UNITED STATES DEPARTMENT OF AGRICULTURE AGRICULTURAL RESEARCH SERVICE Office of Administrator Washington 25, D. C.

March 15, 1954

TO : Participants in Conference on Rumen Punction

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 discussions and notes of the second Conference on Ruman Function, held at the Congress Hotel, Chicago, Illinois, on Pecember 2 and 3, 1953, and a list of persons in attendance during the two-day meeting.

At the close of this Conference the group agreed that a similar conference should be held in 1955 and that the same approximate time end place for the conference was satisfactory. It was also agreed that the same panel chairmen should be continued. The chairmen of the various panels are to be complimented on their work in arranging for a most interesting series of discussions.

The Conference convened at 9:30 AH on December 2, 1953.

Attachments

MAMMANIS

REPORT ON CONFERENCE OF BUILDIN FUNCTION

held at

Congress Hotel, Chicago, Illinois December 2 and 3, 1953

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

NAME:

I, H. Pattison

C. K. Thitchair

P. S. Horg

W. D. Pounden

M. P. Bryant

G. O. Mout

H, H, Cole

J. E. Foster

R. F. Richter

C. F. Haffman

C. D. Meredith

R. H. Roethke

Thomas W. Freeze

Rue Jensen

H. H. Brugman

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G. C. Anderson

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J. O. Grandstaff

E. A. Tunnicliff

U. S. Garrigus

V. G. Kammlade, Jr.

L. E. Childers

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George A. Montgomery

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

(a) Animal Management - Dr. H. H. Cole
(b) Rumen Physiology - Dr. C. F. Huffman
(c) Physio-Pathology - Dr. R. W. Dougherty
(d) Agronomic - Dr. W. K. Kennedy

(e) Microbiology

- Dr. W. D. Pounden

ANDRAL MANAGEMENT PAREL

The Relation of Soil Partility and Fertilizer Treatment to the Incidence of Blost in Cattle - B. F. Parrentine, Mississippi State College

I don't think that there is any doubt that there is a relationship between fertilizer treatment and the incidence of bloat, at least on poor soils, or that certain legumes growing on fertile soils are more likely to cause bloat than those growing on poor soils.

Let us consider the less extreme cases - say where the soil is fertile enough to grow clover as compared with heavy applications of phosphate and potash on this soil. Is bloat any more severe on heavily fertilized pastures than on moderately fertilized? I don't think that there is any doubt that it is. We have many reports to support this idea. One farmer fertilizes heavily and has an acute bloat problem; his neighbor fertilizes moderately and has little or no trouble with bloat, and yet both had a good growth of clover.

Of course, we have to be careful in interpreting such reports, because it is well known that fertilizers such as phosphates can influence the botanical composition of a pasture. We may be comparing a pasture of almost pure clover with a clover-grass mixture.

In the fall of 1951, we decided to study this problem further. We selected a fairly fertile area and divided it into four paddocks and treated as follows:

- 1. Check no fertilizer
- 2. 500# 20% Superphosphate 100# KCL
- 3. 1500# 20% Superphosphate and 200# KCL, no limestone
- 4. 1500# 20% Superphosphate 200# KCL

About 2 tons of limestone was applied to all plots, except 3, and the area was seeded to ladino clover.

In other words, we wanted to study ladino clover with no phosphate or potash, moderate levels of both, and an extremely high level of phosphate and potash with and without limestone,

We didn't get any clover, this was due to dry weather, or an early freeze or a combination of both. So in the fall of 1952, we went back, disced the area and put 1500% more of 20% superphosphate on plots 3 and 4, and planted to ladino clover again. This time we had better luck and got a good stand of clover in all plots.

This past spring, after "hand requeing" the plots for grasses and weeds, we had practically a pure stand of clover in all plots, and the clover was ready for testing.

Here we had some more difficulty. Excessive rains kept, us from grazing the area until late May, We put the heifers in dry lot at night and the next morning allowed them to graze clover for two hours. If an animal bloated, it was taken off and put back on dry lot. That afternoon they were again allowed to graze for 2 hours and returned to dry lot. The same procedure was followed for several days until we had sufficient information to classify an animal as a bloater or non-bloater. Out of the 23 heifers, 15 bloated at least once, and I decided that I could use 10 for the testing.

We then decided to test Plot 1 - the check plot, and Plot 4 - the heaviest fertilized plot. We devided the heifers into two groups of five each, as equally as possible on the basis of their bloat history. We followed exactly the same procedure as in the preliminary work. One group was put on Plot 1 morning and afternoon, and the other group on Plot 4. The testing period lasted for two days. We had 4 cases of bloat on Plot 1 and 12 cases on Plot 4. This is cut of a possible 20 for each group.

Then we reversed the groups allowing a two-day change-over period. In the reversal, we had 7 cases of bloat on Plot 1 and 9 cases of bloat on Plot h.

Adding these together, we get 11 cases of bloat on Plot 1 and 21 cases on Plot 4. This is out of a possible 40.

After a few days, we decided to again test these same plots using the reversal method. However, I did remove one heifer from each group as they had not bloated during the previous total. This left four heifers in each group.

In the second trial let us consider the complete trial including the reversal. We had 7 cases of bloat on Plot 1 and 12 cases of bloat on Plot 4. This was out of a possible 32.

There was no visible difference in the clover of the two plots, or any of the other plots for that matter. There was another thing we observed, we recorded 6 cases of severe bloat on Plot 4 and no cases of severe bloat on Plot 1.

