

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

March 17, 1966

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 Eighth Conference 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 Midland Hotel, Chicago, Illinois, on December 1 and 2, 1965.

The Conference convened at 9:15 AM on December 1 and was concluded at approximately 2:00 PM on December 2.

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 papers.

The participants agreed that a similar Conference should be held in 1967 at approximately the same time of the year and also to be held in Chicago. The Chairman was pleased to announce his successor. Dr. C. R. Richards, Cooperative State Research Service, U.S. Department of Agriculture, Washington, D.C. has agreed to take over the duties of Chairman.

The retiring Chairman wishes to take the opportunity to thank the Panel Chairmen and the participants of the Conference for their continued efforts that have resulted in a most interesting and informative series of conferences.



Enclosures - 2

CONFERENCE ON RUMEN FUNCTION  
held at  
Midland Hotel, Chicago, Illinois  
December 1 and 2, 1965

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

<u>NAME</u>	<u>ORGANIZATION</u>
Allen, R. S.	Dept. Biochemistry & Biophysics, Iowa State Univ., Ames
Allison, M. J.	National Animal Disease Laboratory, Ames, Iowa
Barnes, R. F.	USDA, ARS, Dept. Agronomy, Purdue Univ., Lafayette, Ind.
Bartley, E. E.	Kansas State Univ., Manhattan
Beckett, S.D.	Vet. School, Univ. of Missouri, Columbia
Bensadoun, A.	Ani. Sci. Dept., Univ. of Illinois, Urbana
Bernbe, R.	Kansas State Univ., Manhattan
Brookes, I. M.	Dept. Animal Sci., Univ. of Illinois, Urbana
Bryant, Marion	Dept. Dairy Sci., Univ. of Illinois, Urbana
Carlson, G.E.	USDA, ARS, Crops Res. Div., Beltsville, Maryland
Conrad, H. R.	Ohio Agr'l Res. & Dev. Center, Wooster
Cope, W. A.	Crop Sci. Dept., North Carolina State Univ., Raleigh
Dehority, B. A.	Ohio Agr'l Res. & Dev. Center, Wooster
Diven, R. H.	E. S. Erwin Assoc., Tolleson, Arizona
Dougherty, R. W.	National Animal Disease Laboratory, Ames, Iowa
Dunlop, Robert H.	College Vet. Med., Univ. of Saskatchewan, Saskatoon, Can.
Dyer, J. A.	Washington State Univ., Pullman
Dziuk, H. E.	College Vet. Med., Univ. of Minnesota, St. Paul
Essig, H. W.	Mississippi State Univ., State College
Fina, L. R.	Kansas State Univ., Manhattan
Foote, L. E.	Dept. Vet. Sci., Louisiana State Univ., Baton Rouge
Garrigus, U. S.	Ani. Sci. Dept., College of Agri., Univ. of Illinois, Urbana
Gendrich, R. L.	Abbott Laboratories, North Chicago, Illinois
Goetsch, D. D.	Dept. Physiology, Oklahoma State Univ., Stillwater
Good, A. L.	College Vet. Med., Univ. of Minnesota, St. Paul
Greathouse, T. R.	Dept. Ani. Sci., Univ. of Illinois, Urbana
Hargus, W. A.	Int. Min. & Chem. Corp., Skokie, Illinois
Hatfield, E. E.	Dept. Ani. Sci., Univ. of Illinois, Urbana
Henson, Paul R.	USDA, ARS, Crops Res. Div., Beltsville, Maryland
Hinds, Frank C.	Dept. Ani. Sci., Univ. of Illinois, Urbana
Hodson, H. H.	Aeromed. Res. Lab., Holloman AFB, New Mexico
Hubert, T. L.	College Vet. Med., Univ. of Illinois, Urbana
Hungate, R. E.	Univ. of California, Davis
Ingalls, I. R.	Univ. of Manitoba, Winnipeg, Manitoba, Canada
Ioset, R. M.	Dow Chemical Co., Midland, Michigan
Jackson, H. D.	Dept. Vet. Physio. & Pharm., Purdue Univ. Lafayette, Ind.
Jacobson, N. L.	Iowa State Univ., Ames
Jeffers, Barbara R.	Iowa State Univ., Ames
Kendall, W. A.	Agron. Dept., Univ. of Kentucky, Lexington
Kodras, Rudy	Dept. Physiology, Kansas State Univ., Manhattan

NAMEORGANIZATION

Lippke, Hagen	Iowa State Univ., Ames
Lister, E. E.	Animal Research Institute, Canada Dept. of Agri., Ottawa
Loughheed, T. C.	John Labatt Ltd., London, Ontario, Canada
Martin, J. R.	Abbott Laboratories, North Chicago, Illinois
McArthur, J. M.	Canada Dept. of Agri. Res. Station, Summerland, B.C.
McCarrick, R. B.	Dept. Animal Sci., Iowa State Univ., Ames
McDonald, Ian W.	C.S.I.R.O., Div. of Ani. Physiology, Prospect NSW, Australia
McKinley, R. E.	Food & Drug Administration, Washington, D.C.
Mitchell, G. E., Jr.	Univ. of Kentucky, Lexington
Moose, Marv	International Minerals & Chemical Corp., Skokie, Illinois
Nichols, Ray	College Vet. Sci., Univ. of Wisconsin, Madison
Oliver, T. J.	Abbott Laboratories, North Chicago, Illinois
Phillips, G. D.	Univ. of Manitoba, Winnipeg, Manitoba, Canada
Phillips, R. W.	Colorado State Univ., Dept. of Physiology, Ft. Collins
Purser, D. B.	I.N.F.T., Ohio State Univ., Columbus
Richards, C. R.	Coop. State Research Service, USDA, Washington, D.C.
Reynolds, P. J.	Ani. Husbandry Res. Div., ARS, USDA, Beltsville, Maryland
Roberts, W. K.	Univ. of Manitoba, Winnipeg, Manitoba, Canada
Sheidy, S. F.	School Vet. Med., Univ. of Pennsylvania, Philadelphia
Sellers, A. F.	New York State Veterinary College, Ithaca
Smith, Clyde,	Dept. Microbiology, Michigan State Univ., East Lansing
Smith, I. D.	Abbott Laboratories, North Chicago, Illinois
Stifel, Fred	307 Kildee Hall, Iowa State Univ., Ames
Stephens, R. C.	S.K. & F. Laboratories, Philadelphia, Pennsylvania
Stone, E. J.	Louisiana State Univ., Baton Rouge
Swenson, M. J.	Iowa State Univ., Ames
Thomas, J. W.	Dairy Dept., Michigan State Univ., East Lansing
Thomas, O. O.	Montana State Univ., Bozeman
Tucker, J. O.	Div. Vet. Sci., Univ. of Wyoming, Laramie
Vetter, R. L.	Iowa State Univ., Ames
Vidacs, Gyorgy	Ani. Sci. Dept., Colorado State Univ., Ft. Collins
Whipp, S. C.	National Animal Disease Laboratory, Ames, Iowa
Wolf, Claude	Industrial Bio-Test Laboratories, Northbrook, Illinois
Wright, G. E.	Ruskuna Agri. Res. Center, Hamilton, New Zealand

REPORT ON  
CONFERENCE ON RUMEN FUNCTION  
held at  
Midland Hotel, Chicago, Illinois  
December 1-2, 1965

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

(a)	Agronomic	-	Paul R. Henson
(b)	Rumen Physiology	-	N. L. Jacobson
(c)	Microbiology	-	C. K. Smith
(d)	Physio-Pathology	-	R. W. Dougherty

AGRONOMIC PANEL

Factors Affecting Foam Production In-Vitro With Forage Legumes -  
W. A. Kendall, ARS, USDA, and Kentucky Agricultural Experiment Station,  
Lexington

Foams were produced in the laboratory by beating crude extracts in a food mixer. Recent studies have shown that the greatest foam production with trefoil and lespedeza samples occurs at relatively high pH values, i.e., about pH 7, in contrast to optimum values at pH 5.5 for the clovers and alfalfa. The volume of foam produced with trefoil and lespedeza at the higher pH values may be about equal to the volume of foam produced by the clovers and alfalfa at the lower pH. Crownvetch samples produced a very small amount of foam and it was practically independent of pH. Crownvetch samples from plants considered high in tannin produced less foam than similar plants with low tannin content. Forage samples which contain tannin and normally produce a relatively small amount of foam (trefoil, lespedeza, and crownvetch) produced large volumes of foam when polyvinyl pyrrolidone K-30 was added to the crude extract in phosphate buffer solvent. The volume of foam of many forages, including bluegrass was greatly enhanced when acetic acid was added to the crude extract.

