BLOAT AND BLOAT PROPHYLAXIS AND THEIR RELATIONSHIP TO CERTAIN PROPERTIES OF RUMEN INGESTA AND BLOOD

by

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INTRODUCTION

Although bloat is one of the oldest noninfectious diseases afflicting ruminants and has been subjected to intensive study for a long time, it is, nevertheless, one of the most serious problems facing animal researchers today. The first written account of bloat is credited to an ancient Roman author in 60 A. D. (cited by Parham, 198). Since that time bloat or treatment used for bloat is mentioned frequently in the literature. Beddows (20) describes many observations on bloat taken from agricultural writings between 1716 and 1827. During the last 30 years reports of bloat have appeared in the literature with increasing frequency.

Many attempts have been made to classify the various types of bloat; however, no infallible system of classification has been proposed. One of the best systems suggested to date is described by Cole et al. (46). These authors classify bloat as (a) chronic -- a condition occurring irrespective of the type of feed, (b) subacute -- a condition caused by diet but in which symptoms of distress are not manifested and (c) acute -- a condition caused by diet but in which the animal shows signs of considerable distress. Bloat may also be classified, according to the type of feed, as pasture bloat or feed lot bloat. When an animal is bloated, froth may or may not be present; thus, bloat also has been classified as either frothy or free-gas bloat.

Cases of bloat occurring among animals grazing native grasslands quite likely were due to a variety of causes including such things as mechanical obstruction of the esophagus and selective grazing of plants that had bloat producing properties similar to alfalfa. However, in relatively recent years bloat in grazing cattle has become a problem of considerable magnitude because of the advent of the widespread use of legume pastures. Feed lot bloat was unknown until farmers began to fatten cattle by feeding diets composed of concentrates and low levels of roughage for long periods of time.

The losses from bloat in terms of a percentage of the total animal population appear relatively small; Johns (127) reported that approximately 0.62 per cent of the cattle in New Zealand die from bloat each year. Nichols (189) estimated the annual death loss in Wisconsin to be 0.16 per cent. However, from the standpoint of the individual farmer, the loss of only a few animals may mean the difference between profit and loss for the entire year.

Of course, the value of animals lost from bloat is actually only a part of the overall loss that may be attributed to this syndrome. Total losses due to bloat in the United States have been estimated by Dougherty (72) to be approximately 40 to 50 million dollars annually. Among factors that must be considered in determining the overall loss due to bloat are (a) losses in production from animals that bloat and recover, (b) losses caused by changing the grazing management in attempting to reduce the bloat hazard (these include not grazing the most productive forage at certain periods and in many cases not using legumes for pasture), (c) time that the farm operator must spend treating bloated animals or administering preventives, and (d) the anxiety that the farmer may experience due to the possibility of losing animals from bloat.

If one considers the gain in production due to the use of superior pasture, as compared to pastures used 25 or 30 years ago, the net return per acre, even with bloat losses, may still be considerably higher. However, this does not justify an attitude of complacency, nor is it a comfort to the farmer who has recently lost several of his best animals from bloat.

Without question, a problem of this magnitude should be, and indeed has been, the subject of much research. Currently, bloat is being investigated at many experiment stations in the United States as well as in New Zealand, in Great Britain, and in many other countries throughout the world. Work at Iowa State University has been part of the regional project NC-27, "Chemistry and Physiology of Bloat"; this represents a large inter-institutional effort directed at various phases of the bloat problem.

Many hypotheses concerning bloat have been proposed; at the present time absolute proof is lacking for each hypothesis. However, data are being accumulated constantly that refute some aspects of some hypotheses and confirm others. Questions such as, "Why do cattle bloat?" and "Why do cattle die from bloat?" remain unanswered.

The objectives in the research reported herein were:

1. To measure the effects of the following bloat preventives when cattle were fed alfalfa soilage: (a) penicillin, (b) crude soybean oil, and (c) n-decyl alcohol.
2. To determine the mode of action of these preventives.
3. To study various factors associated with the etiology of "pasture" and feed lot bloat.

4. To determine various physical and chemical changes that take place in rumen ingesta and blood that may provide basic information as to the cause of bloat and cause of death from bloat.

REVIEW OF LITERATURE

During the last four years research in the field of bloat and associated areas has been extremely active. Extensive reviews have been prepared by Blake (23), Cole et al. (46), Dougherty (72), Johns (127, 134), Parham (198), and Pfizer (201). Various aspects of the bloat problem have also been discussed by Johns (133), Johns et al. (136) and Williams (261). The present review is intended to summarize briefly the conclusions reached in the review articles and to discuss in more detail the literature since 1955. A concurrent review has been prepared by Johnson (138) which covers various aspects of bloat etiology, prophylaxis and therapy which will not be considered in detail in this review.

Bloat Theories

The large number of theories as to the cause of bloat can be considered in a few general groups. The excessive gas production theory has generally been disproved. Although certain feedstuffs may produce more gas than others, the volume of gas produced is never more than the animal could easily eliminate by eructation if no other complicating factors were present. Parham (198) stated that the rate of gas production reaches a maximum within 30 minutes after feeding alfalfa either as hay or as green forage. A difference in the composition of rumen gases from bloat-producing and non-bloat-producing diets is not readily apparent. Although it is evident that there is an accumulation of gas during bloat, it is still not clear why this accumulation takes place.

Other theories contend that bloat is due primarily to paralysis of the rumen musculature. Cyanide has been suggested as one toxic factor which may be involved; however, the cyanide content of many plants, such as birdsfoot trefoil which usually does not produce bloat, is many times higher than that of the legumes that often do produce bloat. Likewise, it now appears that flavones, histamine, carbon monoxide, hydrogen sulfide, anticholinesterase and an increase in pH of the rumen ingesta probably are not the primary causes of bloat even though each has been incriminated from time to time.

Many theories have been proposed which involve the physical nature of the feed. It has been suggested that roughage is necessary in the diet to stimulate eructation and salivary flow. Feeding of roughage before grazing has reduced the amount of bloat in some cases (Colvin et al. 49); however, bloat occasionally occurs on quite mature alfalfa pasture or even on hay, either of which should stimulate salivary flow and eructation. Density of the ingested green feed has been incriminated in various ways. One theory states that the ingested green feed is more dense than the mass of the rumen contents, thus it will pile up near the cardia to block eructation. Another theory states that the boluses of green feed will sink to the bottom of the rumen where the main fermentation occurs producing gas and froth to cause the level of liquid to rise above the cardia and to interfere with eructation. However, the latter seems unlikely since Johns (128) reports that the major portion of fermentation in the rumen of cattle grazing clover pasture occurs in the posterior ventral sac. Furthermore, the normal level of ingesta in the rumen is somewhat above the level of the cardia.

Foaming and surface tension theories have been discussed in detail by Johns (127), Johnson (138), and Parham (198).

Lienert (151) and Lienert and Kienel (152, 153, 154) have proposed that the high phosphatase content of rumen ingesta and the rich phosphate content of bloat-producing plants are responsible for an increased rate of gas production in bloating animals. On the other hand, Cooper (56, 57, 58), Cooper and Hall (59, 60), Cooper et al. (61), and Cooper and Woodle (62) have proposed that bloat is more likely to occur when cattle are grazing alfalfa on soil where the level of phosphorus is low in relation to the level of nitrogen. Cooper et al. (61) propose balanced fertilization as a means of controlling bloat.

Another group of theories concerns the rumen microorganisms. Very rapid changes occur in the population of organisms when sudden changes are made in the diet. Jacobson et al. (125) have demonstrated a large increase in encapsulated organisms associated with feed lot bloat. Hungate et al. (119) found that the rate of fermentation per gram of ingesta taken from bloated animals is higher than that from non-bloated animals grazing on the same pasture; however, the proportion of end products is the same.

Cause of Bloat

Most researchers agree that faulty eructation is an important factor in bloat; however, there is little agreement as to the cause of faulty eructation. During the last few years many contributions have been made to the knowledge of the mechanism involved. Some of these are the cinefluorographic studies by Dougherty and Habel (75), the study by Colvin et al. (49) measuring the volume of gas eliminated through eructation, studies of the innervation of the ruminant stomach by Dziuk and

Sellers (79, 80) and the esophageal innervation and eructation reflex in sheep as studied with decerebrated sheep by Dougherty et al. (76).

Johns (134) has made the following observations which lend both clarification and confusion to the reasons for the occurrence of bloat. The occurrence of bloat is not related to the amount of forage eaten, therefore, it is not necessarily the greedy eaters that are most susceptible. Bloat reduces dry matter intake; therefore, severe bloat is not the result of a larger amount of fermentable material. Although most cases of bloat occur on succulent forage, bloat has been observed on legumes in all stages of growth, including dry hay; this proves that succulence is not essential for the production of bloat. Neither belching nor the muscular activity of the fore-stomach appears to be inhibited during the early stages of bloat; in fact, Hancock (102) found an increase. Based on the similarity in behavior of identical twin cows it appears that the tendency to bloat is an inherited characteristic. All effective preventive and treatment measures involve the administration of anti-foaming agents. Furthermore, Johns feels that the primary cause of pasture bloat is foaming of the rumen contents. Dougherty and Habel (75) describe a cranial esophageal sphincter which opens readily to a gas pressure stimulus but which remains closed under considerable pressure of froth or liquid.

Johns also found that bloat will increase eight to nine days after heavy rains; bloat occurs mainly in the spring but generally does not seem to be associated with a particular type of weather. Wilting of legumes reduces the amount of bloat. In New Zealand, prefeeding of hay or concentrate did not decrease the amount of bloat; however, California work by Colvin et al. (50) shows a considerable reduction in bloat when certain grass hays are fed prior to grazing. Johns also found that neither adrenaline nor antihistamine is effective in alleviating bloat.

Nichols (187) proposes that bloat depends upon the rate of froth formation which in turn depends upon available gas-producing organisms and upon an adequate supply of readily convertible media. As the froth forms, the liquid phase is incorporated into the froth making the ingesta more viscous which, in turn, makes it more difficult for the gas to be released.

Barrentine et al. (15) compared the bloat producing ability of various legumes. Ladino and Persian clover and alfalfa are about equal in bloating potential, red clover and white dutch clover produce somewhat less bloat but very little bloat is produced on crimson clover. Troughton (254) reported some differences among three strains of white clover in respect to their bloat producing potential.

Of the current theories regarding bloat, the foaming or surface tension theory is probably most widely accepted. Foam in the rumen during bloat is thought to be caused by both plant and animal factors. Saponins are the plant factors most frequently incriminated; saponins are foaming agents and also may be toxic. The work on saponins has been extensively reviewed by Lindahl et al. (156). More recently, Wilson et al. (263) and Colvin et al. (51) reported that saponins have an adverse effect on rumen motility in vitro and in vivo. Doizaki et al. (70) found that intravenous administration of saponins causes an appreciable rise in the acetylcholinesterase activity of erythrocytes of blood taken from bloated animals. Reber (207) found intraruminal administration of cactus saponins decreases feed consumption, distends the rumen and, if the level is high enough, causes death. Gutierrez et al. (98) found that alfalfa saponins are utilized by certain rumen bacteria with resultant production of acids, gas and large amounts of slime. Coulson (63) and Van Atta and Guggolz (257) describe and discuss analytical procedures for saponins and their constituents. In many cases, work with saponins has been delayed because of lack of a good procedure that is rapid enough to permit analysis of a considerable number of samples.

Virtanen (258) tested the foam potential of red clover and found that the amount of foam and stability of foam increases in proportion to the amount of bloat experienced. This is further evidence of the importance of constituents of alfalfa which may be responsible for foam.

Probably the most important animal factor involved in foam formation is saliva. It has not been established what effect a copious flow of saliva will have on the foaming characteristics of rumen ingesta. Somers (241) found that much more saliva is produced on dry feed than on fresh material. Somers listed the functions of saliva as follows: (a) to transport ingesta, (b) to provide fluid environment for microorganisms, (c) to dilute fermentation products, (d) to stimulate bacterial activity, (e) to serve as bicarbonate and phosphate buffers, (f) to lower surface tension and (g) to recycle nitrogen. Reid and Huffman (216) found saliva to have an average pH of 8.53 and an average surface tension of 47.1 dynes per centimeter; the surface active properties should make it an excellent substance for reducing bloat. However, saliva also contains a large amount of sodium bicarbonate which in turn probably produces a considerable amount of carbon dioxide. Johns et al. (135) reported that for each liter of saliva entering the rumen at pH 5.7, two liters of carbon dioxide are evolved. It takes 21 liters of carbon dioxide to raise the intra-ruminal pressure from 2.5 to 10 mm. Hg, whereas it takes less than 10 liters to raise it from 10 to 40 mm. Hg. Therefore, it is apparent that additional saliva may present a danger to a moderately bloated animal. Phillipson and Reid (205) found that many animals have an increase in salivary flow as pressure in the rumen increases from zero to between 5.0 and 20 mm. Hg. Although the pressure required for stimulating salivary flow varies considerably among animals it was found that if the pressure is increased above this level salivary flow is inhibited. The average pressure at which inhibition occurs varies from 13 to 40 mm. Hg. Clark and Weiss (45) demonstrated an increase in salivary flow due to mechanical stimulation in the area of the cardia.

Hungate et al. (119) found that the rate of fermentation per gram of ingesta is higher in samples taken from bloated animals than in samples from non-bloated animals. They also found that the in vitro production of foam is correlated with bloat severity.

Nichols (188) maintains that if the water level in the rumen is above the cardia it will interfere with eructation and decrease the effective buoyancy of the ingesta.

Cause of Death from Bloat

The exact cause of death from bloat is still not clear. Most animals used in bloat studies are of sufficient monetary value that researchers find it necessary to make every effort possible to save them. Also it is desirable to evaluate various therapeutic measures under conditions of severe bloat. Furthermore, death often occurs so quickly and the workers are so busy at the time that it is impossible to account for all the minute changes that may take place.

Boda *et al.* (28) have described in some detail the sequence of events preceding death of a cow from acute experimental bloat. Johns (127) in his 1956 review summarized the reports of various workers, stating that the pressure in the rumen during acute bloat varies from 60 to 75 mm. Hg. However, in 1958, Johns *et al.* (135) reported maximum pressure in the dorsal rumen to be 45 to 50 mm. Hg. Some animals show marked distress with as little as 35 mm. Hg.

Two groups of theories have been proposed for the cause of death. One maintains that death is due to the absorption of toxic factors from the rumen and the other states that the pressure in the rumen distends the diaphram into the thorax thus mechanically interfering with respiration and circulation. At the present time it appears that death from bloat is probably due to a combination of a great number of factors which may culminate in a reduced oxygen supply.

Experimental Production of Bloat

Over the years the one thing that has probably hampered bloat research the most is the inability of workers to produce bloat at will for experimental purposes. Various techniques have been used to produce bloat for experimental studies; however, in the laboratory it is difficult to simulate bloat under field conditions.

Workers who have studied bloat with animals grazing legumes include Alder and Davies (1), Barrentine (12), Barrentine et al. (13, 14, 15), Blake (23), Blake et al. (24, 25, 26), Jackson et al. (121, 122), Johns (127, 128, 129, 130, 131, 132, 133, 134), Johns et al. (135, 136) and Thomas (251, 252). Probably most of the losses from bloat occur under grazing conditions but in many areas it is impossible to produce bloat consistently in this manner.

Other workers have used soilage techniques to produce bloat experimantally. Included among these are Boda (27), Colvin et al. (48) and Johns (127). Following this procedure, theoretically one can select and cut the forage so that bloat can be produced rather consistently. In fact, the above workers have all reported very good experimental production of bloat when alfalfa tops were selected and fed to cattle. However, considerable care must be used in the selection of forage for soilage because it often is more difficult to produce bloat by soiling than it is by grazing.

Blake (23), Derrickson et al. (69), Ferguson and Terry (87) and Moore et al. (176b, 177) have used forage extracts to produce bloat experimentally. The bloat produced under these conditions may be quite different than bloat observed in the field. Ferguson and Terry (87) used flavones and cyanide in attempts to produce bloat. Blake (23), Moore et al. (176b, 177) and Ferguson and Terry (87) attempted to produce bloat using sugars either alone or in combination with legume or grass extracts. For the most part the use of such chemicals has been unsuccessful in producing bloat. Boda et al. (29) produced bloat by administering large quantities of fresh eggwhite to cows consuming ground dehydrated alfalfa. Shinozaki et al. (236) produced bloat experimentally by obstructing the esophagus of the goat.

Rumen Physiology in Relation to Bloat

Rumen motility

The movements of the rumen probably are necessary for the normal functions of mixing the ingesta, eructating, regurgitating and assisting in the passage of ingesta down the tract. These movements may be influenced by various factors including nature of the diet, blood glycemic levels, bloat and factors present in the rumen ingesta. Colvin et al. (52) reported that the average rate of rumen contractions during fasting is affected by scabrous material in the diet and by bloat. Johns et al. (135) reported averages of 117, 157, and 128 contractions of the reticulum per 100 minutes for resting, feeding and ruminating, respectively, for four non-bloated cows on red clover. Colvin et al. (49) found that both primary and secondary rumen contractions are greater in number when alfalfa tops are fed than when oat hay is fed.

Vallenas (256) reported that rumen motility is inhibited by hyperglycemia and stimulated by hypoglycemia; stimulation can be overcome by intravenous administration of glucose or fructose.

Williams (261, 262) found that an eructation accompanies each intraruminal contraction and that these contractions are absent in frothy bloat. Williams also suggested gas pressure as the primary stimulus for eructation.

Colvin *et al*. (51) found that saponin solutions stop motility almost immediately when administered into the rumen.

Rumen gases

Composition of rumen gases has been discussed from a qualitative standpoint by Blake (23) and from a quantitative standpoint by Cole *et al*. (46) and Parham (198). In general, Cole *et al*. and Parham concluded that under most conditions the average composition of ruminal gases will be approximately 67 per cent carbon dioxide, 26 per cent methane, 7.0 per cent nitrogen and less than 1.0 per cent oxygen.

Carbon dioxide and methane are the natural end products of bacterial fermentation in the rumen. Carbon dioxide can be converted to methane by rumen microorganisms (Cole *et al*. 46). Saliva produces a considerable amount of carbon dioxide due to the bicarbonate content.

In 1958, Conrad *et al*. (55) found that cell wall structures, hemicellulose, and pectic compounds are precursors for rapid ruminal gas formation when incubated *in vitro* with rumen ingesta.

Rumen Metabolism Associated with Bloat

Toxic factors present in rumen ingesta, including hydrogen cyanide, flavones, hydrogen sulfide and histamine, have been cited as possible causes of bloat or causes of death from bloat. This area has been reviewed by Blake (23), Cole *et al*. (46), Johns (127), and Parham (198).

Other factors that are normal end products of rumen digestion conceivably could be involved in bloat.

Nitrogenous compounds

Lush young legumes have a very high nitrogen content; when these nitrogenous compounds are broken down by bacterial fermentation large quantities of ammonia result. If ammonia accumulates in the peripheral blood, one might raise the question of whether animals dying of bloat actually may be subjected to toxic levels of ammonia. Hale (101) reported that 50 per cent or more of a natural protein may be degraded to ammonia or bacterial protein. Head and Rook (106, 107) found average rumen ammonia nitrogen for the eight hours after hay and grain feeding to be 13.2 mg. per cent whereas, after grazing it was 41.3 mg. per cent. Head and Rook also found that the level of rumen ammonia can be reduced by feeding starch. When rumen ammonia is above about 60 mg. per cent the ammonia content of peripheral blood increases in proportion.

There is some question about the normal level of rumen ammonia. The amount of readily available nitrogen in the diet determines the level of ammonia nitrogen in the rumen. Stallcup and Looper (244) fed rations of cottonseed hulls and various protein sources and never found a rumen ammonia nitrogen content above 24 mg. per cent. Christian and Williams (44) feeding dried grass found a maximum rumen ammonia nitrogen level of 33 mg. per cent at about one and one-half hours after feeding. Head and Rook (106, 107) found rumen ammonia nitrogen levels of 10 to 20 mg. per cent under winter feeding conditions and 40 to 60 mg. per cent when fed fresh grass.

Volatile fatty acids

Volatile fatty acids are formed in large amounts as a result of bacterial fermentation of cellulose. The proportions of the various acids are known to vary from one diet to another. However, at the present time volatile fatty acids have not been associated with bloat.

Eructation as Related to Bloat

It appears that a major portion of the difficulty during bloat is the inability of the animal to eructate the gas produced during normal fermentation of fresh legumes. In recent years, several researchers have been working to elucidate the many problems in this area. Habel (100) has reviewed the innervation of the ruminant stomach, the normal motility, eructation, regurgitation, results of stimulation, results of vagotomy, sympathetic stimulation, effects of autonomic drugs and histology of the nerves to the ruminant stomach.

Dougherty and Habel (75) found histamine inhibits eructation and reported the presence of a cranial esophageal sphincter which remains closed to inhibit regurgitation when the rumen is completely full of water and ingesta at a pressure of 40 mm. Hg. They also found that eructation is composed of the following motor events: (a) two contractions of the reticulum, (b) contraction and raising of the ruminoreticular fold, (c) general ruminal tonus or contraction forcing gas forward and downward into the relaxed, relatively empty reticulum, (d) increased contraction and dilation of the esophagus, (e) relaxation of the two caudal esophageal sphincters, (f) gaseous distention of the esophagus, (g) continued closure of the cranial esophageal sphincter retaining the gas in the esophagus until the cardial and diaphragmatic sphincters close and (h) closure of the glottis causing a transient rise in intrapleural pressure which aids the esophageal musculature in rapidly clearing the esophagus of gas through the relaxed cranial esophageal sphincter.

Dougherty et al. (76) studied the motor nerve supply to the esophagus and found receptors around the cardia that are capable of initiating reflexes that stimulate and inhibit eructation. Eructation is stimulated by a variety of gases and inhibited by water, mineral oil, or fluid ingesta. The eructation reflex can be stimulated by gas pressure when the eructation inhibitory reflex is blocked suggesting that the stimulatory receptors are deeper than the inhibitory receptors.

Dziuk and Sellers (79, 80) found the spontaneous motility of the rumen of roughage-fed calves is more regular and contractions stronger than in milk-fed calves. Response of the rumens of roughage-fed calves to vagal stimulation is more marked than that of milk-fed calves. Moderate electrical stimulation of the dorsal vagus is generally associated with increased rates of eructation and ruminal contraction. Stronger stimuli are generally associated with spasmodic contractions of the rumen, reticulum, and cardia. Stimulation of the dorsal vagus still causes an increase in the eructation rate even though the hindquarters are raised and the cardia is covered with six to eight inches of water. Stevens and Sellers (246) applied procaine hydrochloride to the dorsal vagal trunk; this impairs eructation efficiency. They concluded that the dorsal vagus apparently serves as an afferent pathway in an eructation reflex.

Shinozaki and Sugawara (232) described two types of rumen movements and reported the effects of time after feeding, rumination, air insufflation and autonomic drugs upon the two types of movements.

Titchen (253) found that contractions of the reticulum are inhibited by atropine or by severing the dorsal abdominal vagus. The contractions are stimulated by stretching the reticulum, by distending a balloon in the omasal canal, by reducing the pH of the abomasum to 0.9 - 1.0, and by touching the lower part of the thoracic esophagus or the abomasal mucosal surface. Dracy (78) used vagal inhibitors to cause gas accumulation but was unable to produce bloat by using atropine in cattle fed alfalfa hay.

