A STUDY OF SOME CHEMICAL AND PHYSICAL PROPERTIES OF RUMEN FLUID AS RELATED TO FROTHY BLOAT IN CATTLE

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Richard A. Phelps

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A STUDY OF SOME CHEMICAL AND PHYSICAL PROPERTIES OF RUMEN FLUID AS RELATED TO FROTHY BLOAT IN CATTLE

By

RICHARD A. PHELPS

AN ABSTRACT

Submitted to the College of Agriculture of Michigan State University of Agriculture and Applied Science in partial fulfillment of the requirements for the degree of

MASTER OF SCIENCE

Department of Dairy

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ABSTRACT

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Rumen fluid samples from two fistulated dairy steers were subjected to various chemical and physical determinations. The animals were fed one non-froth producing and two froth producing rations to ascertain the possible relationship of frothy rumen fluid to frothy bloat.

The results of this study showed that there was wide variability of rumen fluid nitrogen fractions between animals on the same ration. There was also considerable daily variation of rumen fluid nitrogen.

Total soluble rumen fluid nitrogen per se did not appear to influence froth production.

The determination of nonprotein and protein nitrogen of rumen fluid by trichloroacetic acid precipitation did not appear to be a valid method due to incomplete precipitation of the proteins.

Heat coagulable and non-heat coagulable rumen fluid nitrogen values, when expressed as a percentage of the total soluble nitrogen, were extremely variable between both rations and animals, and they could not be correlated with froth production.

Rumen fluid nonprotein nitrogen as determined by an alcohol precipitation method, displayed considerable correlation with frothing, when it was expressed as a percentage of the total soluble nitrogen.

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Rumen fluid nonprotein nitrogen as determined by an alcohol precipitation method, displayed considerable correlation with frothing, when it was expressed as a percentage of the total soluble nitrogen.

Rumen fluid protein nitrogen, as determined by an

alcohol precipitation method, did not show a positive relationship to frothing.

The flow time of rumen fluid did not appear to be correlated with froth production.

Rumen fluid from the non-froth producing ration was found to yield a more stable foam than in the case of froth producing rations.

Rumen fluid from the non-froth producing ration also yielded the greater column of foam when air was bubbled through the liquid.

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INTRODUCTION

The anticipation of bloat has influenced many livestock men to utilize grass pastures rather than the more productive legume pastures. This practice has resulted in economic losses estimated to be twenty million dollars in the North Central states alone. The feeding of various bloat "preventives," such as hay and chemical compounds, have resulted in additional expense.

The many bloat studies undertaken have yielded considerable information without delineating the physiological and/or chemical cause(s) of the malady. It is generally agreed that: (1) the incidence of bloat is increasing, (2) legumes are the worst offenders, (3) new seedings cause less trouble than the same pastures on the second and subsequent years, (4) legumes cause the most trouble during the periods of lush, rapid growth, (5) bloat occurs only rarely on grasses, (6) the incidence of bloat varies in different years, (7) there is a seasonal variation in the occurrence of bloat, (8) bloat is more likely to occur in certain areas than in others, (9) fatal bloat occurs in the feed lot on grain and hay as well as on pasture, (10) bloat cannot be produced experimentally at will, and (11) some animals have a greater tendency to bloat than others.

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Several basic problems still remain to be solved. First, the specific cause(s) of bloat need to be determined. Secondly, it should be resolved whether fatal bloat results from excessive pressure, toxic materials, or other causes. Thirdly, the difference between frothy and free-gas bloat must be determined, and fourthly, bloat preventives must be developed. Whether the studies should be aimed primarily at determining the cause of bloat and secondly, finding preventives for bloat, or vice versa, remains controversial.

The purpose of this study is to investigate the relationship of some of the chemical and physical properties of rumen liquor to the incidence of frothy bloat.

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REVIEW OF LITERATURE

Introduction

The complex problem of bloat has been a challenge to stockmen for at least nineteen centuries (Dougherty, 1953). Numerous theories of the etiology of this physiological malfunction have been postulated during this long period of time. Proof of this is demonstrated by the following factors which have been reported to influence bloating in ruminants: toxic gases, physical deficiencies, ammonium carbamate, chemical constituents of plant origin, histamines, antigens, anatomical defects, rumen flora changes, excessive gas formation, minerals, saliva, and ingesta buoyancy.

Physiology of Bloat

Bloat, also referred to as hoven or tympanites, is an overdistention of the rumen with gas (Dukes, 1949). It is obvious that this disorder results from the inability of an animal to rid itself of the gas produced through rumenal fermentation. Many studies have been undertaken to determine the cause of this eructation failure. Weiss (1953b) stated that an ordinary rumen contraction, which is essential for the mixing of ingesta, involves a rumen wave which travels backward. An eructation contraction, however, involves a wave which travels forward. Clark and Weiss (1952a) demon-

strated that eructation consists essentially of the movement of free gas from the dorsal rumen forward and downward to the cardia. This is accomplished by: (1) a wave of contraction that starts at the posterior blind sac and moves forward, (2) clearing of the cardia, which is covered by ingesta in a normally full rumen, by a sudden relaxation of the reticulum, and (3) opening of the cardia and passage of the gas up the esophagus.

Dougherty and Meredith (1955) recently completed cinefluorographic studies of the rumen and reticulum and of eructation. They report that the following mechanisms are important in eructation: (1) the clearing of ingesta from the reticulum by two contractions of this organ, (2) contraction of the ruminoreticular fold up to (or nearly to) the level of the cardia, holding the rumen ingesta away from the cardia and preventing it from flowing or falling back into the reticulum, (3) relaxation of the relatively empty reticulum, (4) contraction of the rumen, pushing gas forward around the cardia and down into the relaxed reticulum, and (5) synchronous relaxation of the cardia and diaphragmatic sphincter, permitting gas to rush into the relaxed esophagus. It is obvious, therefore, that a blocking of any one or more of these phases could result in eructation failure and resulting bloat.

Weiss (1952) stated that the main stimulus for eructation is gas pressure in the posterior dorsal sac of the rumen.

He further stated that the efficiency of the reflex may depend on hereditary factors. Some of the factors affecting the reflex are: obstruction of the esophagus, frothing of the ingesta, the degree of filling of the rumen, and the posture of the animal. In regard to obstruction of the esophagus, he believed that the cardia is kept closed by pressure from surrounding organs since there is no evidence of a cardiac sphincter. He also found that animals with a nonfunctional reticulum were particularly susceptible to overfilling. He noted that when two liters of water were added to the rumen, eructation was prevented. This worker also found that alkalosis, as determined by the carbon dioxide combining power of the blood, could cause reticular paralysis and consequent impaired eructation efficiency. Weiss also showed that section of the right ventral branch of the vagus caused abomasal and intestinal distention with accompanying chronic frothy bloat, again due mainly to inhibition of reticular activity. Section of the left dorsal branch of the vagus resulted in only temporary impaired eructation efficiency. It was found that hydrocyanic acid, atropine, histamine, and adrenalin would decrease or inhibit the reflex. Carbamylcholine and veratrine both caused spasm of the rumen and reticulum with consequent interference of eructation. In another study, Clark and Weiss (1952a) reported that the secretion of adrenalin due to a psychic disturbance caused mass bloating in cattle.

Weiss (1953b) noted that the ratio of backward to forward contractions in the rumen changed from 2:1 to 1:1 by elevating the hindquarters of a sheep. The rumen had been first paralyzed by sodium carbonate and then stimulated to eructation by introduction of air. He believed this posture effect to be further support for the theory that receptors in the posterior blind sac (origin of the forward wave of contraction) are more sensitive to pressure. He noted that increasing the rumenal pressure increased eructation frequency, and that complete inability to eructate was remedied by removing three liters of ingesta. As soon as the ingesta were restored, the animal returned to inefficient eructation.

Types of Bloat

It is common practice to refer to bloat as being either the free-gas or frothy type. Unfortunately, it is usually impossible to distinguish between these two types in non-fistulated animals. Consequently, the recent classification by Cole et al (1955) is preferred. This classification outlines three types or degrees of bloat; namely, chronic, subacute, and acute. Chronic bloat is produced in an animal regardless of the nature or quality of the diet. It is therefore obvious that an anatomical abnormality, such as an enlarged thymus acting to constrict the esophagus, or a ruminant suffering from peritonitis, could result in chronic bloat.

Subacute bloat is distention of the rumen resulting

from, and depending upon, a specific diet. The bloated animal must return to a non-bloated state without treatment to be placed in this category. Bloat from legumes and the frothy type of feed lot bloat are examples of subacute bloat. The acute category of rumen distention varies from the subacute only in degree, acute bloat being manifested by more severe distention, frequent urination and defecation, and labored breathing.

Factors Influencing Bloat

Physical deficiency. Cole, Mead, and Kleiber (1942) and Cole and Mead (1943) reported that the cause of bloat is the lack of sufficient irritating material in the rumen to stimulate belching. Distention in the chronic bloater is explained by the animal having a higher threshold of irritability. These workers also stated that bloat on green alfalfa or grain alone occurs because the feed cannot be packed away in the posterior rumen; the cardiac orifice becomes submerged and belching is prevented. Cole et al (1943b) stated that bloat from immature alfalfa pasture was most severe when the animals had been deprived of hay for 48 hours. When alfalfa hay was fed it was not completely effective but did reduce the incidence and severity of bloat. The feeding of Sudan grass hay protected the cows completely. The authors believed that the scabrous leaves of Sudan grass hay induced rumination and belching and thus prevented bloat. They stated, "In our opinion, bloat on legume pasture is largely due to a lack

of sufficient irritating roughage in the rumen."

It is difficult to understand why cows do not bloat on immature succulent grasses if this theory is valid. Further argument against this theory is supported by the work of Newbold (1954) who found that all parts of the red clover plant produced bloat and stated, "It is hard to reconcile the results of the feeding tests reported here with any theory that regards bloat as being due to a lack of coarse material in the feed." Johns (1954) also found that bloat was produced at all stages of wilting of red clover and up to hay of 72% dry matter content. Thus the physical deficiency theory does not appear to satisfy the still unknown etiology of free-gas bloat.

Excessive gas. Cole et al (1945) reviewed the earlier works supporting this explanation for the cause of bloat. They emphasized that this theory is supported by more opinions than facts.

Bloat is rarely produced from grasses; therefore, if the above theory is valid, one would expect more gas to be produced from legumes than from grasses. Espe and Cannon (1940) pointed out that there was little difference in the rate of gas formation between finely cut fresh alfalfa and bluegrass when the forages were placed in rumen fluid. They also pointed out that frosted alfalfa or bluegrass, an environmental condition sometimes acclaimed to increase the frequency of bloat, did not materially change the rate of gas formation. Jacobson et al (1942) also reported that no more

gas was produced *in vitro* from alfalfa than from bluegrass. Cole and Kleiber (1948b) noted that the gas production from alfalfa and Sudan grass, when fed *ad libitum*, was about equal, but that the cows ate about three times as much of the latter. They emphasized that cows bloat on alfalfa but not on an amount of Sudan grass which would yield as much gas as the alfalfa.

Since animals rarely bloat when on a diet of hay, one would also expect, if the theory were valid, that more gas would be formed on green forage than on dry. Cole *et al* (1942) stated that bloating on green alfalfa cannot well be attributed simply to excessive gas formation since more gas was produced from alfalfa hay than from green alfalfa.

Additional studies of gas formation relating to the type and amount of feed ingested have been accomplished. Mead *et al* (1944) reported that the rate of gas formation varies directly with the amount of feed consumed. Cole and Kleiber (1948a) reported from a study of the rate of gas formation in the rumen that the feeding of glucose caused a more rapid increase in gas formation than starch, but the total gas produced was about the same.

The rate of gas production in the rumen also appears to depend upon the activity of the rumen. Stone (1949) reported that the ability of the ingesta from inactive rumens to produce gas is less than that of ingesta from active rumens.

Several workers have studied excessive pressures in

the rumen. Weiss (1953b) reported that animals with excessive rumenal pressure still exhibited powerful eructation contractions. However, abnormal pressure in the rumen during bloat must be defined. Cole and Kleiber (1945) stated that animals with rumen pressure of 60 mm. Hg were in serious condition. They used a tympanometer devised by Kleiber to measure pressure. Olson (1942) found that animals which had died of bloat had rumen gas pressures of 60 to 70 mm. Hg. Animals were insufflated experimentally to 100 mm. Hg with no ill effects. In a later work (Olson, 1944) dealing with both cattle and sheep, similar results were obtained. Barrentine et al (1954) reported that bloated steers yielded tympanometer readings of 20 to 60 mm. Hg. Acutely bloated steers had intrarumenal pressures of 80 mm. Hg. The results of these studies indicate that a mildly bloated animal should exhibit an intrarumenal pressure of approximately 40 mm. Hg. Dougherty (1940), as previously stated, pointed out that intrarumenal pressures in cases of acute bloat did not seem to be as great as in induced bloat, but yet the cattle died suddenly. In a later work (Dougherty, 1941), he insufflated the rumen of a sheep to a pressure of 140 mm. Hg and maintained the pressure for two minutes. In another study (Dougherty, 1942a), he pointed out that intrarumenal pressures on dead animals varied from 72 to 75 mm. Hg, thus 50% of the insufflated pressure.

Dougherty (1941) noted that it took much less air to increase the intrarumenal pressure when air was forced up

through the ingesta than when air was introduced into the top part of the rumino-reticular cavity. Eructation was also more difficult when the former method was used. Weiss (1953a) reported that when air was introduced into the watery ingesta of sheep it was eructated efficiently, but eructation was interfered with when the free air was introduced into viscid ingesta. Clark (1950b) found it very difficult to cause retention of carbon dioxide in the rumen of sheep when the gas was introduced from a carbon dioxide cylinder. This is understandable when one considers the finding of Weiss (1953b) that animals suffering from excessive rumenal pressure still exhibit powerful eructation contractions.

Cole et al (1942) reported that increased pressure, in itself, does not force gas from the rumen through the esophagus. Nichols (1951) indicated that insufflation of the rumen with gas increases belching in sheep. Dougherty (1942b) reported that intrarumenal insufflations of oxygen increased the activity of the experimentally paralyzed or non-paralyzed rumen of a cow.

Recent work by Dougherty et al (1955) dealing with insufflation of the ruminant stomach showed that there were marked individual differences in tolerance to the same gas insufflated at the same pressure. It was also noted that increased intrarumenal pressure caused sharp rises in arterial (carotid) and venous (jugular) blood pressures. The arterial pressure increased during insufflation and dropped again when the intrarumenal pressure was released. It was further noted

that insufflation had a pronounced effect on blood gases. The changes were rapid and were influenced by the immediate intrarumenal pressures. It was observed that even during a short insufflation period the blood gases varied during and after eructation. The rapidity with which bloat symptoms appear and disappear on pasture can be correlated well with this information.

Thus the theory of excessive gas formation and excessive pressure in the rumen does not appear to be supported by factual data. Conversely, the above mentioned studies indicate that living ruminants can withstand much higher pressures than are found in animals which have died of bloat. It also appears likely that ruminants have no difficulty eructating the gas produced in the rumen when the eructation mechanism is functioning properly.

Toxic gas. Dougherty (1942a) analyzed the gas and ingesta of three feed lot steers that had died of frothy bloat and found that all gas and ingesta samples had relatively high concentrations of hydrogen sulfide. The steers had received a mixture of first and second cutting alfalfa the first week in the feed lot. Grain feeding was commenced the second week and at full feed the steers were getting 7 lb. per day of a mixture of 50% ground rye and 50% ground barley, plus supplement added at the rate of 1 lb. to 8 lb. of grain. Olson (1942) stated that he believed hydrogen sulfide played a significant role in the cause and death from bloat. Dougherty (1941) noted that the type of feed being ingested

had a marked influence on the amount of hydrogen sulfide present. When animals were fed good quality legume hay, the hydrogen sulfide content of the rumen ingesta was greatly increased. In a later work, Dougherty (1942a) stated that the hydrogen sulfide content of ingesta could be increased markedly by feeding protein-rich feeds, whereas Cole et al (1942) reported that the type of feed eaten did not alter the composition of rumen gas. Olson (1940c) reported that no striking differences were found between the rumenal gases produced from legume and non-legume pastures. In a later work, Olson (1944) referred to the formation of toxic gases from a high protein diet. Dougherty (1941) also noted that when a cow was fed poor quality hay, and when the rumen contents were dry, the animal could tolerate comparatively large amounts of hydrogen sulfide. When the animal had been fed good quality legume hay or freshly cut ladino clover or alfalfa, and the rumen contents were moist, the hydrogen sulfide tolerance was low. The tolerance was very low when the intrarumenal pressure was increased with air immediately after hydrogen sulfide insufflation. Dougherty also pointed out that the animal recovered much more rapidly from hydrogen sulfide intoxication than from carbon monoxide intoxication. He reported that the frothy ingesta of a heifer which died of bloat had a hydrogen sulfide content much greater than the level considered to be toxic to one experimental cow under average conditions. The total blood sulfur was approximately four times the amount normally found in blood. The animal

had been on the same irrigated ladino clover pasture for six weeks prior to the day it died.

Olson (1944) reported that hydrogen sulfide has been shown to be greatly increased in bloated animals. In attempting to explain the origin and relation of hydrogen sulfide to bloat, he stated that the leaves and finer stems of legumes are relatively higher in sulfur than the stems of the same plants, with the leaves of alfalfa having twice as much sulfur as the stems. He also reported that more hydrogen sulfide was produced when cattle were stable fed fresh green alfalfa than when grazing. The possible connection of rainfall to bloat was pointed out. He reported on a milking herd which was pastured for seven years of low rainfall with no bloat. However, much bloating occurred during a period of normal rainfall. Olson reasoned that the plants contained more sulfur when they grew rapidly during periods of heavier rainfall. He believed that cows will bloat on plants grown in the soils containing the most sulfur.

Kleiber et al (1943) demonstrated that the relatively high hydrogen sulfide concentration in the rumen gas of cows on alfalfa pasture does not necessarily lead to bloat. They stated, "Most likely, therefore, hydrogen sulfide plays no very significant role as a condition producing bloat. Conceivably, on the other hand, this poisonous gas is involved in the fatal consequences of bloat."

Cole et al (1945) reviewed the toxic gas theory and pointed out that the theory lacked substantiation since

hydrogen sulfide had not been shown to be in greater amounts in living bloated animals than in normal animals. They also pointed out that the evidence was to the contrary. However, they concluded with, "Considering the very appreciable amounts of hydrogen sulfide present in the rumen at all times, and the toxic effects when it is artificially introduced into the rumen, hydrogen sulfide may well be a contributing factor in death of bloated animals."

Dougherty (1940) studied the effect of other gases upon the rumen. He stated that carbon dioxide was absorbed from the rumen and produced a marked effect upon the respiratory apparatus with relatively high pressures causing extreme dyspnea. He noted that an increase in intrarumenal pressure caused an increase in the rate of absorption of both carbon dioxide and carbon monoxide from the rumen. Dougherty noted that when carbon monoxide was present in the rumen, even in very low concentrations, and when the intrarumenal pressure was increased moderately with other gases common to the rumen, marked preprostration symptoms were produced. It was also reported that carbon monoxide was found in appreciable amounts in two experimental cows fed freshly cut ladino clover, and that carbon monoxide was the only gas of those used (carbon monoxide, carbon dioxide, methane, and hydrogen) that seemed to inhibit belching. Dougherty noted the significant fact that intra-rumenal pressures in cases of acute bloat did not seem to be as great as in induced bloat, yet animals died suddenly.

Thus the relevance and significance of toxic gases in the etiology of bloat remains obscure.

Flavones and unknown forage extracts. Ferguson (1948a) reported a factor in pastures that inhibited the movement of the rabbit gut. He noted that the factor, containing no nitrogen, was found mainly in legumes (white clover and alfalfa). Orchard grass and some other grasses contained smaller amounts. He thought that grasses contain both inhibiting and stimulating factors with the latter usually more active. He further reported (Ferguson, 1948b) that preliminary tests indicated that the addition of very small quantities of adrenalin to muscle affected by clover juice caused recovery after a brief period. Weiss (1953b) later reported that the administration of adrenalin to sheep produced variable results in sheep with some interference of the eructation mechanism. Johns (1954) reported that adrenalin was "definitely dangerous" when administered as a treatment for frothy bloat produced by stall feeding of fresh red clover.

Ferguson et al (1949) found a muscle-inhibiting compound in alfalfa to be a flavone. They noted that there was much less of the material in the plant in the third cutting than in the first. They later identified the flavone as a tricin (Ferguson et al, 1950). They believed additional flavones exist in alfalfa because other flavone preparations possess greater activity on smooth-muscle movements than the purified isolated flavone.

