

EFFECT OF BOVINE SALIVA, MUCIN, AND SEVERAL ANTIFOAMING AGENTS  
ON ALFALFA SAPONIN FOAMS ASSOCIATED WITH BLOAT

by

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B. V. Sc., Panjab University, India, 1949

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A THESIS

submitted in partial fulfillment of the

requirements for the degree

MASTER OF SCIENCE

Department of Dairy Husbandry

KANSAS STATE UNIVERSITY  
OF AGRICULTURE AND APPLIED SCIENCE

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## INTRODUCTION

Bloat is a disorder of ruminants which occurs in all parts of the world, especially where grazing of legumes is an established practice. It is characterized by an interference with the normal elimination of gas produced by microbial digestion of the food in the rumen. Gas accumulates extending the rumen which exerts pressure on the diaphragm and contiguous blood vessels. Unless bloated animals are treated immediately a large percentage may die from asphyxia accompanied by heart failure.

Although the condition has been recognized for some time, the incidence has steadily increased probably due to the changes in feeding practices. The recognition of the high nutritive value of legumes has led to their increased cultivation which has added to the incidence of bloat. In recent years it is generally conceded that the incidence of bloat is somewhat proportional to the increased use of pure legumes or mixed legume pastures.

The seriousness of the bloat problem can be recognized from the fact that in the United States alone bloat in dairy cattle, beef cattle, and sheep causes animal losses of forty to forty-five million dollars a year (Dougherty, 1955). Merely determining actual losses attributable to bloat is not a satisfactory means of evaluating the seriousness of the problem. The losses in milk and meat production are probably of greater importance than the actual death toll. In addition, expensive preventive measures as well as restrictions on the use of legumes because of the danger from bloat should also be considered.

Attempts have been made to classify bloat as chronic, subacute and acute, (Cole, et al., 1955), according to the degree of ruminal distention and the duration and relapse of the condition. The difficulty with this method of

classification is that some so-called chronic cases may suddenly become "acute." Broadly speaking, acute and subacute bloat are attributed to a condition resulting from a specific dietary regimen, such as the feeding of succulent legumes, and the two differ only in the degree of intraruminal pressure. Chronic bloat is limited to a condition arising from some pathological disturbance of the animal.

Bloat also has been classified as "foaming" or "free gas," according to the amount of foam or free gas present in the rumen. There is a difference of opinion in regard to this classification but it has been accepted generally that legume bloat is always "foamy," though there may be varying amounts of free gas present.

The exact cause of bloat still remains a mystery although considerable effort has been expended by workers in this field. Practically all the factors related directly or indirectly to rumen digestion have been studied and several theories have been advanced by investigators from time to time regarding the cause of bloat.

Formation of a stable foam in the rumen and reticulum has become recognized as the primary cause of the majority of cases of uncomplicated pasture bloat. There is a difference of opinion as to what condition(s) or factor(s) contribute to the formation of a stable foam. It has been suggested that the lack of salivary secretion resulting when cattle graze legumes is responsible for froth formation, but there is divergence of opinion as to whether saliva inhibits stable froth formation or enhances the development of froth.

Weiss (1953) believes that the formation of froth is dependent on the consistency of the ruminal ingesta which in turn is influenced by reflex salivary secretion. Reflex salivary secretion is largely stimulated by the

presence of coarse material in the forestomachs. The amount of saliva secreted, therefore, depends on the physical condition of the feed. When lush green legumes are fed the lack of fiber leads to a lack of salivary secretion. On account of this the rumen contents remain viscid so that gas becomes trapped as a foam and cannot be expelled by belching. The gist of Weiss' hypothesis is that a large salivary secretion helps to prevent bloat.

In the light of Weiss' hypothesis that the low rate of salivary secretion might be a contributing factor to the etiology of bloat, work has been conducted at the Kansas station to determine the relationship of saliva to legume bloat. Mucin, being the most prominent constituent of the organic matter of saliva has been suspected as playing an important role in the regulation of the functions and properties of saliva.

Bartley (1957) added saliva or mucilaginous linseed meal extracts to the rumen contents obtained from bloating fistulated cattle. When these contents were incubated at 39° C. there was a greater release of gas from the rumen contents to which saliva or linseed meal mucin was added than from the rumen contents incubated alone. The feeding of linseed meal to cows before pasturing reduced the incidence and severity of bloat. The author postulated that if froth formation in cattle while grazing legumes was due to a lack of salivary secretion, the mucin in saliva or linseed could be responsible for the protective action of these materials.

Further studies at the Kansas station by Van Horn (1959) showed that both saliva and the mucin extracted from linseed meal significantly decreased the foam produced by alfalfa saponin in vitro.

The work reported here was a continuation of Van Horn's work. In vitro studies were made to determine the effect of saliva, animal and plant mucins, and some antifoaming agents, on the foam produced by alfalfa saponin. It was



hoped that this study might add further information to the role of saliva in foaming as well as locate materials that could be used in the field for preventing bloat caused by legumes.

#### REVIEW OF LITERATURE

It is well known that the microbial digestion of cellulose in the rumen results in the production of large volumes of gases. Under normal conditions these gases are eliminated mostly by eructation, however, rather small quantities are absorbed in the blood and eliminated through the lungs. Contrary to an old belief, that an increased rate of fermentation is a factor in the production of bloat, it has been shown that there is no significant difference in the rate of fermentation in bloated and non-bloated animals, and that under normal conditions, the animal can eliminate large quantities of gas by eructation. From these findings it has been concluded that the trouble lies in the inhibition of the eructation mechanism under certain conditions, rather than in an increased rate of gas production.

While there are several theories as to the cause of bloat, the review of literature presented here is connected mainly with the "frothy" type of bloat which is observed when cattle graze legume pastures. This type of bloat is characterized by the production of "foam" in the rumen, thereby preventing the eructation of the entrapped gas.

#### Foaming or Surface Tension Theory of Bloat

According to this theory, some alteration in the surface tension of the ruminal fluid takes place so that the gases of fermentation tend to accumulate in countless bubbles throughout the ingesta instead of rising to the top and collecting in a gas pocket above the ingesta (Dougherty, 1953).

Although this is a new theory it appears to have its origin in the older "saponin theory." McCandlish (cited by Cole, et al., 1945) claimed that the chemical substance in clover or in potatoes which appeared to cause bloat was saponin, and the ill-effects were induced presumably not by an extra production of gas in the rumen but by the retention of the gas already formed. Olson (1942) also referred to saponin as an explanation of the bloat associated with froth and foam in the rumen.

Amadon (1930) concluded from his experience that most cases of bloat on green legumes were of the frothy type. Quin (1943) proposed that the saponin in lucerne increased surface tension and the tendency towards foam formation, thus impeding the escape of gas from the ruminal mass. He reported that repeated examinations made on fresh ruminal ingesta from sheep kept on different diets revealed a definite tendency towards foam formation in the sheep fed alfalfa, whereas foam was only slight and of transitory nature in sheep fed other rations. The fact that the antifoaming agents are effective in the treatment of bloat, give support to this theory.

Since the theory was suggested by McCandlish (1942), Olson (1942) and Quin (1943), it has gained a large number of supporters (Weiss, 1955; Johns, 1954; Ferguson and Terry, 1955; and Colvin, et al., 1956). Now it is a general belief that bloat in ruminants grazing fresh legumes is due primarily to a foaming of the contents of the rumen and reticulum.

#### Characteristics of Intrarumen Foam

In testing the foaming capabilities of rumen contents it is difficult to decide on what physical determination to make as representing a property that is important in bloat. However, studies have been performed by inves-



tigators using different measures of the characteristics of foam found in the rumina of bloated animals; e.g., surface tension, buoyancy, viscosity and foam strength.

Johns (1956) reported that foam may be of two types: (1) Highly stable foams produced by surface viscous compounds, e.g., proteins and saponins whose solutions show relatively little lowering of surface tension and whose foam stability is correlated with their high surface viscosity. (2) Less rigid foams produced by compounds whose solutions exhibit low surface tension and low viscosity, e.g., soaps.

Nichols, et al. (1957) measured the apparent surface tension and relative viscosity of the rumen fluid samples collected from cows repeatedly subjected to sudden changes from alfalfa hay to fresh ladino clover. These workers reported that the periods of increased and excessive frothing following changes from hay to fresh clover were associated with periods of high surface tension and viscosity.

There is a difference of opinion among workers as to whether the anti-foaming agents used in the treatment of frothy bloat produce their effect by the lowering or raising the surface tension of the ruminal fluid. Clark (1948) stated that very small concentrations of antifoaming agents have a marked effect on the physical consistency of the ingesta, raising surface tension and breaking down the foam.

Contrary to the statement of Clark, Blake, et al. (1957) reported that turpentine, defoaming agents of the methyl silicone and fatty acid types, and two detergents markedly reduced the surface tension of rumen fluid. He found that manual defoaming of rumen fluid samples reduced surface tension of rumen fluid. Johns (1956) considered it to be likely that substances which reduce

surface tension act by displacing the highly viscous type of foaming compounds from the surface layer thus giving rise to an unstable foam.

From in vitro studies of the effects of various surface active agents on the surface tension and other properties of rumen fluid, Nichols et al., (1957b) observed that silicone sulfonate and lecithin reduced the surface tension of rumen fluid.

Nichols, et al. (1956) reported that the feeding of fresh alfalfa caused an increase in surface tension and a decrease in relative viscosity. Water intake of alfalfa fed animals caused no appreciable change in surface tension but was followed by reduced viscosity. Viscosity of the rumen samples taken before feeding during the period when fresh alfalfa was being fed was appreciably higher than that of the samples taken before feeding during the periods of grass or hay feeding.