Colvin et al. (49) found that cattle grazing alfalfa tops have a decreased frequency of eructation, and a smaller volume of gas per eructation, but the frequency of rumen contractions is higher. They also reported a correlation of 0.96 between the ratio of secondary to primary contractions and intraruminal pressure.

Rumen Microbiology as Related to Bloat

Although a great deal of work has been done on microbial digestion in the ruminant, relatively little is known of the changes that take place during bloat. Recently several investigators have examined the possibility that changes in or characteristics of the microflora may be involved in the etiology of bloat. The volume of gas being produced by the microbial fermentation may be of great importance when it becomes entrapped in the ingesta.

Slime production

In 1955, Hungate et al. (119) suggested that the production of slime by rumen bacteria may be associated with frothy bloat. Since then, Jacobson (123) and Jacobson et al. (125, 126) have found a high correlation between encapsulation of organisms and degree of bloat. Bryant et al. (40) characterized approximately 50 strains of rumen bacteria from bloating and non-bloating cattle; non-bloaters have lower anaerobic counts, but otherwise there is little difference. They concluded that the flora of the rumen of bloated and non-bloated cattle are not grossly different.

Gutierrez et al. (98) isolated rumen bacteria capable of degrading alfalfa saponins and producing slime. According to Hungate et al. (119), the rate of fermentation per gram of ingesta is higher in bloated than in non-bloated animals. Hungate (116) also found that the molar ratio of carbon dioxide to other metabolic end-products is higher when cattle are grazing ladino clover than when they are on hay rations.

Gutierrez et al. (99) found an increase in Streptococcus bovis and an LC type organism during the onset of feed lot bloat. Dain (64) also reported S. bovis to be the predominant organism in bloat and overfeeding sickness.

Dain et al. (65) working with experimentally overfed sheep found increasing levels of histamine in the ingesta when the pH is below 5.0.

Methanogenic bacteria in the rumen

In 1955, Carroll and Hungate (43) reported that certain rumen bacteria are capable of quantitatively converting formate to methane and carbon dioxide. Later, Smith and Hungate (239) reported numbers of methanogenic bacteria as great as 2 X 10^8 per ml. The predominating organism is a nonmotile, nonsporeforming, gram-positive, encapsulated rod with rounded ends which can utilize hydrogen and formic acid.

Opperman et al. (195) obtained stabilized cultures from rumen fluid which produced methane from formic and acetic acids. Acetate-fermenting cultures are inhibited by carbon dioxide, dyes and penicillin, but formate cultures are unaffected.

Factors affecting rumen bacteria

Allison et al. (3) reported that strains of three species of bacteria, which are among the most numerous and most active cellulolytic bacteria in the bovine rumen require or are greatly stimulated by volatile fatty acids. Horn et al. (113) found that aureomycin inhibits the rumen bacteria to such an extent that cellulose digestion is decreased, whereas penicillin has no effect on cellulose digestion. The work of Maki and Foster (169) revealed that cows fed concentrates have two to three times higher total rumen bacterial counts than cows fed roughage. About one-third of the organisms from the roughage group produce butyric acid while those from the concentrate group ferment glucose to acetic, lactic and succinic acids. Barrentine et al. (13) stated that the effect of penicillin as a bloat preventive is probably due to slight alterations in the rumen flora. It was pointed out, however, that it is necessary to feed several times the amount of penicillin used for controlling bloat in order to produce unfavorable results in terms of decreased digestion of cellulose.

It is extremely difficult to determine the normal population of ruminal flora which is composed of bacteria, ciliate protozoa and yeasts. Morphological studies by Munch-Petersen (178) have demonstrated 33 bacterial organisms: ten rod forms; seven coccoid; four spiral; two vibrios; two sarcina; two Selenomonas spp.; one each of: diplococcal, rosette, fusiform, crescentic, an oval organism, and Oscollaspira guilliermondii. No doubt the flora are in a continuous state of flux and may change very rapidly with small changes in diet. Bryant et al. (41) have studied the composition of the ruminal flora and fauna of young calves. Hungate (117) studied the microorganisms in the rumen of cattle fed constant rations. Numerous other studies have been conducted with various organisms that have been isolated from the rumen.

Oxford (197) found Epidinium ecaudatum to be the predominant oligotrich in the rumens of two cows fed red clover soilage. Bailey (8) isolated a cell free extract from this organism containing an α- amylase with optimum activity in the pH range 5.3 - 6.5 and in the temperature range 37 - 45° C.

Various aspects of ruminant microbial digestion have been discussed by Munch-Petersen (178), Moir (175) and Mansson and Andersson (172). Conditions within the rumen that make it ideal for rapid and continuous activity of microorganisms include uniform temperature, high moisture, controlled pH and constant removal of end products. Moir (175) reported direct counts of ruminal bacteria of 20 to 100 X 10^9 per milliliter, ciliate counts of 3 X 10^6 per milliliter and flagellate counts of 10^8 per milliliter.

All 22 strains of the Genus Bacillus and 20 coli-form microbic species examined by Mansson and Andersson (172) have the capacity to reduce nitrite in vitro.

Relationship of Bloat and Rumen Fluid Characteristics

Physical characteristics

Surface tension is probably the most universally used and accepted physical measurement of rumen ingesta in bloat research. Nichols (188) and Nichols et al. (190, 191, 194) have studied the effects of various agents on the surface tension of rumen ingesta. These workers found that the feeding of fresh alfalfa or changing from alfalfa hay to fresh ladino increases the surface tension of rumen ingesta. On the other hand feeding alfalfa hay increases surface tension while feeding grass has no effect. Blake (23) found that the surface tension of rumen ingesta collected orally via stomach tube is not materially different from that collected via rumen fistula. Blake also found that the surface tension of rumen ingesta is reduced by a large variety of surface-active agents.

The viscosity of rumen ingesta also has been studied quite extensively by Nichols (188), Nichols et al. (190, 191, 194). They found that the feeding of fresh alfalfa decreases the viscosity of rumen ingesta. Furthermore, the viscosity before feeding is higher during periods when fresh alfalfa is fed than when hay or grass is fed. These workers also concluded that surface tension and viscosity vary independently.

Nichols et al. (192) found that the effective buoyancy of rumen ingesta is reduced following the intake of fresh legumes.

Cellulose digestion time and sediment activity time have been proposed by Nichols and Penn (193) to measure the activity of gas producers and cellulose digesting organisms.

Ammerman and Thomas (5) reported a lower pH of rumen ingesta from animals fed fresh forages than from animals fed hay and grain. They also found that neither alfalfa nor ladino clover cause poorly buffered systems in the rumen.

Blake (23) found the following characteristics of rumen ingesta have increased values during bloat: foaminess, foam half-life, ingesta-volume-increase, surface tension and viscosity.

Broberg (31, 32, 33, 34) measured redox potentials of rumen ingesta and generally found them to be quite stable, but he found considerable variation in the case of bloated animals. On this basis, Broberg concluded that bloat is of variable etiology.

Chemical characteristics

Shinozaki and Sugawara (233) found that red clover and ladino clover produce more gas and volatile fatty acids than trefoil or orchard grass. During the fermentation of red clover and ladino clover there is an increase in non-protein nitrogen and ammonia; the authors felt this suggests the possible importance of soluble proteins in the etiology of bloat.

Dried grass produces more gas than fresh grass, but dried legumes produce less gas than fresh legumes (Shinozaki *et al*. 234).

Shinozaki *et al*. (235) found increased levels of volatile fatty acids, histamine, protein and non-protein nitrogen in rumen ingesta when animals are suddenly changed from a hay diet to ladino pasture.

Dain et al. (65) identified histamine and tyramine from the rumen ingesta of experimentally over-fed sheep. Histamine levels of greater than 70 micrograms per milliliter of ingesta were obtained; illness is directly correlated with the level of histamine in the ingesta. As the acidity becomes lower than pH 5.0, histamine formation increases. Shinozaki (231) concluded that histamine probably is not associated with bloat.

Johns (131) reported maximum rumen ammonia levels vary between 35 and 130 mg. per cent ammonia nitrogen for sheep grazing pasture throughout the year. He noted no marked change in total volatile fatty acids or in acid proportions with changing pasture composition. The level of volatile fatty acids is generally greater on pasture than on hay; the proportion of acetic acid is lower on pasture, while butyric is higher. Rumen ammonia levels do not necessarily follow the pasture nitrogen levels.

Conrad and Hibbs (54) fed alfalfa hay and found the ammonia nitrogen level in the rumen to increase sharply within one-half hour, reach a maximum in one and one-half hours and return to normal within four hours. Urine nitrogen showed two peaks -- one at one and one-half hours and one between three and nine hours.

Looper and Stallcup (161) found that rumen microorganisms can release significant amounts of ammonia from urea and uric acid but not from glycine.

In work with feed lot bloat, Phelps et al. (203) adjusted centrifuged rumen juice to pH 9 with NH_4OH; the precipitate contained $MgNH_4PO_4$. Frothy ingesta has five times more $MgNH_4PO_4$ than non-frothy ingesta.

Methods of Feeding Fresh Legumes

The practice of soiling has been used to some extent for many years; however, only recently with the advent of systems of completely mechanized handling of fresh-chopped forage has such a practice become feasible on many farms. Soiling has been recommended as a method for controlling bloat; it seems reasonable that soilage should reduce the amount of bloat because animals are forced to eat the entire plant rather than selecting the lush tops as they do while grazing. Several researchers, as pointed out in a previous section of this review, have used soilage as a method of producing bloat.

When animals are allowed to graze alfalfa pasture they are rather selective in their grazing behavior. Cattle may select grass and weeds in preference to alfalfa when they start to graze a pasture and tend to graze plant tops rather than consuming the entire plant. Meyer _et al._ (173) found sheep to be much more selective than cattle when grazing alfalfa pasture. Lofgreen _et al._ (158) found that the TDN of pasture is more efficiently utilized than the TDN of soilage although there is very little difference in the digestibility of the dry matter. Lofgreen _et al._ (157) found that cattle spend less time eating when forage is high in TDN.

Results indicate that greater gross returns per acre can be obtained by soilage feeding than by various systems of grazing (Colorado Agricultural Experiment Station, 47; Henderson _et al._, 111; and Larson, 147). Hull _et al._ (115), and Lofgreen _et al._ (158) found cattle on soilage consume more dry matter than cattle on pasture, but found no difference in weight gains. Foreman et al. (89) found cows fed soilage consume more total dry matter and maintain a higher level of milk production than do those fed silage.

Hancock (102) in his study of bloat in relation to grazing behavior found: (a) break grazing does not control bloat, (b) strictly enforced off and on grazing can control bloat, (c) long feed is safer than short feed, (d) cattle on potentially dangerous pasture are more reluctant to graze, (e) bloated animals have reduced rumination time, (f) identical twins have similar bloating behavior indicating that bloat is inherited, and (g) in early stages of bloat, ruminal motility and belching increase in intensity and frequency.

Balch and Line (9) found that the apparent large losses in body weight that occur when cows are changed suddenly to pasture from dry feed are due to a decrease in contents of the digestive tract.

The Effect of Bloat upon Blood Composition

A relatively limited amount of work has been done on changes in blood composition during bloat. Jackson et al. (121, 122) found a considerable increase in plasma cholesterol with bloat produced on alfalfa pasture, while no changes were noted in plasma ascorbic acid and red-cell cholinesterase.

Shinozaki et al. (236) produced bloat by obstructing the esophagus of goats and noted the following changes in the composition of the blood: (a) an increase in the number of neutrophil leucocytes and a relative decrease in lymphocytes, (b) a gradual decrease in eosinophiles and (c) an increase in blood sugar and lactic acid. They found no significant changes in blood ketones or non-protein nitrogen.

Bartlett et al. (17) found no changes in blood pH, serum calcium, sodium, potassium and blood glucose when cattle were changed suddenly from dry feed to pasture. However, they did find a considerable increase in blood serum non-protein nitrogen, urea and ammonia.

In 1951, Bodansky (30) presented an extensive review on methemoglobin and methemoglobin-producing compounds. In normal dogs death results if about 80 percent of the total blood pigment is converted to methemoglobin. Pfander et al. (200) stated that in cases of nitrate poisoning more than 70 per cent of the hemoglobin may be in the form of methemoglobin. Respiration is impaired under conditions present in the critical stages of bloat. Perhaps, if methemoglobin were a factor, the fatal level could be much lower. If the forage is high in nitrate and nitrite, methemoglobin could tend to accumulate in the blood during the day and explain why more bloat is experienced in the afternoon than in the morning.

Lewis et al. (150) found that changes in rumen ammonia are paralleled by changes in portal blood ammonia. At rumen ammonia concentrations of 60 millimoles per liter (84 mg. per cent ammonia nitrogen), no increase in peripheral blood ammonia is noted, however, at levels above this there is an increase in the level of ammonia. These workers and Repp et al. (217) reported that if the level of ammonia in peripheral blood is about 10 micrograms per milliliter, toxic symptoms will be noted.

Feed Lot Bloat

Feed lot bloat is a problem in areas where cattle are finished for market on typical fattening rations consisting of limited amounts of roughage and a liberal feeding of concentrate. Smith *et al*. (238), the first to study this problem experimentally, found that the most severe cases usually occur before feeding in the morning. These workers found methyl silicone and lipase treated cream effective in reducing foam. The appearance of iodophilic streptococci is concurrent with frothing.

Jacobson (123) and Jacobson *et al*. (124, 125, 126) in studying the frothy bloat problem reached the following conclusions:

1. In the case of uncomplicated frothy bloat, sufficient froth is present in the area of the cardiac orifice to block the eructation mechanism.
2. Factors contributing to the stable froth include sufficient adjustment of the rumen microflora to the diet, a high degree of encapsulation of the organisms, high concentrate rations with readily available energy, and the presence of any one or any combination of saponin, glucose and valeric acid.
3. A sufficient amount of stable froth appears to be produced in the rumen to cause bloat when the right combination of a sufficient number of factors is present.
4. The physical nature and volatile fatty acid composition of the rumen contents vary with changes in diet and are constantly changing for 74 days on the bloat producing diet.
5. In terms of the *in vitro* dissimilation of glucose and cellobiose,

the metabolic activity of the rumen bacteria varies with diet and in the case of the bloat producing diet is constantly changing for 74 days following the changeover to this diet.

6. Rumen microorganisms are intimately associated with and contribute to the cause of frothy bloat.
7. The difference in animal susceptibility to bloat is not correlated with eating habits.
8. There is an increase in bloat incidence with the length of time on the diet; this seems to be caused by an additional factor other than lack of roughage and is apparently not rumen atony.

Nichols (186) proposes a method for controlling feed lot bloat which involves increasing the amount of roughage -- possibly mixing chopped hay and concentrate to insure a total intake of one-third roughage.

EXPERIMENTAL

The research reported herein was divided into five experiments. Experiment I was designed to study the effect of procaine penicillin upon the incidence and severity of bloat of cattle fed alfalfa soilage. Experiment II was designed to study the effect of crude soybean oil and n-decyl alcohol upon bloat under conditions similar to those of Experiment I. In Experiment III, various high-concentrate, low-roughage feeding systems were used in attempts to produce and study frothy feed lot bloat. Experiment IV was designed to study the effect of bloat and of grazing upon various blood components and rumen ingesta of cattle grazing alfalfa pasture. Experiment V is composed of three trials related to other phases of the bloat problem.

Experiment I -- Soilage Trials, 1956

Experimental procedure

In 1956, fifteen dairy animals (average initial weight, 520 pounds) from the Iowa State University dairy herd were employed to study the value of oral administration of penicillin as a bloat preventive. In this experiment the animals were confined to a single dry lot and fed fresh alfalfa soilage *ad libitum*. The soilage was chopped fresh each morning with a direct-cut field chopper, hauled to the dairy farm, weighed and fed to the animals at about 8 A.M. The soilage was allowed to remain in the bunk for the entire day and any excess was weighed back prior to the next daily feeding.

The first three days of the soilage feeding program served as a preliminary period during which the animals were observed for susceptibility to bloat. At the end of this period the animals were ranked on the basis of the total three-day bloat score and divided into two groups which appeared to be approximately equal in bloating potential.

Animals used in this phase of the experiment, the total bloat severity score for the preliminary period and the division of the animals into the experimental groups are presented in Table 1.

Table 1. Allocation of animals for Experiment I

Group I			Group II		
Animal	B. S.[a]	Rank	Animal	B. S.	Rank
4143	0	15	4150	1	14
4146	1	13	4152	13	4
4185	7	9	4165	5	10
4187	8	6	4193	8	7
4209	4	11	4194	3	12
4220	14	3	4205	17	2
4224	20	1	4107	8	8
4227	10	5			

[a]B. S. = total bloat severity score for the three-day preliminary period. Bloat severity scores were assigned approximately seven times each day according to the 0 - 5 scale described in Table 3.

The experimental design was of the switchback type. The animals received penicillin or served as controls for three periods of approximately 10 days each and a final period of 24 days. The animals were allowed a recovery period of at least seven days between each period.

It was hoped that the microflora of the rumen would recover from the effects of penicillin during this time. The design is outlined in Table 2.

Table 2. Design of Experiment I

	Experimental periods (inclusive dates)			
	1	2	3	4
	May 22 - June 1	June 9 - 18	July 6 - 16	Sept. 1 - 24
Group I	Penicillin	Control	Penicillin	Control
Group II	Control	Penicillin	Control	Penicillin[a]

[a]This group had been receiving 75 mg. penicillin per head per day since August 7.

A period of drought occurred between periods 3 and 4 which limited the amount and quality of alfalfa available for feed as soilage. During this time the animals were fed a high level of concentrate and a limited amount of alfalfa soilage. This trial was a part of Experiment III which will be described later in more detail. After the late summer rains produced an abundance of lush forage, the animals were again switched to an ad libitum soilage feeding program; this was the beginning of period 4.

The penicillin used in this experiment was "Pro-pen", a 50 per cent antibiotic feed supplement (mixture of 50 per cent procaine penicillin and 50 per cent ground oyster shell meal) manufactured by Merck & Co., Inc., Rahway, New Jersey. The penicillin was prepared for use by mixing one part of the penicillin supplement with 19 parts of glucose (15 grams of supplement and 285 grams of glucose). This material was thoroughly mixed and put into number 12 gelatin capsules so that each capsule contained about 3 grams of the mixture or approximately 75 milligrams of procaine penicillin. A procaine penicillin assay, Grove and Randall (97), pp. 15-31, indicated the presence of an average of 76 (standard error = 1 mg.) of procaine penicillin per capsule. Prior to the daily feeding of alfalfa each animal in the treated group was given one of the capsules with a balling gun.

Following the feeding of alfalfa at about 8 A.M. the animals were observed for bloat at least once each hour. An hourly rating based on the 0 to 5 bloat scale described in Table 3 was recorded for each animal throughout the remainder of the daylight hours. If at any time during the day enough bloat were present to be dangerous to the animals, they were observed continuously and the highest degree of bloat attained during an hour was recorded at the end of the respective hour. When an animal became quite distressed from bloat an attempt was made to relieve it with a stomach tube. If this method failed, approximately 100 ml. of lard oil was injected into the rumen at the left paralumbar fossa.

Six to eight samples of rumen ingesta were collected per day approximately five days each week. An attempt was made to take samples from all animals an equal number of times during a given treatment period. Samples were taken at the time during the day when the amount of bloat was usually maximum. Normally this was approximately two hours following the once daily feeding of fresh alfalfa.

The equipment used for sampling was a hand stomach pump, a piece of

Table 3. Description of scale used in assigning bloat scores

Score	Description
0	No bloat - no distention in left paralumbar fossa
1	Slight - slight distention in left paralumbar fossa; "puffy"
2	Mild - marked distention in left paralumbar fossa; well rounded out between hip and rib on left side; little or no distention on right side.
3	Moderate - well rounded out on left side, drumlike; full on right side; restless
4	Severe - both sides badly distended; left hip nearly hidden; skin tight; defecation; urination; incoordination; protruding anus; mild respiratory distress
5	Terminal - extreme abdominal distention; severe respiratory distress; cyanosis; prostration; death unless treated

plastic garden hose approximately seven to eight feet long and 5/8 inch in diameter, an Emont speculum and a pair of nose tongs for holding the animals. This equipment is more thoroughly described by Blake (23). The stomach tube was inserted into the rumen through the speculum which had been fastened securely in the mouth. The hand pump was then attached to the tube and a sample of the liquid portion of the ingesta was pumped directly into a one pint vacuum bottle. If possible, a one pint sample was collected, however, when an animal was bloated, it often was very difficult to obtain ingesta in this manner and a smaller sample was used. Immediately after the last sample was collected, the samples were taken to the laboratory and examined for the following physical characteristics: surface tension, pH, relative viscosity, foam volume, foam stability, ingesta-volume-increase, specific gravity and per cent solids.

The samples were prepared for analysis by squeezing the ingesta through four layers of cheese cloth to remove course particles. Blake (23) found that this method of purification produced a product that was sufficiently homogeneous to yield fairly reproducible results when measuring surface tension with the DuNouy tensiometer. The feed particles remaining in the liquid portion apparently did not interfere with the measurements. The measurements discussed here, with the exception of ingesta-volume-increase were carried out at room temperature.

After straining through cheese cloth into a large beaker, the ingesta were stirred thoroughly with a glass stirring rod and approximately 35 ml. was poured into a 50 ml. beaker. This aliquot was used first to measure pH and then to measure surface tension. A Beckman model H2 pH meter was used to determine the pH of the sample.

Surface tension was measured with a DuNouy (ring-type) tensiometer. The ingesta were stirred thoroughly, the ring dipped into the ingesta and inserted into the instrument; then six readings were taken as rapidly as possible. Usually the last five readings were relatively close, thus, the first one which was somewhat different, either higher or lower, was discarded. A mean value was computed from the last five and this mean was used in analyzing the data. Between samples, the ring was removed from the tensiometer, blotted on filter paper, rinsed in the next sample and reinserted into the instrument. After use, the ring was cleaned by rinsing first in distilled water, then in methyl ethyl ketone and then by heating thoroughly in the flame of a bunsen burner.

Ingesta-volume-increase, or fermentation capacity, was measured by placing 20 ml. of ingesta (liquid + incorporated foam) into a 50 ml. graduated cylinder. This was incubated at 39° C. until maximum volume was reached. This was recorded as ingesta-volume-increase =

$$\frac{\text{maximum volume}}{\text{original volume}} \times 100.$$

Specific gravity was measured by pouring approximately 50 ml. of ingesta into a 100 ml. graduated cylinder and reading directly with a hydrometer. Free foam which frequently formed on top of the liquid ingesta caused considerable difficulty in reading the hydrometer. Perhaps this could have been overcome by the addition of a small amount of de-foaming agent such as _n_-decyl alcohol.

Foam volume and stability were measured as follows: 50 ml. of liquid ingesta (not including the distinct layer of foam) was measured into a 100 ml. graduated cylinder. This in turn was mixed in a Waring Blendor for two minutes and immediately poured back into the graduated cylinder. The upper meniscus of the foam layer was marked; this reading minus the original 50 ml. was considered as foam volume. After standing one hour at room temperature the volume of foam was again noted. Foam stability then was computed: foam stability = $\frac{\text{final foam volume}}{\text{original foam volume}} \times 100.$

Relative viscosity was measured by determining the length of time necessary for the top of a column of liquid sample to fall from 0 to 15 ml. in a 25 ml. serological pipette held in a stationary vertical position. The time required for a similar drop in a column of distilled water in the same pipette was used as a standard and the relative viscosity was computed: $\text{relative viscosity} = \frac{\text{time for sample}}{\text{time for water}}$.

