UNITED STATES DEPARTMENT OF AGRICULTURE
Agricultural Research Service
Office of Administrator
Washington 25, D. C.

February 17, 1958

TO : Participants in Conference on Rumen Function

FROM : H. W. Marston, Research Coordinator, Agricultural Research Service, U.S.D.A., Washington 25, D. C.

SUBJECT: Report of Conference

Attached is a copy of the notes of the fourth Conference on Rumen Function, held at the Maryland Hotel, Chicago, Illinois, on December 4-5, 1957, and a list of persons in attendance during the two-day meeting.

The Conference convened at 9:30 AM on December 4, 1957.

The chairmen of the various panels are to be complimented for their part in arranging a most interesting program. The speakers also are to be commended for the excellence of the material presented. At the close of this Conference it was agreed that a similar conference should be held in 1959, and that the same approximate time and place for the conference was satisfactory. It was also agreed that the same panel chairmen should be continued, or that they arrange for a successor.

Attachment

REPORT ON
CONFERENCE ON RUMEN FUNCTION
held at
Maryland Hotel, Chicago, Illinois
December 4-5, 1957

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

NAME	ORGANIZATION
N. L. Jacobson	Dairy Husb. Dept., Iowa State College, Ames
C. E. Stevens	College Vet. Med., Uni. Minnesota, St. Paul
L. Meyer Jones	Veterinary Div., Iowa State College, Ames
C. M. Stowe	College Vet. Med., Uni. Minnesota, St. Paul
Clyde K. Smith	Dept. Microbiology, Michigan State Uni., East Lansing
W. K. Kennedy	Dept. Agronomy, Cornell Uni., Ithaca, New York
G. D. Goetsch	Dept. Vet. Sci., Purdue Uni., Lafayette, Indiana
H. D. Jackson	Dept. Biochemistry, Purdue Uni., Lafayette, Indiana
Volney Wallace	Dept. Biochemistry, So. Dakota State College, Brookings
J. M. Scholl	Dept. Agronomy, Iowa State College, Ames
E. A. Hollowell	Crops Res. Div., ARS-USDA, Beltsville, Maryland
J. E. Miltimore	Dept. Dairy Husb., Summerland, B.C., Canada
H. L. Wilkins	Crops Res. Div., ARS-USDA, Beltsville, Maryland
R. E. Davis	Animal Husb. Div., ARS-USDA, Beltsville, Maryland
H. J. Anderson	Cudahy Laboratories, Omaha, Nebraska
George B. Garner	Dept. Ag. Chem., Uni. Missouri, Columbia
E. J. Carroll	Dept. Ag. Chem., Uni. Missouri, Columbia
A. D. Allen	Dept. Vet. Med., Uni. Missouri, Columbia
Roy S. Emery	Dairy Dept., Michigan State Uni., East Lansing
C. Ray Thompson	Western Utilization Res. & Development Div., ARS-USDA, Albany, California
R. S. Allen	Dairy Husb. Dept., Iowa State College, Ames
C. F. Huffman	Dairy Dept., Michigan State Uni., East Lansing
W. E. Thomas	Dept. Animal Industry, No. Carolina State College, Raleigh
W. D. Pounden	Dept. Vet. Sci., Ohio Agr. Expt. Sta., Wooster
M. P. Bryant	Animal Husb. Res. Div., ARS-USDA, Beltsville, Md.
Ray E. Ely	State Expt. Stas. Div., ARS-USDA, Washington, D. C.
James E. Johnston	Dairy Dept., Louisiana State Uni., Baton Rouge
A. B. Nelson	Animal Husb. Dept., Oklahoma State Uni., Stillwater
Allen D. Tillman	Animal Husb. Dept., Oklahoma State Uni., Stillwater
C. K. Whitehair	Dept. Vet. Med., Michigan State Uni., East Lansing
Frank H. Baker	Dept. Animal Husb., Uni. of Kentucky, Lexington
G. K. L. Underbjerg	Dept. Physiology, Kansas State College, Manhattan
C. L. Baldwin	Pitman-Moore Co., Indianapolis, Indiana
C. A. Yarns	Dairy Dept., So. Dakota State College, Brookings
H. R. Conrad	Dairy Dept., Ohio Agr. Expt. Sta., Wooster
C. L. Moore	Dairy Dept., So. Dakota State College, Brookings

NAME	ORGANIZATION
Arthur E. Dracy	Dairy Dept., So. Dakota State College, Brookings
R. E. Nichols	Dept. Vet. Sci., Uni. Wisconsin, Madison
J. F. Sykes	Animal Husb. Div., ARS-USDA, Beltsville, Maryland
R. E. Hodgson	Animal Husb. Div., ARS-USDA, Beltsville, Maryland
Howard W. Johnson	Animal Disease & Parasite Div., ARS-USDA, Beltsville, Maryland
Don R. Jacobson	Dairy Dept., Uni. of Kentucky, Lexington
W. E. C. Moore	Dept. Bacteriology, Virginia Polytechnic Institute, Blacksburg
Ben F. Barrentine	Animal Husb. Dept., Mississippi State College, State College, Miss.
I. L. Lindahl	Animal Husb. Div., ARS-USDA, Beltsville, Maryland
James M. Boda	Dept. Animal Husb., Uni. California, Davis
H. H. Cole	Dept. Animal Husb., Uni. California, Davis
Harry W. Colvin, Jr.	Dept. Animal Industry, Uni. Arkansas, Fayetteville
Wolcott E. Stewart	New York State Vet. College, Cornell Uni., Ithaca
Laurent Michaud	Merck & Co., Inc., Rahway, New Jersey
L. C. Payne	Uni. Nebraska, Lincoln
J. C. Thompson	Raltson Purina Co., St. Louis, Missouri
J. L. Williamson	Ralston Purina Co., St. Louis, Missouri
William B. Hardie	Chas. Pfizer & Co., Terre Haute, Indiana
William H. Hale	Chas. Pfizer & Co., Terre Haute, Indiana
A. M. Lee	Animal Disease & Parasite Div., ARS-USDA, Beltsville, Maryland
Roland A. Gessert	Food & Drug Administration, Washington, D. C.
Richard E. Brown	Dept. Dairy Sci., Uni. of Illinois, Urbana
W. O. Nelson	Dept. Dairy Sci., Uni. of Illinois, Urbana
Ralph A. Shaw	Dept. Biochemistry & Vet. Sci., Purdue Uni., Lafayette, Indiana
G. C. Anderson	Dept. Animal Husb., West Virginia Uni., Morgantown
Bruce Taylor	Animal Sci. Dept., Uni. Arizona, Tucson
R. W. Dougherty	New York State Veterinary College, Cornell Uni., Ithaca, N. Y.
G. O. Mott	Dept. Agronomy, Purdue Uni., Lafayette, Indiana
L. R. Fina	Dept. Bacteriology, Kansas State College, Manhattan
Maurice E. Heath	Dept. Agronomy, Purdue Uni., Lafayette, Indiana
J. C. Shaw	Dairy Dept., Uni. Maryland, College Park
H. W. Marston	ARS-USDA, Washington, D. C.

For the purposes of discussion, the program was divided into panels. The identification of the panels and the chairman of each was as follows:

(a)	Animal Management	-	Dr. H. H. Cole
(b)	Agronomic	-	Dr. J. N. Scholl
(c)	Rumen Physiology	-	Dr. C. F. Huffman
(d)	Microbiology	-	Dr. W. D. Pounden
(e)	Physio-Pathology	-	Dr. R. W. Dougherty

ANIMAL MANAGEMENT PANEL

Froth Production and Incidences of Bloat When Fistulated and Normal Cattle are Grazed on Ladino Clover with Different Grazing Routines - W. E. Thomas, North Carolina Agricultural Experiment Station

During the bloating seasons of 1956 and 1957 there have been considerable losses from bloat in North Carolina in the coastal areas. Rumen fistulated cows were hauled to some of the farms when losses were occurring and the rumen contents studied under these severe bloating conditions. The most satisfactory measure of the bloating hazard was to weigh a pint of rumen contents and if the specific gravity was down to approximately 0.60 the normal cows in the herd were in danger.

The degree of bloat in the normal cows and the froth production as measured by specific gravity of rumen contents of fistulated cows were remarkedly consistent. If the cattle were turned to good pasture hungry there was rapid grazing of clover for 30 to 45 minutes and then a search for grass or briers in hedgerows was underway. Bloating would be at its worst about 45 minutes later or approximately 1-1/2 hours after first being turned to pasture. If the cattle were teased with some 10 minute grazing periods before being permitted to eat what they wanted the froth production and bloating incidence were not great. Hay feeding was not effective in preventing the initial rapid froth production.

One 400 acre area of Ladino clover was managed with the idea that if approximately 200 cattle were confined to just 25 acres the feed supply would be so scarce that bloating would be controlled. Eight animals died with this system even when salt-penicillin was available fresh free choice. This area was very fertile and moisture abundant but the water contained some sodium chloride.