Some workers have correlated the pH of the ruminal fluid with the production of foam. Mangan (cited by Johns, 1958) devised a method of measuring the strength of foam. Using this method the properties of the foams produced from saponins and cytoplasmic proteins were examined, particularly with respect to the effect of pH and salt concentration. On examining samples of rumen fluid from bloating cows, it was found that the pH has to fall to the region of 6.3 for the first signs of bloat to occur. Then, as the fermentation proceeds it may fall as low as 5.7. A determination of the pH for maximum foam strength of the rumen fluid showed it to be in the region of 6, with a salts concentration of 0.15 to 0.2 M.

Myburgh and Quin (1943) reported that the pH of the ruminal ingesta of Merino sheep varied between pH 5.5 and 6.8. The feeding of lucerne hay yielded slightly acid ingesta (pH 6.45 to 6.95), whereas fresh green lucerne as well as mature veld grass hay produced slightly alkaline conditions vary-

ing from pH 7.3 to 7.7. However, they did not study the pH of the rumen contents in relation to the development of bloat.

Nichols et al. (1955) determined the specific gravity of rumen fluid of cattle fed hay, grass, and fresh legumes and expressed it as the effective buoyancy. They concluded that the effective buoyancy of rumen fluid was less following the intake of fresh legumes than following the intake of either hay or fresh grass.

Johns (1954) tested the foam stability of incubated rumen juice (37° C.) obtained from two groups of sheep fed red clover and cocksfoot respectively. The foam from sheep which had been fed red clover was stable for 12 hours and the volume had decreased only slightly after 24 hours. The foam from the grass-fed sheep completely disappeared in 90 to 120 seconds. Johns observed that the foam was never so stable as to retain all the gas and that there would be a slow release to a gas pocket even in the most severe cases of foaming. The more stable the foam the smaller would be the amount of gas released and the smaller the amount eliminated by eructation.

#### Factors Associated with the Production of Foam

Foaming is considered to be a complex mechanism which cannot be attributed to a single factor. Several factors of animal and plant origin have been associated with the production of foam in the rumina of cattle and sheep feeding on fresh legumes, e.g. ✓ plant saponins, plant proteins, saliva, and rumen bacteria. A discussion of the important factors associated with the production of foam follows.

Saponin. McCandlish (cited by Cole et al., 1945), Olson (1944), and Quin (1943) have postulated that saponin alters the surface tension of the ruminal contents and that it might contribute to frothy bloat by the entrap-

ment of gases of fermentation in bubbles throughout the ingesta.

As early as 1919 Jacobsen (1919) isolated a compound from alfalfa that he regarded as a saponin. However, he could not produce any ill-effect in sheep by the feeding of this product.

Thompson et al. (1957) reported that saponins are naturally occurring plant glucosides that have soaplike properties. They may be broadly divided into two classes; in one the nuclei or aglycones are steroids, while those of the other are triterpenoids. The saponins are surface-active compounds because the aglycones or the sapogenins are fat-soluble while the carbohydrate portions of the intact saponin molecules are water soluble. Saponins in legume forages appeared to belong to the triterpenoid type. The total content of saponin in alfalfa is approximately 0.5 to 2 percent of the dry matter (Thompson et al., 1957).

Lindahl et al. (1954) produced bloat symptoms in sheep and cattle by the oral administration of the saponin isolated from alfalfa. Two commercially available saponin preparations isolated from the Yucca plant did not produce any bloat symptoms when administered in a like manner to the same animals.

Weiss (1953) suggested that the presence of saponin in lucerne was not the cause of bloat per se, but it merely contributed to the colloidal state of the ingesta and made it susceptible to foaming. He found that the daily mechanical shaking of samples of expressed lucerne juice over a period of months under different meteorological conditions showed no significant variations in the amount of foam produced daily.

Ferguson and Terry (1955) reported that administration to sheep of lucerne saponin, four other plant saponins and other foaming agents failed to produce bloat. However, Lindahl and Davis (1957) showed from in vitro and in vivo experiments that alfalfa saponin can contribute to the formation

and stabilization of froth of ruminal ingesta. They suggested that probably alfalfa saponin is not the only factor involved in stable froth formation either in experimental or clinical bloat, and that a possible interaction may exist among saponins, proteins and carbohydrates in the formation of froth.

Lindahl et al. (1957b) reported that alfalfa saponins have marked surface tension activity giving rise to stable foam. In addition they also have marked physiological and pharmacological actions. Physiological actions alone may cause an interference in the eructation mechanism. Intravenous administration of alfalfa saponin led to the death of the majority of the animals.

Ferguson and Terry (1955) reported that the fractionation of bloat-provoking lucerne juice showed that the bloat-provoking factor was retained after (a) precipitation of chloroplast material and (b) passage of clear juice through an anion or cation exchange resin. It appeared that the bloat-provoking factor was non-ionic and not absorbed on resins. These properties are exhibited by saponins. They concluded that the formation of stable foam was a major factor in lucerne bloat and that feed constituents other than saponins and the physical condition of the rumen ingesta probably influenced the stability of foam.

Henrici (cited by Cole et al., 1955) conjectured that the bloatiness of some legume pastures were due to increased saponin content of the plant associated with zinc deficiency of the soil. Hoagland (cited by Cole et al., 1955) presented evidence to indicate that zinc deficiency resulted in increased saponin content of legumes.

Plant Proteins. Soluble plant proteins have been suggested as a possible factor which might contribute to the production of froth. Johns et al.



(1957) in a determination of the optimum pH for maximum foam stability for red clover saponins and cytoplasmic protein extracts found that, while the former was about five, the latter was approximately the same as that of the rumen contents (pH 6). From this evidence they suggested that at the pH of the rumen contents at which bloat occurs, proteins were likely to be more important as foaming agents than the saponins. Contrary to the belief that proteins could not be released rapidly enough from the plant to contribute to bloat, they found that proteins were rapidly released from the plant material and a concentration was reached in 30 minutes (20 mg. N/100 ml.) which produced foam of considerable strength.

Ferguson and Terry (1955) subjected alfalfa extracts to heat treatment (80° C.) with 75 percent alcohol and passage through cation and anion exchange resins, without reducing their ability to produce bloat. These facts would suggest that the plant proteins are not important in the etiology of bloat. However, Boda et al. (1957) reported that commercial dehydration procedures destroy the bloat-producing ability of fresh chopped alfalfa. This would suggest that proteins may be of importance if it is assumed that the major effect of dehydration on plant constituents is a denaturation of water soluble proteins.

Lindahl and Davis (1957) suggested the possibility of an interaction existing among saponin, proteins and carbohydrates in the formation of froth in legume bloat.

Conard et al. (1958) attempted to determine the characteristics of alfalfa that were considered a part of the complex causing stable foam formation in the rumina of cattle. Gas production in in vitro rumen fermentations, and stable foam formation after mechanically blending with water were determined using various fractions of alfalfa. The results showed that the plant



substances responsible for the initial rapid gas production obtained with green alfalfa were closely associated with the fiber fraction, but were removed after digesting in vitro for 10 hours with rumen microorganisms or by extraction for 12 hours with hot water. They concluded that the combined effects of physical structure of green alfalfa fiber, pectic substances of alfalfa plants and reducing sugars normally present are capable of causing the formation of stable foam found in pasture bloat.

Saliva. There is difference of opinion regarding the rate of salivary secretion under varying conditions of feeding. Coats et al. (1956) reported that inflation or deflation of the rumina of Merino sheep had no effect on the rate of secretion of the parotid glands.

However, Phillipson and Reid (1956) studied response of rumen pressure on salivary secretion in sheep and calves and reported an increase in salivary secretion with an increase in the intraruminal pressure. Parotid and submaxillary glands varied in the degree of response. The responses obtained with the submaxillary and the sublingual glands were slower in starting than those from the parotid glands. Atropine given one calf during a pressure response stopped secretion of all the salivary glands.

Sharma (cited by Reid and Huffman, 1949) reported that the mastication of green grass by a buffalo cow with a parotid fistula in the left duct produced a secretion of 42 ml. in five minutes. This output increased to a rate of 102 ml. when the animal was masticating bhoosa.

Clark and Weiss (1952) showed a four to five-fold increase in parotid secretion by mechanical stimulation of the cardiac region of the esophagus with a bottle brush. This stimulus was removed, however, by sectioning the vagus nerve.

Elach (cited by Johns et al., 1957) showed that the amount of saliva secreted was directly related to the condition of the feed. He reported that while eating 10 pounds of feed a cow secreted 45 to 55 pounds of saliva with

hay, 12 to 15 pounds with ground concentrates, and 7.5 to 10 pounds with fresh grass.

Several authors have shown that the physical properties of the feed have an important bearing on the activity of the parotid glands (Clark and Weiss, 1952). Dry fibrous feed stimulated a greater output of saliva as compared with succulent green leafy material (Bailey, 1959). These observations give support to the contention of Weiss (1953) that the volume of saliva secreted reflexly is related with the coarseness of the material in the forestomachs.

There are two schools of thought regarding the association of saliva with the formation of stable foam in the rumen. According to one, saliva will inhibit the formation of foam, while according to the other, saliva will increase foaming.