Now this looks pretty encouraging, but this bloat is an elusive thing and I am presenting this as a preliminary observation and I am looking forward to repeating this work next year. If what I have reported is a fact, then it appears to me that it would lend strong support to the "toxic substance theory" as to the cause of bloat. In other words, very fertile soil or highly fertilized soil produces a clover with a higher content of some bloat producing substance than clovers of less fertile soils.

Dr. Gall raised a question as to whether two days was sufficient to allow for change in rumen flora,

Dr. Cole pointed out the need for information on feed consumption during such tests. There is evidence that animals ate about twice as much phosphorus rich hay as they did of phosphorus poor hay. He again suggested the adoption of a standard method of measuring the amount of pressure produced by bloat.

The Role of Animal Management in the Prevention of Bloat - J. E. Foster, University of Maryland

The program of development of improved permanent pastures has made rapid progress in the East and the South but the improvement has been accompanied by an increase in the incidence of bloat. Ladino clover has been an important crop in the pasture improvement program. It has proved its adaptability to the area, it is palatable, has good carrying capacity and high nutritive value but the number of death losses from bloat where it is pastured are in some cases so great as to prohibit its use. Actual death loss from bloat is not the only factor to consider. Other factors are poor performance of animals following racovery, expensive procedures used to prevent bloat and the restrictions on the use of legumes.

In order to get an indication of the extent of the bloat problem in Haryland, questionnaires were sent to farmers in all parts of the State. A total of 1170 farmers responded. On 645 of these farms there were ladino clover pastures and 85% of those reporting the use of ladino clover pasture had had bloat. Individual surveys were made on 85 farms and bloat had occurred on 65. Pastures that were predominantly ladino rere found on 76% of these farms. Feeding hay or other roughage may have helped but did not prevent bloat. Apparently weather and time of day was not a factor in bloat, but the level of fertility may have been.

In a closely observed case of steers on ladino-blue grass posture in May, four steer had died by the 10th day after bleating started. Neither timothy-clover mixed hay or bright wheat straw fed in racks prevented bleating. The cattle were changed to a meadow grass pasture and bleating ceased. When the cattle were returned to the original pasture after the clover was rather mature, no cases of bloat developed. Chemical analyses of forage from bloat producing pasture showed a crude protein content of 20.5%, while the non-bloating forage had a crude protein content of 15.9%.

Dr. Foster also outlined new work being developed in cooperation with the Agricultural Research Service, USDA, and reviewed information he had obtained from a number of State agricultural experiment stations on results or observations on bloat.

Dr. Cole stated that California had experienced no real trouble when alfalfa was cut and hauled to cattle.

Dr. Parsons reported that extracts of birdsfoot trefoil were lethal but did not cause bloat. These extracts contained 10 times as much cyanide as clover and 100 times as much as alfalfa. Sodium thiosulphate counteracted the lethal effect,

The question was raised as to whether there was any relation between bloat and grass tetany. Dr. Barrentine observed that he had seen no relationship. There was a report of bloat on wheat pasture in West Texas,

Dr. Barrentine reported that one farmer in Mississippi was preventing bloat by close folding his cattle.

Dr. Huffman reported that a crude sugar (Nytose) had been tested at Michigan to determine its value in preventing bloat, then the animals are about 1/h lb. of this material on ladino clover - grass pasture there was little trouble from bloat. However, the consumption of the product increased, going as high as 7 lbs. per day, and bloating increased.

Dr. Cole had found no difference in incidence of bloat on alfalfa when Hytose was fed in comparison with animals fed no supplement. He also suggested the need for more information on the value of grasses for preventing bloat.

Dr. Musson stated that in South Dakota there had been death losses from bloat on all'alfa pasture and also some bloat on sweet clover. One animal when switched from sweet clover to alfalfa pasture, bloated and died.

Dr. Grandsteff reported that bloat developed in range ewes fed alfalfa hay in New Mexico. Varying the amount of hay had no effect on the incidence. The heavy, more vigorous ewes were the ones that bloated. Chopping the hay prevented the trouble.

Dr. Huffman stated that finely ground ladino straw, when fed with grain, will produce bloat in sheep.

RUMEN PHYSIOLOGY PANEL

Frothy Bloat - Clyde Smith, Michigan State College

Frothy blost was observed when animals were fed alfalfa hay. This condition is unrelievable with a stomach tube. The frothy mass extends throughout the rumen without formation of a solid mass and there is no regergitation. Hay is apparently a necessary portion of the ration when frothy bloat occurs. Corn and hay alone, or hay alone would not produce froth. Frothy bloat was most severe in early morning. After eating, animals with mild cases of this condition seemed to recover. The more severely bloated animals refused feed and might remain in this condition for two days. Methyl silucone was found to relieve this condition and would prevent it for several days when given in recommended amounts. Lipase-treated cream was also effective, as was additional hay, in treating this condition,

Chemical analysis showed no significant difference in the amount of nitrogen in frothy and normal material from the rumen. However, there was a very significant difference in the non-coagulable proteins from these materials.

Bacteriological examination of rumen content showed no difference that would account for the frothy condition.

In laboratory tests, corn was shown to be an excellent source of foam when beaten in a Waring blender. Soytean oil meal was also a good foam producer. A mixture of corn and soybeen oil meal gave a product in which the gas was trapped in the combined material.