Parsons and co-workers (1952), as mentioned previously, reported that death resulted in sheep 9 to 30 minutes after dosing them with 16 oz. and higher doses of birdsfoot trefoil extract. Death was prevented by administration of sodium thiosulfate. Sub-lethal and lethal doses of birdsfoot trefoil and excessive doses of Kentucky 31 fescue extracts did not produce a bloated condition. Sixteen ounces of alfalfa or ladino clover extract produced slight bloat, while 32 oz. of either extract produced excessive bloat in less than one hour. Larger doses produced scours in the experimental animal. Scouring was also observed when beef cattle bloated on alfalfa-ladino pasture in Michigan (Smith and Emery, 1955b).

Parsons et al (1953) reported that rumen contents from cows that died due to bloat inhibited the motility of the isolated rabbit intestine. The effect was also noted with forage extracts taken from the pastures that these animals were grazing at the time of death. This inhibition was not observed when the rumen contents from normal cattle were used. Acute bloat and death were produced in sheep using an extract of ladino clover. Orally administered legume extracts were noted to inbibit the eructation mechanism. Lindahl et al (1954) reported that no distention of the rumen was produced when sheep grazing on grass pasture were drenched with ladino clover juice or alfalfa juice. Newbold (1954) reported that red clover was fractionated and 16 to 18 l. of the fractions were given by drench. Only feeds containing the hydraulically pressed juices produced bloat. Parsons and co-workers (1955)

reported that an extract of ladino clover, known to cause bloat, inhibited eructation in a fistulated cow. The extract, as in the previously mentioned work by Parsons et al (1953), inhibited the motility of segments of the isolated rabbit gut. The active substance was stable to heat and freezing and the material in one sample was dialyzable. Blake et al (1955) reported that acute bloat in cattle was induced by oral administration of juices expelled from freshly cut alfalfa. Mild bloat was induced with concentrated and with spray dried alfalfa juice.

It is evident that in the majority of studies concerned with bloat produced by forage extracts, the presence or absence of frothiness is unknown. A universal agreement on this factor alone would greatly aid the solution of this particular phase of the etiology of bloat.

Saponins. Quin (1943) studied the pathogenesis of acute bloat in South Africa and found that foam was produced in the rumen of sheep fed freshly cut alfalfa. He found that pressed alfalfa and the watery extract of alfalfa, on shaking, resulted in a foam which was stable for twelve hours. The material responsible for the foaming was found to be a saponin. He pointed out that cases of rapidly fatal bloat in sheep were associated with greedy feeding of the tops of alfalfa. It was not reported from what part of the alfalfa the saponin was isolated. It was noted, however, that the sugar content of alfalfa during an outbreak of bloat increased from 2.5% in early morning to 6% in late afternoon, when the most bloat

occurred. Evans and Evans (1949) reported that saponin in a 1:5000 dilution paralyzed rabbit intestine. They also showed that one liter of clover juice, from about 5 lb. of fresh clover, introduced directly into the rumen of a sheep, paralyzed rumen movement immediately. They tended to incriminate hydrocyanic acid, however, rather than a saponin as the paralyzing factor.

Henrici (1952) studied legumes and grasses grown on different soils in South Africa. This worker isolated two saponins from Tribulus terrestris but found none in plants grown on soil where no bloat had occurred. Henrici believed the saponins are formed on certain soils, presumably those which are unable to supply zinc in sufficient quantity to Tribulus terrestris during periods of flush growth. This investigator reported that zinc deficiency and wilting are factors dissolving starch and leading to the accumulation of sugars. Alfalfa was also studied and yielded results similar to that of Tribulus terrestris. The saponin isolated from alfalfa foamed readily and was hemolytic, as were those from Tribulus terrestris. The saponin was not the same as Jacobson's, which was not hemolytic and contained nitrogen. Henrici reported that none of the alfalfa grown on non-bloat producing soils under similar conditions was ever hemolytic. It was pointed out that the saponin may increase the permeability of the intestines so as to allow sugars and gases to pass with great velocity, or the saponin may be split up in the "stomach" and a large amount of sugars freed, thus

increasing the amount of rapidly fermentable sugars.

Lindahl and others (1954) investigated the role of alfalfa saponin in ruminant bloat. They found that large doses of saponin, 15 gm. with sheep and 75 gm. with a heifer, produced light bloat. These amounts of saponins are believed to exceed the amounts that could be ingested from alfalfa by normal grazing. They noted that distention appeared to be due to gas retention rather than froth. No distention was produced when the water allowance was increased two or three times. Potter and Kummerow (1954) studied the biological activity of saponins isolated from alfalfa and soybeans. They reported that both the purified alfalfa and soybean saponins inhibited the growth of chicks, while their genins did not. They believe that bloat in cattle from untoasted solvent extracted soybean oil meal might be explained by toxic soyasapogenols, which on cooking hydrolyze to non-toxic genins, but may not do so if the cooking process is too mild. Jacobson and Lindahl (1955) studied the rumen ingesta volume increase in frothy bloat. They found a greater increase with saponin plus glucose than with either one alone.

Colvin et al (1955) studied the effect of alfalfa saponin on rumen activity in sheep. They reported that when sheep were on diets of either Sudan grass or alfalfa tops, the administration of 100 gm. of alfalfa saponin dissolved in one liter of 5% glucose solution caused death within 1 1/2 to 2 1/2 hours. The administration of 100 gm. of saponin dissolved in one liter of 0.9% sodium chloride solution produced death

in 2 1/4 hours in a sheep fasted 48 hours. After the administration of the saponin solution, rumen motility ceased almost immediately. Frothy bloat was observed when the glucose and saponin were introduced into the rumen together. Postmortem findings indicated that death was associated with an intense submucosal and subserosal hemorrhagic condition of the gastrointestinal tract. In other trials, rumen motility was markedly depressed by either 25 gm. of alfalfa tops or 12.5 gm. of alfalfa saponin. They noted that when alfalfa tops were fed, the animals became slightly bloated; however, no distention was observed following the use of alfalfa saponin in the animals on an oat hay diet.

The preceding review indicates the possible relationship of saponins to the cause of ruminant bloat. It is obvious, however, that cattle and sheep probably do not ingest as much saponin under normal grazing conditions as was administered in the bloat-producing experiments. It is well to keep in mind, however, that the hemolytic effect of alfalfa saponins disappeared very rapidly after the compounds were extracted (Henrici, 1952).

Hydrocyanic acid. Weiss (1952) stated that small doses of hydrocyanic acid inhibited the reticulum and backward movement of the rumen, with consequent inefficiency of eructation and abolition of the eructation reflex. Clark and Weiss (1952a) noted that sub-clinical doses may cause bloat whereas large doses cause total rumenal paralysis.

Clark and Quin (1945) showed that more than four times the amount of potassium cyanide is required to cause rumenal paralysis in sheep during active fermentation of alfalfa in the rumen than after a fast of fourteen hours. The increased tolerance of potsssium cyanide after feeding was explained on the basis of an accelerated elimination of hydrogen cyanide from the lungs. This results from the greater respiratory exchange, which in turn is caused by the absorption of carbon dioxide from the alimentary tract during fermentation. They reported that sheep showing paralysis of the rumen, caused by potassium cyanide, were able to eructate two liters of gas per minute introduced through the rumen fistula. They concluded with, "These observations, therefore, afford no evidence for incriminating the cyanogenetic factors in plants as being associated with the etiology of acute bloat in ruminants." Later work by Clark (1951) showed that rumen musculature paralyzed by potassium cyanide failed to respond to carbamyl-choline-chloride. The drug combined its effect with prussic acid to increase the severity of the symptoms of prussic acid poisoning.

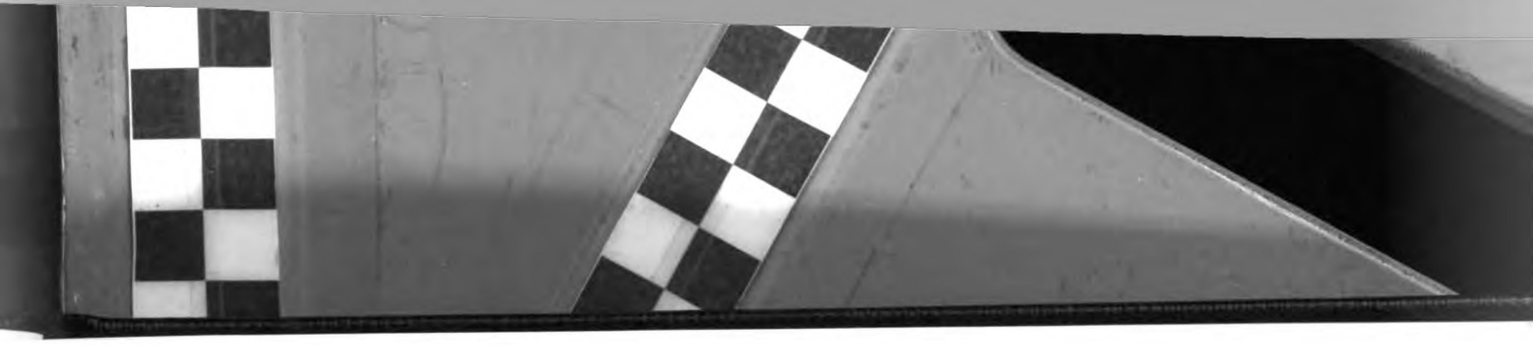
Evans and Evans (1949) showed that one liter of clover juice, from about five pounds of fresh clover, introduced directly into the rumen of a sheep paralyzed its movements immediately. They noted that the condition resembled hydrocyanic acid poisoning. They then starved a bull and two heifers for twelve hours and turned them onto white clover pasture. The animals quit grazing the clover after twenty

minutes and grazed rough herbage other than clover. The bull bloated but the heifers exhibited only mild distention. The bull had 0.1 mgm.% hydrocyanic acid in its blood; the heifers had 0.04 mgm.%.

Heath and Park (1953) studied an irreversible choline-esterase inhibitor in white clover. They stated, "Clover may in some cases produce signs of anti-choline-esterase poisoning as well as cyanide poisoning. We think, therefore, that these observations may have some relevance to bloat." Parsons et al (1952) reported that dosing sheep with 16 oz. of birdsfoot trefoil extract produced death in 9 to 30 minutes. They noted that the symptoms suggested hydrocyanic acid poisoning. Dougherty and Christensen (1953) found that freshly pressed birdsfoot trefoil juice contained at least twenty times as much hydrocyanic acid as freshly pressed alfalfa, ladino clover, or orchard grass. One and one-half liters of each of the juices were introduced into the rumen of sheep. Only the birdsfoot trefoil extract proved lethal, and no symptoms of bloat were noted. The lethal dose of birdsfoot trefoil, for sheep, was found to be between 212 and 318 millliliters. The introduction of 350 ml. of cyanide-free birdsfoot trefoil extract into the rumen of a sheep did not prove lethal. Dougherty and Christensen pointed out that even though hydrocyanic acid is toxic to sheep, there is little or no clinical evidence that either hydrocyanic acid poisoning or bloat occurs in animals pastured on birdsfoot trefoil.

Ammonium carbamate. Hale and King (1955) cited the bloating of sheep, observed by Repp et al (1955), when urea was administered orally in doses that were fatal or near fatal. They believed that conditions in the rumen at this time were similar to those when ruminants suffer from acute bloat on immature legume pastures, since these pastures are high in nonprotein nitrogen. They stated, "In consideration of results in this experiment, it appears plausible that death during acute bloat in cattle and sheep may actually be due to the absorption of ammonium carbamate or some related nitrogen compound."

Clark et al (1951) showed that in acute cases of urea toxicity in sheep, clinical symptoms appeared from 30 to 60 minutes after dosing. Dullness was followed by marked hyperesthesia and severe muscular twitches over the whole body. Moderate to severe bloating frequently occurred at this stage. The introduction of urea into the rumen was followed by a decrease or entire cessation of rumenal motility and a sharp rise in the pH. It is significant that sheep on a diet of poor quality grass hay were more susceptible than those on alfalfa hay, thus tending to contradict the ammonium carbamate theory. However, the cause of rumenal paralysis was associated with an increased alkalinity of the rumenal contents following the formation of ammonia. The paralysis could be prevented or alleviated by administration of an acid. The toxicity of urea was found to depend on the activity of the rumenal flora, as determined by the basic diet, and the presence



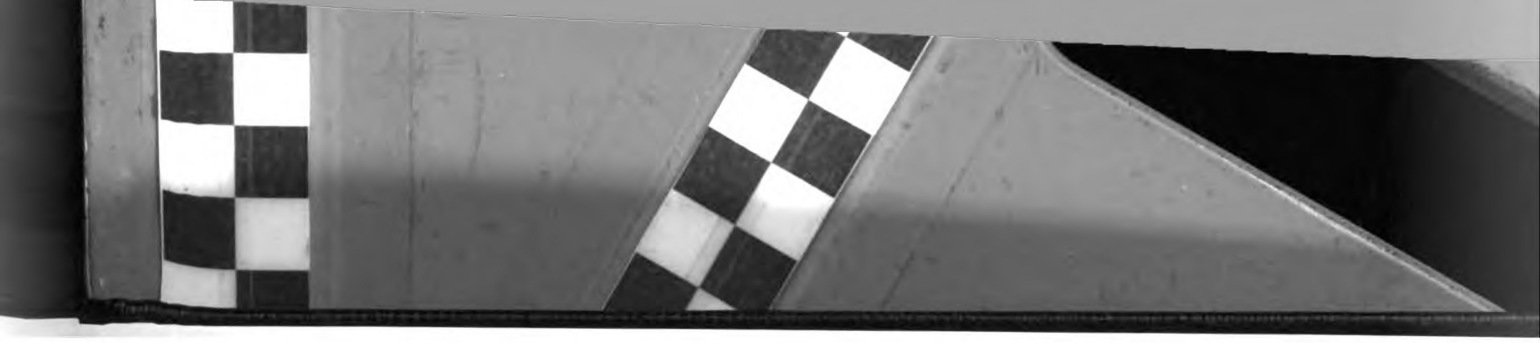
of available carbohydrate.

The validity of the ammonium carbamate theory appears to depend upon the speed with which ammonium carbamate in the blood is hydrolyzed to ammonium carbonate by carbonic anhydrase. Sumner and Somers (1943) stated that carbamate slowly hydrolyzes to carbonate and that hydrolysis is retarded by carbon dioxide. They believed that the first products formed from urea by urease, known to be present in the gastric mucosa of cows and sheep, are carbon dioxide and ammonia. In the absence of buffers, these unite to form ammonium carbamate. If buffers are present, the products are ammonium salts and carbonic acid.

It is noteworthy that carbonic anhydrase is inhibited in the dark by carbon monoxide and hydrogen cyanide (Sumner and Somers, 1943). The latter compound has been incriminated as contributing to the cause of bloat (Clark and Quin, 1945; Evans and Evans, 1949; Clark and Weiss, 1952a; and Weiss, 1952).

Histamines and allergies. Dougherty (1942a) reported that all ingesta samples were relatively high in histamine content, after studying steers that had died of frothy bloat while on feed lot. He reasoned that the histamine may have come from decarboxylation of histidine. Dougherty cited the fact that the histamine content of rumen ingesta can be increased markedly by feeding protein-rich feeds. He also reported (1942b) that histamine and adrenalin had a marked

depressing action on rumenal motility. Petersen (1945) found that the intravenous injection of histamine produced marked bloat. Clark's study (1950a) of the action of histamine on rumenal musculature showed that the intravenous injection of histamine (1 to 2 mg.) caused a prompt and complete cessation of rumenal movements without affecting the general intrarumenal pressure. The large intestine, however, displayed hypermotility. The injection of three antihistaminic drugs reversed the effect of histamine, and prior administration of the drugs prevented histamine action, to prove the above effects were due to that specific compound. Two milligrams of histamine were intravenously injected after left vagotomy. The rumen and reticulum gave no response, but the intestine assumed a wrinkled appearance, due to spasm of the circular muscle layer. The distal end of the left vagus was then stimulated with the previously determined strength of current, and the reticulum and rumen were seen to contract as strongly as they had prior to the injection of histamine. Repeated stimulation at intervals of from 2 to 20 minutes yielded identical results. Contraction was not observed in either the rumen or isolated strips of the rumen. Clark stated, "Rumenal stasis associated with high protein intake may, therefore, either be due to excess alkalinity of the rumen (ammonia) or to the presence of toxic amines (histamines ?), or to a combination of both these factors." Weiss (1953b) reported the blocking of eructation in sheep by the injection of histamine.



Dougherty and Cello (1952) reported a study of toxic factors in the rumen ingesta of experimentally overfed sheep. Coarsely cracked grain, placed in the rumen, caused 5 of 6 sheep to die in 20 to 24 hours. Heparinized blood plasma taken from acutely sick sheep depressed the blood pressure of anesthetized dogs. Freshly drawn normal heparinized sheep plasma did not. Injections of the toxic rumen ingesta material caused a pronounced leukopenia in dogs. The leukopenia coincided with the fall in blood pressure, but occurred in a refractory dog when the blood pressure fall did not occur after an injection of ingesta fluid. There was an indication that the toxic factor was absorbed from both the intestinal tract and the rumen. Three antihistamines gave fair protection. Johns (1954) reported that an antihistaminic was "definitely dangerous" when used to treat frothy bloat produced by feeding red clover. In an earlier work, Dougherty and Cello (1949) reported that the toxic factor depressed blood pressure in dogs and goats, inhibited rumen motility in all intact animals, stimulated motor activity of the lower gut in the intact dog and goat (anesthetized) and the sheep (unanesthetized). Clark (1950a), as previously reported, showed that histamine also stimulated motor activity of the lower gut of sheep. The toxic substance was heat stable, dialyzable and not volatile with steam distillation at varying pH levels.

It is noteworthy that the injection of histamine in humans causes a fall in blood pressure (Anonymous, 1952).

An abnormal distribution of leukocytes results, with leukopenia occurring in the peripheral blood and leukocytosis in the blood of the abdomen (Houssay et al, 1951). A knowledge of the corresponding influence of histamine on the blood of ruminants would aid the study of the etiology of bloat.

The rumen distention produced by Trichomonas fetus antigen injection has resulted in bloat. Kerr and Lamont (1946) and Lamont (1946) reported this phenomenon after the antigen was injected into the uterus of cattle. The symptoms of bloat appeared after the second, or exciting, dose of antigen. They believed the bloat resulted from a spasm of "unstriped muscle at the esophageal opening into the rumen." The allergic shock was relieved by injecting 3 to 5 cc. of adrenalin subcutaneously or administering atropine sulfate in 0.5 grain doses. Shanks (1946) also reported the production of bloat after Trichomonas fetus antigen injection. He stated that this rumen distention in cows in Northern Ireland resulted from a form of protein shock, and it was again relieved by the injection of adrenalin. He noted that adrenalin injection also "cured" cases of naturally occurring bloat. It is noteworthy that Johns (1954) found adrenalin therapy on frothy bloat to be "definitely dangerous," while Weiss (1953b) found that it interfered with eructation, and Clark and Weiss (1952a) reported that adrenalin inhibited the reticulum. Shanks (1946) proposed the theory that young,

luscious pastures and certain foods contain specific proteins which cause protein shock on absorption into the body. He also believed that the shock interferes with belching, possibly by contracting the sphincter at the cardiac orifice.

In regard to feeding certain proteins, Dougherty (1942a) stated that the histamine content of rumen ingesta can be increased markedly by feeding protein-rich feeds. Clark (1950a), as previously cited, reported that rumenal stasis, associated with a high protein intake, may either be due to excess alkalinity of the rumen, or to the presence of toxic amines, or to a combination of both these factors. Quin and Van Der Wath (1938) reported that the type of feed had little influence on the motility of the rumen. It is obvious that the fractionation and separation of proteins must be accomplished before the protein shock theory can be substantiated.