Weiss (1953) reported that the consistency of the ruminal ingesta and the occurrence of frothy bloat after feeding was influenced by the type of lucerne fed. Bloat, caused by frothing of thick, viscid ruminal ingesta, occurred immediately after feeding succulent leafy lucerne in the preflowering stage of growth. When mature stalky lucerne was fed, the ruminal ingesta immediately reverted to a watery consistency even in the absence of available drinking water. Under these conditions bloat ceased. The author concluded that the rapid reduction in consistency of the ruminal ingesta after feeding stalky lucerne was due to reflex stimulation of salivary secretion initiated in the forestomachs by the physical character of the feed. Also, ingestion of the stalky type of lucerne was slow, with the result that proportionately more saliva was secreted per given weight of food; whereas the rapid rate of feeding observed in the case of the immature type of lucerne caused a proportionately smaller amount of saliva to be secreted per given weight of feed. The consistency of the ruminal ingesta therefore increased and, if gas formation was adequate, the ingesta rose up into a tenacious frothy mass.

Denton (1956) reported that feeding of fresh green chopped lucerne caused

a reduction in the daily output of parotid saliva compared with the output when roughage was included in the diet.

Cole et al. (1943) demonstrated that the feeding of coarse hay prior to lucerne pasturing prevents bloat. These workers (Meade, Cole and Regan, 1944) also demonstrated that the incidence of bloat is much higher when cattle are fed ground hay and grain than when fed unchopped hay and grain. These workers attributed the protective action of unchopped hay to the fact that coarse material is necessary to elicit the eructation reflex. Contrary to the statement of these workers that the coarse material was necessary to produce the eructation reflex, Weiss (1953) showed that the physical state of the ruminal contents did not appear to affect the eructation reflex directly, and that the physical condition of the lucerne had a direct bearing on the occurrence of bloat through its action on reflex salivation.

✓ Reid and Huffman (1949) reported that bovine saliva has a low surface tension. Compared with water (71.3 dynes per cm.), saliva had an average value of 47.1 dynes per cm., when measured at a comparable temperature. Johns (1954) believed that this property made saliva a foam promotive agent which may have some bearing on the incidence of bloat in ruminants.

Ferguson and Terry (1955) showed that a synthetic inorganic saliva and cow's saliva did not prevent bloat in sheep. From the single test made with cow's saliva it was not possible to say if saliva increased the severity of bloat.

Phillipson and Reid (1956) reported that an additional salivary flow in response to pressure in the rumen may have been large enough to be an important factor in precipitating a dangerous state in a moderately bloated animal. Carbon dioxide released from the salivary bicarbonate, particularly from parotid saliva, would further increase the intraruminal pressure, while the surface active salivary mucoproteins could increase foaming of the ingesta. In the latter connection it may be significant that the level of mucoprotein in the submax-

illary saliva can reach high levels (Mangan, 1957, cited by Phillipson and Reid, 1958) when excessive distention of the rumen causes respiratory inhibition coupled with marked and fluctuating increases in blood pressure which is probably caused by the release of adrenalin.

Johns et al. (1957) suggested that there may be some difference in the chemical composition of saliva from bloating and non-bloating animals, and also there may be animal differences in the rate of salivary secretion. According to Johns (1958), a large secretion of saliva could, according to its composition, either assist in preventing bloat by buffering a fall in pH, or increase its severity by adding to the  $\text{CO}_2$  evolved and by assisting in foam formation.

✓ Rumen Bacteria. The possibility that bloat may be due to a lack of balance of microorganisms normally found in the rumen or to the presence of an abnormal type has been suggested. Koffman (1957) found a decrease in the number of large protozoa in the rumen of sheep affected with bloat.

Quin (1943) demonstrated that acute gas production in the forestomachs immediately after the consumption of certain feeds was associated with a process of oxidative assimilation. By this process, variable proportions of sugars were rapidly oxidized by a strain of false yeast, Schizosaccharomyces ovis. This organism was present in large numbers in the rumen of sheep, especially when animals were fed lucerne. Attending this oxidation of part of the ingested sugar, large volumes of gas were suddenly generated within the ruminal mass. Simultaneous with this, the rest of the sugar was rapidly assimilated and stored as glycogen by the yeast cells. Complete starvation or inadequate feeding of the animal was promptly followed by suppression leading up to a total disappearance of this yeast strain. Under these circumstances various iodophilic bacteria normally present in the ruminal ingesta were afforded the opportunity of metabolizing the available sugar. Oxidation in this case decreased as was evident from the reduced amount of gas produced.

Later, Quin (1943) concluded that the pathogenesis of acute bloat was

closely associated with the production of gas during rapid oxidation, mainly by yeast cells, of sugar. Normal eructation of gas may be impeded as a result of excessive foam production in the forestomachs especially when animals are restricted to a diet of green lucerne.

However, Jacobson et al. (1942) compared the rate of fermentation of samples of the rumen contents subjected to different treatments. They found that the changes in dilution, temperature and hydrogen-ion concentration, in the ranges normally occurring in the rumen, had little effect on the rate of fermentation and ensuing gas production. The maximum amount of salt which a cow could tolerate in her drinking water also had little effect on gas production in in vitro studies. From these observations the authors concluded that the alteration in the environment of the rumen bacteria has no basis for the development of bloat.

Hungate et al. (1955) observed the production of extracellular mucilagenous material from a number of isolated strains of pure cultures of various cellulolytic bacteria from the rumen. Foaming was noted when certain strains of non-spore forming butyric acid producing rods were incubated 24 hours in a broth culture with various fermentable sugars. The most striking production of mucilagenous material was encountered in cultures of a spore-forming actively growing cellulolytic rod which had been isolated from a large number of cattle grazing on ladino clover pasture. From these studies Hungate et al. postulated that slime production by bacteria may account for the formation of a stable foam.

Jacobson et al. (1957b) in feed-lot bloat studies found that the percentage of encapsulated microorganisms reached a high level on a bloat-producing diet and the quantity of these organisms definitely correlated with the bloat index. In an eight-week period a correlation coefficient of 0.94 was obtained between the percentage of encapsulated microorganisms and the occurrence of bloat.



Dain et al. (1956) studied slime production in Streptococcus bovis, an organism found in considerable numbers in the rumen of sheep and cattle. The organism produced mucoid colonies on sucrose agar in the presence of carbon dioxide and probably dextrin formation was responsible for the sliminess of the colonies. These workers suggested that slime production may be correlated with the development of stable froth formation in ruminants.

However, Bryant (1958) reported that visual observations of the rumina of cattle showed that slime producing bacteria were no more numerous in cattle fed fresh legumes than those fed alfalfa hay.

In a recent study of the bacterial changes in the rumen of cattle, Outierrez et al. (1959) found that the microflora consisted mostly of small single or paired cocci and rods before the onset of bloat. During the development of feed-lot bloat these workers observed an increase in the number of short chained, encapsulated streptococci and LC type organisms (Peptostreptococcus elsdenii). The steers used in these experiments were fed a ration of barley supplemented with either soybean oil meal, cottonseed meal, or linseed oil meal as a protein source. The strains of encapsulated cocci were lactic acid producing and similar to Streptococcus bovis which commonly occurs in the rumen. Surface cultures of these cocci formed white and mucoid colonies. Slimy growth with a ropy, viscous characteristic was produced in liquid yeast extract-peptone medium supplemented with different carbohydrates. The authors suggested that an increase in Streptococcus bovis type streptococci and Peptostreptococcus elsdenii during the onset of feed-lot bloat may play a role in its etiology. ✓

#### The Effect of Rumen Froth on Eructation

Several investigators have suggested that stable froth formation in the



rumen will prevent eructation. Colvin et al. (1958a) reported that acute legume bloat resulted from rapid gas formation accompanied by a frothing of the ingesta. The frothing ingesta physically prevented eructation.

Weiss (1953) suggested that frothing of the ingesta was one of the mechanical factors affecting the eructation reflex. Nichols (1954) reported that legumes, due to their density, would sink in the rumen liquid just after eating causing the level of liquid to rise above the cardia. The bubbles produced by the fermentation lowered the buoyancy of rumen juice which caused an increased trapping of air bubbles. The foam so formed rose high above the cardia preventing eructation.

Dougherty (cited by Johns, 1958) using the isolated reticular pouch technique in sheep, demonstrated the inhibitory effect of liquid on the opening of the cranial esophageal sphincter. Johns (1958) using the same technique, introduced various foams into the pouch, passed in gas, and measured the size and frequency of belches. He found that the reflex receptor controlling the opening of the cranial sphincter could distinguish between free gas and foam as the latter caused inhibition of belching.

#### The Use of Antifoaming Agents in the Prevention and Treatment of Bloat

Turpentine has been found effective in the treatment of bloat, however, substances like formalin, phenol and vegetable oils also have been used with good results (Cole et al., 1955). According to Cole et al. (1945) the therapeutic value of these substances as inhibitors of fermentation or inducers of belching have been known for a considerable period of time.

Clark (1948) has shown that therapeutic concentrations of turpentine and certain phenol preparations commonly used to treat bloat had little or no

inhibitory action on gas formation in ruminal ingesta incubated in vitro. However, very small concentrations of these substances had a marked effect on the physical consistency of the ingesta, raising surface tension and breaking foam. He concluded that the therapeutic value of these drugs depended on their surface tension action rather than on their inhibition of fermentation.

Later Quin et al. (1949) reported the use of a surface-active agent, a highly polymerized methyl silicone for the treatment of bloat. This material effected 115 recoveries out of 155 cases of bloat. The drug was given either by direct injection into the rumen or by stomach tube. Johns (1954) obtained good results with a commercial silicone preparation (Avlinox, I.C.I), turpentine and vegetable oils (linseed oil, mustard oil) in the treatment of bloat. Reid (1955) reported that spraying emulsified vegetable oil on pasture at the rate of two to four ounces of oil per cow per day has been tried with success as a bloat preventive measure in New Zealand and Australia.