Plant Polyphenols that Inhibit Enzymes - W. A. Cope, T. A. Bell, and W. W. G. Smart, Jr., ARS, USDA, and North Carolina Agricultural Experiment Station,  
Raleigh

The water extract of green leaves of the muscadine grape, Vitis roundifolia, has been found to inhibit certain pectinolytic and cellulolytic enzymes. The extract was effective in preventing softening of cucumbers during the brining process of pickling, such softening being caused by fungal enzymes from the cucumber flower. Search for a commercial source revealed that sericea, Lespedeza cuneata, among other species, had a high leaf content of essentially the same substance. Studies with sericea showed that the substance inhibited rumen cellulase. Activity of the sericea extract was related to tannin content in genetic lines differing in content and showed seasonal variation, apparently dependent on light intensity and duration. Forage from genetic lines of sericea, quite low in tannin, were more completely digested than high tannin forage in "in vitro" digestion studies.

The substance has been isolated in relatively pure form. The patented process involves complexing the cold extract with caffeine, removing the caffeine with chloroform, and freeze-drying to obtain the substance. The substance has been identified as leucoanthocyanin, hydrolyzing the leucodelphinidin. It is a polymer of the 15 carbon flavonoid skeleton. The inhibitor substance has the form of a light, fluffy powder, is readily water soluble, is heat stable, and has a molecular weight in the range of 15,000. In solution it is inactivated by certain soluble proteins, nicotine, and caffeine. The concentration of the substance in mature sericea leaves exceeds one per cent of the green weight; other plant species show inhibition at concentrations as low as 0.05 of one per cent. Concentrations of the substance as low as 5 ppm show definite inhibition in the standard test, while 100 ppm gives 95% or more inhibition and also gives excellent control of cucumber flower enzymes in cucumber brining.

The substance may be quite similar to, or the same as, tanning fractions which have recently been reported to bind enzymes and soluble proteins in macerated tissue, preventing the isolation of enzymes. Two questions are raised: (1) Whether the inhibitor substance in sericea, at a concentration of 1% or more of ingested material, may possibly interfere with normal rumen processes, and (2) whether the substance may inhibit enzymes in the plant tissues which could reduce relatively simple pectins to pectic acid, producing a gel to contribute to a bloat-producing system (according to the hypothesis of Gupta and Nichols).

Phenolics, Soluble Proteins and Total Nitrogen Levels of Bloat Producing Alfalfa - B. R. Jeffers, S. H. Synhorst, N. L. Jacobson, D. K. Hotchkiss and R. L. Vetter, Iowa State University, Ames

A bloat assay group of 20 sheep and 20 cattle were fed 6-14 inch tops of alfalfa throughout the summer. The alfalfa soilage was analyzed for total nitrogen (calculated as crude protein, soluble protein, and phenolic (expressed in terms of a "tannin" standard) contents. Linear correlation coefficients were calculated for these chemical constituents and bloat.

The sheep bloat scores had correlation coefficients of +.418 with crude protein, +.422 with soluble protein, and -.420 with a ratio of total "tannin" content to soluble protein. All of the correlation coefficients were significant at the 5% level.

The cattle bloat scores had correlation coefficients of +.705 with crude protein (significant at the 1% level), +.363 with soluble protein (significant at the 5% level) and -.461 with a ratio of total "tannins" to soluble protein (significant at the 1% level).

It was concluded that a meaningful relationship exists between these constituents of alfalfa and the incidence of bloat.

Differences Between the Proteins of Bloating and Non-Bloating Forages as Indicated by Molecular Sieving - J. M. McArthur and J. E. Miltimore, Canada  
Department of Agriculture, Summerland, British Columbia

At the last conference, a protein foaming agent, 18-S protein, isolated from alfalfa was described. It was suggested that this protein was responsible for the foam which develops in the rumen in legume bloat. The protein is well known as the major component of plant protein and as the enzyme involved in carbon dioxide fixation in the photosynthetic cycle. Because of its enzymatic function and because it has been found in a number of plant families, it would appear probable that it occurs in all plants. This leads to the question then, "What is the difference between bloating and non-bloating forage plants?" An investigation was designed to determine if there were qualitative or quantitative differences in the protein content, particularly with respect to the 18-S protein.

The clarified saps of forage plants were passed through molecular sieves one meter long packed with 5% agar gel beads. The effluent was fractionated volumetrically and the fractions analyzed for protein and ribonucleic (RNA) and deoxyribonucleic acids (DNA). The concentrations were calculated per gram dry matter and plotted versus the effluent volume. The total protein, RNA and DNA in the sample were calculated from the sum of the fraction analyses and the 18-S protein from its peak area on the concentration vs. effluent volume plots.

The proteins in the forages did not appear to differ insofar as the molecular size was concerned. The first protein fraction to come off the molecular sieve contained both RNA and DNA indicating nucleoproteins. These were probably the chloroplastic and cytoplasmic ribosomes with molecular weights of 3 to 4 million. These were followed by 18-S protein whose molecular weight is 500,000. Next, several incompletely separated proteins appeared whose molecular weights were approximately 300,000, 100,000, and 60,000. The last fraction contained proteins of molecular weights of less than 50,000. It also contained RNA and DNA. These may be nucleoprotein fragments or nucleotides since the nucleoproteins are unstable and no special precautions were taken to preserve them.

Although the protein composition of the forages did not appear to differ qualitatively there was a quantitative difference in the 18-S protein content of the bloating and non-bloating forages. The bloating forages contained considerably more 18-S protein than the non-bloating forages. This supports the view that 18-S protein is the causative agent in plants which give rise to legume bloat.

## RUMEN PHYSIOLOGY

### The Toxicity of the Fungal Metabolite, Sporidesmin, to Ruminants - D. E. Wright, Ruakura Agricultural Research Centre, Hamilton, New Zealand

Sporidesmin is a toxin produced by the fungus Pithomyces chartarum growing on dead grass. This toxin causes an economically important disease known as facial eczema. This name arises from a photosensitization of the animals' skin. The disease is primarily a liver disease with characteristic gross and microscopic pathological changes.

Sporidesmin is toxic at very low concentrations, 3.0 µg of toxin/ml of culture fluid causing cytopathological changes in Hela cells. An oral dose of 0.8 mg/kg body weight results in severe liver damage and photosensitization. Animals vary greatly in their sensitivity to the poison, the difference presumably being due to differences in detoxication of the poison rather than differences in metabolic action or absorption.

The effect of the toxin has been examined with respect to its action on nucleotides in liver tissue, on the ability of poisoned cells to oxidize a wide range of substrates both in vivo and in vitro and on several enzyme systems. The results have not provided conclusive evidence regarding its action.

Attempts to control the disease by fungicides or by immunization have not been successful. Pasture management is the best method to limit fungal growth and reduce the incidence of the disease.

### Pre-intestinal Destruction of Vitamin A - G. E. Mitchell, Jr., C. O. Little, F. J. Klatte and B. W. Hayes, Kentucky Agri. Expt. Sta., Lexington

The results of several experiments suggest extensive pre-intestinal destruction of vitamin A in both sheep and cattle. Wethers ligated at the pyloric valve were given massive intraruminal injections of vitamin A and sacrificed 24 hours later. Recovery of vitamin A from the pre-intestinal contents was 9.5-26.4%. Serum vitamin A data did not suggest sufficient absorption to account for these losses. Losses observed after incubating vitamin A in ruminal fluids from cattle and sheep anaerobically at 37°C. for 4 hours averaged 36.1% as compared with 13 and 16% of the same quantities incubated in autoclaved ruminal fluid and distilled water, respectively, and 33.6% following incubation in abomasal fluids. Average recovery of 5 commercial stabilized vitamin A products from the abomasal fluid of steers 24 hours after dosing was 26.1% as determined by an indicator: Vitamin A ratio procedure. Including approximately 1% of potassium nitrate in the diets of wethers or steers with abomasal fistulas did not adversely affect recovery of vitamin A from the abomasal fluid 24 hours after dosing.

### Some Effects on Intestinal Infusions of Cellulose - G. D. Phillips, University of Manitoba, Winnipeg, Canada

Experiments were conducted to determine the digestibility of α-cellulose infused into the duodenum of sheep and the effects of such infusions on output of fecal components. Digestibility trials of 8-day duration were conducted with 3 sheep on: a) the basal ration of 1200 gms of hay; b) basal plus 2 l. per day water infused into the duodenum; c) basal plus infusion of 2 l. per day of 0.2% Agar; d) basal plus infusion of 4l gm. α-cellulose

in 2 l. agar solution; e) basal plus 82 gm. - cellulose in 2 l. of agar solution; f) basal plus 166 gm. - cellulose in 4 l. of agar solution.

