THE ROLE OF SALIVA IN THE ETIOLOGY AND PREVENTION OF BLOAT

by

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INTRODUCTION

As nearly as can be determined from the literature there has been a sharp increase in bloat in the last century and especially in the last twenty-five years. Feeding practices and genetics may account for some of the increase but it is generally conceded that the incidence of bloat is somewhat proportional to the increase in use of pure legume and mixed legume pastures. This constant increase in losses is becoming of great economic importance. It is estimated that this disease results in annual losses of over $40,000,000 in the United States. This figure considers the number of cows dying from bloat and in addition estimates the loss of production resulting from the restricted use of legume pastures in areas where bloat prevails.

Bloat is obviously the result of gas being formed in the rumen faster than it can be eliminated. A view held until recently was that only under conditions in which bloat was common was there any appreciable formation of gas in the rumen. From evidence now at hand, however, the feeding of bloat provoking diets does not result in greater gas formation (Cole *et al.* 1945). The main problem seems to center around its expulsion. Gas may be expelled from the rumen by eructation and by being absorbed into the blood stream and expelled with the expired air. Eructation is the more important of the two mechanisms since absorption from the rumen is not an adequate means of gas expulsion when the esophagus is blocked.

Bloat is frequently classified as chronic and acute (Cole *et al.*, 1945). Chronic bloat refers to bloat extending over a considerable period due to an abnormality in the physiological state of the animal. Acute bloat refers to bloat that occurs because of an abnormal feeding regime that in some way interferes with the animal's ability to expel gas. Acute bloat is classified as "frothy" or "free gas" bloat according to whether there is foam or free gas present in the rumen.

While it has been shown that legume bloat is of the frothy type there has been no conclusive evidence as to what mechanisms are involved in froth production. Weiss (1953) found that the amount of froth produced was dependent upon the consistency of the ruminal ingesta. He found that the feeding of succulent legumes resulted in a thick, viscid consistency of the ingesta and the occurrence of frothing, but when coarse, stemmy hay was fed the ingesta reverted to a watery consistency and foaming decreased. He believed that reflex salivation caused by coarse hay scratching the cardia was responsible for the watery consistency.

On the basis of the findings and conclusions of Weiss, work was initiated to find the effect of saliva on legume froth *in vitro* and *in vivo* and to determine what constituent(s) of saliva are important in producing this effect. It is accepted that the amount of saliva produced when ruminants are pastured on succulent legumes is less than when a coarse, dry feed is fed. However, the problem is to determine whether or not reduced saliva flow may be one of the etiological factors of bloat.

Although there are many theories as to the etiology of bloat, the studies and discussion here will be limited to the frothy type bloat and factors which affect it.

REVIEW OF LITERATURE

The Cause and Effect of Foaming in Bloat Production

Mechanism of Foam Formation. Most investigators now agree that stable intrarumen foam interferes with eructation and is responsible for bloat in cattle when grazing fresh legumes. This theory was suggested by Olson (1944), Quin (1943), Weiss (1953), Johns (1954), and Jacobson *et al*. (1957a). Bloat has been classified into two kinds, "foaming" and "free gas." However, Clark and Weiss (1952) and Johns (1954 consider it doubtful that there are two distinct types of bloat. They believe that these supposed differences may be due only to the degree of foaming and that the amount of free gas present is a function of the stability of the foam. According to Lindahl *et al*. (1957a) "feed-lot" bloat is of the frothy type with a varying amount of free gas always present.

Many workers (reviewed by Dougherty, 1953) believe that surface tension is involved in rumen froth formation. The surface tension theory includes any material which will change surface tension so that the gases of fermentation will tend to accumulate in countless bubbles throughout the ingesta instead of rising to the top of the rumen and collecting in a gas pocket above the ingesta.

Investigators differ in their opinions as to the action of surface active agents in the treatment of bloat. Some believe that intrarumen foam is formed because rumen liquids have a very low surface tension. Therefore, to break the foam an agent should be added that will raise the surface tension and thereby reduce the ability of the liquid to foam. The other school of thought is that even though liquids of lower surface tensions are more prone to foam, foams that are formed when there is a low surface tension are unstable and will break readily allowing the gas to escape. Wallace (1958) observed that agents which reduce surface tension reduce the energy necessary to form foam but do not necessarily stabilize foam.

There has been some confusion as to the mechanism of foaming and the manner in which anti-foaming agents affect foaming. Clark (1948) related the beneficial effect of turpentine, which is a common agent for the treatment of bloat, to its ability to break foam by increasing surface tension. Blake et al. (1957) have shown that turpentine decreases the surface tension of ruminal ingesta.

Reid and Johns (1957) believe there are two broad groups of foaming compounds: (1) Solutions of soaps and detergents in which the surface tension is usually low; these foams in general are not particularly stable and are non-viscous; (2) protein and saponin solutions which exhibit strong superficial viscosity and in which the surface tension factor is subordinate to the mechanical and cohesive properties of the stabilizing film. These solutions yield foams of great stability and at the same time exhibit high surface tension values which show little correlation with foam stability. Soaps may lower the surface tension 40-50 dynes per cm., whereas proteins rarely lower surface tension more than 20 dynes per cm.

Reid and Johns (1957) explain further that these surface-non-viscous and surface-viscous type compounds are mutually incompatible. If a soap solution and a saponin solution are mixed, the soap solution, which has the lower surface tension of the two liquids, causes displacement of the saponin from the surface layer of the combined liquids. Since the soap solution occupying the surface layer does not have cohesive properties an unstable foam is produced. Nichols (1954) has shown that detergents can be used to treat bloat. He concluded that the foaming agent in the rumen of animals with frothy bloat is of the surface-viscous type since highly active, non-viscous types of surface active compounds are effective treatments.

Boda (1957) has shown that the introduction of fresh egg-white, a surface-viscous type agent, into the rumen produces moderate bloat in cattle given ground, dehydrated alfalfa.

Wallace (1958) believes that finely divided particles which are poorly wetted by water serve as foam stabilizers. These particles adhere to the water-air interface and the coated bubbles cannot coalesce because of the coating. The rumen contents of a fistulated steer with frothy bloat were noted to show dimpled bubbles, as though the bubbles were adhering to the food particles. When the rumen juice was allowed to stand, the solids rose to the top of the solution, leaving an almost clear solution below. The food particles were not lighter than water as they could be centrifuged down with ease.

In explanation Wallace referred to Reinder's theorem, "If the surface tensions between three phases are such that one value exceeds the sum of the other two, that interface having the high value will not occur in a mixture of these three phases." He postulated that in the non-frothing rumen, the surface tension between air and food particles exceeds the sum of the air-water and water-particle surface tensions. Digestion in the rumen removes carbohydrate from food particles, enriching it in lipid and protein. Analogy of the food particles to simple organic liquids suggested that this digestion lowers the particle-to-air surface tension and raises the particle-to-water surface tension. This process carried far enough leads to the balance of surface tensions that result in adhesion of food particles to gas bubbles leading to the production of froth.

Nichols et al. (1957) found that periods of increased and excessive frothing following changes from alfalfa hay to fresh ladino clover were associated with periods of high surface tension and viscosity.

Nichols et al. (1955) observed that the effective buoyancy of rumen juice is less following intake of fresh legumes than following the intake of either hay or grass. Specific gravity was the measure of effective buoyancy used. He noted that for an hour or so following the change-over from hay to legumes that the fresh legumes were not on top of the paunch contents as hay usually is just after it has been fed but were below what remained of the previously fed hay. The lowered effective buoyancy of the rumen fluid was believed to be a factor in causing this. Nichols also observed that frothing seemed to start in the upper regions of the paunch and extend into the lower regions where it increased in intensity.

The Effect of Saponins on Foaming. Saponins are found in various plants and are abundant in alfalfa and other legumes. Olson (1944) found that saponin alters the surface tension of ruminal contents and that it might contribute to frothy bloat by the entrapment of the gases of fermentation throughout the ingesta.