In severe frothy bloat a cessation of rumen motility was noted. Then animals had access to shavings, no frothy bloat occurred. The dry matter in the rumen content was slightly lower when frothy bloat occurred.

Dr. Huffman reported that hay alone was fed to one of a pair of identical twin calves and grain alone to the other. The calf fed hay was healthy while the calf on grain was sick but did not bloat. However, when hay was added to this calf's ration it bloated.

Chemical and Physical Factors in the Etiology of Acute Ploat on Legume Pasture - H. H. Cole, University of California

It is the purpose of this paper to review the evidence concerned in throwing light upon the rôle which chemical and physical factors play in the etiology of bloats

Physical Factors

A. "Scabrous Deficiency" Theory

According to this theory, soute bloat is due to a lack of sufficient, scabrous material in the diet to elicit the reflex act of eructation. Presumably, rough particles of ingesta rubbed against the ruman wall by ruminal motility excite afforent nerve fiters resulting in implies building up a central excited state. Exactly how the active dilation of the cardiac sphineter is synchronized ith ruman contraction presents an interesting problem in nerve physiology.

The following evidence has been submitted in support of this theory:
(1) It was found that as much gas was produced on alfalfa hay as upon green alfalfa indicating that the rate of gas production is not the sole factor determining the incidence of bloat. If the rate of gas production were the only factor involved, one would expect as much bloat on alfalfa hay as upon green alfalfa tops, which is not the case. This does not mean that the rate of gas production is of no significance,

- (2) It was found that belching occurred at the peak of ruminal contraction. Since belching did not occur at each ruminal contraction and since it is doubtful that the cow is conscious of ruminal activity, it is logical to assume that eructation is a reflex act;
- (3) It was found that a maximum amount of bloat occurred when rumination was at a minimum. This indicates that both rumination and eructation are reflex acts responding to a common stimulus. It was shown earlier that rumination is dependent upon scabrous material in the rumen for its elicitation. This would indicate that the amount of time spent ruminating can be used as an index as to the bloat-inducing capacity of a given diet.
- (4) It was found that 21 cases of bloat occurred on finely ground alfalfa and concentrates as compared to one case of light bloat on whole alfalfa and concentrates. Since the ground and whole alfalfa tere obtained from the same lot, the only difference is in the physical condition of the alfalfa fed. This provides proof that the physical nature of the diet may be an etiological factor in bloat. One cannot decide, however, whether the change in the physical condition of the alfalfa increased the incidence of bloat because of a reduction of its scabrous nature or whether fine grinding merely made some chemical substances involved in bloat production more available.

- (5) It was found that bloat could be prevented by feeding 10 or 15 pounds of Sudan hay to lactating dairy cous pastured on legeme pasture during the day. Both green Sudan and Sudan hay produce a maximum amount of ruminantion testifying to their scabrous nature.
- (6) Acute bloat only occurs on diets lacking in scabrous character -- for example on legume pasture, cabbage or lettuce leaves or on concentrates.

B. Other "Physical" Theories

The evidence cited above supports the view that acute bloat is due to a lack of sufficient scabrous material in the diet to elicit the reflex act of eructation. Others have suggested the piling up theory — namely that cattle eat legumes so rapidly that the material piles up thus occluding the cardiac orifice and making belching impossible. In support of their theory, they present data indicating that under their conditions lactating dairy constate legumes more rapidly than grassen. We have found, under our conditions, that cattle vill eat about three times as much green Sudan as they will of alfalfa tops in a given interval. Inasmuch as bloat never occurs in normal animals on Sudan pasture, this theory would appear to be untenable.

A different physical theory has recently been suggested. According to this hypothesis, legumes sink to the bottom of the rumen causing the water level to raise. This blocks the opening of the rumen and prevents the cow from belching.

Chemical Factors

Presumably chemical factors might be involved in the etiology of bloat by several means. First, the chemical nature of the feed may have an influence upon the rate of gas production in the ruman. Secondly, the chemical substances in the feed or substances produced by micro-organisms in the ruman may exert an influence upon the ruman musculature. Finally, foaming agents in the feed may interfere with the release of gas.

A. Chemical Factors Influencing the Este of Gas Production

In an experiment involving ad libitum feeding over a k-hour period, 4.3 cubic feet of gas were produced from 15.5 pounds of alfalfa and 4.7 cubic feet of gas were produced from 47.6 pounds of Sudan grass. It is reported that no gas was formed on grass hay as compared to a marked gas formation on alfalfa. It was found that the addition of silage to an alfalfa hay and grain ration depressed gas formation of rumen ingesta in vitro. A report states that more gas was produced from sugar than from starch and theorized that high sugar content of plants in the late afternoon was an eitological factor in bloat. We found as much gas formed following drenching with starch as with sugar when cattle were on a basel diet of alfalfa hay. There was a more immediate response in increased gas production u on the introduction of sugar by means of a canula directly into the rumen. We have found that

the rate of gas production on a dict of silage alone is extremely low as one would expect since the silage has already passed through a fermentation process. If silage will depress the amount of gas formed from other constitutents of the ration, this may be an important factor in the control of acute bloat on legume pasture. Certainly studies clearly indicate that the rate of gas production varies with the feed. As is pointed out above, however, the evidence is equally clear that it is not the sole etiological factor in bloat. It is stated that the rate of gas production depends upon the splitting of phosphoric acid esters of carbohydrates by phosphatase and further that arsenates increase the activity of phosphatase. Legumes are rich in arsenates.