Treatment b and c had no effect on the fecal output (and therefore digestibility, as intake remained same) of ash, dry matter, ether extract, fibre, cellulose or nitrogen.

Treatments d, e and f resulted in progressively larger fecal outputs of dry matter. This was virtually entirely due to increased output of cellulose. Digestibility of the infused cellulose was calculated by difference from the mean found for treatments a, b and c where no cellulose was infused. Thus 28.4% was digested of 41 gms. infused, 33.8% of 82 gm. infused and 18.8% of 166 gm. infused. All levels of cellulose infusion caused significantly more nitrogen to be lost in the feces than the basal treatment. The increased losses were .59 gm per day for 41 gm. cellulose infused, .67 gm per day for 82 gm. cellulose infused and .91 gm per day for 166 gm. cellulose infused. As the daily fecal nitrogen output for the basal treatment was 7.49 gm. these increases were 7.9, 8.9 and 12.1%, respectively. The corresponding increases in fecal dry matter outputs were 7.4, 11.3 and 28.8%. Cellulose infusion had no effect on the fecal outputs of ash or ether extract.

Some Effects of Dietary Potassium Upon Nutrient Utilization in Cattle -  
W. K. Roberts and V. V. E. St. Omer, University of Manitoba, Winnipeg, Canada

Balance studies were conducted with 9 Hereford heifers, averaging 240 kg in body weight. A basal low K ration containing the following was formulated: Dried brewers grains, 74.7%; corn starch, 15.0%; barley, 5.0%; animal tallow, 3.0%; dehydrated alfalfa, 1.0%; NaCl, 0.5%; mineral-vitamin A, 0.8%. This ration contained .08% K, .25% Na and 23.7% crude protein, oven-dry. Potassium carbonate was added to make the various ration levels of potassium. Each heifer was fed 2.54 kg of this ration and 439 mEq K daily during a 14-day adjustment period. K intake of some heifers was then abruptly changed to 157 or 1087 mEq daily and balance studies were conducted for 15 consecutive days. The heifers were then returned to floor pens for 10 days and fed the same rations, but excreta was not collected. The heifers then were returned to metabolism crates and the balance studies continued for 15 days. Thus, 3 treatment levels of K were fed (low, 157; medium, 439; high, 1087 mEq daily) to 3 different heifers for 40 days. Tap water was available ad libitum and the daily consumption measured. Jugular blood samples were collected on the 40th day and analyzed for various serum electrolytes.

Heifers receiving the low K ration lost 1.8 kg and those receiving the medium and high rations gained 4 kg during the 40 days. Average nitrogen and sodium retentions were positive for all treatments, and there were no significant differences among treatments. Heifers fed the high K ration consumed more water ( $P < .01$ ) and excreted more urine ( $P < .01$ ) than the other heifers. Thus apparent water balance was not affected by treatment. Apparent digestibilities for energy, crude fiber, ether extract and nitrogen were not affected by treatment. Apparent K balances were significantly ( $P < .01$ ) affected by treatment. Heifers fed the low K ration lost 24.8 mEq K daily, those fed medium K retained 15 mEq and heifers fed high K retained 147.9 mEq K daily. A regression equation was calculated for K intake per 100 kg body weight vs K balance per



100 kg body weight and the regression coefficient was  $.195 \pm .029$ . The daily K intake per 100 kg body weight at zero K balance is 133 mEq, which should approximate the maintenance K requirement for these heifers. Urine ammonia excretion during the last 5 days of the trial was significantly ( $P < .01$ ) higher for heifers fed the low K ration. Serum K was significantly ( $P < .05$ ) lower and phosphorus higher for heifers fed low K. Serum Na, Ca, Mg, Cl were not significantly affected by treatment, although Mg and Ca were higher in heifers fed low K than those fed high K.

Studies on the Origin of Methane in the Rumen Employing the VIVAR Technique - R. Berube, L. R. Fina, and E. E. Bartley, Kansas State University, Manhattan

Methane production in the rumen, using the VIVAR technique, comes mainly from carbon dioxide and formate reduction. The carbons of acetate, propionate and butyrate are minor contributors to the formation of methane. The results with carbon dioxide and formate are not surprising. However, it appears that formate does not merely give rise to carbon dioxide, with subsequent reduction to methane, but that the reactions are much more complex. It is surprising that only the carboxyl carbon of propionate appears in carbon dioxide and methane, and that none of the carbons of acetate, or of the 2nd and 3rd carbons of propionate, or of the 2nd carbon of butyrate appears in the carbon dioxide or methane fraction.

The Effect of Feed Intake Upon Bloating Patterns in Lambs Grazing Green-Chopped Alfalfa - Fred B. Stifel and R. L. Vetter, Iowa State University, Ames

Several trials were conducted studying the effect of feed consumption of green-chopped alfalfa upon bloating patterns in crossbred wether lambs. In two trials, a direct linear relationship appeared between the level of green-chop consumed in the afternoon and the severity and frequency of bloat. In all trials, lambs bloated significantly more in the afternoon, regardless of the level of green-chop consumed. The importance of the level of intake in the afternoon was further shown in trials using different combinations of high and low intake. The treatment combinations were low-low, low-high, high-low and high-high, referring to the level of intake in the morning and afternoon respectively. In both trials, the lambs on the high-high and low-high bloated significantly more than those of the other two treatments.

Correlations Between Bloat Incidence and Heat Units - J. E. Miltimore and J. M. McArthur, Canada Department of Agriculture, Research Branch, Summerland, British Columbia

Cattlemen often report that bloat is more prevalent in the fall when nights are cold. This study was undertaken to test the relationship between temperature and bloat incidence.

Daily temperatures expressed as heat units, were used in the calculations of correlation coefficients. The heat units were determined from a formula used to select areas with a climate suitable for corn production.

$$\text{Heat units} = \frac{\text{Minimum temp. } -40^{\circ}\text{F.} + (4.39 \times \text{max. temp.}) - (.0256 \times \text{max. temp.}^2)}{-155.18}$$

Three sets of bloat data were collected for five years from two herds of cattle. One set using a daily bloat score was obtained from a control herd of six cows. A second set was also obtained from the control herd but the number of animals treated each day was used for the calculation. A third set of bloat data was obtained by adding to the second set the number of cows treated daily in an experimental herd of 16 cows that was used in a test of bloat preventives. The data for each year were divided into three seasons - spring, summer and fall. The data for all years were combined into a single calculation for each season.

In addition to correlating the bloat score and the number of cows treated each day with the respective heat units for that day (Stage I), weather preceding the occurrence of bloat was considered. In Stage II the bloat score each day was correlated with the heat units from the preceding day. In Stage III the average of the heat units from the three preceding days was correlated with the bloat score.

Bloat score was significantly correlated with heat units for the same day but the coefficient of  $-.22$  is too small to have much biological significance. The number of cows treated for bloat in the control herd and the number of cows treated in both herds were also significantly correlated with heat units,  $r = -.29$  and  $-.25$  respectively. The other correlation coefficients were small and statistically insignificant.

The data suggest that bloat incidence may be influenced by cooler fall temperature. Similar temperatures prevail in the spring but the correlations were not significant in the spring and this would be a contradiction. As only five to eight per cent of the variation in bloat could be attributed to a reduction in temperature in just one season, temperature expressed as heat units does not appear to be a major factor in determining either the susceptibility of cattle or the bloat potential of alfalfa. If bloat is more prevalent in the fall it is probably due to the very small proportion of grass in the aftermath on alfalfa fields.

Tyrosine and Tryptophan Metabolism in the Normal and Bloater Bovine -  
I. A. Dyer and R. J. Johnson, Washington State University, Pullman

The complexity of the etiology of tympanites has become increasingly evident during the past decade. Most researchers realize that there is a multiplicity of factors involved in this malady. Our work on tympanites has been centered largely around biochemical differences in the normal and tympanitic bovine.

A series of experiments were conducted, using normal and bloater to determine (a) the effect of temperature on tyrosine and tryptophan metabolism; (b) the effect of Tapazole (a potent goitrogen) on L-aspartate; (c) the effect of increased tryptophan on urinary 5-hydroxyindole acetic acid excretion; and (d) hepatic monoamine oxidase activity in the normal and bloater bovine.

In general normal steers maintained a more constant excretion of tyrosine and tryptophan metabolites when subjected to temperature changes than did the bloaters. At the levels fed, Tapazole caused an increase in the 5-hydroxyindole acetic acid excretion in both normal and bloater animals, but in the incidence and severity of bloat, among bloaters, was increased during Tapazole administration. The administration of D, L-tryptophan resulted in

an increase urinary excretion of 5-hydroxyindole acetic acid in both normal and bloater steers. The hepatic monoamine oxidase activity was decreased in the bloater steers.