Lindahl *et al.* (1957b), using sheep, appear to be the first to have produced bloat with a saponin isolated from a pasture legume. Saponin had a toxic effect when administered to sheep at high levels. Several sheep treated with 100 g. alfalfa saponin and 50 g. dextrose mixed in one liter of water collapsed after a short time. In instances where marked bloat symptoms were obtained the animals displayed frequent urination and defecation, labored respiration, extreme discomfort, and just before collapse suffered from an extreme drop in blood pressure. Sheep with slight or no bloat did not exhibit these symptoms even when they received the same amount of saponin. Autopsy revealed that the ruminal ingesta was frothy in all fatalities. *In vitro* and *in vivo* experiments indicated that alfalfa saponin can contribute to the stabilization of rumen froth. However, it is also evident that alfalfa saponin is not the only factor involved in stable froth formation.

Jacobson *et al.* (1957b) found that lucerne saponin and glucose can aid greatly in formation of stable froth in rumen fluid.

Ferguson and Terry (1955) found that the frothing capacity of juice pressed from fresh lucerne with a triple roller gave a fair indication of the bloating potential of the lucerne. They found a loss of foaming capacity on drying the herbage, which suggested either a breakdown of a frothing constituent or a change in the physical state of the constituent. Since extraction of saponins has been carried out on dried material, this may be important.

Boda (1958) found that dehydration greatly reduced the bloat producing ability of green alfalfa and suggested that denaturation of the water-soluble plant protein may be a partial explanation for this effect.

There has been some work done to try to isolate the frothing factor(s) from fresh legumes. Ferguson and Terry (1955) found that bloat was produced with lucerne juice after the chloroplastic compounds had been precipitated and the filtrate passed through an anion and a cation exchange resin. This suggests that the bloat provoking factor(s) are nonionic and are not absorbed on resins. Saponins exhibit similar properties.

Mangan (as quoted by Johns, 1958) studied solutions of saponins and proteins to determine the conditions necessary to form foams of optimum strength. The pH optimum for saponins was found to be 4.5-5.0 whereas cytoplasmic protein of red clover had a pH optimum of 6.0. The protein optimum pH corresponded to the pH optimum of rumen fluid after ingestion of clover. At a pH 6.0 saponin foams had little strength. Reid and Johns (1957) found that bloat could be produced on high protein grass. These data led Mangan to conclude that protein may play a more important part in bloat than saponins. Head (1958) has indicated that the bloat provoking factor is a non-protein, non-nitrogenous substance. This information conflicts with Mangan's conclusion.

Conrad et al. (1958) have shown that the plant substances responsible for the initial rapid gas production in ingested alfalfa were closely associated with the fiber portion of the plant but that these substances were removed after 4-10 hours with rumen microorganisms or by extracting 12 hours with hot water. It was indicated that the combined effects of the physical structure of green alfalfa fiber, pectic substances, galacturonic acid obtained from hydrolysis of pectic substances, and reducing sugars normally present are capable of causing the stable foam found in legume bloat.

The Effect of Saliva on Foaming. Weiss (1953) suspected saliva as a factor in frothy bloat. Working with Merino sheep fed green lucerne, he found that the ruminal ingesta had a tendency to foam but the type of foam formed was directly dependent upon the consistency of the ingesta. When rumen contents were watery, gas bubbles rose freely to form free gas and an unstable foam. As the consistency increased there was a greater tendency for gas bubbles to become entrapped in thick viscid material causing ingesta to rise up in a frothy mass. Bloat caused by frothing of thick, viscid, ruminal ingesta, occurred immediately after succulent, leafy legume was fed. When mature stalky legume was fed, the ruminal ingesta immediately reverted to a watery consistency and bloat ceased even in the absence of drinking water. He attributed this effect to reflex salivation caused by coarse, stemmy material scratching the cardia and the walls of the forestomachs around the cardia.

Other workers have shown that the volume of saliva secreted reflexly can be related to the coarseness of the material in the rumen and reticulum. Coates (1956) showed that reflex salivation was produced by tactile stimulation of the esophagus, and tactile stimulation of the cardiac and reticulo-omasal orifices. Denton (1956) found that feeding fresh, green-chopped lucerne caused a reduction in the daily output of the parotid saliva compared with the output when roughage was included in the diet. Somers (1957) believes that these differences in salivary secretion may be accounted for by reduced regurgitation and remastication in the case of green feed, but also believe that the receptors in the forestomachs may not be stimulated to the same extent as with coarse roughage. Somers (1957) believes that irrespective of how the reduction in salivary output is brought about, the consistency of the ingesta will be influenced by this reduction.

Bailey (1959) found that fibrous foods were eaten more slowly than less fibrous foods and stimulated a larger flow of saliva per unit weight of food consumed. In work with four dry, fistulated Shorthorn cows he showed that only 0.94 g. saliva per g. food was secreted while eating fresh grass as compared to 3.63 g. saliva per g. food when eating dry hay.

In order to test the effect of a drug that blocked salivation, Moore *et al.* (1957) drenched cattle with alfalfa juice and with alfalfa juice plus atropine. Bloat was produced 15% more often on the alfalfa juice plus atropine drench than when alfalfa juice was given alone.

Jacobson *et al.* (1957b) in working with feed lot bloat, contradicted Weiss's work in that they did not find a difference in the consistency of the rumen ingesta between bloat-producing and non-bloat-producing diets. However, they found a significant difference in the ingesta-volume-increase between the two diets. Ingesta-volume-increase was measured as the percentage increase in volume of rumen ingesta placed in cylinders and incubated at 39° C. for one hour.

Cole *et al.* (1943) reported that feeding Sudan grass hay the night before grazing bloat provoking legumes prevented bloat. They also found some correlation between rumination and eructation and postulated that both acts were apparently initiated by the same stimulus. Cole and Kleiber (1945) supported these observations but found that it was necessary to feed at least 17 lb. of Sudan grass hay the night before grazing alfalfa to effectively control bloat. Colvin *et al.* (1958) observed that the feeding of 12 lb. of oat hay per cow per day prior to grazing alfalfa significantly reduced the incidence and severity of bloat. Grass hays were the only scabrous materials that gave protection from bloat. Mead, *et al.* (1944) advanced the theory that coarse material is necessary in the rumen to provide the stimulus for the eructation reflex and that the absence of such material leads to defective eructation and bloat. These workers believe that scabrous materials may prevent bloat in the following ways: (1) They physically prevent the formation of froth; (2) they contain a chemical anti-frothing agent; (3) their scabrous nature causes stimulation of the eructation reflex. They do not consider reflex salivation as one of the beneficial effects of roughage feeding.

Many workers believe that saliva is an agent which promotes foaming. Bovine saliva has a low surface tension (Reid and Huffman, 1949). Compared with the surface tension of water (71.3 dynes per cm.), saliva has an average surface tension value of 47.1 dymes per cm. when measured at the same temperature. Blake et al. (1957) however, found in testing the effect of various compounds on the surface tension of rumen fluid that water and saliva were the only materials added that raised surface tension.

Johns (1954, 1958) considers that saliva has several properties that can contribute to bloat. The large secretion of saliva, according to its composition, could either assist in preventing bloat by buffering a fall in pH or increase its severity by adding to the CO_2 evolved and by assisting in foam formation. The low surface tension of saliva is one factor that would augment foaming. He also believes that mucoproteins present in saliva may assist in forming a stable, viscous type foam which would not involve a great change in surface tension.

Ferguson and Terry (1955) administered to sheep a synthetic saliva containing 9.8 g. $NaHCO_3$ and 9.3 g. Na_2HPO_4 (the quantity present in 1 liter of sheep's saliva) dissolved in 200 ml. water together with 2.5 liters of moderately bloat provoking lucerne juice. Six sheep received lucerne juice only and six sheep received lucerne juice plus saliva. The degree of bloat was similar in both groups. A sheep that frequently bloated was given 750 ml. of cow's saliva plus 2.5 liters of lucerne juice. Two control sheep were given 2.5 liters of lucerne juice alone. All three sheep became well bloated. Under the conditions of these tests synthetic and natural saliva did not influence the severity of bloat.