Blake et al. (1956) observed a significant reduction in bloat incidence and severity by the administration of an alkyl aryl sodium sulfonate type detergent to cattle grazing alfalfa. They found that the surface tension and viscosity of rumen fluid were decreased by detergent administration. Specific gravity and pH were slightly lower during bloat and increased after treatment. Brown et al. (1958) found that a water dispersible oil markedly reduced the incidence of bloat even during periods of very severe bloating, when fed at levels of one and two percent in the drinking water of Holstein steers grazing alfalfa pasture.

Johnson et al. (1958) reported that soybean oil at the rate of 0.25 pounds per animal prevented bloat for three to four hours when fed in the grain ration immediately before grazing. Corn lecithin was highly surface-

active and appeared to give good protection when 0.25 pound was fed in spent germ flakes and grain. A similar quantity (0.25 pound) of a mixture of lecithin (75 percent) and methyl esters of fatty acids (25 percent) was slightly effective but was not sufficiently dependable.

Blake et al.<sup>3</sup> (1957) administered a detergent "ultravet K" via gelatin capsules at the rate of 20 and 30 gm. daily per 1,000 pounds of body weight and observed a significant reduction in the incidence and severity of bloat. During bloat the ingesta volume increased and foam stability and degree of foaminess of rumen ingesta markedly increased. Surface tension and viscosity moderately increased, while the specific gravity and pH were lowered moderately. All these properties were altered in the opposite direction during detergent treatment. Bloat-provocative alfalfa juice was prepared and administered by stomach tube to young dairy animals. Neither cholesterol nor the detergent when added to the alfalfa juice before administration effectively reduced bloat or greatly altered the rumen fluid characteristics measured above.

Colvin et al. (1959) reported that the administration of vegetable oil before alfalfa feeding prevented the development of bloat symptoms. Oil administered during the development of bloat increased the rate of eructation and the volume of gas expelled and reduced intraruminal pressure within 15 minutes.

Schumacher (1959) suggested that higher silicone doses are necessary for the destruction of foam in acute cases of bloat than for prophylaxis. The use of these agents in acute cases is of value only if the gases liberated from the foam and from the continuing fermentation can escape either by eructation or through an artificial outlet.

Johnson et al. (1958) observed that soybean oil sprinkled on chopped alfalfa soilage at a level of approximately 0.25 pound per 1,000 pounds body weight of animal 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 free gas in a short time.

Bartley (1957) reported that the feeding of linseed meal to cows before pasturing appeared to reduce the incidence of bloat and caused the bloat to subside more rapidly.

Johns et al. (1957) stated that a wide range of surface-active agents effective as antifoaming agents can be used for relieving bloated animals. However, a number of them have undesirable characteristics that will adversely affect the health of the animal or its milk production. Silicones, which are widely used in industry as defoamers, are non-toxic and might be expected to be very useful in treating bloated animals. However, they have proved unreliable. The most effective and harmless agents tried thus far were stated to be peanut oil and tallow. Antifoaming agents have been found to be effective in cases where the animals are not in a distressed condition. Complete deflation can be expected to occur in some 30 minutes. However, when the animal is in a seriously distressed condition a trocar or knife appears to be the only treatment.

#### APPARATUS, MATERIALS, AND METHODS

##### Measurement of Foam

Two ml. of an alfalfa saponin solution (1 gm. in 50 ml. of distilled

water) was placed in a 100 ml. graduated cylinder and diluted by the addition of 20 ml. distilled water. The solution thus formed was aerated with a glass tube with a fine tip for two minutes at a pressure of 10 mm. water. The tip of the glass tube was placed one inch from the bottom of the cylinder. The amount of foam produced was measured in milliliters as it rose in the graduated cylinder. The saponin solution (diluted) mentioned above and the amount of foam produced by its aeration, was used as control in all foam measurement studies. The effect of various substances on the foam produced by saponin was tested by adding fractions of their solutions to the saponin solution prior to aeration. The standards of time, temperature, and pressure of aeration were maintained throughout the study.

The apparatus used in the measurement of foam produced by aeration of alfalfa saponin solution is illustrated in Plate I. A 100 ml. graduated cylinder (1) contained the saponin solution. A glass tube (2) twelve inches long, bent at one end and with a fine tip at the other, hung in the solution with the fine tip one inch from the bottom of the graduated cylinder. A rubber tubing joined the bent end of the glass tube to a Y-shaped cast aluminum connecting tube. The remaining two ends of the Y-shaped connecting tube were joined with rubber tubing respectively to a compressed air outlet and a water manometer (3). The compressed air passed through the Y-shaped connecting tube at a definite pressure (10 mm. water) controlled by manometer (3) and bubbled in the saponin solution in the graduated cylinder (1) through the tip of the glass tube (2). A stop watch was used for the control of aeration time (2 minutes).

#### Preparation of Solutions of Mucin and other Substances

Solutions of alfalfa saponin, mucin and other substances used for test-

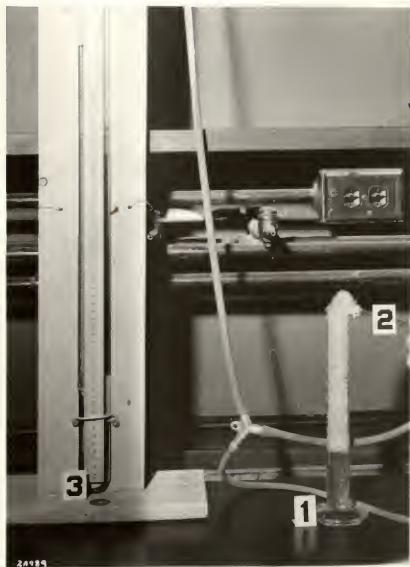
#### EXPLANATION OF PLATE I

Apparatus used to test the effect of saliva, plant and animal mucins, and several other substances, on the foam produced by alfalfa saponin.

1. Graduated cylinder (100 cc.) containing 2 ml. alfalfa saponin solution (1 gm. in 50 ml. distilled water) in 20 ml. distilled water; showing the foam produced by aeration of solution.
2. A glass tube bent at one end and finely tipped at the other.
3. A water manometer.



## PLATE I



ing antifoaming activity were prepared in distilled water at room temperature. Saliva, obtained from a cow was used undiluted and linseed meal mucin was extracted according to the procedure described below. Substances used in this experiment; concentration of their stock solutions; their solubility; and the source of supply are given in Table 1. Animal mucin from Swift and Company (hereafter referred to as animal mucin-S), a by-product extracted from bones and cartilages in the preparation of glue and another animal mucin from Thompson-Hayward Company (hereafter referred to as animal mucin-T), extracted from the fundic part of hog stomachs, were tested for antifoaming properties after subjecting their stock solutions to further treatments.

The different treatments tested are summarized as follows:

1. Decanted portions of the stock solutions.
2. Filtrates of the stock solutions of the substances found effective in the preceding test (except saliva and linseed mucin).
3. Filtrates (at room temperature) and residues of the stock animal mucin-S solution using filter papers of different porosities.
4. Supernatants of the centrifuged stock animal mucin-S solution using different speeds of centrifugation ranging from 5,000 r.p.m. to 40,000 r.p.m.
5. Top, middle and bottom layers of the solutions obtained from prolonged and successive centrifugation of the stock animal mucin-S solution. The stock solution was either decanted or filtered (by suction through a Buchner funnel). The filtrate and supernatant then were centrifuged to yield top, middle, and bottom layers. Details of the different treatments mentioned above will be discussed under experimental design.

#### Extraction of Linseed Mucin

Linseed mucin was extracted in the laboratory according to the procedure described by Mason and Hall (1948) with minor alterations. Linseed meal (12.5 gm.) was added with vigorous agitation to 500 ml. of distilled

Table 1. Concentration of substances used to test antifoaming ability against alfalfa saponin foam, their solubility in water, and source of supply.

Test substances	Concentration of stock solutions		Solubility	Source
	Quantity of test substance	Water (ml.)		
Linseed mucin	12.5 gm.	500	high	Extracted in the lab.
Irish moss	12.5 gm.	500	very low	Extracted in the lab.
Peyllium mucilloid	5.0 gm.	100	insoluble	G. D. Searle and Co.
Acacia gum	5.0 gm.	100	low	American Cyanamid Co.
Locust bean gum	5.0 gm.	200	low	American Cyanamid Co.
Arrow root	5.0 gm.	100	low	American Cyanamid Co.
Bovine saliva	undiluted	--	--	Collected from a fistulated cow
Fluid mucin-S	20 ml.	100	low	Swift and Co.
Animal mucin-S	5.0 gm.	100	very low	Swift and Co.
Animal mucin-T	5.0 gm.	100	very low	Thompson Hayward and Co.
Intestinal mucin	5.0 gm.	100	very low	Thompson Hayward and Co.
Bentonite	10.0 gm.	500	low	Thompson Hayward and Co.
Silicone defoamer (Antifoam B)	10 ml.	100	fair	Dow Corning
Silicone defoamer (Antifoam C, 120 B)	10 ml.	100	fair	Dow Corning
Dimethyl silicone + Procaine Penicillin (Stop Bloat)	5.0 gm.	100	fair	Specifide, Inc.

water at 60° C. containing 2.5 gm. of sodium chloride. The temperature was maintained at 60° C. at all times as too low a temperature retarded extraction while too high a temperature degraded the product. The pH of the mixture was maintained between 7.0 and 7.2 during the extraction period by the addition of sodium bicarbonate. As the extraction proceeded, the mixture became more acidic, and it was necessary to add small amounts of the alkalinizing agent from time to time to neutralize the mixture. Extraction was continued for six hours. The supernatant liquid was decanted and was used as the linseed mucin solution in these studies.