The Use of Resistopen -- A Semisynthetic Penicillin in the Control of Clover Bloat in Cattle - L. E. Foote, R. E. Girouard, Jr., E. J. Stone, A. J. Guidry and W. H. Willis, Louisiana Agricultural Experiment Station, Baton Rouge

Antibiotics have been used singly, sequentially and in combination to control leguminous bloat in cattle. Their effectiveness may have been limited by inactivation by penicillinase and/or acid hydrolysis. Resistopen, a semisynthetic penicillin, is resistant to both. Two groups of yearling Hereford steers, 13 in each group, were grazed on Ladino clover twice daily. The study consisted of pretreatment, treatment, and posttreatment phases. During the treatment phase the 13 treated steers were each administered 250 mgs of Resistopen in a gelatin capsule every other day for 4 periods of 7 days each. The control steers received no treatment. Bloat incidence and severity observations were made immediately following each grazing period, and ratings of 0 (none), 1 (slight), 2 (moderate), 3 (severe), or 4 (lethal) were assigned each animal. Percentages incidence of bloat during treatment phase were as follows: Controls, 80.1 (AM); 76.1 (PM); 78.1 (AM + PM). Treated, 9.6 (AM), 16.3 (PM) and 12.9 (AM + PM). Chi square analysis indicates highly significant differences ( $P < .005$ ) between the treated and control groups, and analysis of variance of the data for bloat severity indicates highly significant differences ( $P < .01$ ) for weekly periods. Resistopen proved very effective in the control of clover bloat in cattle.

Terramycin and Dimethylpolysiloxane in Molasses Blocks for Ladino Clover Bloat Control - H. W. Essig and C. B. Shawver, Mississippi Agricultural Experiment Station, State College

Research has indicated that molasses blocks are a satisfactory vehicle for inducing consumption of various compounds. Work at the station has shown that oxytetracycline was effective, for short periods of time, in reducing severity and incidence of bloat in beef cattle grazing Ladino clover. Dimethylpolysiloxane and oxytetracycline have both been approved by FDA as "an aid in the prevention and control of bloat" when used singly. The silicone product is believed to function primarily as an antifoaming agent which prevents or reduces the tendency for gasses to be trapped in stable foam within the rumen. The antibiotic, on the other hand, is believed to function as a bloat preventative by reducing the activity of organisms which are gas producers, in excessive amounts within the rumen, in the presence of legumes. Because the two materials are likely functioning for bloat control in a separate manner, it is deemed probable that a higher degree of efficacy may be obtained by using the two in combination than that observed for each one used singly, particularly when fed in a supplement on a continuous free choice basis.

This test was primarily to determine the efficacy for this particular combination of drugs at two levels of intake and the feasibility of a free choice method of administration. The 43-day test consisted of the following

three periods: (1) 8-day control, where all steers were given access to Ladino clover; (2) 17.5-day period in which one treatment group of 5 steers was given access to molasses blocks containing 0.1765% (TM - 50) oxytetracycline and 0.22% dimethylpolysiloxane (DMPS); and (3) 17.5-day period in which one group of 5 steers was given access to a molasses block which contained 0.88% DMPS and 0.7060% TM-50 while the other group of 5 steers was given access to a plain molasses block.

There was no difference for severity and incidence between treatments or between grazing times for the steers during the 8-day control period. The severity and incidence of bloat was decreased in steers given access to molasses blocks containing the lower level of TM-DMPS, however, this decrease was not enough to be considered of value in bloat prevention since it was about equal to severity and incidence for the control period. During the period when the steers were given access to the molasses block containing the high level of TM-DMPS their severity and incidence was slightly lower than that for the steers given access to the plain molasses blocks. Two steers in the treatment group given the high level of TM-DMPS died, indicating that even at high levels these compounds would not prevent loss of animals, therefore, being of no value in bloat prevention of steers grazing Ladino clover.

A Non-Ionic Surfactant in the Control of Clover Bloat by Cattle -  
L. E. Foot, J. E. Johnston, R. E. Girouard and W. H. Willis, Louisiana  
Agricultural Experiment Station, Baton Rouge

Antifoaming agents, tallows, mineral oils, vegetable oils, silicones, detergents and other surfactants have been used to control leguminous bloat in cattle. One study using poloxalene-containing pellets and a second study using poloxalene-containing blocks were conducted to determine the effectiveness of poloxalene in the control of clover bloat of cattle. Each study consisted of a pretreatment, treatment and posttreatment phase. Bloat incidence and severity observations were made periodically after the animals grazed Ladino clover; and ratings of 0 (none), 1 (slight), 2 (moderate), 3 (severe), or 4 (lethal) were assigned to each animals.

Study 1 involved 53 Hereford steers, one and two years of age, which were divided initially into three groups. Group I, the controls, consisted of 19 steers; Group II, the poloxalene-treated cattle, consisted of 18 steers; and Group III, the penicillin-treatment-control cattle, consisted of 16 steers. Group I steers were provided placebo-grain pellets, 210 gm./animal/day for 69 days and Group II steers received 220 gm. poloxalene pellets/animal/day. The poloxalene pellets consisted of 10 gm. poloxalene incorporated into 210 gm. of the same grain given Group I steers. Groups I and II were grazed on the same clover plot each morning and afternoon for varying periods of 45 minutes to two hours. Each steer in Group II was given daily treatment of 125 mg. procaine penicillin in a gelatin capsule for 17 days starting April 22, 1965. When the study involving Group III was terminated, 10 steers from this group were selected to form Group IV. Group IV steers were grazed continually on Ladino clover and were provided poloxalene-containing pellets at the rate of 220 gm./animal/day for 55 days.

Following the posttreatment phase of Groups I and II, a preliminary experiment, Study 2, was initiated to learn if cattle would lick molasses blocks containing 30 gm./poloxalene/lb. in sufficient amounts to control clover bloat. Nine steers were placed in control Group V, nine in treated Group VI and six in Group VII.

Molasses blocks without poloxalene were provided Group V steers in one holding lot, and molasses blocks containing poloxalene were available to Group VI steers in a separate holding lot. Steers in Groups V and VI were grazed together on the same clover plot for 1 to 2 hours each morning and afternoon for 17 days.

Each of the six steers in Group VII was grazed continually on individual Ladino clover paddocks for 17 days. Each steer was provided one poloxalene-containing molasses block; each also had free access to salt, steamed bone meal and water throughout the study.

Analysis of variance of the Group I and Group II study indicates the differences between grazing times and between treatments for both incidence and severity of bloat were significant ( $P < .01$ ).

Analysis of variance of the Group II and Group III study indicates the differences for incidence of bloat were significant between days ( $P < .05$ ), grazing time ( $P < .01$ ) and treatment ( $P < .01$ ). Analysis of variance for bloat severity was also significant between days ( $P < .01$ ), grazing time ( $P < .05$ ) and treatment ( $P < .01$ ).

Analysis of variance of the Group V and Group VI study indicates the differences in bloat incidence and severity were significant at ( $P < .01$ ).

Individual consumption of the treated blocks varied from 0 to 1.8 lbs./animal/day, but the average daily consumption of the treated blocks by the 6 animals continually grazed on separate cover pastures varied from 0.66 to 1.10 lbs./animal/day. Group consumption of blocks without poloxalene was 1.52 lbs./day as compared to 0.9 lb./day of the blocks which contained poloxalene.

Poloxalene Residue in Milk and Meat and Its Effect on Animal Health and Well-Being - R. M. Meyer, L. G. Helmer and E. E. Bartley, Kansas State University, Manhattan

Previous studies have demonstrated the efficacy of poloxalene in controlling legume pasture bloat. Several studies have been conducted to test the safety of poloxalene administration. When a single 40 g. dose of poloxalene containing over 8,000  $\mu$ c of  $C^{14}$  labeled poloxalene was administered to a lactating cow, no  $C^{14}$  activity appeared in milk or in body tissues nine days following administration of the drug. Since 94.3% of the  $C^{14}$  activity administered was recovered in feces and 4.0% in urine, it was concluded that the poloxalene is poorly absorbed from the digestive tract.

Results from six poloxalene residue studies indicate that no detectable residues can be found in milk or meat from animals receiving recommended prophylactic doses (10 g./1000 lb. body weight) of poloxalene. When poloxalene was administered at a rate of ca. 75 g./1000 lb. weight for several days, trace quantities of poloxalene could be detected in milk and meat. A 4-8 hr. withdrawal period lowered milk residues to insignificant values and a 7-day withdrawal period likewise lowered tissue residues to insignificant values.