Per cent solids was determined by pouring approximately 50 ml. of ingesta into a 50 ml. graduated centrifuge tube, centrifuging for 30 minutes at approximately 3000 r.p.m. in an International number 2 centrifuge and then expressing the volume of sediment as a per cent of the total volume.

Samples of the forage were taken at the time of feeding at weekly intervals and analyzed for total nitrogen, non-protein nitrogen, two nitrate fractions, saponin, reducing sugar and invert sugar. Sampling and analyses were done by Professor Lester Yoder and associates of the Iowa State University Chemistry Department.

Results

A summary of the effect of penicillin treatment upon bloat and average daily weight gains by periods is presented in Table 4. These data were analyzed statistically first as a paired comparison; the effect of pairing was significant only in the first period. The data were analyzed further, assuming a completely randomized design using both average daily maximum bloat and average daily bloat severity. The same levels of significance were obtained regardless of whether the average daily maximum bloat or the average daily bloat severity was used. The results are summarized in Table 4.

The average bloat severity was less for animals that received

Table 4. Effects of the penicillin treatment on bloat and weight changes

	Experimental period							
	1		2		3		4	
	T[a]	C[a]	T	C	T	C	T	C
Bloat severity[b]	0.24[c]	0.50	0.17	0.20	0.04	0.14	0.50[d]	0.26
Bloat incidence[e]	19	36	13	11	3	11	34	20
Average maximum[f]	0.73	1.35	0.51	0.54	0.18	0.38	1.62	0.97
Average daily gain (lb.)	5.17	3.87	-0.92	0.42	2.38	2.66	1.61	1.14

[a]T = penicillin treatment of 75 mg. per animal per day, C = control.

[b]Bloat severity based on an hourly rating from 0 (no bloat) to 5 (severe bloat). Bloat severity = $\frac{\text{sum of hourly ratings}}{\text{number of hourly observations}}$.

[c]According to an analysis of variance using a paired comparison this difference was significant at $P < 0.05$.

[d]According to an analysis of variance assuming a completely randomized design, this difference was significant at $P < 0.005$.

[e]Bloat incidence = $\frac{\text{times bloated}}{\text{number of observations}} \times 100$.

[f]Average maximum = average daily maximum bloat = $\frac{\text{sum of maximum bloat values for each animal each day}}{\text{number of animal days}}$.

penicillin than for the controls in each of the first three periods, but only in period 1 was the difference statistically significant at $P < 0.05$. However, during period 4 bloat severity, average daily maximum bloat and bloat incidence all were considerably higher in the treated group. The difference between treated and control groups was significant at $P < 0.005$. Animals with bloat typical of that occurring during this phase of the experiment are shown in Figure 1.

Penicillin had very little, if any, effect upon weight gains; it appeared to increase weight gains in periods 1 and 4 and to reduce them in periods 2 and 3. There were no statistically significant differences in weight gains (at $P = 0.05$) in any of the four periods.

Daily forage consumption, forage "quality", average daily maximum bloat scores, bloat severity and bloat incidence are summarized in Table 5.

It is evident that the amount of bloat was highest in period 4 and lowest in period 3. Incidence of bloat was quite high during period 1 but was low during period 2 and very low during period 3. The weight gains were good except during periods when the forage was very high in moisture or when a large amount of bloat occurred. The visual subjective measurement of forage quality was not closely associated with either forage consumption or bloat.

During the early part of the summer there was little relationship between forage consumption and bloat, however, during period 4 this relationship was quite high ($r = 0.66$). A summary of the analyses of rumen ingesta is presented in Table 6. These data have been analyzed by multiple regression and levels of significance determined using Students' t test. The regression of treatment, bloat at time of sampling, daily maximum bloat, period, group and treatment-period interaction on each of the measurements made on the rumen ingesta was computed. Periods 1 and 2 were compared to periods 3 and 4 since each pair constituted a

Figure 1. Animals bloated on alfalfa soilage during period 4 of experiment I; control (left), penicillin-treated (right).

Table 5. Summary of animal responses in Experiment I (data include both treated and non-treated groups)

Date	Forage: Quality[a]	Forage: Consumption[b] (lb.)	Bloat: Max.[c]	Bloat: Severity[d]	Bloat: Incidence[g]	Weight[e] (lb.)	r[f]
			Period 1				
5-22	Excellent	119	1.27	0.55	47	517	-0.31
5-23	"	124	1.33	0.65	48		
5-24	"	127	1.47	0.70	46		
5-25	"	120	1.20	0.54	42		
5-26	"	143	0.87	0.33	26		
5-27	"	124	1.07	0.39	29		
5-28	Good	117	0.93	0.23	18		
5-29	"	138	0.80	0.18	13		
5-30	"	168	0.93	0.18	13		
5-31	"	136	0.80	0.23	19		
6-1	"	134	0.53	0.20	15	567	

[a]Forage quality based upon visual observation at time of feeding.

[b]Forage consumption per 1000 pounds body weight.

[c]Max. = average maximum bloat per animal as calculated in Table 4.

[d]Bloat severity as calculated in Table 4.

[e]Average body weight on days of weighing.

[f]r = correlation of average daily maximum bloat and feed consumed per 1000 lb. body weight.

[g]Bloat incidence as calculated in Table 4.

Table 5. (Continued)

Date	Forage		Bloat			Weight[e]	r[f]
	Quality[a]	Consumption[b] (lb.)	Max.[c]	Severity[d]	Inci-dence[g]	(lb.)	
			Period 2				
6-9	Fair	152	0.73	0.24	19	576	0.06
6-10	"	128	0.67	0.20	18		
6-11	"	125	0.47	0.12	11		
6-12	"	99	0.20	0.09	9		
6-13	Excellent	157	0.33	0.10	8	593	
6-14	Excellent	102	0.67	0.24	17		
6-15	"	118	0.67	0.17	12		
6-16	"	136	0.47	0.11	8		
6-17	"	131	0.47	0.12	11		
6-18	"	111	0.60	0.18	12	574	
			Period 3				
7-6	Good	101	0.60	0.12	9	594	0.17
7-7	"	78	0.20	0.06	5		
7-8	"	76	0.20	0.07	6		
7-9	"	102	0.20	0.10	7		
7-10	Excellent	96	0.20	0.07	6	610	
7-11	Excellent	123	0.27	0.07	6		
7-12	"	81	0.27	0.07	6		
7-13	"	104	0.20	0.12	9		
7-14	"	90	0.33	0.14	11		
7-15	"	70	0.27	0.08	7		
7-16	"	75	0.27	0.12	9	621	

Table 5. (Continued)

Date	Forage		Bloat				
	Quality[a]	Consumption[b] (lb.)	Max.[c]	Severity[d]	Incidence[g]	Weight[e] (lb.)	r[f]
			Period 4				
9-1	Very good	26	0.33	0.28	16	725	0.66[h]
9-2	"	65	0.60	0.27	17		
9-3	"	89	0.40	0.15	12		
9-4	"	124	0.53	0.17	15		
9-5	"	138	0.67	0.22	17		
9-6	"	127	1.53	0.50	32	700	
9-7	"	126	1.47	0.44	34		
9-8	"	108	1.07	0.42	31		
9-9	Fair	110	1.47	0.49	35		
9-10	Very good	124	1.33	0.57	43		
9-11	Very good	153	1.87	0.54	44	704	
9-12	"	154	1.67	0.51	37		
9-13	"	156	2.07	0.53	37		
9-14	"	141	2.00	0.66	48		
9-15	"	154	1.80	0.83	60		
9-16	"	126	1.93	0.72	52		
9-17	"	171	1.33	0.74	54	702	
9-18	"	136	1.53	0.71	51		
9-19	Poor	117	1.53	0.61	42		
9-20	"	109	1.13	0.36	29		
9-21	Fair	83	0.87	0.25	22		
9-22	Good	112	0.47	0.18	14	764	
9-23	Very good	127	1.07	0.31	21		
9-24	"	113	1.87	0.54	35		

[h] This value of r is statistically significant at $P < 0.01$.

Table 6. Rumen fluid measurements in Experiment I

Comparisons	Rumen fluid measurements: Surface tension (dynes/cm.)	pH	% solids	Foam stability	Foam volume	Viscosity	Specific gravity	Ingesta-volume increase	No. of observations
Effect of penicillin treatment									
Treated	57.4	6.52	9.9	81	37	1.003	1.003	124	96
Control	56.6	6.62	9.6	83	35	0.999	1.002	123	136
P <[a]		0.005		0.050	0.100				
Effect of bloat at time of sampling									
Unbloated	55.9	6.55	8.4	82	38	1.001	1.003	121	178
Bloated	60.3	6.68	14.0	82	30	1.000	0.998	132	54
P <	0.005	0.100	0.050						
Effect of daily maximum bloat score									
Daily maximum bloat score 0	55.5	6.54	7.6	83	39	1.002	1.004	119	130
Daily maximum bloat score 1	57.2	6.58	10.4	82	35	0.995	1.004	125	41
Daily maximum bloat score 2	59.6	6.69	13.6	81	30	1.004	0.997	131	50
Daily maximum bloat score 3	61.7	6.55	14.6	83	27	1.002	0.996	137	11
P <	0.001		0.001		0.005		0.050	0.010	

[a]Level of statistical significance of the effect as determined by multiple regression.

Table 6. (Continued)

	Rumen fluid measurements								
Comparisons	Surface tension (dynes/cm.)	pH	% solids	Foam stability	Foam volume	Viscosity	Specific gravity	Ingesta-volume increase	No. of observations
<u>Effect of period</u>									
Periods 1 and 2	56.9	6.57	8.4	81	34	1.003	1.001	120	100
Periods 3 and 4	57.0	6.58	11.1	84	38	0.998	1.004	128	132
$P <$			0.005	0.001	0.001	0.100	0.100	0.001	
<u>Effect of group</u>									
Group 1	56.0	6.58	8.8	82	37	0.999	1.004	122	128
Group 2	58.2	6.58	10.8	82	33	1.003	1.001	126	104
$P <$	0.025				0.100				
R^2 [b]	0.32	0.07	0.29	0.07	0.19	0.04	0.06	0.18	

[b] Fraction of $\sum y^2$ due to regression.

complete switchback.

Surface tension was unaffacted by treatment, but the regression of surface tension on bloat at time of sampling and upon daily maximum bloat was significant. The difference in surface tension between groups was significant. Penicillin reduced the pH of rumen ingesta, however, the regression of pH upon bloat at time of sampling and upon daily maximum bloat was non-significant. Per cent solids was higher when animals were bloated than when they were not bloated. Solids were also higher in periods 3 and 4 than in periods 1 and 2. Although foam stability was not associated with bloat, it was decreased somewhat by the penicillin treatment and was lower in periods 1 and 2 than in periods 3 and 4. Foam volume tended to decrease with increasing bloat, and was higher in periods 3 and 4. Viscosity and specific gravity were affected very little, if any, by treatment, bloat, period or group. However, ingesta-volume-increase tended to increase with bloat and was higher in periods 3 and 4.

Although treatment had very little effect on most of the characteristics of rumen ingesta that were measured, it did reduce the pH. Bloat at time of sampling increased surface tension and per cent solids and tended to increase pH and ingesta-volume-increase. Daily maximum bloat was associated more closely with the characteristics measured than was bloat at time of sampling. As bloat increased there were corresponding increases in surface tension, per cent solids and ingesta-volume-increase; there was also a decrease in foam volume and specific gravity. Periods 1 and 2 were different than periods 3 and 4 in per cent solids, foam volume and stability and ingesta-volume-increase. With the exception of surface tension there was little difference due to groups in any of the rumen fluid characteristics measured.

The correlation of average daily bloat severity with each of the following forage components was determined: total nitrogen, non-protein nitrogen, protein nitrogen, total saponins, reducing sugar, invert sugar, and reducing and invert sugar. The correlation coefficients are presented in Table 7; none of these was significant at P = 0.05.

Table 7. Correlation coefficients of bloat severity with various components of the forage

	Forage component						
					Sugar		
	Total nitrogen	Non-protein nitrogen	Protein nitrogen	Saponin	Reducing	Invert	R + I[a]
Correlation coefficient	0.325	0.185	0.348	0.012	0.151	0.097	0.390

[a] R + I = reducing + invert sugar.

Discussion

The reduction of bloat due to penicillin during period 1 is in agreement with the work of Barrentine (12), Barrentine *et al.* (13, 14, 15), Thomas (252), Johnson and Bailey (137), Heinemann *et al.* (108) and Reid (214). Summary of data from periods when neither group of animals was receiving penicillin indicated that group 2 had a greater bloating potential than group 1. Although penicillin apparently loses its bloat preventive effect after prolonged feeding, it is improbable that continued administration will cause an increase in bloat. Therefore, the fact that the treated group had a considerably higher incidence of bloat than the control group during period 4 may be additional evidence that the bloating potential of the groups was not equal.

If one can assume that the bloating potential of the groups did not change between periods 2 and 4 then there actually may have been some reduction in bloat due to penicillin during period 2 even though the mean bloat values during this period were approximately equal. During period 3, bloat was less in the treated group than in the control group; however, the amount of bloat during this period was low and the animals of the control group had a higher bloating potential. Penicillin did not control bloat during period 4; these results are in agreement with the reports of Johnson et al. (141), Emery et al. (83) and Johns (132).

Johnson et al. (141), the first to demonstrate the transitory effect of penicillin as a bloat preventive, found penicillin loses its bloat preventive effect after approximately 10 days of daily administration. Emery et al. (83) and Johns (132) also express some doubts as to the effectiveness of penicillin as a bloat preventive over an extended period of time.

Barrentine et al. (13) and Thomas (252) reported control of bloat for two to four days after a single treatment with 50 to 100 mg. procaine penicillin. Heinemann et al. (108) and Johnson and Bailey (137) reported control of bloat over long periods of time by the daily administration of 32 and 38 mg. of penicillin per day, respectively. However, in the experiment by Johnson and Bailey the dosage was 62,500 units of penicillin, which is equivalent to 62 mg. of procaine penicillin. Heinemann et al. did not specify the type of penicillin used. It may be that the actual dosage in the two later reports was approximately equal to dosages used by other workers. Barrentine et al. (14) in 1958 also agree that penicillin has less effect as a bloat preventive after 10 to 14 days of continuous administration.

At the present time it is not clear whether the initial bloat preventive effect of penicillin is due to the inhibition of certain rumen microorganisms or to alteration of microbial metabolic pathways. It is apparent that after cattle receive a daily penicillin treatment for a considerable period of time, certain changes take place and the antibiotic is of no further value as a bloat preventive. These changes probably occur regardless of the level of antibiotic used or the method of administration.

Weight gains were unaffected by the penicillin treatment. Ten day experimental periods probably are not long enough to detect changes in body weights unless the treatment has a very drastic effect upon weight. From most reports in the literature as indicated in the review by Lassiter (148) and by the work of Heinemann et al. (108), one would not expect an increase in weight gains due to penicillin treatment.

The three-day preliminary period apparently was a poor basis to use for dividing the animals into experimental groups. It probably takes at least two days and perhaps somewhat longer for cattle to begin to bloat on alfalfa soilage. Furthermore, the bloating behavior of cattle tends to change somewhat during the season. Thus, it would probably be better experimental procedure to allow a short preliminary period at the beginning of the season to permit the cattle to become accustomed to the diet and then assign them to experimental groups by pairing them according to size, breed and other characteristics and then randomly allotting one of each pair to each experimental group.

When this experiment was designed it was hoped that the microflora of the rumen would recover from the effects of the penicillin treatment during the seven day recovery period. However, later work by Johnson et al. (139, 141) has shown that antibiotic resistance does develop and will not be overcome even in a considerably longer period of time. According to Johnson's work there will be no reduction in bloat due to penicillin treatment after about two weeks.

Although the animals were allotted to experimental groups in pairs, the pairing was effective only during the first period. This may be interpreted as meaning that the bloating behavior of the animals changed quite early in the season.

In general, the amount of bloat decreased almost continually from the beginning of period 1 in May until the end of period 3 in July. This decrease undoubtedly was due to the dry weather and the consequent slow growth and general lack of lushness of the forage. For the most part the alfalfa was quite pure, fine stemmed and of general good quality, but it lacked the extra quality necessary for producing bloat when fed as soilage. Period 4 was subsequent to a considerable amount of late summer rain; thus the alfalfa was lush and growing very rapidly.

The criteria used for evaluating the quality of forage included size of stems, leafiness, moisture content and purity, all based entirely upon visual inspection. However, based upon either weight gains or bloat it appears that this was in fact a poor estimate of forage quality.

For many years it has been the feeling among laymen that animals that are greedy eaters are more likely to bloat than animals that seem quite finical in their eating habits. However, the work reported by Hancock (102) and Johns (128) indicates that the rapidity with which an animal eats has little or nothing to do with the bloat that may occur. However, an extremely slow eater may never get enough of the forage to cause bloat. The correlation of forage consumption and bloat during periods 1, 2 and 3 of this experiment is in agreement with the work of Hancock and Johns. The significant positive correlation of forage consumption and daily maximum bloat during period 4 at first would indicate that as the animals consume more forage they tend to bloat more. However, the forage during this period was much more lush than it had been during the other three periods. Although dry matter determinations were not made on this forage it is quite reasonable to assume that the amount of dry matter consumed per day during this period was not materially greater than during the other three periods.

The results of the rumen fluid analyses are the basis for much conjecture. Blake (23), Nichols <u>et al.</u> (190, 191) and the concurrent work of Johnson (138) demonstrated that the surface tension of bloated animals is higher than that of non-bloated animals. The results of this experiment agree with those data. Furthermore, it appears that the surface tension, as measured in this experiment, increases with bloat severity; Johnson (138) also found this to be true.

There was a difference in surface tension between the two groups independent of bloat. Group 2 had a higher surface tension and a greater bloating potential than group 1. These data suggest that the ingesta of bloat-susceptible animals may have higher surface tensions than the ingesta of non-bloat-susceptible animals.

The fraction of the variation in the various rumen fluid measurements that was accounted for by regression on treatment, bloat at time of sampling, daily maximum bloat, period, and group indicates that surface tension and per cent solids were influenced to a greater degree than were the other rumen fluid factors. However, since approximately 70 per cent of the variation remains unaccounted for, several other factors are probably involved. Somewhat less of the variation in foam volume and ingesta-volume-increase was attributed to the regression. Nearly all of the variation in pH, foam stability, viscosity and specific gravity were unaccounted for by the regression. Other factors besides treatment, bloat at time of sampling, daily maximum bloat, treatment and groups must account for the remainder of the variation in rumen fluid characteristics; these factors may include such things as composition of the forage, weather, rumen microorganisms, size of the rumen and salivary secretion.

In agreement with Blake (23) and Johnson (138), specific gravity was lower in bloated than in non-bloated animals. Blake (23) found that pH is lower in bloated animals than in non-bloated animals; however, this experiment and the work of Johnson (138) did not substantiate this. Blake also reported a marked increase in foam stability associated with bloat, whereas in this experiment and in Johnson's work there was no effect.

Per cent solids increased with bloat severity; this is in agreement with Johnson (138). This increase is probably due to the fact that more solid matter is incorporated in the ingesta of bloated animals due to the formation of froth which makes straining of the ingesta considerably more difficult and as a net result more solid material is forced through the cheese cloth into the working sample.

Under the conditions of this experiment foam volume tended to decrease as bloat severity increased. This is in agreement with the work of Blake (23) and is not refuted by Johnson (138). Increasing bloat with decreasing foam certainly does not seem reasonable because, without question, foam is involved in almost all cases of bloat. More correctly this decrease should be taken as evidence of a poor method for measuring the foam volume. In reality, this method measures the increase in foam volume due to processing two minutes in the Waring Blendor rather than the actual foam volume of the sample. It may be that rumen ingesta are capable of producing only a given amount of foam under a particular set of conditions; if, as in the case of bloat, some of this "foaming capacity" is used up it may well be that the amount of foam that can be formed artificially will be inversely proportional to the amount that has been formed already.

Ingesta-volume-increase was greater in bloated animals than in non-bloated animals and increased with bloat severity. This is in agreement with Blake (23) and Johnson (138).

A total of seventeen forage samples were analyzed during this experiment. Bloat during this time ranged from very mild to quite severe. The reducing + invert sugar and the nitrogen components were positively correlated with bloat severity. However, the values did not approach significance. It appears that the chemical composition of bloat producing forage is not grossly different than non-bloat producing forage of the same species.

Experiment II -- Soilage Trials, 1957

Experimental procedure

After terminating Experiment I near the end of the 1956 pasture season the same animals were used in a preliminary trial in which various oils were added to the forage on alternate days. Using a power sprayer, the oils were sprayed on the forage in the wagon before feeding and mixed in thoroughly with a fork. Crude soybean oil was used at 0.50 lb. and 0.25 lb. per animal per day; lard oil[1] in both the emulsified and unemulsified forms was also used at the rate of 0.25 lb. per animal per day. On another day, soybean oil was applied with a sprinkling can at the rate of 0.25 lb. per animal per day.

In 1957, twenty-two dairy animals were used in Experiment II. The cattle consisted of a group of eight large animals with an initial average body weight of 998 pounds and 14 small animals with an initial

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[1]Lard oil is a derivative of lard, prepared by Midwest Dried Milk Co., Dundee, Illinois.

average body weight of 331 pounds. Each of these two groups was divided into two comparable groups approximately equal in size and, insofar as possible, with an equal number of animals of the same breed in each group. The breed, sex, experimental number, herd number, group division and initial weights are given in Table 8.

Each group was confined to a separate dry lot and fed fresh chopped soilage _ad libitum_. The feeding procedure was the same as that used in Experiment I except that the soilage not consumed by 6:00 P.M. on the day it was fed was removed from the bunks, weighed and discarded. Observations of bloat were taken and recorded as in Experiment I.

Treatments used included crude soybean oil and _n_-decyl alcohol. At the time of feeding the oil was sprinkled over the forage with a sprinkling can and was mixed in with a fork; _n_-decyl alcohol was administered orally in gelatin capsules. All animals were weighed at weekly intervals. All experimental periods were 14 days except period 9 which was three days. Treated and control groups were alternated for each succeeding period. The experimental design is presented in Table 9.

Rumen fluid samples were taken in periods 1 through 7 by the method described in Experiment I. In the laboratory the surface tension, foam volume, foam stability, ingesta-volume-increase, and rumen ammonia nitrogen of these samples were determined. The methods employed were the same as those described in Experiment I, except for ammonia which was not measured in that experiment. The technique used for rumen ammonia was a modification of the aeration method for determination of urine ammonia as described by Hawk _et al_. (105), p. 889 (a modification of the method

Table 8. Animals used in Experiment II

Experimental number	Herd number	Breed[a]	Sex[b]	Initial weight (lb.)
		Group 1		
37	4205	G	M	782
39	4224	H	M	775
47	3947	A	F	1260
51	4193	BS	F	1038
		Group 2		
41	4125	H	F	1086
46	4146	H	M	1120
52	4152	H	M	1020
53	4388	BS	M	820
		Group 3		
38	4438	J	M	195
43	4343	H	M	590
53	4388	BS	M	368
55	4406	J	M	291
56	4416	BS	M	243
57	4427	H	M	265
58	4394	G	M	302
		Group 4		
40	4340	A	M	468
42	4311	H	M	548
44	4387	BS	M	379
45	4382	J	M	237
48	4417	BS	M	242
49	4428	J	M	174
50	4391	G	M	333

[a]G = Guernsey, H = Holstein, A = Ayrshire, B. S. = Brown Swiss, and J = Jersey.

