponin contents of the leaves were 2.13, 3.84, and 3.37 for the first, second, and third crops respectively. The saponin content of the leaves of Lahontan alfalfa was 2.26 compared with 3.43 for DuPuits.

The saponin contents of the stems were 0.94, 1.56, and 1.46 for the first, second, and third crops respectively. For the entire season, Lahontan stems averaged 1.11 percent saponin compared with 1.33 for Du Puits.

The season-average saponin contents of the foliage (both stems and leaves) were 1.82 for Lahontan and 2.58 for Du Puits.

Highest saponin values were obtained on the earliest dates of harvest for the second and third crops. Saponin content declined as the plants became older. This trend was not observed on the first crop, harvested on May 10. Possibly high saponin values occur earlier in the season. This is supporting evidence for the saponin factor in bloat.

The failure of the two varieties to have a similar pattern of saponin values for the different harvest dates resulted in a significant date by variety interaction. As Du Puits matures faster than Lahontan, this may have been responsible.

Saponin content in the foliage is controlled to a large extent by the percentage of leaves because of the high percentage of saponin in the leaves. The decrease in saponin content associated with maturity is linked with the percentage of leaves.

A correlation of .84 between percent saponin in the foliage and percent leaves was obtained by using values from both varieties for the season. Thus, a sample with 40 percent leaves would have a calculated saponin content of 1.02 percent compared with a sample with 60 percent leaves which would have a calculated value of 2.36 percent saponin.

Looking at the problem from the plant breeder's viewpoint, we are in the fortunate position of having naturally occurring breeding material with a wide range in contents of a toxic substance, but are in the unfortunate position of not having adequate evidence on varietal comparisons for toxicity. A full scale bloat test of the two varieties is needed in order to evaluate properly the importance of the differences in saponin content.

Foaming Properties of Selected Varieties of Alfalfa - S. H. Synhorst, D. K. Hotchkiss, R. E. Worthington, C. P. Wilsie and R. S. Allen, Iowa State University, Ames.

Alfalfa varietal differences and the effect of nitrogen fertilization on the foaming properties of 4-inch tops, as measured on a static foam meter, were studied during the 1962 growing season. In addition, the influence of solar radiation, moisture stress, and growth period were considered. The twelve winter-hardy varieties used in this work were selected to include a wide range of genetic diversity. All varieties were grown in a similar environment with half of each plot being fertilized with nitrogen before the first and third growth periods.

The basic test was an in vitro measurement of the foam stability of buffer aqueous extracts of 4-inch alfalfa tops using static foam meters under standard conditions as described by Pressey et al. (J. Animal Sci., 22:970-79, 1963).

Forage samples were processed 6 days per week during the first, second, and third growth periods. Twelve varieties were studied during the first and second growth periods; supplementary nitrogen fertilizer (ammonium nitrate, 120 lbs N/acre) was added to four of these varieties prior to the first growth period. During the third growth period, direct comparisons of the fertilized (200 lbs N/acre applied after the second cutting) and non-fertilized plants of eight varieties were made.

Statistical analysis was accomplished by least squares analysis necessitated by missing observations in the original partially balanced imcomplete block design. Most varieties (Ranger, Narragansett, Arnim, Cody, Vernal) showed a significant ($P < .05$) increase in foaming potential with nitrogen fertilization; however, others (Travois, Culver) showed virtually no change due to fertilization. Differences were accentuated during periods of rapid growth. During the first and second growth periods Alfa showed a significantly ($P < .05$) lower foaming potential than other non-fertilized varieties, while Cody had a foaming potential significantly higher ($P < .05$) than other varieties studied.

Adjustment for varictal differences was accomplished by covariance to permit an evaluation of the influence of environmental factors. Any influence of soil moisture stress on foaming scores was not detectable in this study. This may be attributed to the limited number of days when moisture stress occurred.

A significant quadratic trend in foaming potential over stage of growth was noted during both the first and second cuttings, shown as a general increase in foam potential up to plant differentiation for flowering, followed by a rapid decline. This trend occurred during a much shorter period of time for the second cutting as compared to the first.

Soluble Protein Content of Alfalfa and Birdsfoot Trefoil - S. H. Synhorst, C. P. Wilsie, and H. E. Worthington, Iowa State University, Ames

Samples of 4-inch tops of Alfa alfalfa (Medicago sativa) and Viking birdsfoot trefoil (Lotus corniculatus), grown on adjacent plots, were analyzed for Kjeldahl nitrogen, soluble protein nitrogen, and dry matter. The stability of foam produced from buffered aqueous extracts was also determined using the static foam meter described by Pressey et al., (J. Animal Sci., 22:970, 1963).

Sampling was initiated when the plants had attained a height of 6 inches following the first cutting, and was continued for a period of 26 days. The samples were collected at 9 a.m., with processing starting within an hour.

A Serval Omni-Mixer was used to blend 25 g. of fresh plant material in 60 ml. of 0.2 M, pH 6.5 phosphate buffer for 2.5 min. at 39°C. The resulting slurry was strained through cheese cloth and centrifuged for 5 min. at 1000 x g; the supernatant was used in measuring the foam potential. An additional aliquot of the supernatant was centrifuged for an additional 30 min. at 30,000 x g and an aliquot of the resulting supernatant analyzed for Kjeldahl nitrogen. A second aliquot of the latter supernatant was treated with an equal volume of 20% trichloroacetic acid, centrifuged to remove precipitated protein, and analyzed for Kjeldahl nitrogen. The difference between the two nitrogen values was accepted as the value for protein nitrogen. Total Kjeldahl nitrogen was determined on material which had been dried for 18 hours at 110°C. and subsequently ground in a Wiley mill.

The Kjeldahl nitrogen content of alfalfa varied from 5.5% on a dry weight basis (0.9%, fresh weight basis) at the beginning of the test period to about 3.5% on a dry weight basis (0.7%, fresh weight basis) near the end of the period. The soluble protein content of alfalfa, which was approximately 9.6% of the dry weight (1.5% fresh weight basis) at the beginning of the test period, increased to about 12% of the dry weight (3%, fresh weight basis) during the bud stage, and declined thereafter to approximately 9% of the dry weight (2.2%, fresh weight basis) near the end of the test period. Foaming potential also reached a maximum during the bud stage and declined thereafter.

The Kjeldahl nitrogen content of birdsfoot trefoil varied from an initial value of approximately 5% of the dry weight (0.7%, fresh weight basis) to about 2.5% of the dry weight (0.6%, fresh weight basis) near the end of the test period. The initial soluble protein content of birdsfoot trefoil was about 3% of the dry weight (0.5%, fresh weight basis) but declined to trace amounts during the first 4 days of the test period and remained at this low level for the remainder of the period. The foaming potential of the birdsfoot trefoil samples also declined quite rapidly, and during the latter half of the test period frequently failed to produce measurable stable foam.