Relationship of Plant Composition, Weather, and Prophylactic Measures to Intensity of Alfalfa Bloat - R. H. Johnson, L. R. Brown, N. L. Jacobson, R. S. Allen, and P. G. Homeyer, Iowa State College

In one series of trials, 36 steers (av. initial wt. 556 lb.) grazed alfalfa pasture of high bloat-producing potential. Animals grazed for 3-1/2 hrs. daily during each of two periods, one in the AM and one in the PM. Immediately preceding grazing, all groups received equal amounts

of grain or grain mixed with preventive. During and for several hours after each grazing period the animals were under constant observation. Preceding each trial animals were ranked by bloat susceptibility and randomly divided into three groups of approximately equal numbers and bloating potential. Body weights were taken at the beginning and completion of each trial. Bloat was greatly reduced by (1) soybean oil (0.25-0.53 lb. per head) in each feeding of grain, (2) lard oil, a water dispersible lard derivative, (0.25 lb.) in grain, (3) lard oil (2%) in the drinking water, and (4) lecithin (0.19-0.27 lb.) plus lard oil or soybean oil (0.06-0.09 lb.) in grain. The effect was very marked for 3-4 hours after treatment; thereafter the effect diminished rapidly at low dosage levels but more slowly at higher levels. Penicillin (75 mg. per head per day) reduced bloat sharply for 9 days but subsequently bloat increased to the level of the controls. Increasing the level of penicillin to 125 mg. again depressed bloat but the effect subsided after 2 days.

Daily gains of the animals receiving the various oil treatments were 0.07 to 0.75 lb. greater than the respective controls. Daily gains of animals fed penicillin were somewhat greater than the controls.

When animals bloated severely an attempt was made to release any free gas with a stomach tube. This was usually unsuccessful; then 150-250 ml. of lard oil was introduced into the rumen by stomach tube. Soon after administration of the oil large quantities of gas were released from the rumen via stomach tube and/or eructation. In most instances, relief from the critical stage occurred within a few minutes, and complete recovery within an hour.

In a second series of trials, 22 dairy animals, varying in size, sex and breed were divided into two comparable groups, confined to dry lot, and fed alfalfa soilage ad lib. Observations of bloat were taken at least hourly after feeding the soilage. All animals were weighed at weekly intervals, and the treated and control groups were alternated every two weeks. Crude soybean oil sprinkled daily over the soilage at the rate of 0.25 lb. per 1000 lb. body weight of animal effectively controlled bloat. Administration of n-decyl alcohol, 18 ml. per 1000 lb. body weight of animal daily, reduced bloat but the effect was of too short duration for satisfactory prophylaxis.

The same procedure was followed in treating severe cases of bloat, except that the agent used was n-decyl alcohol administered in approximately 25 ml. doses by stomach tube or intraruminally by hypodermic needle. Decyl alcohol caused a prompt reduction in bloat, but the effect lasted for only about 2 hours.

Rumen ingesta samples were taken at regular intervals from all animals via stomach tube, and prepared for analysis by straining through 4 layers of cheese cloth. Surface tension was determined by a ring-type tensiometer. Fifty ml. of ingesta was placed in a Waring Blendor and agitated for 2 minutes; the increase in volume was considered to be foam volume, and the

amount of foam remaining at the end of one hour was considered as the foam stability. To determine ingesta volume increase (I.V.I.), an additional 50 ml. of ingesta was incubated in a 100 ml. graduated cylinder at 37°C. for 2 hours. Ammonia was determined by an aeration technique.

Rumen ingesta samples which had high surface tension, I.V.I. and ammonia values were associated with high bloat severity. Foam volume and foam stability as measured in this experiment did not seem to be associated with bloat severity. Treatments that were effective in reducing bloat severity reduced surface tension considerably, reduced I.V.I. somewhat and either slightly reduced or did not affect rumen ammonia.

Forage samples were taken twice weekly from the pasture forage and from the soilage by random sampling techniques. Samples were immediately frozen and held in that state until analyzed for dry matter, total protein, non-protein nitrogen, amino nitrogen, reducing sugars, phosphorus and ash. Forage high in moisture and high in phosphorus was associated with high bloat severity. No other relationships between forage composition and bloat were apparent.

Weather observations (including temperature, relative humidity, rainfall, cloudiness, wind velocity and moisture on the forage) were taken daily at 6 AM and 6 PM. Bloat severity was less on rainy days and increased 1-2 days following significant rainfall. On hot days bloat severity was less; otherwise, there was little or no relationship between weather observations and bloat severity.

The Influence of Tallow on the Incidence and Severity of Cattle Bloat - E. S. Erwin, C. B. Roubicek, L. Rosenblatt, and F. Pritchard, University of Arizona

Because fat provides energy for the animal as well as being a possible bloat prophylactic, a tallow supplement was fed to steers to test its possible benefit. Fifty-nine yearling steers were randomly allotted to ten pens and fed fresh field-chopped alfalfa ad lib. twice daily throughout the experimental phases. During Phase A (36 days) four, four and two randomly selected pens of steers were fed twice daily a barley, fat, and cotton gin trash supplement. While the fat and barley supplement was fed at the rate of 0.9 lb./100 lbs. body weight, the amount of gin trash fed was the same in all groups.

The amount of supplement was increased from 0.9 to 1.5 lb./100 lbs. body weight in two of the four pens fed the fat and barley supplements during Phase B (56 days); the remaining treatments were unchanged.

The bloat observations were made on all animals during the daylight hours only. Since bloat varies in degree of severity, an index 1, 2, 3 and 4 was established; 1 being mild bloat and 4 being death resulting from bloat.

The maximum bloat period commenced with the last week of Phase A and continued throughout Phase B. Of the 273 cases of bloat observed during Phase A, 41.3, 35.9, and 22.8 percent of the bloat (based on steer days) were attributed to gin trash, barley, and fat supplemented groups of steers respectively. However, the severity of bloat was slightly greater in those animals supplemented with tallow.

During Phase B (56 days) 573 cases of bloat were observed. The incidence of bloat was markedly less in those steers supplemented with tallow. In addition, the few cases of bloat that were observed in the tallow fed animals were largely of a mild type.

Prevention of Bloat on Alfalfa Tops with Animal and Vegetable Fats,
J. M. Boda and Thomas Wegner, University of California

A series of trials have been conducted to study the effectiveness of added vegetable oil and of supplemental concentrates containing animal tallows in preventing bloat in dairy cattle fed freshly harvested alfalfa tops.

Effect of Added Vegetable Oil - Eight cows, selected for bloat susceptibility, were fed fresh alfalfa tops ad lib. for a 2-hour period at 8 AM and again at 1 PM. The alfalfa tops were harvested in the pre-bloom stage of maturity at approximately 7 AM each morning. At the end of each 2-hour feeding period, alfalfa consumption was determined for each animal. Thirty minutes after the start of each period and at 15-minute intervals thereafter, until intrarumen gas pressure returned to the pre-feeding levels, the degree of bloat was estimated with a tympanometer with the rumen relaxed. After a 4-day preliminary period, the animals had attained a fairly stable alfalfa intake and moderate to severe bloat was observed in most of the animals. They were divided randomly into two groups. At the morning feeding period, one group received fresh alfalfa containing vegetable oil (Wesson) added at the rate of 1 ml. per pound of fresh alfalfa. The other group served as controls. Oil was not given at the afternoon feeding period to either group. This procedure was followed to allow the disappearaance of any residual effect of the oil by the subsequent morning and to determine if the effect of the oil on bloat would carry over to the afternoon period. The following morning, the two groups were reversed, the second group receiving oil, the first group serving as controls. This reversal procedure was continued for 4 days.

It was found that the addition of oil to the alfalfa at the morning feeding period prevented bloat at that period. The average, maximal, intrarumen, gas pressure attained in the cattle receiving oil was approximately 1 mm. Hg. above atmospheric pressure. In the control cattle, the average pressure was 27 mm. Hg. In one case, when the intrarumen pressure reached 50 mm. Hg., the animal was treated effectively by introducing (via stomach tube) 100 ml. of vegetable oil into the rumen.

There was little difference in average alfalfa consumption, at the morning feeding period, between the animals receiving oil and the controls. The average consumption in the afternoon was the same for both groups. There

appeared to be little residual effect of the oil in preventing bloat at the afternoon feeding period although bloat in all animals was much less severe in the afternoon. The average pressure attained in the animals which had received oil in the morning was 11 mm. Hg. as compared with 14 mm. Hg. for the controls. No explanation can be given for the marked reduction of the severity of bloat in all animals in the afternoon as compared with the morning periods. Wilting of the alfalfa was slight and alfalfa consumption nearly the same at both periods.

Effect of Supplemental Concentrates Containing Animal Tallows - Two types of animal tallow were used in these trials, commercial yellow grease with a titer of approximately 38°C and fancy bleachable tallow with a titer of 41.5°C. Saponification value of both tallows was 196. The experimental plan was similar to that outlined above except that the tallows were incorporated into a concentrate mixture consisting of 9 parts ground barley and 1 part dried beet pulp. The concentrate mixtures were fed approximately 15 minutes preceding the morning feeding period of alfalfa. Control animals received an equivalent amount of concentrates. Concentrates were not given at the afternoon feeding period to either group. The concentrate intake per animal varied from 2 to 5 lb., the amount of tallow added to the concentrate from 2 to 10%. Reversal type trials were conducted at each level of concentrates and added tallow. The number of morning observations at each level varied from 9 to 28. Control observations were equivalent in number.

Tallow intakes exceeding 0.06 lb. per animal, regardless of the type of tallow, completely prevented bloat at the morning feeding period. A few mild cases of bloat were observed in animals receiving 0.06 lb. and one case of severe and several cases of moderate bloat occurred in cattle receiving 0.04 lb. yellow grease. Statistically highly significant increases of alfalfa consumption at the morning feeding periods were associated with the prevention of bloat by the tallow. The average alfalfa consumption by the tallow fed animals was 39 lb. compared with 27 lb. for the controls. The average alfalfa consumption by the animals receiving yellow grease at levels greater than 0.04 lb. per head, was 39 lb. compared with 34 lb. by the controls, a difference which was statistically significant at the 5% level.