[b]M = male and F = female.

Table 9. Experimental design of Experiment II

Period	Dates (inclusive)	Soilage	Treatment groups: 1 and 4 treatment[a]	Treatment groups: 2 and 3 treatment[a]
1	5/16 - 5/29	Alfalfa	Control	S B O
2	5/30 - 6/12	Alfalfa	S B O	Control
3	6/13 - 6/18	Alfalfa	Control	S B O
	6/19 - 6/26	Birdsfoot trefoil	Control	S B O
4	6/27 - 7/10	Alfalfa	S B O	Control
5	7/11 - 7/24	Alfalfa	Control	S B O
6	8/3 - 8/16	Alfalfa	D A	Control
7	8/17 - 8/30	Alfalfa	Control	D A
8	8/31 - 9/13	Alfalfa	S B O	Control
9	9/14 - 9/16	Alfalfa	Control	S B O

[a]S B O = soybean oil given at the rate of approximately 0.25 lb. per 1000 lb. body weight per day. D A = n-decyl alcohol given at the rate of 18 ml. per 1000 lb. body weight per day.

of Van Slyke and Cullen). It was found that the saturated carbonate solution used to release the ammonia from urine was not basic enough to release it from rumen fluid. The addition of a 50 per cent solution of sodium hydroxide released the ammonia quickly and efficiently with an apparent minimum deamination of other compounds. Therefore, in this experiment ammonia was released with a 50 per cent solution of sodium hydroxide.

Forage samples were taken twice weekly. Large samples were collected at random from the forage in the wagon before feeding; this material was thoroughly mixed and a small sample was taken from this, placed in a one quart plastic freezer bag, sealed and frozen immediately. As time and laboratory help permitted, some of these samples were selected to be representative of both high and low bloat periods and were analyzed for dry matter, total nitrogen, non-protein nitrogen, amino nitrogen, reducing sugars, ash, phosphorus, potassium and calcium.

When an animal bloated severely, an attempt was made to relieve it with a stomach tube. If this was unsuccessful, as it often was, 25 to 100 ml. of n-decyl alcohol was administered either by hypodermic needle or by stomach tube to break the foam.

Results

The results of the preliminary trial are presented graphically in Figure 2. Without exception, the oil treatment considerably reduced the incidence of bloat. These results plus the favorable results obtained with oil by Reid (209) and Reid and Johns (215) in New Zealand were the basis for setting up Experiment II.

A summary of the effects of preventives and the average daily weight gain is presented in Table 10. These data were analyzed statistically using the following analysis of variance, Snedecor (240):

Source of variation	Degrees of freedom
Rations	1
Pens/rations	2
Animals/pens	18
Total	21

Results of these analyses are summarized in Table 10. Soybean oil sprinkled over the forage at the rate of 0.25 lb. per 1000 lb. body weight per day considerably reduced the average bloat severity and the average daily maximum bloat in six of seven periods. Bloat severity and average daily maximum bloat were lowest in the one period that soybean oil did

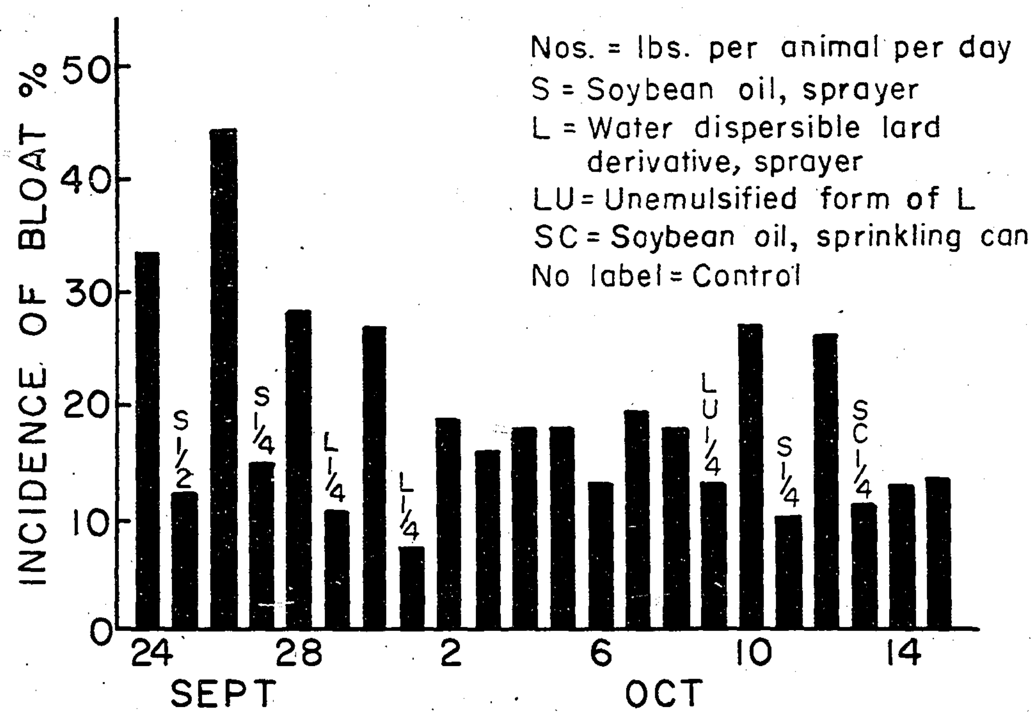

Figure 2. Effects of various oils (sprayed or sprinkled on alfalfa soilage) on incidence of bloat.

Table 10. Response of animals to treatments in Experiment II

Period	Treatment	Bloat severity[a]	Average maximum[a]	Average daily gain (lb.)
1	Soybean oil[b]	0.03	0.12	4.92
	Control	0.13	0.46	4.61
2	Soybean oil	0.03	0.11	1.56
	Control	0.13	0.44	1.16
3	Soybean oil	0.01	0.05	0.51
	Control	0.01	0.03	0.03
4	Soybean oil	0.00[c]	0.03[c]	0.49
	Control	0.20	0.93	0.47
5	Soybean oil	0.02	0.14	1.45[d]
	Control	0.06	0.28	2.36
6	N-decyl alcohol	0.05	0.20	0.76
	Control	0.08	0.29	0.83
7	N-decyl alcohol	0.07	0.17	0.76
	Control	0.05	0.14	0.91
8	Soybean oil	0.09	0.28[e]	2.18[e]
	Control	0.24	0.72	1.40
9	Soybean oil	0.07[f]	0.24[f]	3.42[f]
	Control	0.72	2.30	-5.47

[a]See explanation of terms in Table 4.

[b]Soybean oil sprinkled over the forage at the rate of 0.25 lb. per 1000 lb. body weight per day.

[c]This difference was statistically significant at $P < 0.05$.

[d]This difference was statistically significant at $P < 0.025$.

[e]This difference was statistically significant at $P < 0.10$.

[f]This difference was statistically significant at $P < 0.005$.

not reduce bloat. Birdsfoot trefoil soilage was fed during the last eight days of this period and no bloat occurred during that time; therefore, the oil treatment reduced bloat in all periods where bloat was serious. In every instance this method of treatment eliminated the problem of bloat; the cattle were able to consume, without danger of bloat, forage that when untreated was capable of producing severe bloat. In periods 8, 4 and 9 the difference in bloat severity between treated and untreated groups was significant at $P < 0.10$, 0.05 and 0.005, respectively.

The oil treatment increased weight gains to various degrees in six of seven periods. The difference in weight gains were statistically significant in periods 8 and 9 at $P < 0.10$ and 0.005, respectively. However, in period 5 the weight gains of the control animals were significantly higher than those of the treated animals at $P < 0.025$.

Bloat during the two n-decyl alcohol feeding periods was quite mild. This treatment had very little, if any, effect upon bloat, but during both periods it apparently reduced the average daily gains slightly.

A summary of the daily forage consumption, daily maximum bloat, and average weights at weekly intervals is presented in Table 11. The correlation of forage consumption with average daily maximum bloat was computed for each period and over the whole experiment. In eight of nine periods the correlation was positive; however, the correlation coefficient was significant in only one period. Over the entire experiment, the correlation of forage consumption and average daily maximum bloat was significant at $P < 0.01$.

Table 11. Summary of animal responses in Experiment II

Date	Forage consumption[a]	Daily maximum[b]	Weight[c] (lb.)	r[d]
Period 1				
5-16	96	0.18	570	
5-17	110	0.27		
5-18	87	0.27		
5-19	142	0.32		
5-20	108	0.27		
5-21	88	0.18		
5-22	132	0.41		
5-23	134	0.18	618	
5-24	118	0.36		
5-25	139	0.32		
5-26	113	0.36		
5-27	122	0.32		
5-28	106	0.36		
5-29	113	0.27		0.418
Period 2				
5-30	141	0.59	636	
5-31	157	0.18		
6-1	112	0.36		
6-2	110	0.32		
6-3	112	0.23		
6-4	93	0.27		
6-5	108	0.27		
6-6	104	0.14	645	
6-7	120	0.32		
6-8	135	0.41		
6-9	139	0.23		
6-10	116	0.09		
6-11	124	0.14		
6-12	113	0.27		0.214

[a]Daily forage consumption per 1000 lb. body weight.

[b]Average maximum bloat per animal.

[c]Average body weight on days of weighing.

[d]r = correlation of average daily maximum bloat and feed consumed per 1000 lb. of body weight.

Table 11. (Continued)

Date	Forage consumption[a]	Daily maximum[b]	Weight[c] (lb.)	r[d]
Period 3				
6-13	91	0.04	656	
6-14	166	0.14		
6-15	116	0.04		
6-16	170	0.14		
6-17	93	0.09		
6-18	132	0.14		
6-19[e]	40	0.00		
6-20	34	0.00	647	
6-21	76	0.00		
6-22	109	0.00		
6-23	54	0.00		
6-24	87	0.00		
6-25	130	0.00		
6-26	112	0.00		0.712[f]
Period 4				
6-27	127	0.68	659	
6-28	140	0.59		
6-29	116	0.54		
6-30	128	0.54		
7-1	143	0.41		
7-2	152	0.27		
7-3	168	0.45		
7-4	106	0.54	664	
7-5	118	0.41		
7-6	117	0.59		
7-7	112	0.41		
7-8	151	0.27		
7-9	132	0.50		
7-10	131	0.41		-0.448

[e]Birdsfoot trefoil was fed from 6-19 to 6-26 inclusive.

[f]This value of r is statistically significant at $P < 0.01$.

Table 11. (Continued)

Date	Forage consumption[a]	Daily maximum[b]	Weight[c] (lb.)	r[d]
Period 5				
7-11	126	0.32	666	
7-12	123	0.14		
7-13	125	0.18		
7-14	123	0.18		
7-15	121	0.14		
7-16	123	0.27		
7-17	112	0.27		
7-18	126	0.23	673	
7-19	109	0.23		
7-20	101	0.09		
7-21	117	0.23		
7-22	107	0.23		
7-23	130	0.23		
7-24	113	0.18	693	0.306
Period 6				
8-3	115	0.09	712	
8-4	138	0.27		
8-5	144	0.27		
8-6	151	0.32		
8-7	135	0.36		
8-8	152	0.45		
8-9	151	0.36		
8-10	142	0.04	706	
8-11	129	0.00		
8-12	143	0.32		
8-13	118	0.09		
8-14	154	0.14		
8-15	130	0.41		
8-16	126	0.36		0.379

Table 11. (Continued)

Date	Forage consumption[a]	Daily maximum[b]	Weight[c] (lb.)	r[d]
Period 7				
8-17	163	0.45	723	
8-18	179	0.09		
8-19	173	0.09		
8-20	166	0.09		
8-21	164	0.18		
8-22	148	0.09		
8-23	161	0.18		
8-24	139	0.18	735	
8-25	178	0.10		
8-26	124	0.05		
8-27	198	0.10		
8-28	150	0.05		
8-29	189	0.33		
8-30	193	0.19		0.219
Period 8				
8-31	157	0.19	758	
9-1	154	0.05		
9-2	146	0.10		
9-3	134	0.19		
9-4	136	0.33		
9-5	160	0.43		
9-6	179	0.38		
9-7	156	0.57	774	
9-8	200	0.48		
9-9	179	1.05		
9-10	173	0.76		
9-11	192	0.52		
9-12	187	0.90		
9-13	167	1.24		0.499
Period 9				
9-14	137	1.19	783	
9-15	152	1.29		
9-16	104	1.24	780	0.980
				0.269[g]

[g]Over-all correlation of average daily maximum bloat and forage consumption. This value is significant at $P < 0.01$.

It may be noted that bloat was high only during the latter part of period 8 and in period 9. During the feeding of birdsfoot trefoil in period 3, there was no bloat; on many other days there was very little bloat.

During most of the periods the rate of gain was fairly good; however, the initial unpalatability of trefoil caused a considerable loss of weight during this period.

A summary of the analyses of rumen ingesta is presented in Table 12. These data have been analyzed by multiple regression and levels of significance determined using Student's t test. The regression of treatment, bloat at time of sampling, group, animal size and period upon each of the measurements made on the rumen ingesta was computed. These data include no information taken when bloat incidence was high because rumen ingesta samples were not taken during periods 8 and 9.

Based on the small number of samples taken from bloated animals during the soybean oil treatment period, it appeared that bloat increased surface tension and rumen ammonia and decreased foam volume. The soybean oil treatment decreased surface tension, foam volume, foam stability and ingesta-volume-increase. During periods 1 and 2, surface tension, foam stability and ingesta-volume-increase were higher and foam volume was lower than in periods 4 and 5. There was no appreciable effect due to animal size or group during that time.

Surface tension was higher during the periods of higher bloat but was decreased by soybean oil on alfalfa or birdsfoot trefoil; it was unaffected by group or size of animals.

Table 12. Rumen fluid measurements in Experiment II

Comparisons	Rumen fluid measurements S. T.[a]	F. V.[b]	F. S.[c]	I.V.I.[d]	NH_3[e]	N[f]
	Periods 1, 2, 4 and 5[g] (Soybean oil treatment)					
Effect of bloat at time of sampling						
Unbloated	52.9	31	82	109	-	50
Bloated	57.3	35	85	108	-	5
P<[h]	0.100	-	-	-		
Effect of soybean oil treatment						
Treated	51.2	27	80	107	-	29
Control	55.6	36	84	111	-	26
P<	0.001	0.001	0.100	0.100		
Effect of group						
Group 1	53.9	29	82	110	-	29
Group 2	52.6	34	83	107	-	26
P<	-	0.025	-	-		
Effect of animal size						
Large	53.2	29	80	108	-	24
Small	53.3	33	84	109	-	31
P<	-	0.100	0.100	-		
Effect of period						
Periods 1 and 2	56.3	27	86	115	-	16
Periods 4 and 5	52.0	33	81	106	-	39
P<	0.001	0.050	0.100	0.001		
R^2[i]	0.72	0.33	0.16	0.37		

[a]S. T. = surface tension in dynes/cm.

[b]F. V. = foam volume.

[c]F. S. = foam stability.

[d]I.V.I. = ingesta-volume-increase.

[e]NH_3 = mg. NH_3-N per 100 ml. rumen ingesta.

[f]N = number of observations.

[g]Periods from Table 9.

[h]P < = level of statistical significance.

[i]R^2 = fraction of Σy^2 due to regression.

Table 12. (Continued)

Comparisons	Rumen fluid measurements S.T.[a]	F. V.[b]	F. S.[c]	I.V.I.[d]	NH_3[e]	N[f]
			Periods 4 and 5 (Soybean oil treatment)			
Effect of bloat at time of sampling						
Unbloated	52.0	32	80	106	30.6	38
Bloated	56.5	29	81	109	35.0	5
P <	0.100	0.100	-	-	0.100	
Effect of soybean oil treatment						
Treated	50.3	28	78	106	31.6	21
Control	54.6	35	83	107	30.6	22
P <	0.001	0.050	0.050	-	-	
Effect of group						
Group 1	52.1	29	80	107	31.8	21
Group 2	52.9	33	80	107	30.4	22
Effect of animal size						
Large	51.4	31	79	106	29.2	16
Small	53.2	31	81	107	32.2	27
P <	0.005	-	-	-	-	
R^2[i]	0.73	0.16	0.12	0.08	0.14	
			(N-decyl alcohol treatment)			
Effect of bloat at time of sampling						
Unbloated	53.3	38	78	109	31.6	36
Bloated	54.8	48	84	113	30.0	2
P <	-	-	-	0.100	-	
Effect of n-decyl alcohol treatment						
Treated	52.0	32	75	110	32.8	19
Control	54.7	40	82	108	30.2	19
P <	0.001	-	0.050	-	-	
Effect of animal size						
Large	52.9	35	76	109	29.4	14
Small	53.6	41	79	109	32.7	24
P <	-	0.100	-	-	-	
R^2[i]	0.44	0.18	0.24	0.14	0.07	

Table 12. (Continued)

Comparisons	Rumen fluid measurements S. T.[a]	F. V.[b]	F. S.[c]	I.V.I.[d]	NH_3[e]	N[f]
(Soybean oil treatment with trefoil soilage)						
Effect of soybean oil treatment						
Treated	48.4	29	72	106	12.7	12
Control	55.6	29	77	107	12.7	12
P <	0.025	-	-	-	-	
Effect of animal size						
Small	52.6	27	70	106	13.0	16
Large	50.7	32	83	106	12.0	8
R^2[i]	0.35	0.06	0.08	0.02	0.04	

Foam volume was not affected by bloat but was reduced considerably by the soybean oil treatment. Foam stability was reduced by the treatment, but was affected little by bloat, group, animal size or period.

There was little difference in ingesta-volume-increase except that it was considerably higher in periods 1 and 2 than in periods 4 and 5. Rumen ammonia was higher in bloated than in non-bloated animals but was unaffected by treatment, group or animal size.

During the n-decyl alcohol treatment period it was again apparent that the bloated animals had a greater ingesta-volume-increase than non-bloated animals. The n-decyl alcohol treatment also reduced the surface tension and the foam stability.

A correlation coefficient of average daily bloat severity with rumen ammonia, surface tension, ingesta-volume-increase, foam volume and foam stability was computed. These results are summarized in Table 13. There

Table 13. Correlation coefficients of bloat severity with rumen ingesta and forage composition determinations

	Correlation coefficients	Number of observations
Rumen ingesta		
NH_3	0.288	18
Surface tension	0.307	18
Ingesta-volume-increase	0.342	18
Foam volume	-0.173	18
Foam stability	0.186	18
Forage composition		
Dry matter	-0.335	22
Total nitrogen	0.876[a]	22
Non-protein nitrogen	0.380	22
Amino nitrogen	0.467[b]	22
Reducing sugar	0.052	22
Ash	0.289	22
K	0.701[b]	9
P	0.798[a]	22
Ca	-0.787[b]	8

[a]This value is statistically significant at $P < 0.01$.

[b]This value is statistically significant at $P < 0.05$.

was a positive correlation of bloat severity with rumen ammonia, surface tension, foam stability, and ingesta-volume-increase; however, none of these values was significant at P = 0.05.

In an attempt to associate bloat severity with various forage components, correlation coefficients were computed for bloat severity and each of the following forage components: forage dry matter, total nitrogen, non-protein nitrogen, amino nitrogen, reducing sugars, ash, potassium, phosphorus and calcium.

Dry matter and calcium were negatively correlated with bloat severity; the correlation of calcium and bloat was significant at $P < 0.05$. All other constituents had a positive correlation with bloat severity; total nitrogen, amino nitrogen, potassium and phosphorus were significant. These correlation coefficients are summarized in Table 13.

In severe cases of bloat the oral administration of 25 ml. of n-decyl alcohol was soon followed by the release of large quantities of gas from the rumen via stomach tube and/or eructation. Recovery was usually complete within 30 to 45 minutes. Animals so treated showed no adverse after effects; in fact, they would often begin to eat immediately and sometimes were showing some bloat again in as little as two hours.

Discussion

Throughout most of the season the bloat was quite mild; however, for a few days near the end of the season when extremely young, lush forage was fed the bloat was severe. Throughout the season the animals on alfalfa pasture in the concurrent study conducted by Johnson (138) showed a considerably higher incidence and severity of bloat although the quality of the forage available was approximately equal to that used for soilage. This is additional evidence that soilage feeding will tend to reduce the amount of bloat, probably because the animals are forced to eat the entire plant rather than selecting primarily the lush tops as they tend to do when grazing. This experiment also indicates that bloat can be produced quite effectively in this manner if one is able to select the very lush, rapidly growing forage by chopping only the top few inches of the plant.

New Zealand workers, Johns (128) and Reid (211) reported very successful control of bloat by spraying pasture plots with peanut oil. The results of the preliminary trial of Experiment II, conducted at the close of the 1956 season, indicated that applying oil to the forage also would be quite successful in controlling bloat with a soilage feeding program. The results of this experiment demonstrated conclusively that 0.25 lb. of crude soybean oil per 1000 lb. of body weight per day applied to the soilage at feeding time will eliminate the danger of bloat.

With the increasing size of farms and more complete mechanization, many farmers now have sufficient equipment available, including a direct cut chopper, a self-unloading wagon and an extra tractor, to adopt this feeding system. During most of the season one would not have to add oil because the soiling process will reduce the amount of bloat considerably. Using a little ingenuity one could devise a means of adding the oil automatically as the soilage is being placed in the feed bunk from the self-unloading wagon. This method of feeding also has other advantages in that larger yields of forage are obtained than are possible with any system of grazing. Also, the animals are kept near the farmstead so they can be observed frequently for any abnormal behavior.

In terms of effectiveness this method compares quite favorably with the method of pasture spraying as described by Johns (128) and Reid (211). There are no time limitations upon either of these treatments except that fresh forage must be fed each day. In both cases the animals continue to receive the preventive as long as they continue to eat the forage. Likewise, the treatment will continue to be effective over extended periods of time; there is no possibility that the rumen microorganisms can develop resistant strains causing the preventive to become ineffective as is the case with antibiotics.

However, the soilage method as well as the pasture spraying method has some disadvantages. The process of soiling, although it may not require a lot of time with modern equipment, does require a given amount of labor each day. At certain times during the season and/or on some days of the week it may be extremely inconvenient to do this job. This method also requires a large capital investment in special machinery. An individual contemplating such a change in management would need to consider carefully all economic aspects involved.

Such changes in management also can cause problems for the farmer. Cattle confined to a small area are more subject to disease; sanitation and manure removal become problems. However, probably the largest factor that will prevent adoption of this method on a large scale is that it involves a change of considerable magnitude in management of the farm animals. In general, farmers are reluctant to make such changes;

undoubtedly this change will come about slowly, if at all.