The Use of In Vitro Techniques for Estimating Forage Digestibility and Intake - R. F. Barnes, Indiana Agricultural Experiment Station, Lafayette

The development of reliable laboratory methods for estimating forage quality is a most challenging problem The use of in vitro rumen fermentation techniques for determining the digestibility and intake for forages has advanced extraordinarily since 1955. The in vitro techniques attempt to simulate the digestive process in the rumen by which structural carbohydrates are digested and converted into soluble products by enzymes of the rumen microorganisms. The main criteria for the in vitro analyses are dry matter and cellulose disappearance, although gas production and volatile fatty acid production are also utilized. The combination of in vitro fermentation and enzymatic breakdown by pepsin has also shown considerable promise. The precision or reproducibility of the in vitro method is one of its greatest problems, and a standard forage is usually employed in an attempt to control some of the variability. The accuracy of predicting in vivo results is approximately $\pm$ 2.0 digestibility units and $\pm$ 5.0 intake or digestible potential units (e.g. nutritive value index). One of the greatest deterents to the accuracy of the in vitro methods is the large variability inherent with the in vivo measurements, upon which the in vitro results must be based. The in vitro rumen fermentation technique appears best adapted to the classification of the digestibility or intake of forages relative to one another and where differences are marked.

Estimation of the Digestibility and Nutritive Value of Forages by Cellulose and Dry Matter Solubility Methods - Burk A. Dehority and Ronald R. Johnson, Ohio Agricultural Experiment Station, Wooster

Based on the solubility of forage cellulose in cupriethylene diamine (CED) a laboratory method was developed which showed a remarkably close association between the amount of cellulose dissolved from a grass hay and its in vivo dry matter digestibility (DMD), cellulose digestibility (CD), and energy digestibility (ED). However, attempts to apply this method to legume samples were unsuccessful. Subsequent studies revealed that if the legume were extracted with 1.0 N H_2SO_4 prior to treatment with CED, the amount of cellulose dissolved approximated the amount of cellulose digested by rumen bacteria in vitro. Using this "improved" CED method, fairly high correlations were obtained with DMD, CD, and ED for grasses, legumes and mixed forages. On the other hand, correlations with relative intake (RI) and nutritive value index (NVI) were not high enough to be of practical use. In hopes that the readily soluble material from a forage might be more closely associated with relative intake, the dry matter solubility of forages in 1.0 N H_2SO_4 was determined (DMS method). This laboratory value proved to be more closely associated with relative intake. In vivo digestibility trials were conducted on a total of 65 forages (22 grasses, 26 mixed forages and 17 samples of alfalfa), and these data were correlated with CED values, DMS values, and the product of CEDxDMS values. The data were correlated with each class of forages separately and for all forages together. The highest correlations obtained for the combined 65 forages, and the method giving this correlation were: DMD, 0.87 (CEDxDMS); CD, 0.89 (CED); ED, 0.87 (CEDxDMS); NVI, 0.79 (DMS); and RI, 0.75 (DMS).

A Comparison of the True Protein and Element Contents of Alfalfa Hay from Bloating and Non-Bloating Farms - J. E. Miltimore, J. M. McArthur, J. L. Mason and R. B. Carson, Canada Department of Agriculture, Summerland, British Columbia

Alfalfa samples at the hay stage of development were harvested by hand from 35 farms where bloat was a serious problem and from 38 farms where bloat did not occur even on lush legume pastures. The samples were analyzed for nitrogen, phosphorus, potassium, sulphur, calcium, magnesium and true protein. There were no differences in the mean contents of these constituents between bloating and non-bloating farms. There were wide ranges within both types of farm; true protein for example varied from 7.3 to 14.6 per cent in alfalfa from bloating farms, with a similar range from 7.7 to 16.9 per cent from non-bloating farms. Calculated digestible dry matter content using the equation, 16.4 + (11.8 x % N) + (34.2 x % P + (13.2 x % S) + (.204 x % K), varied from 52 to 77 per cent with similar means for both types of farm. Correlation coefficients revealed a significant association between nitrogen and phosphorus (.488) in alfalfa from non-bloating farms. There were significant associations between sulphur and nitrogen (.366) and between sulphur and protein (.707) in alfalfa from bloating farms.

These data do not provide information on which to base correctivefertilizer practices where bloat is a problem. In order to clarify the interactions between the elements suggested by differences in association between minerals observed in this study, further study is warranted.

PHYSIO-PATHOLOGY PANEL

Effects on Blood Flow to the Reticulorumen of Absorption, Motility Changes and Other Factors - A. F. Sellers, New York State Veterinary College, Cornell University, Ithaca

The right ruminal artery is a major supply for the posterior and ventral parts of the rumen. Chronic implantation of blood flow probes in 14 adult dairy cattle, for periods of 3 weeks to 3 months, allowed use of unanesthetized animals for study of relative changes in flow of blood during imposition of changes in contents and motility of the reticulorumen. Estimates of absolute flow were made after applying a correction for sensitivity change with impedance change, and utilizing 3 independent zero-setting procedures. Calibration of the flow probes was *in situ* at autopsy and *in vivo* in some animals.

Transport of Sodium and Chloride by the Isolated Rumen Epithelium - C. E. Stevens, New York State Veterinary College, Cornell University, Ithaca

A study was made of Na and Cl transport across the isolated rumen epithelium of the cow and goat. The transepithelial potential and the current necessary to completely short-circuit this potential were measured. From these tissue resistance was determined. Early experiments showed that the procedure for collecting tissue greatly affected resistance and had much less effect on the magnitude of the current. Net Na and Cl transport were measured in the absence of electrochemical gradients and the rumen epithelium of both species demonstrated an active transport of both ions in the direction of lumen to blood. Short-circuit current and net ion flux decreased with time, but the decrease was sufficiently slow and linear to allow a comparison of generated tissue current and transport of the two ions studied.

Electrolyte Absorption from the Small Intestine of Sheep and Calves - W. E. Roe, New York State Veterinary College, Cornell University, Ithaca

The net fluxes of sodium, potassium chloride, and total CO_2 were studied in the small intestine of sheep and calves. Chronic isolated intestinal segments (Thiry-Vella fistulae) were used as the experimental preparation. A balanced electrolyte solution, including methyl cellulose as a volume marker, was inserted into the fistulae for a standard interval of time. Net fluxes then were determined by difference. One-way fluxes of sodium also were determined using Na^{24} in the experimental influsion solution. A variation of the electrolyte absorption pattern along the small intestine was found. Administration of desoxycorticosterone at levels significantly altering salivary electrolyte constituents had no demonstrable effect upon intestinal absorption.