Saliva. Smith (1954) showed that frothy bloat could be induced in an animal by cannulating the parotid glands and thus preventing a portion of the serous saliva from entering the rumen. It was postulated that the mucoid type of saliva caused the ingesta to froth, whereas the serous saliva decreased froth formation. McGilliard (1955) found two distinctly different types of saliva in cattle. When collection was made at the cardiac end of the esophagus, the saliva was decidedly more viscous than when collected orally from the same cow. Komarov and Stavraky (1940) studied the submaxillary saliva of cats. They reported finding two kinds

of saliva, one produced by chorda tympani stimulation and the other by administration of adrenalin. The two kinds of saliva contained a different and characteristic glucoprotein. They believed that chorda tympani stimulation caused the secretion of one glucoprotein from mucous cells of the submaxillary gland, and that adrenalin administration caused a different glucoprotein to be secreted by the serous cells of the gland. They found that urea represented quantitatively the main fraction of the nonprotein nitrogen of submaxillary saliva by either form of stimulation. They believed that prolonged chorda tympani stimulation caused a decrease in the permeability of the submaxillary gland to the passage of nonprotein nitrogen, whereas adrenalin increased the permeability. Quin and others (1951) reported a finding that may well be attributed to saliva. They noted that rumenal ingesta invariably became progressively more watery during fasting, despite the fact that the water consumption was always greatly reduced. Clark and Weiss (1952b) studied reflex salivation in sheep and goats. They noticed that ingesta became more watery after the feeding of hay, even in the absence of drinking water. They felt that the decrease in viscosity could only be explained by increased salivation and proceeded to determine if an increase occurred. They first located the afferent arc of the salivation reflex by stimulating the proximal and distal ends of the severed left vagus nerve. Electrical stimulation of the proximal end of the left vagus yielded profuse parotid gland secretion whereas

there was none when the distal stump was stimulated. Manipulation of the reticulum also caused an increased flow of saliva. Mechanical stimulation of the throat caused a two-fold increase in parotid secretion. Mechanical stimulation of the cardiac region produced a four to five-fold increase, provided the vagi were intact. Division of the vagi abolished this reflex and reduced the resting flow. Thus it can be seen how feeding may decrease the viscosity of the ingesta through the action of the parotid gland. Weiss (1953a) studied the significance of reflex salivation in relation to froth formation and acute bloat in ruminants. He found that frothiness was produced in sheep when the rumenal ingesta became thick and viscid; conversely, the amount of froth was insignificant when the rumenal ingesta were watery. He noticed that the feeding of crisp, succulent, prebloom alfalfa caused the rumen ingesta to be thick and viscid with bloat resulting from frothing of the thick ingesta. When mature stalky alfalfa was fed, the rumenal ingesta immediately reverted to a watery consistency, even in the absence of drinking water, and bloat ceased. Similar results were obtained by placing chopped hay in the rumen. He stated, "The conclusion is justified that the rapid reduction in consistency of the rumenal ingesta after feeding stalky lucerne was due to reflex stimulation of salivary secretion initiated in the forestomachs by the physical character of the feed. Ingestion of stalky alfalfa was also slower with the result that proportionately more saliva is secreted per given weight of feed." One may

argue that the decrease in consistency of the ingesta could have been due to increased serous saliva secretion, and the increase in consistency due to increased mucoid saliva, rather than difference in total saliva as Weiss postulated. Weiss (1953a) also pointed out that free air was eructated efficiently when introduced above both viscid and watery rumen ingesta. Air introduced into watery ingesta was also eructated efficiently, but eructation was interfered with when introduced into viscid ingesta.

Several other reports indicate possible relationships between saliva and bloat. Cole et al (1945) pointed out that saliva is a potential source of rumen carbon dioxide and may transport daily to the rumen an equivalent of 120 liters of carbon dioxide. Clark (1944) pointed out that a sufficently fluid state of the rumenal contents is also essential for maintaining rumination. Jacobson and others (1942) and Espe and others (1943) attempted to answer the question of why cattle appeared to bloat more on frosted pasture and pasture covered with dew. They postulated that less saliva was added to the rumen when these types of pasture were ingested. Balch et al (1953) later reported the amounts of saliva produced under slightly different conditions. They found that a cow, during eating, produced saliva at the rate of 5.8 lb. per lb. of hay, when receiving limited amounts of water, but only 4.6 lb. of saliva per lb. of hay, when receiving water at will. This work appears to confirm the findings and beliefs of other workers who have been previously

mentioned (Quin et al, 1951, Clark and Wiess, 1952b, Weiss, 1953a).

Buoyancy. This explanation for bloat is similar to an obsolete theory reported by Jacobson et al (1942), Espe et al (1943), and Cole et al (1942). The difference lies in the belief that in the modern theory, some ingested material is buoyed on the surface of the rumen liquor, whereas other ingested material sinks, raising the rumen liquor level and thus exerting a corresponding blocking influence on the cardiac orifice (Anonymous, 1953). In the older theory, there was simply a physical blocking of the cardiac orifice by compacted rumen ingesta. Nichols et al (1955) attempted to substantiate the modern theory by studying the effective buoyancy of the rumen juice of cattle fed hay, grass, and fresh legumes. They postulated that if enough small bubbles are produced in the lower regions of the rumen, they may be retained in the rumen fluid with the resulting development of a frothy mass. They believed that the effective buoyancy of rumen juice can be expressed on the basis of its specific gravity. They also believed that the frothy ingesta has less density and less buoyancy. They reasoned that the contents of the ventral sac of the rumen should be lower in specific gravity following the intake of fresh legumes than following the intake of hay or fresh grass. Their determinations showed that approximately one-half hour after the intake of feed and water, the specific gravities of ventral sac liquor were significantly lower than

those of the corresponding samples taken before feed and water ingestion. Specific gravities of centrifuged samples one-half hour after feeding were altered the least when legumes were fed and water intake was lowest, and were significantly reduced when hay and grass were fed and water intake was greatest. Specific gravities of strained rumen liquor one-half hour after intake of legumes were significantly lower than after the intake of either hay or grass. This lowering was evident even in the absence of the apparent lowering effect of water intake, as observed on the specific gravities of centrifuged half-hour samples.

Abnormal rumen flora. Hungate et al (1952) studied microbiological changes associated with acute indigestion in sheep. They found that when an excess of grain or glucose was introduced into the rumen, it caused a marked change in the rumen microorganisms. The cellulolytic bacteria were greatly decreased in number, the protozoa were killed, and the relative number of gram-positive bacteria increased. They also noted that nonvolatile acids accumulated and the amount of volatile acids diminished. Rumen motility was partially or completely inhibited. Dougherty and Cello (1949) reported an increase in volatile acids of the rumen may be associated with acute indigestion.

Quin et al (1951) studied the effect of fasting on the activity of the rumenal flora of sheep and cattle. They reported that fasting caused a marked decrease in the ability

of the rumenal flora to ferment glucose, and thus presumably decreased gas production. Cellulose digestion returned to a high rate rapidly, after fasting, if grass hay was fed. The recovery was slow in sheep, however, if alfalfa hay was fed. Stone (1949) reported that during fasting the volatile acidity of bovine rumen ingesta decreases, and is also subnormal in atonic rumens. Jacobson and Lindahl (1955) studied the biochemical, physical, and bacteriological factors involved in feed lot bloat. They found that when cattle were on a hay and corn silage diet, the ratio of acetic, propionic, and butyric acids was roughly 6:2:1. After the cattle were on a diet of 4 lb. of hay and 6 lb. of a bloat producing mix for four weeks, the ratio was altered to approximately 7:1:1. They reported that the bacterial content of the rumen markedly increased when the animals were on the bloat diet. They also noted a high degree of encapsulation of the rumen bacteria which increased with the incidence of bloat. They believed this encapsulation played a part in the stability of the froth. Cole et al (1955) recently reported similar observations and conclusions. Smith et al (1953) reported the appearance of an iodophilic streptococci concurrent with frothing of rumenal ingesta. The frothy bloat was experimentally produced with a grain mix containing corn and soybean oil meal.

Attempts to find the cause of bloat through rumen microflora studies have generally been discouraging. The

possible significance of increased bacterial encapsulation concurrent with frothy bloat may, however, contribute to the solution of the bloat problem.

Minerals and soil factors. Henrici (1952) is convinced that a soil factor is the primary cause of the involved phenomenon of bloating. This investigator believes, specifically, that the cause of bloat is related to a zinc deficiency in bloat-producing plants at the time of lush growth. It was stated that zinc deficiency only occurs under high illumination and with high temperatures, never in a foggy country. It was also pointed out that the role of zinc in the plant is not fully understood, but that only the results of the deficiency are known. They are: stoppage of growth, resulting in dwarf plants; increase of peroxidase in the leaves; destruction of the auxin and chlorophyll, accelerated by peroxidase activity; dissolution of starch, but no decrease in sugars; and the appearance of phytosterine and polyphenolic substances. Henrici grew Tribulus terrestris in plots where no bloat had ever been produced, with rain as the only source of water. Thus, a zinc-free condition was presumed to have existed. Analysis of the fresh plants showed that the stems contained more starch than the leaves; sugars were present in approximately the same quantity as in fresh summer legumes; but starch occurred in much larger amounts, particularly in the stems. It was noted that in fresh Tribulus terrestris, there was a tendency for monosaccharides to prevail. A saponin-like glucoside was extracted for the plants that

caused bloat in ruminants. The glucoside was not present in fresh plants from ordinary soil in an area where no bloat had been known to occur. Henrici stated that the saponin was formed on certain soils, presumably those which are deficient in zinc or which are unable to supply zinc in sufficient quantity to Tribulus terrestris in flush periods. It was further hypothesized that at the end of the season, no zinc deficiency occurs, as growth is slow, and no saponin is formed. The possibility of the saponin being a phytosterol was expressed, and Reed and Dufrenoy (1942), who made mention of a phytosterin being formed when a zinc deficiency and strong illumination occur simultaneously, were cited. McIntosh (1941) reported that cattle could be pastured on alfalfa grown on fertilized and "properly farmed" land without encountering bloat. He cited cases where cows that had been bloating on poor soils were moved to well-fertilized soils and bloating ceased.

Duckworth and Godden (1939) found that inorganic phosphorus, total calcium, ionic calcium, calcium in a phosphate-carbonate complex, calcium in an unknown complex form, protein bound calcium, and total magnesium of the blood appeared in lesser amounts when the cow was in a bloated state than when the animal was apparently normal.

Other workers have studied minerals and soil factors relating to bloat. Espe and Cannon (1940), in an attempt to prevent bloat, found that salt, soda, hydrated lime, or

combinations of the three which the cow would tolerate in her drinking water increased rather than suppressed gas formation. Olson (1940b) reported that bloating of cattle on alfalfa in Argentina was not considered a serious menace when rock salt was available at all times. In another work (1940a), he reported that steamed bone meal, salt, and baking soda in the drinking water of cattle had no effect on bloat production.

Cattle were observed eating dirt just prior to bloating while on alfalfa-ladino clover pasture (Smith and Emery, 1955.) Other cattle were observed eating dirt after they had been bloated by a grain mixture (Huffman, 1955).

Heredity. The possibility that the tendency to bloat may be inherited has been reported by several workers. Knapp et al (1943) reported that a highly significant difference in the number of steers showing excessive bloat was observed among the progeny of various bulls. Their analysis was based upon the number of steer days during which bloat occurred. Johns (1954) studied bloat on red clover with two pairs of identical twins and a single cow. He observed similarities between twins and differences between pairs in bloating behavior, reaction to different periods of starvation, and reaction to change in dry matter content of the clover. Hancock (1954) studied bloat in relation to grazing behavior. He also stated that there was evidence of bloat being inherited. Some twins did not bloat on either controlled or "break" grazing, even though most of the animals did.

Hancock postulated that the incidence of bloat may have been due to differences in the rate of grazing and also to the type of herbage selected. He disproved this idea by feeding two sets of twins in stalls. The twins that were highly susceptible bloated forty-five minutes after being fed 25 lb. of preflowering alfalfa, but the non-susceptible twins did not bloat. Lindahl and Davis (1954) studied some factors in feed lot bloat. They noted a significant difference among the test animals in their susceptibility to bloat. Dougherty et al (1955) studied the physiological effects of insufflation of the stomachs of sheep. They observed marked individual differences in tolerance to the same gas, insufflated at the same pressures. Weiss (1953b) studied the physiology of eructation in ruminants. He postulated that the eructation reflex is initiated by gas pressure in the rumen, and that reflex variations among individual animals is due to differences in the level of nervous reaction.

Anatomical defects. Cole et al (1945) pointed out that bloat in some cases might be due to a partial obstruction of the esophagus by enlarged mediastinal lymph glands. Ascott (1946) reported five cases of bloat resulting from blocking of the esophagus by tuberculosis and other diseases.

Preventive Measures

Prior feeding of hay. One of the most publicized measures of bloat prevention is the feeding of hay prior to pasturing ruminants. Cole, Mead, and Regan (1943b) reported

the production and prevention of bloat in cattle on alfalfa pasture. They noted that bloat from immature alfalfa pasture was most severe when the animals had been deprived of hay for 48 hours. They found that feeding alfalfa hay prior to pasturing reduced the incidence and severity of bloat, but was not completely effective as a preventive. Prior feeding of Sudan grass, however, protected the cows completely. They also found that pasturing on Sudan grass at night almost completely prevented bloat in cows pastured the following day on alfalfa. Cole et al (1943a) studied the prevention of bloat and found that supplemental feeding of hay in dry lot at night and in the pasture reduced the incidence of bloat. Cole and Kleiber (1945) reported that Sudan hay was only effective in preventing bloat when animals had all they would eat overnight before being pastured on alfalfa. They found that 17 lb. of Sudan grass hay completely prevented bloat. They also noted that supplemental feeding of Sudan grass hay generally increased consumption of green alfalfa, if no concentrates were fed. Mead, Cole, and Regan (1944) reported that the feeding of barley straw prior to pasturing was not effective in preventing bloat. They noted that bloat occurred sooner after animals were turned into pasture if grain was fed just previously. Johns (1954) studied bloat in cattle on red clover and found that the feeding of hay was not effective in preventing bloat. He noted bloat was produced at all stages of wilting of clover up to hay of 72% dry matter content. Olson (1940a) also reported that prior feeding of hay was not effective in

preventing bloat. It is well to keep in mind that most legumes produce bloat and most grasses do not. Consequently, one might expect a grass hay to prevent bloat, but not a legume hay. The previously reported studies seem to bear this out.

Soiling. Another measure that has been employed to prevent bloat is soiling, or cutting the feed green and hauling it to the cattle. Newbold (1954) reported that red clover pastures producing bloat with grazing would also produce bloat when cut and stall fed. Johns (1954) reported that bloat was produced when red clover at all stages of wilting was stall fed. Thus it can be seen that the soiling method is not completely effective in preventing bloat.

Alternate grazing of grass and legume pastures. Cole et al (1943b) found that overnight pasturing on Sudan grass proved effective in preventing bloat on alfalfa pasture the following day. Simultaneous pasturing of Sudan grass and a legume did not prove practical, however, as a method of preventing bloat (Regan and Mead, 1945).

Intermittent versus continuous grazing. Hancock (1954) reported more cases of bloat when the cattle were allowed to graze over several periods rather than one. The bloat, however, was mostly mild. Six out of eight days of observation, the break-grazed cows suffered from more bloat than the continuously grazed control group. Thus the break-grazing method of preventing bloat did not appear to be effective.

Proper grass-legume ratio. Cole et al (1943a) reported that bloat rarely occurs if grasses make up 50% of the pasture mixture. Hancock (1954) reported in his study of bloat in relation to grazing behavior that, "We still need to know the proportion of grasses necessary to make a mixed clover-grass sword safe," and "We need to know whether there are specie or strain differences among grasses and clovers in their relative bloat danger."

Grazing mature plants. A sixth measure employed to prevent bloat is prohibiting grazing until legumes reach a mature stage. Beef cattle in Michigan bloated on seed stage alfalfa and ladino clover pasture (Smith and Emery, 1955). Hancock (1954) reported less bloat on the most mature pasture, but admitted that the critical stage of maturity was unknown. Johns (1954) reported bloat on red clover hay of 72% dry matter.

Oil sprays. The spraying of pastures with a peanut oil emulsion has resulted in the prevention of bloat in New Zealand (Petersen, 1955). Two ounces of corn oil were emulsified with 2 qt. of water and sprayed on 9 by 14 yd. plots in Michigan (Smith and Emery, 1955a). Bloat was not prevented, but there was an indication that froth formation was impaired. Additional studies must be completed before the effectiveness and practicability of this method can be ascertained.

Feeding antifrothing compounds. Barrentine, Shawver, and Williams (1954) reported that the administration of 20 gm. capsules of methyl silicone before morning grazing helped to

prevent bloat in the morning but not in the afternoon. A recent study in Michigan (Smith and Emery, 1955a) showed that prior feeding of 5 gm. of methyl silicone or spraying a methyl silicone emulsion on small plots was not effective in preventing bloat. Household detergents have also been fed to cattle in attempts to prevent bloat (Anonymous, 1953).

Feeding minerals. Most of the data indicate this practice to be of doubtful value. Olson (1940b) reported that the bloating of cattle from alfalfa in Argentina is not considered a very serious menace when rock salt is available at all times. Espe and Cannon (1940) reported that the amounts of salt, soda, and hydrated lime, or combinations of the three, which cows will tolerate in their drinking water increase rather than suppress gas formation.

Regulating water consumption. Lindahl et al (1954) produced bloat in ruminants by feeding large doses of saponins, but no distention was produced if the amount of water was increased two to three-fold. Olson (1940a) reported that cows were watered four times in ten hours while on pasture and still bloated. Cole, Mead, and Regan (1943b) studied the production and prevention of bloat in cattle on alfalfa pasture. They stated, "Although bloat has often been attributed to either the presence or the absence of water, we observed no differences in the extent of bloat which we could ascribe to this factor. Our cows always had free access to water when they were taken off pasture, and very few bloated after that time." Weiss (1953b) reported a study in which

test animals were deprived of water for twenty-four hours before pasturing. Half of the animals were watered just prior to turning on pasture. A greater incidence of bloat among the watered group was noticed. In another study, Weiss (1953a) found that frothiness occurred in sheep, when the rumen ingesta were thick and viscid. The froth was insignificant, however, when the rumen ingesta were watery. If this observation is valid in all cases of bloat, then one might expect thick and viscid froth-producing ingesta to become non-frothy when the animal takes in water. It is also apparent that when ingesta are too dry to produce froth, the ingestion of water may create froth within the rumen. It is also well to keep in mind that the rumen ingesta may become more watery in the absence of drinking water (Quin et al, 1951, Weiss, 1953a).

Correcting anatomical abnormalities. It is obvious that bloat resulting from anatomical abnormalities and faulty nervous mechanisms can best be controlled by medically correcting the respective abnormalities.

Treatments for Bloat

Turpentine or kerosene. Whether the above compounds act as irritants to stimulate belching, or alter the surface tension of rumen liquor to allow the gas to escape, or both, is still controversial. Dougherty and Meridith (1954) reported an *in vitro* study of the efficacy of silicone products compared to turpentine for the treatment of bloat. They found

that the silicone products dispersed rumen liquor foam better than turpentine. The reverse was true, however, when the materials were judged against the reconstitution of foam produced by reshaking the rumen liquor. Clark (1948) found that the in vitro administration of turpentine breaks up foam and facilitates the movement of gas through rumen ingesta. Blake et al (1955) reported that the in vivo administration of turpentine decreased the surface tension of frothy rumen ingesta. Clark (1950b) reported that turpentine, administered to sheep bloated by 100 gm. of sucrose and 2 gm. of saponin, resulted in a moderate rise in rumen pressure.

Formaldehyde. This compound has also been successfully used to treat bloated animals (Huffman, 1955).

Lipase treated cream. Smith et al (1953) reported that lipase treated cream was effective in reducing the froth of experimentally produced frothy bloat.

Silicone compounds. Dougherty and Meridith (1954) found that silicone suspension products were effective in dispersing in vitro rumen liquor foam preparations. They were not as efficacious as turpentine, however, in preventing reconstitution of the foam. Quin et al (1948) reported the successful treatment of bloat by intrarumenal injection of methyl silicone. Smith et al (1953) reported the reduction of froth in experimentally produced frothy bloat by administering methyl silicone. Barrentine and Shawver (1954) reported that treating bloated animals with methyl silicone appeared to be of doubtful value for bloat produced by pasturing

animals on ladino clover.

Household detergents. These cleaning compounds have been used to treat and prevent bloat (Anonymous, 1953). Blake et al (1955) found that the in vivo administration of a detergent decreased the intrarumenal surface tension.

Adrenalin and atropine sulfate. These compounds have been used successfully to relieve specific cases of bloat produced by injection of Trichomonas fetus antigen (Kerr and Lamont, 1946, and Lamont, 1946). Shanks (1946) also relieved the antigen type of bloat and also "naturally occurring" bloat by injecting adrenalin. Clark and Weiss (1952a), however, reported that a mass outbreak of bloating in cattle resulted from adrenalin secretion, caused by psychic disturbance of the animals. Johns (1954) reported that the administration of adrenalin to cattle bloated by stall fed red clover was "definitely dangerous." Treatment with an antihistaminic agent yielded similar results.