#### Collection of Saliva

The apparatus used for the collection of saliva consisted of a 50 ml. conical pyrex centrifuge tube with 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 rubber teat cup inflation. The upper portion of the teat cup inflation was cut off before inserting the centrifuge tube so that the rubber covered only the cone portion of the tube. The end of the teat cup inflation was joined to a four foot piece of rubber tubing through a three inch piece of nine mm. glass tubing. A Y-shaped cast aluminum connecting tube served to join this tube to another four foot rubber tube which led to a glass tube inserted through a No. 8 rubber stopper in a 2 l. vacuum flask. Rubber vacuum tubing connected the vacuum flask to a milking machine vacuum pump. A trap was placed between the vacuum flask and the vacuum line. A fistulated cow was used for the collection of saliva. The rumen contents were removed and the centrifuge tube was inserted through the oardia into the posterior part of the esophagus so that the vacuum might draw saliva into

the 2 l. collecting 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.

#### Animals Used

Two pairs of identical twin cows were used in this study. Each animal had a permanent rumen fistula fitted with a plastic cannula and cap. One pair were five-year old Holstein cows (15 and 16) and the other pair were five-year old Jersey cows (81 and 82). The cows were grazed on alfalfa pasture a few days prior to treatment. During the treatment period the cows were kept in the dry lot in the afternoon and night without feed and pastured on alfalfa in the morning. Drinking water and salt were available to the cows while in the dry lot. When treatments were administered, one animal of each pair received treatment and her twin served as the control.

#### EXPERIMENTAL DESIGN

##### Effect of Saliva, Mucins and other Substances on Saponin Foam

Saliva, linseed mucin, mucins from animal sources, and some other substances were tested in vitro for antifoaming properties. Aliquots of solutions of these substances were added to the alfalfa saponin solution in a 100 ml. graduated cylinder. The solution so formed was aerated at a uniform rate through the glass tip one inch from the bottom of the cylinder. 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. Aeration of the alfalfa saponin solution above served as a control. The series of

experiments conducted in testing different substances are described below:

1. In the first study fractions of saliva and of the decanted portions of the stock solutions of other substances were used for determining their efficacy in combating saponin foam.
2. Stock solutions of the substances in treatment No. 1, exhibiting some antifoaming properties were filtered through Whatman No. 12 filter paper. Filtrates thus obtained were tested further for antifoaming activity. Saliva and linseed mucin were not included in this test.
3. Animal mucin-S solution found most effective in treatment No. 2 was filtered at room temperature through filter papers of different porosities. The types of filters used were: Seitz filter and Whatman papers number 1, 12, 41, and 42. Both the filtrates and the liquid residues were examined for antifoaming properties.
4. The supernatant portion of the stock animal mucin-S solution was decanted and centrifuged for 30 minutes at several speeds ranging from 5,000 r.p.m. to 40,000 r.p.m. Supernatants obtained were tested.
5. It was apparent from treatment No. 4 that there was a tendency for the formation of three distinct layers of solution after centrifugation: top thin layer was greyish and fat-like; a clear middle layer comparatively larger than the top one; and a large, turbid bottom layer. In this treatment, an attempt was made to separate these layers by ultracentrifugation, and test them individually for activity. The stock animal mucin-S solution was divided in two parts. Part 1 was filtered by suction through a Buchner funnel, the filtrate was centrifuged at 2,000 r.p.m. for 20 minutes and the top thin layer was removed by floatation with a similarly treated mucin solution. Part 2 was decanted to obtain the supernatant. The supernatant was then treated similarly as the filtrate obtained in part 1.

Solutions obtained from parts 1 and 2 were centrifuged for 30 minutes at 30,000 r.p.m. The supernatants obtained in each case were separated in two portions with a pipette, one (A) having the top and middle layers and the other (B) the bottom layer. The bottom layers (B) in both cases were not treated further and were kept for testing. The top and middle layers (A) from parts 1 and 2 were divided into two portions, keeping one-half for the test, and subjecting the other half to centrifugation at 40,000 r.p.m. for one hour. From the supernatants three layers could be clearly differentiated



as top, middle and bottom. These were separated into two portions (C and D) in each case. Portion C contained the top and bottom layers and D the middle layer.

A portion of A from both parts 1 and 2 was centrifuged at 40,000 r.p.m. for three hours. The supernatants obtained were designated as E in each case. At the end of these treatments each of the original parts 1 and 2 yielded portions A, B, C, D, and E which were used for the antifoaming tests. A brief outline of the various steps taken to arrive at these solutions appears in Table 2.

#### Effect of Changes in Temperature and pH on the Antifoaming Activity of Animal Mucin-S Solution

A Beckman potentiometer was used for the determination of the pH of animal mucin solutions. The following mucin solutions were tested for antifoaming activity after subjecting them to temperature or pH changes.

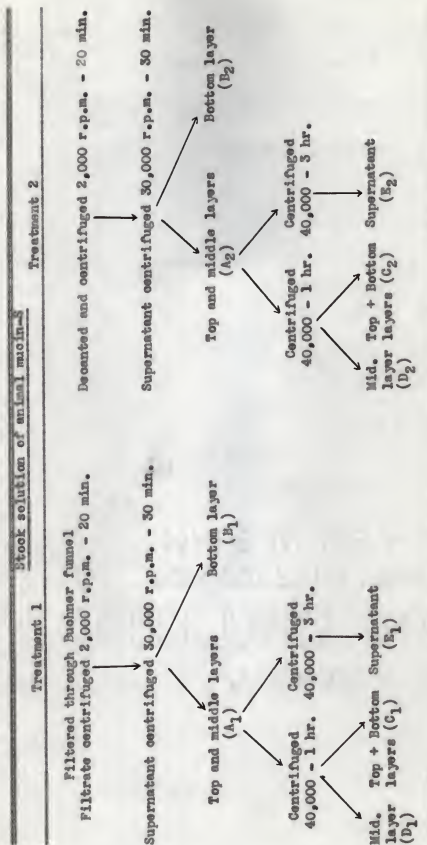
1. The stock animal mucin solution at room temperature and pH 6.4.
2. Stock solution at room temperature changed to pH 7.7 by the addition of sodium bicarbonate.
3. Stock solution heated to 110° F. (pH did not change).
4. Stock solution heated to 200° F. and again having no change in pH (most of the residue dissolved when heated to 200° F.).

#### Antifoaming Properties of Animal Mucins as Affected by Incubation with Fresh Rumen Fluid

Rumen fluid was obtained from a fistulated cow grazing on alfalfa pasture by squeezing the rumen ingesta through two layers of cheese cloth. The fluid obtained was treated as follows:

1. Ten gm. of animal mucin-S was mixed with 200 ml. rumen fluid.

Table 2. Various steps taken in separating fractions of mucin solution by centrifugation<sup>1</sup> for testing the antitumescing ability.



<sup>1</sup> Spinco Ultracentrifuge - Model L.

2. Ten gm. of animal mucin-T was mixed with 200 ml. rumen fluid.

3. Two hundred ml. of untreated rumen fluid served as control.

All the three samples were incubated at 40° C. in an incubator and tested for antifoaming activity at three hour intervals during a twelve hour incubation period. The incubates were tested in two ways. In one, 10 ml. of incubate was diluted with 10 ml. of water in a graduated cylinder and aerated. While in the other 1.8 ml. of incubate was added to 20 ml. of water having two ml. of saponin solution. It was also aerated and the foam was measured.

#### Effect of Drug Action on Rumen Froth

Since reduced salivary secretion is suspected as one of the causative factors in bloat, trials were conducted using drugs which either stimulated or inhibited salivation. Two pairs of fistulated identical twin cows (15, 16 and 81, 82) were used in this trial. The cows were grazed on alfalfa pasture a few days prior to and during the period of treatment. The alfalfa pasture available for grazing during the period of treatment was weedy and not in itself bloat provoking. Atropine sulfate was injected subcutaneously in doses varying from 30 to 60 mg. per day (dissolved in five cc. of distilled water) into cows No. 16 and 82. Cows No. 15 and 81 served as controls. After injections in the morning, cows were grazed on pasture (for five hours) and observations made at intervals to find out whether reduced salivation caused by the action of atropine sulfate in treated animals resulted in a greater production of froth. This treatment was continued for a period of five days. The groups were reversed and treated for three days.

In another trial, using the same animals, Lentin<sup>1</sup> was injected subcutaneously to cows 16 and 82 to determine if increased salivation resulting from the influence of the drug would reduce the amount of froth produced in the rumen. The condition of alfalfa pasture during this period was bloat-provoking. Treatment was continued for three days using a dose of 4 cc. per day. The animals were injected in the morning before being turned out to pasture. The period of grazing was five hours.

## RESULTS

### Effect of Saliva, Mucin, and Other Substances on Saponin Foam Production

A number of substances were tested for their antifoaming action on the foam produced by alfalfa saponin. The effect of the substances tested on saponin foam is shown in Table 3 and Fig. 1. The volume of foam produced by the saponin solution after the addition of the various test substances was compared with the volume of foam produced by the alfalfa saponin solution alone. Saliva, linseed mucin, animal mucin-S, intestinal mucin, and animal mucin-F, though varying in their effectiveness, appeared to inhibit definitely saponin foam production. Dow Corning antifoam B and Dow Corning C. 120 B, totally prevented saponin foam formation, whereas Stop Bloat had only a slight effect. Other substances tested did not exhibit any antifoaming activity on saponin foams.