The effect of the tallows on bloat and alfalfa consumption, even at tallow intakes of 0.5 lb. per head, did not carry over to the afternoon feeding period to any appreciable extent. The average intrarumen pressures of the animal which had received fancy tallow at the morning feeding period was 10 mm. Hg. The average control pressure was 13 mm. Hg. Even though the difference between these means was statistically significant, there were a number of moderate cases of bloat in both groups and it is doubtful if the difference is of practical importance. In the animals receiving yellow grease in the morning, there was no residual effect of the oil in the afternoon. Both the tallow fed and control groups had average intrarumen pressures of 12 mm. Hg. above atmospheric pressure.

These data demonstrate that the use of animal and vegetable fats will effectively prevent bloat, at least for a limited period of time after administration, in cattle fed freshly harvested alfalfa. The marked increase of alfalfa consumption associated with the prevention of bloat by animal tallow is of obvious practical importance. In management operations where freshly harvested legumes are fed and where the fats can be added directly to the fresh material or be fed with supplemented concentrates, these findings have direct application. However, because of the brief residual effect of these fats in preventing bloat, a period of less than four hours after administration, their use under pasture conditions is limited, unless methods are available for feeding the fats periodically through the day.

Continuous Feeding of a Salt-Penicillin Mixture to Steers Grazing Ladino Clover - B. F. Barrentine, C. B. Shawver and L. W. Williams, Mississippi State College

Paddocks of Ladino were used in this study. Steers grazed these paddocks for 90 minutes morning and afternoon and between grazing periods, the steers were kept in dry-lot with shade and water but no feed. Nine steers which bloated regularly under this program were selected for this study. The selected steers bloated 100% of the time the day preceding starting the penicillin-salt feeding.

The penicillin-salt mixture contained 0.175% procaine penicillin which was approximately 50 mg. of procaine penicillin per ounce of salt. The penicillin-salt mixture was offered free-choice to the steers while they were in the dry-lot for a period of 28 days. Since there were 9 steers grazing twice daily the maximum number of bloat cases that could occur was 18. As previously stated, there were 18 cases of bloat in this group of steers the day preceding the penicillin-salt feeding. On the first day of treatment there were 3 cases of bloat. Bloat varied from 3 to 6 cases until the 10th and 11th day at which time only 1 case of bloat occurred each day. There was a gradual increase in the cases of bloat until the 16th day at which time 9 cases of bloat occurred. Bloat decreased again until feeding was stopped and there was a rapid increase of bloat. On the 6th day after stopping the penicillin-salt feeding, there were 12 cases of bloat and one steer died and another required treatment. The study was discontinued at this point. Penicillin-salt consumption was high, averaging about 5 oz. per steer per day.

The incidence of bloat was about 28% during the 28-day period. It is estimated that the incidence of bloat would have been about 90% without penicillin-salt treatment.

Feeding Penicillin for Control of Bloat in Grazing Cattle and Its Effect on Milk Production, R. S. Emery, C. K. Smith, and C. F. Huffman, Michigan State University

The occurrence of bloat was followed on 20 farms in four counties for 150 days (May 12 to Oct. 8, 1957). The trial includes a total of 110,790 cow days or an average of 739 cows for 150 days. One herd was yearling Hereford heifers while the others were Holstein, Guernsey, or Jersey dairy herds. The herds were chosenon the basis of interest and a past history of problems with bloat. Fifteen herds were divided into approximately equal groups and one of these groups was fed 100 mg. of procaine penicillin either daily or every third day. The remaining cows in these 15 herds served as a control group and were managed in the same manner as the treated group with the exception of not receiving penicillin. The penicillin was fed on top of the grain mixture in a soybean oil meal carrier containing 850 mg. procaine penicillin per lb. or in trace mineralized salt carrier containing 3 g. of procaine penicillin per lb. The other 5 herds were offered loose salt containing 3 g. of procaine penicillin per lb. on a free-choice basis. The salt was usually placed daily and in no case was it allowed to remain in the box longer than 3 days. All herds were visited about every 2 weeks to evaluate pasture conditions, observe feeding practices, and obtain histories on any occurrence of bloat.

In addition, 40 cows in the University herd were assigned to 5 rations and 4 cows on each ration were fed procaine penicillin mixed into their grain at the rate of 8 mg. per lb.

The bloat incidence was 9.7% with a mortality of 4.2% of the bloated animals. There was no bloat in the University herd during the 6 week duration of the trial. Bloat cases were rated from 1 to 3 on the basis of severity. A rating of 1 indicates a mild bloat with spontaneous recovery while a 2 rating describes a cow in definite distress which was usually treated in some manner to relieve the bloat and a 3 rating describes a death due to bloat or a cow which obviously would have died if prompt treatment had not been initiated. In the above groups there were 39 cases of bloat with a rating of 1, 26 cases with a rating of 2, and 7 cases with a rating of 3. Three deaths occurred.

The bloat incidence was usually greatest on pastures with an abundant growth and a high percentage of legumes. However, some bloat was observed on pastures with 10% or less of legumes. Cows were particularly prone to bloat when changed to a new and better pasture. The 20 farms seemed to be characteristic as to their incidence of bloat and the pattern it followed. These farms can be separated into 2 groups, those with considerably more or less bloat than the 9.7% incidence given above. A continuously declining incidence of bloat occurred throughout the season, but individual farms varied greatly from this pattern.

The 144 monthly butterfat production records for the penicillin treated cows averaged 38.5 lb. as compared to 38.7 lb. for the 137 records from control cows. The difference of 0.2 lb. is not significant. A mean daily

4% fat corrected milk production of 27.2 lb. was observed for the penicillin treated cows in the University herd as opposed to 27.5 lb. for the controls. Neither this difference nor the difference attributable to rations was significant. Incidently, it should be noted that results obtained in the 2 separate trials showed a slight depression of milk production in penicillin fed groups. The value of this milk must be added to the cost of the treatment if this is the case.

Experience obtained in other experiments with penicillin by the authors indicates that a marked depression of milk production often occurs for several days after inclusion of 400 mg. procaine penicillin in the ration. This depression of milk production may be accompanied by loss of appetite and other symptoms including discharge at the mostrils and hyperemia of the vaginal mucosa. All symptoms disappeared in a few days and production returned to normal although penicillin feeding was continued. These symptoms were noticed in 1 herd on the field experiment in cows receiving only 100 mg. procaine penicillin. The problem was largely overcome in this herd by feeding only 50 mg. procaine penicillin for several days until the cows became adjusted to the treatment.

The incidence of pasture bloat was reduced by a factor of about two-thirds by feeding 100 mg. procaine penicillin per cow per day either with the grain or in the salt on a free-choice basis. The efficiency of treatment and the bloat incidence appeared to decrease as the season progressed. The bloat incidence on individual farms was distinctly above or below this average. It is recommended that farms with a bloat incidence higher than the average feed 50 mg. procaine penicillin per cow per day and gradually increase the dose to 100 mg. as the season progresses.

AGRONOMIC PANEL

The Occurrence of Bloat on Various Legumes, B. F. Barrentine, C. B. Shawver and L. W. Williams, Mississippi State College

The incidence of bloat on various legumes was compared to Ladino Clover. In 1956 common white, persian, red and crimson clover and alfalfa were compared to Ladino Clover. In 1957 the same legumes were used and in addition S-1 white clover was compared to Ladino Clover. The results of this work are shown in Table 1.

Selected steers which were bloating regularily on Ladino Clover were used in this study. The forages were clipped and the steers fed all the fresh forage that they would eat during a 90 minute period morning and afternoon.

A group of three to five steers were fed clipped Ladino for four or five days, and at the same time another group of steers of comparable bloat history were fed the legume under study. The number of cases of bloat in each group was recorded. An index of 100 was set for Ladino Clover and the index calculated for the other legumes. The Bloat Index referred to

in Table 1, is based upon the number of cases of bloat that occurred during a feeding period for each legume as compared to Ladino Clover. This Index does not reflect the severity of bloat that occurred.

Table 1. COMPARISON OF DIFFERENT LEGUMES

Legume	Bloat Index 1956	Bloat Index 1957
Ladino	100	100
Common White	64	79
S-1		93
Persian	111	100
Alfalfa, Pre-bloom	108	
Bloom		68
Red Clover, Pre-bloom	83	47*
Bloom		48
Crimson, Pre-bloom		75
Bloom	7	12

* This value is not in agreement with 1956 results, also it is difficult to explain why the Index for red clover (pre-bloom) was no higher than red clover (bloom).

The Free-Reducing and the 0.4 Normal Hydrochloric Acid Hydrolyzable Sugar Content of the Legumes Fed in the Bloat Tests of April - May, 1957, at Mississippi State College - Herbert L. Wilkins, Crops Res. Div., ARS-USDA

The legume forages which were cut and fed to the steers in the bloat trials at Mississippi State College were sampled at the morning and afternoon feedings of April 30, and May 1, 1957; and at all feedings from May 7 P.M., to May 9, P.M. inclusive.

During each of these two trial periods samples of the Ladino Clover and of one other legume under test were taken every four hours for 24 hours. A few other samples were taken for other purposes.