B. Chemical Factors and Muscle Inhibition

The production of toxic metabolites in the rumen may interfere with the normal functioning of the rumen musculature. The evidence is clear-cut that carbon monoxide, hydrogen sulfide and other chemical substances, normally present in the rumen in small concentrations, are toxic when given in sufficient quantities. Reports show a muscle-inhibiting factor in lucerne and evidence has been submitted that the substance in clover juice causing paralysis of smooth muscle is cyanide. Limited evidence has indicated that hydrogen cyanide is high in scutely bloated animals. However, cyanide depresses muscular activity, and we have found in our studies that rumen musculature is very active in early stages of acute bloat. Furthermore, it would appear that the rapid recovery of bloated animals after the excess gas is removed is contrary to what one would expect in cyanide poisoning.

Reports have shown that extracts of birdsfoot trefoil are lethal in lambs with symptoms suggesting cyanide poisoning. Extracts of alfalfa or ladino clover, on the other hand, produced acute bloat accompanied with scouring in less than an hour after drenching.

C. Chemical Factors and Palatability

The chemical constitution of the plant has an important influence on its palatability and thus upon the total amount of a given feed that is consumed. Thus in order for an outbreak of bloat to occur, it appears that not only must the alfalfa or legumes be at a succulent stage, but also it should be palatable so that copious quantities are consumed. The greater the amount consumed, the greater will be the gas production and thus the greater the difficulty in expelling the gas as rapidly as it is formed.

D. Chemical Factors and the Release of Gas from the Rumen Ingesta

It is suggested that the high saponin content of alfalfa may cause excessive bubble formation with a minimum amount of free gas. The presence of the gas in the form of bubbles within the ingesta makes eructation impossible or difficult. Data are available indicating that the saponin content of alfalfa is higher than that of birdsfoot trefoil and these findings are in line with the fact that acute bloat is frequent in alfalfa and rare on birdsfoot trefoil. Thus, the possibility that saponins are involved as an etiological factor in bloat in certain instances should be considered.

Summary and Conclusions

Evidence has been submitted indicating that both the physical and chemical factors may be responsible as etiological agents in bloat. Considerable evidence is available to indicate that the lack of scabrous material in the diet is a contributing factor in acute tympany. I am fully convinced that it is not the only etiological factor. The more fact that, although a very high percentage of animals can be bloated on any succulent alfalfa pastures, fatal bloat does not necessarily occur, indicates to me that the lack of scabrous quality of the feed is not the only factor involved.

There is convincing evidence that the rate of gas production varies depending upon the nature of the diet — that is upon its chemical composition and this may be an important factor in the etiology of bloat. There is evidence that toxic constituents or metabolites of legumes may be important as causative agents of bloat. The influence of chemical composition upon the palatability of feed may be an important factor.

Mr. Lindahl reported that he has produced bloat in sheep with 15 to 20 gms. of saponin and in a heifer with 3 times that amount. In all cases the bloat was moderate. All animals had been previously dosed with alfalfa and ladino juice to ascertain that the animal would bloat. Bloat produced with saponin occurred within 35 to 45 minutes after administration, while bloat produced with alfalfa occurred within 10 minutes. The use of two commercially prepared saponins gave no reactions. It was noted that rumen motility increased in the early stages of bloat.

Dr. Maclay reported the results on growth and feed efficiency studies with chicks, showing the depressing effect as the amounts of saponin increased. Evidence has been accumulated to show differences in growth inhibiting effects and saponin content of different alfalfa varieties and atdifferent stages of cutting. The saponin content was high alfalfa, ladino and trefoil. Results indicate that there is more saponin in the leaves than in the stems of plants. It has been demonstrated that there is an enzyme in plants which hydrolyzes the saponin. Laboratory tests have demonstrated that saponin inhibits the movement of the smooth muscles:

PHYSIO-PATHOLOGY PANEL

The Effective Buoyancy of Rumen Juice of Cows Fed Hay, Grass and Legumes, R. E. Nichols, University of Wisconsin

Specific gravities of samples of strained and of centrifuged juice from the ventral sac of the rumen of 3 fistulated cows were determined at rumen temperature before, shortly after and a few hours after intake of water, hay, young fresh grass, prebloom alfalfa and mixed clover. Drinking water reduced the specific gravity of the centrifuged juice. Eating fresh green feed apparently reduced the effective buoyancy of strained rumen juice even when the liquid portion of the juice was not diluted by water intake.

The reduction in buoyancy was greatest in samples with the most bubble formation. It was much more evident with legumes than with grass or hay and extended over to the next day more with legumes than with grass or hay. It is felt that the position in the paynch in which the major part of gaseous digestion takes place can be influenced by plant density, by reduction in the buoyancy of rumen juice resulting from dilution by water intake and the degree of bubble formation taking place in the lower portions of the paunch, this formation being greatest shortly after the intake of fresh yours, legumes. Since every particle of food sinking in it and every tubble formed in it displaces an equal volume of juice, it is felt that the resulting rise in fluid level, if of sufficient magnitude, can allow the possibility of mechanical blockage of the cardia. Depending on the agent used and amount administered, surface tension reducing agents not only can clear excess froth from the surface of paunch contents but apparently can also control the degree of bubble retention and/or formation within the juice of the ventral sac-This was evidenced by smaller reductions in the buoyancy of juice following consumption of fresh young legumes when detergents or surface active agents were administered. The significance of low level intake of these agents as a practical means of taking more advantage of legumes' superior yields was discussed.