Six cows were fed varying levels of poloxalene (10, 20, or 40 g. per cow per day) for 12 weeks and 6 cows served as controls. Poloxalene had no deleterious effect on milk production, milk fat test, body weight, feed consumption, conception rate, or animal health. When 10 g. poloxalene per day was fed to fistulated identical twin cattle, no significant differences between poloxalene-fed and control animals were observed in rumen ammonia concentration, rumen pH, rumen lactic and volatile fatty acid concentration, or cellulose digestion.

The results appear to justify the conclusion that feeding poloxalene in quantities up to 40 g. per day does not deleteriously affect cattle.

Poloxalene in Corn for Control of Bloat of Beef Cattle Grazing Ladino Clover -  
H. Werner Essig, Charles B. Shawver and Vernon D. Godsey, Mississippi Agricultural Experiment Station, State College

The objectives of this study were: 1) to study the efficacy of feeding 10 gm. of poloxalene in 454 gm. of ground shelled corn fed once daily before the morning grazing period. 2) To measure the response of daily administration of 10 gm. poloxalene in a capsule plus giving access to 454 gm. of ground shelled corn. 3) to determine the benefit of twice daily feeding of 454 gm. of ground shelled corn containing 10 gm. poloxalene.

The 89 day trial was divided into seven periods as follows: 1) Seven day control period of grazing Ladino clover. 2) All animals were fed 454 gm. ground shelled corn per head daily before the a.m. grazing for 14 days in order to assure consumption of corn by all animals. 3) Animals in treatment groups 1 and 4 were fed 454 gm. ground shelled corn and those in treatments 2 and 3 were fed 454 gm. of the corn-poloxalene mixture (10 gm. poloxalene per 454 gm.) for 44 days. 4) For seven days all steers were fed 454 gm. ground shelled corn before the a.m. feeding and those steers in treatments 2 and 3 were each given 10 gm. of poloxalene by capsule before the a.m. feeding of corn. 5) Same as period 3 except each steer in treatment 1 and 4 were given 10 gm. of poloxalene by capsule before the a.m. feeding of corn for five days. 6) Same treatment as period 3, but for only six days. 7) A 17-day period in which the treatment was the same as in period 3 except that all animals were fed twice daily (before each grazing time).

There was no difference for severity and incidence of bloat for the steers in treatment 1 and 4 when compared to those in treatments 2 and 3 during the control period when the steers were allowed to graze Ladino clover. When 454 gm. of ground shelled corn per head daily was fed before the a.m. grazing period the severity and incidence of bloat for the a.m. was significantly decreased as a result of feeding the corn. The a.m. feeding of corn appeared to have little effect on the p.m. severity and incidence of bloat. The a.m. feeding of 454 gm. of ground shelled corn-poloxalene mixture (10 gm. poloxalene

per 454 gm. of mix) significantly decreased the severity and incidence of bloat, however, the following p.m. severity and incidence was significantly higher than the a.m. severity and incidence. The p.m. severity of bloat for steers fed corn-poloxalene before the a.m. grazing was lower than either the a.m. or p.m. severity of bloat for the steers fed only corn before the a.m. grazing. The administration of 10 gm. of poloxalene by capsule in addition to the steers being fed 454 gm. of corn before the a.m. grazing essentially eliminated bloat in the a.m., however, some bloat severity and incidence was observed the following p.m. which was generally less than the a.m. severity and incidence of steers fed the corn-poloxalene mixture. The feeding of the corn-poloxalene mixture twice daily, immediately before each grazing time, eliminated any difference between a.m. and p.m. severity and incidence of bloat. Poloxalene was effective in reducing severity and incidence of bloat for 68 days during this test.

Different Levels of Poloxalene in Molasses Blocks for Control of Ladino Clover Bloat in Beef Cattle - H. Werner Essig, Charles B. Shawver and Vernon D. Godsey, Mississippi Agricultural Experiment Station, State College

This work showed that feeding 10 gm. of poloxalene twice daily in corn was more effective in controlling bloat severity and incidence than when 10 gm. of poloxalene was fed once per day in the corn. Since beef cattle are not generally fed grain while grazing legumes, a more suitable means of administering poloxalene was desired. Previous work has indicated that molasses blocks were satisfactory vehicles for inducing consumption of various compounds. Poloxalene was incorporated into the molasses block at two different levels and fed to beef cattle to determine the suitability of administering poloxalene in this manner.

Ten steers of beef breeding showing bloating tendencies when grazing Ladino clover were allotted to two treatments with steers in one treatment being given access to molasses blocks containing 10 gm. poloxalene per 454 gm. and the other treatment group of steers being given access to molasses blocks containing 30 gm. of poloxalene per 454 gm. Each treatment group consisted of two periods: (1) A 17.5 day control period and (2) a 40-day treatment period.

After five days access to the molasses blocks during the time of drylot confinement, there appeared to be no effect on severity and incidence of bloat of cattle grazing Ladino clover. Steers given access to the molasses blocks which contained 30 gm. poloxalene consumed a greater quantity and spent more time "apparently consuming" the blocks than did the steers given access to the 10 gm. poloxalene level blocks. The steers spent more time "apparently consuming" the block at night than during the daytime. The average daily consumption of 13.93 gm. of poloxalene reduced the severity and incidence of bloat below that of the control period. The consumption of 46.69 gm. of poloxalene was more efficient in controlling bloat than the 13.93 gm. poloxalene level or the control period; however, the high level did not completely prevent bloat under the extreme conditions of this test. All steers receiving poloxalene (both levels) showed a higher severity and incidence of bloat after the p.m. grazing time than after the a.m. grazing time. The block containing 30 gm. poloxalene was more efficient in controlling severity and incidence of bloat than was the block containing 10 gm. poloxalene. Molasses blocks containing 30 gm. poloxalene appeared to be an acceptable method for reducing severity and incidence of bloat in steers grazing Ladino clover.

Effect of Poloxalene Administered in Various Farms on the Prevention and Treatment of Cattle Bloat - E. E. Bartley, R. M. Meyer, H. B. Pfost and L. G. Helmer, Kansas State University, Manhattan, and H. Lippke and W. I. Jacobson, Iowa State University, Ames

Half of the cows in each of four Kansas dairy herds served as controls and half were fed poloxalene. All were provided succulent alfalfa. The treatments were reversed after 7 to 10 days. Poloxalene prevented bloat nearly 100% of the time. In a second experiment all cows in four Kansas herds were fed poloxalene before being fed succulent alfalfa. Again 100% control of bloat was achieved when cows consume sufficient poloxalene. In a third experiment, 36 dairy steers were used at Iowa State University. Half served as control and half received poloxalene before being provided succulent alfalfa. During the 113-day trial complete bloat control was achieved when animals consumed at least half the poloxalene offered. In the three experiments poloxalene was fed as a supplement, incorporated in the regular herd ration, or sprinkled over grain. In all experiments the animals readily consumed poloxalene after becoming accustomed to it. Mixing poloxalene in the entire herd grain ration for lactating cows presented problems in administration. Some cows received more poloxalene than necessary to control bloat; others, not enough to prevent bloat. Mixing poloxalene with an inert carrier such as vermiculite and using the mixture as a top dressing for grain or as a supplement seems more practical.

Four sets of identical twin dairy heifers were divided into two groups and pastured on adjacent strips of alfalfa. One group was offered molasses-salt blocks containing 15 g. poloxalene per lb. while the other group was offered a similar molasses-salt block containing no poloxalene. The treatments were reversed after 7 to 8 days. The animals were pastured from 7 to 9 a.m. and then returned to a drylot where the blocks and water were available. They were pastured again between 3 to 5 p.m. and then returned again to drylot. The intake of the poloxalene and control blocks usually exceeded 0.5 lb. per head per day. This level of poloxalene (ca. 15 g.) controlled bloat. However, on two occasions when the animals were not shut in with the blocks, intake was reduced and the incidence of bloat increased. It is apparent that proper management of the block is important in regard to consumption and bloat prevention.

Eleven cases of severe alfalfa bloat in cattle were treated by administering a mixture of poloxalene and alcohol (5:1 v/v). The poloxalene and alcohol mixture was added to either 0.5 or 1.0 gal. of water and given by stomach tube or drench bottle. In each case bloat subsided in 10 to 25 min. Six severely bloated cattle were administered the poloxalene mixture by injecting it directly into the rumen. Bloat subsided in 50 to 70 min. It is apparent that poloxalene is an effective treatment for bloat. However, ruminal injection is not so effective as oral administration.



Effect of Poloxalene on Rumen Fluid Characteristics and Feed Intake of Cattle -  
H. Lippke and N. L. Jacobson, Iowa State University, Ames

These experiments were undertaken to study more closely the bloat preventive characteristics of poloxalene, a non-ionic surfactant, which when consumed in sufficient amounts affords complete protection from pasture bloat.