Moisture, ash, ether extract, crude protein, crude fiber, free-reducing sugars and total sugars were determined in these samples. Nitrogen-free extract and hydrolyzable sugars were calculated from the appropriate data and the completed analysis converted to the moisture-free basis.

Averages of certain of these data on the materials fed during the specified periods are shown in the following table, in which the arrangement is in the descending order of the bloat scores, here shown as percentages.

Dates (Inclusive)	Material	Bloat Score (%)	Free-Red. Sugar (%)	Hydrolyz. Sugar (%)	Total Sugar (%)	Crude Fiber (%)
5/7 - 5/9 P.M.-P.M.	La. S-1 Clover	85.0	5.7	2.8	8.5	15.3
5/7 - 5/9 P.M.-P.M.	Ladino	85.0	5.1	3.1	8.2	12.9
4/30-5/1 A.M.-P.M.	Ladino	77.8	4.4	1.9	6.3	14.6
4/30-5/1 A.M.-P.M.	Alfalfa	38.9	2.0	2.6	4.6	24.2
5/7-5/9 P.M.-P.M.	Red Clover	35.0	5.7	3.3	9.0	23.2
4/30-5/1 A.M.-P.M.	Crimson Clover	5.5	4.9	1.6	6.5	29.6
5/1-5/1 A.M.-P.M.	Persian Clover	0.0	8.0	1.9	9.9	26.4

Sugar content alone is probably not a prime factor in bloat. Our two highest total sugar percentages occurred in separate trial periods and in materials having a low bloat rating. The fact that in the week of 4/15, when the regular test of Persian clover was run, it bloated in 38 out of 40 feedings and that a specimen taken on April 17th showed a total sugar of 15.16%, gives a hint that within species the higher sugar contents may be associated with the higher bloating potential.

No large or consistent trends are observed in the series of samples of those legumes which were sampled at four-hour intervals for a period of 24 hours.

There is an inverse relationship between the corresponding averages of the bloating scores and the crude fiber contents of the legumes tested, since the four materials with the lower bloat scores all had the higher crude fiber values.

The limited weather data available from this study indicate that it may have a considerable influence on the bloating potentiality of forages. In the period of April 29 to May 2, 1957, there was no rain on the first day but varying amounts fell on each of the other days.

For Ladino clover the bloat scores were: 1st day, 90%; 2nd day, 80%; 3rd day, 75%; 4th day, 50%; for alfalfa: 1st day, 70%; 2nd day, 70%; 3rd day, 0%; and the 4th day, 25%; for Crimson clover: 1st day, 10%; 2nd day, 0%; 3rd day, 12%; 4th day, 12%; and for Persian clover: (1st and 2nd days, no test); 3rd day, 0%; 4th day, 16%.

It is not known what it is in the weather complex that has this effect. A recently published observation that water will leach carbohydrates from growing leaves may be applicable to this situation.

Some Factors Affecting Chemical Composition of Alfalfa, R. S. Allen, Iowa State College

In connection with studies on causes of bloat in cattle consuming alfalfa pasture and alfalfa soilage, a series of factors affecting composition of alfalfa was investigated in the summer of 1957.

Sampling of alfalfa pasture was accomplished by a stratified random procedure. Each sample was a composite of either 6 sets of 25 or 9 sets of 20 tops (4 in. lengths) which represented a given plot of alfalfa. The individual samples were quickly frozen and stored in a frozen state. Just prior to analysis they were chopped in a cold room and mixed well before sub-sampling for various analyses. The alfalfa soilage was sampled from the wagon at feeding time, usually within 1 hour after cutting and chopping. A number of portions were taken from each wagon and, after thorough mixing, a representative sample was taken and quickly frozen. Subsequently these samples were treated as described above.

All samples were analyzed for dry matter, nitrogen (non-protein, amino and total), reducing sugars, phosphorus and ash. Certain samples were analyzed for succinic, fumaric, malic, malonic and citric acid and also for total non-volatile organic acids.

The effect of time after irrigation on the composition of prebloom alfalfa pasture was studied early in May. Irrigation of duplicate plots was accomplished by sprinkling with 5.5 inches of water over a 7-8 hour period, and was completed ½, 9, 18, 27 and 37 hours prior to sampling. Non-irrigated plots served as controls. Irrigation had little effect on reducing sugars, total nitrogen, phosphorus and ash values, whereas there was a significant increase in non-protein nitrogen with increasing time after irrigation. A gradual but limited increase in amino nitrogen also was observed.

A study was made of the effect of time of day on the composition of alfala tops. Triplicate plots were sampled at 6 AM, 10 AM, 2 PM, 6 PM, and 10 PM. The diurnal variation was greatest for reducing sugars, phosphorus and ash. Reducing sugars increased to a maxiumum at 2 PM, then declined; phosphorus, ash and total nitrogen values each declined to a minimum at 6 PM, then increased. Total non-volatile organic acids were lowest at 6 AM, reached a maximum at 10 AM and remained relatively high. Malic, malonic and citric acids predominated in all samples. Succinic, fumaric and several unidentified acids were present in small amounts.

The effect of age of alfalfa on its composition was studied by taking samples of soilage over a 4-week period starting May 17. There were general declines in the values for total nitrogen, non-protein nitrogen, amino nitrogen, phosphorus, and ash. Reducing sugar values were variable.

Comparisons were made of values for the various constituents in alfalfa tops (PM sampling) and soilage (AM sampling) from the same field. Two samples of each type were taken each week for a 3-week period in late May and early June. Total nitrogen, phosphorus and ash values were consistently higher in the tops, whereas the levels of reducing sugars were highest for soilage samples. For the most part trends in values were about the same for each type of alfalfa sample.

First and second crop soilage samples (three of each crop taken over an 8-day period and essentially at the same stage of growth) from the same field were compared. Except for ash, all mean values were greatest in the first crop soilage.

A study was made of seasonal changes in composition of alfalfa tops taken from the same field. Analyses of four samples taken during the first two weeks each of June, July, August and September from plots being strip grazed by cattle were compared. Mean values for the September samples were greatest for all constituents studied except for reducing sugars and ash. Reducing sugar values were lowest during the September period.

Organic Acids and Saponin Content of Some Legumes in Relation to Bloating Response, C. Ray Thompson, Western Utilization Res. & Dev. Div., ARS-USDA, Albany, California

The purpose of the present studies was to determine whether correlation(s) existed between the organic acids and/or saponin content of legume forages and its bloating potential. It has been theorized that organic acids could be a ready source of ruminal gas. Possibly the feeding of green legumes would furnish rumen organisms so much of these readily metabolized compounds that the evolution of gas would be rapid enough to cause bloat. Saponins, which have been shown to give bloat symptoms when fed in large doses, may cause bloat by reducing surface tension, thus encouraging foam formation in the rumen or by acting physiologically to inhibit eructation or other nervous response necessary for normal elimination of gas.

Samples of ladino clover, alfalfa, crimson clover and persian clover were obtained from the Mississippi Station in Spring of 1957. These samples were from fields from which animals, selected for susceptibility to bloat, were being fed the freshly-cut material. Bloat scores and total non-volatile organic acids were compared. Generally at this period bloat occurred readily on ladino clover and alfalfa and seldom on crimson and persian clovers. Organic acids were remarkably constant in all samples analyzed. Ladino clover had the highest bloat score (27 out of 36 chances) and the highest organic acids but accumulation of much more data will be necessary before it can be established that these compounds are positively correlated with bloat.

The studies on saponins have shown that several saponins occur in each of the legume forages. Alfalfa seems to have the wides spectrum of compounds.

In general, the forages which seem to have the most saponins such as alfalfa, ladino clover and black medic also cause bloat while the non-bloating plants, birdsfoot trefoil and vetch, are lower. However, the methods available for assay are crude and the number of samples is inadequate to establish these tentative conclusions. The saponin mixture isolated from alfalfa or ladino clover inhibits peristalsis of excised rabbit ileum when added to a bath in which the tissue is suspended.

Present studies have shown that the saponins can be hydrolyzed to yield several sugars, glucose, xylose, arabinose, rhamnose, plus fat-soluble sapogenins. The sapogenins identified so far include soyasapogenol A, B and C from ladino clover and alfalfa. Medicagenic acid, a new sapogenin, was characterized in a cooperative study with Wayne State University, Detroit, Michigan.

Methods for staining both saponins and sapogenins, which have been separated on paper chromatograms, have been devised. A method of assay is being developed and attempts are being made to separate the individual saponins so that their physiological effects can be determined. In a cooperative study with the Uni. of Arizona, green alfalfa was cut and fed as "green-chop" to steers in feed lot. The bloating potential was observed. Samples were then dried and analyzed for total sapogenins. No positive correlations between bloat and amount of sapogenins could be found. However, when the sapogenin fraction was separated on paper chromatograms and stained with the Liebermann-Burchard reagent a good correlation was found with a fraction which stained red and which had an Rf of about 0.5. Further work on this particular fraction is in progress.

Report on Bloat Prevention, J. E. Miltimore, Experimental Farm, Summerland, B. C., Canada

Immature alfalfa and Ladino clover were freshly cut once daily into self-feeding wagons. Forage was harvested shortly after 8 AM and hauled to feeding yards, where shade, water, and cobalt-iodized salt were made available free choice.

Purebred lactating and dry Jersey females, ranging in age from three to fourteen years were used as experimental animals. Purebred Holstein and Jersey steers were included in some experiments after they had reached 8 months of age.