Detection of Reticulo-Ruminal Contraction in Sheep by Skin Surface and Implanted Visceral Electrodes - Arthur R. Graham, Ontario Veterinary College, Guelph, Ontario

Patterns of electrical potential change associated with reticulo-ruminal contraction were surveyed using a grid of electrodes applied to the surface of the skin on the left side and belly. Visceral electrodes were aseptically

implanted in the walls of the reticulum, rumen and abomasum in one animal and the electrode leads exteriorized; some of these electrodes were functional for more than two months. Simultaneous records from skin surface and visceral electrodes showed there is a good correspondence in the wave form of activity recorded from visceral electrodes and from the electrodes on the skin overlying the reticulum and rumen; major deflections can often be correlated with intraruminal pressure changes recorded from a rumen fistula.

Significant deflections (75-500 uv.) in the electrical records correspond to major contractions of the reticulum and rumen. During rumination, reticular skin records show a sequence of three deflections; the bolus reaches the mouth in the interval between the first and second deflection. Immediately following the third reticular deflection one or two major deflections occur in the ruminal electrical records. The "first" reticular electrical deflection is not evident in the non-ruminating animal. There is a significant increase in intraruminal pressure during the first major ruminal electrial deflection; smaller increases may coincide with secondary deflections in the ruminal electrical records.

Preliminary investigations suggest similar results can be obtained in the cow and goat.

Some of the Enzymatic Aspects of Bloat - Dawson Deese and Roy E. Nichols Wisconsin Agricultural Experiment Station, Madison

The possibility that the chief cause of legume bloat lies in a phase or phases of the enzymatic ruminal digestion of the legumes when they are consumed in the fresh growing state is considered. Evidence is presented in favor of this approach. The enzymic system receiving major emphasis is the pectin methyl esterase system. Additionally, an effective control of the bloating process by the use of an enzyme inhibitor is discussed.

A Quantitative Study of Rumen Microorganisms Aerosolized and Transported to the Respiratory System During Eructation - C. H. Mullenax, M. J. Allison, and J. R. Songer, National Animal Disease Laboratory, Ames, Iowa

It has been shown that eructated gases penetrate deeply into the respiratory system. This study was undertaken to quantitate microorganism transfer by this means from the reticulorumen to the respiratory systems of cattle and sheep.

Eructated gas was collected from the fistulated trachea by means of a cuffed endotracheal catheter to which were attached various collection devices (bags, impingers or filters). A variety of traps and/or pre-impingers was used to preclude contamination of samples with secretions from the trachea.

Aerosolized organisms were collected in anaerobic dilution fluid. To maintain their viability, it was necessary to keep dissolved oxygen at a minimum by bubbling CO_2 through the fluid during collection. Under these conditions, colony counts in an anaerobic rumen fluid medium indicated a concentration of 450 to 500 viable organisms per liter of eructated gas.

Escherichica coli B T-3 bacteriophage, injected through the rumen wall, were recovered from eructated gas within 30 minutes and persisted in the rumen at high levels for 24 hours. Rumen juice samples were negative for phage 48 hours postinjection. No viable phage were recovered from fecal samples collected at intervals up to 72 hours after rumen inoculation.

Attempts will be made to determine the depth of penetration of microorganisms into the lungs by culturing homogenized suspensions of lung tissue.

Intestinal Obstruction in Cattle - H. E. Dziuk, P. B. Hammond and E. A. Usenik - Veterinary Medicine, University of Minnesota, St. Paul

Ligation of the duodenum of calves resulted in hypochloremia, hypokalemia, alkalosis, dehydration, weakness, anorexia, and prostration. Plasma alterations and symptoms were not as severe following colonic ligation. Determinations of water and electrolyte concentrations in the ruminoreticulum and abomasum and of the NAAP and SCN spaces in fistulated calves with duodenal obstruction indicated that plasma alterations resulted from an accumulation of water and chloride ions in the stomach compartments. Limited studies of the possible causes for the hypokalemia resulting from duodenal ligation were done by estimation of total exchangeable body potassium and renal excretion of potassium. Although renal potassium loss occurred during obstruction, the hypokalemia could not be satisfactorily explained on this basis.

Effect of Ruminal Insufflation on the Cerebral Circulation of the Goat - Lloyd E. Davis, Veterinary Medicine, University of Missouri, Columbia

The effects of nitrogen insufflation of the cannulated rumen were studied in ten goats. The parameters which were measured included cerebral blood flow, cerebral vascular resistance, cerebral oxygen utilization, mean carotid pressure, torcular pressure, cerebrospinal fluid pressure, blood-gas contents, packed cell volume and hemoglobin concentration. Increased rumen pressure had little effect on cerebral blood flow and cerebral vascular resistance. The cerebral oxygen consumption was markedly reduced as a result of increased intraruminal pressure. This decrease was caused by a marked reduction in the arterial oxygen content and a consequent decrease in the cerebral arteriovenous oxygen difference. The mean arterial, torcular and cerebrospinal fluid pressures were greatly elevated as a result of the increased intraruminal pressure.

It was concluded on the basis of the experimental evidence that the mental changes which are observed in ruminant animals suffering from acute ruminal tympany may be attributed to cerebral hypoxia. The general nature of the hypertensive changes which are associated with increased introruminal pressure were demonstrated but due to multiple factors influencing the various fluid compartments, no general conclusions could be made as regards the mechanism of these pressor responses. On the basis of pilot studies in a limited number of subjects, it was suggested that mechanical factors played a major role in the pressure responses.

Normal Values of Rumen Motility - P. A. Klavano, Washington State University, Pullman

Three hundred and twenty-six experiments on 21 animals totaling 766 hours were analyzed. The mean rate during rumination was 8.63 ± 1.35/5 min., during non-rumination it was 7.79 ± 1.55/5 min. There was a significant difference between animals and between experiments for an individual animal. Length of time in harness or time of day were not significant. Rate during rumination increased slightly with age but not the non-ruminating rate.

The Physiology of Eructation in Ruminants. Recent Work on the Physiological Disposition of Eructated Gases - R. W. Dougherty, C. H. Mullenax and M. J. Allison, National Animal Disease Laboratory, Ames, Iowa

Small quantities of $C_{14}O_2$ were insufflated into the rumens of sheep with rumen and tracheal fistulas and carotid artery cannulas.

High counts appeared in the continuously sampled arterial blood a few seconds after eructation. High counts also appeared very quickly in continuously sampled parotid saliva.