Twenty in vitro fermentation trials, in which a 25 g sample of fresh alfalfa was incubated with 150 ml of strained rumen fluid for 3 hr at 39°C, were conducted in the summer of 1964 using three pairs of poloxalene-fed and control steers as rumen fluid sources. Samples of rumen fluid were taken 1 hr after poloxalene feeding. During the in vitro fermentation, gas evolution and ingesta volume increase were measured at 20-minute intervals. The fresh alfalfa, which was used as a fermentation substrate, was cut into  $\frac{1}{4}$ -inch segments prior to introduction into the fermentation tubes.

For approximately 2 months prior to and during the in vitro fermentation trials the steers from which rumen fluid was collected were fed good to excellent quality alfalfa soilage ad libitum with the poloxalene-fed steers each receiving 10 g of poloxalene daily. The in vitro rumen fluid comparisons were made only within pairs; each pair consisted of one control and one poloxalene-fed steer. Following six in vitro fermentations, the surface tension of the fluid from the fermentation tubes was measured using a DuMouy tensiometer.

The fermentation trials and surface tension determinations of the previous year were repeated in 1965 in an experiment utilizing three control and three poloxalene-fed (10 g/animal/day) steers. These animals were fed medium to poor quality alfalfa soilage ad libitum during the standardization period (3 weeks) and the comparison period (4 weeks). Daily feed intake was measured for each animal.

The procedure for the in vitro fermentation trials varied somewhat from that of the 1964 trials. For each trial rumen fluid was collected from all six animals, and equal volumes were composited within treatment groups. The fresh alfalfa substrate was ground through a food chopper necessitating the use of 50 ml of an artificial saliva solution to quantitatively transfer the 25 g alfalfa sample into the fermentation tube. The surface tension of fresh rumen fluid as well as of fermentation fluid was measured.

In the 1964 trials an intensive streaming action, which was not evident in control fermentation tubes, was noted in the liquid phase of poloxalene tubes. It appeared that particles which had settled to the bottom of the fermentation tubes would accumulate gas causing them to rise to the liquid-foam interface. Here the gas was apparently released, allowing the particles to return to the bottom of the tubes. This phenomenon indicated the possibility of an altered surface tension of the fluid in poloxalene tubes.

Surface tension measurements indicated that poloxalene feeding reduced the surface tension of fresh rumen fluid by 6.7 dynes/cm ( $P < .01$ ) and of fermentation fluid by 8.3 ( $P < .01$ ) and 3.5 ( $P < .01$ ) dynes/cm in the 1964 and 1965 trials, respectively. The smaller difference in surface tension between the fermentation fluid from control and poloxalene-fed steers in the 1965

trials is attributed to the addition of artificial saliva solution to the fermentation tubes.

Maximum ingesta volume achieved during the 3-hr fermentation was reduced 16% ( $P < .05$ ) and 4% due to poloxalene feeding in 1964 and 1965, respectively. The smaller reduction in ingesta volume (effected by poloxalene) in the 1965 trials is thought to be due to the poor quality alfalfa soilage fed to the experimental animals, i.e., poloxalene could not reduce foaming because a serious foam-producing condition was not present.

Gas evolution was slightly increased ((8% and 15% ( $P < .10$ ) in 1964 and 1965, respectively)) by poloxalene feeding. If this is a real effect, one might postulate that the reduction in surface tension produced by poloxalene enables a greater mobility of rumen microorganisms and metabolites.

There was no difference in feed intake due to poloxalene feeding. Among animals, variations in relative intake, using each animal as its own standard, were surprisingly small. It was concluded that poloxalene feeding (1) reduces surface tension of rumen fluid and thereby reduces foaming within the rumen; (2) has no effect on feed intake at the level used; and (3) may slightly increase gas evolution.

Toxicologic Properties of Poloxalene - C. W. Leaf, Wyandotte Chemical Corporation, Wyandotte, Michigan, C. H. Wolf and J. C. Calandra, Industrial Bio-Test Laboratories, Northbrook, Illinois

The results of a 90-day study to determine the possible toxicologic effects of feed poloxalene to albino rats are reported. Groups of 50 weaning albino rats, each equally divided with respect to sex, were fed poloxalene at three levels in the diet, viz 40, 200 and 500 mg/kg of body weight. A fourth group fed stock diet served as controls.

All diets were freshly prepared each week by adding the calculated amount of poloxalene to a pre-weighed portion of the stock ration and thoroughly blending the mixture. The dietary levels were adjusted periodically in order to maintain the correct dosage on a mg/kg body weight basis. The amount of food allotted per week to each animal was sufficient for ad libitum feeding. Water was also allowed ad libitum.

Initially, the body weight of each rat was determined and recorded. Thereafter weighings were conducted weekly, the data being recorded as an index to growth. Weight gains over the 90-day test period were computed and subjected to statistical analyses. Analysis of Variance was conducted first and any effects disclosed by that treatment were further studied by "t" test. Checks were made daily for mortality and untoward behavioral reactions.

Blood studies, including determinations of hemoglobin concentration, erythrocyte count, fasted blood glucose concentration and both total and differential leukocyte counts were conducted on five male and five female rats randomly selected from the control groups and the 200 and 500 mg/kg dose groups immediately prior to the inception of the study and after 77 days of testing. Urine analyses for reducing substances, protein and microscopic elements were conducted at the same time and on the same rats as the blood studies.

Following 90 days of feeding, all surviving animals were sacrificed and subjected to complete gross pathologic examination. At this time, a complete set of organs and other tissues was removed from each rat for histopathologic study. Also, the body weight of each rat at necropsy was recorded along with the weight of the liver, kidneys and testes. Statistical analyses were conducted on these organ weights and upon their corresponding ratios to the body weight.

Complete microscopic examinations were conducted upon ten male and ten female animals from the control group and from the 200 and 500 mg/kg dose groups to ascertain the presence or absence of histopathologic change as a result of poloxalene ingestion.

In order to determine whether the lowered food consumption and resultant weight depression noted among 500 mg/kg animals was a result of diet palatability or a toxic manifestation of the material, a special paired-feeding study was conducted. In this study, a group of five male and five female rats was paired with an equal number of rats from the 500 mg/kg dose group. Animals with equal body weights were used in pairing. The paired-fed control rats were fed daily amounts of stock ration equivalent to the amount of test diet consumed by their 500 mg/kg test group partner during the preceding 24-hour period. This procedure was followed for 17 days during which time the animals were weighed twice weekly and the weights recorded.

Animals fed poloxalene at levels of 40 and 200 mg/kg/body weight exhibited growth patterns comparable to control. Rats fed the material at a level of 500 mg/kg/body weight exhibited a moderate growth depression as compared to controls. This depression was statistically significant at the 95 per cent confidence level.

The special paired-feeding study confirmed the fact that the weight depression was a result of poor diet acceptance rather than toxic manifestation of the test material.

One control male succumbed after 66 days of testing while one male and one female in the 500 mg/kg group died after 20 and 52 days of the study, respectively. Deaths were attributed to acute respiratory infection. No untoward behavioral reactions were displayed by any of the rats employed in the study.

Values for all hematologic, urinary and clinical blood chemistry parameters studied were within the normal range for the albino rat and were comparable to those of control animals. Organ weight and ratio data collected from animals in the 40 and 200 mg/kg groups were essentially comparable to those of control. A few random inter-group differences were seen, none of which could be related to ingestion of poloxalene. Organ weight and ratio data for 500 mg/kg animals revealed elevations in liver weight and liver to body weight among females. The male liver and testes weights were comparable to or higher than control; however, none of these elevations was statistically significant. Kidney weights of the male and female 500 mg/kg animals were lower than control; however, this finding is expected in view of the body weight depression observed, and only the male kidney weights and

kidney to body weight ratios were significantly lower than control statistically. No significant gross or microscopic alterations were noted among organs and tissues examined from test animals (200 and 500 mg/kg) which could be related to ingestion of poloxalene.

In summary, ingestion of poloxalene at levels up to and including 200 mg/kg produced no untoward effects in any of the parameters investigated.

#### MICROBIOLOGY PANEL

##### Studies on the Relative Importance of Isovalerate Carboxylation Reactions in Biosynthesis of Leucine by Rumen Microorganisms - Milton J. Allison, National Animal Disease Laboratory, USDA, Ames, Iowa

Ruminococcus flavefaciens, a cellulose digesting anaerobe from the rumen, synthesizes leucine using the carbon skeleton of isovalerate. Isovalerate is carboxylated by an unknown mechanism so that leucine synthesized from isovalerate-1-C<sup>14</sup> is labeled in carbon number 2. Carbon dioxide, but not formate, contributes to the carboxyl carbon of leucine. This pathway of leucine biosynthesis is distinct from the pathway that has been described in E. coli, Neurospora, Salmonella and other aerobic or facultative microorganisms. The present studies were designed to assess the relative importance of the isovalerate carboxylation pathway in the rumen.