The experimental design consisted of four by four balanced latin square with at least four mature females per square except for one experiment where only two mature females were available for each square. Squares in later experiments consisted of one Holstein and one Jersey steer in addition to the mature Jersey females.

Treatment periods lasted for one week and data collected for the first two days was not considered in the final analysis unless there was no carry-over effect.

Data was recorded hourly from nine in the morning until nine at night. Records consisted of the degree of bloating and whether the animals were eating or chewing their cuds. Bloating was evaluated visually with a value of 1 being considered normal, ranging up to 5 where a trocar and canula were required to prevent death.

Preventive treatments were fed daily as follows: 75 mgs. and 100 mgs. of procaine penicillin, 4 and 9 ounces of mineral oil, 40 grams Antifoam AF Emulsion, 3 grams of Methyl Silicone XC-120, 140 gms. of Monosodium phosphate, 1½ oz. of turpentine, and a combination of 75 mgs. of procaine penicillin plus 4 oz. of mineral oil. Treatments were administered in the barn following the morning milking. Each experiment consisted of three treatments and a control.

Results indicate that none of the treatments completely prevent bloat. In the first experiment both 9 ounces of mineral oil and 75 mg. of penicillin were significantly effective but these results could not be repeated in a further experiment. It appears that 9 ounces mineral oil and penicillin show a trend to reduce bloat incidence.

Treatments in a feeding management experiment consisted of:

1. Feeding 24 hours daily plus grain.
2. Feeding 24 hours daily without grain.
3. Feeding during daylight
4. Feeding at night.

In this experiment the animals fed during the night bloated significantly less than the animals that had access to feed for 24 hours each day. Other treatments did not differ significantly.

It is desirable that materials for bloat prevention should be reliable since failure of the preventive treatment would immediately cause a serious loss. Research workers must give more careful consideration to endorsing a bloat preventive than to endorsing other livestock treatments where failure of the treatment would result in a loss equal to approximately the value of the treatment material.

RUMEN PHYSIOLOGY PANEL

The Effect of a Scabrous and a Non-Scabrous Diet on Rumen Motility and Intra-Rumen Pressure, H. W. Colvin, Jr., Uni. of Arkansas; S. D. Musgrave and G. F. Williams, Oklahoma State Uni.

Four Ayrshire bulls were fed a non-scabrous diet consisting of a concentrate mix and a roughage in the form of an almost pure wood cellulose product, Solka-Floc BW-20, from six months to 41 months of age. At 41 months of age, the Solka-Floc BW-20 was removed from the ration by increments and prairie hay introduced gradually. Both diets satisfied the requirements suggested by the National Research Council for all nutrients except phosphorus which was deficient.

When the bulls were 35 months old, trials were undertaken to study the effect of a non-scabrous diet on rumen motility. The periodic changes in total intra-rumen pressure, caused by contractions of the rumen, were recorded on a kymograph. The technique for measuring intra-rumen pressure included a rumen puncture with a trocar-cannula (6 mm I.D.). After the trocar was removed, the cannula was connected to a Buchner funnel (4 in. diameter) by means of plastic tubing. The system was made air-tight by stretching a piece of thin rubber latex over the top of the funnel. Mounted on the latex was the ink-writing lever arm and appurtenances. With such a system, the following can be determined: rumen contraction frequency, rumen contraction amplitude, and intra-rumen pressure.

In 19 non-scabrous trials with 4 bulls, the fasting rumen motility record indicated an average rumen contraction frequency of 2.1 contractions per minute, an average contraction amplitude (large, non-eructation contraction) of 9.1 cm. of water, and an average intra-rumen pressure (IRP) of 1.8 cm. of water. Not only were the bulls bloated during the motility trials but also previous to this and up to the time scabrous material was included in the diet.

Six months following the initial trials, prairie hay replaced the Solka-Floc BW-20 in the diet according to the schedule shown below. The following table indicates the results of the dietary change on fasting rumen contraction frequency, amplitude, and intra-rumen pressure:

Dietary Roughage			Av. Contr.	Av. Contr.	Av. IRP
Hay(%)	Solka-Floc(%)	Date	Freq/Min.	Amplitude (Cm water)	(Cm water)
0	100	1-27-57	2.4	8.0	0.8
25	75	1-29-57	2.6	9.5	1.2
50	50	1-31-57	2.2	8.9	3.0
75	25	2-2-57	2.2	12.6	2.8
100	0	2-12-57	2.4	9.0	2.7
100	0	2-19-57	2.2	9.4	1.0
100	0	3-14-57	1.6	12.2	-4.9
100	0	4-16-57	1.8	9.0	-3.0

Since it is known that the normal intra-rumen pressure is sub-atomospheric, it can be seen that this condition existed within six weeks after scabrous prairie hay replaced the non-scabrous Solka-Floc BW-20 in the diet. Furthermore, the amplitude of the rumen contractions was increased and the frequency of the rumen contractions returned to that observed in normal healthy animals. An additional series of trials conducted a month later confirmed the sixth week observations.

On the basis of these results, it is suggested that scabrous material in the diet is necessary to maintain the tonic activity of the central pool of motor neurons responsible for rumen motility. A constant stream of afferent impulses probably arrives at the motility center from tactile receptors in the rumen wall over sensory components of the vagi. That receptors responding to tactile stimuli are present in the rumen has previously been demonstrated for the rumination reflex. The presence of vagal afferents from the rumen has also been shown.

The results also demonstrated that if scabrous material is absent from the diet for relatively long periods of time, rumen motility becomes abnormal, with respect to frequency and amplitude, and bloating occurs with marked regularity.

Frothing of Reticulo-ruminal Contents and Some of its Associated Measurements and Variations, Roy E. Nichols. Uni. of Wisconsin

The frothing of reticulo-ruminal contents is a normal process and only endangers the animal when it becomes excessive. Many techniques for measuring this process have been tried with varying degrees of success. One technique which is simple and usually more than 80% reliable is the measurement of the maximum froth volume, maximum froth gas volume and 2 hour gas volume of an incubated sample of reticulo-ruminal content strained through one layer of cheesecloth to remove coarse particles. With this method it has been possible to demonstrate that frothing is least in samples taken before feeding, is increased by feeding and decreased by drinking. Frothing is greatest on fresh feed, particularly legumes. Rain results in increases in the frothing of samples when animals are grazing. Increases in the amount of particulate material in samples increase viscosity and froth stability. Comparison of the reclaimed liquid of artificially frothed portions of samples of rumen content with that of unfrothed portions suggests that the liquid of the bubble skins is more dense, less viscous and lower in total nitrogen than that of the unfrothed portions of the same samples.

Factors Controlling Rumen Fermentations, W. E. C. Moore, Virginia Agr. Expt. Station

Indirect evidence has been obtained that the ruminant animal as an individual has considerable control over the rumen fermentation activity.

One main limiting factor appears to be the rate of absorption of fatty acid end products from the rumen. Increased fatty acid levels in the rumen depressed cullulose digestion as measured in vivo.

The physical nature of the feed, especially ground roughage has been found to increase gas production rate, shift fermentation products in vitro and cause the disappearance of certain proteolytic bacteria from the rumen.

With pure cultures, substrate (carbohydrate) greatly influenced slime production which has been suspected as a contributing factor in bloat.

Evidence has been obtained for the presence of a rumen cellulase inhibitor in many rumen fluid samples. This appears to be a fourth factor in control of rumen fermentation rates.

Cellulolytic activity and composition of the ingesta of each organ of the digestive tract indicate the possibility that activity of these organs may also indirectly influence rumen activity.

Interactions between factors controlling rumen fermentations are obviously exceedingly numerous. Our group has been interested in attempting to determine major relationships to help explain significant control mechanisms in the rumen fermentation.

Rumen Function Investigations in New Zealand, R. W. Dougherty, Cornell University

These investigations and observations were made in New Zealand during the spring and summer (autumn and winter, northern hemisphere) of 1956-57. The work was confined to the northern island where the dairy cattle population is greatest and was done with Dr. A. T. Johns and other members of the staff of the Plant Physiology Laboratory, Palmerston North, New Zealand.

A number of cases that had been bloating daily in the Massey College herd were treated with 100 mg. procaine penicillin given orally every third day. When penicillin treatment was discontinued, bloat reappeared in these individuals. Eventually procaine penicillin was tried in one member of several pairs of identical twins having uniform bloating behavior. It was again found to be quite effective. Finally it was tried successfully in many herds with histories of severe bloating. Penicillin did not appear in the milk in measurable amounts and did not affect milk production appreciably. One 100 mg. oral dose was effective from two to three days.

In a country where little grain or stall feeding is practiced and where labor is scarce and restraining stocks are practically non-existant, the oral administration of procaine penicillin is rather impractical. If better methods are established of getting the antibiotic into the rumen, then it has a definite future in the control of bloat.

Oil spraying of pastures was observed and found to be effective in preventing bloat. An emulsion of peanut oil was used and was applied to each fresh pasture 'break' daily, at the rate of 2 ounces of peanut oil per cow per day. The cost and the extra labor have inhibited the widespread use of this method of bloat control.

Work at the Plant Chemistry Laboratory and at Massey College has shown that paraffin oils are effective in preventing bloat, but are impractical because they interfere with the absorption of carotene.

New Zealand is an excellent place to study bloat because of the high incidence in dairy cattle. In a country having about 2,000,000 dairy cattle the annual losses vary between 10,000 and 20,000. The less obvious losses are probably of greater importance.