Saliva and linseed mucin solution affected the stability of foam. Saponin alone formed a stable, viscous foam that rose up in the cylinder and was very slow in breaking down when aeration of the solution was

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<sup>1</sup> The trademark of Merck and Co., Inc., Rahway, N. J. for the drug carbachol.

Table 3. Antifoaming activity of various substances on alfalfa saponin foam.

Test substances	Quantity of stock soln. added to saponin soln. (ml.)							
	0	0.2	0.6	1.8	1	2	4	8
	Amount of foam produced <sup>1</sup>							
	(ml.)							
Bovine saliva	90				30	5	7	8
Linseed mucin	90				88	64	32	28
Irish moss	80				80	82	82	86
Bentonite	80				42	58	64	66
Arrow root	80	86	86	84				
Psyllium mucilloid	80	50	45	40				
Fluid mucin-S	80	80	84	86				
Animal mucin-S	80	0	0	0				
Animal mucin-T	80	10	5	2				
Intestinal mucin	80	50	46	24				
Acacia gum	56	50	54	74				
Locust bean gum	56	60	55	48				
Silicone defoamer (Antifoam B)	90	0	0	0				
Silicone defoamer (Antifoam C, 120 B)	90	2	0	0				
Dimethyl silicone + Procaine Penicillin (Stop Bloat)	90	68	64	55				

<sup>1</sup> Each value is an average of three replicates.

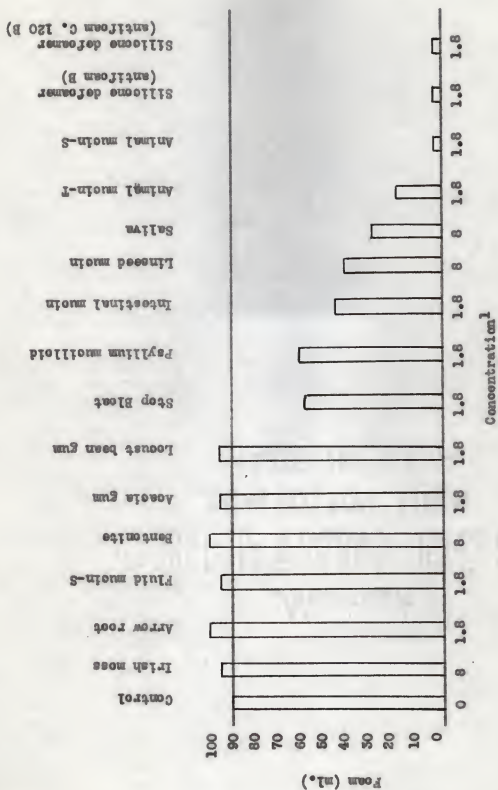


Fig. 1. Antifoaming activity of various test substances on alfalfa saponin foam (only concentrations exhibiting maximum activity shown).

1 Concentration: milliliters of stock solution added to saponin solution.



stopped. However, the foam formed when saliva or linseed mucin solution was added was unstable and broke down rapidly when aeration of the solution was stopped. Of all the mucilaginous substances tested, animal mucin-S was the most effective in preventing the formation of saponin foam. This mucin totally prevented saponin foam formation even when added in minute quantities (0.2 ml.).

#### Effect of Filtration of Mucin Solutions on Saponin Foam Production

Stock solutions of two animal mucins-S and T and intestinal mucin found effective in the previous study, were filtered (without suction) through Whatman No. 12 filter paper. Small quantities of filtrates obtained, as well as the liquid residues were tested for their antifoaming activity (Table 4). The filtrates did not exhibit any effect on saponin foam production, whereas the residues maintained the antifoaming activity. It appeared that the active ingredient(s) responsible for the antifoaming activity did not pass through the filter paper.

Filtrates of the animal mucin-S solution obtained after filtration at room temperature through filter papers of varying porosities were also found devoid of antifoaming activity. Results of the effect of filtrates as well as liquid residues on saponin foams are given in Table 5. Here, again, residues were found to retain the antifoaming property.

#### Effect of Centrifugation of Animal Mucin<sup>1</sup> Solution on Saponin Foam Production

The effects on saponin foams of the supernatants obtained from cen-

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<sup>1</sup> Swift and Co.

Table 4. Effect of filtration<sup>1</sup> of mucin solutions on saponin foam.

Quantity of filtrate: or liquid residue: added to saponin solution (ml.)	Animal mucin-F		Foam produced (ml.)		Animal mucin-S	
	Filtrate:	Liquid residue:	Filtrate:	Liquid residue:	Filtrate:	Liquid residue:
0						80
0.2	85	10	85	55	60	0
0.6	75	5	85	45	37	0
1.8	75	2	75	25	25	0

<sup>1</sup> Whatman No. 12<sup>2</sup> Foam produced by bubbling air through a solution containing 20 ml. water and 2 ml. saponin solution.

Table 5. Effect on saponin foams of filtrates of animal mainin-S stock solution using filters of different porosities.

Filter	Porosity of filter	Quantity <sup>1</sup> added to saponin solution (ml.)	Foam (ml.)	
			Filtrate : Liquid residue : Control <sup>2</sup>	
Seitz	Fine, bacterial filter, used with vacuum.	0.6 1.8	80 78	2 0
Whatman - 41	Very rapid	0.6 1.8	80 73	0 1
Whatman - 42	For finest pyts.	0.6 1.8	85 85	0 0
Whatman - 12	Rapid filter	0.6 1.8	85 90	1 0
Whatman - 1	Moderately rapid	0.6 1.8	90 86	0 0

<sup>1</sup> Quantity of filtrate or liquid residue added to control saponin solution.

<sup>2</sup> Foam produced by bubbling air through a solution containing 20 ml. water and 2 ml. saponin solution.

trifugation at several speeds ranging from 5,000 r.p.m. to 40,000 r.p.m. are shown in Table 6. The supernatants were effective in preventing saponin foam formation to approximately the same extent as exhibited by the decanted solution in the first study. Apparently the active ingredient(s) did not leave the supernatant to any appreciable extent.

In the above mentioned trial there was a tendency for the supernatants to separate into three distinct layers during high speed centrifugation. Though the three layers could not be distinctly separated for individual testing, sufficient material was obtained from the three layers for preliminary testing. It was observed that the lower (viscid) layer had comparatively greater antifoaming activity than the other layers. By prolonged and successive centrifugation it was found that the upper two layers retained only a little activity, while the lower layer possessed most of the activity. Upon further centrifugation of the middle layer again three layers were differentiated. Of these the lower layer exhibited antifoaming activity while the upper and middle layers had only a little activity (Table 7). From this study it may be concluded that the active ingredient(s) in the mucin solution responsible for the prevention of saponin foam is a suspensoid or colloid in nature which has a tendency to settle to the bottom of the solution on prolonged centrifugation.

#### Effect of Change in Temperature and pH on the Antifoaming Activity of Animal Mucin Solution<sup>1</sup>

The pH of the original animal mucin solution was 6.4. No change in antifoaming activity was observed when the solution was made slightly al-

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<sup>1</sup> Swift and Co.

Table 6. Effect of the supernatants obtained from centrifugation of stock animal moin-S solution on saponin foam.

Quantity of supernatant added to saponin solution :	Speed of centrifugation (r.p.m.) :				
	5,000 : 10,000	15,000 : 20,000	25,000 : 30,000	35,000 : 40,000	Control <sup>1</sup>
	Foam				
(ml.)	(ml.)				
0					100
0.6	0	0	1	2	0 1
1.8	0	0	0	0	0 0

<sup>1</sup> Foam produced by bubbling air through a solution containing 20 ml. water and 2 ml. saponin solution.

Table 7. Effect of prolonged and successive centrifugation of stock animal main-S solution on saponin foam.

Quantity of supernatant added to saponin solution :	Supernatants obtained from treatments 1 and 2 (Table 2)										Control <sup>1</sup>
	A <sub>1</sub>	B <sub>1</sub>	C <sub>1</sub>	D <sub>1</sub>	E <sub>1</sub>	A <sub>2</sub>	B <sub>2</sub>	C <sub>2</sub>	D <sub>2</sub>	E <sub>2</sub>	
	Foam										
(ml.)	(ml.)										
0											100
1.8	74	4	12	94	84	74	2	24	74	85	

<sup>1</sup> 20 ml. water + 2 ml. saponin solution.



kaline (pH 7.7) by the addition of sodium bicarbonate (Table 8). The antifoaming activity also was not altered on heating the mucin solution to 110° F. or to 200° F. There was no appreciable change in the pH of the mucin solution when heated to these temperatures.

#### Antifoaming Properties of Animal Mucins as Affected by Incubation with Fresh Rumen Fluid

The antifoaming properties of animal mucins were not affected by incubation with rumen fluid (Table 9), and were maintained at the same level throughout the 12 hr. incubation period.

#### Effect of Drug Action on Rumen Froth

For the sake of convenience in expressing the difference in degree of foaming and bloating in the experimental animals, the system of classification described below was adopted.

- 0 - No foam
- 1 - Mild foam
- 2 - Low foam
- 3 - High foam
- 4 - Mild bloat
- 5 - Moderate bloat
- 6 - Severe bloat

In the first trial, injections of atropine sulfate were given to even-numbered (16-82) cows of each twin pair for a period of five days, followed by a reversal of the treatments for three days. The degree of foaming observed in the treated and non-treated cows is shown in Table 10.

No clear-cut difference in foaming was observed between the treated

Table 8. Effect of change in temperature and pH on the antifoaming activity of animal mucin-S solution.