Dr. Dougherty suggested that bubbles can be broken by either increasing or decreasing surface tension.

Dr. Gall suggested that the microorganism population be followed carefully to check the effect of detergents on total numbers and activity of the population.

Dr. Nichols estimated that bloat was costing the State of Misconsin \$2,000,000 annually. This is based on the number of cows treated by Veterinarians. It does not take into account losses due to animals going off feed, etc.

Cardiovascular and Blood Gas Changes in Experimental Bloat, C. D. Meredith and R. F. Barrett, Cornell University

A series of experiments have been conducted to determine the changes in arterial blood pressure and in volumes per cent CO_2 and O_2 under conditions of raising intraruminal pressure with various gases. O_2 and CO_2 were used, and also a 60 per cent CO_2 , hO per cent methane mixture as approaching more nearly the natural gas composition of the rumen. The results obtained may be briefly stated as follows:

- (1) Using O2 or air, sheep can tolerate intrarominal pressures of 100 mm Hg. without showing more than discomfort. There is a moderate elevation of blood pressure which is subject to fluctuation -- falling with each eructation of ges and increasing as the pressure builds up again.
- (2) Using CC2 pure, or CO2:CH1 mixture, there is immediate distress, hyperphoea, cyanosis, marked elevation of bood pressure and eventual collapse and death. These results are obtained with different pressures,

and times for which pressure is maintained, in individual sheep as there is apparently marked variation in susceptibility. In some cases 40 mm Hg. pressure will produce these effects, in others up to 80 mm is required. Tolerance will vary from two to five minutes.

- (3) Blood gas analyses show increases of vol, per cent CO₂ in arterial blood corresponding to the enset of symptoms and blood pressure rise. This is more pronounced with CO₂ than CO₂:CH₁ mixture, as would be expected. Then vol, per cent CO₂ shows a material increase, a reduction of per cent O₂ appears which in one case amounted to an 87 per cent fall in O₂ when the CO₂ level rose 47 per cent. This combination can be expected to produce histotoxic and anoxic aroxia.
- (h) Two other phenomena are worth recording. A fall in vol per cent CO₂ subsequent to insufflation with CO₂ pure or as a mixture; to less than the preinsufflation level; and a simultaneous depression of the arterial blood pressure. A drop of lh-18 per cent has been recorded. A possible explanation is that of hyperventilation of the lungs and blood following CO₂ stimulation of respiration and blood pressure.
- (5) An experiment on an anesthetized dog to observe whether partial or total occlusion of the post vena cava by external pressure was a factor in raising blood pressure gave negative results.

Inferences

It would appear that the percentage CO₂ present in the rumen is highly significant since with higher percentages, and hence partial pressure, correspondingly lower total pressures may produce toxemic blood levels of absorbed gases. Thus histotoxic and anoxic anoxia may paralyze the respiratory center and cause death very rapidly. Should highly poisonous gases, e.g. H₂S, be present in significant concentrations, then their absorption under conditions of increased intraruminal pressure can be expected to reinforce or even overshadow the effect of CQ₂ absorption.

There these conditions are fulfilled --- viz., inability to cructate freely, high percentage of CO2 in the rumen and possibly poisonous gases, death may ensue without excessively high pressures developing in the rumen.

This paper was followed by a very lively discussion.

Isloated Gut and Fumen Hotility as Affected by Forage Juices and Other Helated Substances, A. R. Parson, University of Illinois

The Illinois study is being conducted with the idea that bloat may result because of a toxic substance in the forage and/or rumen contents that paralyzes the eructation mechanism of the rumen and causes an accumulation of gases in the rumen. Ferguson reported the use of forage juices with respect to their effect on the activity of isolated smooth muscle, and this suggested the use of not only forage juices that had caused bloat but to also test the effect, on smooth muscle, of rumen contents of cattle

that died from bloat. The test substances were tested by using the jejunum section of the rabbit intestine in order to obtain a more consistent reaction of the smooth muscle.

Kymograph tracings were presented that showed the reaction of sections of intestine to normal ruman contents and also to ruman contents taken from bloated animals. Similar recordings were shown of the reaction of the gut to juices taken from forages that were causing serious bloat. Normal ruman investe did not inhibit the intestinal motility while ruman contents of bloated animals and also the juices from bloat producing forages did inhibit the motility of the intestine. Considerable variation was noted in the motility of the isolated rabbit gut when a variety of legume and grass extracts as well as some selected chemical compounds were used, particularly urea of saponins. Both alfalfa sponin and cateus saponins inhibited the motility of the intestine. The inhibiting influence of urea was stimulated by the addition of urease to the test portion of urea.

Acute bloat and death were produced in sheep using an extract of ludino clover although considerable variations in response were obtained by orally administering the different forage juices to sheep. A fistulated cow was used to study the effect of forage juice on ruman motility. An orally administered legume juice was noted to inhibit the cructation mechanism of this cow. Further studies have indicated that the inhibiting substance in legumes seems to be heat stable, water soluble and also dialyzable.