Phillipson and Reid (1958) have found there are individual differences in saliva flow when pressure is produced in the rumen. They have found that pressure in the rumen usually causes a marked increase in the rate of secretion of the parotid and submaxillary glands and the residual saliva flowing from the mouth. The pressure to cause this stimulation varies from 8-20 mm. Hg. Once stimulation has occurred further increase in rumen pressure causes inhibition of salivary secretion. They found considerable variation in this effect, however, some animals did not produce an increased saliva flow in response to rumen pressure stimulation. Their experiments suggest that additional salivary flow in response to pressure in the rumen may be important in causing a moderately bloated animal to become worse, since they conclude that saliva contributes to foaming. They also noted that the level of mucoprotein in the submaxillary saliva could reach high levels when excessive distention of the rumen caused respiratory inhibition coupled with marked and fluctuating increases in blood pressure. They attributed these effects to the release of adrenalin. Sectioning of both cervical vagi allowed higher pressures than before to be induced in the rumen without this adrenalin-like crisis appearing.

The Effect of Bacterial Slimes on Foaming. Hungate *et al.* (1955) suggested that slime production by bacteria may be associated with frothy bloat. Slimes might function by slowing down the bubble movement thus retarding the coalescence of bubbles.

However, Bryant *et al.* (1958) observed that the flora of the rumen of different groups of cattle studied did not vary to a significant degree. Visual observations showed that slime producing bacteria were no more numerous in cattle fed fresh legumes than those fed alfalfa hay.

Jacobson *et al.* (1957b) found that in feed-lot bloat the rumen samples of animals that had been on a bloat producing diet for a period of time had a slimy consistency and the degree of encapsulation of these slime bacteria was a significant factor. The degree of encapsulation increased with the length of time on bloat producing diet. The correlation between the average percentage encapsulation and the average bloat index was 0.94 and highly significant.

The Effect of Rumen Froth on Eructation. Most investigators now agree that stable intrarumen foam interferes with eructation and is responsible for bloat on fresh legumes. However, very little work has been done to show how this mechanism inhibits eructation. Dougherty and Habel (1955) demonstrated the presence of a cranial esophageal sphincter. Dougherty (as quoted by Johns, 1958) using an isolated reticular pouch technique, demonstrated the inhibitory effect of liquid on opening of the cranial esophageal sphincter. Johns (1958) introduced various foams into the pouch and gas was passed into the pouch. Evidence was obtained that the reflex receptor controlling the opening of the cranial sphincter could distinguish between free gas and foam because foam caused inhibition of belching.

The Use of Antifoaming Agents in Treatment and Prevention of Bloat

There are many methods that have been used for treatment of bloat but none have been more successful for the treatment of frothy bloat than the use of antifoaming or surface-active agents.

Clark (1948) has related the beneficial effect of turpentine, which is a common agent used for the treatment of bloat to its surface-active properties. Neither turpentine nor coal tar derivatives had any effect on gas formation in the rumen in therapeutic concentration, but they did have an effect on surface tension. The addition of small amounts of turpentine, coal tar preparations, or thin oil brought about an immediate and marked change in the physical properties of frothing ingesta, breaking the foam and preventing its reformation.

Quin *et al.* (1949) have reported on the clinical use of several surface-active agents. Of 155 cases of bovine bloat treated, 115 made complete recoveries. The best results were obtained by injecting the agents directly into the dorsal vault of the rumen; 95 percent of the cases made complete recovery by this treatment, 80 percent recovered when agents were dissolved in water and administered as a drench or with a stomach pump.

Blake et al. (1956) administered an alkyl aryl sodium sulfonate type detergent alternately to two groups of cattle grazing alfalfa. The detergent was administered daily in capsule form. Although lower levels had little effect, a significant reduction in bloat incidence and severity occurred at a daily rate of 3 g. of detergent per 100 lbs. of body weight.

Blake _et al_. (1958) found that detergent administered at a level of 20 g. per 1000 lb. cow was only slightly effective but at 30 g. it greatly reduced bloat incidence and severity.

Dougherty and Meredity (1954) evaluated _in vitro_ the antifoaming properties of turpentine, two products of silicone suspensions, and a turpentine mixture. The efficacy of silicone products was higher than turpentine products based on foam dispersal. However, the turpentine products were more effective when judged on ability to prevent reconstitution of foam when the rumen liquor was reshaken. Dougherty and Meredity conclude that surface-active agents should relieve most cases of bloat if: (1) All (or most) bloat were frothy in nature; (2) there were no inhibition other than mechanical of the eructation mechanism; (3) surface-active agents were active in all or most cases of bloat; and (4) wide dispersal of the agent in a severely distended rumen were possible.

Johns (1956) has reported that vegetable oils have been effective in bloat treatment and in reducing the incidence of bloat. Effective control of bloat was reported when pastures were sprayed with peanut oil at the rate of 1½ gallons of oil per acre (each cow was expected to consume about 2 ounces of oil per day). Emulsified tallow was also used with good results.

Brown et al. (1958) found that a water-dispersible oil markedly reduced the incidence and severity of bloat when fed at levels of 1 to 2% of the drinking water. The effect was less in feed-lot bloat but the addition of oil to the drinking water was beneficial.

Johnson et al. (1958) administered various prophylatic agents to steers on alfalfa pasture. Soybean oil, lard oil and lecithin mixed with soybean oil greatly reduced bloat for several hours when fed at the rate of 0.25 lb. or more per animal in the grain at each feeding or at a rate of 2 percent in the drinking water. It also increased weight gains. In trials with dairy animals, crude soybean oil sprinkled on the soilage at a level of 0.25 lb. per 1000 lb. of body weight per day effectively controlled bloat. The effect of n-decyl alcohol was of too short duration for satisfactory prophylaxis. Lard oil and n-decyl alcohol administered intraruminally were successful in relieving very severe cases of bloat apparently by breaking the foam and releasing large quantities of gas in a short time. Lard oil was more satisfactory because of duration of effect and ease of administration.

Reid and Johns (1957) have found that bloat in cows on red clover could be successfully treated with antifoaming agents including vegetable oils, vegetable turpentine, emulsified tallow, whale oil, cream, lanolin, five grades of liquid paraffin and paraffin wax emulsions. Treatments without antifoaming agents failed to give relief.

Southcott and Hewetson (1958) found that peanut oil in the drinking water (12 ounces per head per day) or in the drinking water and on a hay supplement (14 ounces of oil per head per day) reduced the incidence and severity of non-fatal bloat in cattle grazing clover-rich pasture. However, it failed to prevent sporadic deaths.

Johns (1954) listed silicones as among the effective agents in bloat control. The silicones tried at that time were dissolved in a paraffin and he believed that perhaps the paraffin was the effective agent. Reid and Johns (1957) found that silicones were often quite unreliable in bloat control.

Since it is desirable that a material for therapeutic use for bloat should not adversely affect the health of animals or the properties of milk and butterfat, work has been conducted to determine to what extent various oils fulfill these requirements. Reid (1957) found temporary reductions of 40-90% in feed intake following a single dose of 200 ml. of a very light grade of paraffin such as odorless kerosene. It was concluded that it is undesirable to administer very light paraffins to ruminants. McDowall _et al_. (1957) found that the ingestion of heavy liquid paraffins did not affect the yield of milk, the fat and solids-non-fat contents of milk, or the iodine and saponification values of the butterfat. However, there was a drop in the carotene and vitamin A levels of the blood and butterfat.

APPARATUS, MATERIALS, AND METHODS

Animals Used

Two pairs of identical twin heifers were used in this study. Each animal had a permanent rumen fistula fitted with a plastic cannula and cap. The first pair were four-year old Holstein heifers (15-16) and the second pair were four-year old Jerseys (81-82). When pasturing alfalfa the animals were kept in the dry-lot in the afternoon and night without feed and pastured in the morning. When treatments were administered, one animal of each pair received treatment and her twin served as the control.