In goats, high counts appeared in the milk in 3 minutes and in the lactose of milk in 20 minutes.

The same procedures were applied to intraruminal insufflations of $C_{14}H_4$. Relatively high counts appeared in blood after eructation and in some counts appeared in the milk.

Blocking the entrance of labeled eructated gas into the lungs permitted only small counts to appear in the blood.

Effect of Feeding High Saponin Alfalfa Hay to Four Month Old Holstein-Friesian Bulls - Wayne Binns and Marion W. Pedersen, Animal Disease and Parasite Research Division and Crops Research Division, Agricultural Research Service, U. S. Department of Agriculture, Logan, Utah

DuPuits variety of alfalfa hay containing 2.62% saponin and Lahontan variety with 1.72% saponin were each fed to a separate group of four Holstein-Friesian bull calves for six months. The hay was chopped and fed free choice to each group with no other supplements added to the ration.

The amount of hay eaten by each group was recorded daily and every fourteen days the bromsulfalein liver test was run on each animal and blood samples were obtained for the determination of blood glucose, red blood cell volume, blood cholesterol, and blood protein fractions.

The average value in each group for red blood cell volume, blood cholesterol, blood glucose, blood protein functions and bromsulfalein liver function tests remained within the normal range throughout the feeding trial. No abnormal clinical signs of excess salivation, regurgitation, anorexia, bloating, diarrhea, or indigestion was observed. The appetite of all animals remained good and they made normal increases in body weight and growth.

ANIMAL MANAGEMENT PANEL

Sugar Cane Bagasse - Blackstrap Molasses Rations for Beef Cattle - Paul B. Brown, Louisiana State University and Agricultural and Mechanical College, Baton Rouge

Seven feeding trials were conducted in which dehydrated sugar cane bagasse and blackstrap molasses rations were fed to beef cattle. Four roughages - sugar cane bagasse, grass hay, rice straw and cottonseed hulls - were compared in rations fed to 650 lb. beef steers in a 154-day feeding trial. The roughages comprised 62.5%; and the molasses, 20% of the rations. The daily gains, in lbs., were .88, 1.51, 1.88, and 1.49, in the order listed above. In the second trial, the same roughages composed 27.5%; and the molasses, 40% of the rations. They were fed to 1000 lb. beef cows at the rate of 8 lbs/cow/day on winter range, an amount calculated to supply 40% of the daily feed requirement with the range furnishing the remaining 60%. The cows completed the test in excellent condition. In the remaining five trials, only bagasse - molasses combinations were fed in drylot to 500-600 lb. steers for 112 to 140 days. The combinations ranged from 45% bagasse - 25% molasses to 20% bagasse - 50% molasses, for a total of 70% of the ration. The remaining feed ingredients, 30%, supplied additional protein, energy, vitamins, and minerals. In the last two trials, a 20% bagasse - 50% molasses ration combination, supplemented with diethylstilbestrol (DES), and chlortetracycline (Aureomycin), was fed in each trial. The treatments were: I - basal ration; II - basal plus 10 mg. DES/steer/day; III - basal plus 75 mg. Aureomycin/steer/day; and IV - basal plus 10 mg. DES plus 75 mg. Aureomycin/steer/day. The daily gains, lbs., for the two trials were: 1.71, 1.74; 1.98, 1.51; 1.60, 1.28; and 1.95, 1.63, for the respective treatments. The differences in daily gains between treatments within each of the trials were not statistically significant.

RUMEN PHYSIOLOGY PANEL

Measuring the Flow of Digesta from the Abomasum of Sheep - G. D. Phillips and G. W. Dyck, University of Manitoba, Winnipeg

A method has been developed for measuring the flow of digesta from the abomasum of sheep, based on a market dilution technique. A solution of Polyethylene glycol 4000 was continuously infused at a constant rate into the abomasum via a permanent fistula, and the dilution of this marker was determined in samples of digesta taken at intervals from a duodenal cannula. In 16 digestibility trials using 4 sheep on 4 rations, abomasal flow measurements were made by infusing PEG for 6 days and taking samples of duodenal digesta at hourly intervals during the last 5 days. Fecal collections were made at the same time. In the rations, starch was substituted for straw to give two levels of readily fermentable carbohydrate, and urea was added to give two levels of nitrogen, in a factorial arrangement. The sheep were fed once daily. There was diurnal variation in the volume of abomasal outflow, with the highest flow rates occurring at, or just prior to, feeding, and the lowest rates appearing about 9-12 hours after feeding. Analysis for PEG of hourly fecal collections on the fourth day of each trial also showed diurnal variations in PEG concentration in feces, thus indirectly

confirming the abomasal outflow results. Furthermore, there was diurnal variation in the weight of feces produced, the highest output occurring concurrently with highest abomasal flow rates.

The average of the coefficients of variance of the mean flow rates was 4.8%. Covariance analysis of the mean hourly flow for each animal on each ration showed that volume of abomasal outflow was affected by both the composition of the rations and the quantity consumed. After adjusting for quantity of food consumed, the highest average flow was obtained with the high straw - low urea ration, followed in descending order by high straw - high urea, high starch - low urea, high starch - high urea rations.

Further statistical analysis of the data indicated that a modified schedule for sampling duodenal contents would result in reasonably reliable estimates of average flow. Samples would be collected 4 times a day, one sample being taken at an hour selected at random within each of four 6-hour segments of the day, for a total of 10 days. Thus, the total number of samples taken would be reduced to one-third, while the sampling period would be increased to a more desirable length, compared with the present experiment. In such an experiment, estimation of abomasal outflow could be determined with minimum disturbance to the animals.

Effects of Feeding Vermiculite on Ion Concentrations in the Rumen - R. S. Emery and D. H. Steyert, Michigan State University, East Lansing

Preliminary studies were conducted to determine the effect of feeding 0.5 lb. special verxite #4 (Zonalite feed grade vermiculite specially asphalt coated) on feed-lot type bloat. Twin Holstein cows with rumen fistulas were fed 16 lb. of grain (corn, soybean oil meal and minerals) and 4 lb. hay once per day until frothy bloating was initiated. Bloating was observed 4 to 5 days per week over the next 3 weeks and rated by the scale of Bartley and Yadava (J. Animal Sci., 20:648, 1961). Verxite (0.5 lb./day) was then added to the ration for one twin and observations were continued for an additional 3 weeks.