MICROBIOLOGY PANEL

Dissimilation of Alfalfa Saponins by Rumen Bacteria, J. Gutierrez, R. E. Davis, and I. L. Lindahl, Animal Husbandry Res. Div., ARS-USDA

Saponin-digesting bacteria were isolated from steers on a fresh alfalfa diet with a peak number of 680 million per ml. of ruminal fluid. Quantitative experiments showed that CO_2, acetic, butyric, formic and lactic acids were produced from alfalfa saponins by bacterial strains tentatively identified as Butyrivibrio. Large amounts of cell and slime material from bacterial saponin fermentation were harvested by centrifugation at the end of the incubation period, while scant growth occurred in control medium without saponins. Rapid microbial decomposition of legume saponins with the concomitant production of gas and slime indicates the interaction which exists between the saponins and certain rumen bacteria, and demonstrates how these plant compounds may contribute to bloat.

Predominating Bacteria in the Rumen of Bloating and Non-Bloating Cattle Pastured on Ladino Clover, M. P. Bryant, Animal Husbandry Res. Div., ARS-USDA, and B. F. Barrentine, C. B. Shawver and L. W. Williams, Mississippi State College

Recent research on bloating cattle has suggested that the kinds of bacteria present in the rumen may be one of the primary causative factors in bloat. This study was undertaken to determine the kinds of predominant culturable bacteria in the rumen of three bloating and three non-bloating cattle and in the bloat-prone cattle prevented from bloating by penicillin therapy.

Yearling steers were pastured on Ladino clover for 90 minute periods beginning at 7:30 A.M. and 2:30 P.M. daily. Animals were selected for study on the basis of their being good or poor bloaters - based on visual observations. Rumen content samples were collected at 1 P.M. Fifty mg. procaine penicillin in capsules was given at 1 P.M. the day before sampling.

Samples of rumen content were obtained by stomach tube, taken immediately to the laboratory and cultured in anaerobic rumen fluid-glucose-cellobiose agar. Other media inoculated were cellulose broth; aerobic medium identical with the anaerobic agar medium above except that cysteine-HCl, Na_2CO_3, and CO_2 were eliminated; and Rogosa's selective lactobacillus medium. A gram stain of each sample was observed and the pH of each sample was taken within 10 minutes after it was collected.

Approximately 50 strains of bacteria were randomly isolated from tubes of the anaerobic agar medium inoculated with each sample. The strains were placed in 11 groups on the basis of the following characteristics: colonytype, morphology, motility, gram reaction, final pH and appearance of growth in glucose broth, relations to oxygen, H_2S production, gas production, and cellulose digestion.

There was no significant difference in pH, MPN (most probably numbers) cellulolytic bacteria, or total anaerobic count in the 3 groups of animals studied. The difference in anaerobic count between bloaters and non-bloaters approached significance.

The anaerobic counts were much higher than are found in animals on the usual high roughage ration. Animals fed rations high in concentrates have shown counts as high and higher than the present animals.

The counts of bacteria able to grow under aerobic conditions were higher for the bloaters than for the same animals treated with penicillin. Observations on colony type and gram reaction indicated that this effect was mainly on streptococci. The small numbers of these bacteria found indicate that they are of relatively little importance in Ladino clover-fed animals.

Counts on Rogosa's medium were always less than 1,000 per g. indicating that lactobacilli capable of growth under aerobic conditions were of no importance in these animals.

No significant differences were noted in the groups of bacteria isolated from the three groups of animals. Also, when the groups of bacteria were further subdivided on the basis of variable characteristics within the groups, no significant differences could be found.

All of the bacterial groups have been isolated from animals on various rations at Beltsville. This indicates that the flora of animals at Mississippi and Beltsville are similar. However, when data on percentage of total isolates of individual bacterial groups isolated from animals fed fresh alfalfa or alfalfa hay was compared with data from the animals fed Ladino clover, some differences were apparent. Cellulolytic bacteria presumptively identified as Bacteroides succinogenes and Ruminococcus were lower and bacteria similar to Butyrivibrio and Lachnospira were higher in the clover-fed animals.

The fact that detailed studies on both of these groups show that they produce considerable amounts of CO_2, H_2, and formic acid from glucose suggests that the flora of the rumen of Ladino clover-fed cattle is capable of a greater production of gas than the flora found when many other rations are fed.

In view of the theory that slime production in the rumen is one of the causative factors in bloat, the authors were interested in determining if slime-producing bacteria were more prevalent in the clover-fed animals than in animals fed other rations. Based on visual observations for slime

in glucose medium, slime producing strains were no more numerous in the Ladino clover-fed animals than in animals fed other rations such as alfalfa hay or fresh alfalfa. Bacterial strains similar to Bacteroides sp. have been the most evident slime producers in animals fed alfalfa. Slime producers of this group were less evident in the Ladino clover-fed animals. The most numerous slime producers belonged to the groups presumptively identified as belonging to the genus Butyrivibrio.

The present methods of demonstrating slime production are very crude. It is possible that an increase in knowledge on the nature of the slime and on factors affecting its production by ruminal bacteria will result in better methods of assay which might show differences in slime producing capacity of bacteria from bloating and non-bloating cattle.

The facts that the numbers or predominant groups of bacteria cultured were not significantly different when the non-bloating, bloating, and penicillin-treated cattle were compared suggest that some significant component of the flora was not cultured or that some strains within one or more of the bacterial groups cultured differed in their metabolism in ways not detectable with the few characteristics studied. However, the data show that the flora of the rumen of the groups of cattle studied were not grossly different.

Effect of Nitrate in the Ration and a Study of Digestibility With Sheep Inoculated With Psuedomonas Aeruginosa, G. B. Garner, Uni. of Missouri

The characteristic of oat hay poisoning was established several years ago. Following the drought of 1954, Missouri forages contained excessive amounts of nitrate which was shown to produce symptoms not typical of classical oat hay poisoning. Experimental findings indicate a more rapid conversion of nitrate to nitrite and nitrite to ammonia when energy is added to the ration. The effect of energy becomes negligible whenever the concentration of nitrite exceeds certain levels. The ration of the animal influences the rate of conversion, and may lessen the effect of added energy as in the case of alfalfa fed sheep compared to timothy fed sheep, thus indicating a different type of microflora.

Experiments were conducted in which sheep on two rations were inoculated with Psuedomonas aeruginosa. The course of the infection was followed by counting the blue pigment producing colonies. Digestibility of cellulose and nitrogen indicates possible modes of action. Cellulose digestion was repressed and nitrogen absorption improved when the sheep were fed timothy. Cellulose digestion was not affected and the nitrogen absorption was only slightly changed on the alfalfa ration. Contamination of a water tank lead to further experiments in which it was noted that with a prolonged period of inoculation the alfalfa fed sheep developed counts as high as the timothy fed sheep. Polymyxin B sulfate (41 mg per sheep) given intraruminally, effectively removed the Psuedomonas aeruginosa. After treatment with polymyxin a noticeable frothing of rumen fluid was noted. Sheep given polymyxin remained on feed and exhibited no ill effects. In vitro cellulose digestion was stimulated slightly when polymyxin was added at twice and four times the recommended antibiotic level.

Production of Gas and Stable Foam in Rumen Fermentations from Alfalfa Fiber, Pectic Substances and Their Precursors, H. R. Conrad, W. D. Pounden and A. W. Fetter, Ohio Agr. Expt. Sta., Wooster

Recent evidence presented concerning stable foam formation firmly establishes that pasture bloat in cattle results primarily from the formation of abnormal quantities of stable foam in the rumen, particularly during periods of rapid gas production. Previous results have shown that alfalfa and Ladino clover fibers promote a more stable type of foam than grasses and birdsfoot trefoil.

The aim of the present studies has been to isolate some of the physical and chemical characteristics of alfalfa which might predispose cattle to pasture bloat. The results of experiments carried out show gas production by rumen microorganisms acting on newly cut growing alfalfa or a fraction of alfalfa. Also, the effects on stable foam formation of various purified substances normally present in alfalfa have been studied.

Rumen contents were obtained by stomach tube from dairy calves weighing 400 to 500 lbs. Feeding procedures for the calves were standardized to avoid as much as possible day to day variations in the inoculum used.

The following procedure was worked out for the short time in vitro rumen fermentations. Fresh rumen contents were strained through a double thickness of 90 gauge cheesecloth. Thirty-five milliliters of strained rumen juice were added to each fermentation flask to which had previously been added 35 ml. of 1/15 N phosphate buffer solution. A temperature of 38.5°C was maintained throughout the fermentations by means of a constant-temperature water bath. Fermentation time was for 2 hours. The amount of gas produced was measured as the volume of water displaced in simulated manometers attached to each fermentation flask in a gas-tight system, the base of the water column being in equilibrium with the atmosphere.

The proportion of carbon dioxide in the total gas was measured as the per cent absorbed by a 40% solution of sodium hydroxide after bubbling through the total volume of gas produced in the fermentation flasks.

The following procedures were used for preparing various materials tested for total gas producing capacity in short time in vitro fermentations. Newly cut green alfalfa was mascerated in a Hobart food cutter. The washed alfalfa fiber was obtained by repeatedly extracting green alfalfa with cold water in a Waring blender until all visible traces of green material were removed. The digested washed fiber was obtained by digesting with rumen microorganisms 2 to 10 hours in the fermentation flasks and rewashing with cold water. Alfalfa cellulose was prepared from the green plant by treatment with the Crampton and Maynard analytical procedure.