Saliva Collection

The apparatus for collecting saliva consisted of a 50 ml. conical pyrex centrifuge tube that had a hole cut in the bottom of the cone. The cone shaped end of the tube was inserted and glued into the cup shaped portion of a teat cup inflation. The upper portion of the teat cup inflation was cut off before inserting the centrifuge tube so that the rubber covered the cone portion of the tube. A 3 in. piece of 9 mm. glass tubing was used as a connecting joint between the end of the teat cup inflation and a 4 ft. piece of rubber tubing. A Y-shaped cast aluminum connecting tube served to join this tube to another 4 ft. rubber tube which led to a glass tube inserted through a No. 8

rubber stopper in a 2 l. vacuum bottle. Rubber vacuum tubing connected the vacuum bottle to the milking machine vacuum line in the milking barn. A trap was placed between the vacuum bottle and the vacuum line. Fistulated animals were used for collecting saliva. The rumen contents were removed and the centrifuge tube was placed through the cardia into the posterior esophagus so that the vacuum might draw saliva into the 2 l. collection flask. The free arm of the Y-shaped connecting tube was used as a bleed tube to allow air to enter the line to keep the vacuum from becoming too strong and pulling the mucosa of the esophagus into the centrifuge tube and preventing saliva from being drawn into the flask.

Mucin Extraction

The mucin was extracted in the laboratory using the procedure described by Mason and Hall (1948) with minor alterations. One hundred g. of linseed meal was added, with vigorous agitation, to 4 liters of water at 60° C. containing about 20 g. of sodium chloride. Temperature was maintained at 60° C. at all times as too low a temperature retarded the extraction while too high a temperature degraded the product. During the extraction period the pH of the mixture was maintained at 7.0-7.2 by the addition of sufficient sodium carbonate or sodium bicarbonate. As the extraction proceeded, the mixture became more acidic, and it was necessary to add small amounts of the alkalizing agent from time to time to neutralize the mixture. The supernatant liquid was decanted from the residue and the supernatant was used in mucin treatment studies.

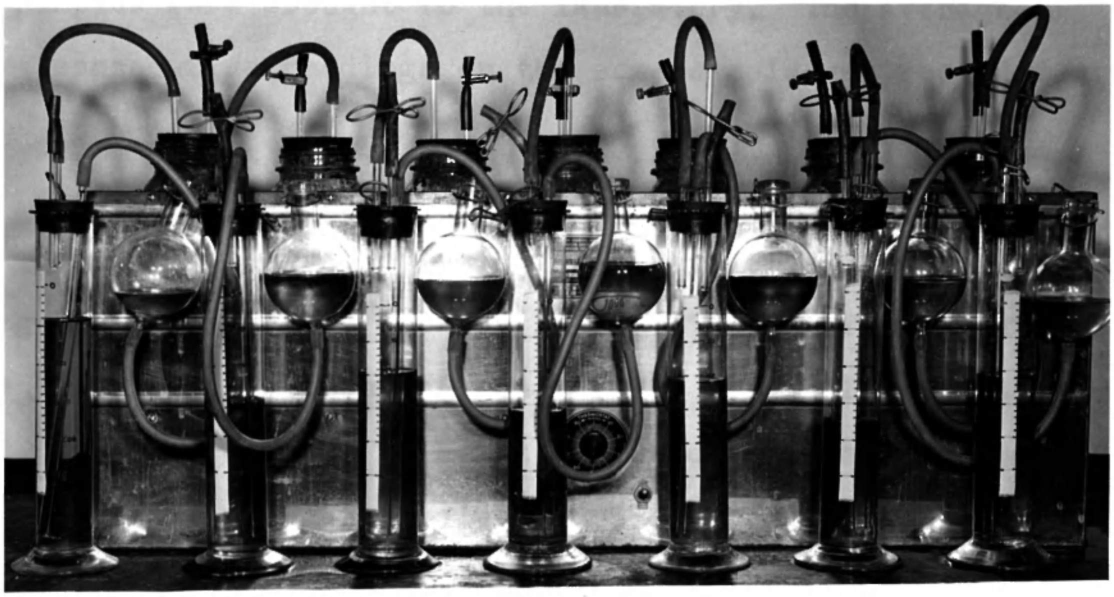
Gas Analysis

The technique used for measuring gas escape was described by Browning (1958). Two hundred g. samples of rumen ingesta (frothing or normal) were collected for determining the total amount of gas released. Samples were weighed into quart jars, which were then placed in a constant temperature (39 $\pm$ 1° C.) water bath and connected by a rubber tube to a gas collecting cylinder (Plate I). The gas collecting vessel consisted of a 450 ml. glass cylinder containing water. The cylinder was calibrated from 0-250 ml. and fitted with a No. 10 rubber stopper. The rubber stopper was fitted with two pieces of 5 mm. glass tubing 6 cm. long and 1 piece 30 mm. long that reached within 2 cm. of the bottom of the cylinder. The rubber tubing connecting the quart jar to the gas collecting cylinder was attached to one of the short pieces of glass tubing. A short piece of rubber tubing closed with a pinch clamp was attached to the other short piece of glass tubing. Rubber tubing 35 cm. in length connected the gas-cylinder via the long glass tube to a leveling bulb. A 200 ml. round bottom flask with a piece of glass tubing (5 mm. x 1.5 cm.) fused to the bottom served as the leveling bulb. As gas escape progressed, the pressure of the evolving gas caused displacement of water from the gas collecting cylinder into the leveling bulb. At the end of the gas collection period the aforementioned pinch clamp was opened, allowing the gas to escape and the displaced water to flow back into the cylinder to the zero point. Gas volume readings were made at 10, 15, 30, 45, and 60 minutes.

PLATE I

Gas collecting apparatus used to determine the total quantity of gas released during the *in vitro* analysis of frothing and non-frothing rumen contents.

PLATE I



pH

A Beckman potentiometer was used for the determination of pH of the rumen samples. The pH readings were made as soon as the samples were brought to the laboratory. The average of three consecutive readings on the same sample was recorded.

Free Volatile Fatty Acids (VFA)

Molar proportions of volatile fatty acids were determined using the procedure of Keeney (1955). The only modifications were that 5 ml. portions of rumen fluid was analyzed instead of 2 ml. and 0.5% butanol in hexane solution was used instead of 1.0% solution in eluting valeric and butyric acids. The latter was done so that there would be a greater distance between the acid bands which made possible a more distinct separation of these acids. Samples of VFA determination were obtained by squeezing by hand the whole rumen ingesta. The samples were then brought to the laboratory and frozen by placing them in a deep-freeze unit. At the time VFA determinations were made, the samples were thawed and centrifuged at 500 r.p.m. for approximately 10 min.; the supernatant was then used for VFA determinations.

Experimental Design

Effect of Saliva and of Mucin on Gas Escape from Rumen Froth. A study was conducted using fistulated animals grazing alfalfa pasture to test *in vitro* the effect of saliva and linseed meal mucin solution on the release of gas trapped in frothing rumen contents. Frothing rumen contents were collected from bloated fistulated animals in a large three-gallon container and taken quickly to the laboratory where 200 g. samples were weighed into quart jars in preparation for gas escape analyses. Bovine saliva or linseed meal mucin solution was added at levels of 20 and 80 ml. to the jars. A control jar received no additives. Trials were conducted in duplicate. Water and mucin or saliva at levels of 160 ml. were also tested in a few trials.

In a similar study gas analyses were conducted using non-frothing rumen contents. These trials were made to determine whether saliva or mucin solution added at 80 ml. levels would affect gas production of rumen microorganisms. The amount of gas released from froth represents gas from two sources, that produced by the microorganisms during the time of incubation and gas that had been previously produced and trapped in rumen froth. This study was made to determine if saliva or mucin would affect fermentation during the time of incubation.

<u>Effect of Saliva and of Mucin on Saponin Foam</u>. Saliva and linseed meal mucin solution were tested <u>in vitro</u> for anti-foaming properties. Uniform glass tips were prepared by heating a glass tube in the center and pulling the ends so that the center was drawn to a fine diameter. The tube was cut at the point of smallest diameter forming two tips. Air was bubbled at a uniform rate through the glass tip one inch from the bottom of a 500 ml. cylinder. The cylinder contained 1 g. of alfalfa saponin in 100 ml. water. To the cylinder various levels of saliva, linseed meal mucin solution, other mucin sources, or water

were added so that the liquid volume in each case was equal. The amount of foam produced in the cylinders was measured and the stability observed by noting the time taken for the foam to break down.