Table 1. Incidence of Bloat

Cow	T19 (Verxite)				T20 (Control)			
Bloat severity	0	1	2	3	0	1	2	3
	(No. of observations)				(No. of observations)			
Control period	2	3	7	1	0	5	4	4
Test period	0	8	5	2	0	0	11	4

The verxite appeared to float on the surface of the rumen contents for not more than 5 hours. Bloat control was negligible. Similar observations were made with three other cows fed regular verxite #4 and results were comparable. Acid insoluble ash was measured in the rumen of one cow and increased from 2% before verxite feeding to 4% after feeding verxite for 10 days. The verxite did not produce a measurable change in the ruminal total ash or dry matter.

The primary study consisted of three 2-week periods with 0.5 lb. of verxite #3 being fed in alternate periods. One pair of Holstein cows with rumen fistulas was fed a ration of 15 lb. alfalfa-brome hay plus 10 lb. grain and a second pair was fed a ration of 2 lb. hay plus 18 lb. grain. One member of each pair started the trial with the verxite treatment while the other member started with the control treatment. The cows were fed between 7 a.m. and 8 a.m. Rumen contents were mixed in situ and sampled at 10:30 a.m., 12:30 p.m. and 6:30 p.m. on the last day of each period. Samples were centrifuged at 2000 RPM for 10 minutes and the supernatant adjusted to pH 2.

The verxite feeding apparently depressed ruminal pH and ammonia concentrations. Sodium ion activity (as measured by sodium sensitive electrode) was depressed at 12:30 and 6:30 by feeding verxite but this trend was reversed at 10:30 (P = 0.12). Calcium ion concentration may have been somewhat depressed with the higher hay ration plus verxite but the trend was reversed with the restricted roughage ration.

Table 2. Concentrations of several constituents in the rumen (mean of 18 values)

	Verxite	Control	P
pH	6.23	6.35	0.15
Ammonia-N (mg/100 ml)	20.0	23.8	0.30
Sodium (mg/100 ml)	107.0	109.0	0.50
Calcium (mg/100 ml)	12.6	12.9	0.50
Lactic acid (mg/100 ml)	1.0	1.0	0.50
Total volatile fatty acids (uM/ml)	128.0	122.0	0.40
Acetic acid (Molar %)	64.0	65.0	----

Chemical Control of Appetite - D. R. Jacobson, University of Kentucky, Lexington

The correlations with voluntary intake obtained from 19 lactating Holstein cows fed good quality alfalfa hay ad libitum for 5 weeks were 0.11, for milk production; 0.17, for body weight; and 0.16, for barrel circumference. Multiple correlation of milk production and body weight with dry matter intake was 0.56.

Four sets of identical twins used in 20 trials of intravenous infusion of saline to the control and metabolite to the treatment two 8 hours for 3 days provided data which show that acetate and propionate administered at levels corresponding to normal rumen production cause a cessation of prehensive activity and voluntary intake.

It has subsequently been shown that the rumen C_2, C_3, and C_4 volatile fatty acids increase in concentration in the rumen, and that the C_2 and C_3 acids as a percent of C_2 to C_5 acids increase, following the consumption of hay. Blood acetate at 0.88 mmoles/liter before eating increased significantly to 1.32 mmoles/liter after eating, and then dropped to 1.13 mmoles/liter three hours later.

Diluting the ration with inert polyethylene cubes did not significantly influence voluntary intake of nutrient-containing dry matter, nor did the addition of cubes in a nylon mesh container or water in a rubber bag tied to the fistula plug reduce voluntary intake of dry matter. However, removal of rumen contents after eating did cause a significant increase in feed intake.

All these observations point strongly to a mechanism of control of feed intake involving a chemoreceptor response to threshold levels of blood metabolites which is mediated by the central nervous system.

A Technique for Studying the Rate of Cellulose Digestion in the Rumen Using C^{14} Labeled Alfalfa - C. L. Alexander, E. E. Bartley, L. R. Fina, C. L. Keith, J. L. Morrill, R. N. Meyer and E. L. Sorensen, Kansas State University, Manhattan

It has been difficult to determine when cellulose is digested in the rumen because cellulose from previous feedings may be present in the rumen at any given time. A good estimate of the rate of cellulose digestion might be obtained by feeding a common roughage such as alfalfa hay in which only the cellulose is labeled with C^{14}. Since selective labeling of the components of a roughage is presently not possible, studies were initiated with C^{14} labeled alfalfa hay to determine how the unaltered plant is degraded in the rumen. The criterion used to determine the rate of degradation was the detection of C^{14} activity in the rumen VFA. It was recognized that there would be labeled VFA resulting from the degradation of the noncellulose portion of the labeled hay. Therefore, activity in the VFA would indicate the rate of breakdown of alfalfa hay but not necessarily of cellulose. However, if cellulose breakdown proceeds at a rate slower than that of the more available nutrients, it should be possible to obtain an estimate of the rate of cellulose degradation in the rumen.

In preliminary trials, labeled hay was placed in the rumens of fistulated cows fed unlabeled alfalfa hay initially and at 12-hour intervals. An initial major peak in specific activity of VFA occurred between 1 and 12 hours and a second smaller peak at about 37 hours. It was assumed that the first peak was from soluble carbohydrates and the second from insolubles such as cellulose. To test this, labeled hay was partitioned into a hot water-soluble extract and an insoluble residue. Specific activity peaked 1 hour after feeding the water-soluble extract and between 1 and 6 hours after feeding the insoluble residue. The decline in specific activity before the next feeding may have resulted from dilution by VFA produced from feed residues from previous feedings. To test this, a rumen was emptied and refilled with labeled hay, a normal feeding of unlabeled hay, and strained rumen fluid. The animal was fasted for 48 hours. Specific activity peaked between 3 and 12 hours, then declined for 48 hours. Drop in specific activity may have

been due to labeled hay being degraded at a different rate from unlabeled hay. However, labeled hay and unlabeled hay fermented in vitro at similar rates. Work is in progress to determine why specific activity declines. Since specific activity apparently was not a suitable measure for studying the rate of cellulose breakdown, a measure of total activity was obtained by using polyethylene glycol as a means of estimating the total weight of rumen content. Total activity curves of VFA and specific activity were similar. After 48 hours the animal was fed unlabeled hay and specific activity dropped precipitously. The total activity curve was unchanged.

It was concluded that total activity was a more reliable measure than specific activity for indicating the rate of digestion of alfalfa hay. However, measurement of total activity did not appear to be suitable for determining when cellulose is specifically degraded in the rumen. Consequently, a method was developed which determined the total amount of activity in cellulose present in the rumen at regular intervals.

By using PEG to estimate the weight of rumen contents and by extracting cellulose from the rumen at regular intervals, it was possible to determine the C^{14} activity of cellulose present in the rumen following a single feeding of labeled hay. The degradation of cellulose commenced shortly after the feeding of the labeled hay, and then proceeded at a rapid rate for the first 24 hours. At 36 hours, the digestion of cellulose ceased.