Cell-free extracts of R. flavefaciens, bacteria obtained from rumen contents, and E. coli were assayed for the enzyme  $\alpha$ -hydroxy- $\beta$ -carboxy-isocaproate dehydrogenase. This is an enzyme present in the aerobic leucine biosynthetic pathway. The ratio of enzymatic specific activity (units/mg of protein) in extracts from E. coli, mixed rumen bacteria, and R. flavefaciens was 200:4:1, respectively. This suggests the pathway that is operative in E. coli is relatively unimportant in the rumen. Whole rumen contents were fermented for 90 minutes in vitro in acetate-2-C<sup>14</sup>. Amino acids from the bacterial protein were isolated by column chromatography and their specific activities were determined. Most of the C<sup>14</sup> in amino acids was in alanine, aspartic acid, and glutamic acid (21%, 19%, and 17%, respectively). About 6.6% of the C<sup>14</sup> in the amino acids from the rumen bacteria was in leucine and the specific activity of leucine was .56 of the specific activity of alanine. In the pathway operative in E. coli, a 2-carbon unit from acetate becomes carbon 1 and 2 of leucine and when organisms employing this pathway are grown in labeled acetate, leucine is often one of the most highly labeled amino acids. Since this was not the case in the rumen, it is suggested that the pathway operative in E. coli is not the predominant pathway in the rumen.

Our survey of pure cultures of rumen bacteria is incomplete, but important rumen bacteria other than R. flavefaciens that incorporate isovalerate into cell protein include Bacteroides ruminicola and Peptostreptococcus elsdenii. Organisms that do not incorporate significant amounts of isovalerate-1-C<sup>14</sup> during growth include strains of Selenomonas ruminantium, Lachnospira multiparus, and Butyrivibrio fibrisolvens.

The Relationship of Aerobic and Anaerobic Mucinolytic Bacteria to Bloat in Cattle - L. R. Fina, E. E. Bartley, B. D. Mishra, and T. J. Claydon, Kansas State University, Manhattan

Several aerobic bacterial isolates from the rumen readily utilized bovine salivary mucin as the sole source of organic nutrients. Also, saliva was found to be a potential source of aerobic mucinolytic bacteria (average counts were 87,000 per milliliter). Identical twins that received hay before pasturing had fewer aerobic (facultative) mucinolytic bacteria in rumen contents than twin mates receiving pasture only. Rumen contents from bloat-susceptible twins were more mucinolytic than were contents from nonsusceptible twins when inoculated into a saliva substrate. Bloat may occur more readily when mucin concentration in the rumen is lowered by reduced salivation on succulent feeds and/or by destructive action of mucinolytic bacteria. Preliminary results using anaerobic bacteria, isolated from the rumen, are similar.

Production of Gas Bubbles by Rumen Protozoa from Bloating Cattle - R. Kodras, Kansas State University, Manhattan

In a recent study, rumen protozoa isolated from cattle bloating on high grain rations or fresh alfalfa tops were observed to contain and expel gas bubbles. The production of a gas bubble by a protozoan, described here for the first time, occurred by accumulation of gas in the protozoan, passage of the gas through the esophagus and cystostome, and formation of a shape like a bowling pin, then a dumbbell, and a sphere as the gas bubble was expelled. Protozoa from non-bloating animals did not produce gas bubbles. This observation suggests that protozoa may be the cause of bloat in ruminants. As the number of protozoa increase with the approach of bloat, a greater number of protozoa die or burst and consequently produce a highly viscous rumen fluid. This coupled with the production of gas bubbles by protozoa could result in a stable foam or bloat.

Bloating cattle treated with copper sulfate remained free from bloat for approximately 5 to 10 days. Bloat returned with the return of a high ciliate population. One animal on a high grain ration became spontaneously defaunated when it went off feed. The rumen pH dropped to about 5. The animal has not bloated for 3 weeks.

The volume of protozoa (obtained by settling) in the rumen contents showed a positive correlation with the severity of bloat. The ciliate volume from bloaters measured about 8 to 11%, while non-bloaters had about 4% or less. This variation in number and also genera of protozoa found in individual animals suggests an association with the sporadic incidence of bloat in a herd.

Addition of one ml of alfalfa juice to ten ml of rumen contents containing protozoa resulted in the release of 2.6 ml of gas in 10 minutes. Rumen contents freed from most of the ciliates produced about 0.4 ml of gas.

Hydrogen cyanide at 0.01% concentration and copper sulfate at 0.01 to 0.02% concentration were lethal to protozoa in vitro.

When rumen contents obtained from a bloating and non-bloating animal were compared, a color difference was observed. Rumen contents of a bloater had a yellow green color while those of a non-bloater had a deep green color. Protozoa readily ingest plant material or chloroplasts. It appears that when the chlorophyll becomes colorless upon ingestion or oxidation the yellow pigments, xanthophyll and carotene, are unmasked and give the rumen contents a yellow-green color

Food particle size affected the nutrition of different protozoa. Rich starch granules, 3 to 8 microns in size, were readily ingested by isotrichs and the larger oligotrichs but not by the smaller protozoa. Corn and wheat starch granules, 5 to 30 microns, were ingested by the large oligotrichs. However, the large potato starch granules, up to 100 microns, were not ingested by any of the protozoa.

Methods for cultivating *Casytricha ruminatum* - R. E. Hungate, California Agricultural Experiment Station, Davis

One culture was maintained in vitro for a period of 65 days during which there were many cell divisions and a definite growth of the protozoa, indicating that if the time and effort had been expended it would have been possible to grow them continuously in vitro. The medium contained rumen fluid, and an extract of rumen holotrich protozoa was required in order for division to occur. This requirement could also be met by bovine serum. It was necessary to feed sucrose during only a portion of the day in order to avoid excessive bacterial growth. It was not possible to obtain conclusive evidence as to the ingestion or necessity of bacteria but a positive correlation between success of a culture and the formation of methane was observed.

PHYSIO-PATHOLOGY PANEL

A New Technique for In Vivo Metabolic Studies in Large Animals - R. W. Phillips, Colorado State University, Fort Collins

Ruminants make excellent experimental animals in that multiple sampling may be performed on a single animal, minimizing the effect of individual variation. A method has been developed which expands the use of such animals by implanting a vinyl zipper in the body wall allowing relatively unlimited access to the peritoneal cavity. Multiple biopsys of liver were removed and assayed for glycogen concentration and the activities of glucose-6-phosphate dehydrogenases, aldolase, and glutamic oxalacetic transaminase. The concentrations of these enzymes did not vary under prolonged fasting. Portions of the liver which were examined histologically showed considerable fat infiltration in a fasting pregnant ewe and little fat infiltration in a non-pregnant fasting ewe.

Acute Bovine Pulmonary Emphysema and Its Possible Relationship to the Ruminant Digestive Tract - James O. Tucker, University of Wyoming, Laramie

Pulmonary emphysema of cattle is an acute, sometimes chronic, noncontagious, afebrile disease. It is usually characterized by a sudden onset with severe expiratory dyspnea which is exaggerated by exercise. Acute bovine pulmonary emphysema can occur anytime during the year, but the occurrence is greatest during the summer and fall seasons and is associated with a change in forage. Usually, if the disease is to occur, it will be manifested within ten days after cattle have been transferred from a dry summer pasture to an improved green or ungrazed "lush" type of pasture.

Mention was made of bovine pulmonary emphysema as it occurs and is reported in various areas of the world. The inherent structural weakness of the bovine lung is discussed as a possible cause for so many conditions resulting in an emphysematous condition of the organ.

Theories as to the cause of A.B.P.E., which have tentatively been discarded, were enumerated and a multiphased postulate advanced.

Effect of Rumen Insufflation Upon Renal Function of Calves - S. D. Beckett, University of Missouri, Columbia

The rumen of conscious Holstein heifer calves were insufflated to 40 and 50 mm Hg pressure with nitrogen gas and the following parameters studied: Heart rate, blood pressure, hemoglobin, blood glucose, oxygen use, renal blood flow, glomerular filtration rate and potassium excretion. The heart rate, mean blood pressure and hemoglobin levels increased during rumen insufflation. The arterial oxygen increased 0.45 vol percent at 40 mm Hg but decreased 2.11 vol percent at 50 mm Hg. When the oxygen was expressed on a per gram of hemoglobin basis, the milliliters of O<sub>2</sub> decreased during insufflation. This indicates that the oxygen saturation of hemoglobin decreases during insufflation even though the oxygen per 100 milliliters of blood is increased. The arterial glucose levels were fairly constant if the animal remained quiet through the experiment but increased if the animal struggled.