Effect of Drug Action on Rumen Froth. Since reduced saliva production was suspected as one of the causative factors of bloat production, trials were conducted using drugs which either stimulate or block salivation. One set of fistulated identical twins (15-16) were used in this trial and were fed a bloat provoking ration. The ration consisted of 25% whole dehydrated alfalfa pellets, 25% ground dehydrated alfalfa pellets, 20% chopped alfalfa hay, 30% ground corn, 1% salt, and 2% steamed bone meal. The ration was fed free choice to both animals and usually produced an intrarumen froth but did not produce actual bloat in which the rumen was distended. Atropine sulfate was administered to the animal selected for treatment to see if reduced salivation caused by the action of this drug would result in a greater production of rumen froth. The drug was injected subcutaneously in 50 mg. doses twice daily at 8:00 a.m. and 4:00 p.m. Later treatments were made using Lentin[1] to see if increased salivation due to drug action would reduce the amount of froth being produced in the rumen. Two mg. doses were given twice daily as above.

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[1]The Trademark of Merck & Co., Inc. Rahway, N. J. for the drug, carbacol.

Effect of Saliva and an Animal Mucin[2] on Rumen Froth In Vivo. A final study was made using two sets of fistulated identical twins (15-16 and 81-82). One twin of each set was treated and the other served as a control. In the first trial with these animals, 2 liters of bovine saliva previously collected from the fistulated animals was added to the rumen via the rumen fistula just before turning the animals on pasture. In 15 and 16, levels of 4 and 6 liters were also administered. Samples of rumen ingesta were taken before turning on pasture and 3-4 hrs. after turning on pasture.

The work of Jacobson et al. (1958) showed a drop in the proportion of acetic acid produced and an increase in the proportion of propionic acid produced in switching from the control diet to a bloat provoking diet. Rumen ingesta samples were checked in this study for variations in pH and molar proportions of volatile fatty acids to see if the same change occurred when pasturing legumes as that reported by Jacobson et al. in the feedlot. Differences between treatments were also measured.

The trial outlined above was repeated using an animal mucin for treatment instead of saliva. The mucin was added in solution and also in dry form. In both trials water was added to the control animals' rumen contents in amounts equal to the liquid volume of saliva or mucin solution used.

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[2]Provided through the courtesy of Swift & Co.

RESULTS

Effect of Saliva and Mucin on Gas Escape from Rumen Froth

The amount of gas evolved from 200 g. samples of frothing rumen ingesta is shown in Table 1. The values listed for each time of each treatment represent the mean of 12 trials. In these 12 trials it was found without exception that more gas escaped from the frothing rumen samples to which saliva (0.011 g. solids/ml.) or mucin (0.017 g. solids/ml.) was added. Other trials shown in Table 6 of the Appendix indicated that 160 ml. of saliva or mucin did not release as much gas as the 80 ml. level but more than the 20 ml. level. The addition of water had no effect on gas release.

An analysis of variance showed a highly significant difference among the treatments of the frothing rumen samples shown in Table 1. In the 60 min. test period, the 80 ml. levels of saliva and of mucin both released significantly more trapped gas than was released from the control samples. The 20 ml. levels of saliva and of mucin were not as effective and differences only approached significance. In the early periods of the gas release trials, the saliva treated samples released trapped gas faster but as time progressed the release of gas from the mucin treated samples began to approach the saliva treatments.

The amount of gas released from froth represents gas from two sources, that produced by the microorganisms during the time of incubation and gas that had been previously produced and

Table 1. Effect of saliva and linseed meal mucin upon the release of gas from frothing rumen contents and the effect of saliva on the production of gas by non-frothing rumen contents.

Treatment	No. Trials	Amount of gas released in mls. from 200 g. rumen contents.				
		10 min.	15 min.	30 min.	45 min.	60 min.
		Frothing Rumen Contents				
Control	12	35.1 ± 8.4[1]	51.2 ± 12.0	93.2 ± 21.2	129.0 ± 31.0	149.7 ± 30.3
20 ml. mucin	12	* 38.4 ± 8.1	** 57.7 ± 13.1	** 105.3 ± 22.0	** 147.2 ± 31.0	** 175.4 ± 34.4
20 ml. saliva	12	42.8 ± 8.9	61.8 ± 12.4	107.2 ± 24.3	146.3 ± 31.0	170.9 ± 31.6
80 ml. mucin	12	43.8 ±15.5	68.0 ± 21.1	124.5 ± 40.5	174.0 ± 48.8	196.8 ± 43.4
80 ml. saliva	12	49.8 ± 7.7	71.2 ± 13.0	128.7 ± 25.6	177.0 ± 37.0	202.5 ± 27.7
		Non-frothing Rumen Contents				
Control	4	20.0 ± 8.9	31.5 ± 5.8	65.0 ± 14.7	91.8 ± 16.3	115.5 ± 19.0
80 ml. saliva	4	19.0 ± 6.5	29.8 ± 6.9	63.8 ± 19.8	94.0 ± 22.2	121.8 ± 26.4

1 Standard Deviation
* Significant at 5 percent level
** Significant at 1 percent level

trapped in the rumen froth. As is shown by the data in Table 1 from the non-frothing rumen samples, saliva had no influence on the amount of gas produced by the microorganisms during the incubation period. This being the case, saliva may cause more gas to be released from the frothing contents by an effect on the froth and not by an effect on the rumen microorganisms. Saliva may cause more gas to be released by causing the bubbles in the froth to break down.

Effect of Saliva and of Mucin on Saponin Foam

Various levels of saliva or linseed meal mucin were added to 100 ml. of water containing one gram of alfalfa saponin and air was bubbled through the solution. It was found that 50 ml. of saliva or linseed meal mucin solution added to the saponin solution reduced the amount of foam produced (Plate II). The stability of the foam was also affected. Saponin alone formed a stable, viscous foam that rose up in the cylinder and was slow in breaking down when air bubbling was stopped. The foam formed when saliva or mucin solution was added was unstable, breaking down quickly when air bubbling was stopped. The addition of 10 ml. of an animal mucin solution completely stopped foam formation.

Effect of Drug Action on Rumen Froth

The administration of atropine subcutaneously had no apparent effect on intrarumen foaming in an identical twin as compared to her control twin mate. The injections were not

PLATE II

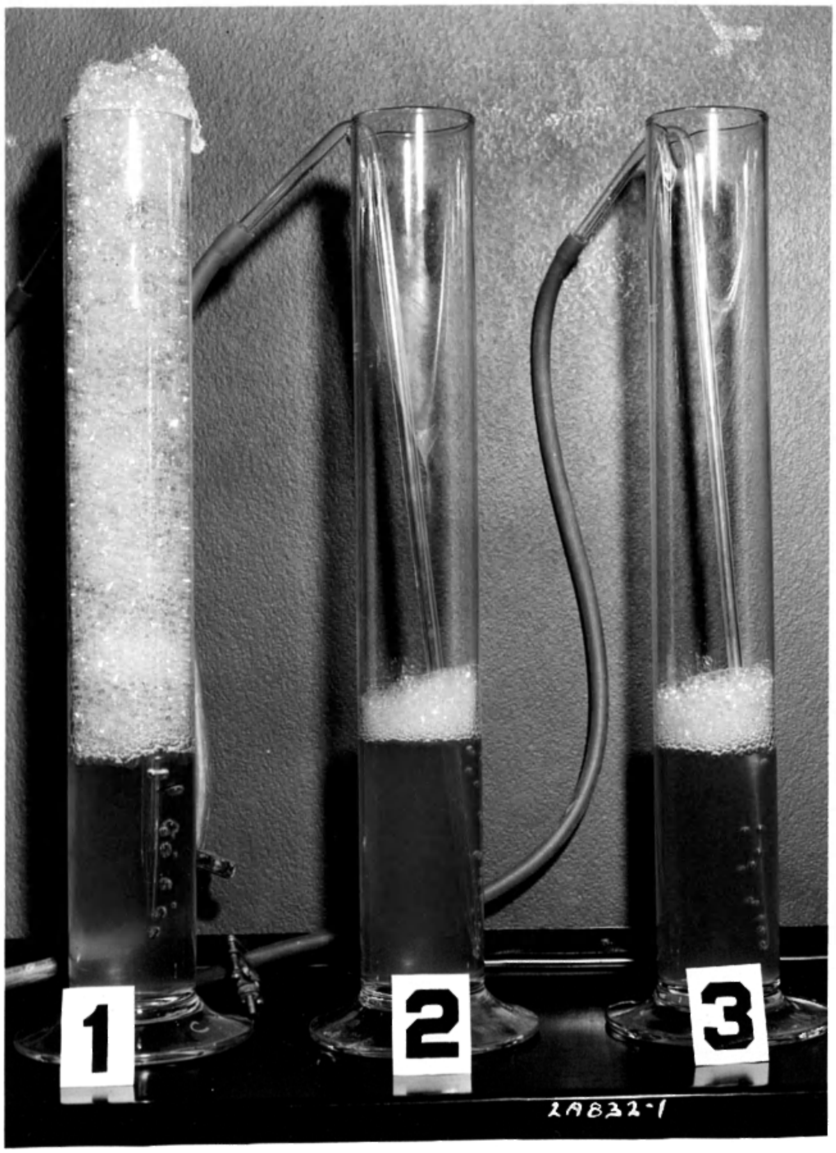
Apparatus used to test the anti-foaming properties of saliva and of linseed meal mucin solution.