Mechanical treatments. The administration of a stomach tube, or trocar and cannula, to a bloated animal is commonly employed (Cole et al, 1945). The latter treatment should be attempted only if a bloated animal is down and unable to get up. Emergency rumenotomies sometimes have to be resorted to to relieve severe cases of frothy bloat (Cole et al, 1955). Mild cases of bloat have been relieved by simply walking the animal or placing a stick or rope in the animal's mouth (Petersen, 1950).

EXPERIMENTAL PROCEDURE

Steer number C-707, a rumen fistulated, five year old, 1,450 lb. Guernsey, was placed on each of two rations two weeks prior to the initial sampling date. The first ration consisted of 24 lb. of first cutting alfalfa hay plus 50 gm. of salt daily. The second ration consisted of 4 lb. of second cutting alfalfa hay, 50 gm. of salt, and 10 lb. of the following concentrate mixture daily, subsequently referred to as T-7 mix:

77.0 % ground corn
20.0 % soybean oil meal
1.0 % dicalcium phosphate
1.0 % salt
1.0 % calcium carbonate
25 gm./100 lb. dry vitamin A
5 gm./100 lb. irradiated yeast

Steer number CS-126, a rumen fistulated, 13 month old, 550 lb. Holstein, was placed on each of the three rations two weeks prior to the initial sampling date. The first ration consisted of 18 lb. of second cutting alfalfa hay plus 50 gm. of salt and 5 mg. of cobalt sulfate daily. The second ration consisted of 3 lb. of second cutting alfalfa hay, 12 lb. of T-7 mix, plus 50 mg. of salt daily. The third ration was the same as the second except that 3.26 % urea "262" was substituted for the 20 % soybean oil meal.

Rumen ingesta was obtained from both animals, ventromedial to the fistula plug. The ingesta was chilled in a deep-freeze for 30 minutes and then forced through cheese-

cloth to remove the larger feed particles. The smaller feed particles were then removed by passing the rumen liquor through glass wool. The rumen liquor was then centrifuged at 1,052 relative centrifugal force. The supernatant was pipetted off for use in the fractionation and determination of physical measurements.

The following determinations were performed in duplicate:

(1) Five milliliters of supernatant were pipetted into a 500 ml. Kjeldahl digestion flask for total soluble nitrogen determination.

(2) Twenty-five milliliters of supernatant were pipetted into a 110 ml. volumetric flask for non-heat coagulatable nitrogen determination. Seventy-five milliliters of distilled water were added and the solution boiled for twenty minutes. It was then cooled to room temperature and filtered through Whatman No. 42 filter paper. Twenty-five milliliters of the filtrate were then pipetted into a 500 ml. Kjeldahl digestion flask.

(3) Twenty-five milliliters of supernatant were pipetted into a 250 ml. pyrex beaker and 100 milliliters of 15% trichloroacetic acid added. The mixture was allowed to stand 30 minutes and then filtered through Whatman No. 42 filter paper. Twenty-five milliliters of the filtrate were then pipetted into a 500 ml. Kjeldahl digestion flask for trichloroacetic acid nonprotein nitrogen determination.

(4) Twenty-five milliliters of supernatant were pipetted into a 250 ml. pyrex beaker and 100 milliliters of 95% ethanol

were added. The mixture was then filtered through Whatman No. 42 filter paper. Twenty-five millliliters were then transferred to a 500 ml. Kjeldahl digestion flask for alcohol nonprotein nitrogen determination.

Fifteen millliliters of concentrated sulfuric acid were added to the contents of each digestion flask. Fifteen grams of catalyst (43.75 gm. mercuric oxide plus 1000 gm. sodium sulfate) were added to the flasks. The contents were allowed to digest over a gas flame until 30 minutes after they became clear.

The solutions were then cooled to room temperature and 200 millliliters of distilled water were added to each flask. Sixty millliliters of 50% scdium hydroxide solution (containing 125 gm. sodium thiosulfate per 500 gm. of sodium hydroxide) and zinc were then added to each flask and the contents boiled over a Kjeldahl electric distillation rack. The distillate was collected in 250 ml. Erlenmeyer flasks containing 10 ml. of 4.5% boric acid, 15 ml. of distilled water, and 5 drops of Kjeldahl indicator. The distillations were allowed to proceed until the distillates reached the 125 ml. level in the Erlenmeyer flasks. The distillate-boric acid solution was then titrated to the endpoint with standard 0.02 N. sulfuric acid.

The alcohol protein nitrogen fraction and the trichloroacetic acid protein nitrogen fraction were determined by subtracting the respective nonprotein nitrogen fraction values

from the corresponding total soluble nitrogen determinations. Heat coagulatable nitrogen was determined by subtracting non-heat coagulatable nitrogen values from the corresponding total soluble nitrogen determinations.

The rumen liquor supernatant was cooled to 10 degrees C. and passed through a modified 25 ml. pipette to determine the flow time.

Twenty-five milliliters of the supernatant were bubbled through a frittered glass foammeter to determine the breaking height of the foam column and the stability of the foam. The stability was ranked relatively.

RESULTS AND DISCUSSION

The main objective of this study was to determine whether specific nitrogen fractions of rumen fluid could be correlated with froth production in ruminants.

Total Soluble Nitrogen

The total soluble nitrogen (TSN) found in the supernatant layer of strained, centrifuged rumen fluid obtained from steer number CS-126 is illustrated in Figure I. The non-froth producing ration of alfalfa-brome hay resulted in a TSN level below that of the froth producing ration, T-7, but greater than the TSN level of the other froth producing ration containing urea. The latter ration was the same as the T-7 ration, except that urea replaced the soybean oil meal on a nitrogen equivalent basis. Since froth was present when the animal was on either the T-7 or the urea ration, and it was not present when the animal was on the hay ration, it might be concluded that either TSN does not produce frothiness, or that there is a froth inhibiting factor(s) in alfalfa-brome hay. It was found that considerable dirt in the feed manger inhibited froth production. The dirt had been brought in by a burrowing rat. Difficulty was also experienced when froth production was attempted without feeding hay. When three pounds of second cutting alfalfa-brome hay were added to the T-7 or urea ration, the animals began frothing in almost all cases. The feeding of cobalt also appeared to aid froth production.

The mean values for the mg.% of TSN for the three rations were as follows: T-7, 93.66 mg.%; hay, 65.99 mg.%; and urea, 54.12 mg.%.

Similar information for another animal, C-707, is represented by Figure II. The TSN level again is greater for the T-7 ration than for the hay ration. The mean mg.% level of TSN for the hay ration was approximately 70% of the mean mg.% level of the T-7 ration for both animals. The mean mg.% of the TSN was the same for steer C-707 on the hay ration as for steer CS-126 on the urea ration. Frothing was never observed when the hay ration was fed, whereas repeated frothing was observed with the urea ration.

The pronounced cycling demonstrated the necessity for sampling at frequent intervals over an extended period of time.

Nonprotein Nitrogen from Trichloroacetic Acid Treatment

The nitrogen obtained by treating 100 ml. of centrifuged rumen fluid with 25 ml. of 15% trichloroacetic acid is shown in Figures III and IV. The results have been expressed as mg.% in Figures IIIB and IVB, and as a percentage of the TSN in Figures IIIA and IVA. A study of Figures IIIA and IIIB revealed that the non-froth producing hay ration resulted in a higher level of nonprotein nitrogen (NPN) than did the urea or soybean oil meal froth producing rations. The higher level for the hay was maintained regardless of whether the results were expressed as total mg.% or as a percentage of the TSN (with two exceptions).

The NPN level of the urea ration for steer CS-126, expressed as mg.%, was much lower than that of the other two rations.

However, the level was between that of the hay and soybean oil meal rations when the data were expressed as a percentage of the TSN. The low NPN level, when expressed as mg.%, might be explained by rapid consumption of the nitrogen by the rumen microorganisms. It would be expected that a ruminant on a urea-rich ration would exhibit a high percentage of NPN. This is demonstrated by Figures IIIA and IIIB. The NPN of the urea containing ration was 67% of the TSN, whereas the NPN of the other concentrate ration was only 57% of the TSN.

The difference between animals in NPN content of the rumen fluid, as determined by trichloroacetic acid precipitation, is shown by comparing Figures IIIA and IIIB with Figures IVA and IVB. The hay ration resulted in the highest level of NPN for steer CS-126, but the lowest level for steer C-707.

Thus, NPN determined by trichloroacetic acid precipitation, as shown in Figures IIIA, IIIB, IVA, and IVB, did not appear to significantly influence froth formation and was not accurately measured by trichloroacetic acid precipitation.

Protein Nitrogen from Trichloroacetic Acid Treatment

The values for the trichloroacetic acid method of protein nitrogen (PN) determination were obtained by subtracting the trichloroacetic NPN values from the corresponding TSN values. Steer CS-126, when receiving the T-7 ration, produced a higher level of rumen fluid PN than did the animal on either the urea or hay ration as revealed in Figures VA and VB. This appeared true regardless of whether the values were expressed as mg.% or as a percentage of the TSN. The feeding of the other froth producing ration that contained urea resulted in a level of

PN approximately one-half that of the T-7 ration, but greater than the non-froth producing hay ration. The feeding of the latter ration resulted in a PN level approximately one-third that of the T-7 ration. It was observed that the higher the level of PN, the greater the day to day variation.

An inverse relationship for the trichloroacetic acid PN values for steer C-707 as compared to CS-126 are revealed in Figures VIA and VIB. These limited data show that the PN level resulting from the feeding of the hay ration exceeded the T-7 ration, regardless of whether the values were plotted as mg.% PN or as a percentage of the TSN.

The "t" test revealed that the trichloroacetic PN values of the rumen fluid of these animals, when fed the T-7 ration, were significantly different to the 0.1% level. This was not true when they were fed the hay ration.

Non-heat Coagulable Nitrogen

The amount of nitrogen that did not coagulate when rumen fluid was boiled for twenty minutes is shown in Figures VIIA and VIIB. Figure VIIB revealed very erratic lines when the non-heat coagulable nitrogen was plotted as mg.%. However, when these values were plotted as a percentage of the TSN, a more logical picture was presented. The graph reveals that the non-froth producing hay ration produced an almost steady line between 95 and 98.5% of the TSN, whereas both the T-7 ration and urea ration lines appear erratic. However, the first four observations plotted on the T-7 ration line were obtained when the animal was slightly "off-feed" and producing very little froth. Conversely, the last four observations

occurred when the animal was frothing heavily. Thus, the line reveals four non-frothing observations with a mean of about 100% of the TSN, and four frothing observations with a mean of about 88% of the TSN.

A study of the urea ration line revealed a pattern approximating that of the T-7 ration line. The first two and the last four observations occurred when the animal was frothing. The third, fourth, and fifth observations occurred when the animal was either not frothing or only very slightly. The mean of the frothing was approximately 90% of the TSN, and the mean of the non-frothing observations was approximately 81% of the TSN.

It should be noted that the mg.% non-heat coagulable nitrogen graphs, Figures VIIB and VIIIB, and the TSN graphs, Figures I and II, can be almost superimposed upon each other.

The non-heat coagulable nitrogen graphs for the rumen fluid from steer C-707 present a pattern somewhat similar to those for steer CS-126. The non-heat coagulable nitrogen values from the T-7 ration are higher than those from the hay ration, regardless of whether they are plotted as mg.% or as a percentage of the TSN. The hay ration produced a more variable picture with steer C-707, when plotted as a percentage of the TSN, than with steer CS-126.

Heat Coagulable Nitrogen

The heat coagulable nitrogen values for the rumen fluid from steer CS-126, plotted as a percentage of the TSN and as mg.-% heat coagulable nitrogen, are illustrated in Figures IXA and IXB. It is apparent that the heat coagulable nitrogen values from the hay ration remain almost constant from one

sampling to the next. The urea and T-7 rations, however, demonstrate variable results that can only be partially explained by studying the behavior of the animal. The graph for the T-7 ration shows the first four values to be low compared with the last four values. The animal's rumen was either not frothing or frothing only very slightly during the first four sampling periods, but was frothing considerably during the last four periods, when the heat coagulable values were much higher. When the urea samples were obtained, the animal's rumen contents were exhibiting considerable froth during the first two and last four sampling periods, but either no froth or very little during the third, fourth, and fifth sampling periods.

The graphs for the heat coagulable nitrogen of the three rations illustrate that when the animal was frothing, the heat coagulable nitrogen values were usually higher and represented a larger percentage of the TSN, than when the animal was on the non-frothy ration.

Heat coagulable nitrogen values for the rumen fluid of steer C-707 are plotted in Figures XA and XB. They demonstrate an inverse relationship from those of steer CS-126. It was noted that, with one exception, when plotted as a percentage of the TSN, the non-froth producing hay ration yielded higher heat coagulable nitrogen values than the froth producing T-7 ration.

Nonprotein Nitrogen by Alcohol Precipitation

The values obtained by precipitating rumen fluid with 95% ethyl alcohol are shown in Figures XIA and XIB. The data

display considerable variability between samples from the same ration and also between rations. The froth producing rations generally yielded more NPN, when expressed as a percentage of the TSN, than the non-froth producing hay ration.

The NPN values for rumen fluid from steer C-707, which was fed a hay ration, are illustrated in Figures XIIA and XIIB. The mean values obtained for NPN from C-707 and CS-126 were 27.76 mg.% and 25.74 mg.%, respectively. However, when the data were expressed as a percentage of the TSN, the difference was greater, 50.50% for C-707 and 39.23% for CS-126. These values were found to be significantly different at the 5% level when the "t" test was applied.

Protein Nitrogen by Alcohol Precipitation

The PN obtained by precipitating the rumen fluid of steer CS-126 with 95% alcohol is shown in Figure XIIIA. This graph is an inverse of Figure XIA, which illustrates the NPN. Thus, when the PN is plotted as a percentage of the TSN, the higher values tend to favor the non-froth producing ration.

The alcohol precipitated rumen fluid PN for steer CS-126, expressed as mg.%, is shown in Figure XIIIB. The data point out that the non-froth producing hay ration yielded PN values midway between the values for the froth producing rations.

Figures XIB and XIIIB appear very similar despite the fact that the former figure illustrates the data for NPN and the latter represents PN. In both graphs, the froth producing T-7 ration containing a high percentage of soybean oil meal yielded the greatest amount of rumen fluid nitrogen, the froth producing urea ration containing the theoretical equivalent

of nitrogen yielded the least rumen fluid nitrogen, and the non-froth producing hay ration yielded values midway between the two froth producing rations.

The alcohol precipitated PN of the rumen fluid from steer C-707, plotted as a percentage of the TSN, is illustrated in Figure XIVA. The mean percentage of the values from the hay ration was 49.50, compared with 59.76 for steer CS-126.

The alcohol precipitated PN of the rumen fluid from steer C-707, expressed as mg.%, is shown in Figure XIVB. The PN level remained constant, despite the fact that the TSN level varied considerably. A comparison with the values obtained for steer CS-126 on a similar hay ration showed that the rumen fluid of steer C-707 yielded 26.41 mg.% PN, whereas steer CS-126 yielded 40.26 mg.%. Thus, the considerable difference between animals is again illustrated.

Flow Time

The seconds required for a specific amount of rumen fluid to flow through a graduated pipette is shown in Figure XVA. The results show that the rumen fluid from the T-7 ration required the longest flow time, that from the urea ration the next longest, and the rumen fluid from the non-froth producing hay ration the shortest length of time. Thus, the rumen contents from the two froth producing rations appeared more viscous than those from the non-froth producing hay ration. The data revealed that there was significant difference to the 0.1% level between the flow times from the rumen fluid of the non-froth producing hay ration and those from the froth producing T-7 ration.

Foam Stability and Foam Height

Twenty-five milliliters of rumen fluid were placed in a frittered glass tube and bubbled with air to determine foam height and foam stability. The results, as shown in Figures XVB and XVC, reveal that the foam from the rumen fluid of steer CS-126, when on the hay ration, was more stable and rose to a greater height than when the animal was on the froth producing rations. However, the data are too variable to be conclusive.

Flow time, stability and foam height for the rumen fluid from steer C-707 are illustrated in Figures XVIA, XVIB, and XVIC respectively. The data for this animal closely approximate those for steer CS-126. The rumen fluid from the froth producing T-7 ration yielded a slower flow time than that from the non-froth producing hay ration for both animals. The non-froth producing hay ration yielded more stable foam and greater foam height than the froth producing T-7 ration. The foam height and stability data may be explained by reasoning that the rumen fluid from the hay ration still possessed the froth producing fraction(s), and thus yielded greater foam height and greater stability. The froth producing T-7 ration, however, may have already exhausted the froth producing fraction(s), and thus yielded a shorter column of foam with less stability.

The various rumen fluid nitrogen fractions from steer CS-126, when it was receiving the hay ration, were analyzed in the foam measuring device. Negligible foam was produced from all fractions except the non-heat coagulable fraction. Aliquots as small as 1 ml. produced a column of foam in excess of 48 mm., the limit of the measuring tube. Twenty-five milliliters of strained,

centrifuged rumen fluid yielded only a 12 mm. column of foam. Thus, it appeared that either a foam inhibiting factor(s) was present in the strained, centrifuged rumen fluid, or that heating it to 100°C. for 20 minutes altered the liquid in such a way as to cause greater foam production.

Chemical analysis of the foam from frothy rumen ingesta yielded the following results:

Dry Matter	90.65%
Crude Protein	25 %
Ether Extract	13 %
Ash	16 %
Acid Hydrolyzable Polysaccharides	3.5 %
Starch	2 %

The pH of the foam was 6.6.

Mean values, variance, standard deviation, and standard error of the mean for the rumen fluid from all rations of both steers are shown in Table I.

The levels of significance for the "t" values, obtained by comparing the rations for each animal, and by comparing different steers on the same ration, are illustrated in Table II.