At 40 mm Hg intra-ruminal pressure (IRP), the renal blood flow decreased 27.6 percent and glomerular filtration rate 17.9 percent. Even though the urine volume was reduced 71 percent, the total potassium excreted was reduced only 27 percent. The A-V difference or oxygen extraction per 100 milliliters of renal blood flow increased, although the milliliters of oxygen per gram of hemoglobin decreased, and in this way the kidney could maintain a relatively constant oxygen consumption.

At 50 mm Hg IRP, there were much greater changes in the various parameters. The renal blood flow decreased 55.9 percent and glomerular filtration rate decreased 72.4 percent. The total amount of potassium excreted decreased 85% although the urinary concentration of potassium increased. The milliliters of oxygen extracted by the kidney per 100 ml of renal blood flow increased over the control level; however, due to reduced renal blood flow the total oxygen available to the kidneys was less.

Under the conditions of this experiment the kidney were able to compensate at 40 mm Hg IRP and maintain a fairly normal renal function. At 50 mm Hg IRP, the renal function was greatly reduced from the control values.

Absorption of Lactic Isomers From Ovine Intestinal Loops - R. H. Dunlop  
and E. M. Stefaniak, University of Saskatchewan, Saskatoon, Canada

Earlier work has shown that blood lactate can rise as rapidly as 1.6 mM/1/hour in cattle engorged on grain or corn. Most of the rise was attributable to D-lactate which is not usually detectable in bovine blood. Since the rise in lactate was accompanied by a pronounced fall in blood pH, it appeared that the undissociated forms of lactic acid were absorbed more rapidly than their respective salts. Experiments were designed to test this theory in cattle having rumen fistulae and sheep equipped with Thiry-Vella intestinal loops. Lactic acid disappeared at about 25% per hour with respect to the marker, polyethyleneglycol 4000, when placed in the washed bovine rumen at pH 3.90 and an initial concentration of 200 mM/L. This disappearance of lactic acid was observed only if rumen motility was sustained; if the rumen was static, absorption could not be demonstrated. Absorption of pure isomers of lactic acid at both high and low pH was studied in the ovine intestinal loops by measuring the concentration of lactate and marker. A significant difference in absorption rates for the two isomers was not demonstrable. However very marked differences were found between absorption of the ionized lactate of each isomer and its undissociated lactic acid. The former was slowly absorbed at about 15% per hour from a solution having an initial concentration of 50 mM/L. The undissociated acid was absorbed very rapidly at an initial rate of over 30% in 15 minutes; the final rate reduced to 65% in 1 hour as a result of the rising pH and the declining concentration of lactic acid. It was concluded that both rumen and intestine could contribute to the absorption of lactic acid.

Electrical Impedance Changes Associated with Reticulo-Rumen Motility -  
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The electrical potentials due to movement of the reticulum were recorded in sheep using a pair of silver chlorided electrodes (1.5 cm. diam.) applied to the skin over the sternum and dorsal left side of the thorax, while the impedance of a pair of stainless steel electrodes (8 x 1.5 cm) applied to the skin on opposite sides of the ventral thorax near the coastal margin was measured simultaneously. The magnitude and phase-angle of the impedance of the stainless steel electrodes to a 100 kc. signal were measured against an external standard using an impedance comparator (GR 1605-A). With optimum positioning of both sets of electrodes, the waveform of the potential due to movements of the reticulum corresponds to changes in phase-angle and magnitude of the impedance of the stainless steel electrodes.

Some Physiological Effects of Feeding Pellets to Sheep - Ivan L. Lindahl and  
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A series of studies were initiated to determine some of the effects of feeding pelleted rations to sheep, particularly over extended periods of time. From 1958 through 1961, crossbred lambs were fed from birth pelleted alfalfa hay ad libitum in comparison with other rations, including chopped alfalfa hay



and a normal feeding regimen. The ewes received the same rations as their lambs until weaning at 120 days of age. The pellet and chopped hay lambs remained in dry lot at all times; those on the normal feeding regimen were allowed to graze and to consume creep feed and long hay. The pellet fed groups received no other dry feed during their lifetimes. The pellets were made from commercial U.S. No. 2, leafy, 45 percent green alfalfa hay.

Prior to weaning, lamb weight gains in all groups were similar; ewes allowed pellets free choice, however, gained appreciably prior to weaning. After weaning, the ad libitum pellet fed lambs gained appreciably more rapidly than either the chopped hay or normal regimen controls, and weighed, as yearlings, about 25 percent more. These differences were associated with a greater consumption of the pellets offered free choice.

Death losses in the ad libitum pellet fed groups were greater at all ages. Causes of death in these groups were overheating, rectal prolapse, gastroenteritis, liver degeneration, and congestion of the lungs. The cause of the gastroenteritis and liver degeneration and their pathologies have not been determined.

Observations on rumen development were made on a number of animals from the various groups. Ruminal musculature, especially the pillars, were not nearly as well developed in the pellet fed lambs. Rumen papillae of the pellet fed sheep were smaller, thinner and had rolled edges giving them a cupped-shaped appearance. Also, there were fewer papillae per unit area, particularly in the dorsal blind sac.

Rumeno-reticular volumes, measured by filling to a 3 cm over pressure with water, were not different in the various groups. Average percentage dry matter of the rumen contents was 19.4 percent in the pellet fed group, 11.6 percent in the chopped hay group, and 14.0 percent in the normal regimen group. Molar percentages of VFA in the rumen contents measured on four occasions, two of which were at slaughter, were not different among the various groups.

The ad libitum pellet fed group spent 45 percent less time eating and 43 percent less time ruminating than those of the chopped hay group. Average parotid salivary gland weight was significantly greater in the ad libitum pellet group than in the chopped hay group; the hypothesis is that this is a compensatory hypertrophy related to a reduced salivary flow which in turn is related wholly or in part to reduced eating and ruminating time.

Sheep fed pellets consume appreciably more salt, offered ad libitum as loose salt, than either the chopped hay or normal regimen groups, and the bedding requirements of the pellet groups were considerably greater. At less than ad libitum levels of intake, the pellet fed sheep developed a craving for fibrous material as shown by their eating bedding or chewing on wood.

The salt intake phenomenon was investigated with wethers that had not been fed pelleted rations previously. When chopped or pelleted alfalfa intakes were limited to 80 gm per day per W<sup>0.73</sup><sub>kg</sub>, and salt consumption limited to 8 gm per day, there was no differential effect of forage form on water consumption or

urine volume. When chopped or pelleted alfalfa was limited at that level and salt allowed free choice, salt consumption was appreciably greater by the pellet fed group, and water consumption and urine output were also significantly increased. A reversal of the two forage forms within a level of salt intake confirmed the results of the first in the limited salt group, but suggested that the greater free choice salt intake of the sheep in the first period persisted for a time into the second period on chopped hay

Studies of wood chewing by pellet fed sheep indicate that a number of interacting factors affect this craving, among them age of animal, ration, level of feeding, and ambient temperature. Presently under study are the effects of adding 0.5 percent sodium bicarbonate to the drinking water of sheep fed pelleted rations. Results are preliminary but water consumption is appreciably increased on this regimen.

Metabolism of Carbon-Labeled Methane by Sheep - R. W. Dougherty, J. J. O'Toole, and M. J. Allison, National Animal Disease Laboratory, Ames, Iowa

Sheep plasma (50 ml) was "saturated" with  $C^{14}H_4$  at atmospheric pressure and infused into the abdominal aorta of sheep through permanently implanted catheters. The  $C^{14}H_4$  "saturated" plasma was infused at 1 cc per minute for 1 hour.

Expired respiratory gases were monitored constantly by passing a fraction of the same through a  $^4$  Pi gas flow counter. Expired gas was also passed through soda lime to trap  $CO_2$ . Carbon dioxide in the soda lime was freed with acid and then trapped and precipitated as  $BaCO_3$ . Dried  $BaCO_3$  was suspended in toluene plus  $CB-o-Sil$  for measurement of radioactivity. It was estimated that of the  $2.5 \times 10^8$  counts approximately  $2.3 \times 10^4$  counts were retrieved in the soda lime trap as barium carbonate.

It is assumed from this work that a relatively small percentage of methane that is formed in the rumen is metabolized.

After infusion of plasma containing  $C^{14}H_4$  stopped, radioactivity in expired gas and in arterial blood rapidly declined. The decline of radioactivity in venous blood was, however, much slower, indicating a relatively large  $CH_4$  pool size in body tissues.