Because the animal was fed only one feeding of hay, the rate of cellulose digestion indicated above would not be normal since the microorganisms did not have available cellulose from previous or subsequent feedings. It would appear that this technique might be used in determining the rate of cellulose breakdown in the rumen when an animal is fed normally provided an accurate estimate of the weight of rumen contents can be obtained at each sampling period.

A Protein Foaming Agent in Alfalfa and Its Significance in Pasture Bloat-
J. M. McArthur, J. E. Miltimore and M. J. Pratt, Canada Department of Agriculture Research Station, Summerland, British Columbia

When cattle bloated on alfalfa the in vivo rumen pH was 5.4 to 5.8. This coincides with the optimum pH for viscosity of foams stabilized by alfalfa protein. Observations on the development of bloat in fistulated cattle led to the conclusion that the significant properties of the foams are their stability and yield stress, i.e. force required to make the foam flow.

The foam stabilizing protein was obtained from alfalfa leaves by disintegrating in pH 7 phosphate buffer, precipitating with sodium sulfate, and chromatographing a solution of the precipitate in phosphate buffer on agar gel. Examination of the protein by ultracentrifuge and electrophoresis indicated that it was monodisperse. The molecular weight calculated from the sedimentation and diffusion coefficients was 515,000. This indicates that it is Fraction I protein. Examination of crude alfalfa extract confirmed that it was Fraction I. Electron microscope and streaming birefringence examination of the protein indicated that the molecule was almost spherical. The protein is precipitated from solution by foaming, indicating that it is surface denatured. The alfalfa sedimentation pattern appeared to be the same as that of Ladino clover. However, a comparison of grass and alfalfa indicated that the grass Fraction I was a little larger.

The above indicates that the protein adsorbs at the surface where it denatures and stabilizes the bubble films. The rate of denaturation depends upon the pH of the solution. The amount of denatured protein on the surface is determined by the rate of denaturation and rate of coagulation. The rate of coagulation is affected by mechanical agitation of the films. Thus, foaming and mechanical agitation destroy the foaming properties of the solution.

Rideal states that the stability of a foam is a function of the drainage rate; the extension of the films due to shock, the compressional surface modulus, the flow rate of the surface monolayer, and the adsorption rate. These in turn are affected by a number of factors such as: Bulk and surface viscosities, solute concentration, suspended solids, pH, rates of formation and removal, optimum concentrations, etc. Because these factors all have an effect on foam formation, it would appear unlikely that a high correlation would be found between any one factor and bloat.

Metabolism of Tryptophan by Normal and Bloater Cattle - I. A. Dyer, R. J. Johnson, Constance Richards, Judith Templeton and John Clark, Washington State University, Pullman

Metabolic pathways of amino acids in the ruminant have not been elucidated. Because of the metabolic complexity of this intact organism, accepted pathways for simpler species have been assumed to be applicable also for the ruminant. The normal bovine has been reported to secrete higher levels of epinephrine, measured both as epinephrine and as 3-methoxy-4-hydroxy mandelic acid, than the bovine subject to tympanites (Dyer et al., 1962). In previous tests, the tympanitic bovine was unable to secrete as much epinephrine, even under stress, as the normal bovine. The normal and tympanitic bovine appear to differ in certain metabolic processes, thus simultaneous determinations of the different measurements were made on the normal and tympanitic bovine.

In a series of experiments, metabolites of tryptophan were measured in urine from two normal (28 samples) and two tympanitic (47 samples) bovine. Metabolites of tryptophan included urinary 5-hydroxy indoleacetic acid (Underfriend and Weisbach, 1955), 3-methoxy-4-hydroxy mandelic acid (Sunderman et al., 1960) and tryptophan (Greenstein and Winitz, 1961). In addition, 13 μc 3-C^{14} tryptophan was administered intramuscularly and the urinary excretory pattern of C^{14} measured.

Excretion of certain tryptophan metabolites, free tryptophan are presented in Table 1.

Table 1. Urinary 5-hydroxy indoleacetic acid, 3-methoxy-4-hydroxy mandelic acid and tryptophan excretion of normal and tympanitic bovine.

Comparisons	BOVINE	
	Normal	Tympanitic
Indoleacetic acid, mg/ml.	0.52	1.06
Mandelic acid: creatinine ratio, mcg/mg.	2.63	1.58
Indoleacetic acid: creatinine ratio, mg/mg.	0.52	0.73
Tryptophan, mg/ml.	0.27	0.33

The above data corroborates an earlier report by Dyer et al. (1962) that mandelic acid excretion is reduced in the tympanitic bovine. This difference in tryptophan metabolism might implicate an impairment in the tryptophan pyrrolase system in the tympanitic bovine. Oral administration of 50 gm. DL-tryptophan (1 gm/8 kg. body weight) resulted in an increase in 5HIAA from 0.52 to 1.98 mg/ml. in the normal and 1.06 to 2.71 mg/ml. in the tympanitic bovine. This supports the contention that tryptophan pyrrolase is impaired in the tympanitic bovine since administration of large quantities of tryptophan has been reported (Sakami and Harrington, 1963) to increase hepatic tryptophan pyrrolase several fold in the rat. This should result in a concomitant reduction in 5HIAA. In this test, oral tryptophan resulted in a higher 5HIAA in the normal bovine (increased by a factor of 4) than in the tympanitic bovine (increased by a factor of 2.5) showing a relative shift from the 5HIAA pathway in metabolism of tryptophan in the tympanitic bovine.

The tympanitic bovine further showed an increased excretion of tryptophan per se.

Data collected from this series of experiments suggest: (1) One of the main pathways in metabolism of tryptophan is through 5HIAA; (2) the normal and tympanitic bovine do not quantitatively metabolize tryptophan comparably. More tryptophan is excreted as 5HIAA by the tympanitic bovine, which suggests a less well developed tryptophan pyrrolase system; and (3) the concentration of free tryptophan in the urine is greater in the tympanitic than in the normal bovine.

Evaluation of the Effectiveness of Antibiotic Containing Boluses in Controlling Clover Bloat - Lon E. Foote, R. D. Thompson, A. J. Guidry, C. P. Breidenstein, W. H. Willis and S. L. Hansard, Louisiana State University, Baton Rouge

In the spring of 1962, a study of bloat control of cattle grazed on Ladino clover was made. Fifteen head of cattle were administered antibiotic containing boluses (Eli Lilly and Co.). An additional fifteen head of cattle served as controls.