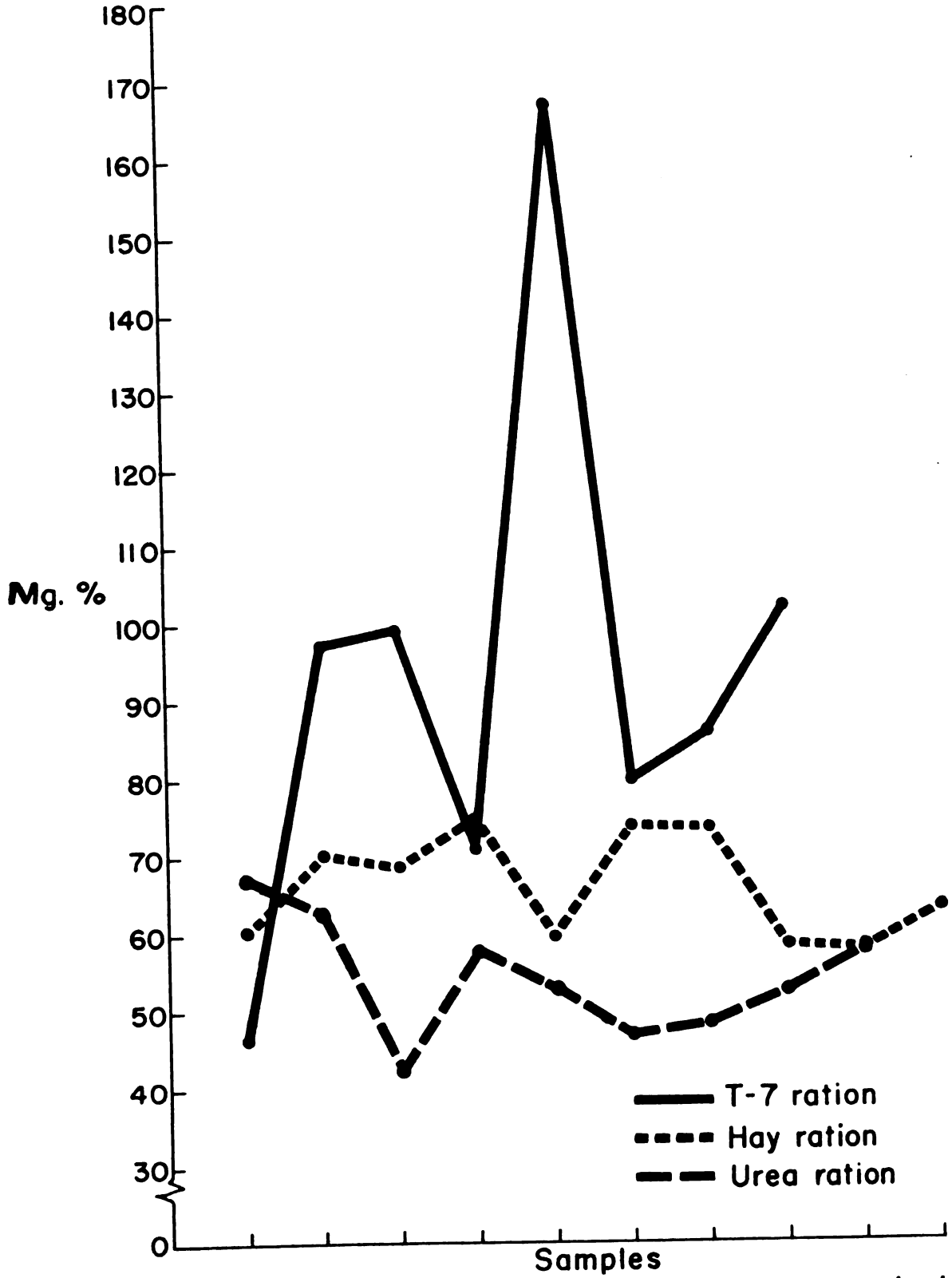


Fig. I. Steer CS-126, total soluble nitrogen content of rumen fluid

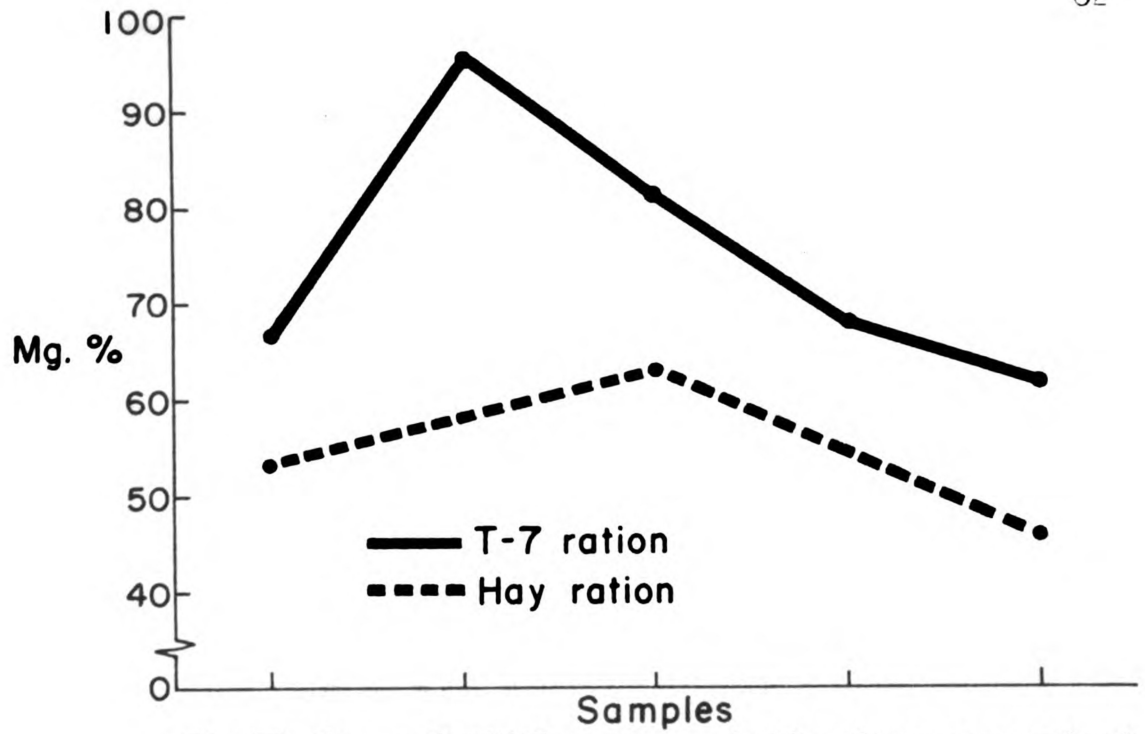


Fig. II. Steer C-707, total soluble nitrogen content of rumen fluid

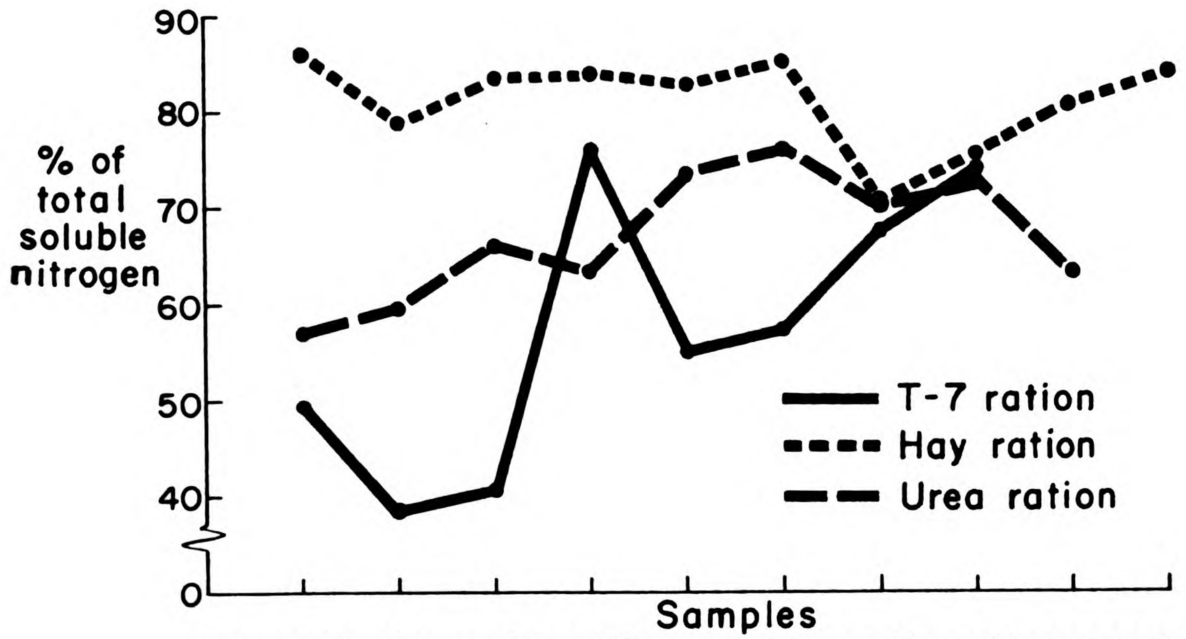


Fig. III A. Steer CS-126, trichloroacetic acid nonprotein nitrogen content of rumen fluid

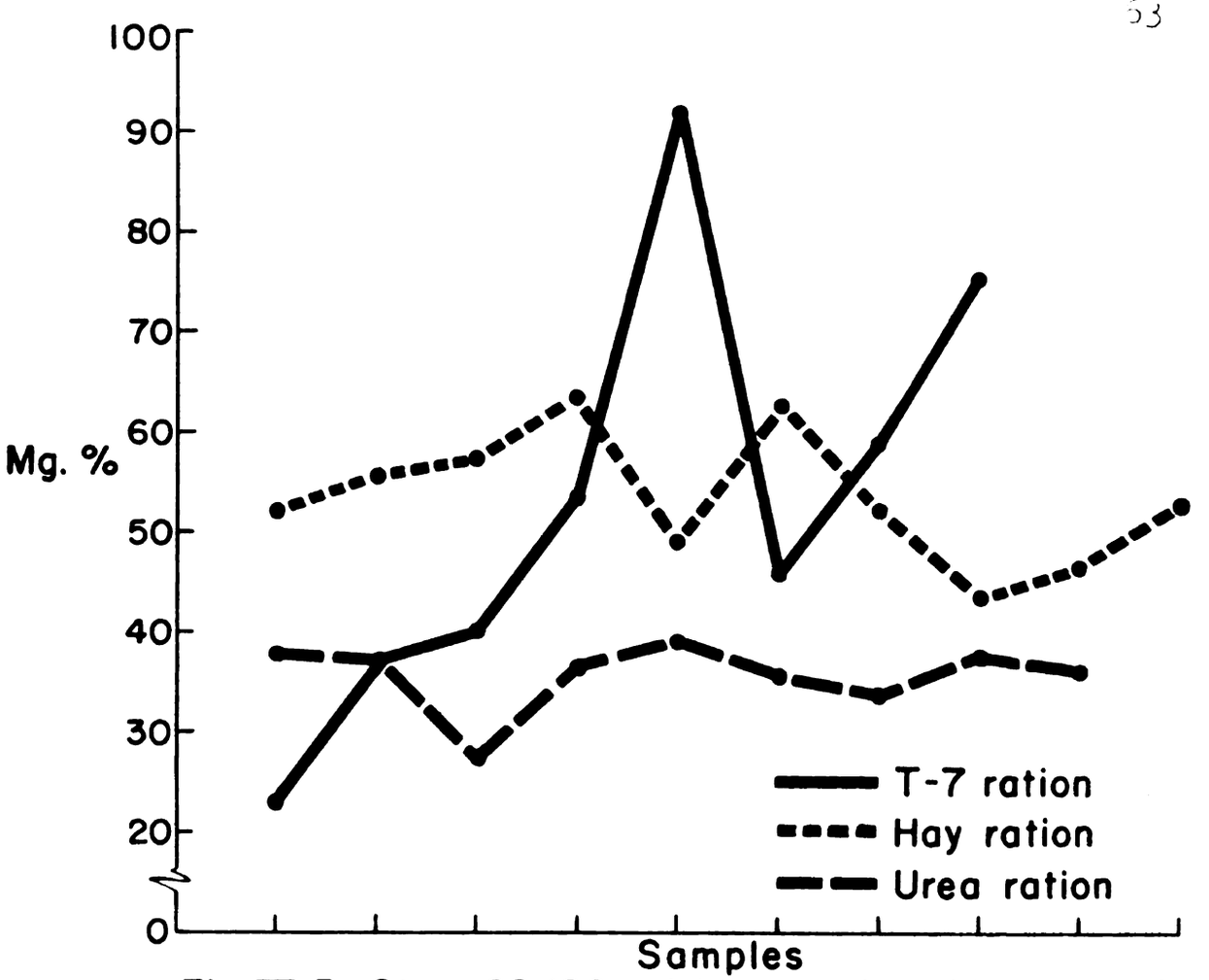


Fig. III B. Steer CS-126, trichloroacetic acid nonprotein nitrogen content of rumen fluid

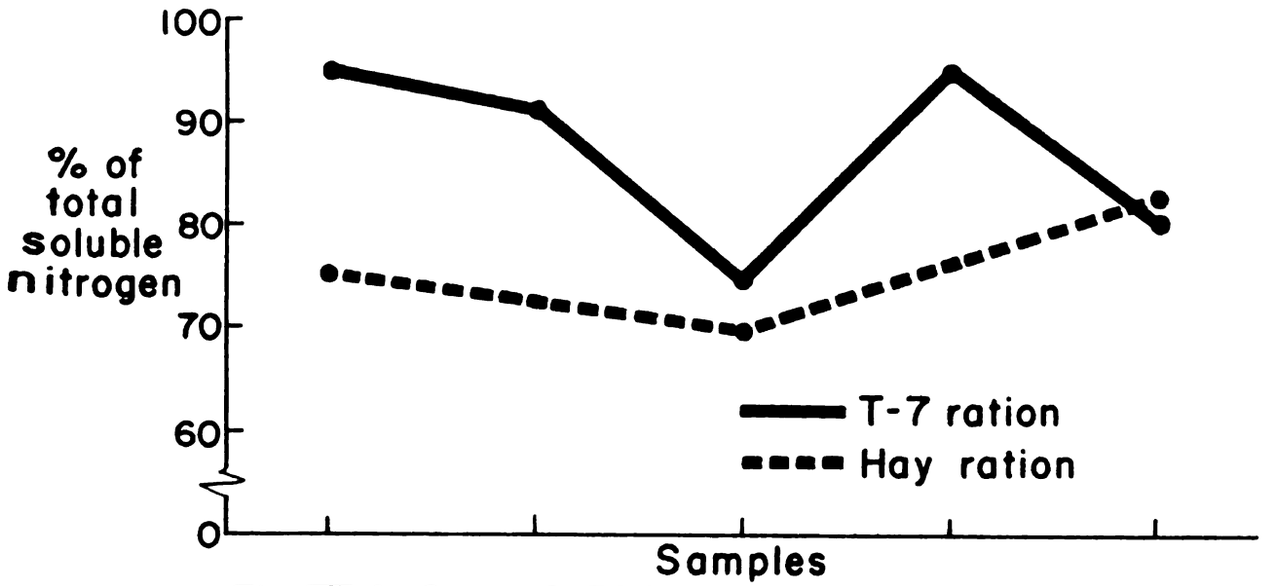
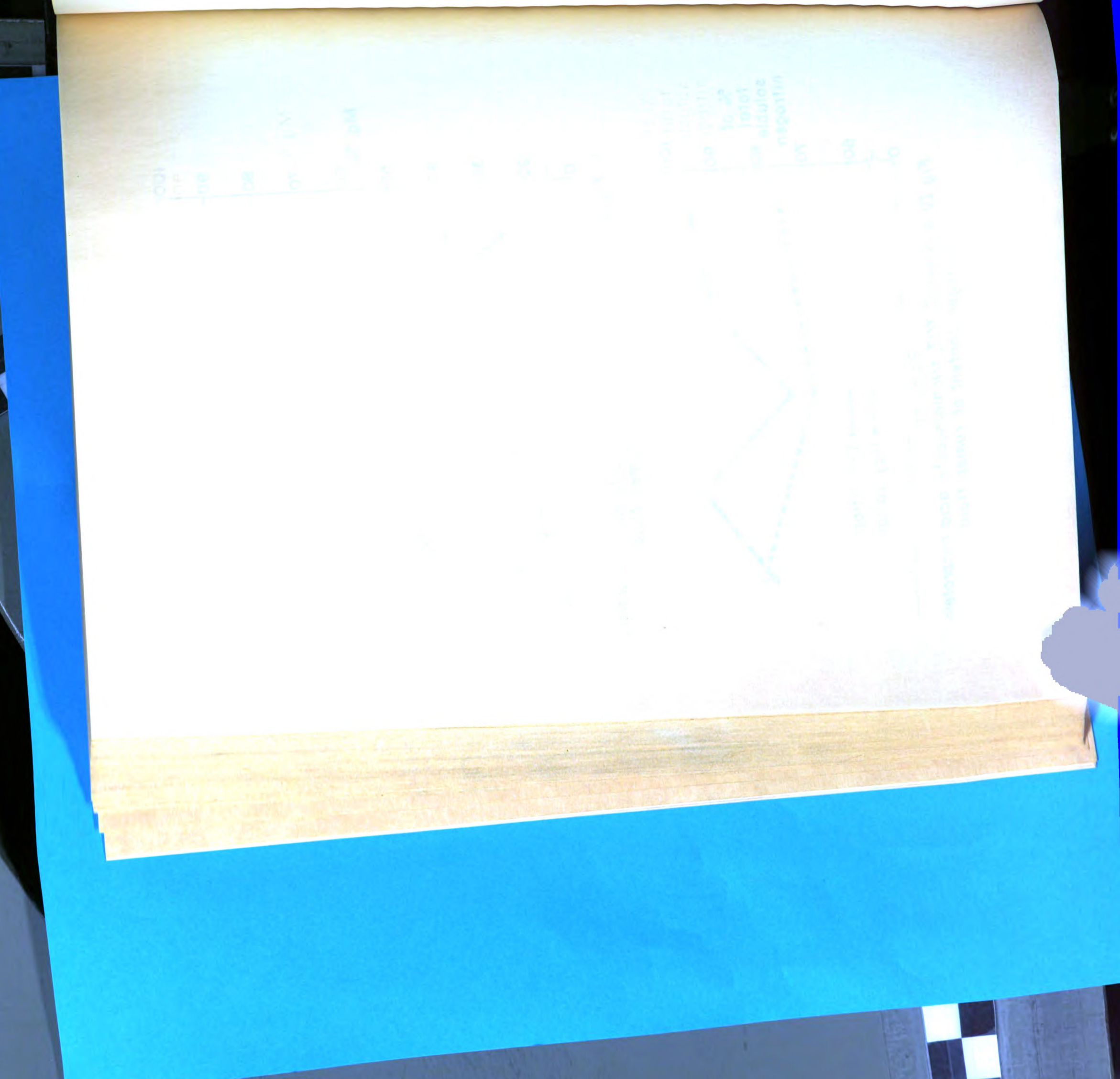


Fig. IV A. Steer C-707, trichloroacetic acid nonprotein nitrogen content of rumen fluid



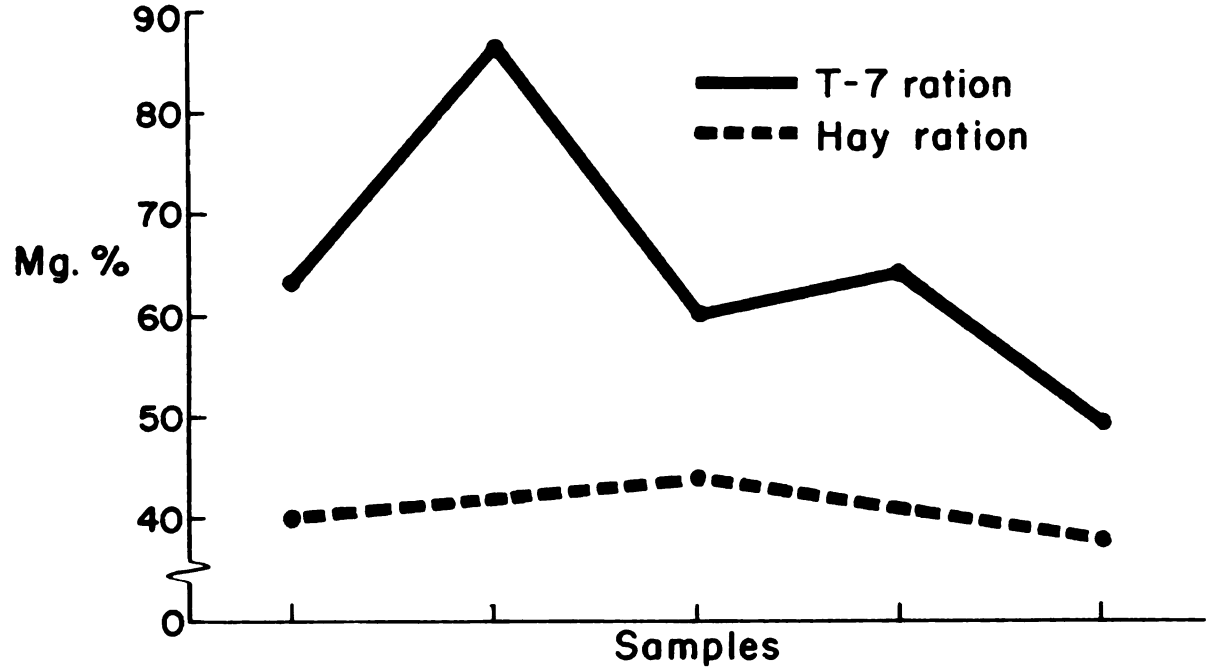


Fig. IV B. Steer C-707, trichloroacetic acid nonprotein nitrogen content of rumen fluid

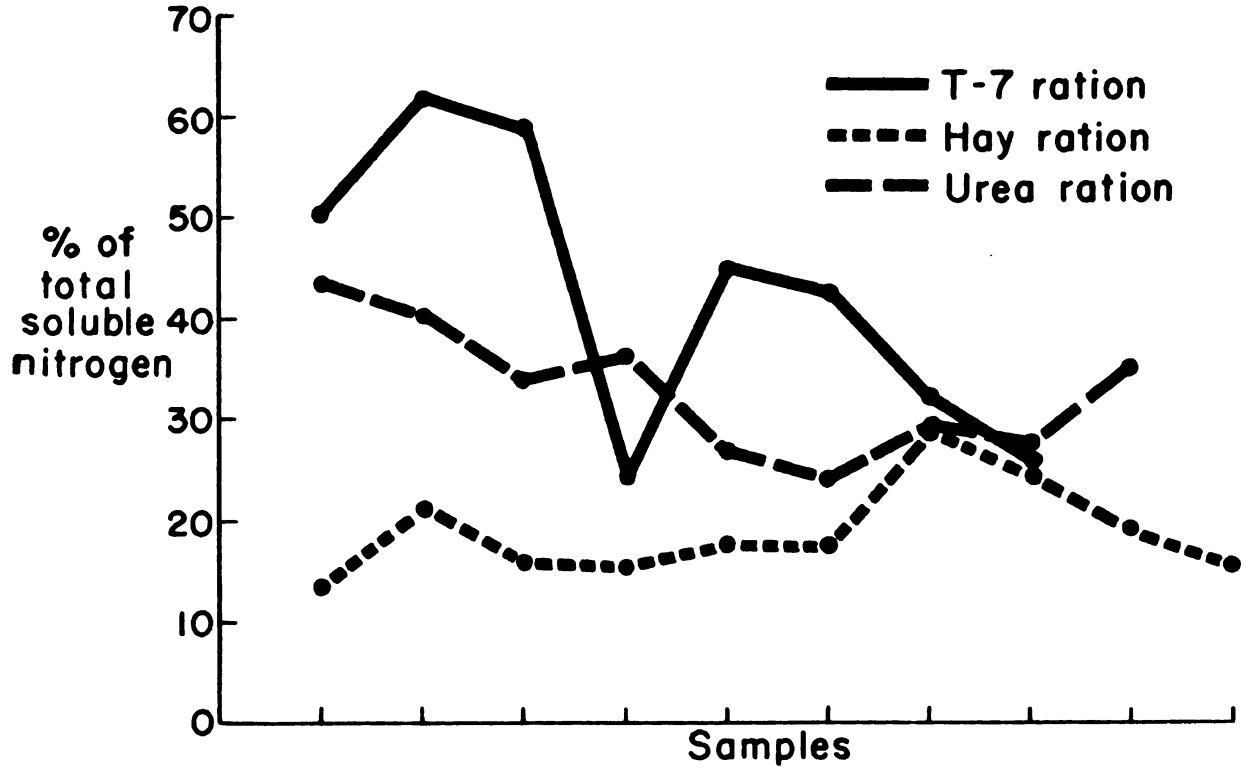


Fig. V A. Steer CS-126, trichloroacetic acid protein nitrogen content of rumen fluid