Depectinated alfalfa was obtained by extracting mascerated alfalfa with distilled water at 80° C for 12 hours. Both the extract and depectinated residue were saved for rumen fermentations.

A hydrolysate of methyl ester of poly-galacturonic acid was obtained by incubating purified pectin at 37° C for 24 hours with 1/10 part of 5% pectinase solution buffered at pH 6.8.

Other products tested were commercial crude pectin, purified pectin, glucose and dextrose.

Results of rapid gas production studies show that compounds responsible for the initial rapid gas production obtained with green alfalfa were intimately associated with the fiber but were removed after 4 to 10 hours digestion with rumen microorganisms and by hot water extraction. Purified pectin added to washed alfalfa fiber enhanced gas production. Crude pectins, glucose, and galactose also increased gas production. Hydrolyzed pectin produced the most marked effect yielding 2.25 times as much gas as washed fiber. Gas production of mascerated alfalfa was increased by adding pectinase to the fermentation mixture. This suggests that galacturonic acid may play an important role in excess rumen gas production from alfalfa.

Twenty to forty per cent of the total volume of gas produced was determined to be carbon dioxide. It is assumed that the remaining 60 to 80% of the gas was mostly methane.

The stable foam test was used to measure the ability of alfalfa plant material to simulate the stable foam in the rumen contents of bloated cattle. Green alfalfa plants were chopped in fractions 1 to 2 inches in length, added in 2 to 3 gram quantities to 100 ml. of water in a Waring blender, and beaten in the blender until a stable homogenous gassy mass was produced. A test of the effect of pectin on stability of the mass produced showed that on the average 52 grams of green alfalfa produced a stable foam in 8 minutes beating time. Whereas when 0.5 gram of crude pectin was added a stable foam was produced with only 43 grams of plant material in seven minutes beating time. Using 1 gram of crude pectin 40 grams of plant material produced a stable foam in five minutes beating time.

In measuring stable ingesta volume in vitro, it was found that the stable ingesta volume of 100 ml. of untreated rumen contents increased 27 ml. in 45 minutes. When 3 grams of pectin were added to this amount of rumen contents, stable ingesta volume increased 56 ml. Also, the liquid layer below the stable foamy mass was only 26 ml. when pectin was added contrasted to 58 ml. of liquid layer in the untreated rumen contents. The total stable foamy mass was 69 ml. in 100 ml. of untreated rumen contents after 45 minutes fermentation and 130 ml. when 3 grams of pectin were added.

Analysis show that alfalfa plants contain 5-8% pectic substances and 6-10% reducing sugar. When the results of these experiments are considered, it appears that the combined effect of the physical structure of alfalfa fiber, pectic substances of alfalfa plants, galacturonic acid obtained on hydrolysis of pectic substance, and reducing sugars normally present is capable of causing the formation of the stable foam found in pasture bloat.

Mechanisms of Foam Stabilization, Volney Wallace, South Dakota State College

Surface Active Agents - Saponin was observed to displace egg albumin preventing it from forming a surface layer of denatured protein. Certain of these surface films -- proteins and saponins -- are known to have high viscosity. Agents which reduce surface tension diminish the energy necessary to form a foam, but do not necessarily stabilize foam. If there is a strong lateral attraction between surfactant molecules on the surface of a solution, the surface is elastic or viscous. This characteristic of surfactants does stabilize foam.

Particulate Matter - Finely divided particles which are but poorly wetted by water serve as foam stabilizers. These particles adhere to the water-air interface and the coated bubbles cannot coalesce because of this coating. According to Reinders' theorem, that particles will adhere to the air-liquid interface if the surface tensions between the three phases are such that one does not exceed the sum of the other two.

A fistulated steer at this experiment station developed frothy bloat on grain and alfalfa hay ration. The rumen contents (strained free of coarse matter) was examined and it appeared that this mechanism was responsible for the froth. Under the microscope, the bubbles appeared coated with food particles and could approach no closer than about 5 microns. The bubbles appeared dimpled, as though they were adhering to the food particles. When the rumen juice was allowed to stand, the solids rose to the top of the solution, leaving an almost clear solution below. The food particles were not lighter than water as they could be centrifuged down with ease. The centrifuged juice produced an unstable foam and was not observed to be gelatinous or unduly viscous. This flotation of food particles was noted subsequently in IVI tests when there was a measurable ingesta volume increase.

According to Reinder's theorem, if the surface tensions between three phases are such that one value exceeds the sum of the other two, that interface having the high value will not occur in a mixture of these three phases. It follows that in a non-frothing rumen, the surface tension between air and food particles exceeds the sum of the air-H_2O and H_2O-particle surface tensions. Digestion in the rumen removes carbohydrate from food particles, enriching it in lipid and protein (we have found up to 17% crude fat and 57% crude protein in suspended rumen solids). Analogy of the food particles to simple organic liquids suggests that this digestion lowers the particle-to-air surface tension and raises the particle-to-water surface tension. This process carried far enough leads to the balance of surface tensions that results in adhesion of food particles to gas bubbles. In this regard, we have reports that feed lot bloat is worse several hours after feeding.

Another consequence of adhesion of food particles to gas bubbles is that the bubbles are more easily trapped in the coarse rumen contents.

Gels and Thickening Agents - Bacterial slimes have been suspected as frothing agents in frothy bloat. These might function by two mechanisms. First, high viscosity slows down bubble movement thus retarding coalescence of bubbles. Secondly, gels hold bubbles in place until the forces to move the bubble exceeds the elastic limit of the gel. The elastic limit need not be large to immobilize small bubbles.

A Comparison of the Factors Causing Legume and Food Lot Types of Bloat,
Don R. Jacobson, Kentucky Agr. Expt. Sta.

The primary cause of both legume and feed-lot bloat is the excessive formation of stable froth in the reticulo-rumen. However, the factors affecting the rate of stable froth formation in legume bloat appear to be somewhat different from the factors affecting the rate of stable froth formation in feed-lot bloat. The physical nature of the rumen contents in legume bloat includes a definite stratification with about 30% of the volume made up of a liquid phase. The froth or foam formed is much more fluffy in nature and has a tendency to accumulate in the top of the rumen. The per cent of the rumen microorganisms encapsulated is low (0-16) and bears little relationship, if any, to the amount of froth formed or the incidence of legume bloat. The presence of a readily available carbohydrate or a fresh legume juice extract always led to rapid froth formation. The following factors appear to have a marked effect on the rate of stable froth formation of rumen contents in vitro.

(1) Number of days the animals have grazed the legume.
(2) Length of grazing period daily.
(3) Time at which the sample is taken during the grazing period.
(4) The intensity with which the animal grazes.
(5) Method of sampling (the use of vacuum is particularly undesirable).
(6) Nature of rumen sample obtained (specific gravity, per cent water, etc.).

Even though the above factors may be maintained constant, it remains difficult to obtain a representative rumen sample.

The Effects of Penicillin on the Activities of Ruminal Microflora,
C. K. Smith and R. S. Emery, Michigan State University

Centrifuged cells were incubated in vitro in a CO_2 atomosphere, using 5 gm. of the ration that the donor animal was receiving and 100 ml. of inorganic salt solution as substrate. Changes in viscosity of the liquid layer was determined at intervals during the five hour incubation period. Crystalline penicillin was added to the flasks immediately prior to the addition of the cells. These data show that when the donor animal is not receiving penicillin the addition to the in vitro fermentation will tend to prevent an increase in viscosity, however after the penicillin has been fed to the donor animal (100 mg daily) for as short a period as 8 days, this control is no longer effective.

TABLE I

Percent increase in viscosity after five hours incubation in vitro.

	Before feeding penicillin	8 days after feeding penicillin
1. Substrate	6	6
2. Substrate + cells	20	55
3. Substrate + cells + 150 units pen.	6	66
4. Substrate + cells + 300 units pen.	6	83

This effect could not be demonstrated when procaine penicillin was used in vitro.

Samples of rumen liquid were taken before and at intervals following the feeding of penicillin to determine the level of penicillin in the rumen liquid and the length of time that it remained in the rumen. These animals were on three different feeding regimes. The results show that when the animal had not previously been fed penicillin the drug reached the posterior of the rumen fifteen minutes after ingestion and the levels (1.0-0.1 unit/ml) were maintained without an unusual decrease for four hours. After the animals had received 100 mg. of penicillin daily for three weeks the level had decreased markedly by two hours and penicillin was not detectable in most cases at three hours after ingestion.

TABLE II

Levels of Penicillin in the Rumen of Penicillin Fed Dairy Cattle

	1st Day of Feeding 100 mg. of Penicillin	3 Weeks on 100 mg Daily Intake of Penicillin		
	Range Over a 4-hr. Period	15 min.	2 hr.	3 hr.
700# Yearling	0.05 - 1.0*	0.5	0.04	0.0
1200# Cow	0.05 - 0.13	0.09	0.04	0.02
1200# Cow	0.05 - 0.13	0.04	0.02	0.00

* Values expressed as units/ml of rumen fluid.

Rumen fluid was taken from the above animals two hours after ingestion of the penicillin. This liquid was then subjected to the following experimental treatments: (a) incubated at 39°C; (b) centrifuged at 32,000XG for 15 minutes and the supernatent incubated at 39°C; and (c) Seitz filtered and the filtrate incubated at 39°C. Procaine penicillin was added to all samples to a final concentration of 1.0u/ml. It was found that the penicillin destroying principle was filterable and was present in the Seitz filtered liquid in about 80% of the potency of the original rumen liquid.