Cylinder 1--contains 1 g. alfalfa saponin in 150 ml. water.

Cylinder 2--contains 1 g. alfalfa saponin in 100 ml. water plus 50 ml. of saliva.

Cylinder 3--contains 1 g. alfalfa saponin in 100 ml. water plus 50 ml. of linseed meal mucin solution.

PLATE II



effective in reducing the amount of saliva produced to a large degree as a saliva collection was made four hours after injection and a normal flow of saliva seemed to be produced. The drug injections were made for three consecutive days at the 50 mg. level per injection and then increased to 75 mg. This increase provoked extreme nervousness and irritability in the animal so the level was reduced to 50 mg. on the fifth day.

The administration of Lentin subcutaneously had no effect on the rumen froth produced by the diet fed. From previous work on the effect of saliva on the release of trapped gas and from its ability to break saponin foams, it was suspected that an increased saliva production would stop the intrarumen foaming but Lentin did not produce this effect.

Effect of Saliva and an Animal Mucin on Rumen Froth In Vivo

In Table 2, a summary of the effect of the administration of saliva and of an animal mucin on rumen froth is presented. Since bloating did not always occur, the degree of foaming was used as an estimation of the ability of these substances to control bloat. The administration of 2 liters each day before pasturing of saliva controlled frothing for three consecutive days in cow number 82 but when the treatment was switched to cow number 81 no control was observed. In cow number 16 no control of frothing with saliva was noticed until the level was increased to 6 liters on the fourth day. At this time a marked difference was observed as the treated animal, 16, showed a complete absence of froth

Table 2. Effect of saliva and of mucin on the formation of intrarumen foam in cattle on alfalfa pasture

Twin pair	Cow No.	Date	Treatment before pasturing	Degree of foaming*	
				2 hrs.	4 hrs.
1	15	4/15	None	+	+
	16	4/15	2 liters saliva	+	+
2	81	4/15	None	+[a]	+[a]
	82	4/15	2 liters saliva	0	0
1	15	4/16	None	+	+
	16	4/16	4 liters saliva	+	+
2	81	4/16	None	+[a]	+[a]
	82	4/16	2 liters saliva	0	0
1	15	4/17	None	+	+
	16	4/17	4 liters saliva	+	+
2	81	4/17	None	+	+
	82	4/17	2 liters saliva	0	0
1	15	4/18	None	+[b]	+
	16	4/18	6 liters saliva	0	0
2	81	4/18	2 liters saliva	+	+
	82	4/18	None	+	+
1	15	4/19	None**	0	0
	16	4/19	None	+	+
2	81	4/19	2 liters saliva	+	+
	82	4/19	None	+	+
1	15	4/20	None	+	+
	16	4/20	40 g. Mucin	0	+[a]
2	81	4/20	None	+	+
	82	4/20	40 g. Mucin	0	+[a]
1	15	4/21	None	+[b]	+
	16	4/21	40 g. Mucin	0	+
2	81	4/21	None	+	+
	82	4/21	40 g. Mucin	0	+
1	15	4/22	40 g. Mucin	0	+
	16	4/22	None	+	+
2	81	4/22	40 g. Mucin	0	+
	82	4/22	None	+	+

* Degree of foaming
0 No foam
\+ Foam, stable
+[a] Foam, non stable
+[b] Foam, bloat producing
** Saliva collected from this animal before pasturing

Table 3. Effect of alfalfa pasture on pH of rumen fluid, molar proportion of rumen volatile fatty acids (VFA), and total VFA production in 100 ml. of rumen fluid.

Cow no.	Date	Feed	Treat-ment		Molar percent VFA C_2	C_3	C_4	C_5	Total acid[1]
15	4/14	Hay + silage	0	6.0	73	14	11	2	11.3
16	"	"	0	5.7	65	20	13	3	12.8
81	"	"	0	6.0	70	16	12	2	12.8
82	"	"	0	6.4	72	16	9	2	11.5
15	4/15	Alfalfa pasture	0	6.5	64	21	11	4	10.5
16	"	"	2 l. S	6.4	62	24	10	4	9.5
81	"	"	0	6.7	66	20	9	5	7.1
82	"	"	2 l. S	6.9	66	19	12	5	7.7
		"							
15	4/16	"	0	6.4	68	19	10	3	9.1
16	"	"	4 l. S	6.6	65	20	11	5	8.2
81	"	"	0	6.8	65	19	12	4	6.0
82	"	"	2 l. S	6.5	66	22	11	1	8.4
15	4/17	"	0	6.5	65	20	11	4	7.2
16	"	"	4 l. S	6.5	67	18	11	4	4.5
81	"	"	0	6.5	68	18	10	4	11.2
82	"	"	2 l. S	6.6	68	15	11	5	10.4
15	4/18	"	0	6.2	69	17	11	3	11.3
16	"	"	6 l. S	6.3	67	20	10	3	9.7
81	"	"	2 l. S	6.4	70	19	8	3	11.0
82	"	"	0	6.5	65	20	11	5	9.2
15	4/21	"	0	6.0	61	22	-17-[2]		15.5
16	"	"	40 g. M	6.4	61	23	-15-		10.5
81	"	"	0	6.2	64	21	-15-		11.3
82	"	"	40 g. M	6.5	70	17	-14-		9.6
15	4/22	"	40 g. M	6.1	65	22	-13-		12.2
16	"	"	0	6.0	61	23	-16-		11.9
81	"	"	40 g. M	6.4	65	21	-15-		11.7
82	"	"	0	6.2	63	21	-17-		13.2

[1] Total acids listed in m. mol./100 ml. rumen fluid.

[2] Butyric and valeric bands did not separate so were titrated together.

S--saliva

M--animal mucin

while the control twin, 15, exhibited a moderate case of bloat with a stable intrarumen froth present. Cow number 15 which served as a control was used to collect saliva for administration to 81 on the last day of saliva administration. Therefore, 15 and 16 were not treated on the last day but were pastured on the alfalfa with 81 and 82. Frothing was observed in 16 but no frothing was observed in 15, the animal from which the saliva collection was made. Since it is possible to collect only a part of the saliva secreted by the cow during stimulation and collection, the extra saliva produced may have had an influence on the control of frothing that day.

In the mucin trials, it was found that the addition of 40 g. animal mucin completely controlled foaming for the first two hours of the feeding period but after four hours on pasture foaming began to occur. During the first two days of the mucin trial 40 g. of mucin was mixed in one liter of water and the solution poured through the rumen fistula. When treatments were switched on the third day, dry mucin was added through the fistula to see if a longer effect would be given by adding the substance dry rather than in solution. The dry mucin gave the same results as the liquid in that both controlled foaming for the first two-hour period with foam beginning to form after four hours.