Clover pastures grazed consisted of a 22-acre field divided into six approximately equal plots each planted with Ladino clover. Two acre-inches of water were applied to each of the six plots once weekly except when ample rainfall was received.

Prior to the start of the bloat preconditioning phase of the study, the cattle grazed Landino clover for 15 days. The preconditioning period extended from April 11 to May 1, and the cattle grazed 2 hours each morning and 2 hours each afternoon throughout the study. Between grazing periods, the cattle were held in a lot with shade trees without feed but with fresh water. The cattle were weighed once every 2 weeks beginning on April 24.

The percentage of incidence of bloat was computed for each animal during the preconditioning phase, and the severity of bloat for each animal after each grazing period was evaluated and rated 0, 1, 2, 3, or 4. Five animals were lost during the preconditioning phase; therefore, 25 of the 30 cattle had 40 opportunities to bloat, 2 had 18, 1 had 4, and 2 had no opportunity to bloat. Of the 30 cattle, 13 were yearling steers, 9 were 2-year-old steers, 1 was a Brown Swiss cow, 3 were Jersey cows, and 4 were Holstein cows.

The 30 animals were placed alternately in Group A (control) and Group B (treated) on the basis of incidence of bloat. Each animal in Group B was administered two STEP boluses with a balling gun on May 1; none of the cattle in Group A were given any medicinal treatment. The STEP boluses contained (S) streptomycin sulfate, (T) tylosin phosphate, (E) erythromycin thiocyanate, (P) procaine penecillin.

The two groups were combined and managed as one group during the study terminated June 5 because of stoppage of clover growth. Three grazings (one on May 1 and two on May 2) were not included in these data to allow for the initial disintegration of boluses administered cattled in Group B.

During the experiment, three cattle in Group A died; therefore, cattle in this group had 888 opportunities to bloat. Since no cattle in Group B were lost, this group had 1,020 opportunities to bloat. The incidence of bloat for Group A was 56.4% compared to 10.2% for Group B. The analysis of variance and the "t" test on incidence of bloat indicated that treatment differences were highly significant ($P < .01$). However, the treatment caused no significant reduction in bloat severity. This probably was because of lack of sufficient obeervation due to early termination of clover growth.

An important aspect of this study was that four boluses were found in the holding lot the morning of May 3. Either two, three, or four of the cattle given boluses May 1 regurgitated the boluses after one, two, or three clover grazing periods.

Two animals in Group B were each administered two additional boluses on May 18, and five steers in this same group were each administered one bolus on May 29, because after grazing periods previous to these dates the designated cattle had shown an increased tendency to bloat.

The cattle in Group A had an average daily gain of 1.54 pounds, compared to 2.26 pounds for the cattle in Group B administered STEP boluses. This is a significant difference in body weight gain per animal per day in favor of the treated cattle.

Some Physiological Effects of Hypocalcemia, Induced by Hemodialysis, in Ruminants - W. E. Stewart, H. F. Downey, J. C. Smith, Dairy Department, University of Maryland, College Park; and R. G. Cragle, University of Tennessee - A. E. C., Agricultural Research Laboratory, Oak Ridge, Tennessee

The parturient paresis syndrome of dairy cows has been closely reproduced in conscious sheep by depleting calcium from the plasma by hemodialysis to terminal plasma calcium levels of 2.6 to 4.0 mg.%. The two primary symptoms are central nervous system depression and skeletal muscle tetany. These two responses represent two apparently, completely separate abberrations in calcium's physiological function in the body.

Complete rumen atony gradually sets in as the plasma calcium level drops, but rumen motility quickly returns, within 3 - 5 minutes after starting calcium therapy, and definitely precedes the return to consciousness. However, even after the sheep is again standing (about 90 minutes) the motility pattern is of normal amplitude, but contains atypical double major contractions. Eructation contractions begin within 25 minutes. During rumen atony the rumen cannula was opened at intervals to release accumulated gases.

Effect of a Combination of Antibiotics on Incidence of Alfalfa Bloat - P. R. Shellenberger, N. L. Jacobson, P. A. Hartman, and A. D. McGilliard, Iowa State University, Ames

During the 1962 pasture season, 203 cattle and 445 sheep were used to study the effects of a bolus containing a combination of streptomycin sulfate (S) tylosin phosphate (T), erythromycin thiocyanate (E) and procaine penicillin (P). Each cattle bolus weighed 64 g. and contained 6 g of antibiotic in the ratio of 7 S : 7 T : 7 E : 4 P. In cattle, the initial administration of one, two or three boluses reduced bloat for 3 to 4 weeks. Administration of one or two boluses 6 weeks after the first boluses usually reduced bloat for about 1 week. Average weight gains in 70 animals receiving boluses were greater (0.14 lb. per animal per day) than in 44 controls.

In sheep, the initial administration of one-half bolus reduced bloat for 3 to 4 weeks, whereas the same dosage 4 weeks after the first bolus effected no reduction. In one flock, wool loss from the dorsal surface of the body was noted in many of the treated sheep.

Studies with fistulated steers, receiving boluses by balling gun, demonstrated that the majority of the boluses are depoited initially in the anterior blind sac of the rumen. Subsequently, most of the boluses migrate to the reticulum. The bolus has a half-life (weight basis) of about 2 months, but most of the antibiotic activity disappears within 6 weeks.

In 1963, the effect of 250 mg. of the same combination of antibiotics in gelatin capsule, administered to cattle at 1-, 2-, or 3-day intervals was evaluated. Bloat was reduced 89% during the first 4 weeks; frequency of administration had no appreciable effect on efficacy during this 4-week period. Because of insufficient bloat after this period, subsequent observations were inconclusive.

It has been demonstrated that certain transient undesirable side effects occasionally occur when the antibiotics are fed to cattle on a high grain diet. The syndrome appears to be more common in females than in males. In studies during the past year, no adverse effects were seen when a high hay diet was fed for at least 4 days prior to the initiation of antibiotic administration.

Our data suggest that administration of antibiotics at daily intervals affords protection against bloat for a longer period than that provided by the bolus. Moreover, there is some indication that administration at 2- or 3-day intervals may extend the effective period further.

MICROBIOLOGY PANEL

Accumulation of Ethanol in the Rumen Following Overfeeding with Readily Available Carbohydrate - M. J. Allison, R. W. Dougherty, J. A. Bucklin, and E. E. Snyder, National Animal Disease Laboratory, Ames

A neutral volatile substance in ruminal contents from sheep suffering from acute overfeeding indigestion has been identified as ethyl alcohol. Cunningham and Brisson found alcohol in the rumen and blood of lambs fed purified diets and Krogh noted an alcohol-like odor in the rumen of sheep fed sucrose but to our knowledge this is the first identification and quantitation of ethanol in ruminal contents of overfed sheep.