Treatment of deoanted animal mucin-S solution	Temperature	pH	Quantity added to saponin solution	Foam <sup>1</sup> (ml.)	Control <sup>2</sup> (ml.)
None	80° F.	6.4	0.2 0.6 1.8	10 3 0	80
pH adjusted to 7.7 by the addition of sodium bicarbonate	80° F.	7.7	0.2 0.6 1.8	0 0 0	
Heated to 110° F.	110° F.	6.3	0.2 0.6 1.8	10 2 0	
Heated to 200° F.	200° F.	6.4	0.2 0.6 1.8	10 2 0	

<sup>1</sup> Foam produced on bubbling air through the solution.

<sup>2</sup> 20 ml. water + 2 ml. saponin solution.

Table 9. Antifoaming activity of animal mucins-S and T as affected by incubation at 40° C. for 12 hours with fresh rumen fluid.

Incubate	Treatment <sup>1</sup>	Period of incubation (hr.)					Foam <sup>2</sup>	(ml.)
		1	3	6	9	12		
		:	:	:	:	:		
10 gm. animal mucin-S in 200 ml. rumen fluid	1		0	0	0	0	0	0
	2		0	0	0	0	0	0
10 gm. animal mucin-T in 200 ml. rumen fluid	1		40	30	30	30	20	20
	2		4	4	5	10	10	10
200 ml. rumen fluid (control)	1		0	0	0	0	0	0
	2		90	85	40	30	30	30

<sup>1</sup> Treatment: 1 = 10 ml. incubate + 10 ml. water.

2 = 1.8 ml. incubate + 20 ml. water + 2 ml. saponin solution.

<sup>2</sup> Foam produced by bubbling air through the solutions.

and non-treated twin-mates on the first day of treatment using a 50 mg. dose of atropine sulfate. Mild foaming was visible in all the four cows. A marked difference was observed on the second day of the treatment when one of the treated cows (No. 16) developed severe bloat and the other (No. 82) had a high degree of foaming. The controls (15 and 81) developed only a mild foam. Since on the second day the treated cows (16 and 82) exhibited some uneasiness and nervousness due to the effect of the drug, it was decided to reduce the dose of atropine to 30 mg. on the next day. This reduced dose did not appear to be active since there was no difference in foaming among the treated and untreated animals. All animals exhibited a mild foam. The dose of 50 mg. was resumed on the fourth day. A clear difference in foaming again was observed; those treated (16 and 82) had a high foam and the controls had only a low foam. Approximately similar results were observed on the fifth day of the treatment and on the next three days of the reverse treatment, with the exception of cow No. 82 that continued to develop high foam and bloat on the first two days of the reversal period. The condition of cow No. 82 did not permit grazing her on pasture any more. She was moved to a dry lot on the third day of the reversal period. The possible cause of continued bloat in cow No. 82 could have been the result of a carry-over effect of the drug or the establishment of a bloat producing rumen microflora.

Considering the results of the two twin-pairs separately, the Holstein twin pair (15 and 16) exhibited an appreciable difference in the development of foam between the control and atropine treatment. This difference was apparent throughout the trial, except on the first day when the treatment was started and the third day when the dose was reduced from 50 mg. to 30 mg. Similar results were observed in the Jersey twin pair (81 and

Table 10. Effect of atropine sulfate on the degree of foaming of rumen ingesta of cows grazing alfalfa pasture.

Date	Cow <sup>1</sup> No.	Quantity of atropine sulfate administered subcutaneously	Degree of foaming and bloating <sup>2</sup>
(mg.)			
5/2/59	15	0	1
"	16	50	1
"	81	0	1
"	82	50	1
5/3/59	15	0	1
"	16	50	6
"	81	0	1
"	82	50	3
5/4/59	15	0	1
"	16	30	1
"	81	0	1
"	82	30	1
5/5/59	15	0	2
"	16	50	3
"	81	0	2
"	82	50	3
5/6/59	15	0	0
"	16	60	2
"	81	0	0
"	82	50	3
5/7/59(Reversal)	15	50	3
"	16	0	1
"	81	50	5
"	82	0	5
5/8/59	15	50	3
"	16	0	0
"	81	50	3
"	82	0	5
5/9/59	15	60	3
"	16	0	2
"	81	50	3
"	82	0	-
(Removed from the expt.)			

<sup>1</sup> Pairs of identical twins (15-16 and 81-82).

<sup>2</sup> Degree of foaming and bloating; 0 = no foam; 1 = mild foam; 2 = low foam; 3 = high foam; 4 = mild bloat; 5 = moderate bloat; 6 = severe bloat.

82), with the exception of a possible carry-over effect in No. 82, which appeared to have developed a tendency for continued bloating. In general, the rumen ingesta of the treated cows who were under the influence of the antisialagogue action of atropine sulfate appeared to foam to a greater degree than the non-treated ones.

In another trial of three days' duration, using the same fistulated twin cows, injections of 4 cc. of Lentin were given daily to odd-numbered cows (15 and 81) before grazing them on alfalfa pasture in the morning. Observations of the development of foam are shown in Table 11. The results of the Holstein twin pair (15 and 16) showed that Lentin had some foam preventing effect. However, the Jersey twin pair (81 and 82) developed a high degree of foam during all three days of treatment. The animals salivated profusely for a few minutes after being injected with Lentin. However, it is possible that the effect of Lentin is too short-lived to add sufficient saliva to the rumen to prevent foaming. The results, as a whole, do not indicate that Lentin by accelerating the secretion of saliva has any preventive effect on the development of foam.

#### DISCUSSION

Water, saliva, microorganisms, food and its breakdown products are the main components of the ruminal contents. There is always a quantitative and qualitative variation in these components (excluding water) in the rumen. Microbial fermentation is the main function of the rumen and "foaming" is a property of fermentation. It is the quality of food and its breakdown products along with an interaction of these with other components of ruminal contents that determine whether the foam produced is stable or non-stable. If it is non-stable it disappears readily without any harm to



Table 11. Effect of Lentin on the degree of foaming of rumen ingesta of cows grazing alfalfa pasture.

Date	Cow <sup>1</sup> No.	Quantity of carbachol administered subcutaneously (cc.)	Degree of foaming and bloating <sup>2</sup>
5/12/59	15	4	0
"	16	0	2
"	81	4	4
"	82	0	2
5/13/59	15	4	0
"	16	0	2
"	81	4	4
"	82	0	4
5/14/59	15	4	4
"	16	0	6
"	81	4	4
"	82	0	6

<sup>1</sup> Pairs of identical twins (15-16 and 81-82).

<sup>2</sup> Degree of foaming and bloating: 0 = no foam; 1 = mild foam; 2 = low foam; 3 = high foam; 4 = mild bloat; 5 = moderate bloat; 6 = severe bloat.

the animal; if stable it may cause bloat.

Bloat, especially the frothy type, is observed more frequently in cows fed green legume plants than on any other feed. However, stable foam and bloat are not always observed in cows fed green legumes. This indicates that either the compound(s) responsible for stabilizing the foam is not always present in sufficient quantity in a particular feed, or, its effect is neutralized by foam inhibiting compound(s) that might be present in the ruminal contents. It seems more likely that there are present in the rumen, compounds that favor stabilization of foam as well as the compounds which have an antagonistic effect on foam production. If, during the complex chemical interaction and physical association of different compounds in the rumen, the compounds which stabilize foam, under the influence of their increased quantity or potency, overpower the effect of compounds which prevent stabilization of foam, bloat will result.

Saliva, being an important component of ruminal contents is likely to have properties which either aid or prevent the stabilization of foam. Weiss (1953) suggested that salivation may be an important factor in the prevention of bloat. Van Horn (1959) has shown that the addition of saliva to frothing rumen contents increases the release of gas trapped in the froth. He also observed that saliva had an antifoaming activity on saponin foam. The results presented herein on the effect of saliva on saponin foams support the latter finding of Van Horn and indicate that reduced salivary secretion in the presence of compounds that aid in the formation of stable foam may be an important factor in bloat production.

The fact that bloat is not produced as frequently when cows pasture succulent grass as when they pasture succulent legumes would indicate that

saliva is not the only factor involved. 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 a decrease in the antifoaming factor.

Bailey (cited by Johns, 1958) reported a 75 percent decrease in the amount of saliva secreted while cows were eating succulent grass as compared to dry hay. If mucin in saliva is the agent which gives saliva its antifoaming property and its ability to release trapped gas from frothing rumen contents then decreased saliva production in cows eating succulent legumes would be critical. Mucin is produced by the submaxillary and sublingual salivary glands and their activity is greatest during mastication (Dukes, 1955).

Bartley (1957) reported that the number of bloat cases and the severity of bloat in cows grazing alfalfa was only slightly lower among cows fed linseed meal as a source of mucin than among control cows. However, after a period of two to three hours after removal of the cows from pasture 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 antifoaming effect of linseed meal mucin thereby causing trapped gas in froth to be released. The work in this thesis has shown that saliva and mucins from linseed meal and animal sources when added to a saponin solution reduced the amount and stability of foam. A mucin from an animal source (animal mucin-S) was found the most effective in preventing the formation of saponin foams even when used in very low concentrations. The centrifugation of animal mucin-S dissolved in water

showed that the active ingredient(s) was not soluble in water but remained in the mixture as a colloid or suspensoid. The effectiveness of mucin from linseed meal and from animal sources in the prevention of saponin foams would indicate that mucin in saliva might be the agent responsible for the antifoaming property of saliva.