The data collected on pH and molar proportions of volatile fatty acids (VFA) are given in Table 3. The pH values and VFA values given are from the 10 a.m. sampling period which was four hours after the cows were put on pasture. The molar proportions of VFA were determined once daily, according to Shaw (1959) the molar proportions of VFA remain the same throughout the day even though the total acid present may vary with the time of sampling.

The pH values are in the normal range with no observed difference between treatments. The pH was higher in the samples taken when the cows were first put on alfalfa pasture after transferring from the hay plus silage ration, but no consistent difference was maintained.

The addition of saliva or mucin to the rumen did not affect the molar proportions of VFA. However, there was a drop in acetic acid and an increase in propionic acid in changing from the hay and silage ration to alfalfa pasture. The amount of total VFA in m. mol./100 ml. rumen juice is also given in Table 3. There was a drop in the total VFA immediately after starting to pasture alfalfa but the total VFA rose to the beginning levels near the end of the experiment. The large variation among samples makes it impossible to draw any definite conclusions.

DISCUSSION

The role of salivation in the prevention of bloat as suggested by Weiss (1953) may be an important factor in bloat. The results presented herein on the effect of saliva on the release of gas trapped in frothing rumen contents and by its anti-foaming activity on saponin foam further support this theory. This work would indicate that reduced saliva flow in the presence of compounds that aid in the formation of froth may be the main factor in bloat production.

If saliva were the only factor involved, bloat would be produced when pasturing succulent grass in the same manner as produced on succulent legumes. Frothy bloat is evidently stimulated by the presence of compounds that augment froth formation, but there is some evidence to indicate that some of these froth promoting compounds are present in the dried forage in nearly the same concentrations as in the green forage. Bloat, then, would seem to be the result of the presence of froth promoting compounds with an absence of an antifrothing factor.

The work reported in this paper in which saliva was added to the rumen contents <u>in vivo</u> needs further investigation before any definite conclusions can be drawn. Saliva did not increase the incidence of foaming and in several cases controlled foaming. When saliva was added at the 6 l. level foaming was markedly reduced, however, due to a shortage of saliva this level was used only once. Even 6 l. of saliva would be a small quantity to add considering that a cow normally secretes from 125-200 pounds of saliva per day on a hay ration (Balch, 1958).

Bailey (1959) reported a 75% decrease in the amount of saliva secreted while cows were eating succulent grass as compared to dry hay. Data were not given on the amount of reduction in saliva secreted reflexly. If mucin in saliva is the agent which gives saliva its anti-foaming properties and its ability to release trapped gas from frothing rumen contents then the decreased saliva production during the time of mastication and remastication would be critical. Mucin is produced by the submaxillary and sublingual salivary glands and their activity is greatest during mastication.

Bartley (1957) found that the number of bloat cases and the bloat indexes were only slightly lower among cows fed linseed meal as a source of mucin than among control cows, but after a period of 2 to 5 hrs. in the dry-lot the number of bloat cases was fewer and the bloat index was lower among the cows fed linseed meal than among those fed none. This might be explained by the effectiveness of linseed meal mucin in causing trapped gas in froth to be released.

Johns (1954) considers that the low surface tension of saliva is a factor that could contribute to foaming by lowering surface tension of the rumen ingesta. Phillipson and Reid (1958) and Johns (1958) believe that mucoproteins present in saliva would cause the formation of a stable viscous type foam. Blake et al. (1957) have shown that saliva actually increases the surface tension of rumen fluid so it would seem unlikely that saliva would contribute to foaming by an effect on surface tension. The work in this paper has shown that when saliva and mucin from linseed meal and animal sources are added to an alfalfa saponin solution the amount of foam and the stability of the foam are reduced. Animal mucin controlled foaming in vivo for a two-hour period. However, there was some indication that the concentration of the mucin was critical in relation to its effect on foam. The 160 ml. level of saliva or of mucin was not as effective as the 80 ml. level.

The various trials made in this experiment were made to try to find different ways of testing the effect of saliva and mucin on the formation of intrarumen froth. The trials in which drugs were injected did not give conclusive results. However, the diet used may not have had the potential of producing bloat because the froth formed was unstable. Further studies using alfalfa pasture would be helpful in clarifying the action of these drugs.

The drop in the proportion of acetic acid and the increase in the proportion of propionic acid in changing the cows from a hay plus silage ration to alfalfa pasture is in agreement with the change reported by Jacobson *et al.* (1958) in feed-lot bloat studies. The effect of this change on the frothing ability of the rumen ingesta remains to be explained. However, this change in acid production does seem to indicate a change in the microbial population of the rumen.

SUMMARY

The effect of saliva and linseed meal mucin solution on the release of trapped gas from frothing rumen contents was tested *in vitro*. The effectiveness of these compounds as anti-foaming agents was also tested. Atropine and Lentin were administered to an animal to see if the stimulatory or inhibitory effect on salivation would have an effect on the froth produced in the rumen. Saliva and an animal mucin were tested *in vivo* on alfalfa pasture as agents for prevention of bloat. The pH, molar proportions of volatile fatty acids (VFA) and total VFA per 100 ml. rumen juice were determined.

The addition of saliva or linseed meal mucin was found to increase the rate at which trapped gas was released from 200 g.

samples of frothing rumen contents. The addition of either 80 ml. of saliva or linseed meal mucin solution was effective in releasing trapped gas. The addition of 160 ml. of either solution did not release as much gas as the 80 ml. level.

Saliva or linseed meal mucin when added to an alfalfa saponin solution markedly reduced the amount and stability of foam produced by bubbling air through the solution.

Atropine injected subcutaneously did not affect the formation of intrarumen foam. The injection of Lentin to stimulate salivation was ineffective in controlling intrarumen foam.

The administration of saliva <u>in vivo</u> at a 2 liter level controlled foaming in for three consecutive days in one animal but when the treatment was switched to her twin mate no effect on foaming was observed. In the other twin pair no anti-foaming effect was observed until the level was increased to 6 liters. At this level a marked difference was observed between the treated and control animals. The addition of 40 g. of animal mucin to the rumen contents completely controlled foaming in every treated animal for the first two-hour period of pasturing, after four hours on pasture the mucin began to lose its effect and foaming occurred.

The pH and total VFA of the rumen ingesta did not differ among treatments and no consistent difference was observed in the change from a hay plus silage ration to alfalfa pasture. The molar proportions of VFA were not different among treatments but did show a drop in the proportion of acetic acid and an increase in the proportion of propionic acid in the change from the hay plus silage ration to alfalfa pasture.

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APPENDIX

Table 1. Effect of 20 and 80 ml. levels of saliva or linseed meal mucin solution on the release of trapped gas from frothing rumen contents in a 10 min. period.

Trial no.	Amount of gas released in ml. from 200 g. frothing rumen contents				
	Control	20 ml. mucin	20 ml. saliva	80 ml. mucin	80 ml. saliva
1	31	25	38	23	50
2	24	31	34	19	46
3	31	34	33	29	41
4	25	31	39	35	41
5	44	48	51	42	52
6	42	45	56	38	52
7	38	39	40	56	40
8	27	32	28	52	44
9	36	51	47	52	62
10	30	47	43	50	45
11	43	39	55	60	58
12	50	39	49	70	63
Av.	35.1 $\pm$ 8.4[1]	38.4 $\pm$ 8.1	42.8 $\pm$ 8.9	43.8 $\pm$ 15.5	49.8 $\pm$ 7.7

[1] Standard deviation

Table 2. Effect of 20 and 80 ml. levels of saliva or linseed meal mucin solution on the release of trapped gas from frothing rumen contents in a 15 min. period.