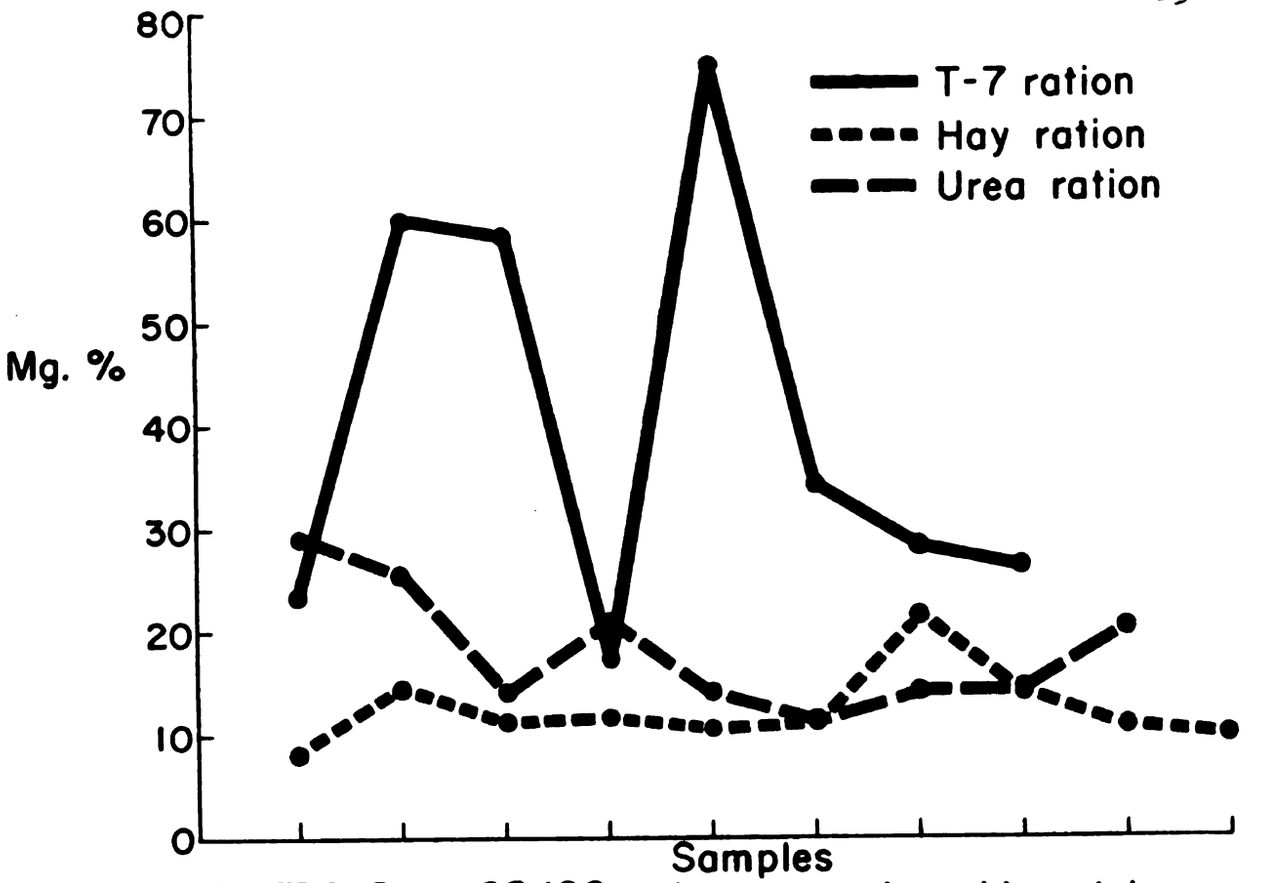


Fig. V B. Steer CS-126, trichloroacetic acid protein nitrogen content of rumen fluid

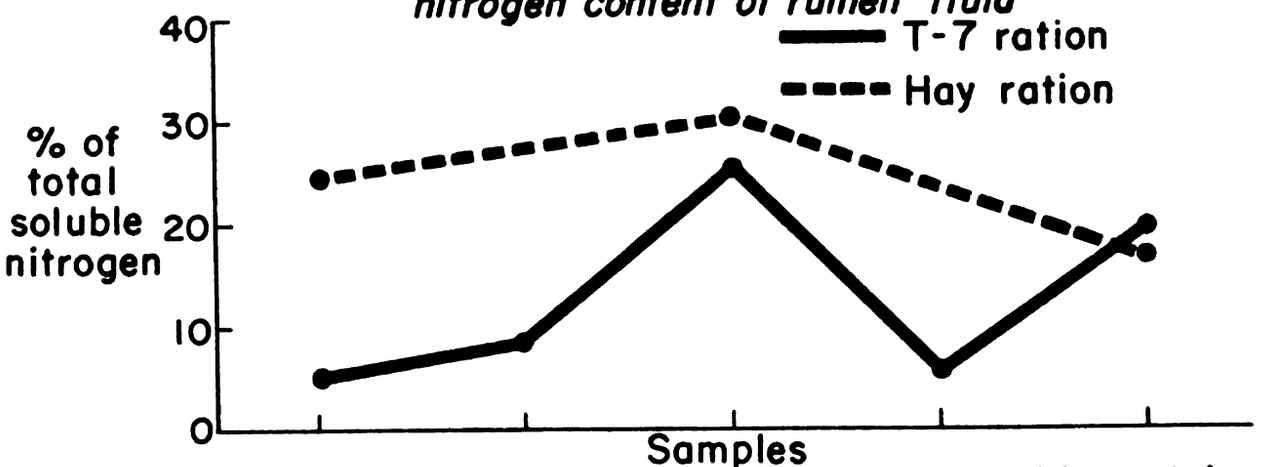


Fig. VI A. Steer C-707, trichloroacetic acid protein nitrogen content of rumen fluid

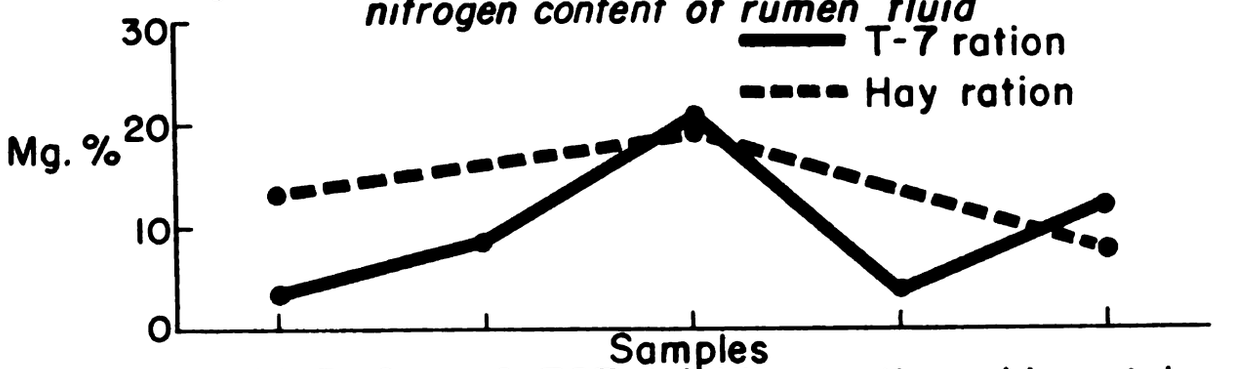


Fig. VI B. Steer C-707, trichloroacetic acid protein nitrogen content of rumen fluid