According to others, saliva is considered to increase rumen froth. Johns (1956) considers that the low surface tension of bovine saliva is a factor that could contribute to foaming by lowering the surface tension of rumen ingesta. In support of Johns hypothesis Magan (1959), reported that mixed bovine saliva when aerated formed a very stable foam. However, Weiss (cited by Nichols, 1959) believes that despite its own froth stability, saliva assists in reducing the stability of digesta froth. Van Horn (1959) showed that large amounts of saliva did not release as much gas trapped in frothing rumen ingesta as did smaller amounts of saliva. Also, in the study reported here, it was found that saliva when aerated formed a large and stable foam but when added in small quantities to saponin solution prevented, to a considerable extent, the development of foam. It would appear that a normal flow of saliva might be necessary for the prevention of stable foam in the rumen, whereas a reduced flow and probably also an increased flow might not successfully prevent frothy bloat. An increased flow of saliva might enhance the formation of stable foam in the presence of foaming compounds, whereas a reduced flow might not be able to counteract foam stabilizing compounds.

Phillipson and Reid (1958) and Johns (1958) believe that mucoproteins present in saliva cause the formation of a stable foam. Whereas Blake et al. (1957) reported that saliva actually increases the surface tension

of rumen fluid. It would seem unlikely that saliva would contribute to foaming by an effect on surface tension. However, Reid and Huffman (1949) have shown that bovine saliva has a surface tension much lower than water when measured at the same temperature. Nichols (1959) reported that an increased surface tension and viscosity, and a decreased density and pH were some of the major physical differences in digesta encountered when bloat-provoking fresh legumes were fed. It appears from some of these results that the question of surface tension of saliva, its relationship with the surface tension of digesta and how it effects the development of foam is still not very clearly understood.

Van Horn (1959) reported that animal mucin-S controlled foaming in vivo for a two hour period and thereafter the effect diminished. No explanation was given for the failure of mucin to prevent foaming for more than two hours. Bartley (unpublished, 1959) found that animal mucin-S in doses (80 gm. per cow per day) that were larger than those used by Van Horn (40 gm. per cow per day) prevented foaming in vivo for periods varying from three to four hours. Although foaming started to develop after three or four hours of grazing, bloat did not usually occur for another two hours. Controls under similar conditions developed foam and bloat much sooner. These studies indicate that animal mucin-S has a marked antifoaming effect in vivo also, but the effect disappears or decreases after a few hours. The loss of effectiveness of mucin in vivo may be due to mucinase in the rumen or the localization of the effect of mucin when the rumen is full.

The work reported here showed that the antifoaming effect of animal mucin-S when incubated in vitro with rumen fluid did not disappear in 12 hours. Although the results of this work do not explain the conditions

existing in vivo they suggest a course for further study. When the cause of the destruction of mucin in vivo has been determined, a method to prolong its effect in preventing the formation of rumen froth should be forthcoming.

The administration of drugs which either increase or decrease salivary flow were tested for their effect on the production of intrarumen froth in animals grazing alfalfa pasture. Atropine injected subcutaneously to reduce saliva production appeared to increase intrarumen froth. The injection of Lentin to increase salivary flow did not exhibit an effect on froth production. The drugs, atropine sulfate and Lentin, while possessing antisialagogue and sialagogue action respectively, also effect other parts and organs of the body. These two drugs are known to affect motility and secretions of the digestive organs. Thus, the use of these drugs give rise to an abnormal condition of the animal by their action on several organs and it is difficult and unwise to interpret the results on the basis of their action on the salivary glands alone. Drugs having a localized effect on the salivary glands to meet the conditions desired are probably not known. The condition of increased salivation in an animal may, to some extent, be duplicated by the administration of saliva obtained from another animal. However, the addition of saliva from another animal at periodic intervals might not parallel the normal salivary flow and its subsequent effect on the physical state of the rumen ingesta.

#### SUMMARY

The effectiveness of bovine saliva, linseed mucin, mucins from animal sources, and some other compounds as antifoaming agents against foams pro-



duced by alfalfa saponin was tested in vitro. A mucin (animal mucin-S) from an animal source was tested in some detail. Its solution was submitted to filtration, centrifugation and variations in temperature and pH. These solutions were tested for antifoaming properties. The antifoaming activity of animal mucin-S as affected by incubation with fresh rumen fluid at 40° C. was tested. Atropine and Lentin were administered to animals to see if the stimulatory or inhibitory effect on salivation would have an effect on the froth produced in the rumen.

Saliva, linseed mucin, mucins from animal sources and three commercial silicone antifoaming preparations when added to an alfalfa saponin solution markedly reduced the amount and stability of foam produced when air was bubbled through the solution. Of all the mucilaginous compounds tested, animal mucin-S was the most effective in preventing the formation of saponin foam produced by aeration. Animal mucin-S was only slightly soluble in water. When this mucin solution was filtered, the antifoaming property did not appear in the filtrate and was apparently retained in the filter paper. When the mucin solution was centrifuged, the fraction containing the antifoaming activity settled down in the bottom of the tube. This indicated that the fraction responsible for the antifoaming activity of animal mucin-S is not soluble in water but exists as a colloid or suspensoid. Slight variations in pH and temperature of the animal mucin solution did not affect its antifoaming activity. The antifoaming activity of animal mucin was not altered when the mucin solution was incubated at 40° C. with fresh rumen fluid for 12 hours. Since the antifoaming activity appears to be unaffected by the action of rumen bacteria, it would appear that mucin has the bloat-preventing potential for extended

periods of time in animals grazing legume pasture.

✓ Atropine injected subcutaneously into animals to inhibit salivation resulted in an increased intrarumen foam. The injection of carbachol to stimulate salivation was not effective in controlling intrarumen foam.

## ACKNOWLEDGMENT

The author wishes to express his sincere gratitude to Dr. E. E. Bertley for his guidance and assistance in the planning of this investigation, the preparation of this thesis, and the helpful suggestions throughout graduate study.

The author is grateful to Dr. C. L. Norton, Head of the Department, for providing facilities in carrying out the investigation.

Appreciation is also expressed to Dr. R. E. Clegg of the Chemistry Department for providing facilities for ultracentrifugation of the mucin solutions.

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EFFECT OF BOVINE SALIVA, MUCIN, AND SEVERAL ANTIFOAMING AGENTS  
ON ALFALFA SAPONIN FOAMS ASSOCIATED WITH BLOAT

by

INDRAJIT SINGH YADAVA

B. V. Sc., Panjab University, India, 1949

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AN ABSTRACT OF A THESIS

submitted in partial fulfillment of the

requirements for the degree

MASTER OF SCIENCE

Department of Dairy Husbandry

KANSAS STATE UNIVERSITY  
OF AGRICULTURE AND APPLIED SCIENCE

1960

Most investigators agree that the formation of a stable intrarumen foam is the primary cause of bloat when cattle graze legume pastures. It has been recognized that there is a marked decrease in the amount of saliva secreted by animals grazing succulent pasture. Some workers attribute foam production to the decreased salivary flow. However, other investigators are of the opinion that saliva enhances stable foam formation. If additional salivation is necessary to prevent frothy bloat it might be conjectured that the antifoaming activity of saliva is due to mucin since this compound constitutes the major portion of the organic matter present in saliva.

The work reported herein was initiated to determine the effect of saliva and mucin on the formation of foam. Saponin, a surface-active compound obtained from alfalfa and suggested to be one of the possible factors associated with the formation of stable froth in legume bloat, was used as a foaming agent in in vitro studies.

The effects on saponin foam of saliva, four mucins from animal sources and several mucilaginous plant materials (linseed meal, acacia gum, Irish moss, arrow root, psyllium seed and locust bean gum) were tested in vitro. Also bentonite and three preparations containing silicone were tested. Saliva or the solutions of the substances listed above were added at various levels to an alfalfa saponin solution (40 mg. saponin in 22 ml. water) in a 100 ml. graduated cylinder. Aeration of the control saponin solution produced a stable froth. The addition of saliva or linseed mucin solution to the saponin solution markedly reduced the production and stability of foam. Solutions of animal mucins and the silicone preparations prevented foam formation. The other substances tested did not exhibit an antifoaming effect and some of them promoted foaming.

An animal mucin<sup>1</sup> found most effective in the above test was examined further by subjecting its solution to filtration, centrifugation, and variations in temperature and pH. Filtrates obtained by filtration of the stock mucin solution through a Seitz filter and Whatman filter paper did not show any antifoaming activity. The liquid residues from all of them effectively prevented the formation of saponin foam. Supernatants obtained after centrifugation of the stock solution (5,000 to 40,000 r.p.m.) retained the antifoaming activity. The supernatant was separated into three layers by prolonged and successive ultracentrifugation. The antifoaming activity was present only in the lowest layer.

The pH of the stock animal mucin solution was 6.4. The antifoaming activity was not affected when the pH was adjusted to 7.7 by the addition of sodium bicarbonate. The solution of animal mucin retained its antifoaming activity on heating to 200° F. Ten grams of animal mucin when incubated at 40° C. for 12 hours with 200 ml. of fresh rumen fluid did not lose its antifoaming activity.

The administration of drugs which either increase or decrease salivary flow were tested for their effect on the production of intrarumen froth in animals grazing alfalfa pasture. Atropine injected subcutaneously to reduce saliva production appeared to increase intrarumen froth. The injection of Lentin to increase salivary flow did not exhibit an effect on froth production.

These studies indicate that saliva and animal mucins have a marked antifoaming effect on saponin foam. Since the animal mucins tested exhibited

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<sup>1</sup> Animal mucin - extracted by Swift and Company in the preparation of glue from bones and cartilages.



marked antifoaming activity, mucin in saliva also might have a similar effect. Further study may yield more information concerning the role of saliva in legume bloat and thus indicate suitable preventive measures.