Trial no.	Amount of gas released in mls. from 200 g. frothing rumen contents				
	Control	20 ml. mucin	20 ml. saliva	80 ml. mucin	80 ml. saliva
1	42	45	51	41	65
2	38	37	45	35	59
3	42	45	50	51	57
4	36	43	51	49	63
5	70	75	80	68	80
6	58	64	76	62	75
7	55	58	61	85	64
8	43	50	50	77	57
9	50	69	67	81	69
10	47	65	61	72	90
11	61	67	71	90	95
12	72	74	79	105	81
Av.	51.2 ± 12.0	57.7 ± 13.1	61.8 ± 12.4	68.0 ± 21.1	71.2 ± 13.0

Table 3. Effect of 20 and 80 ml. levels of saliva or linseed meal mucin solution on the release of trapped gas from frothing rumen contents in a 30 min. period.

Trial no.	Amount of gas released in mls. from 200 g. frothing rumen contents				
	Control	20 ml. mucin	20 ml. saliva	80 ml. mucin	80 ml. saliva
1	73	77	82	73	103
2	65	83	77	69	96
3	76	82	86	89	93
4	69	80	91	80	107
5	115	130	128	126	142
6	108	118	130	115	137
7	94	100	101	149	130
8	72	89	79	144	117
9	100	130	123	150	128
10	97	123	115	132	154
11	118	123	139	171	151
12	131	129	135	196	186
Av.	93.2 ± 21.2	105.3 ± 22.0	107.2 ± 24.3	124.5 ± 40.5	128.7 ± 25.6

Table 4. Effect of 20 and 80 ml. levels of saliva or linseed meal mucin solution on the release of trapped gas from frothing rumen contents in a 45 min. period.

Trial no.	Amount of gas released in mls. from 200 g. frothing rumen contents				
	Control	20 ml. mucin	20 ml. saliva	80 ml. mucin	80 ml. saliva
1	99	113	107	109	138
2	89	109	108	103	131
3	108	116	126	130	149
4	95	111	129	121	143
5	157	185	179	181	194
6	155	168	180	177	188
7	122	135	112	201	167
8	98	130	139	195	159
9	142	171	155	203	180
10	141	165	147	178	210
11	162	185	189	240	220
12	180	179	185	250	253
Av.	129.0 ± 31.0	147.2 ± 31.0	146.3 ± 31.0	174.0 ± 48.8	177.7 ± 37.0

Table 5. Effect of 20 and 80 ml. levels of saliva or linseed meal mucin solution on the release of trapped gass from frothing rumen contents in a 60 min. period.

Trial no.	Amount of gas released in mls. from 200 g. frothing rumen contents				
	Control	20 ml. mucin	20 ml. saliva	80 ml. mucin	80 ml. saliva
1	125	145	141	150	175
2	113	139	129	132	167
3	130	145	157	159	183
4	119	139	159	155	175
5	192	225	230	230	240
6	195	308	210	210	230
7	153	168	145	247	197
8	125	165	174	233	203
9	172	205	188	242	240
10[1]	173	215	176	210	215
Av.	149.7 ± 30.3	175.4 ± 34.4	170.9 ± 31.6	196.8 ± 43.4	202.5 ± 27.7

[1] The reason for only ten trials at the end of 60 min. is that more than 250 ml. of gas was released which was the maximum level that could be read.

Table 6. Comparative effect of different levels of saliva or linseed meal mucin on the release of trapped gas from frothing rumen contents.

Treatment	Trial no.	Amount of gas released in mls. from 200 g. frothing rumen contents				
		10 min.	15 min.	30 min.	45 min.	60 min.
Control	1	40	60	91	137	170
Control	2	41	53	94	135	166
20 ml. saliva	1	43	65	104	150	188
20 ml. saliva	2	50	67	119	167	202
20 ml. mucin	1	40	58	103	148	190
20 ml. mucin	2	35	47	88	135	176
80 ml. saliva	1	52	79	136	188	230
80 ml. saliva	2	53	81	139	195	235
80 ml. mucin	1	40	65	120	181	220
80 ml. mucin	2	38	59	106	157	209
160 ml. saliva	1	47	71	103	155	201
160 ml. saliva	2	50	76	118	164	207
160 ml. mucin	1	45	67	107	162	205
160 ml. mucin	2	44	64	104	158	197

Table 7. Effect of saliva on the production of gas by non-frothing rumen ingesta.

Treatment	Trial no.	Amount of gas released in mls. from 200 g. non-frothing rumen contents				
		10 min.	15 min.	30 min.	45 min.	60 min.
Control	1	30	39	80	110	137
Control	2	22	32	75	100	125
Control	3	13	25	48	72	94
Control	4	15	30	57	85	106
Avg.		20.0 ± 8.9	31.5 ± 5.8	65.0 ± 14.7	91.8 ± 16.3	115.5 ± 19.0
80 ml. saliva	1	25	36	81	114	151
80 ml. saliva	2	22	33	80	110	142
80 ml. saliva	3	10	20	42	70	93
80 ml. saliva	4	19	30	52	82	101
Avg.		19.0 ± 6.5	29.8 ± 6.9	63.8 ± 19.8	94.0 ± 22.2	121.8 ± 26.4

THE ROLE OF SALIVA IN THE ETIOLOGY AND PREVENTION OF BLOAT

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Most investigators now agree that the formation of a stable intrarumen foam is the primary cause of bloat on legume pastures. Some workers have indicated that saliva may be necessary to prevent foaming, but others believe that saliva is an agent which augments foaming. The work herein reported was initiated for the purpose of furnishing more information concerning the effect of saliva on foam formation *in vitro* and *in vivo*. Since saliva is a source of mucin the effect of linseed meal mucin and an animal mucin on foam formation were also tested.

In the first study the effect of bovine saliva and linseed meal mucin on gas escape from frothing rumen contents obtained from fistulated animals on alfalfa pasture was tested *in vitro*. Two-hundred g. samples of frothing rumen contents were weighed into quart jars and placed in a water bath at 39° C. Rubber tubes connected the jars to gas collecting cylinders containing water. The amount of gas released was determined by water displacement. In 12 trials it was found without exception that more gas escaped from the frothing rumen samples to which mucin (0.017 g. solids/ml.) or saliva (0.011 g. solids/ml.) was added than from the controls. Results for respective treatments after a 60 min. period in ml. gas released were: control, 150; 20 ml. saliva, 170; 20 ml. mucin solution, 176; 80 ml. saliva, 203; 80 ml. mucin solution, 197. Other trials indicated that 160 ml. of mucin or saliva did not release as much gas as the 80 ml. level but more than the 20 ml. level. When non-frothing rumen contents were used instead of frothing contents the addition of saliva or mucin did not increase gas escape.

The effect of saliva and of mucin on saponin foams was tested *in vitro*. Saliva or linseed meal mucin solution were added at various levels to 100 ml. of solution containing 1 g. alfalfa saponin. When air was bubbled through the control solution a stable froth was formed which rose up in the cylinder in which the solution was placed. The addition of 50 ml. saliva or linseed meal mucin solution prevented the formation of stable froth.

The administration of drugs which either increase or decrease saliva production were tested *in vivo* for their effect on intra-rumen froth. Atropine injected subcutaneously to reduce saliva production did not show any effect on froth production. Neither did the injection of Lentin to increase saliva production show any effect on froth production.

Saliva and an animal mucin were tested as agents for prevention of bloat in animals grazing alfalfa pasture. The pH, molar proportions of volatile fatty acids (VFA) and total VFA per 100 ml. rumen juice were determined. Two sets of four-year old fistulated identical twins were used. The daily introduction of two liters of saliva into the rumen of one animal before grazing controlled foaming for three consecutive days but when the treatment was switched to her twin mate, no effect was observed. In the other twin pair, no effect was observed until the level was increased to 6 liters. At this level a marked difference was observed. The introduction before pasturing of 40 g. animal mucin into the rumen of one member of each twin pair controlled foaming in every treated animal for the first two-hour period of pasturing. After four hours on pasture the mucin began to lose its effect and foaming occurred. No change was observed among treatments in pH or the total VFA of the rumen ingesta, however, the molar proportions of VFA did show a drop of acetic acid and increase in the proportion of propionic acid in the change from the hay and silage ration to alfalfa pasture.

These studies support the theory that saliva prevents bloating by preventing the formation of a stable froth and permitting trapped gas to escape from frothing rumen contents.