With the exception of four of the large animals that had been used in Experiment I the animals were assigned to treatment groups with no knowledge of their susceptibility to bloat. From Experiment I it was evident that one animal, 4152, bloated with such frequency that he might approach the classification of a chronic bloater. Another animal, 4146, had bloated only very infrequently. These two animals were assigned to one group and the other two animals, both of which were known to be somewhat susceptible to bloat, were assigned to the other group.

Because animals of the same age, size, sex and breed are likely to respond similarly, the animals were grouped on this basis. With the exception of 4152, whose behavior was very much like that of a chronic bloater, the groups were quite comparable in their susceptibility to bloat.

Dr. Robert Hansen of the Iowa State University Chemistry Department suggested the 8 to 12 carbon, straight chain alcohols as possible defoamers of rumen ingesta and possible bloat preventives. In a preliminary trial with a sample of frothy rumen ingesta it was found that n-decyl alcohol was a very effective defoamer. In a later trial performed by adding very small increments of n-decyl alcohol to a given volume of rumen ingesta, it was found that a level of 0.03 per cent alcohol by volume produced a maximum response in lowering the surface tension. This level also was effective in defoaming frothy ingesta as well as reducing the amount of foam produced as measured by the ingesta-volume-increase technique. If the rumen of an adult animal has a capacity of 60 liters, then an 18 ml. dose of this alcohol should be effective in breaking the foam or preventing its formation and thus preventing bloat.

After testing n-decyl alcohol *in vitro*, it was tested for possible toxicity by feeding to a Jersey calf. The alcohol was administered orally via gelatin capsule first at 2.0 ml. and later at 4.0 ml. twice daily. This calf weighed 81 pounds initially and received a maximum of 8.6 ml. n-decyl alcohol per 100 pounds body weight. The calf gained an average of 1.53 pounds per day on the milk, hay and grain feeding program. On this basis it was concluded that the n-decyl alcohol was non-toxic at levels sufficiently high to control bloat.

Since n-decyl alcohol is very irritating to mucous membranes and has quite a repulsive odor it was decided that to assure consumption of a known amount of preventive and not to interfere with palatability of the forage the best experimental method of administering this preventive was via gelatin capsule.

It is evident that the administration of the entire day's treatment in a single dose is not as effective as continuous administration in which the animal receives the treatment as forage is eaten. Although the amount of bloat was quite mild during the time this preventive was employed the animals receiving treatment appeared to eat more than non-treated animals. Furthermore, they tended to bloat less during the early part of the day and to bloat more late in the day than non-treated animals.

Apparently n-decyl alcohol is removed from the rumen, metabolized by the rumen bacteria or both in a relatively short period of time so that it soon becomes ineffective as a preventive. In the concurrent studies with cattle grazing alfalfa pasture as reported by Johnson et al. (141) the bloat preventive effect of oil administered in the grain tends to decrease with time after treatment. Two ways of overcoming this difficulty are apparent: first a system whereby a preventive is continuously administered or second a method whereby the preventive can be released slowly within the rumen and thereby exercise its effect over a prolonged period.

The statistically significant effect of the oil treatment in increasing weight gains during periods 8 and 9 was probably due to the decrease in bloat. Although the difference in weight gains due to the soybean oil treatment was significant only during two periods, animals receiving the soybean oil treatment gained slightly faster in six of the seven periods it was used as a preventive. This indicates that there may have been some beneficial effect of the oil treatment on weight gains. This was confirmed in most of the trials involving oil feeding as a preventive for pasture bloat by Johnson (138). The weight gains in this experiment are based upon single day weights; since the weight of the contents of the digestive tract tend to vary considerably this was probably a factor causing considerable variation in apparent body weights. The variability could easily mask any effect that the oil may actually have had on weight gains.

When birdsfoot trefoil feeding was started, the animals at first were very reluctant to eat; in fact, this forage was so unpalatable that for the first few days they consumed only about one-third the amount of forage that they had been eating.

Consumption gradually increased until by the end of a week the trefoil consumption was approximately equal to the previous alfalfa consumption.

Although trefoil consumption reached a maximum of 130 lb. per 1000 lb. body weight per day, no deleterious effects, including signs of cyanide poisoning or bloat, were noted. It is the general feeling among scientists and laymen that bloat cannot be produced on trefoil. Perhaps in some areas more extensive use of this forage can offer a partial solution to the bloat problem.

In Experiment II as in Experiment I there was a significant positive correlation between forage consumption and average maximum bloat. However, dry matter was not determined on the forage as it was consumed; it is thought that the positive correlation probably was due to a decrease in dry matter of the forage and a consequent increase in total pounds consumed during periods when forage was young and lush and bloat was most prevalent.

Although the amount of bloat during the time rumen ingesta were taken was quite low, ingesta from bloated animals were higher in surface tension than those of non-bloated animals. This is in agreement with the results of Experiment I, the concurrent results of Johnson (138) and the results of Blake (23). At the present time it is difficult, if not impossible, to separate cause and effect. No doubt a high surface tension is associated with the froth present during bloat; however, the exact relationship between surface tension and froth formation is not known.

Contrary to the results in Experiment I the foam volume, in most phases of this experiment, tended to be higher in the ingesta of bloated animals than in those of non-bloated animals. However, during periods 4 and 5 foam volume was higher in ingesta from non-bloated animals than in those from bloated animals. The ability of rumen ingesta to form large volumes of stable foam or froth is an important factor in the etiology of bloat. It must be concluded that this measurement as determined in these experiments does not give a true representation of the relationship that must be involved between the formation of froth and bloat.

During Experiments I and II and in Johnson's work (138), contrary to Blake's report (23), foam stability was associated little, if any, with bloat severity. Possibly the situation would be considerably different if one could measure the stability of the froth in the rumen.

Throughout this experiment the ingesta-volume-increase measurements were low. If one accepts the results of Experiment I and the experiments of Johnson (138) bloat severity is associated with ingesta-volume-increase. Since the values for bloat and for ingesta-volume-increase in this experiment are both quite low, these data tend to confirm the results of Experiment I and of Johnson.

In this experiment and in the concurrent work of Johnson (138), rumen ammonia values tended to become higher with increasing bloat severity. There is some question as to whether any significance can be attached to this fact since the levels attained are for the most part well below the toxic levels.

Since soybean oil was an effective bloat preventive one would expect it to have an effect on rumen fluid characteristics opposite to the effect of bloat. Generally this is true -- oil decreased surface tension, decreased foam volume and stability and decreased ingesta-volume-increase. This has been confirmed by Johnson (138) using soybean oil and lard oil.

According to the R^2 values, the regression of surface tension on the factors considered accounts for a high proportion of the variation encountered; in two of the analyses the regression accounted for more than 70 per cent of the variation.

The effects of n-decyl alcohol treatment are much less marked; this is probably because of the low level of bloat during that treatment period.

The comparison of forage composition data and bloat severity is the basis for much conjecture. In this experiment there was a significant positive relationship between bloat severity and total nitrogen, amino nitrogen, potassium, and phosphorus, but a negative relationship between bloat severity and calcium. However, these results are largely in disagreement with the results of forage samples taken from alfalfa pasture in the concurrent study by Johnson (138). In the soilage samples the correlation of bloat severity with total nitrogen in the forage was 0.88 and significant at $P < 0.01$ whereas, in the pasture samples the correlation was -0.06. In the pasture samples the correlation of bloat severity and reducing sugars was 0.50 and significant at $P < 0.01$ while in the soilage samples the correlation was 0.05. Both sets of samples showed a relationship between bloat severity and calcium content; however, it was negative in the soilage samples and positive in the pasture samples.

It is possible that neither of these studies involved enough data to show the true relationships between bloat and chemical composition of the forage. However, they do indicate that the chemical composition of forage that will cause bloat is not greatly different from that which will not cause bloat.

N-decyl alcohol was an effective therapeutic agent for frothy alfalfa bloat. When treatment is given via stomach tube pressure in the rumen may cause some of the agent to be spilled or sprayed into the air; if this gets into the eyes or on the skin it is very irritating. From this standpoint it may be undesirable or even dangerous to use *n*-decyl alcohol in this manner. Lard oil has been equally or perhaps more effective than *n*-decyl alcohol and therefore is preferable. Other oils including corn oil (Boda *et al.*, 29) and peanut oil, tallow and mineral oil (Johns, 127) also have been used effectively to relieve cases of severe bloat.

Experiment III -- Feed Lot Bloat

Phase 1. High levels of concentrate with alfalfa soilage

Experimental procedure The first phase of Experiment III was conducted during the summer of 1956. At that time 15 animals were on an *ad libitum* feeding of alfalfa soilage. A drought during the latter part of July drastically reduced the amount of alfalfa available for soilage. The feeding program then was changed to a full feeding of concentrate mixture 1 (Table 14), while the animals continued to receive all the alfalfa soilage they would eat. On most days they did not eat more than 20 to 30 pounds of alfalfa per animal.

Table 14. Concentrate mixtures used in Experiment III

Components of mixtures	Mixture 1	2	3	4	5	6	7
	(Per cent)						
Corn	70.0	75.0	65.2	49.5	53.6	60.0	72.0
Soybean oil meal	27.0	22.0	19.1	34.8	20.0	20.0	25.0
Calcium carbonate	1.0	1.0	0.9	0.9	0.8	-	1.0
Dicalcium phosphate	1.0	-	-	-	-	-	-
Salt	1.0	1.0	0.9	0.9	0.8	1.0	1.0
Steamed bonemeal	-	1.0	0.9	0.9	0.8	-	1.0
Chopped alfalfa	-	-	13.0	13.0	16.0	-	-
Dextrose	-	-	-	-	8.0	-	-
Dried skim milk	-	-	-	-	-	19.0	-
Total	100.0	100.0	100.0	100.0	100.0	100.0	100.0

The soilage was fed, penicillin was administered, bloat observations were taken, animals were weighed and rumen ingesta samples were collected and measurements taken as described in Experiment I. The concentrate mixture was fed at 8 A.M. and 4 P.M. each day. The animals of group 2 of Experiment I (Table 1) received the penicillin treatment throughout this phase. This group had received penicillin for a 10-day period ending 50 days prior to this experiment.

<u>Results</u> Table 15 is a comparison of the bloat and weight gains of the two groups. From this it is evident that penicillin was ineffective

Table 15. Animal response to penicillin treatment

	Penicillin treated	Control
Bloat severity	0.48	0.10
Bloat incidence	30	4
Average maximum bloat	0.92	0.34
Average daily gain	3.04	3.24

in controlling bloat produced under this feeding program. The difference in average daily bloat was significant at $P < 0.05$. It should be pointed out that the total number of animals bloating was not large; only two animals in the control group and four animals in the penicillin treated group bloated to an appreciable extent. There was no difference in weight gains between the two groups at $P = 0.05$.

A summary of daily forage and grain consumption, average daily maximum bloat, average daily bloat severity, average daily bloat incidence, and average body weight on days of weighing is presented in Table 16. Since the grain was hand-fed, the consumption was quite constant. The forage consumption varied considerably from day to day, probably depending upon the lushness of the alfalfa. The correlation of forage consumption with average daily maximum bloat was -0.57; this value was statistically significant at $P < 0.01$. The correlation of concentrate consumption with average daily maximum bloat was 0.76; this value also was statistically significant at $P < 0.01$. However, these

Table 16. Summary of animal responses in phase 1 of Experiment III (data include all animals)

Date	Consumption[a]		Bloat			Average weight (lb.)
	Forage	Grain	Maximum[b]	Severity[c]	Incidence[d]	
8-6	37.4	26.0	0.53	0.20	11	642
8-7	35.2	26.4	0.47	0.18	12	
8-8	33.0	26.3	0.33	0.11	10	
8-9	32.0	26.3	0.27	0.11	10	
8-10	44.2	26.2	0.27	0.14	10	
8-11	38.0	26.2	0.47	0.19	14	649
8-12	35.9	26.1	0.53	0.25	17	
8-13	42.9	26.0	0.40	0.20	15	
8-14	37.7	27.5	0.73	0.28	21	
8-15	32.4	27.4	0.80	0.28	20	
8-16	17.7	28.1	0.67	0.33	21	659
8-17	43.3	28.7	0.80	0.29	19	
8-18	23.0	28.5	0.93	0.44	26	
8-19	30.4	28.3	0.73	0.26	17	
8-20	17.8	29.8	0.67	0.30	20	
8-21	30.6	29.6	0.87	0.49	30	675
8-22	19.6	29.5	0.80	0.47	33	
8-23	19.5	29.3	0.87	0.41	25	
8-24	12.1	27.7	0.73	0.37	24	
8-25	37.6	26.5	0.67	0.34	20	
8-26	27.4	26.4	0.73	0.32	20	
8-27	38.2	26.7	0.47	0.28	15	699
8-28	35.4	26.5	0.53	0.30	16	
8-29	46.0	26.7	0.40	0.29	13	
8-30	31.6	25.6	0.53	0.31	15	
8-31	41.5	24.4	0.40	0.28	16	723

[a]Pounds per 1000 lb. body weight.

[b]Average daily maximum bloat per animal.

[c]Average daily bloat severity per animal.

[d]Average daily bloat incidence per animal.

calculations were based upon totals for the lot rather than upon individual animals.

The results of the rumen fluid measurements made during this phase of the experiment are summarized in Table 17. The penicillin treatment had no effect upon the characteristics measured. However, there were concurrent increases in bloat, surface tension, pH and ingesta-volume-increase but a decrease in specific gravity.

Discussion Feeding limited amounts of alfalfa soilage with a full feed of concentrate was a satisfactory method of producing feed lot bloat. Work in phase 3 of this experiment indicates that when animals receive limited amounts of roughage and liberal amounts of concentrates and have access to bedding such as wood shavings, little bloat is likely to occur. One might also expect such a reduction in bloat from ingestion of wood or dirt in unpaved lots. Although the animals in this experiment were in an unpaved lot with wooden fences and buildings, the roughage intake was sufficient to prevent excessive ingestion of non-feed materials and consequent reduction in bloat. The negative correlation of daily maximum bloat and forage consumption is evidence that one may have been able to obtain more bloat by limiting forage consumption. The high positive correlation of concentrate consumption and daily maximum bloat indicates that the more animals eat the more they bloat. However, Hancock (102) and Johns (128) found greedy eaters no more likely to bloat than others. Feed consumption data are for the entire lot of animals and therefore do not take into account variation among individual animals.

During this experiment, both bloat and feed consumption increased

Table 17. Rumen fluid measurements in Experiment III

Comparisons	Surface tension (dynes/cm.)	pH	% solids	Foam stability	Foam volume	Viscosity	Specific gravity	Ingesta-volume increase	No. of observations
	Rumen fluid measurements								
Effect of penicillin treatment									
Treated	53.1	6.01	18.2	71	10	1.023	1.003	143	45
Control	50.4	5.96	19.1	72	13	1.033	1.003	127	72
Effect of bloat at time of sampling									
Unbloated	48.4	5.86	18.4	76	14	1.030	1.003	121	89
Bloated	60.9	6.36	20.0	59	7	1.026	1.002	174	28
P < [a]	0.001	0.005	-	-	0.05	-	0.10	0.001	
Effect of daily maximum bloat score									
Daily maximum bloat score 0	48.2	5.86	18.7	75	13	1.031	1.003	120	79
Daily maximum bloat score 1	53.7	6.03	16.4	78	15	1.021	1.003	136	15
Daily maximum bloat score 2	61.2	6.34	18.0	54	6	1.024	1.004	175	16
Daily maximum bloat score 3	59.9	6.33	26.8	58	6	1.041	0.997	177	7
P <	0.001	-	-	-	-	-	0.005	0.001	
R^2 [b]	0.78	0.27	0.02	0.10	0.15	0.01	0.10	0.77	

[a] P < = level of statistical significance.

[b] $R2$ = fraction of the variation accounted for by regression.

with time. Jacobson _et al._ (124) reported an increase in bloat with time on a given diet. This time factor may be one reason why Blake (23) was unsuccessful in producing bloat on some diets during periods of two weeks or longer.

The changes in rumen fluid associated with feed lot bloat are quite similar to those changes found during alfalfa bloat in Experiments I and II and in the concurrent pasture and feed lot studies by Johnson (138). Surface tension and ingesta-volume-increase are the two measurements most affected by bloat. The increase in surface tension is probably associated with the increased incorporation of gas into the ingesta and consequent formation of a stable froth. At the present time it is not known whether surface tension as such is the cause or effect of the froth formation. The high ingesta-volume-increase found in bloated animals agrees with the work of Johns (127, 133, 134) and Johns _et al._ (135) who feel that the formation of the stable foam is the key to the question of the cause of most cases of bloat.

It should be noted that in the feed lot bloat produced during this phase of Experiment III ingesta from bloated animals were higher in pH than ingesta from non-bloated animals. In the case of alfalfa bloat, pH is not affected by bloat. The reason for this difference is not clear, but may be associated with changes in the microflora of the rumen.

Phase 2. High levels of concentrate with limited alfalfa hay, 1956-57

Experimental procedure At the close of the 1956 forage season, five animals were retained for studying feed lot bloat. These cattle were placed in individual pens in the nutrition laboratory at the Iowa

State University Dairy Farm and bedded sparingly with wood shavings. Later as the amount of roughage, in the form of alfalfa hay, was limited they began to eat the shavings and were then put on screens so that they would have no access to any other roughage.

Good quality chopped alfalfa hay was fed at the rate of six pounds per head per day. Concentrate mixture 2 from Table 14 was fed at rates which were increased gradually each day until the animals were receiving about all the grain they would consume readily. At the end of 12 days some animals were beginning to bloat; bloat observations then were taken several times each day so that a maximum for each animal was recorded each day. Animals were weighed at weekly intervals.

At the end of 28 days this treatment was interrupted for a 14-day period during which a special high quality "bloat producing" hay was fed ad libitum. Cases of fatal bloat are reported occasionally among cattle receiving high levels of legume hay; one such case was reported from the Story City, Iowa area at this time. Some of this hay was obtained and fed to these five animals for a 14-day period; however, no cases of bloat were noted even though the feeding program was essentially duplicated by feeding a limited amount of whole oats plus the alfalfa hay ad libitum. Early in 1957, another farm in this area reported bloat on good alfalfa hay; some of this hay was obtained and fed to four other animals, but no bloat was produced under these conditions.

The original five animals then were fed the high level of concentrate mixture 2 and limited to six pounds of hay for an additional 21 days. Following this, no hay was fed for seven days, then for the remainder of a 46-day experimental period they were limited to three pounds of hay per animal per day.

The remainder of the season was divided into three periods; during this time concentrate mixtures 3, 4 and 5 from Table 14 were used as the only feed for periods of 27, 29 and 56 days, respectively.

Results From the summary of results in Table 18, it may be noted that during the feeding of concentrate mixture 2 weight gains were relatively poor and quite variable. The average daily maximum bloat produced under this regime was somewhat less than was produced on concentrate mixture 1 with the soilage feeding program. However, very little bloat was noted during the time that no hay was fed and the amount of bloat increased as the season progressed.

Results from feeding concentrate mixture 3 indicate that weight gains were slightly better, but there was less bloat than with concentrate mixture 2. This was the only exception to an increase in bloat, irrespective of rations, as the season progressed. For this reason these data are not a good evaluation of the ability of the various diets to produce bloat.

Somewhat more bloat was observed on concentrate mixture 4 and the weight gains were slightly better than on either of the previous two diets.

Average daily maximum bloat was highest on concentrate mixture 5, but average daily gains were intermediate between those of rations 3 and 4.

Table 18. Animal responses to concentrate mixtures 2, 3, 4, and 5 used in Experiment III

	Concentrate mixture			
	2	3	4	5
Animal no. 4205				
Concentrate[a]	18.0	16.8	18.0	22.0
Bloat[b]	0.33	0.33	0.78	1.48
Weight[c]	0.77	0.37	2.86	1.78
Animal no. 4224				
Concentrate	20.0	18.1	20.0	21.8
Bloat	0.54	0.18	0.20	0.21
Weight	1.47	2.59	2.65	1.54
Animal no. 4146				
Concentrate	15.0	18.2	19.6	20.7
Bloat	0.13	0.59	1.02	0.80
Weight	0.78	0.52	5.34	-0.50
Animal no. 4152				
Concentrate	16.4	17.2	21.1	22.8
Bloat	1.46	1.22	1.94	2.32
Weight	1.00	1.30	2.62	2.93
Animal no. 4193				
Concentrate	14.1	19.4	21.6	22.4
Bloat	0.33	0.00	0.07	0.00
Weight	-0.33	2.15	3.94	3.51
Average for all animals				
Concentrate	16.7	17.9	20.1	21.9
Bloat	0.56	0.46	0.80	0.96
Weight	0.74	1.39	3.48	1.85

[a]Pounds concentrate consumed per 1000 lb. of body weight per day.

[b]Average daily maximum bloat.

[c]Average daily body weight changes, pounds.

<u>Discussion</u> The type of bloat occurring when ruminants are fed high levels of legume hay is very difficult to study experimentally. Possibly, as in the case of feed lot bloat produced by feeding high levels of grain, it is a condition that may take a considerable amount of time to develop on a given feeding program. Certainly it cannot be produced simply by short-term feeding of large amounts of the hay that has been incriminated; therefore, other factors probably are involved.

In considering the response to the four concentrate mixtures used during the 1956-57 season, one must consider the general tendency for bloat to increase as the season progresses. The major exception to this was concentrate mixture 3 on which three of the five animals' average daily bloat score decreased from that of the previous diet. However, bloat was produced on each of these diets which indicates that bloat possibly is associated more nearly with management, particularly the limitation of roughage intake, rather than the actual composition of the ration.

Nichols (186) has proposed mixing of hay and concentrate to insure an adequate intake of roughage to prevent feed lot bloat. The results of this phase of Experiment III indicate that cattle would have to consume more than 3.5 lb. of hay per 1000 lb. body weight per day in order to prevent bloat even when the concentrate is incorporated into the roughage.

From the observations made during this experiment, it appears that with some roughage in the diet more bloat will be produced than when there is no roughage. On this basis it seems likely that factors other than mechanical irritation by roughage to stimulate eructation must be involved. Perhaps the hay supplies one or more factors that contribute to the formation of a stable froth. However, it has been demonstrated that large amounts of froth can be produced with no hay in the diet.

Phase 3. High levels of concentrate with limited alfalfa hay, 1957-58

Experimental procedure During the winter of 1957-58, 15 cattle were used at various times on two different concentrate mixtures (6 and 7 from Table 14) in a feed lot bloat trial. These cattle were confined to individual pens and bedded sparingly with wood shavings. Several levels of alfalfa hay were fed, varying from *ad libitum* to as little as two pounds per animal per day. Concentrate was fed twice daily at a level approaching that of full feed. Body weights were taken once weekly and the animals were checked several times each day for signs of bloat.

Results Very little bloat occurred on either of these two grain mixtures during the entire time this system of management was employed. The animals used, the average daily gain on the two rations, and number of days on experiment are summarized in Table 19.