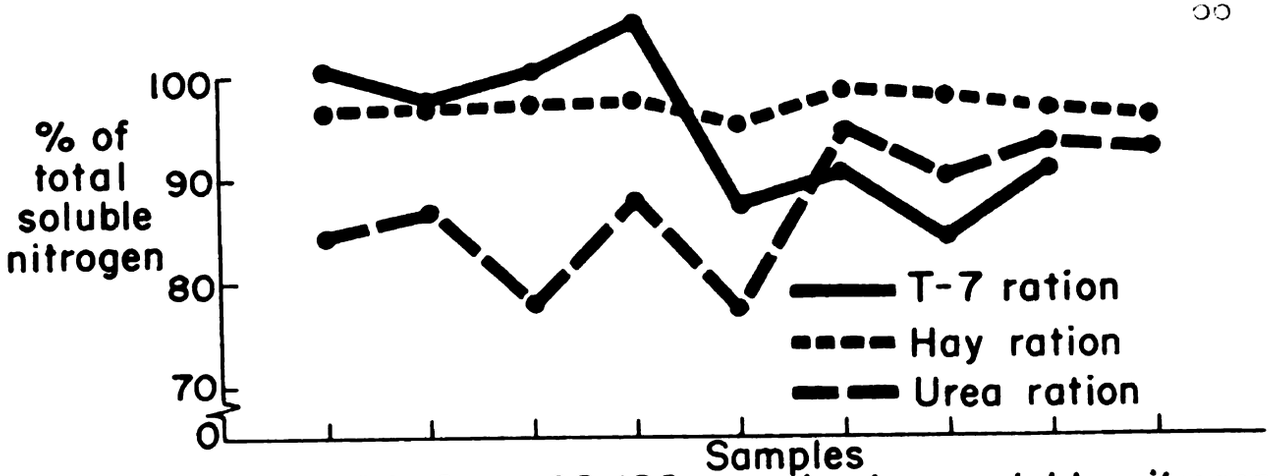


Fig. VII A. Steer CS-126, non-heat coagulable nitrogen content of rumen fluid

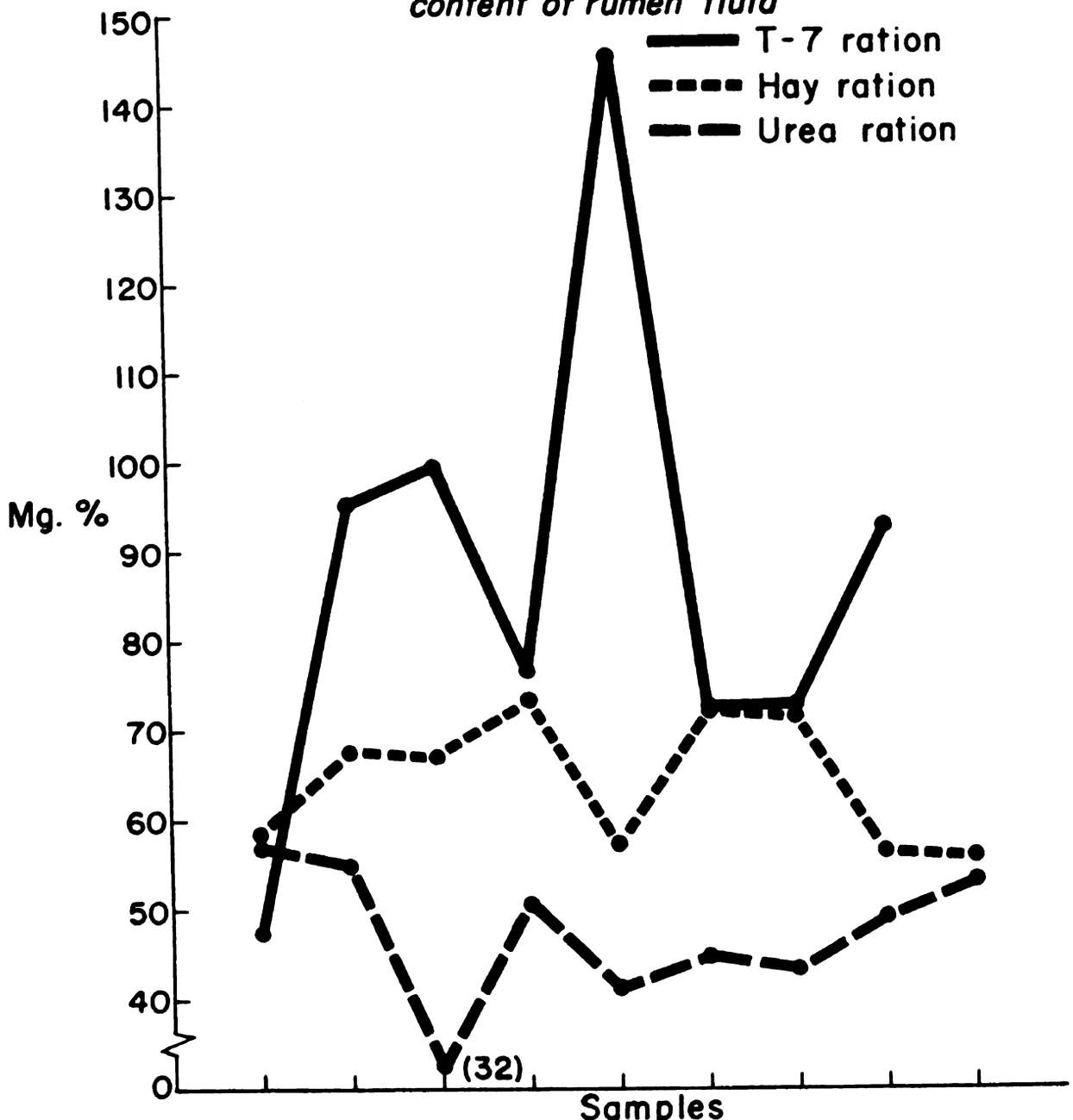


Fig. VII B. Steer CS-126, non-heat coagulable nitrogen content of rumen fluid

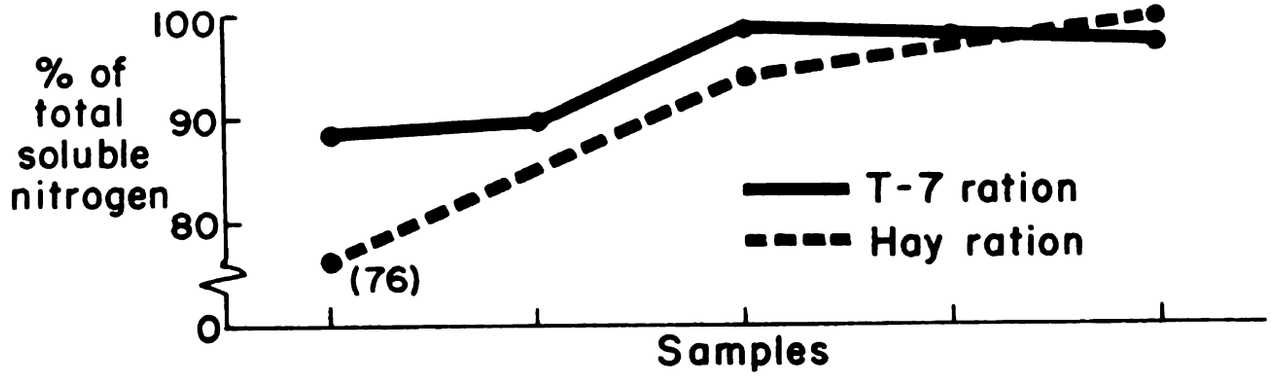


Fig. VIII A. Steer C-707, non-heat coagulable nitrogen content of rumen fluid

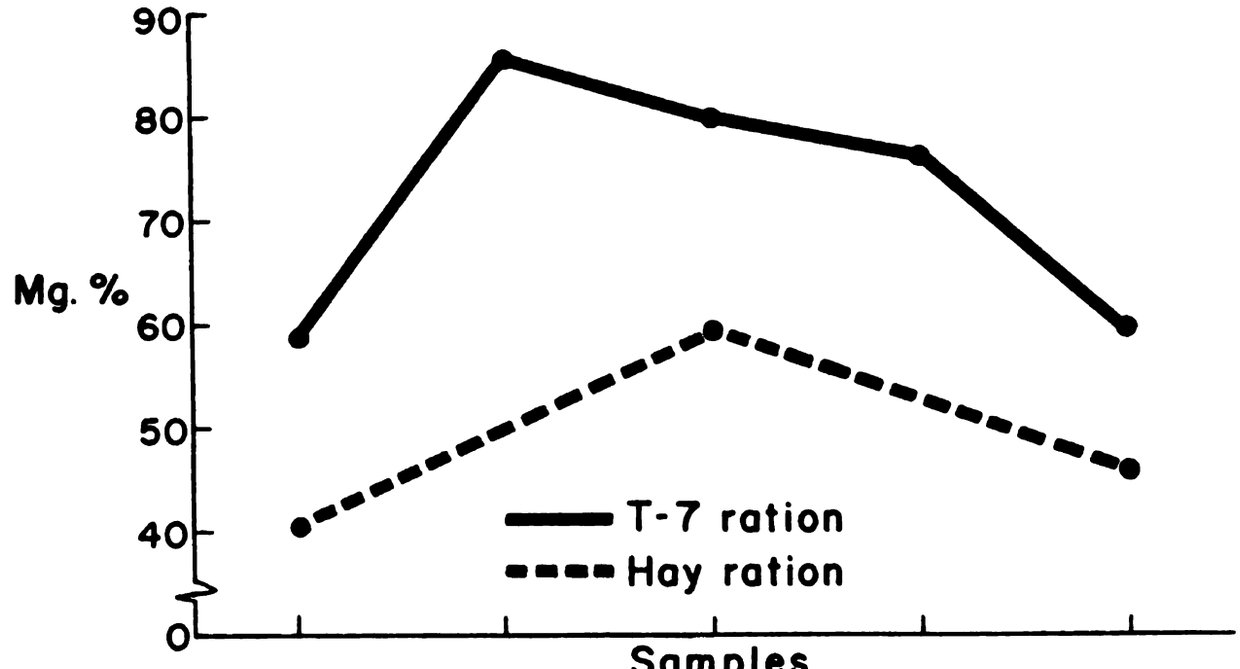


Fig. VIII B. Steer C-707, non-heat coagulable nitrogen content of rumen fluid

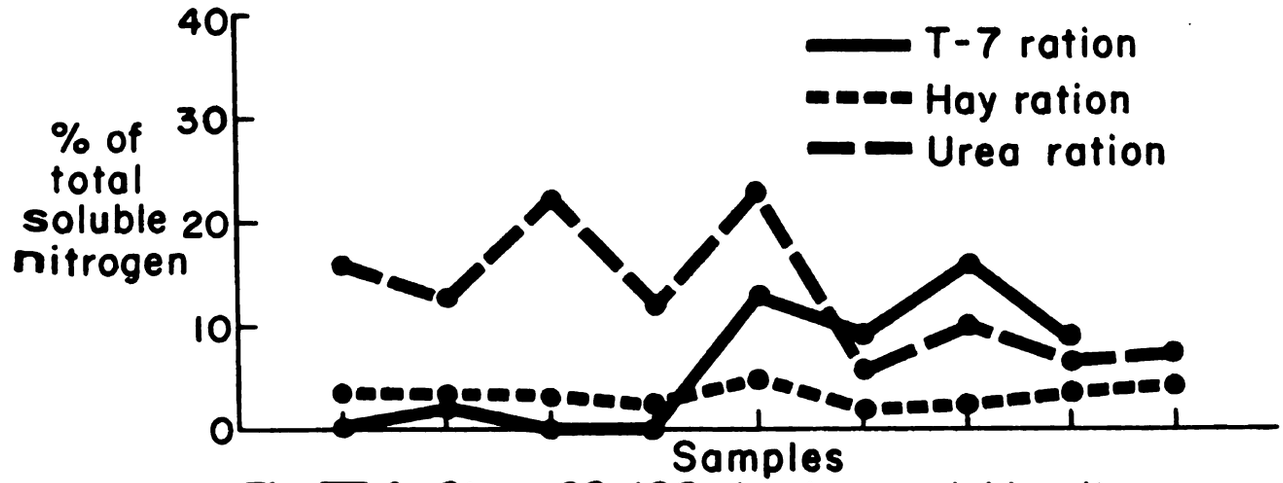


Fig. IX A. Steer CS-126, heat coagulable nitrogen content of rumen fluid

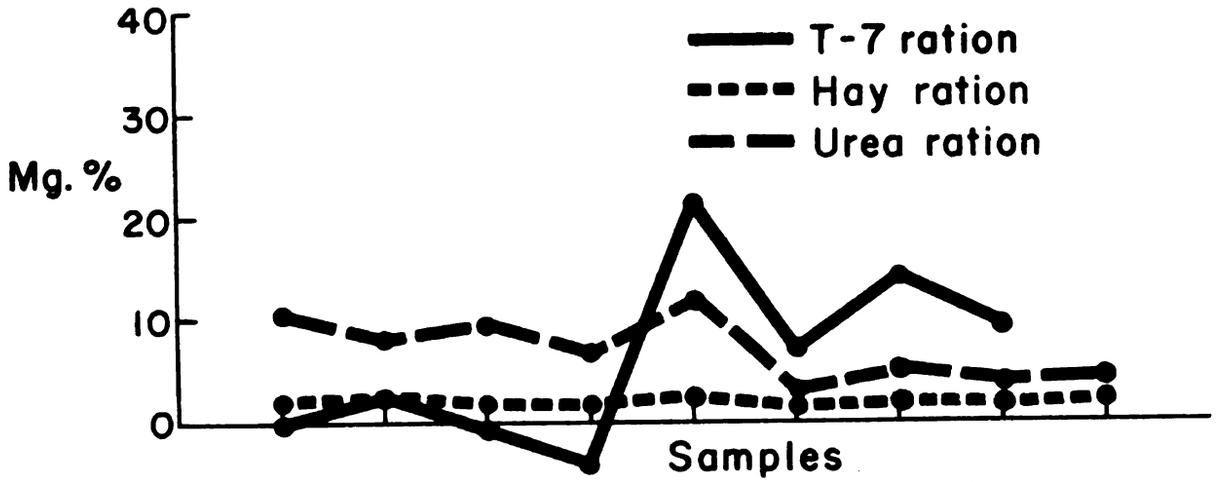


Fig. IX B. Steer CS-126, heat coagulable nitrogen content of rumen fluid

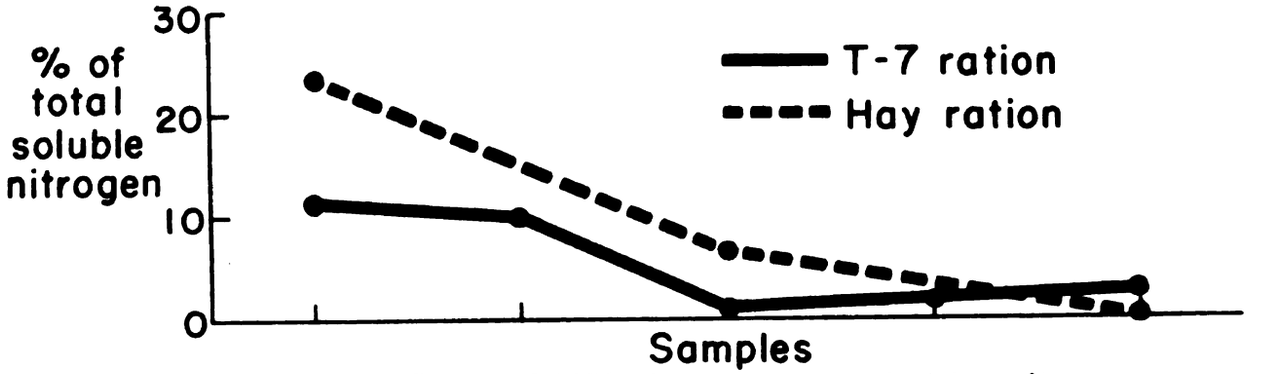


Fig. X A. Steer C-707, heat coagulable nitrogen content of rumen fluid

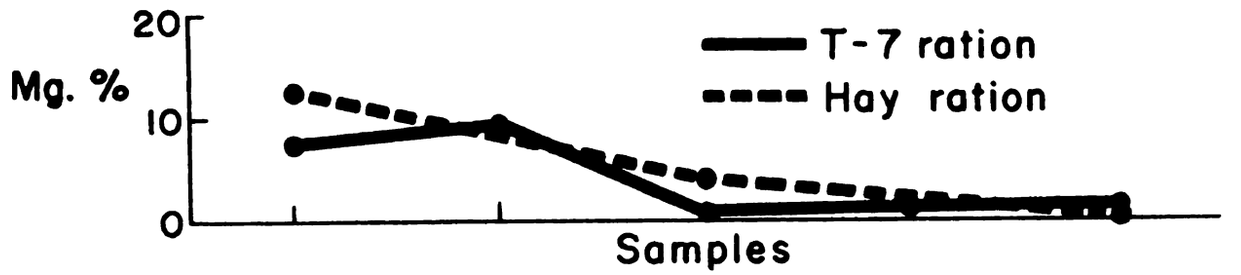


Fig. X B. Steer C-707, heat coagulable nitrogen content of rumen fluid

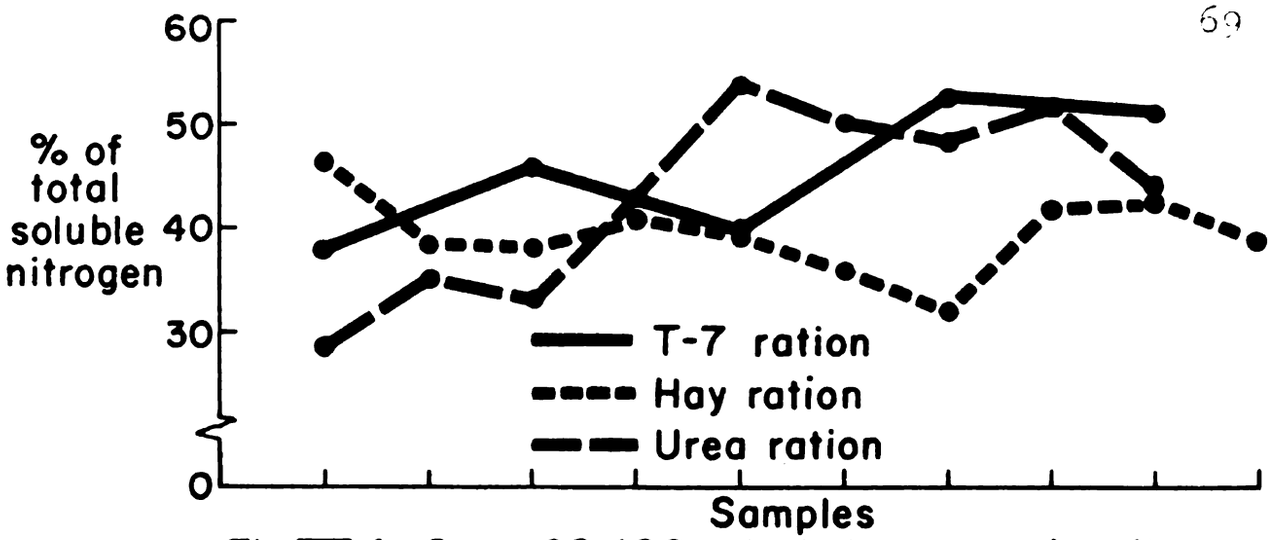


Fig. XI A. Steer CS-126, alcohol nonprotein nitrogen content of rumen fluid

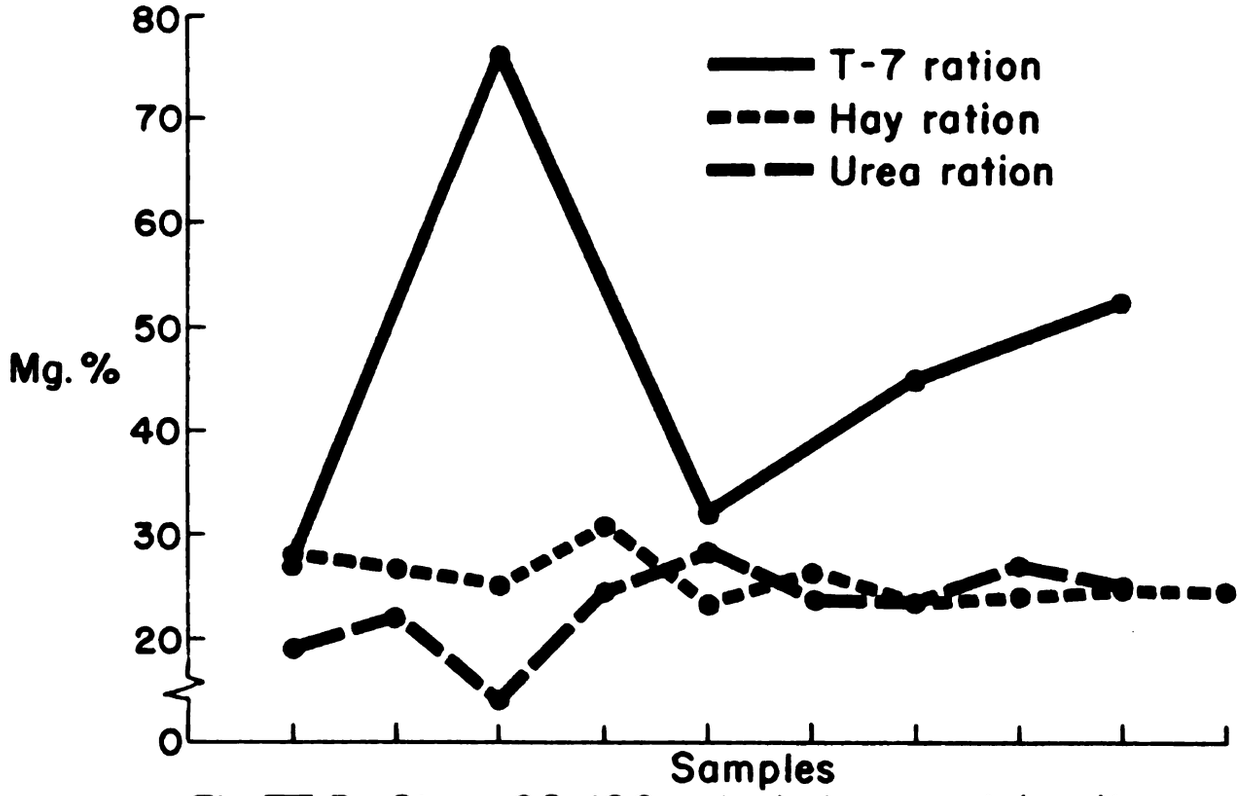


Fig. XI B. Steer CS-126, alcohol nonprotein nitrogen content of rumen fluid

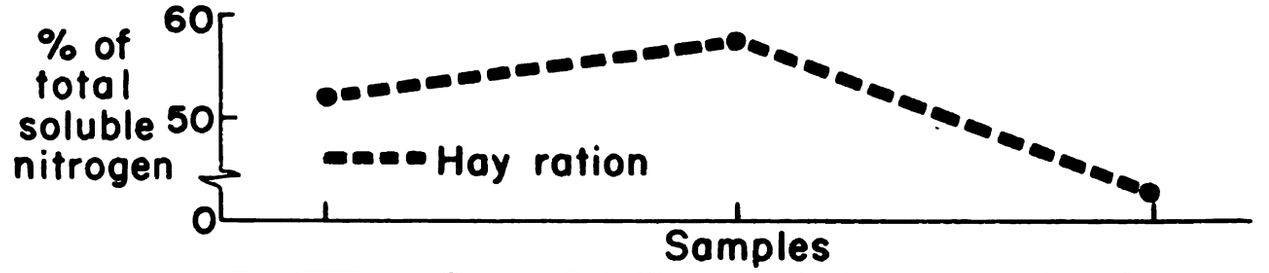


Fig. XII A. Steer C-707, alcohol nonprotein nitrogen content of rumen fluid

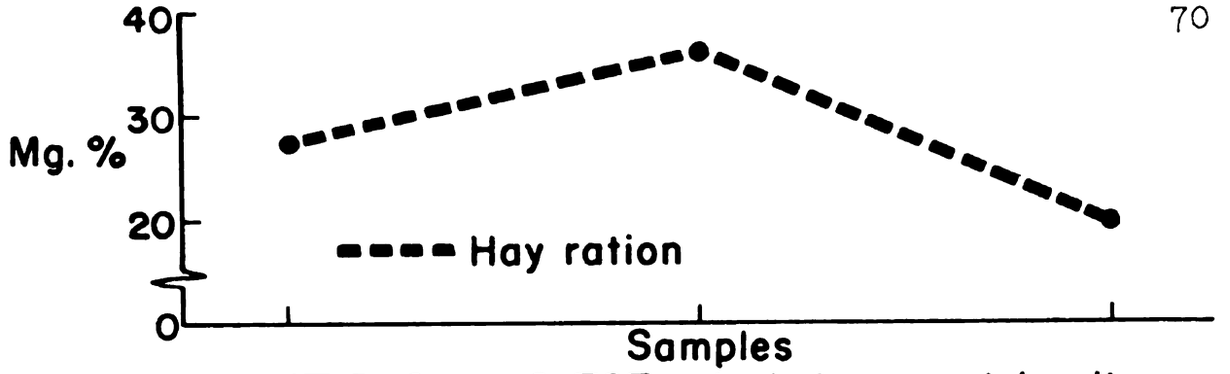


Fig. XII B. Steer C-707, alcohol nonprotein nitrogen content of rumen fluid

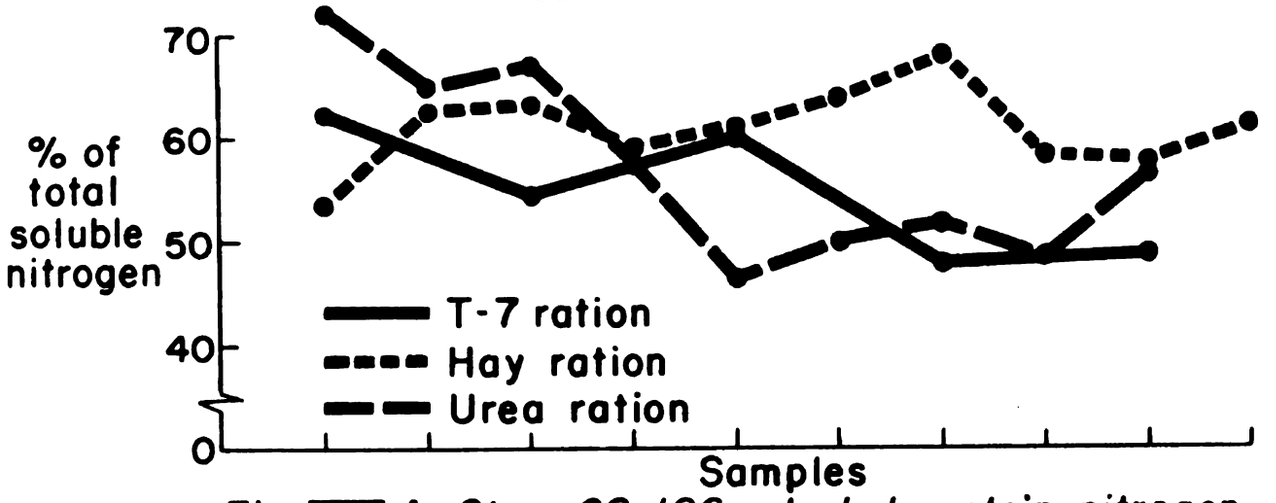


Fig. XIII A. Steer CS-126, alcohol protein nitrogen content of rumen fluid

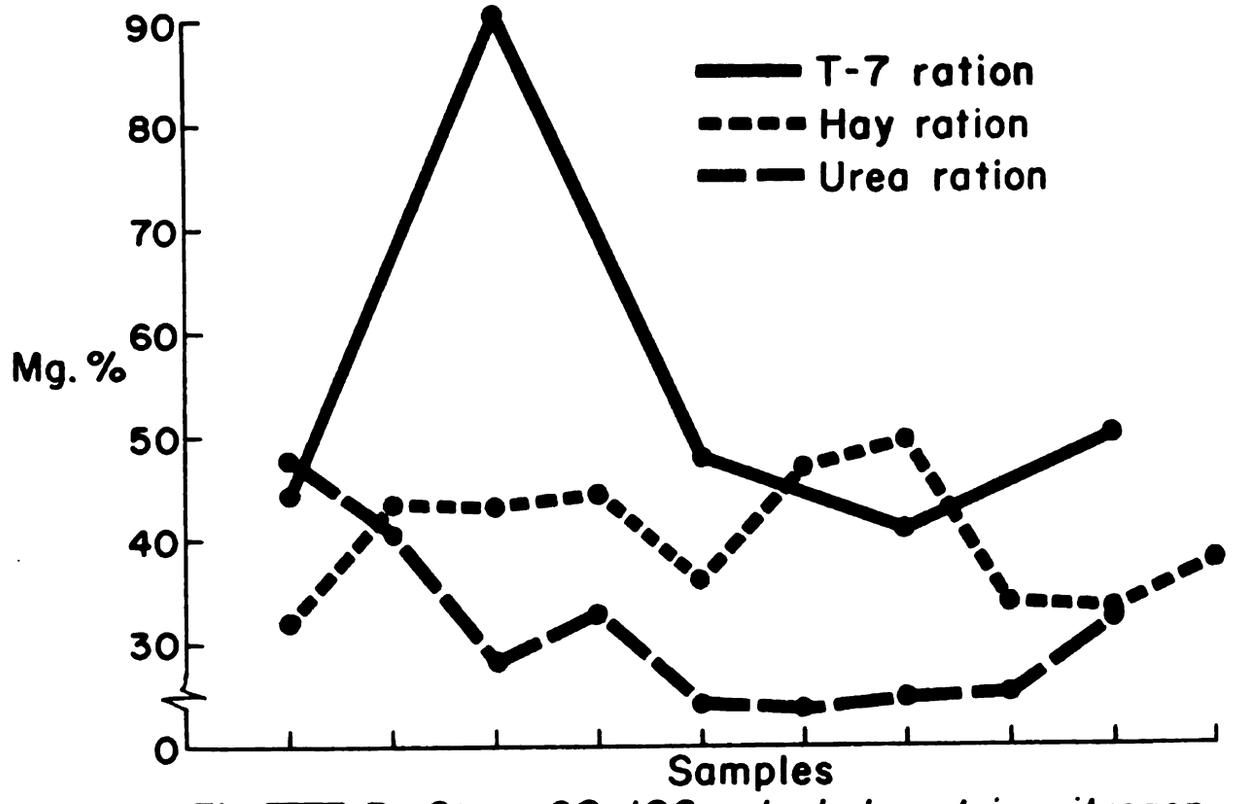


Fig. XIII B. Steer CS-126, alcohol protein nitrogen content of rumen fluid

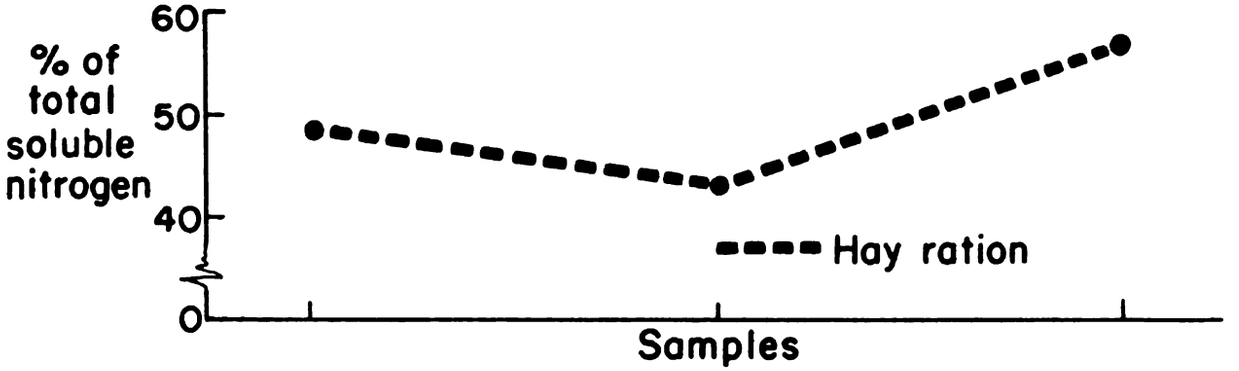


Fig. XIV A. Steer C-707, alcohol protein nitrogen content of rumen fluid

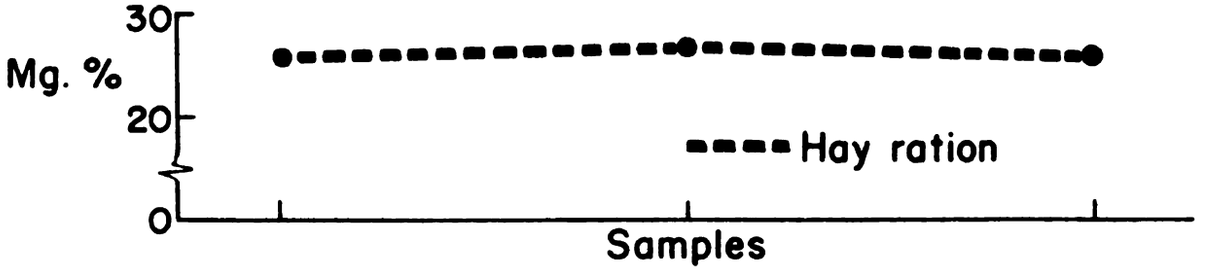


Fig. XIV B. Steer C-707, alcohol protein nitrogen content of rumen fluid

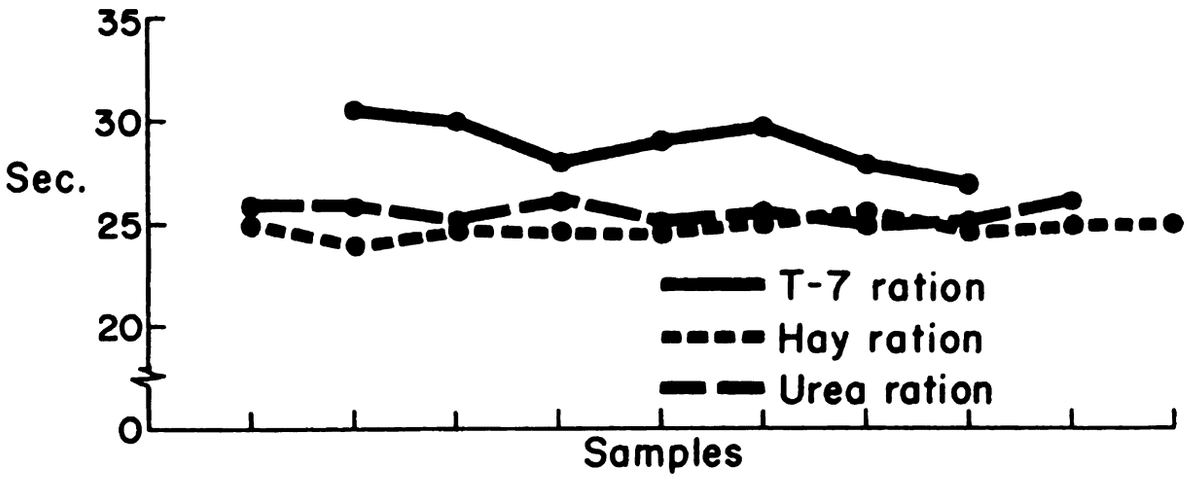


Fig. XV A. Steer CS-126, rumen fluid flow time

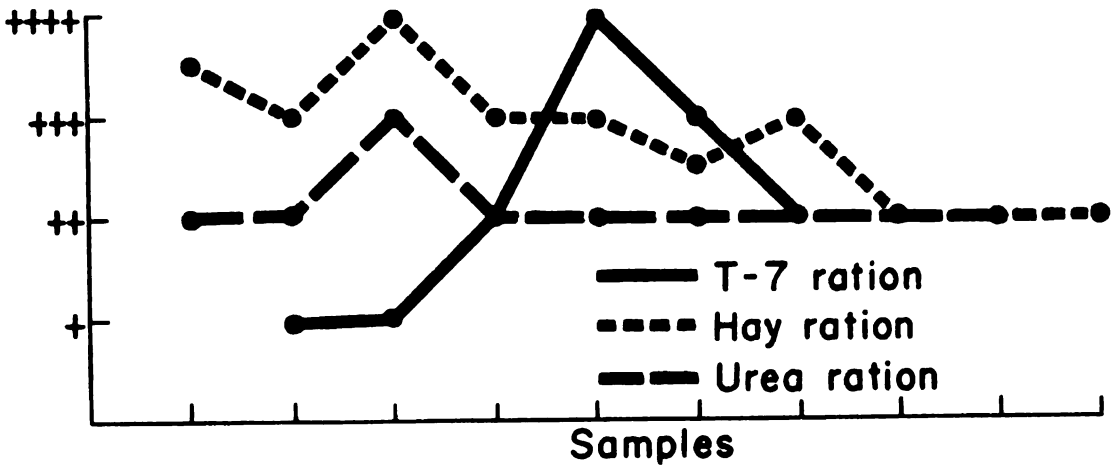


Fig. XV B. Steer CS-126, foam stability of rumen fluid

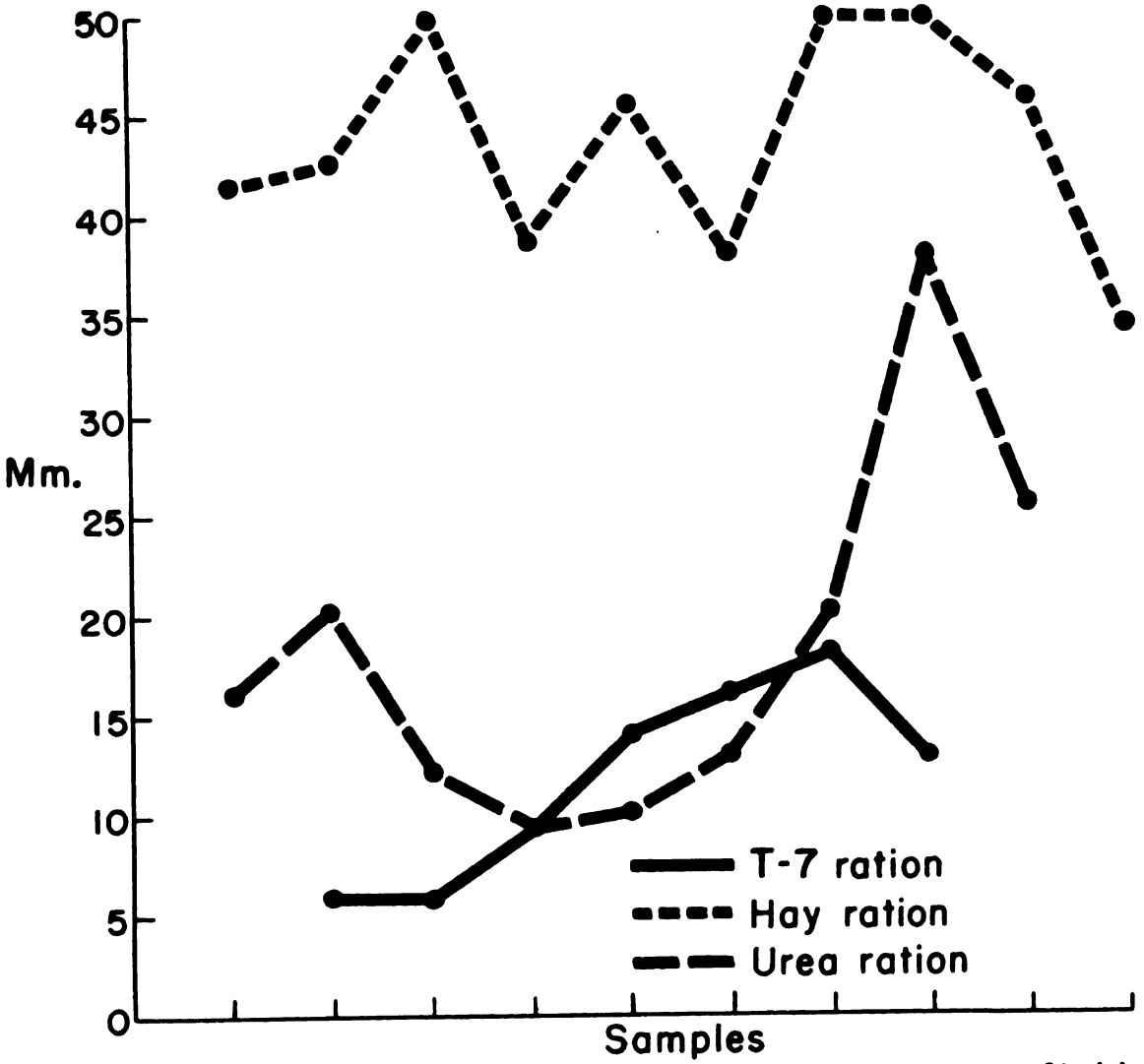


Fig. XV C. Steer CS-126, foam height of rumen fluid

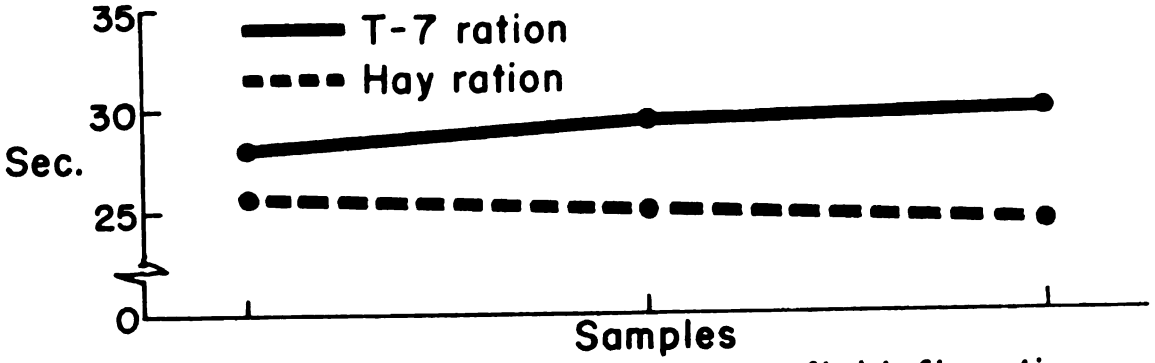


Fig. XVI A. Steer C-707, rumen fluid flow time

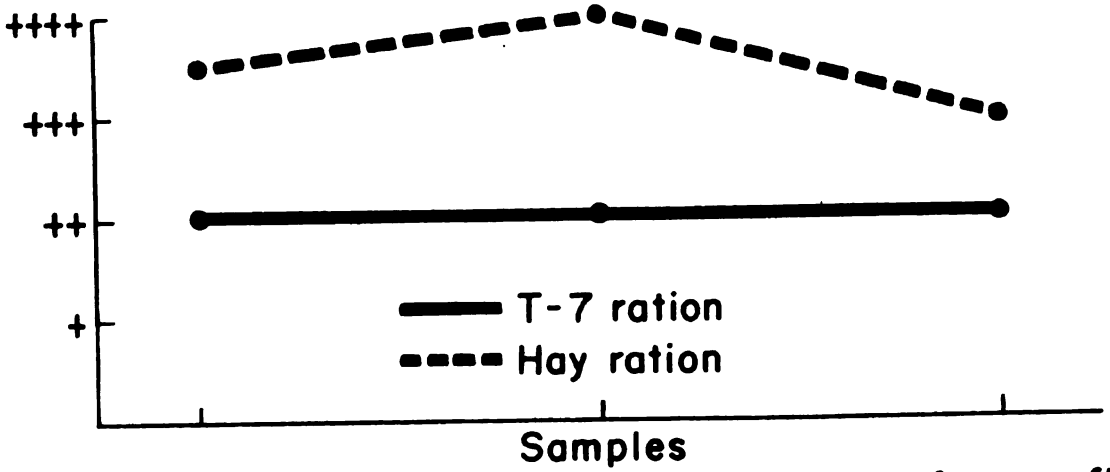


Fig. XVI B. Steer C-707, foam stability of rumen fluid

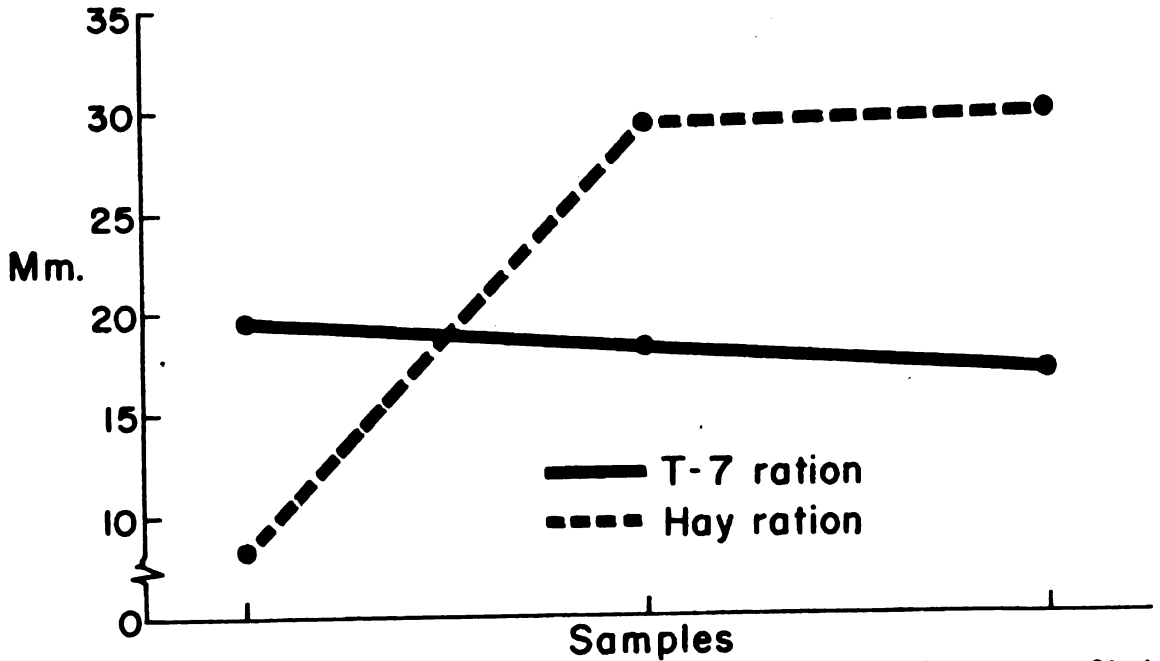


Fig. XVI C. Steer C-707, foam height of rumen fluid

TABLE I

MEAN VALUES, STANDARD ERROR, AND NUMBER OF SAMPLES FOR THE CHEMICAL AND PHYSICAL MEASUREMENTS OF RUMEN FLUID

	TSN - mg. %	Trichloro-acetic Acid NPN - mg. %	Trichloro-acetic Acid PN - mg. %	Trichloro-acetic Acid NPN - % of TSN	Trichloro-acetic Acid PN - % of TSN	Non-heat Coagulable Nitrogen-mg.%
Hay Ration						
Steer C-707						
X	54.17	40.74	13.43	75.89	24.11	48.70
S.E.	5.02	1.79	3.24	3.76	3.76	5.55
N	3	3	3	3	3	3
Steer CS-126						
X	65.99	53.58	12.41	81.20	19.05	64.40
S.E.	2.20	2.02	1.15	1.53	1.16	2.55
N	10	10	10	10	10	9
Urea Ration						
Steer CS-126						
X	54.12	35.84	18.28	66.88	33.12	47.22
S.E.	2.67	1.14	2.04	2.22	2.22	2.57
N	9	9	9	9	9	9
T-7 Ration						
Steer CS-126						
X	93.66	53.29	40.38	57.30	42.69	87.72
S.E.	12.36	7.85	7.52	5.10	5.09	10.24
N	8	8	8	8	8	8
Steer C-707						
X	74.40	64.75	9.65	87.08	12.92	72.08
S.E.	5.21	5.17	3.20	4.12	4.12	5.48
N	5	5	5	5	5	5

X---Mean

S.E.--Standard Error of the Mean

N---Number of Samples (Duplicate Analyses for Each Sample)

TABLE I (Continued)