Ethanol was detected and quantitated by gas-liquid chromatography and was invariably present in ruminal contents of three sheep after dosage with cracked wheat and was also present in the rumen of a heifer 8 hours after it was fed 1 kg. of glucose. The concentration of ethanol in the rumen of the sheep varied inversely with the pH of ruminal contents.

Small quantities of ruminal ethanol (4 to 6 μmoles/ml.) were noted 2 hours after feeding two sheep pelleted alfalfa but most of this had disappeared at the time the next sample was taken, 5 hours after feeding. It is suggested that measurable quantities of ethanol may appear shortly after feeding ruminants normal rations.

When a relatively large dosage of cracked wheat (50 g./kg. body weight) was placed in the rumen of these sheep, the pH of the ruminal material dropped to less than 5 and the concentration of ethanol exceeded 14 µmoles/ml. In the one sheep that died following overfeeding, the concentration of ethanol in the rumen at the last sampling period before the animal died was 33 µmoles/ml.

Blood samples were taken through a catheter in the carotid artery at the same time ruminal samples were obtained. The level of ethanol in deproteinized plasma did not exceed 0.7 µmoles/ml., suggesting that ethanol per se does not contribute significantly to the pathology of this condition. It will be of interest to examine portal blood from overfed sheep.

Further Studies with the VIVAR (An In Vivo Artificial Rumen Technique) - L. R. Fina and E. E. Bartley, Kansas State University, Manhattan, Kansas

A ten minute movie (silent) of the VIVAR technique was presented. The movie illustrates the ease of using this technique and also demonstrates several adaptations.

The data obtained for in vivo (VIVAR) experiments indicated a generation time for Entodinium bursa Stein, 1958 to be 7-9 hours, Epidinium ecaudatum (Fiorentini, 1889, syn. Diplodinium ecaudatum) forma caudatum, to be 5-8 hours, Polyplastron multivesiculatum (Dogiel and Fedorowa, 1925) to be 12-13 hours and Isotricha prestoma Stein, 1959 to be 18-20 hours. In vitro comparisons indicated the duplication time for E. ecaudatum was 20 hours and for P. multivesiculatum was 35 hours. E. bursa and I. prestoma could not be grown in vitro.

From results of preliminary experiments it can be reported that mixed populations of "bacterial-free" rumen protozoa apparently digest only small amounts of cellulose. The major cellulose digestion in cattle appears to be a result of the action of bacteria. The protozoa were obtained from fistulated cattle and were added to VIVAR's in concentrations approximately 10 X that found in the rumen (as determined by visual count). One half of the protozoal samples were washed in antibiotic containing buffers. The antibiotic treated protozoal samples were compared to a second series of samples washed in the same buffer, but minus the antibiotic. A third control set of VIVAR's contained only strained rumen fluid. To all three were added C^{14} labeled cellulose extracted from alfalfa - C^{14} uniformly labeled. Experiments were continued for about 72 hours.

Survival of Selected Exogenous Microorganisms in the Rumen of Cattle - J. C. Adams, J. A. Gazaway, P. A. Hartman, P. R. Shellenberger and N. L. Jacobson, Iowa State University Ames

Serratia marcescens (2G12) rapidly lost viability when introduced into the rumens of fistulated cattle. Counts were reduced by about 99% within nine hours. Survival of the organism depended in part upon the size of the inoculum introduced into the rumen: Viability was lost less rapidly when large inocula were used than when small inocula were used. Cells were protected when they were placed in vivar jars (in vivo artificial rumens), presumably because they were shielded from ingestion by protozoa, were not exposed immediately to high levels of antagonistic compounds, and were not subjected to normal dilution in the rumen.

Chromobacterium violaceum lost viability more rapidly than S. marcescens. Counts of C. violaceum were reduced by about 99% within three hours. Effects of variation in the size of the inoculum and protection by the vivar jars were similar to those described for S. marcescens (2G12).

Vegetative cell preparations of Bacillus thuringiensis and B. stearothermophilus remained viable in slightly greater proportions than the two gram negative bacteria, but their resistance in vivo was otherwise similar. Colonies of the two spore formers were indistinguishable from "background" colonies that probably arose from exogenous microorganisms in the food of the animals. Background, or control, counts were higher just after the animals had eaten than later in the day.

Serratia spp. were frequently recovered from the rumens of fistulated animals that had not received an inoculation of laboratory-grown cells for some time. Serratia spp. were present in rumen fluid from nonfistulated and uninoculated animals about one third of the time rumen samples were examined. Either these organisms are present continuously in the digestive tract (sometimes in very low numbers), or there is a frequent and substantial inoculation of cells into the rumen. Over 60% of the rumen Serratia isolates were not S. marcescens (2G12) because the rumen strains were insensitive to S. marcescens (2G12) phage. Levels of Serratia spp. in samples of soil, food, and water were very low, and their presence in these materials could not account for the levels found in the rumen. It is concluded, therefore, that Serratia is a frequent, albeit a minor, component of the rumen microflora.

Counts of S. marcescens (2G12) phage indicated that the number of infectious units remained essentially constant in the rumen over a seven hour period. When cells susceptible to the phage were inoculated simultaneously into the rumen, the phage apparently attached to the cells and were lost when the cells died. No burst (release of phage) was observed during the experimental period.

A Serratia spp., isolated from the rumen and a rumen Serratia phage were studied in vivo. The rumen Serratia strain was found to survive longer than S. marcescens (2G12), indicating that the former strain is relatively more resistant to the rumen environment. The phage results were equivalent to those of S. marcescens (2G12) phage.

In vitro studies of both Serratia species and phage show that the phage will attach to the cells, but will not cause lysis at 39 C. The viable cell counts of both species of the organism remained essentially constant over a seven hour period, when nutrient broth cultures were grown in the presence of phage at 39 C.

The results of this study lead to the following conclusions: 1) The general belief that microorganisms exogenous to the rumen will not survive for extended periods of time in the rumen has been confirmed. 2) The types of exogenous organisms present in the "background" depends in part upon the time samples are taken following feeding. It is conceivable, however, that an organism may be established in the digestive tract and gain entrance into

the rumen, or appear there in appreciable numbers, sporadically. Such is the case in Serratia spp. 3) Bacteriophage do not account for the rapid disappearance of the two species of Serratia from the rumen; however, certain bacteriophage may be important in the dynamics of rumen microbial populations. 4) Temperature in itself does not cause the reduction of viable cells of the two species of Serratia, but it has a profound effect on the reproduction of the bacteriophage in these organisms.