From the results of these tracings it may be postulated that the increased ruminal pressure, apparently resulting from a paralyzed eructation mechanism, affects the absorption, of a substance or substances, into the blood stream. If the animals body is unable to adjust to this absorption of an as yet unidentified material, a critical threshold is reached that causes death to the "bloated" animals

MICROPIOLOGY PANEL

The Factoria of the Rumen and Some Observations on Their Possible Relationship to Bloat, R. P. Bryant, Agricultural Research Service, USDA

Methods now available for determining the numbers and kinds of bacteria present in the rumen leave much to be desired. Direct microscopic methods are poor because morphologically similar organisms may vary greatly physiologically and microscopic methods give little knowledge on the physiological reactions carried out by the bacteria. Cultural methods to evaluate the flora of the rumen give better data on the kinds of organisms present under a given set of conditions but much is as yet unknown as to the physiological reactions carried out by the various bacteria or of the kinds of bacteria present. Cultural methods now available allow the cultivation of large numbers and a wide variety of different kinds of bacteria but few selective methods are available so that the individual kinds can be isolated if present in small numbers.

Although large differences have been shown in the ruminal flora of animals fed purified rations or rations deficient in minerals as compared to the normal flora, the flora of animals on the usual farm type ration high in roughage appears to remain fairly constant. Animals on high level grain rations appear to support a more acid tolerant flora that causes the production of large amounts of lactic acid, thich may lead to a lowering of the pH in the rumen.

Bloat as it occurs in high grain rations may be due to the lowering of the pH in the rumen and subsequent rumen atony that would tend to cause faulty eructation.

The small amount of data collected at Beltsville on three animals that blooted on high hay or fresh clover rations showed that many of the groups of bacteria and the protozoa commonly found in large numbers in normal animals were also present in large numbers in the bloated animals. This suggested that there are no large differences in the kinds of microorganisms present in bloated and normal cows. It was suggested that certain reactions of the normal flora may be accentuated when bloat producing feeds are available and those may be a factor in causing bloat.

Rate of Volatile Acid Production in the Rusen, R. h. Hungate, State College of Washington

The rate of production of acid fermentation products has been studied by removing rumen contents from two fistula steers and an unfistulated bull and incubating in vitro under conditions simulating those of the rumen. The contents were analyzed for volutile acids at the time of removal from the rumen and after 1, 2, 3 and 4 hours, respectively, of incubation. From the average volatile acid content a curve was drawn showing the amount of vol tile acid at different times. The slope of the curve at time zero was estimated graphically and this rate of acid production was assumed to be the rate of acid production in the rumen.

Rumen contents were removed at various times of day in order to obtain representative values and the rates of production of volatile acids on different diets were determined. Eleven experiments were completed on rumen contents of a hay-fed fistula steer; three experiments on a grain-fed (2/3 grain, 1/3 hay) bull; and three experiments on animals on grass pasture, two of them with a second fistula steer and one with the bull.

The estimated average rates of production of volatile acid were: 36 equivalents per day per 100 kg of rumen contents for the hay-fed animal (range 20 - 66); 55.2 equivalents for the grain-fed animal (range 53 - 58); and 22.8 equivalents for the grass-fed animals (range 19.3 - 25).

The amounts of acetic, propionic, and butyric acids, respectively, in the volatile acid at zero time and four hours here determined for six of the experiments with the hay-fed animal. The average volatile acid production

in these six experiments during the four hours of incubation was 4.18 milliaquivalents per 100 grams ruman contents. The differences between the milliaquivalents of the individual acids initially and after 4 hours were: acetic acid - 2 11, propionic acid - 0.84, and butypic acid - 0.74, or a total of 3.99 milroquivalents. This agrees within 5 per cent with the amount of total volatile acid increase (4.18 meq).

If it be assumed in these six experiments that these individual acids were produced in the same proportion during the first hour of incubation when production was more rapid (1.48 meq/100 g/hour) the amount of each acid would be: acetic acid - 0.9 meq; propionic acid - 0.31 meq, and butyric acid - 0.27 meq per 100 g per hour or a total of 0.22 lb, of acid per hour. Through absorption and oxidation of these acids the amount of energy per 100 kg rumen contents available to the bovine per day would be: acetic acid - 4460 calories; propionic acid - 2750 calories, and butyric acid - 3460 calories. In individual experiments the volatile acid production in the hay-fed animals was estimated to range between 0.12 and 0.4 pounds per 100 kg per hour.

In the above experiments with hay it was possible that the ratios in which the individual acids were produced during the first hour of incubation differed from the average ratios found for the 4-hour period. In the experiments with animals on grass pasture analyses of individual acids were made at zero and one hours. The differences in the individual acids were acetic - 0.61 meq, propionic - 0.24 meq, and butyric - 0.17 meq/100 g, a total increase of 1.02 meq as compared with an increase of 0.91 meq in total volatile acid as determined by steam distillation. The discrepancy is of about the same magnitude as the experimental error.

On the hay diet the milliequivalents of the individual acids supplied per hour were in the ratio of acetic acid ~ 61 per cent, propionic acid ~ 21 per cent, and butyric acid ~ 18 per cent. On the pasture diet the ratios were quite similar, namely: acetic acid ~ 60 per cent, propionic acid ~ 23.5 per cent, and butyric acid ~ 16.5 per cent.