An In Vivo Artificial Rumen, L. R. Fina, G. W. Teresa and E. E. Bartley, Kansas Agr. Expt. Sta.

The artificial rumen technique has been used in rumen function studies for a number of years with varying success. At times the use of this technique has been criticized because one could never be sure that in vitro experiments duplicated in vivo rumen functions. For this reason in vivo artifical rumen technique for studying utilization of cellulose and other nutrients by rumen microorganisms has been developed. The in vivo artificial rumen consists of a porcelain test tube attached to a glass frothing tube and gas escape mechanism. The substrates and inocula are placed in porcelain tubes and the apparatus suspended in the rumen of a fistulated animal.

The porcelain test tubes were first tested to insure their suitability for use. They were found to be permeable to fatty acids (C_2 - C_6 tested) and glucose but not permeable to the enzyme urease, the tetracyclines, and bacteria.

Using the in vivo artificial rumen technique no cellulose was decomposed, even after 10 days, in non-inoculated tubes placed in the rumen. In inoculated tubes digestion of 500 mg. of cellulose was completed in about 48 hours. An initial inoculum continuously digested added increments of cellulose at an unabated rate to the extent of about 90 per cent over an extended period of time (10 days). An increase in the quantity of the organisms in a diluted inoculum was shown to occur during active cellulose digestion.

In other artificial rumen techniques changes which might occur in a 24 hour period in the rumen are not considered. Fluctuations in pH from 7.6 to 5.4 and also temperature differences ranging from lows of 25° C to highs of 41° C have been reported by other workers and confirmed at the Kansas Station. These difficulties are minimized and this technique more closely approximates actual rumen conditions. Application to many types of determinations in the study of bloat, feed utilization, near in vivo pure culture studies, and possibly others seem potential.

PHYSIO-PATHOLOGY PANEL

Studies of the Eructation Mechanism in Cattle, C. E. Stevens and A. F. Sells, Uni. of Minnesota

Adult, fistulated cows were used to study the reflex regulation of the ruminant stomach. Pressure events occurring in the rumen, reticulum and cardia were then recorded under various experimental conditions. Some of these animals were surgically equipped with electrodes applied to the vagal nerves. In a few cases, two electrodes were placed in tandem on the dorsal vagal trunk and each supplied with an empty polyethylene tube which allowed the injection of procaine onto the nerve.

It was found that stimulation of the dorsal thoracic vagal trunk increased eructation rate while procainization markedly decreased eructation efficiency. In addition, stimulation of the more cranial electrode after procainization of the nerve caudel to this, resulted in an increased eructation rate. The reverse of this procedure had no effect, suggesting that afferent nerve fibers were being stimulated.

An attempt to place electrodes in certain branches of the abdominal vagus supplying the reticulum and rumen was successful in one of three animals. Stimulation of the reticular branch of the ventral vagus had no effect in eructation but stimulation of the branches of the dorsal abdominal vagus behind the cardia did markedly increase eructation rate. This suggested that receptors for eructation might be located in the rumen itself.

In one series of experiments, a number of anesthetics were applied topically to the rumeno-reticular mucosa for the purpose of blocking receptors. Since these appeared to have little or no effect, a series was started in which procaine was injected into the walls of the reticulum and rumen in an attempt to block certain branches of the abdominal vagi. It was found that: (a) injection ventral to the cardia had no effect on rumen rate or eructation although reticular motility was practically stopped. (b) injection into the medial border of the anterior dorsal sac stopped or markedly decreased both rumen and eructation rate although reticular motility remained normal. This again suggested that receptors might be in the rumen.

A number of experiments were carried out to try to determine the nature and location of the receptors for eructation. These included the testing of the effect of generalized and localized tactile, electrical and pressure stimuli. It was observed that eructation occurred only in conjunction with a contraction of the rumen and that the rate of each increased with an increase in rumeno-reticular pressure. Decreasing ingesta consistency decreased eructation efficiency but the attempt to localize the receptors by local application of the various stimuli was unsuccessful.

It was concluded from these results that the receptors for eructation are either stimulated directly by the contraction of certain rumen muscle or indirectly to the stimulation resulting from movement of ingesta during the rumen contraction.

Some Aspects of Research on Pasture Bloat in New Zealand; A. T. Johns, C. S. W. Reid, and J. L. Mangan, Plant Chemistry Div., Dept. of Scientific and Industrial Research, Palmerston North, New Zealand

Research work on bloat has convinced us that the primary cause of legume bloat in New Zealand is due to a foaming of the rumen contents. Work is proceeding to try and identify the plant and animal factors involved in the production of the condition. The foam appears to be of the surface viscous type such as is formed by proteins and saponins and in which surface tension values show little correlation with foam stability.

An apparatus has been devised for measuring foam strength and used for determining the conditions for the formation of foams of maximum strength with rumen contents and with solutions of proteins and saponins. It appears that the balance of foaming and antifoaming agents released from plant material following ingestion, may be important in the etiology of bloat.

Investigations on bovine saliva include chemical analyses of parotid and submaxillary salivas, the effect of increased pressure on their secretion and composition, and a consideration of their possible role in bloat.

The methods of controlling bloat by pasture spraying with emulsified oils or tallow has been devised and applied with marked success in the field. The use of penicillin for control of bloat has been tried and found to be partially effective. It has been shown that penicillin acts by slowing down the final stages of the bacterial fermentation, giving a temporary accumulation of lactate, a decreased rate of volatile fatty acid and gas production. Administration of penicillin regularly to dairy cows at the rate of 200 mg. per dose has not resulted in any detrimental effects on appetite, milk production or the quantity and quality of butter fat.

Using transducers and a Sanborn four channel recorder investigations have been made on factors effecting rumen activity and the mechanism of belching especially under bloating conditions.

Records so far have not revealed any evidence of inhibition of reticulorumen contractions during bloat.

It has been found in cows that although belching may occur following the reticular contractions, it usually occurs at other points during the interval between successive reticulum double contractions. Foam itself has been shown to interfere with belching.

Post-Mortem Findings in Steers that Died of Bloat, B. F. Barrentine and J. W. Scales, Mississippi State College

Ten steers that died of bloat were posted as soon as possible after death. The only consistent abnormal finding was the condition of the lungs of these steers. In every case the lungs were very congested. Hemorrhage in the alveoli was common, and one steer had blood in the trachea. Portions of the lungs of all steers showed a condition that was tentatively called pneumonia.

Sixteen steers which had been on the bloat studies were slaughtered. These steers varied in age from two to five years and all had bloated many times. The lungs of these steers were essentially normal. A few of the steers showed lung scar tissue and lung-to-diaphragm adhesions were observed in some cases. It was concluded that the lungs of these steers were normal.

During the past four years a total of 7541 cases of bloat have occurred in yearling steers, and 47 of these steers died of bloat. During the past three years "older steers" (two to five years old) have bloated 4115 times and none have died. These results are summarized in the table below. It appears that some type of protection from death due to bloat is acquired during the first year that a steer is exposed to bloat.

	Yearling Steers		Older Steers	
Year	Bloat Cases	No. Died	Bloat Cases	No. Died
1954	1575	7	--	--
1955	646	2	997	0
1956	2739	18	1325	0
1957	2581	20	1793	0
Total	7541	47	4115	0

The Physiology of Eructation in Sheep, R. W. Dougherty, R. E. Habel and H. E. Bond, Cornell University

Most of the work done in this laboratory on the physiology of eructation has been based on two methods. Cinefluorography was used to study the mechanics of eructation in sheep. Under the conditions of the experiments, eructation was most active after a double contraction of the reticulum which cleared this structure of most of its ingesta. The rumeno-reticular fold and the anterior pillar contracted, keeping the ingesta away from the cardia and emptied reticulum. The cardia and prediaphragmatic sphincter relaxed, gas passed into the esophagus dilating it throughout its entire length. The cranial esophageal sphincter remained closed at all times except during swallowing, eructation and regurgitation. When the esophagus had filled with gas, the cardia and prediaphragmatic sphincters closed, the cranial esophageal sphincter relaxed and the esophagus contracted throughout its entire length forcing the trapped gas out into the pharynx where eructation was completed. Just preceding eructation there was a fleeting closure of the glottis and a transient rise in intrapleural pressure. Later work showed that eructation can occur at any phase of the rumen cycle, but in the animals used and under the conditions of the experiments, it was most active following the double contraction of the reticulum. Two things were obvious, the cardia had to be cleared and the esophagus relatively empty of liquids or solids before eructation could occur.

The second phase of the work involved the use of decerebrate sheep. It was learned from this work that the principal motor nerves supplying the esophagus are the pharyngoesophageal nerve, the recurrent laryngeals and the dorsal vagus, all branches of the vagus nerves.

Sufficient receptors are located in a relatively small area around the cardia which when properly stimulated will initiate the eructation reflex. This does not mean that receptors are located only in this area.

Receptors were found in a relatively small area around the cardia which, when stimulated by the presence of water, ingesta, mineral oil or foam, would initiate reflexes which partially or completely inhibited eructation. These receptors could be inactivated by bathing the area with a 1% solution of butyn sulfate. Following the inactivation of the inhibitory receptors anything in the vicinity of the cardia was 'eructated', whether it was gas, fluids, solids or foam. In other words, the positive phase of the eructation mechanism seemed to be unaffected by the butyn sulfate.