Heat Coagulable Nitrogen-mg.%	Non-heat Coagulable Nitrogen % of TSN	Heat Coagulable Nitrogen % of TSN	Alcohol NPN - mg. %	Alcohol PN - mg. %	Alcohol NPN-% of TSN	Alcohol PN-% of TSN	Flow Time (sec.)	Maximum Foam Height (mm.)	Stability
5.47	90.10	9.90	27.76	26.41	50.50	49.50	25.00	22.58	3.50
3.65	6.95	6.95	4.74	0.40	4.14	4.14	0.29	7.18	0.29
3	3	3	3	3	3	3	3	3	3
1.94	96.99	3.00	25.74	40.26	39.23	59.75	24.75	43.05	2.30
0.17	0.35	0.35	0.74	1.97	1.26	1.59	0.13	1.53	0.23
9	9	9	10	10	10	10	10	10	10
6.90	87.19	12.80	22.99	31.13	42.97	54.62	25.50	18.28	2.11
1.13	2.16	2.16	1.47	2.82	3.01	3.01	0.17	3.04	0.11
9	9	9	9	9	9	9	9	9	9
5.94	94.99	9.83	46.50	54.85	45.38	54.62	28.89	11.64	2.14
3.13	2.85	2.32	8.73	9.23	2.91	2.91	0.48	1.82	0.41
8	8	5	5	5	5	5	7	7	7
4.99	93.62	6.38	-	-	-	-	29.17	18.25	2.0
2.22	2.64	2.64	-	-	-	-	0.60	1.26	0
4	4	4	-	-	-	-	3	2	2

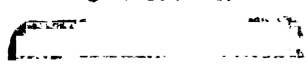


TABLE II

LEVELS OF SIGNIFICANCE USING THE "t" TEST

	TSN	Trichloro-acetic Acid NPN	Trichloro-acetic Acid PN	Trichloro-acetic Acid NPN-% of TSN	Trichloro-acetic Acid PN-% of TSN	Non-heat Coagulable Nitrogen
Steer CS-126						
Hay Ration vs. T-7 Ration	5[a]		1[b]	0.1[c]	0.1	5
Hay Ration vs. Urea Ration	1	0.1	5	0.1	0.1	0.1
T-7 Ration vs. Urea Ration	1	5	5			1
Steer C-707						
Hay Ration vs. T-7 Ration	5	1				5
Hay Ration						
Steer CS-126 vs. Steer C-707	5	0.1				5
T-7 Ration						
Steer CS-126 vs. Steer C-707			1	0.1	0.1	

[a] Significant at the 5% level
[b] Significant at the 1% level
[c] Significant at the 0.1% level

TABLE II (Continued)

Heat Coagulable Nitrogen	Non-heat Coagulable Nitrogen % of TSN	Heat Coagulable Nitrogen % of TSN	Alcohol NPN	Alcohol PN	Alcohol NPN % of TSN	Alcohol PN % of TSN	Flow Time	Maximum Head Height	Stability
		5					0.1	0.1	
	0.1	0.1	5	5			1	0.1	5
	5		5	5			0.1		
							1		5
				0.1	5	5		5	
								5	

SUMMARY

The results of this study to determine whether specific nitrogen fractions of rumen fluid could be correlated with froth production in ruminants have yielded several observations. It was found that there was considerable variability of rumen fluid nitrogen fractions between animals on the same ration. There was also considerable daily variation between rumen fluid nitrogen samples.

Total soluble rumen fluid nitrogen per se did not appear to influence froth production.

The determination of nonprotein and protein nitrogen of rumen fluid by precipitation with trichloroacetic acid did not appear to be a valid method due to incomplete protein precipitation.

Heat coagulable and non-heat coagulable rumen fluid nitrogen values, when expressed as a percentage of the total soluble nitrogen, were extremely variable between both rations and animals and could not be correlated with froth production.

The nonprotein nitrogen content of rumen fluid, as determined by an alcohol precipitation method, displayed considerable correlation with frothing when it was expressed as a percentage of the total soluble nitrogen.

The protein nitrogen content of rumen fluid, as determined by alcohol precipitation, did not show a positive relationship to frothing.

Rumen fluid flow time did not appear to be correlated with froth production.

Rumen fluid from the non-froth producing ration was found to yield a more stable foam than in the case of froth producing rations.

Rumen fluid from the non-froth producing ration also yielded the greater column of foam when air was bubbled through the liquid.

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