Discussion The system of management employed during the 1957-58 season was unsatisfactory for production of feed lot bloat. The failure to produce bloat on these two rations is probably due to the fact that the animals were bedded with shavings thus making it impossible to control the roughage intake. It may be that the consumption of a limited amount of shavings is equivalent to several pounds of hay in terms of the bloat preventive effect. From Table 14, it may be noted that concentrate mixture 7 is very similar to mixture 2; thus it is apparent that the difference in bloat in reality was due to the management rather than the ration

Table 19. Summary of animal responses to concentrate mixtures 6 and 7 in Experiment III during 1957-58

Animal herd no.	Concentrate mixture 6		Concentrate mixture 7	
	Days[a]	A.D.G.[b]	Days[a]	A.D.G.[b]
4461	140	2.44		
4458	112	2.78		
4497	42	0.74	42	0.40
4479	35	1.43	70	1.58
4483	28	2.39	77	2.13
4488			77	2.34
4491			70	2.17
4495			70	2.56
4505			84	2.24
4507			28	1.64
4511			84	2.71
4514			84	2.49
4515			84	1.89
4524			42	3.10
4516			56	2.54
Average daily weight gain		2.25		2.19

[a]Days = days on experiment.

[b]A.D.G. = average daily weight gains, pounds.

composition.

Although feed lot bloat has not been studied extensively, it has been produced on many different concentrate mixtures. As evidence of this, some bloat was produced on each of the seven concentrate mixtures used in this experiment. This type of bloat is a problem when cattle are finished for market by feeding high levels of concentrate and low levels of roughage. Apparently the most critical factors in the experimental production of feed lot bloat include (a) very careful control of the roughage intake and (b) length of time on the diet.

Experiment IV — Observations on Blood and Rumen Ingesta from Cattle Grazing Alfalfa Pasture

Experimental procedure

This experiment, conducted during the summer of 1958, was designed to study some of the possible changes in the chemical composition of venous blood that may be associated with bloat. Two general groups of blood constituents (phosphorus and nitrogen) that have been associated with bloat were selected for concentrated study.

According to the theory proposed by Cooper (58), Cooper et al. (61), and Cooper and Woodle (62), a relatively high level of phosphorus in the soil should prevent bloat if it will produce forage with a nitrogen to phosphorus ratio of 11:1. If this were true, one might expect to find some physiological basis for this theory in that levels of phosphorus in the blood of animals not bloating because of a favorable nitrogen-phosphorus ratio might be higher than in animals that do bloat. This assumes that the higher level of phosphorus in the soil will produce plants that are higher in phosphorus which will in turn, when ingested by the animal, increase the level of phosphorus in the blood. Reports by Gerwig (92), Gross (96) and Power et al. (206) indicate that phosphorus fertilization of alfalfa will increase the phosphorus content of the plant.

The results of Experiment II and the concurrent work of Johnson (138) indicated that the rumen ingesta of bloated animals are higher in ammonia than those of non-bloated animals. It is conceivable that this ammonia increase could also be carried over into the blood and if it were high enough could cause ammonia toxicity.

For the above reasons, the levels of the following blood constituents were compared in bloated and non-bloated animals: total blood phosphorus, plasma inorganic phosphorus, plasma trichloroacetic acid soluble phosphorus, plasma trichloroacetic acid insoluble phosphorus, blood ammonia nitrogen and blood non-protein nitrogen.

Preliminary observations also were made on the effect of grazing alfalfa pasture upon: total blood phosphorus, plasma inorganic phosphorus, plasma trichloroacetic acid insoluble phosphorus, blood ammonia nitrogen, blood non-protein nitrogen, blood urea, total hemoglobin, methemoglobin, amide nitrogen of rumen ingesta, ammonia nitrogen of rumen ingesta and in vivo oxidation-reduction potential of rumen ingesta.

Blood samples were taken from the jugular veins of cattle soon after the bloat reached a maximum during any given grazing period. For the comparisons made in this study, an animal was considered to be bloated only if it rated a two or above on the bloat scale as described in

Table 3. Likewise, non-bloated animals were those that definitely showed no visible distention. Samples were not taken from animals if their bloat was between these scores. Since more bloat usually was experienced during the evening grazing period most of the samples were collected when the cattle were taken off pasture at the end of the evening grazing.

All animals were sampled at least once in a non-bloated state for determination of blood phosphorus. Samples were collected from both bloated and non-bloated animals as often as possible for determination of ammonia nitrogen, non-protein nitrogen and the various phosphorus components. Immediately after collection, samples were taken to the laboratory and prepared for analysis. The various phosphorus fractions were separated and stored for later analysis.

The procedure of Nathan and Rodkey (180) was used to avoid changes in the ammonia content. The protein was precipitated as the samples were drawn; to do this, 5.0 ml. of blood was added to 5.0 ml. of cold 20 per cent trichloroacetic acid and stored in an ice bath until taken to the laboratory. In the laboratory the samples were centrifuged immediately and the supernatant decanted into a test tube. Aliquots of the supernatant were taken for the ammonia analysis which was performed within 12 hours. The remaining portion of the sample was stoppered and stored in the refrigerator for determination of non-protein nitrogen at a later date.

Ammonia nitrogen was determined by the micro-diffusion technique of Brown et al. (38). In this method, the ammonia was released from the solution by addition of a saturated solution of sodium carbonate. The reaction was carried out in a glass dropping bottle and the ammonia collected on a drop of sulfuric acid which was held on a glass rod in the center of the bottle. The bottles were rotated on a wheel at 7 r. p. m. for 30 to 60 minutes so that the ammonia was completely released from the solution. The solution on the rod was washed into a volumetric flask with distilled water. The color was developed with sodium hypochlorite, sodium nitroprusside, sodium carbonate and sodium phenolate; readings were made with the Klett-Summerson colorimeter. Later in the season this method was modified slightly according to the method of Nathan and Rodkey (180). In this method, color was developed with ninhydrin and readings made with the Beckman model B spectrophotometer.

The method of Shahani and Sommer (229) for the determination of non-protein nitrogen in milk was modified for the determination of blood non-protein nitrogen. The blood was diluted with an equal volume of 20 per cent trichloroacetic acid and a 1.0 ml. aliquot of the filtrate used for the determination.

Total phosphorus in the blood was determined by the method outlined by Hawk et al. (105), p. 635. All blood samples were not analyzed for total phosphorus because it was impossible to store the samples of whole blood over an extended period of time and reagents and laboratory help were not always available to keep the analysis current.

Lipid phosphorus was separated from the plasma by precipitation with 10 per cent trichloroacetic acid according to the method of Zilversmit and Davis (266). The precipitation was carried out directly in a 30 ml. micro-kjeldahl flask; the entire flask was centrifuged and the supernatant decanted so that the precipitate would not have to be transferred. The supernatant was used to determine total acid soluble phosphorus and inorganic phosphorus.

Inorganic phosphorus was determined by the Method of Fiske and Subbarow as described by Hawk et al. (105), pp. 630-632. It was found that the concentration of trichloroacetic acid soluble phosphorus and the concentration of inorganic phosphorus were within experimental error of being equal. Therefore, in the studies to determine the effect of grazing upon various blood components, the acid soluble phosphorus fraction was not determined.

Results

A comparison of the levels of ammonia nitrogen and non-protein nitrogen in the blood of bloated and non-bloated cattle grazing alfalfa pasture is presented in Table 20. There was no difference in the levels of non-protein nitrogen between bloated and non-bloated animals. Although the ammonia content of the blood of bloated animals appeared to be higher than that of non-bloated animals, this difference was not significant at $P = 0.05$. The variation was quite high in both bloated and non-bloated groups. All ammonia determinations were carried out at least in triplicate, but there was considerable variation among the replicates. Any individual determinations in which the results were widely discrepant from the other replicates were discarded. It is possible that this may have caused some errors in the mean values as they were recorded. Certainly this technique needs some revision so that greater accuracy can be achieved in the future.

Table 20. A comparison of the levels of nitrogen compounds in bloated and non-bloated animals

	Bloated	Non-bloated
NH_3- N[a]	0.36	0.29
No. observations	27	15
Non-protein N[a]	56.4	56.5
No. observations	23	16

[a]Expressed as mg. per cent nitrogen.

Summaries of the blood phosphorus analyses are presented in Tables 21, 22 and 23. The three tables differ as to the bloat-susceptibility and state of bloat of the animals. In Table 21, all samples have been used to compare the various phosphorus components of bloated animals to those of non-bloated animals. The blood of bloated animals was higher in all phosphorus components measured than was the blood of non-bloated animals. The difference was significant at $P < 0.005$ in the two plasma fractions. The difference in total blood phosphorus was not significant (at $P = 0.05$); however, a smaller number of samples was analyzed for this component.

In order to prepare Tables 22 and 23, the average daily maximum bloat scores for all animals in the herd were summarized for the entire period during which these samples were collected. The animals were ranked by total bloat scores. Almost all animals bloated to some degree during

Table 21. A comparison of blood phosphorus levels between bloated and non-bloated animals after grazing alfalfa pasture

Phosphorus component[a]	State of animals	
	Bloated	Non-bloated
Total blood	23.3[b]	21.8
No. observations	30	13
Plasma inorganic	9.35[c]	7.73
No. observations	34	41
Plasma lipid	7.21[c]	6.12
No. observations	29	38

[a]All values are expressed as mg. per cent phosphorus.

[b]The difference between bloated and non-bloated animals was significant at $P < 0.25$.

[c]The difference between bloated and non-bloated animals was significant at $P < 0.005$.

the experiment but some were definitely bloat-susceptible while others were relatively unsusceptible to bloat. The blood phosphorus levels for the 15 animals with the highest bloat scores were compared to the values of the 15 animals with the lowest bloat scores. In Table 22, "bloat-susceptible" animals only were used to compare the blood phosphorus levels when bloated and not bloated. Although these observations were based entirely on the "bloat susceptible" group, all of the blood phosphorus components of the bloated animals were considerably higher than those of the non-bloated animals.

In Table 23, samples taken from animals that were not bloated at the

Table 22. A comparison of blood phosphorus levels between bloated and non-bloated "bloat-susceptible" animals after grazing alfalfa pasture

Phosphorus component[a]	State of "bloat-susceptible" animal	
	Bloated	Non-bloated
Total blood	23.3[b]	21.2
No. observations	30	4
Plasma inorganic	9.35[c]	8.11
No. observations	34	12
Plasma lipid	7.21[d]	6.33
No. observations	29	10

[a]All values are expressed as mg. per cent phosphorus.

[b]The difference between bloated and non-bloated animals was significant at $P < 0.10$.

[c]The difference between bloated and non-bloated animals was significant at $P < 0.005$.

[d]The difference between bloated and non-bloated animals was significant at $P < 0.05$.

end of the regular grazing period were used to compare the levels of the various phosphorus components in the blood of "bloat-susceptible" animals to those of "non-bloat susceptible" animals. In this case the "bloaters" tended to have higher plasma phosphorus values than "non-bloaters". The "non-bloaters" of Table 23 also had somewhat lower levels of the plasma phosphorus components than the non-bloated "bloat-susceptible" animals of Table 22.

During the latter part of the summer when little or no bloat

Table 23. A comparison of the blood phosphorus levels of "bloaters" and "non-bloaters" while in the non-bloated state after grazing alfalfa pasture

Phosphorus component[a]	"Bloaters"	"Non-bloaters"
Total blood	21.7	21.7
No. observations	3	10
Plasma inorganic	8.23[b]	7.54
No. observations	11	30
Plasma lipid	6.33[c]	6.04
No. observations	10	28

[a]All values are expressed as mg. per cent phosphorus.

[b]The difference between "bloaters" and "non-bloaters" was significant at $P < 0.05$.

[c]The difference between "bloaters" and "non-bloaters" was significant at $P < 0.25$.

occurred, a preliminary study was conducted to determine the effect of grazing upon levels of blood ammonia, urea, non-protein nitrogen, the various phosphorus fractions, ammonia and amide nitrogen of rumen ingesta and *in vivo* oxidation-reduction potentials of rumen ingesta. For the blood analyses, samples were taken from five selected animals before, during and immediately after the grazing period. Additional samples were taken one and one-half and three and one-half hours after the end of the grazing period and before, during and immediately following the second grazing period. Rumen ingesta samples were taken at the same time, relative to grazing but on different days, from the six fistulated steers. None of the animals used in these studies was bloated at any time during the days of sampling.

The effect of grazing upon blood ammonia nitrogen is shown in Figure 3. There was no apparent difference in the quality of July and August forage. It should be noted that the blood ammonia nitrogen did increase considerably during grazing and the peak values reached at the end of the grazing period were approximately equal to the average value for non-bloated animals obtained earlier in this experiment.

Blood urea was determined by the direct Nesslerization method of Koch and Hanke (144), pp. 182-183. The effect of grazing upon blood urea nitrogen and non-protein nitrogen is shown in Figure 4. There was a small rise in urea during the actual grazing period; however, there was a considerable increase for two or three hours following grazing. Grazing appeared to cause a small increase in blood non-protein nitrogen.

Apparently there was considerable difference in the quality of forage that the animals were grazing on the two days the urea, non-protein nitrogen and phosphorus analyses were conducted. Blood ammonia was not determined while the animals were grazing the better quality forage. The so-called better quality forage was growing faster, plants were taller and the plants appeared to be thicker on the ground than in the case of the poorer quality forage. The blood phosphorus levels of the animals on the good forage tended to be considerably higher than those of animals on the poorer quality forage.

The effect of grazing upon the various phosphorus components is summarized in Figure 5. The peak levels of all blood phosphorus components when the animals were grazing good alfalfa pasture were reached

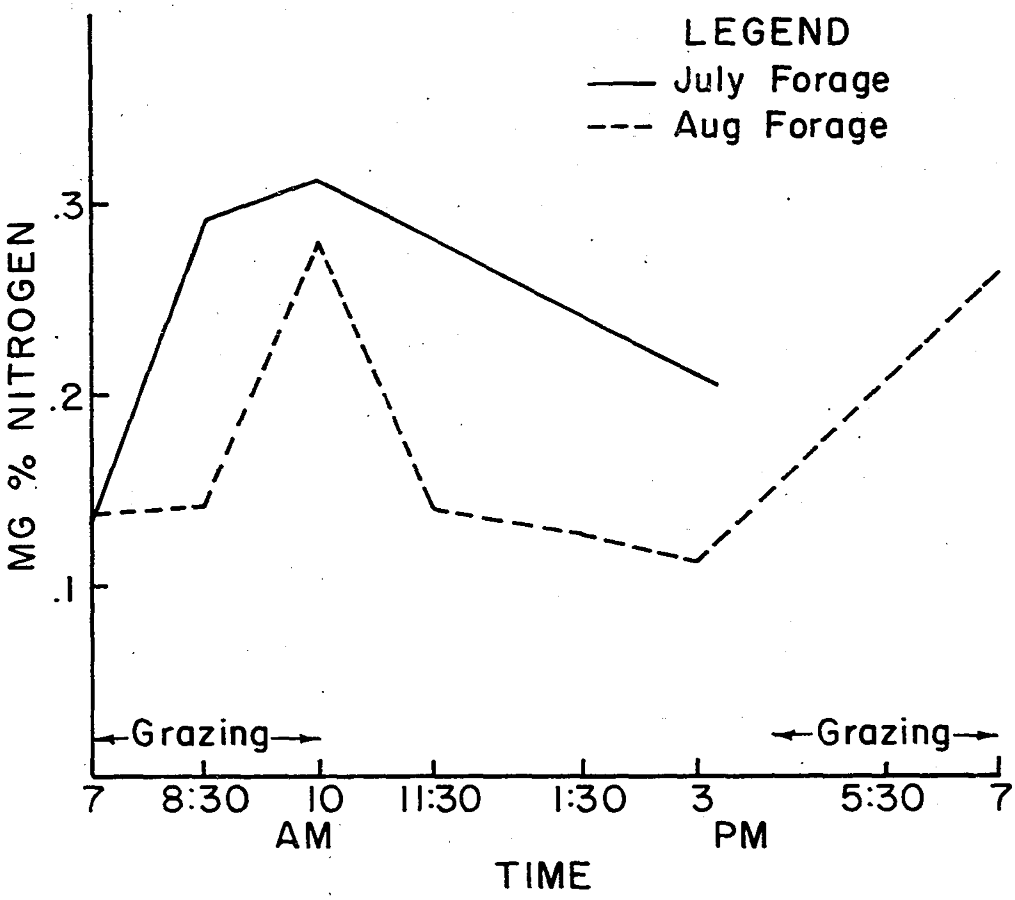

Figure 3. The effect of grazing alfalfa pasture upon blood ammonia nitrogen.

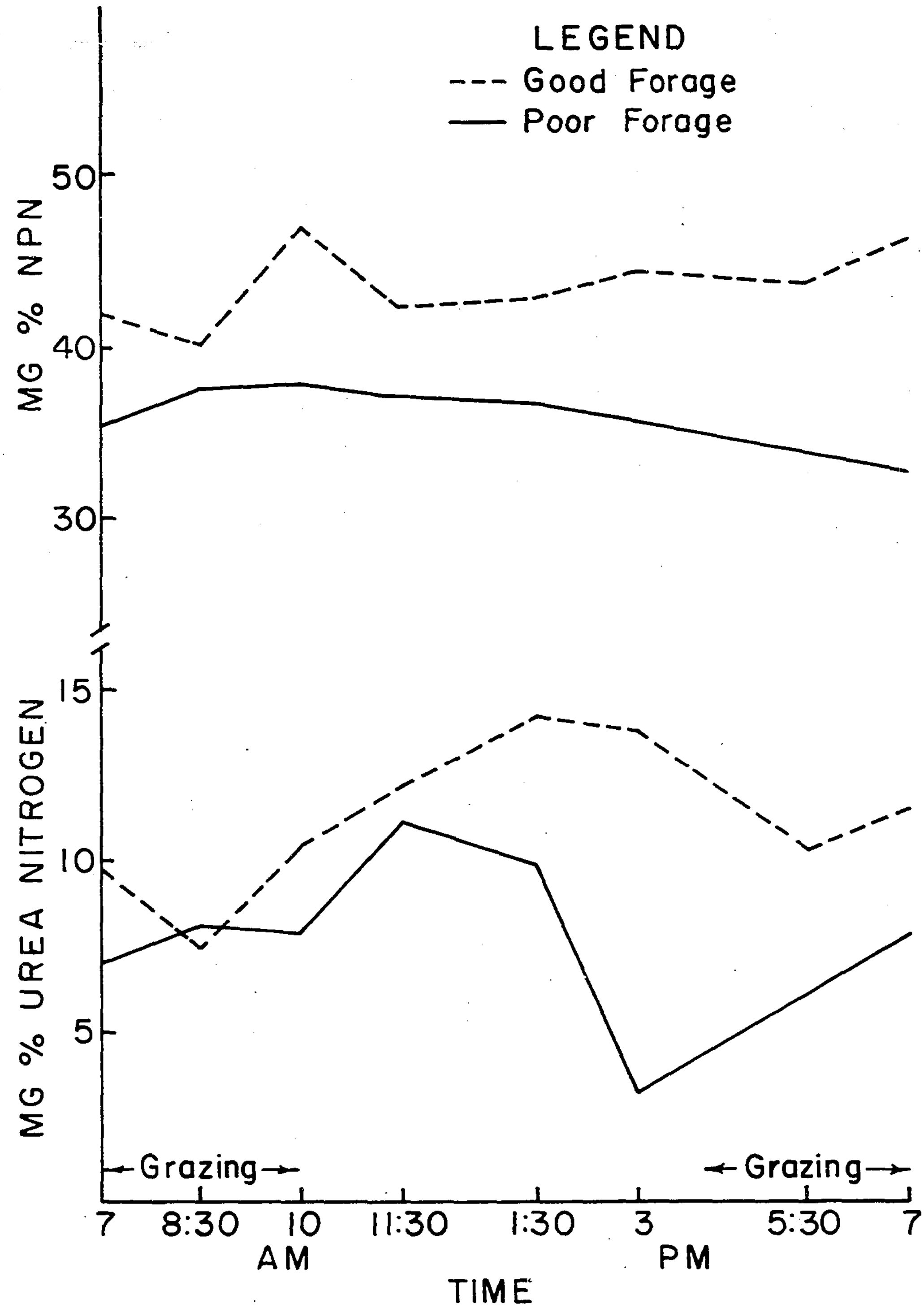

Figure 4. The effect of grazing upon blood urea and non-protein nitrogen.

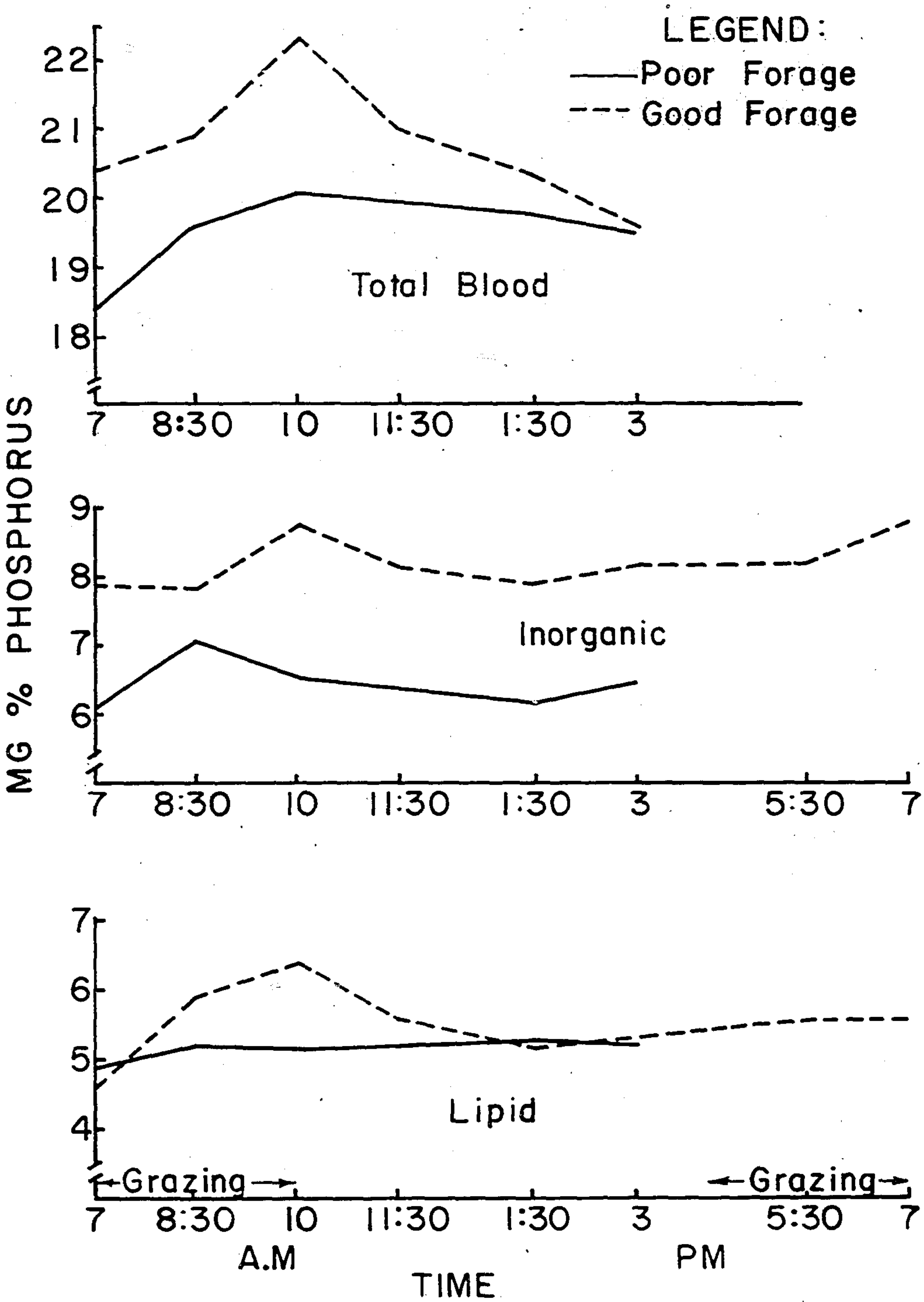

Figure 5. The effect of grazing upon total blood phosphorus, plasma inorganic phosphorus and plasma lipid phosphorus.

at the end of the grazing period.