The analyses for the grain diet were made at zero and two hours. The sum of the amounts of acetic, propionic, and butyric acids at zero hours in one experiment and at two hours in another was in such disagreement with the amount of total volatile acid that the results could not be used. The one successful experiment showed an increase of 0.73 meq acetic acid per 100 kg per hour, 0.38 meq of propionic acid, and 0.31 meq of butyric acid. These are in the proportions of acetic - 51 per cent, propionic - 27 per cent, and butyric - 22 per cent. Using these percentages and the rate of volatile acid production during the first hour as determined by difference in steam distillation values the amounts of the acids available per hour to a bovine with 100 kg of rumen contents would be: acetic acid - 1.01 equivalents (60.6 g), propionic acid - 0.53 eq (39 g), and butyric acid - 0.43 eq (38 g), a total of 0.3 pounds per hour. On a caloric basis these would provide each 24 hours a total of acetic acid - 5070 calories, propionic acid - 4660 calories, and butyric acid - 5300 calories.

The above rates of microbial production of volutile acids are about those which rould be expected if the caloric requirements of the bovine are satisfied by the oxidation of volutile acids absorbed from the rumen. Although the results are not extensive enough to permit wide generalizations, they suggest that the rate of volutile acid production is highest on a grain diet, intermediate on hay, and lowest on a grass pasture diet. Relatively more propionic and butyric acids were formed on the grain diet than on grass pasturage or hay. There are wide variations in the rate of volutile acid production at different times on the same diet.

In view of the magnitude of the microbial fermentation in the rumen it seems feasible to undertake experiments to determine in cases of bloat whether there is an abnormally high rate of production of fermentation products. Information on the acidity in ruman contents of bloated animals will be useful in ascertaining whether changes in the magnitude of microbial activity in the ruman are ctiological factors in bloat.

Dr. Gall stated that there is a pointed rod type of organism usually present in number 100 times greater in animals that have bloated than in normal animals. This organism is a heavy gas producer. Cas producing organisms are always in greater numbers in bloating animals. A 10 times difference is necessary in order to be significant when working with ruman material.

Dr. Pounden observed that there were fewer cases of bloat in Chio this year, as compared to last year. Differences in weather accounted for differences in growth of grasses and legumes.

Dr. Hungate, in response to a question, stated that no information was available on the possible toxic effects of organic acids produced by fermentation. He thought it might be possible.

Dr. Pounden raised a question as to why we do not make differential diagnoses in bloats that appear to respond differently to treatments. His experience has indicated no value to commercial preparations intended to give a desirable rumen flora.

AGRONOMIC PANEL

The Influence of Nitrogen Fertilizer on Botanical Composition, Palatability and Feeding Value of Forage, P. G. Hogg, Delta Branch Experiment Station, Mississippi

The agronomist has a major responsibility in the development of satisfactory feed and pasture crops for the livestock industry of the country.

The animal husbandman desires a pasture that will have sufficient available T.D.N. so that a dairy cow can produce at a high level without supplementary feeding and at the same time maintain animal body weight; or, looking through the eyes of the beef producer, a pasture which will be good enough to produce 2 to 2.5 pounds daily gain on young steers.

For a pasture to meet the above requirements, it must have a high protein and carbohydrate content with a reasonable amount of dry matter. Callulose and lighth must be fairly low.

Under Mississippi Delta conditions, such a pasture can be obtained only by the proper mixture of legumes and grasses. Pure or near pure legume pastures appear to have such a high moisture content that animals do not consume sufficient dry matter to make satisfactory gains. I have been unable to get anything resembling accurate animal yields on pure stands of ladino clover, because the bloat problem is so severe that animals cannot be left on such a pasture. We did, however, obtain strong indications that animals grazing on mixed pasture where ladino clover was dominant were not ingesting sufficient carbohydrates to make satisfactory gains. This points up the most difficult problem in pasture management in the Mississippi Delta and most of the rest of the United States, that is, maintaining a desirable mixture of grass and legume in a pasture. This is greatly complicated by the long growing season and uneven rainfall. Aside from the bloat problem, the cost and productivity of the pastures are of great importance.

There are three general methods of controlling the percentage of grass and clover in a sward: (1) The balance of fertility (in our case the use of nitrogen); (2) method of seeding; and (3) the intensity of grazing and mowing. Then other soil conditions are favorable, nitrogen is a powerful stimulant to the grasses and has little effect on the rate of growth of the clover. It is thus possible by the judicious use of nitrogen to stimulate grass growth and prevent the legume from dominating the clover. In practice this is not as simple as it sounds. Over-fertilization will result in the legume being crowded too much and result in lower animal production and higher pasture costs. If perennial grasses are stimulated during the peak of the clover season, they will likely crowd the legume when the growing season advances and growing conditions favor the grasses.

The study of accurate placement of grass and legume seeds intended to grow in association has long been neglected. In the case of such grasses as Bermuda grass, bluegrass, and bromegrass, the creeping nature of the plant makes controlled seeding of little value. However, in the case of tall fescue and Dallis grass, these bunch type grasses do not spread by creeping root stalk and do not thicken from natural reseeding to any extent in an established pasture.

Yield data, where fescue was seeded broadcast and in 1-, 2-, and 3-foot drills and all plots were over-seeded with ladino clover broadcast, indicate the marked effect method of seeding may have on total yield, percentage of clover in the sward, and protein content of the grass growing in association with the clover. In this experiment, no nitrogen fertilizer was used, and the differences in protein content were due entirely to the nitrogen fixed by the clover.

Data indicate that the crude protein content of grass can be readily raised by nitrogen fertilizers to a level equal to or above that obtained by growing clover with the grass. Mitrogen is quickly taken up by the grass. There 50 poends of nitrogen were used, the crude protein production reached a maximum 7 meeks after fertilization. This would indicate that if the protein content of grass is to be kept at a high level by nitrogen fertilization, the fertilizer must be applied about every 6 weeks during the growing season.

Even though the crude protein percentage and total yield of dry matter may be good, the yield of animal products may be estimated by actual grazing tests. This is a point where many agronomists have gone astray when interpreting data from small plot studies.

In graving experiments, nitrated pure fescue with a protein content ranging from 16 to 18 percent gave less than I pound daily gains, even though the chemical picture of the herbage was excellent and an adequate level of nitrogen was used. There no nitrogen was used but where as much as 25 percent of the sward was made up of ladino clover, the daily gains jumped to 2 pounds a day. The cost of the nitrogen for the fescue was about \$20 per acre. What is more important, the animals on the mixed pasture continued to gain, while those on the nitrated grass became unthrifty, declining in general condition as well as daily gains.

The reason for the great difference in animal response to the two types of pasture is not clear. It is likely several factors are involved, including low palatability and possible deficiencies in the pure grass diet.

Most of the bloat in the Delta occurs on so-called summer permanent pastures where the grasses are Dallis and/or Bermuda. For all practical purposes, clover is responsible for all the trouble. These grasses remain dormant until late in the spring, by which time the clover has made a heavy growth and in years favoring the legume will be completely dominant to the grasses. The common practice in the past has been to broadcast annual ryegrass on this sod in the fall. This practice has not proved satisfactory. First, because it frequently does not make sufficient growth, and second because ryegrass appears to be one of the poorest grasses to reduce bloat in a grass-clover combination.

In recent years the development of the so-called "Pasture Dream" machine, which makes it possible to put out sled and fertilizer in one operation in a permanent pasture, has been developed in Mississippi. Numerous experiments with this type of pasture planting have been very satisfactory in reducing bloat by the seeding of a winter-growing grass or cereal in the summer sod. About 60 pounds of nitrogen are required to make this practice satisfactory. This seeding must be done after the warm-seasoned species are dormant; otherwise the fertilizer may be used by the summer grass. By allowing the winter-growing cereal to make sufficient growth in late winter, the cereal-clover mixture may be grazed off without difficulty during the season when the clover is most dangerous.

The cereal then matures in early May about the time that Dallis, Johnson, and Fermuda grass become active. This practice, while not in general use at the present time, rerits a great deal of consideration. It will cost about \$10 per acre, and in addition to giving bloat control, increase the total annual production of the pasture. The cereal, or ryegrass, as the case may be, should be seeded at a rather low rate of seeding; and the nitrogen fertilizer, while essential, should not be used in excess. Again, the pasture must be grazed at the proper time to prevent the cereal from over-growing the clover, which will damage summer production of the pasture.

The intensity and method of grazing have a profound effect on the botanical composition of the pasture sward. Either the grass or legume may be proofly suppressed or even completely eliminated by grazing management alone. Improper grazing is responsible not only for much bloat but greatly reduced pasture yields. For example, by over-grazing summer pastures in late summer and fall, the grass in the mixture will be weakened and food reserve lowered to such an extent that spring growth will be slower and the actual ground covered by the grasses reduced; leaving more space for the winter growing clover to become established.

In the fescue-clover combination, the same thing may occur. On the other hand, undergrazing will allow the tall-growing fescue to get above and shade out the clover. Either condition may ruin the pasture, the first by eliminating or weakening the grass will cause an over-growth of clover which will result in bloat losses and poor gains; the latter will greatly reduce the clover and results in nitrate-starved grass of poor quality and low animal yields. The proper grazing management must be worked out for each pasture combination. In the case of fescue and clover, it appears to be, roughly, this: Graze moderately thrubut the fall and winter, maintaining the grass at from 3 to 4 inches in height; shorter if the clover is weak. In the spring intensive rotational grazing will likely give somewhat better yields. However, the pasture must not be allowed to get more than 10 to 1? inches in height or the fescue will lose palatability and be refused by the animals. If this occurs, the pasture should be closely clipped with a mower to the height of 2 to 3 inches. This will favor the clover and allow a desirable regrowth of a more palatable grass and clover.

To summarize the bloat conditions in the Mississippi Delta, I would list the following points:

- (1) Clover is the cause of practically all the bloat in the area.
- (2) The frequency of bloat is definitely correlated with the percent of clover in the pasture. Less than 50 percent clover infrequently causes bloat under our conditions.

- (3) So-called methods of controlling bloat are either too expensive, that is, feeding dry feed; or are of little or no value, i.e., keeping cattle off pastures until the dew dries or rain has dried, feeding mineral supplements, feeding yeast, etc.
- (4) There is a great deal of difference in the frequency of the occurrence of bloat at different times of the year, i.e., spring and fall:
- (5) Bloat is more frequent and more severe on warm, sunny afternoons than on cold, wet, dark days,

Soil fertility level and the use of nitrogen as a grass stimulant are important in the maintaining a desirable mixture. The use of nitrogen as a substitute for the legume is: (1) Too costly, (2) has not given satisfactory animal yield, (3) requires too frequent application unless the pastures are very carefully managed and frequent rotational grazing is used, and (4) the most profitable pasture is the grass-legume combination properly fertilized, seeded, and managed.

The participants agreed that a similar conference should be held in two years, that is in 1955, at a time and place comparable to the present conference. It was also agreed that the same panel chairmen should be continued.