Peaks while grazing the poorer forage were somewhat lower and tended to occur during the grazing period. Grazing apparently did not affect the level of plasma lipid phosphorus on the poorer quality forage; however, on the better quality forage, there was a considerable increase due to grazing.

Samples of rumen ingesta were collected from the fistulas of six steers; the liquid portion was separated immediately by straining it through four layers of cheesecloth. The fluid was collected in 250 ml. Erlenmeyer flasks, stoppered and chilled immediately in an ice bath to prevent any changes in the ammonia content due to enzymatic activity. Preliminary tests early in the season indicated that this was a satisfactory method for avoiding changes in the ammonia content for at least 24 hours.

In a preliminary experiment it was found that ammonia in rumen ingesta could be determined by steam distilling the ingesta with a saturated solution of sodium tetraborate. Using this method there was no hydrolysis of asparagine added to rumen ingesta; however, about five per cent of the amide nitrogen of added glutamine was found in the ammonia. The amount of glutamine in rumen ingesta is probably quite low in relation to the total ammonia content so the error introduced from this source should be insignificant. Furthermore, this technique is simple and rapid when compared to others that have been used. If the steam distillation is carried out with a solution of 50 per cent sodium hydroxide instead of the tetraborate solution, all of the amide groups are hydrolyzed. Thus, both amide and ammonia nitrogen can be determined quite simply and rapidly.

In this experiment rumen ammonia nitrogen was determined by steam distilling a mixture of 5.0 ml. of rumen ingesta and 10 ml. borate buffer, pH 10. The distillate was collected in 10 ml. of 4.0 per cent boric acid and titrated with standardized HCl. Rumen ammonia nitrogen plus amide nitrogen was determined by steam distilling a mixture of 5 ml. of rumen ingesta and 10 ml. of 50 per cent NaOH, collecting and titrating as with the ammonia. Amide nitrogen was then computed by difference.

The effect of grazing upon the levels of amide and ammonia nitrogen in the rumen is shown in Figure 6. Amide nitrogen was relatively constant throughout the day. Ammonia nitrogen increased considerably with grazing and continued to increase for about one and one-half hours following the grazing period.

Oxidation-reduction potentials in the six fistulated animals grazing alfalfa pasture were measured <u>in vivo</u> by using a Beckman model N pH meter equipped with a standard calomel reference electrode and a platinum electrode which were attached to the meter with lead wires six feet in length. Readings were obtained before, during, and immediately after grazing; one and one-half and three and one-half hours following grazing; and again before, during and immediately after grazing. These are shown graphically in Figure 7. With the exception of the reading prior to the first grazing period these values seem to be in the range of the <u>in vivo</u> redox potential of sheep receiving a high hay, low concentrate diet as reported by Broberg (34). In Experiment IV, the pre-grazing reading may not be correct

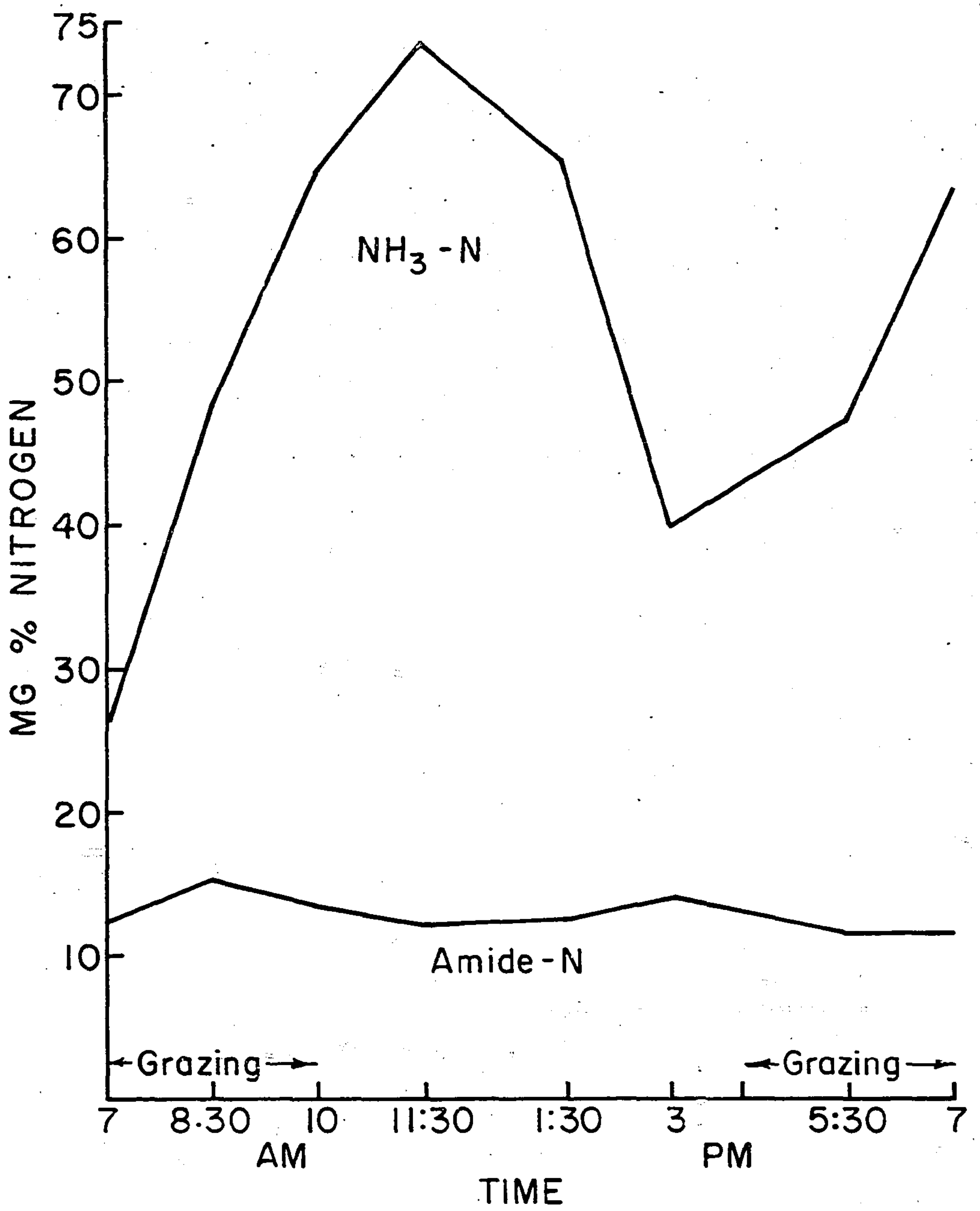

Figure 6. The effect of grazing alfalfa upon rumen ammonia and amide nitrogen.

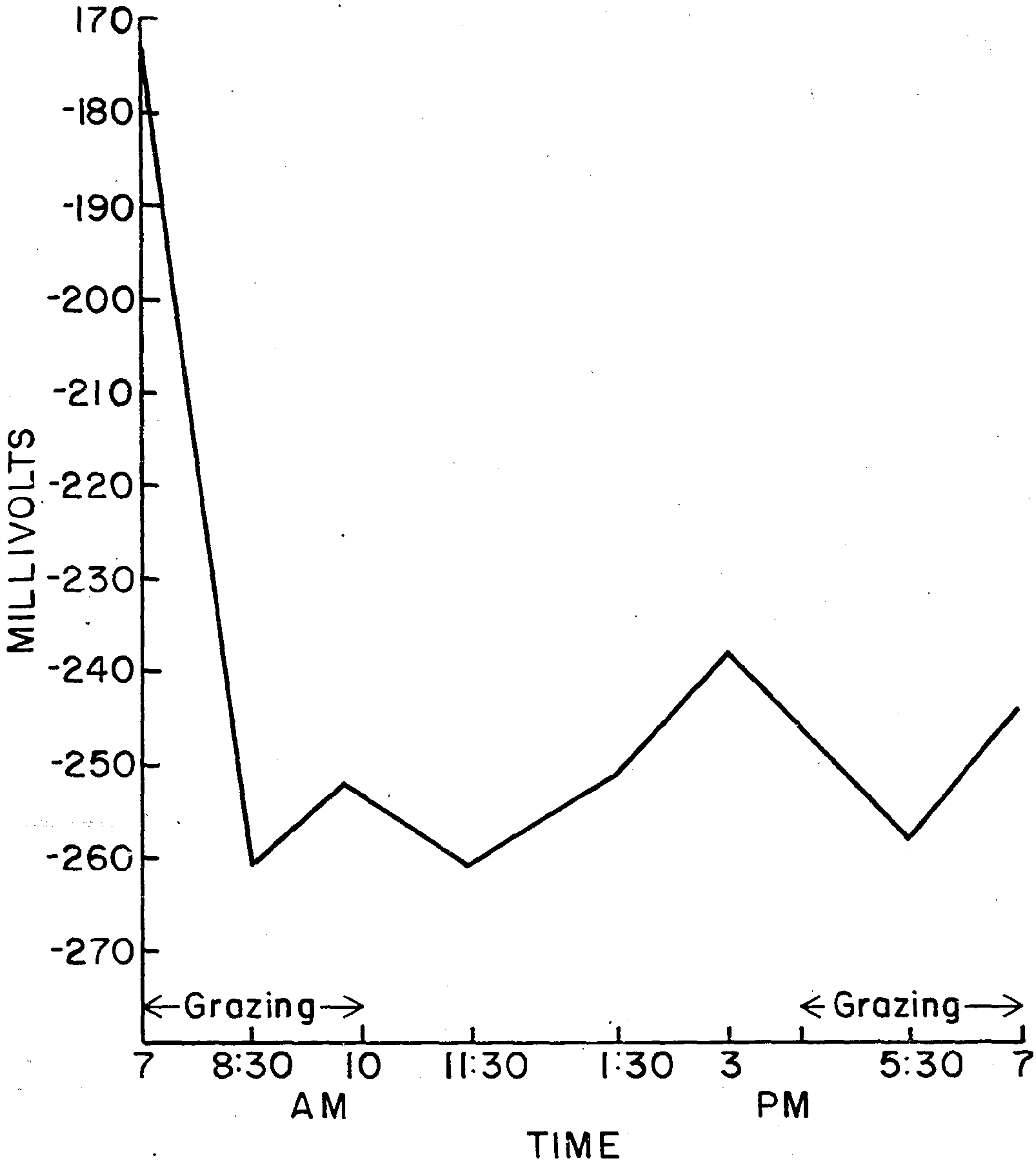

Figure 7. The effect of grazing alfalfa upon the in vivo oxidation-reduction potential of rumen ingesta.

because it is probable that the instrument was not allowed sufficient time to come to equilibrium. The quality of the forage was relatively poor the day these measurements were made and grazing seemed to have little or no effect upon the measurements.

For determination of methemoglobin and total hemoglobin, samples of venous blood were collected from each of eight animals before and after grazing good quality alfalfa pasture. The blood was drawn with a California bleeding needle which had a 10 inch piece of small polyethylene tubing attached to the posterior end. With the aid of the tubing, the blood was collected under paraffin oil in an oxalated test tube. By these precautions, exposure to the atmosphere was minimized and thus valid determinations of any of the hemoglobin compounds could be carried out.

Total hemoglobin and methemoglobin were determined by the method of Evelyn and Malloy (85). The results of determinations for the individual animals are shown in Table 24. Although there were considerable differences among animals, it appeared that grazing did not affect total hemoglobin but did tend to increase the methemoglobin concentration.

Discussion

Blood nitrogen components, especially ammonia, are maintained within relatively narrow limits in the normal animal. However, under certain conditions, as in the feeding of high levels of urea, the capacity of of the liver to detoxify the ammonia is apparently exceeded. In this instance, blood ammonia levels may increase considerably and a condition of urea toxicity results. A situation similar to this could exist in

Table 24. Effect of grazing upon the concentration of total hemoglobin and methemoglobin

Animal no.	Before grazing: Total hemoglobin[a]	Before grazing: Methemoglobin[a]	After grazing: Total hemoglobin[a]	After grazing: Methemoglobin[a]
1	14.88	0.570	11.87	0.414
2	11.43	0.414	11.43	0.829
4	12.90	0.000	12.31	0.518
5	12.68	0.466	12.90	0.829
6	11.95	0.518	11.07	0.673
7	12.31	0.518	12.90	0.570
11	13.41	0.623	13.05	0.570
13	14.15	0.725	16.27	0.623
Mean	12.84	0.479	12.72	0.628

[a]Values are expressed in grams per 100 ml.

the case of bloat on legume pastures -- although it has not been shown that high levels of blood ammonia are involved in bloat. Young, rapidly growing legumes are very high in protein and in non-protein nitrogen compounds. If these compounds were released very rapidly by bacterial fermentation in the rumen it is possible that the animal could be subjected to quite high levels of ammonia which in turn could be a contributing factor to the bloat syndrome.

In the present study the number of samples analyzed and the sensitivity of determination were not sufficient to detect a difference in the ammonia content of blood from bloated and non-bloated animals. However, these data are evidence that the difference in ammonia content of the blood of bloated animals is not appreciably different from that of non-bloated animals. Furthermore, it probably is not consistent from animal to animal on the same treatment or from day to day within the same animal. However, these data are not sufficient to indicate that the ammonia content of the blood is not involved in bloat.

From the data in this experiment it appears rather certain that the non-protein nitrogen content of the blood of bloated animals is not different than that of non-bloated animals.

Normally, blood mineral components are present in relatively constant amounts. Therefore, the changes and differences observed in blood phosphorus during this experiment are the basis for conjecture, are somewhat difficult to explain and seem to raise more questions than they answer. One would not expect animals on the same dietary regime to differ appreciably in levels of blood phosphorus. However, it may be that certain other conditions such as stress at the time of bloat may readily influence the phosphorus content of the blood. Unfortunately, hematocrit values were not determined during this study so it is not known to what extent some of the observed changes may be due to changes in the concentration of the formed elements of the blood.

If one were to accept the hypothesis of Cooper (58), and assume that phosphorus fertilization will reduce the incidence and severity of bloat and that small changes in the phosphorus content of the diet will be accompanied by changes in the levels of blood phosphorus, one would also expect an accompanying increase in blood phosphorus level when bloat is reduced by such a system. However, in this experiment it has been demonstrated that there was an increase in blood phosphorus during bloat. Since hematocrit values were not determined, it is not known how much of this may have been due to hemoconcentration. However, if the effect is real, it is in agreement with what one would expect if one accepts the hypothesis of Lienert (151) and Lienert and Kienel (152, 153, 154) which proposes that the high concentration of phosphorus in the forage plus the high level of phosphatase activity in the rumen is a primary factor involved in bloat.

The difference in blood phosphorus levels is not limited to the difference between bloated and non-bloated animals. There is also a difference between animals that are susceptible to bloat and those not susceptible to bloat. Bloat-susceptible animals tended to have higher levels of blood phosphorus than animals that were not susceptible to bloat. Perhaps this means that bloat-susceptible animals have more phosphatase activity in the rumen which in turn may cause an increase in blood phosphorus. On the other hand, there could be a difference in hematocrit values between bloat-susceptible and non-bloat-susceptible animals which in turn could cause a difference in the phosphorus concentration in whole blood and plasma.

The nature of physiological changes that may be caused by such differences in blood phosphorus levels is not clear. It would seem unreasonable to suppose that bloat itself or death from bloat could result from the observed changes in blood composition.

Field observations indicated that phosphorus fertilization increased the rate of plant growth which in turn caused an increase in bloat. Any time the balance of nutrients in the soil is improved, there should be an accompanying increase in plant growth. Undoubtedly bloat is associated with rapid plant growth; if the rate of plant growth increases, bloat is also likely to increase. Therefore, it is unreasonable to suppose that bloat can be reduced by a fertilization practice that will increase the rate of plant growth.

After finding changes in blood components due to bloat, it was thought that some changes could be associated with periods of grazing independent of bloat. The observations of the effect of grazing upon blood components in this experiment were based on a few animals and very few replications; therefore, they must be considered as preliminary in nature.

There was a two-fold increase in blood ammonia during the grazing period. Since the values at the end of the grazing period were approximately equal to the average value found for non-bloated animals it is probable that blood ammonia is not affected by the quality of the forage. This assumes that forage quality was best when bloat was prevalent and was poorer during the period when this study was made because no bloat occurred during this time.

Blood non-protein nitrogen was materially affected by quality of the forage. On forage that did produce bloat the average value for non-protein nitrogen was considerably higher than the peak values found in this phase of the study for forage that did not produce bloat. Blood urea nitrogen also seemed to be influenced somewhat by the quality of forage.

The change in urea nitrogen occurred after, rather than during, the grazing period. This indicates that urea continued to be synthesized rapidly after grazing. Most of the change in non-protein nitrogen occurred during the grazing period, but most of the change in urea nitrogen occurred after the grazing period. Therefore, other differences in the levels of nitrogenous compounds of the blood must be evident during the grazing period. These constituents are probably affected by grazing and by forage quality.

In Figure 5 one can compare the effect of forage quality upon the levels of the various phosphorus components of the blood. It is evident that good forage causes a considerable increase in blood phosphorus. However, neither forage used in this phase of the experiment was of sufficient quality to produce bloat. If one compares the peak values found here with the values found for bloated and non-bloated animals from Table 21, it is evident that the values for bloated animals are higher than the peak values while the values for non-bloated animals fall in between the peak values from the two different types of forage. According to the standard of quality used, the forage that produces bloat should be of better quality than that which does not produce bloat. If one considers the blood phosphorus levels of non-bloated animals to be normal, then either blood phosphorus levels were not affected by forage quality or forage quality was not properly evaluated during this experiment. A great many more observations are needed in order to properly determine the effect of forage quality on blood phosphorus levels. Undoubtedly this is also true of the effect of bloat upon blood phosphorus levels.

The changes observed in the rumen ingesta of cattle grazing alfalfa pasture are probably what one would expect. Since green alfalfa is high in protein and non-protein nitrogen, one would expect a considerable increase in ammonia as the concentration of nitrogenous material increases in the rumen and ammonia is released by increased microbial activity. This increase continues throughout the grazing period and for a time following grazing. More complex changes in the microbial metabolism of nitrogenous materials would be necessary before one could expect a change in amide nitrogen due to bacterial metabolism.

There is an apparent decrease in the oxidation reduction potential as cattle graze after an overnight fast. There is some question about the validity of the first values because these were the first measurements made and it is possible that the instrument was not allowed sufficient time to come to equilibrium. However, the values throughout the rest of the day are in agreement with the <u>in vivo</u> measurements made by Broberg (34) in sheep receiving a high hay-low concentrate diet.

Methemoglobin could possibly be a contributing factor to death from bloat. It is reasonable to suppose that methemoglobin could be produced by the high concentrations of nitrite and nitrate in green legumes. Furthermore, blood flow and respiration are restricted somewhat during bloat; thus any decrease in the oxygen-carrying capacity of the blood, as would be the case if methemoglobin were produced, could contribute to the cause of death by asphyxiation. In this experiment methemoglobin was increased by grazing while hemoglobin concentration was unaffected. However, this work is preliminary and further work is needed to determine whether this is true. The report of Moore and Dracy (176a) indicates that neither hemoglobin nor methemoglobin is associated with bloat.

Experiment V -- Other Trials Related to Bloat

Phase 1. Effect of monosaccharides on the in vitro fermentation of rumen ingesta

Experimental procedure and results During the early spring of 1956, a preliminary trial was conducted in an effort to determine the effect of monosaccharides on the fermentation of rumen ingesta. On two different days, samples of rumen ingesta were collected via stomach tube from cattle on a normal ration of hay and grain. Ingesta were mixed with an equal volume of alfalfa juice which had been obtained from fresh alfalfa by processing with an Anderson Expeller the previous fall -- the juice had been frozen and stored over the winter. Samples of 400 ml. of this mixture plus 0.0, 1.25, 2.5 and 5.0 grams of glucose and 0.0, 1.25, 2.5 and 5.0 grams of fructose were incubated in the artificial rumen. Samples were taken at zero, one-third, one, two, four and six hours for the determination of surface tension, foam volume, foam stability and ingesta-volume-increase. Results were inconclusive and it appeared that the technique would need considerable modification in order to obtain valid results.

Discussion It has been known for some time that sugars in general will stimulate the activity of rumen bacteria. The preliminary experiment conducted in this phase of Experiment V was an attempt to determine whether glucose or fructose is more effective in the stimulation of rumen bacteria. The technique used in this trial was a rather crude attempt to measure differences which are apparently of a rather small magnitude. From the results of chemical analyses of bloat-producing and non-bloat-producing forage as reported in Experiments I and II, it is doubtful whether differences in the monosaccharide content are responsible for differences in bloat. However, if one were interested in pursuing this line of investigation, it would be desirable to use several replications and perhaps to limit the measurement of response to one criterion such as ingesta-volume-increase.

Phase 2. Observations with malonic acid and fresh eggwhites

Experimental procedure and results In a series of preliminary observations with malonic acid it was found that the addition of malonic acid to rumen ingesta produced an abundance of relatively unstable foam. The addition of an equal volume of filtered human saliva seemed to increase the stability of this foam. When a small amount of dilute acetic acid was added to rumen ingesta the foam produced by the addition of malonic acid was much more stable.

The administration of 10 grams of malonic acid via gelatin capsule to a 125-pound Jersey calf receiving a normal ration of milk, hay and grain produced no bloat or signs of excess gas production. A few days later, 10 grams of malonic acid was dissolved in water and given to this calf via stomach tube. Again there was no outward sign of excess gas production.

Later, 50 grams of malonic acid was dissolved in a quart of water and administered to a 750-pound Holstein steer that was on a bloat-producing concentrate ration. The steer was slightly bloated before the administration and showed a definite increase in distention within 10 minutes afterward. One week later, 25 grams of malonic acid and the whites of one dozen eggs were given to the same animal. No bloating was observed. Three days later, the whites of two dozen eggs and 50 grams of malonic acid were administered to the same steer. Again no bloating was observed.

Two weeks later, this steer was given the whites of four dozen eggs at a time when his bloat was rated a 2. One and one-half hours later, no change in bloat was noted; he was then given 50 grams of malonic acid. No increase in bloat was observed for some time; however, one and one-half hours after the malonic acid was given, the animal was in considerable distress (scored a 4) but within an additional one-half hour had returned to a bloat score of 2.

Attempts to produce bloat by administering eggwhites to cattle receiving alfalfa soilage were unsuccessful both when eggwhites were mixed with the forage and when one to three pints of fresh eggwhites were given by stomach tube one hour after the feeding of alfalfa soilage.

Discussion Rosen *et al.* (218) associated malonic and other organic acids with the production of large amounts of gas by rumen micro-organisms. This was confirmed in a qualitative fashion by the work in this phase of Experiment V. At about the same time, Boda *et al.* (29) reported the production of bloat by administration of fresh eggwhite. In the trials conducted in this phase of the experiment it was hoped to be able to combine these two techniques to produce bloat. However, several trials resulted in the production of very few cases of bloat and it appears that certain necessary physiological conditions must be present in order to produce bloat by the administration of reasonable amounts of the "catalysts". This is confirmed by the increase in severity of bloat that was produced in the one case where eggwhite and malonic acid were administered to a bloated animal.

Phase 3. Effect of penicillin feeding on feed efficiency and weight gains

Experimental procedure Twelve calves were divided into pairs and the animals of each pair were randomly allotted to each of two groups. All animals received four pounds of a concentrate mixture (corn 37.0 per cent, oats 30.0 per cent, wheat bran 20.0 per cent, soybean oil meal 10.0 per cent, steamed bone meal 2.0 per cent, and salt 1.0 per cent) per day and good quality alfalfa hay *ad libitum*. One group received 0.1 mg. penicillin per pound of body weight every fourth day; this was given orally via gelatin capsule. Feed consumption and animal weights were measured daily.

Results Results are summarized in Figures 8 and 9. There were no significant differences in feed efficiency, weight gains or feed consumption, but the weight gains of the penicillin treated animals were consistently lower than those of the controls.

Discussion This phase of the experiment was conducted to determine the effect of penicillin administration according to a system similar to that Barrentine *et al*. (13) used for bloat control. These workers reported efficient control of bloat by the administration of penicillin

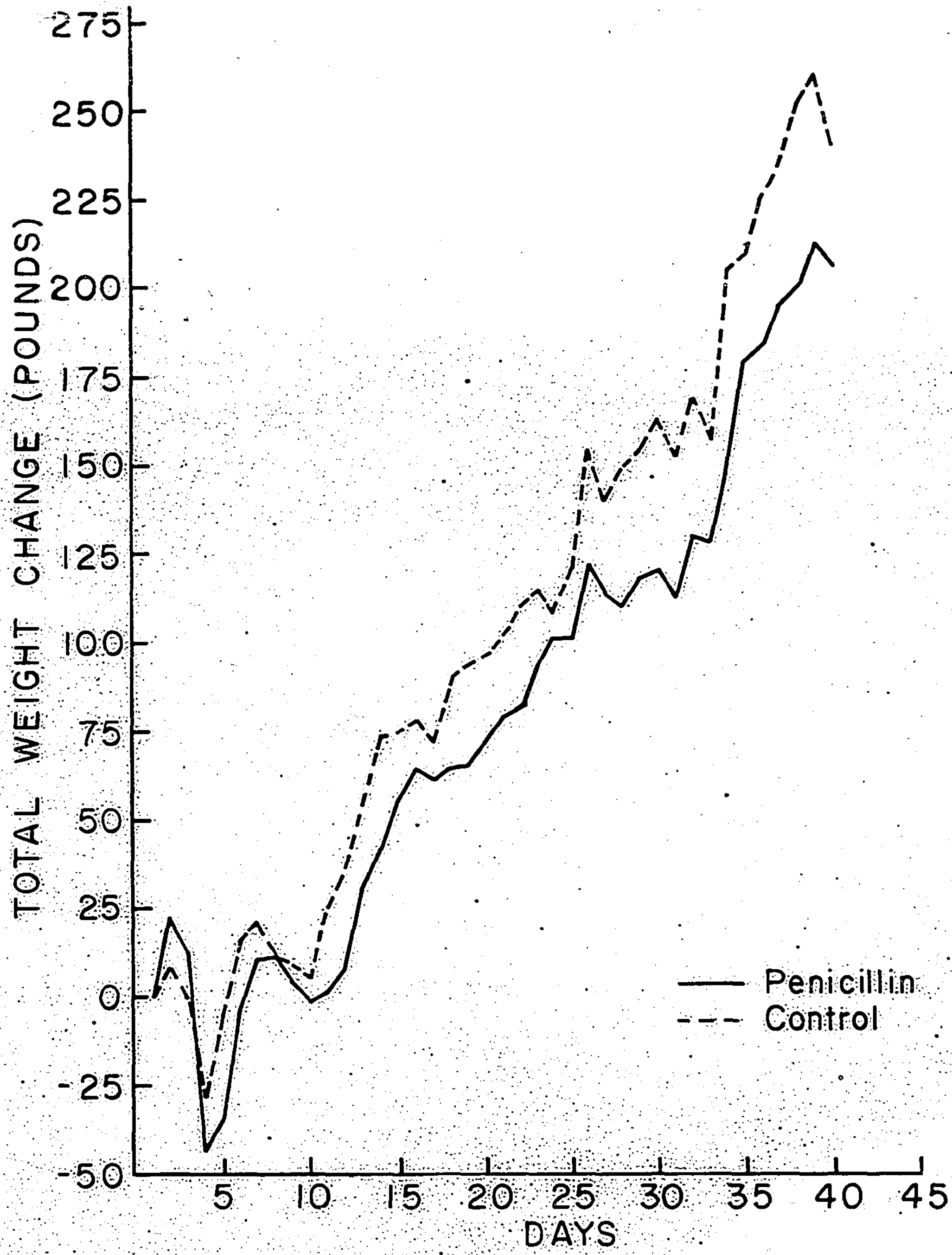

Figure 8. The effect of penicillin upon body weight changes of calves fed a ration of hay and grain.

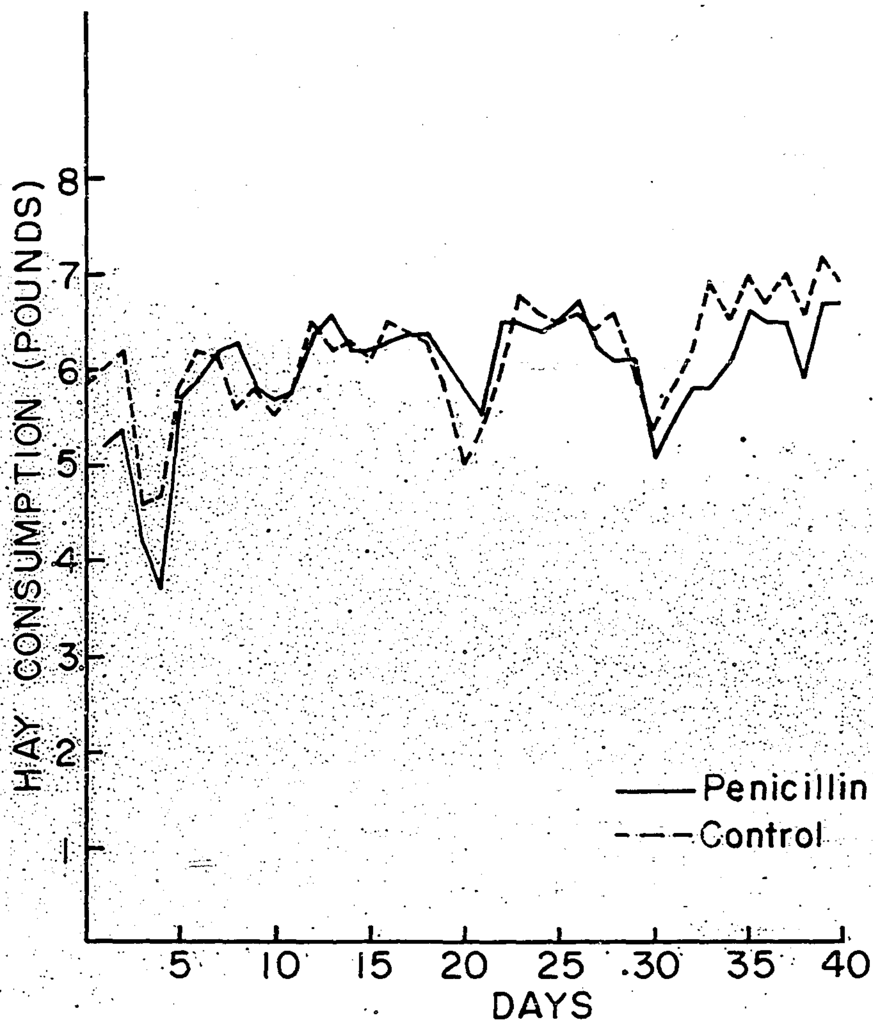

Figure 9. The effect of penicillin upon hay consumption of calves fed a limited amount of grain and *ad libitum* alfalfa hay.

about every third day; however, daily administration of penicillin, as reported in Experiment I, was an ineffective means of controlling bloat. Penicillin administered daily had little or no effect upon weight gains during Experiment I. There was no statistically significant effect on weight gains due to the administration of penicillin every fourth day in this experiment, but the weight gains of penicillin treated calves were consistently lower than those of the controls. The review of antibiotics as growth stimulants for dairy cattle (Lassiter, 148) points out the fact that penicillin has no beneficial effect upon the weight gains of calves.

It apparently makes little difference in the average daily gains whether penicillin is administered daily or every fourth day. This may indicate that the overall effects of the two methods of administration are quite similar. In fact, at the present time, it appears that there is little difference in the length of the bloat preventive effect of the two methods of administration. The bloat preventive effect diminishes after 10 to 14 days regardless of the method of administration or the level of antibiotic used.

GENERAL DISCUSSION

In spite of the large amount of research on the bloat problem in recent years, the two most basic questions concerning bloat, i.e., (a) the cause of bloat, and (b) the cause of death from bloat, remain unanswered. The research reported herein provides some help in the practical aspects of controlling bloat and points out some of the basic physiological changes that take place during bloat, but it poses many more questions than it answers.

There are many phases of the agronomic aspects of the bloat problem that are not understood. For example, there is little discernible difference in a pasture that will cause bloat and one of the same plant species that will not cause bloat. Further, more bloat is produced on legume pasture when an abundant supply of rapidly growing forage is present; this indicates that plant factors are involved in bloat etiology. At times, nearly all animals in the herd will bloat while on other occasions only a few animals bloat indicating that plant factors are more intense at some times than at others.

The identity of the plant factors that influence bloat is not known. Little relationship has been noted between chemical composition of the forage and bloat incidence or severity even though quite extensive chemical analyses have been conducted. Plant factors responsible for bloat may be the structure of cell walls and the form of, rather than the presence of, certain compounds.

There also may be considerable differences in bloat-producing ability among the varieties of alfalfa and other legumes. It may be possible to find varieties that are less bloat provocative than the ones used at present. The differences, if any, may provide some insight to the identity of the plant factors involved in bloat. The investigation of varietal differences in forages is a complex project, but may be one of the most practical approaches to the final solution of the problem.

Cooper (58) stated that bloat occurs because of fertilization programs which do not include sufficient phosphorus. He proposes that phosphorus be added to the soil so that the alfalfa plants will have a nitrogen:phosphorus ratio of 11:1. From all indication, however, phosphorus fertilization is likely to increase the rate of plant growth. The optimum rate of plant growth will probably produce the greatest amount of bloat. However, it may be possible to achieve such a balance of nutrients by fertilization that plant growth may be satisfactory and the amount of bloat decreased. This of course is only speculation at the present time.

The actual cause of death from bloat is another question that remains unanswered. The pressure in the rumen during bloat obviously causes many physiological changes. For example, circulation of blood in the area of the rumen may be inhibited because of the pressure. However, the most important factor involved in death from bloat may be that the gas-extended rumen forces the diaphragm into the thorax, thus considerably restricting the volume of the lungs and hampering the ability of the diaphragm to assist in respiration. During bloat, the partial pressure of carbon dioxide in the rumen is great; this tends to increase the concentration of carbon dioxide in the blood. If the lungs are unable to remove the carbon dioxide from the blood rapidly enough, death by asphyxiation may result. Animals dying of bloat appear to die of asphyxia. It is also possible that toxic factors which will further reduce the oxygen carrying capacity of the blood and hasten the asphyxia may be absorbed from the rumen.

Changes in the composition of the blood during bloat may be caused by the physical effects of pressure, the absorption of "toxic" substances or a combination of many other conditions present during bloat. The effect of increased pressure in the rumen upon blood composition has been investigated to some extent by Shinozaki et al. (236). These workers reported some changes in leucocytes, an increase in blood sugar and lactic acid, but no changes in blood ketones or non-protein nitrogen. However, it is possible that many changes take place which have not been observed. The question to be answered is whether observed changes take place as a result of the physical effect of pressure or because of other factors.

It appears that increased pressure in the rumen should change the absorption gradient so that substances would be absorbed into the blood stream more rapidly. However, other factors must be considered. In the case of bloat, air is incorporated into the ingesta which, in turn, forces more of the ingesta into contact with the rumen mucosa thus increasing the effective absorptive surface. Opposing this, the pressure at the time of bloat is likely to restrict blood flow in the area around the rumen. The decreased rate of blood flow may tend to decrease the rate of absorption due to the higher concentration of blood components present in the immediate area. The increase in blood phosphorus during bloat in the present study and the results of Shinozaki et al. (236) indicating higher levels of blood sugar and lactic acid could mean that the overall rate of absorption is increased. However, as Shinozaki pointed out, other blood components do not show this increase.

At the present time it is not known how the observed changes in blood phosphorus are related to bloat. One could test this by increasing the level of phosphorus in the diet of a group of animals grazing alfalfa pasture to determine whether this has an effect on bloat. If one can maintain higher levels of blood phosphorus by this method and if blood phosphorus is a causative factor of bloat, then theoretically, animals with higher levels of blood phosphorus should bloat more. However, if bloat is the cause of higher levels of blood phosphorus there would be no difference in bloat. Blake (23) fed trisodium phosphate and found no increase in bloat; however, blood phosphorus levels were not measured.

In order for any method or product to be a successful bloat preventive, its use must be compatable with farm management programs. When cattle are given daily allowances of concentrates, bloat preventives can be incorporated into the concentrate mixture. However, many cattle on legume pastures are not fed concentrates; in these cases a practical bloat preventive could be administered in the drinking water or in the salt. However, if a single administration of the preventive were effective for long periods of time, it could be given by capsule, injection, implantation or other means. At the present time, it appears that one possible solution is an antibiotic or combination of antibiotics that are effective at practical levels over a long period of time.

In the selection of bloat preventives to be administered either in the drinking water or in the salt other factors to be considered include: (a) the preventive must exert its effect for a sufficient length of time to provide protection throughout the day, (b) dosage levels must be high enough so that the animal receives sufficient preventive even though salt or water consumption may become quite irregular at times, (c) the cost must be reasonable, and (d) the product must not lose its effectiveness when administered in the salt or drinking water.

Many of the practices that have been recommended as bloat preventives by researchers have not been thoroughly tested by controlled experiments. For example, grazing of grass and legume mixtures has been widely recommended; however, farmers frequently report serious bloat when grasses make up a large proportion of the pasture mixture. Research workers have not yet established what mixtures are successful or in what proportions one could expect control of bloat. Another practice that has been recommended for bloat control is providing hay for the animals to eat while on pasture. Some workers have controlled bloat successfully in this manner, but others have found no reduction in bloat. Perhaps more hay or a specific type of hay is required.

Animals on the same ration may be greatly different in their susceptibility to bloat; at the present time it is not clear why these differences exist. However, when the rations are controlled carefully it is apparent that these differences must be due to animal factors. These factors probably are the ones that control the environment of the microorganisms in the rumen -- for example, differences in salivary secretion or composition of saliva, differences in rates of absorption of volatile fatty acids or differences in rate of passage down the tract.

More basic work is needed to determine the quantity and composition of saliva secreted on various diets and to ascertain the relationship of saliva to bloat. Similar comparisons should be made on differences among animals in the rate of absorption of volatile fatty acids, rate of passage of ingesta from the rumen and differences in microbial population. In reality, we have little understanding of the complete function of the rumen bacteria. Much fundamental work is needed in this area to provide answers which will be helpful not only in understanding phases of the bloat problem but also in solving many of the perplexing problems in the nutrition of ruminants.

The results of the foam measurements in the present study should not be taken as evidence that foam volume and stability are not important *in vivo* factors involved in bloat etiology. Johns (127, 133, 134) and Johns *et al.* (135) feel that the formation of a stable foam is an important factor in most cases of bloat. Anyone observing ingesta removed from a bloated animal would agree that foam is involved. The physical chemistry of foam formation as it occurs under conditions existing in the rumen poses a challenging area of research.

The large volume of froth formed in the rumen during bloat may almost completely fill the rumen. Several workers have proposed that froth blocks the cardia in some way so the animal cannot expel the free gas as it accumulates. Dougherty and Habel (75) and Dougherty *et al.* (76) demonstrated a cranial esophageal sphincter which is very active in the eructation reflex and is readily stimulated by gas pressure but which will remain closed under considerable pressure from water or ingesta. If ingesta in the form of froth completely fill the area of the cardia, the action of this sphincter may make it impossible for the animal to obtain relief by eructation; thus, it may be necessary for the gas to be released from the froth before the animal can relieve the pressure. However, there are exceptions to this; some animals are able to vomit ingesta when severely bloated and obtain relief in this manner.

On the other hand, if some free gas is present the animal often can remove it by eructation. If the foam is not too stable it may break down rapidly enough so the animal can eructate again in a short time thus keeping the ruminal pressure relatively low. Then, too, eructation may reduce the pressure enough to physically rupture some of the bubbles in the froth, releasing free gas.

Apparently a great many factors including some from plants and others from the animal must be present in order for the stable froth to be formed. Some of the plant factors that may be involved include saponins and soluble proteins. The plant factors, although necessary for the formation of the stable froth, are likely to vary but little from animal to animal on the same pasture, therefore animal factors must account for the differences in tendency to form a stable froth in the rumen when animals are grazing legume pastures.

It has been observed that animals that have eaten large quantities of certain types of hay and animals that have been grazing non-bloat-producing pasture are not likely to bloat the first time they are allowed to graze a bloat-producing pasture. There may be several reasons for this, including the following: (a) scabrous material present in the rumen may stimulate eructation so that the gas cannot accumulate, (b) scabrous material may prevent by physical means the formation of a stable froth, (c) certain changes in the ruminal flora may be necessary before bloating can occur, and (d) animals may preferentially select non-bloat-producing plants during the first grazing.

At the present time, feed lot bloat poses numerous questions needing considerable research. As cattle feeders strive for more rapid and efficient gains, the problem of feed lot bloat claims an ever-increasing share of the profit. Current interest in complete rations and pelleted feeds may accentuate this problem. To date, no method short of a drastic change in the feeding program has been successful generally, in preventing these losses. Feeders are naturally reluctant to alter feeding regimes if it means later marketing time, an increase in labor requirements, and/or an increase in physical facilities.

Jacobson (123) and Jacobson et al. (125, 126) have reported the most significant work on feed lot bloat to date. These workers found that a considerable length of time is required for most animals to begin to bloat on a given feed lot ration and that encapsulated organisms are associated with feed lot bloat. However, little is understood about the etiology and prophylaxis of feed lot bloat at the present time.

In feed lot bloat, as in pasture bloat, the formation of a stable foam is intimately involved. At the present time it is not known whether the encapsulated organisms are necessary for the formation of a stable foam. It may be possible to prevent the formation of these organisms with antibiotics which in turn, may prevent the formation of stable foam. In this study, penicillin did not prevent bloat; however, the animals used in this experiment had been treated previously with penicillin and the antibiotic may have lost its effectiveness.

Smith (238) found that methyl silicone and lipase treated cream are effective in reducing foam during feed lot bloat; however, Blake (23) was unable to reduce feed lot bloat with methyl silicone. Preliminary trials conducted during this study indicate that fats, including soybean oil and lard oil, are ineffective in reducing this type of bloat. Further research is needed to elucidate more effective foam inhibitors for control of feed lot bloat.

There may be some means whereby animals can be tested to determine whether they are susceptible to bloat. Most researchers feel that the heritability of the tendency to bloat is quite high; however, on many farms bloat occurs only infrequently and no records are kept of animals that do bloat. Thus animals are not selected on the basis of bloat-susceptibility and selection on this basis may not be practical. The present study suggests two possible indications of whether an animal is susceptible to bloat. The phosphorus work indicates that bloat-susceptible animals have higher blood phosphorus values than non-bloat-susceptible animals. Rumen ingesta studies suggest that the ingesta of bloat-susceptible animals have higher surface tension values than those of non-bloat-susceptible animals. If either of these measurements is highly correlated with bloat, then it could be used as a means of selecting animals on the basis of bloat-susceptibility.

There has been much progress in bloat prophylaxis in recent years. The incorporation of oil into the concentrate mixture of cattle grazing alfalfa is a practical means of controlling bloat for relatively short grazing periods. Bloat can be completely controlled if the farm operator can use a soilage feeding program and add a small amount of oil to the forage when bloat is likely to occur. The control of bloat with antibiotics, although still far from perfect, is an important contribution to bloat prophylaxis and etiology. Further work may develop the use of these and other preventives sufficiently so that they will eliminate the danger of bloat when used properly.

Future progress in the control of bloat also will come about through research on many of the basic problems that need to be answered in ruminant nutrition. A satisfactory solution to the bloat problem is likely to depend upon extensive cooperative research among nutritionists, biochemists, physiologists, bacteriologists, agronomists, physical chemists, veterinarians, and many others. If the large basic problems can be solved, the answers should point the way to final solution of the bloat problem.

SUMMARY

Five experiments were conducted on various phases of the bloat problem. In Experiments I and II, cattle were confined to dry lots and were fed alfalfa soilage. When only the top few inches of the alfalfa plant were used, or when the forage was young, lush and growing very rapidly, bloat was produced quite effectively by this system. However, when the plants were cut only a few inches above the ground, the animals were forced to eat more of the stemmy portion of the plant and less bloat resulted than when cattle were grazing comparable forage. There was no consistent difference in the chemical composition of bloat-producing and non-bloat-producing forage.

During the early phases of Experiment I, oral administration of penicillin was quite effective as a bloat preventive. However, after animals had been given penicillin over a considerable period of time, there was no reduction in bloat due to penicillin.

The feeding of 0.25 lb. of crude soybean oil per 1000 lb. body weight per day with the alfalfa soilage was a very effective means of controlling bloat. In fact, on several days when severe bloat was evident, nearly all animals in the control group bloated while there was essentially no bloat in the group that received soybean oil. Once daily administration of n-decyl alcohol appeared to have little bloat preventive effect; however, the amount of bloat during this period was very low. This product has not been tested as a preventive under conditions of severe bloat.

Lard oil and n-decyl alcohol were effective therapeutic agents for

treatment of animals suffering from severe bloat.

Feed lot bloat was studied during Experiment III. This type of bloat was produced on a variety of concentrate mixtures. The most critical factors in the experimental production of feed lot bloat seem to be careful control of the roughage intake and length of time on the diet. No effective method of preventing feed lot bloat was found.

Rumen fluid characteristics that were associated with bloat both on alfalfa and in the feed lot included surface tension, ingesta-volume-increase and per cent solids. These three measurements were significantly higher in ingesta from bloated animals than in ingesta from non-bloated animals. Ammonia increased with bloat on alfalfa but was not measured with cattle on the feed lot bloat diets.

Chemical changes in the composition of the blood associated with bloat and grazing were measured during Experiment IV. Blood ammonia and non-protein nitrogen changed but little during bloat. Whole blood phosphorus tended to increase during bloat. Plasma inorganic phosphorus and plasma lipid phosphorus were significantly higher in bloated animals than in non-bloated animals. All of these constituents increased somewhat during grazing and were higher when the animals were grazing good forage than when grazing poor forage.

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